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PhD Thesis

Approaching the industrialisation of volatile fatty acids production from urban organic wastes

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DOCTORAL THESIS

APPROACHING THE INDUSTRIALISATION OF VOLATILE FATTY ACIDS PRODUCTION FROM URBAN ORGANIC WASTES

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El nacimiento de la ciencia
fue la muerte de la superstición

Thomas Henry Huxley

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ABSTRACT

In a context where the world population is increasing, so is the generation of urban waste streams such as sewage sludge and biowaste. In recent years, the European Union's efforts have focused on promoting the circular economy model, with the aim of reducing greenhouse gas emissions associated with current production systems based on the consumption of fossil fuels. In this context, the concept of biorefinery is acquiring great relevance to replace the production methods of consumer goods with petrochemical origin by others based on renewable sources. In particular, the carboxylic platform is proposed as an interesting approach to produce volatile fatty acids, intermediate products of the anaerobic digestion process which are accumulated when the methanogenic stage is inhibited.

Volatile fatty acids are organic compounds with six or fewer carbons in their structure and have a wide variety of industrial applications in the pharmaceutical, food and chemical sectors. The main ones are acetic, propionic, butyric, valeric and caproic acid and the market size as well as the demand for all of them are growing remarkably. Considering the immediate need to fight climate change, bio-based volatile fatty acids must achieve commercialisation by overcoming technical, economic, legal and environmental barriers.

This doctoral thesis addresses the scale-up of high-purity volatile fatty acid production technology from sewage sludge and food waste. Thus, the potential of both wastes to be subjected to a fermentation process to give rise to those bioproducts is presented, which need to be further concentrated and purified to meet end users' requirements. Fermentation stage was scaled from laboratory experimentation (0.5 and 5 L volume reactors) to the pilot plant fermenter (1.5 m^3), always with the industrialisation of the technology in mind, evaluating the influence of pre-treatments and selecting the optimal operating conditions with each of the substrates tested.

Although the fermentation stage is complex due to the large number of variables which come into play, the purification and concentration of volatile fatty acids are still at an earlier stage in terms of technological maturity. This work also addresses this part, evaluating the capacity of a membrane train prior to a liquid-liquid extraction with organic solvents and subsequent distillation to generate a final product with purities like those of the acids marketed today.

All the information obtained in the experimentation made it possible to create a tool which includes all the mass and energy balances of the integral process. This tool served to identify the optimal operating conditions from a global perspective. Moreover, it allowed to calculate the amount of waste necessary to make viable the construction of a biorefinery to produce volatile fatty acids with each of the substrates, apart from assessing economically the construction of a centralised plant in the Autonomous Community of Galicia. Furthermore, through an environmental evaluation, the hotspots of the process were studied, also comparing the carbon footprint of bio-based volatile fatty acids with those produced by petrochemical routes.

By carrying out this work, it was possible to go a step further in the process of industrialisation of the technology, with special emphasis on the scaling of the fermentation process, as well as on the optimisation of the unitary operations of concentration and purification of the fermented effluent. In this way, it has been demonstrated that there is a technical feasibility to produce high-purity volatile fatty acids from these organic streams, although the pre-concentration step with membrane technology requires additional experimentation to reduce its associated costs. In any case, a high availability of substrates is needed to obtain reasonable economic indicators, being therefore important to maximise selective collection rates for biowaste.

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RESUMO

Capítulo 1. Introdución xeral

Hoxe en día, o contexto de cambio climático que estamos a vivir debido á actividade antropoxénica relaciónase coa emisión de enormes cantidades de gases de efecto invernadoiro que fan que a temperatura terrestre non deixe de aumentar. De cara a loitar contra esta realidade, a Unión Europea deseñou estratexias como o Pacto Verde Europeo que buscan reducir as emisións nun 45 % para o 2030, acadando así a neutralidade climática no ano 2050.

Tendo en conta que o sector petroquímico é responsable de arredor do 4 % das emisión globais totais, surxe a necesidade de substituír os métodos de produción de bens de consumo actuais baseados nos combustibles fósiles por outros orixinados a partires de biomasa renovable, dando lugar ás denominadas biorrefinerías.

Ao problema do cambio climático hai que engadirlle o derivado do incremento de poboación que soporta o planeta, dando lugar á xeración masiva de correntes residuais orgánicas tales como lodos de depuradora e biorresiduos.

Os lodos de depuración son un subproduto resultante dos procesos de depuración das augas residuais tanto urbanas como industriais, con gran contido en materia orgánica e nutrientes, polo que precisan dunha xestión axeitada de cara a evitar problemas ambientais importantes. Actualmente, a maior parte dos lodos xerados na Unión Europea destínase a agricultura, aínda que isto varía notablemente entre países. En canto aos biorresiduos, aínda que inclúen os restos de podas e de xardíns, pódese afirmar que case a totalidade dos mesmos son restos de comida de procedencia tanto doméstica como de plantas de procesamento de alimentos. Na actualidade, unha grande parte dos restos de comida xestiónase ben a través da compostaxe ou a través da dixestión anaerobia e aínda que a lexislación estableceu a recollida selectiva como obrigatoria antes do 2024, existen lugares nos que aínda está en proceso de implantación.

Coa aparición do concepto de biorrefinería, surxe a posibilidade de dar solución aos dous problemas citados con anterioridade mediante a xeración de bioprodutos a partires de residuos orgánicos. En concreto, os lodos de depuración e os restos de comida preséntase como substratos adecuados para producir ácidos graxos volátiles a través de procesos fermentativos, posicionándose como unha alternativa con potencial para substituír a súa produción actual (practicamente na súa totalidade baseada en vías petroquímicas).

Estes bioprodutos posúen alto valor engadido, dado que son moléculas con menos de seis carbonos na súa estrutura que gozan de infinidade de usos en diversos sectores industriais, destacando as súas propiedades como conservantes, potenciadores de sabor, antimicrobianos, etc. O tamaño de mercado destes ácidos graxos volátiles (sendo o acético, propiónico e butírico os principais) está a medrar de forma notable pola alta demanda que posúen, sendo o acético o que conta con maior volume de mercado, aínda que tamén o menos valorado. O segundo máis demandado sería o ácido propiónico, seguido do butírico, con rangos de prezos en torno aos 1.500 €/t e 2.500 €/t, respectivamente.

Desta maneira, se ditos residuos se someten a un proceso fermentativo no que se establezan as condicións de operación axeitadas para inhibir a última das etapas da dixestión anaerobia (metanoxénese), os ácidos graxos volátiles acumúlanse grazas á actividade das bacterias

acidoxénicas e acetoxénicas, debendo ser posteriormente purificados para dar lugar a solucións de ácidos individualizadas. Así, nesta tese doutoral proponse unha tecnoloxía para producir ácidos graxos volátiles de alta pureza que avalía tanto o proceso fermentativo cos substratos en cuestión como as etapas de concentración e purificación dos mesmos (mediante tecnoloxía de membranas seguida dunha extracción líquido-líquido e destilación).

Aínda que as limitacións técnicas resultan unha barreira importante a superar á hora de comercializar os ácidos graxos volátiles producidos a partires de procesos fermentativos (en particular as relativas á purificación), tamén existen barreiras económicas, ambientais e legais que afrontar. Isto é, deben ser producidos a un custo competitivo con respecto aos de orixe petroquímica, sendo necesaria tamén a implicación das autoridades públicas e administracións de cara á unificación da tan heteroxénea lexislación existente nos distintos estados membros.

Capítulo 2. Obxectivo, metodoloxía e estrutura

O obxectivo principal desta tese doutoral é presentar unha tecnoloxía para producir ácidos graxos volátiles de alta pureza a partires de correntes residuais orgánicas, en concreto lodos de depuradora e restos de comida, avaliando a viabilidade técnica e económica de construír plantas a escala real. Os primeiros capítulos céntranse na etapa fermentativa na que se producen os ácidos graxos volátiles a partires dun ou doutro substrato, mentres que despois se abordan as operacións unitarias precisas para concentrar e purificar o efluente fermentado de cara a obter o produto final. Posteriormente lévase a cabo unha integración global do proceso buscando as condicións de operación que permitan acadar os óptimos económicos, avaliando a viabilidade da potencial construción dunha biorrefinería en Galicia para tal fin. O proceso global tamén foi analizado dende o punto de vista ambiental, podendo comparar o impacto dos ácidos graxos xerados cos obtidos polas vías tradicionais.

Capítulo 3. Produción de ácidos graxos volátiles a partires de lodos de depuradora

Na actualidade, os lodos de depuración xerados nas plantas de tratamento de augas residuais sométense a un proceso de dixestión anaerobia para producir biogás, que pode aproveitarse para xerar electricidade ou satisfacer as necesidades caloríficas da instalación. Pero como se plantexou con anterioridade, este substrato tamén podería ser utilizado para producir ácidos graxos volátiles.

Durante o proceso fermentativo, a primeira das etapas denominada hidrólise adoita ser a limitante, na cal se transforma a materia orgánica particulada en compostos máis sinxelos. É por iso que, de cara a mellorar esta etapa, instaurouse no mercado o uso de diversas tecnoloxías de pretratamento tales como a hidrólise térmica. Aínda que o efecto na mellora da produción de biogás foi amplamente reportado, a influencia deste pretratamento na produción de ácidos graxos volátiles non está tan estudada.

Neste capítulo avaliouse o potencial dos lodos de depuración para a produción destes bioprodutos, comezando a escala laboratorio e continuando coa operación dunha planta piloto de 1,5 m^3 de volume de fermentador.

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A escala laboratorio, en concreto en modo batch, observouse que o lodo hidrolizado obtivo menores rendementos que o lodo crudo, sendo 8 o pH máis favorable. En cambio, na operación dos reactores continuos de 5 L de volume detectouse que os rendementos foron iguais, independentemente de utilizar lodo hidrolizado ou crudo (arredor de 15 % g DQO-AGV/g DQO substrato). Ademais, a mestura de ácidos obtida tampouco se viu modificada polo pretratamento. Esta similitude nos resultados puido comprenderse grazas á análise das comunidades bacterianas destes reactores, que mostrou como a abundancia relativa dos filos principais involucrados na fermentación foi moi similar (con *Firmicutes* cun 70 % de presenza relativa), así como o perfil predito das enzimas relacionadas coa produción dos ácidos. Estes resultados reafirmaron a similitude tanto dos rendementos como da selectividade do proceso acidoxénico independentemente de usar lodo pretratado termicamente ou non.

Tendo en conta que a hidrólise térmica non tivo influencia en modo continuo a escala laboratorio, operouse a planta piloto con lodo hidrolizado polos beneficios en canto a hixienización que aporta este pretratamento. Así, en dita operación elixiuse un pH de 8,5 e un tempo de retención hidráulico de 10 días, igual que os reactores de 5 L. Nas condicións máis favorables, isto é, aplicando unha velocidade de carga orgánica de arredor de 4,6 kg DQO/(m^3·d), obtívose un rendemento promedio de 12 % g DQO-AGV/g DQO substrato. En canto á selectividade da fermentación, o ácido acético foi o predominante con cerca do 70 % de presenza na mestura en base másica. Polo tanto, puido destacarse a estabilidade do proceso fermentativo, sendo capaz de xerar un efluente fermentado con concentracións próximas aos 10 g/L.

<u>Capítulo 4</u>. Produción de ácidos graxos volátiles a partires de restos de comida

De igual maneira que se fixo con lodo de depuradora como substrato, neste capítulo avaliouse a potencialidade dos restos de comida para producir ácidos graxos volátiles, seguindo unha metodoloxía análoga ao capítulo anterior.

Neste caso, as mostras utilizadas tentaron imitar os residuos orgánicos xerados nos fogares europeos, dando lugar a unha mestura que contiña un 50 % en peso de froitas e vexetais, un 25 % de pan e pasta e un 25 % de carne e pescado aproximadamente. En xeral, a DQO das mostras estivo arredor dos 280 g O_2/kg, cunha sequidade do 20 %.

A operación en modo batch mostrou que non houbo apenas diferenza entre os rendementos obtidos a pH 8 e 10 con aqueles a pH 6,5, polo que se elixiu operar os reactores de 5 L en modo continuo nun rango de pH entre 5,5-7 (de cara a reducir o custo en reactivos químicos necesarios dada a natureza ácida dos restos de comida), obténdose os mellores resultados a pH neutro. Ademais, viuse tamén que a velocidade de carga orgánica aplicada non tivo influencia algunha no rendemento da fermentación nin na mestura de ácidos obtida, aínda que si na taxa de produción, sendo esta maior ao aplicar unha velocidade de carga orgánica máis alta.

Na operación da planta piloto obtivéronse practicamente os mesmos indicadores de rendemento do proceso que nos reactores de 5 L, o cal proporciona grande confiabilidade á hora de escalar a tecnoloxía. En concreto, o rendemento promedio foi do 35 % g DQO-AGV/g DQO substrato, sendo a taxa de produción 3,4 g DQO-AGV/(L·d). En canto á mestura obtida, resultou máis variada que a acadada con lodo, sendo un 50 % do reparto acético, seguido de propiónico e butírico cun 20 % de presenza cada un en base másica.

<u>Capítulo 5</u>. Tecnoloxía de membranas para a concentración de ácidos graxos volátiles

Unha vez levada a cabo a fermentación e eliminada a materia particulada a través de sistemas de separación sólido-líquido, obtense un efluente no cal tamén se atopa materia orgánica soluble que non son ácidos graxos. Así, de cara a adecuar esta corrente antes de ser sometida a outras operacións unitarias, recorreuse a procesos con membranas baseados en presión co obxectivo de aumentar a concentración dos mesmos.

Neste caso, a experimentación foi levada a cabo nunha planta piloto na que se instalou un tren de membranas. Primeiramente colocouse un filtro cilíndrico con tamaño de poro no rango de microfiltración, seguido das membranas de ultrafiltración, nanofiltración e ósmose inversa.

A primeira fase da experimentación executouse con medio sintético, que consistiu na mestura de auga potable á que se lle engadiu unha concentración de 15 g/L (10 de acético, 3 de propiónico e 2 de butírico) coa finalidade de traballar nunhas condicións que ben poderían darse cos substratos testados. Estes experimentos mostraron que os ácidos graxos volátiles permearon a membrana de NF VEOLIA DK2540F30 independentemente do pH da alimentación e da presión transmembrana aplicada, obténdose caudais másicos no permeado superiores ao 80 %, sendo os fluxos de permeado máis elevados ao operar a pH 3. Ademais, ao operar con dúas membranas de ósmose inversa (RO VEOLIA AG2540TM e DOW FILMTEC SW302540) de cara a acadar resultados máis concluíntes, observouse que a recuperación dos ácidos de cadea máis longa como o butírico foi superior que para o acético, véndose o factor de concentración apenas afectado polo pH da alimentación.

Operando co mesmo medio sintético, tamén puido validarse que a presenza de nutrientes na alimentación (en concreto NH_4^+ e PO_4^{3-}) non tivo influenza no comportamento dos ácidos nas membranas cando se alimentou o permeado da nanofiltración á ósmose inversa, obténdose maiores porcentaxes de recuperación para o PO_4^{3-} que para o NH_4^+, seguramente debido ao maior tamaño da molécula. Por último, nas últimas probas con medio sintético viuse que, utilizando a membrana de ósmose inversa AG2540TM cun procedemento de doble paso (alimentación da corrente de rechazo alimentada de novo á membrana), acadáronse concentracións de ácidos de arredor de 50 g/L a pH 3. Este resultado, xunto coa redución significativa rexistrada nos fluxos de permeado a pH 5 e 7, permitiron elixir este pH para levar a cabo a experimentación con medio real.

Cando se operou o tren de membranas incorporando os catro rangos de filtración, partiuse dunha concentración de ácidos graxos volátiles de 9 g/L no sobrenadante derivado da fermentación con lodo de depuración. Ao aplicar unha recuperación hidráulica do 80 % nas membranas, acadouse unha concentración no rechazo da ósmose inversa de 21 g/L. Este experimento reportou parámetros de filtración máis favorables que ao operar con medio sintético, seguramente debido ás forzas electrostáticas derivadas do efecto matriz desta alimentación.

A experimentación con medio real permitiu determinar os fluxos de permeado de cada unha das membranas, notablemente inferiores caos rexistrados con medio sintético. Ademais, facilitou a validación da efectividade dos métodos de limpeza das mesmas, tanto hidráulicos como químicos, de forma que foi posible cuantificar de forma aproximada tanto a frecuencia das mesmas como o consumo de químicos asociado. En calquera caso, resultaría necesario

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seguir investigando de cara a optimizar esta operación e enriquecer a recompilación de datos a través de experimentos de maior duración no tempo.

Capítulo 6. Procesos de purificación

Tendo en conta que as purezas dos ácidos graxos volátiles esixidas polos usuarios finais adoitan ser superiores ao 90 %, despois da concentración dos mesmos a través de tecnoloxías de membrana é necesario someter dita corrente a operacións unitarias adicionais, por unha parte para eliminar auga e pola outra para separar os ácidos entre si. Desta maneira, propúxose utilizar a extracción líquido-líquido con disolventes orgánicos acoplada á destilación, testando dous disolventes diferentes: o acetato de etilo, con baixo punto de ebulición, e o octanoato de etilo, con alto punto de ebulición. Mentres que a extracción líquido-líquido foi validada experimentalmente, a destilación simulouse co programa ASPEN HYSYS.

De cara a avaliar a idoneidade da extracción líquido-líquido, utilizouse un medio sintético imitando as concentracións de ácidos que se obterían á saída do tren de membranas (50 g/L). Primeiramente executáronse experimentos a escala laboratorio en modo batch, que serviron para validar que o pH 3 resultou ser o que maiores porcentaxes de recuperación obtivo. Tamén puido demostrarse que a concentración inicial de partida na alimentación non tivo efecto algún na recuperación, así como tampouco a presenza doutros ácidos na mesma, descartando polo tanto o efecto matriz. Ademais, estes experimentos serviron para estudar a evolución das porcentaxes de recuperación co número de etapas, de grande utilidade á hora de deseñar satisfactoriamente a columna de extracción da planta piloto.

Unha vez avaliado o comportamento de ambos disolventes a escala laboratorio leváronse a cabo os experimentos na columna de extracción en modo contracorrente con ambos disolventes. Así, na planta piloto testáronse tres relacións disolvente/alimentación diferentes con cada disolvente, destacando a relación de proporcionalidade directa que existe entre este parámetro e as porcentaxes de recuperación. Aplicando a mesma relación disolvente/alimentación, os resultados obtidos con acetato de etilo foron mellores que con octanoato. Utilizando acetato de etilo para unha relación disolvente/alimentación de 1 kg/kg, reportáronse porcentaxes de recuperación do 70 % para o acético, 98 % para o propiónico e recuperación total para o butírico, observándose unha caída na recuperación de acético ao reducir dita relación ata 0,5, pero manténdose constantes as recuperacións dos outros dous ácidos. Desta maneira, validouse que as porcentaxes de recuperación tamén foron maiores cando se trata de ácidos de cadea máis longa. En xeral, dende o punto de vista técnico, o acetato de etilo obtivo porcentaxes de recuperación máis elevados que o octanoato, tanto a escala laboratorio como nas probas a escala piloto.

En calquera caso, observouse que as porcentaxes de recuperación en modo continuo foron inferiores que as obtidas en batch para o mesmo número de etapas, o que indicou que o comportamento de columna non foi o agardado; é por iso que se fixeron modificacións de mellora na mesma que consistiron no cambio de recheo e na instalación de redistribuidores, o que fixo que se acadaran porcentaxes de recuperación totais con acetato de etilo como disolvente.

Sendo o extracto da columna de extracción o produto de interese no cal se atopan os ácidos graxos volátiles, resulta necesaria a destilación de cara a obter produtos finais de alta pureza. Para analizar esta operación unitaria, leváronse a cabo simulacións co programa ASPEN

HYSYS, deseñando a disposición das columnas de destilación necesarias con cada un dos disolventes contemplando a súa diferente solubilidade.

Tendo en consideración as particularidades de cada disolvente, validouse a necesidade de incorporar unha destilación adicional no caso de utilizar acetato de etilo, dado que resulta preciso recuperar o disolvente perdido na corrente de refinado para recircular ao proceso. Esta destilación adicional evitaríase no caso de utilizar octanoato considerando a súa baixa solubilidade en auga, polo que as necesidades enerxéticas son menores no caso de traballar con este disolvente.

Grazas ás simulacións levadas a cabo, obtivéronse as necesidades de enerxía calorífica de cada unha das columnas de destilación propostas con cada un dos disolventes, información esencial para o cálculo dos custos de operación do proceso integral levado a cabo no Capítulo 7.

Capítulo 7. Integración global do proceso

Nos capítulos anteriores xerouse a información de interese de cada unha das operacións unitarias propostas no proceso de obtención de ácidos graxos volátiles de alta pureza. Así, os capítulos 3 e 4 aportaron información en relación aos rendementos obtidos nas fermentacións a partires de ambos substratos, así como o consumo de químicos necesarios. O capítulo 5 permitiu determinar os parámetros relacionados coa preconcentración con membranas, os fluxos de permeado acadados con efluente real así como a periodicidade e consumo de reactivos asociado á limpeza das mesmas. No capítulo 6 obtivéronse as porcentaxes de recuperación con cada un dos disolventes testados a escala piloto, así como os consumos enerxéticos asociados ás destilacións precisas en cada un dos escenarios. É por iso que, neste capítulo 7 recompiláronse todos estes datos para deseñar unha ferramenta en formato de folla de cálculo na que se incluíron todos os balances de masas e as necesidades enerxéticas do proceso global. Ademais de proporcionar a capacidade de produción de ácidos graxos volátiles dependendo da cantidade de substrato a tratar, esta ferramenta tamén inclúe o cálculo dos custos tanto de capital como operacionais. A maiores, é capaz de determinar os principais indicadores económicos asociados á construción de ditas biorrefinerías.

Tendo en conta que as condicións de operación máis favorables para unha operación unitaria en concreto non teñen por que ser as mesmas que para o proceso dende un punto de vista integral, o uso da ferramenta permitiu elixir o disolvente máis axeitado dende a perspectiva económica, que resultou ser o acetato de etilo, así como a relación S/F máis favorable, que foi 0,75 tanto para o lodo como para os restos de comida.

Sacando proveito da capacidade da ferramenta para estimar os gastos de capital asociados á biorrefinería, comprobouse que o equipamento relacionado coa tecnoloxía de membranas foi a partida máis destacada para ambos substratos. Ademais, o maior gasto operacional tamén resultou ser o relativo ao consumo de químicos necesarios para a limpeza das mesmas, o que puxo de manifesto que esta unidade resultou ser a debilidade do proceso, sendo precisa ben a súa optimización ou a identificación de alternativas para a súa substitución.

Unha vez establecidos os óptimos, a ferramenta permitiu determinar a escala mínima viable para construír unha biorrefinería de ácidos graxos volátiles a partires de ambos substratos, precisándose o lodo xerado por unha poboación de arredor de 871.500 habitantes para recuperar

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a inversión en 20 anos e 438.000 habitantes no caso dos restos de comida (considerando unha porcentaxe de recollida selectiva do 25 % do total xerado).

A ferramenta tamén foi útil para avaliar economicamente a construción dunha planta tratando todo o residuo xerado na comunidade autónoma de Galicia. Se tratase todo o lodo de depuradora producido, requiriríase unha inversión de capital de arredor de 50 millóns de euros, cuns custos operacionais cercanos aos 30 millóns anuais, e produciríanse 6.308 toneladas de ácidos graxos volátiles ao ano (período de recuperación da inversión de 9 anos). No caso de tratar o 25 % dos restos de comida xerados, precisaríanse uns 18 millóns de euros de custos de capital e 4,8 millóns ao ano de custos operacionais, sendo o período de retorno da inversión lixeiramente inferior aos 4 anos.

En conxunto, demostrouse que a produción de ácidos graxos volátiles a partires de lodos de depuradora e restos de comida resultou ser un bioproceso non só viable dende o punto de vista técnico, senón que tamén reflexou uns indicadores económicos razoables cando se trata de instalacións industriais desta magnitude. En calquera caso, o método de cálculo factorial para os custos de capital, así como a extrapolación de datos cunha marxe de optimización importante como os da operación con membranas, poñen de manifesto a necesidade de traballo adicional para reducir a incerteza asociada á industrialización do proceso.

Capítulo 8. Avaliación ambiental

Unha vez analizado o proceso dende o punto de vista técnico e económico, levouse a cabo unha avaliación ambiental do mesmo co obxectivo de coñecer os puntos críticos das biorrefinerías propostas para construción en Galicia. Así, para a realización da análise de ciclo de vida dividiuse a instalación en subseccións e seleccionouse como unidade funcional 1 tonelada de ácidos graxos volátiles producida. Observouse que independentemente do substrato utilizado, o transporte do mesmo ata as biorrefinerías foi o elemento con maior peso en case todas as categorías de impacto, seguido do consumo de químicos. En calquera caso, ao igual que ocorreu dende o punto de vista económico, a biorrefinería tratando restos de comida acadou uns indicadores ambientais máis favorables.

A comparación da pegada de carbono dos ácidos graxos volátiles de base bio xerados no proceso con outros procedentes da fermentacións de substratos renovables e tamén da industria petroquímica resultou complexa, dada a variabilidade de metodoloxías e límites do sistema aplicados na bibliografía. En calquera caso, ao comparar as pegadas coas dos ácidos procedentes de procesos fermentativos tendo en conta a purificación dos mesmos, os datos estiveron aliñados con outros estudos. En cambio, á hora de comparalos coas poucas referencias que existen dos ácidos obtidos a partir da petroquímica, viuse que claramente os obtidos nas biorrefinerías propostas neste traballo duplicaron as cifras de pegada de carbono (arredor de 2,5 kg CO_2 eq./kg acético contra os 5,5 kg CO_2 eq./kg acético a partires de restos de comida sen considerar o transporte). En canto ao ácido propiónico, a pegada de carbono obtida foi de 8,6 e 5,5 kg CO_2 eq./kg de propiónico para lodo e restos de comida sen transporte, que estaría máis preto dos 4,4 kg CO_2 eq./kg acético reportados pola vía tradicional.

Polo tanto, neste traballo demostrouse a necesidade de recorrer a metodoloxías coma a análise de ciclo de vida para afirmar que o proceso baseado en substratos renovables é máis desfavorable dende o punto de vista ambiental que o tradicional baseado nos combustibles fósiles. En calquera caso, ponse de manifesto a urxencia de continuar coa optimización dos procesos de valorización de correntes residuais, especialmente coas etapas de purificación, dado

que se observou que no caso de prescindir do seu impacto en termos de pegada de carbono, o desempeño ambiental da tecnoloxía veríase notablemente mellorado.

Capítulo 9. Discusión xeral

Neste capítulo incluíuse un compendio dos principais resultados derivados da tese doutoral, así como dos seguintes pasos a seguir para acadar a implementación da tecnoloxía a escala industrial.

Neste traballo púxose de manifesto o potencial de ambos substratos testados para xerar ácidos graxos volátiles, obténdose rendementos máis elevados cos restos de comida que cos lodos de depuración. Ademais, a selectividade da fermentación tamén foi diferente, dando lugar a unha mestura máis variada con maior presenza de propiónico e butírico.

Por outra parte, ratificouse a capacidade da tecnoloxía de membranas para preconcentrar os ácidos, aínda que o efluente obtido seguiría a ter unha gran cantidade de auga que sería preciso eliminar. Así, corroborouse tamén a idoneidade da extracción líquido-líquido con disolventes orgánicos para tal fin, obténdose os mellores resultados con acetato de etilo. A maiores, as simulacións en ASPEN HYSYS aportaron a información de utilidade para a creación da ferramenta en formato folla de cálculo do proceso integral, que permitiu calcular os indicadores económicos da construción de biorrefinerías.

Aínda que os resultados obtidos foron de gran axuda na carreira pola industrialización da tecnoloxía, precisaríase reducir incerteza continuando coa investigación cos seguintes obxectivos: optimizar a operativa dos procesos de membranas, validar experimentalmente a destilación, avaliar a co-fermentación de ambos substratos e atallar as barreiras legais que existen actualmente para a comercialización destes bioprodutos.

Unha vez identificadas as limitacións que supoñen as etapas posteriores á fermentación, tanto pola súa complexidade tecnolóxica como polo custo que implican, é importante subliñar que existen alternativas á produción de ácidos de alta pureza tales como o *upgrading* de biogás para a súa inxección en rede como biometano (escenario que implicaría un maior beneficio neto ao dixerir lodos de depuración) ou a búsqueda da formulación de produtos que conteñan ácidos na súa composición tales como desinfectantes, pero que non requiran purezas tan elevadas.

Capítulo 10. Conclusións xerais

Esta tese doutoral demostrou que a produción de ácidos graxos volátiles de alta pureza a partires de lodos de depuración e restos de comida é unha alternativa con viabilidade técnica de cara a substituír os actuais métodos de produción baseados nos combustibles fósiles.

En concreto, os restos de comida obtiveron tanto mellores rendementos de conversión como unha selectividade favorable ós ácidos de maior valor de mercado. A experimentación do proceso fermentativo comezou a escala laboratorio, observándose coherencia na similitude dos resultados cos acadados na planta piloto, o que confire robustez de cara a unha futura industrialización.

Resulta evidente o feito de que a etapa de purificación e concentración é a que goza de menor grado de madurez tecnolóxica, sendo os fluxos de permeado das membranas reportados con

medio real moi inferiores aos especificados nas fichas técnicas das mesmas. Isto impactou enormemente na contía dos custos de capital e de operación ao avaliar as biorrefinerías a escala industrial mediante a ferramenta que integrou o proceso global de produción dos ácidos. Ademais, o cálculo da mínima escala viable indicou que son necesarias instalacións que traten grandes fluxos de residuo para facer a inversión rendible, precisándose a xeración de lodo e de restos de comida equivalente a 871.500 e 438.000 habitantes, respectivamente.

A análise de ciclo de vida levada a cabo mostrou que os puntos críticos do proceso dende o punto de vista ambiental foron o transporte dos substratos ás instalacións e o consumo de químicos. Ademais, a pegada de carbono obtida para os ácidos graxos volátiles que se obterían nas biorrefinerías propostas resultou ser notablemente maior que a reportada a partires de procesos petroquímicos. É por iso é preciso continuar optimizando o proceso (sobre todo a etapa de purificación) de cara a reducir ditos impactos.

Adicionalmente, aínda que esta tese doutoral abordou a produción de ácidos graxos volátiles de alta pureza de cara a substituír os comercializados na actualidade obtidos a través da petroquímica, existen outras alternativas de interese valorizando as correntes residuais estudadas para outros fins: a produción de biometano para inxectar en rede ou a formulación de desinfectantes con alto potencial de comercialización.

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ABBREVIATIONS AND ACRONYMS

AD: anaerobic digestion
AC: acidification
BES: 2-bromoethanesulfonate
BMP: biomethane potential
CAPEX: capital expenditure
CC: climate change
CCA 4.0 IL: Creative Commons Attribution 4.0 International License
CHP: combined heat and power
COD: chemical oxygen demand
CSTR: continuous stirred-tank reactor
EU: European Union
FE: freshwater eutrophication
FRS: fossil resource scarcity
FW: food waste
GHG: greenhouse gases
HKC: heavy key component
HRT: hydraulic retention time
IPCC: Intergovernmental Panel on Climate Change
IRR: internal rate of return
LCA: life cycle assessment
LCI: life cycle inventory
LKC: light key component
LLE: liquid-liquid extraction
LMH: litres of permeate per square metre of membrane area per hour
ME: marine eutrophication
MF: microfiltration
MRS: mineral resource scarcity
MW: molecular weight
MWCO: molecular weight cut-off
NF: nanofiltration
NPV: net present value
OD: ozone depletion
OLR: organic loading rate
OPEX: operational expenses
OTU: operational taxonomic unit
P&ID: piping and instrumentation diagram
RO: reverse osmosis
S/F: solvent/feeding ratio
SS: sewage sludge
TAN: total ammonia nitrogen
THP: thermal hydrolysis pre-treatment
TMP: transmembrane pressure
TN: total nitrogen
TP: total phosphorus
TS: total solids
UF: ultrafiltration
VFA: volatile fatty acids
VS: volatile solids
WC: water consumption
WWTP: wastewater treatment plant

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1) GENERAL INTRODUCTION

1.1. CHANGE OF PARADIGM: NET ZERO 2050 STRATEGY

1.1.1. Climate change context

The Earth's atmosphere is mainly composed of N_2 and O_2, but it also contains other gases such as CO_2, which is the most important greenhouse gas (GHG). These gases absorb heat radiating from the Earth's surface and release it back in all directions, including towards the Earth's surface. Without CO_2, Earth's natural greenhouse effect would be too weak to keep the average global surface temperature above freezing. However, by adding more CO_2 to the atmosphere, human activity is overloading the natural greenhouse effect, causing global temperatures to rise. According to observations from NOAA's Global Monitoring Laboratory, in 2021, CO_2 alone was responsible for approximately two-thirds of the total thermal influence of all human-produced GHGs (Friedlingstein et al., 2022).

CO_2 is also important in the Earth system because it reacts with the water molecules of the ocean producing carbonic acid, lowering its pH and increasing its acidity. Since the start of the Industrial Revolution, the pH of ocean surface waters has dropped from 8.21 to 8.10. This drop in pH is called ocean acidification (Lan et al., 2022). But CO_2 is not the only gas which provokes climate change, there are others such as CH_4 and N_2O which also have been increasing from anthropogenic activity (Figure 1.1A), which have unequivocally caused global warming, with global surface temperature reaching in 2011-2020 1.1 ºC above 1850-1900 as shown in Figure 1.1B (IPCC, 2023).

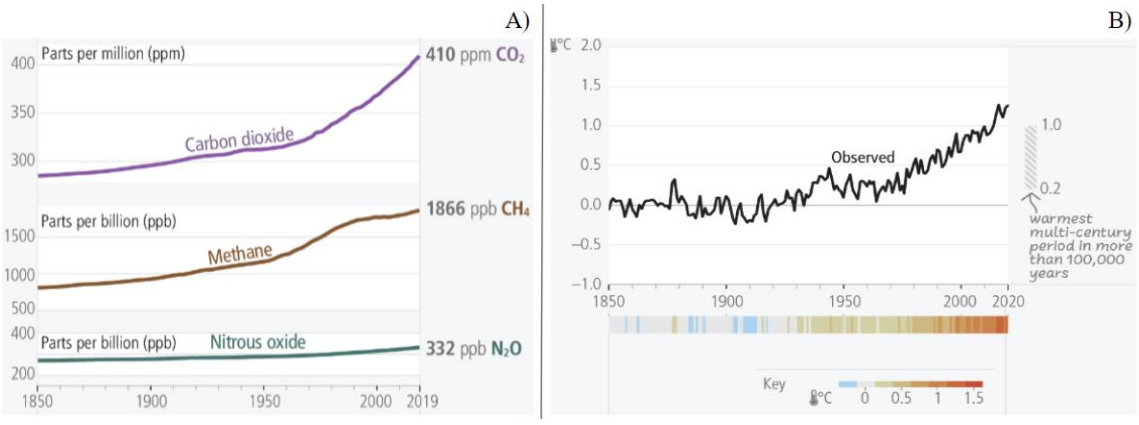

Figure 1.1. Global surface changes related to climate change. A) Evolution of GHG's concentration in the atmosphere. B) Evolution of global surface temperature from 1850-2019. Source: IPCC, (2023) under CCA 4.0 IL.

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According to the 2022 annual report from NOAA National Centre for Environmental Information, the global average atmospheric CO_2 was 417.06 parts per million in 2022, setting a new record. The increase between 2021 and 2022 was 2.13 ppm, the eleventh consecutive year in which the amount of CO_2 in the atmosphere increased by more than 2 ppm.

While the rate of growth in GHG emissions in the past decade slowed compared to the previous decade, average GHG emissions were the highest on record (Figure 1.2); total global GHG emissions averaged 54.4 gigatons of CO_2 equivalent (Gt CO_2 eq.) between 2010 and 2019 and reached a high in 2019.

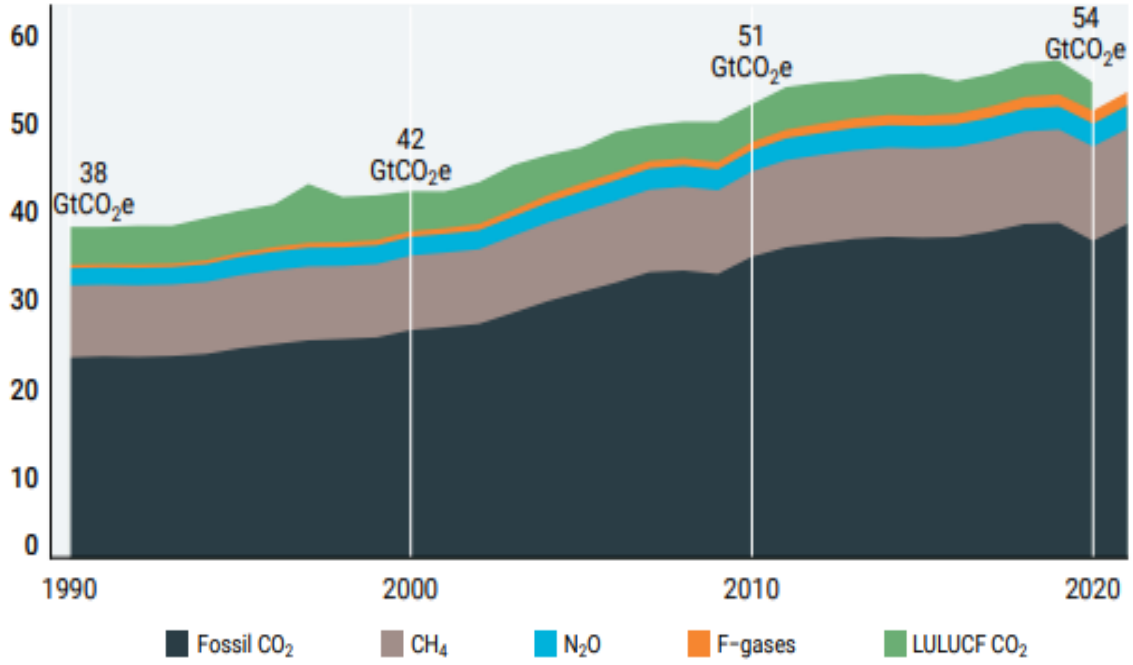

Figure 1.2. Evolution of total GHG emissions between 1990-2021 (Gt CO_2 eq./year). Source: Friedlingstein et al., (2022) under CCA 4.0 IL.

Widespread and rapid changes in the atmosphere, ocean, cryosphere and biosphere have happened. Human-caused climate change is already affecting many weather and climate extremes in every region across the globe. This has led to universal adverse impacts and related losses and damages both to nature and people. Vulnerable communities who have historically contributed the least to current climate change are disproportionately affected (IPCC, 2023). Figure 1.3 shows the observed impacts on ecosystems and human systems attributed to climate change at global and regional scales and it can be seen how most of the changes have a high or very high confidence in attribution to climate change, and most of these impacts are also adverse.

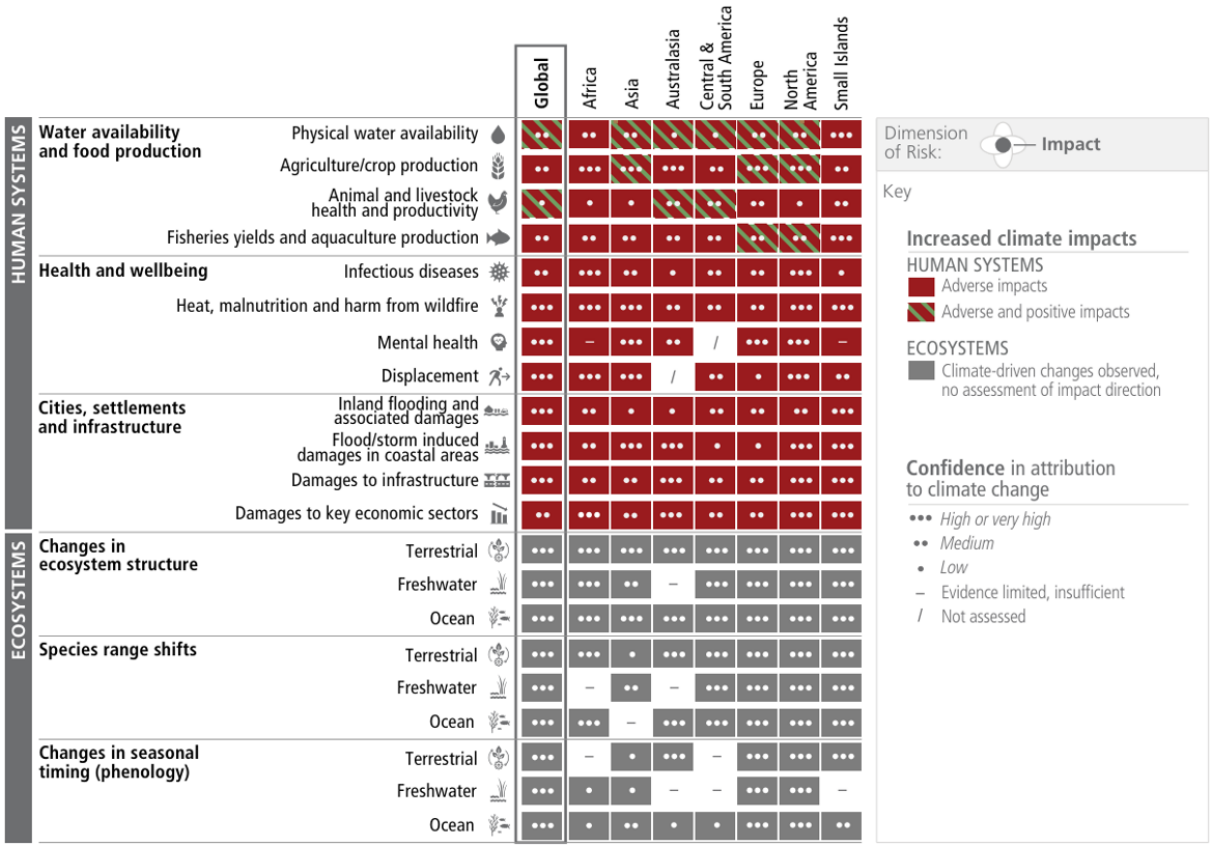

Figure 1.3. Observed impacts and related losses and damages of climate change. Source: IPCC, (2023) under CCA 4.0 IL.

Given that in the 2015 Paris Climate Agreement the world pledged to work together to limit total global temperature rise to well below 2 °C and pursue efforts to stay below 1.5 °C, the Intergovernmental Panel on Climate Change (IPCC) cautioned that the transition away from fossil fuels must be done quickly. Efforts to achieve this goal and the strategies to do so have focused overwhelmingly on transforming energy and transportation systems, which account for 39 % of annual GHG emissions, being both transitions very important. In contrast, emissions from the industrial sector, which represent 30–40 % of total global GHG's emissions every year, have received much less attention (CIEL, 2019).

1.1.2. European Green Deal

This is a decisive moment to fulfil the society's commitments under the Paris Agreement in the interest of well-being, health and prosperity of the human beings. That is the reason why the European Union (EU) has set an example by establishing ambitious targets to reduce net emissions at least 55 % by 2030 in comparison to 1990 levels and to become the first climate neutral continent by 2050. These obligations are well defined in the first European Climate Law.

Today's proposals build on policies and legislation that the EU has already put in place. In fact, the European Green Deal laid out the pathway for this transformative change, establishing the pillars of tomorrow's economy with landmark strategies on biodiversity, circular economy, zero pollution, sustainable and smart mobility, sustainable food and so on (Figure 1.4). Moreover, unprecedented resources have been allocated to support the transition, either through

the EU Recovery Plan (NextGenerationEU), which will contribute at least 37 % to the green transition, the next long-term EU budget for 2021-2027, such as continued attention to sustainable financing and the unblocking of private investment.

Figure 1.4. Main objectives of the European Green Deal. Source: own elaboration from European Commission, (2020).

Figure 1.4 shows that two of the targets of the European Green Deal are the transition to a circular economy, as well as achieving climate neutrality. Both objectives are related, which is why new models must be proposed in order to break through the linear economy and find new ways of producing consumer goods which we use in our day-to-day life.

1.1.3. Biorefinery concept

Meeting the proposed climate targets will demand dramatic reduction of emissions in the industrial sector, considering its high weight on the emissions distribution. In particular, the chemical industry and the production of important basic chemicals is a key sector to consider because this is highly dependent on fossil resources for using it as both fuel and feedstock to produce chemicals or energy.

The chemical industry demands natural gas and petroleum fractions, the first for using it for methanol and ammonia production and the second one as feedstock to make polymers and basic chemicals (such as benzene, polyethylene, carboxylic acids, etc.). In fact, the sector is associated with both energy-related emissions and large process emissions. According to Bauer et al. (2022), in 2020 direct GHG emissions from the petrochemical sector amounted to 1.8 Gt

CO_2 eq., (often known as Scope 1), which is equivalent to 4 % of global GHG emissions. Indirect GHG emissions derived from the activities of other input supply industries for the petrochemical industry represented another 3.8 Gt CO_2 eq. Therefore, the petrochemical industry is associated with a total of 5.6 Gt CO_2 eq. of GHG emissions, equivalent to around 10 % of global emissions. Figure 1.5A shows the evolution of the total GHG emissions from petrochemicals (both direct and indirect) as well as how this number has more than doubled since 1995 (Figure 1.5B).

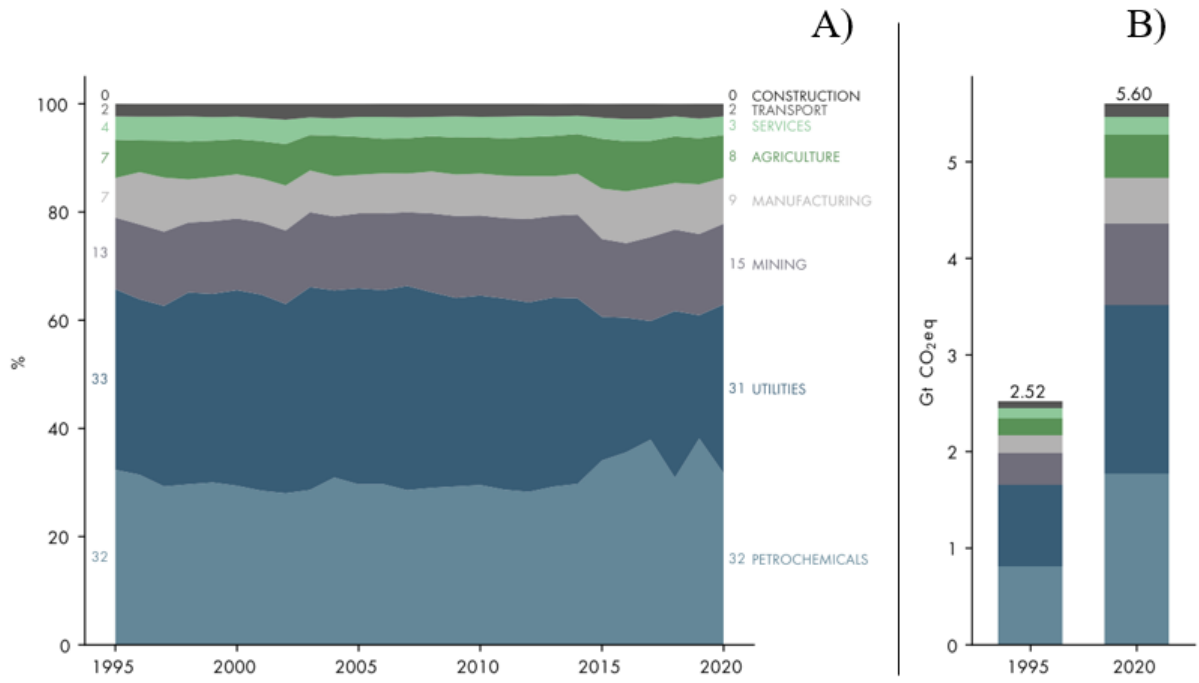

Figure 1.5. GHG emissions coming from petrochemicals production by sector. A) Evolution over time. B) Comparison between 1995 and 2020. Source: Bauer et al. (2022) under CCA 4.0 IL.

Once the importance of the industry was reported, and particularly the relevance of GHG emissions from the petrochemicals, the new policies and laws are driving a process of decarbonisation in all industries, with a critical shift from a fossil fuel-based economy to one based on biomass. This new paradigm implies not only a phase out of fossil fuels as an energy source, but also a move away from crude oil as a source of platform chemicals, polymers, medicines and many other critical materials and consumer goods which we use in our daily life. Therefore, for achieving the United Nations Sustainable Development Goals, crude oil must be replaced by renewable sources, and in this evolution, biorefineries emerge as the critical alternative to traditional refineries to produce fuels, chemical components and materials from renewable sources and waste streams (Calvo-Flores, 2022).

Biorefineries make it possible to use renewable biomasses in an appropriate way to ensure benefits to society and the environment, as well as long-term economic viability compared to fossil fuels. Therefore, a biorefinery is a facility with efficient, integrated and flexible conversion of renewable biomass feedstocks into multiple products through a combination of physical, chemical, biochemical and thermochemical processes. The same as the complex oil refineries were conceived, the concept of biorefinery was created applying the principles of process engineering to its designs such as integration, process flexibility, feedstock fractionation and multiple value-added productions (Kok et al., 2014).

Biorefineries, like petroleum refineries, should follow the strategy of feedstock separation into more treatable and useful fractions, the precursors or platforms. After that, each of these fractions must create a production line in order to further increase adaptability and profit. In this way, the feedstock is converted into commodities (biofuels and electricity) as well as other products such as chemical building blocks which replace petrochemicals. Thus, a complex system is created, and it can exchange material and energy streams to supply its requirements and achieve self-sufficiency, reducing wastes and emissions as well as water consumption (Kok et al., 2014).

There are two currently consolidated biorefinery models, which are the sugar platform, in which purified enzymes convert biomass into five- and six-carbon sugars that can in turn be fermented to fuels, and the other example is the syngas platform, through the which biomass is also converted into synthesis gas which can be transformed into fuels through catalysis (Agler et al., 2011). Another model of biorefinery platform, which has a lower degree of maturity, is the "carboxylate platform", which converts waste streams into short-chain carboxylates that can be separated or further used as intermediate chemicals to produce other added-value products. In this carboxylate platform, it is very common the use of undefined mixed cultures because they can tolerate being fed with a wide variety of waste streams due to their metabolic flexibility (Kleerebezem and van Loosdrecht, 2007).

1.2. CURRENT SITUATION OF URBAN ORGANIC WASTE STREAMS

More than half of the world's population currently lives in cities and by 2050 this proportion is expected to increase to two thirds. Therefore, the demand for energy, water and food in urban areas will not stop growing. At the same time, city inhabitants generate large amounts of wastewater daily (around 715 million m^3 according to Mateo-Sagasta et al. (2015)) and more than 1.6 million tons of organic solid waste (Kaza et al., 2018).

However, organic waste presents opportunities to develop systems which apply the resource recovery concept in order to take advantage of nutrients, energy, water and other materials contained in these streams (Andersson et al., 2016). The implementation of circular economy approaches to waste management can alleviate resource pressures and boost water, energy and food security, as well as reduce risks from diseases related to poor waste management and lack of sanitation (Andersson et al., 2016). Therefore, sewage sludge (SS) and biowaste are organic waste streams with high potential for producing high added-value products.

Environmental issues have been regulated by the EU through the drafting of directives and regulations, which are subsequently transposed to the legal framework of each Member State. In general, the EU directives relating to wastewater treatment, SS and food waste (FW) management, have standardised concepts and requirements but in some countries, there are more ambitious targets. Those most relevant legislation concerning these waste streams are:

A. Directive concerning urban wastewater treatment (Directive 91/271/EEC, as amended by Regulation (EC) No. 1882/2003, Regulation (EC) No. 1137/2008 and Directive 2013/64/EU) and its sludge. It aims to protect the environment from the adverse effects of urban wastewater discharges and discharges from certain industrial sectors.

B. Sludge Directive (Directive 86/278/EEC, by Regulation (EC) No. 807/2003, by Regulation (EC) No. 219/2009, by Decision (EU) 2018/853 and by Regulation (EU) No. 2019/1010) concerning the protection of the environment and the soil, when SS is used in agriculture. EU rules on SS consider the nutritional needs of plants and ensure that the quality of soil, surface water and groundwater is not negatively affected. This includes how farmers

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can use SS as fertiliser, the sampling and analysis of sludge and soil, keeping detailed records of the quantities of sludge produced and used in agriculture, the type of treatment and where the sludge is used, and the composition and properties of the sludge.

C. Waste Framework Directive (Directive 2008/98/EC, as amended by Directive 2018/851). This Directive establishes a new definition of biowaste apart from setting date for certain milestones to be met as the obligation to separate and recycle at source, or collect separately the biowaste by December 31, 2023.

1.2.1. Sewage sludge

The water used in households is collected and conducted from the place of generation to a wastewater treatment plant (WWTP) where is submitted to a treatment process in order to remove nutrients and organic matter. This water is composed by faeces, urine and other domestic streams from human drains and sinks. Moreover, during its way to WWTP, this household water is usually mixed with water run-off and in some cases with industrial water, composing the so-called urban wastewater (Figure 1.6).

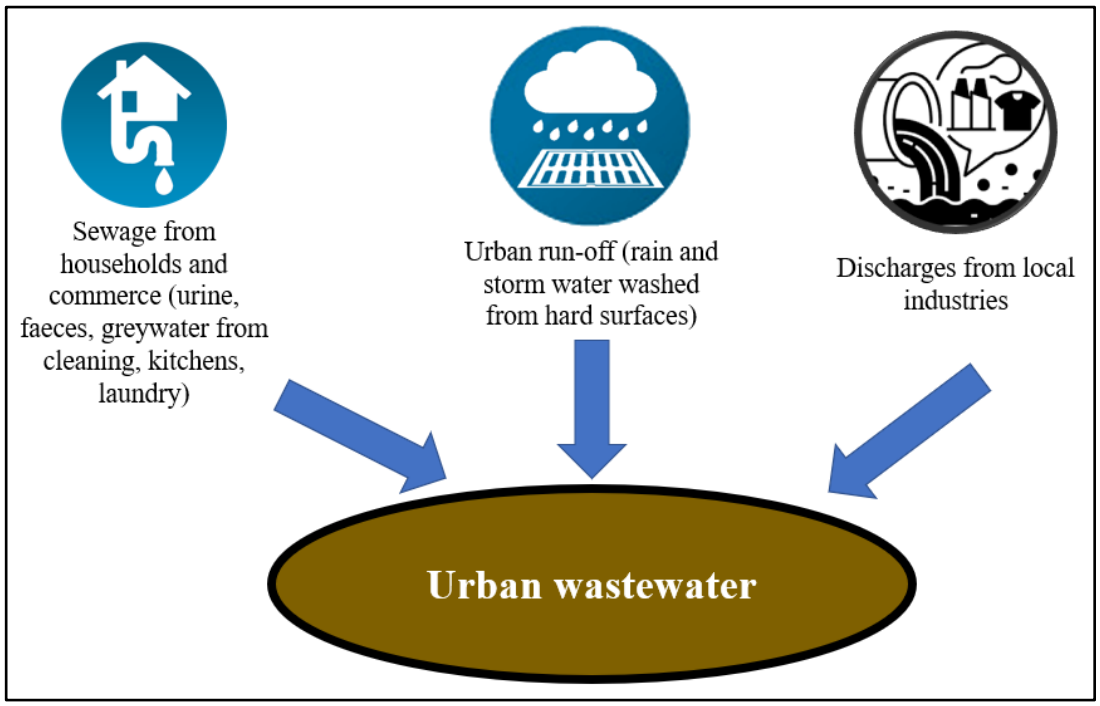

Figure 1.6. Different waste streams composing urban wastewater. Source: own elaboration from European Environmental Agency, (2022).

The wastewater has compounds and microorganisms which need to be treated to avoid damages caused in people and in the environment. So, it must be cleansed before it goes back to the water cycle. During this wastewater treatment, it is generated a stream called raw SS which is mainly composed by water, nutrients and organic matter, containing resources as carbon (25-35 % in total solids (TS)), oxygen (20-25 %), nitrogen (4-5 %), phosphorus (2-3 %) and other residuals as trace elements and metals (EurEau, 2021). SS can have an unpleasant odour but when they are treated in WWTPs, they are categorised as non-hazardous waste in the European List of Wastes (European Commission, 2014).

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Each person produces around 70-100 kg of dewatered sludge per year, which is around 20-25 kg of TS/year (European Comission, 2014). In the EU, 8.7 million tons TS/year are produced, and its destination is shown in Figure 1.7 (EurEau, 2021): agriculture (4.1 million t TS/year), incineration (2.4 million t TS/year), recultivation/land reclamation (0.7 million t TS/year), landfill (0.5 million t TS/year) and other destinations (1 million t TS/year).

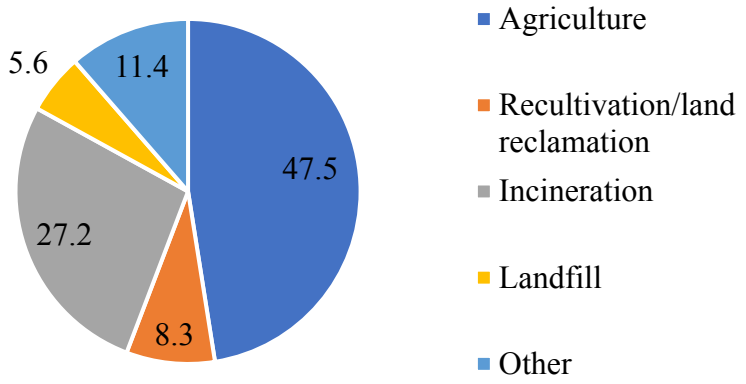

Figure 1.7. Percentages of sewage sludge destinations in the EU. Source: own elaboration from EurEau, (2021).

Depending on their local needs, opportunities or priorities, each country treats and manages its sludge through a particular way. Southern Europe countries which usually need organic matter prefer destining the SS to agriculture, whilst in other countries the problems of excess of manure, eutrophication of water bodies or problems with sludge quality implied a preference for incineration processes (for example, in countries as the Netherlands is banned the recycling of the sludge). The particularities of each country imply important variations in the disposal of the different Member States as is shown in Figure 1.8, but the two main destinations for Europe are agriculture and incineration as it can be observed in the right bar.

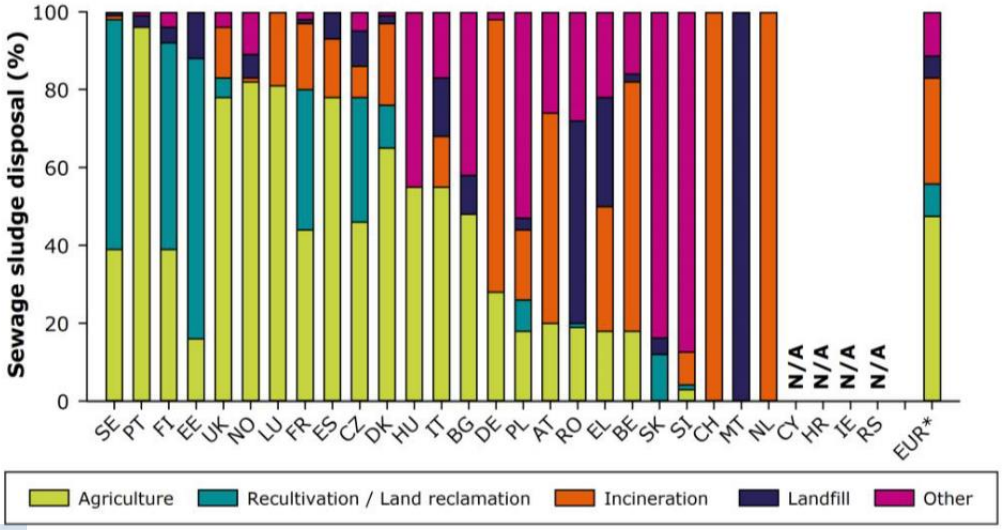

Figure 1.8. Percentage of sewage sludge destinations per county in Europe. Source: EurEau, (2021); authorised reproduction. N.A.: No answer available.

The treatment of wastewater in WWTPs generates SS which is often treated (through processes such as thickening and dewatering) to ensure that it can be further used or disposed, but it can be identified three different types of sludge:

-Primary sludge: solids which settle during primary treatment of wastewater (generated from physical separation such as screening).

-Secondary sludge: composed by organic cells which are produced by bacteria during biological treatment.

-Chemical sludge: it is formed by the addition of chemicals and contains both biological and some residual chemical product, representing a small part or production. It comes from a specific treatment such as the addition of ferric chloride to boost phosphorus removal.

In any case, the SS treatment always has two objectives, which are the recovery of materials or energy from sludge using its resource potential and the reduction of the amount of SS to be managed. Through sludge dewatering, volume of wet sludge can be reduced, increasing its solid content (European Environmental Agency, 2022).

WWTPs need high amounts of energy to work, but theoretically wastewater contains between five and ten times more energy (chemical and thermal) than the needed for treating it. It is clear that only a small part of this energy can be recovered, but when referring to large WWTPs, these could even be net energy producers (Riley et al., 2020). The thermal, chemical and hydrodynamic energy contained in SS can be recovered in the form of electricity, biogas or steam, being the energy content of sludge similar to that which contains a low-grade coal (Abd et al., 2016). The ways of generating energy from SS is through anaerobic digestion (AD) to produce biogas and through incineration, pyrolysis and gasification. Biogas has a content of CH_4 of 60-70 % by volume, CO_2 around 30-40 % and trace amounts of gases such as N_2, H_2S and H_2. When biogas is purified in order to increase its CH_4 content it is called biomethane and it can be utilised in gas engines, as vehicle fuel or for electricity and heat generation.

1.2.2. Biowaste

The Waste Framework Directive (2008/98/EC) states that biowaste comprises biodegradable park and garden waste, food and kitchen waste from households, restaurants, caterers and retail premises as well as similar waste from food-processing plants. It is important to say that from this point, when referring to biowaste, reference is also made to food and garden waste from households and similar public and commercial activities as food services.

Biowaste is the main and most abundant component of municipal waste but is also produced by the agricultural and industrial sectors. Depending on the local conditions and capabilities, several policies in European countries regarding the management of this waste can be found. Although there are countries which have already implemented the separate collection of biowaste, others are in the process of searching for the right means of implementation (although in breach of the law given that it is now obligatory in all Member States, as indicated in Directive 2018/851). Moreover, amending Directive EU 2018/851 implies some important changes regarding biowaste and some of them are:

-EU Member States must redact a report with the biowaste generated per year, apart from adopting prevention programmes.

-An ambitious target to reduce this waste to accomplish Sustainable Development Goal 12.3 until the half by 2030.

-New targets for reusing and recycling of municipal waste which are not going to be met with the current management scheme.

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-The duty for all EU Member States of collecting biowaste separately or ensuring recycling at source from the end of 2023 onwards.

Biowaste has important relevance in the European municipal waste generation. Figure 1.9 shows how in the EU each person generated annually around 488 kg of total municipal waste, of which 321 were non-biowaste and 167 were biowaste; within biowaste, almost 60 % was FW.

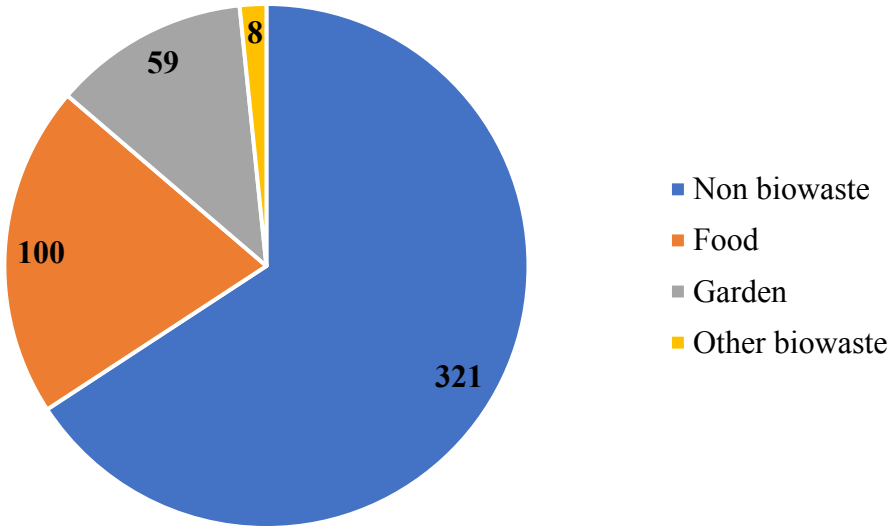

Figure 1.9. Mean amount of biowaste (kg/person) in municipal waste in EU (2017). Source: own elaboration from European Environmental Agency, (2020).

Considering its noticeable volume, the common objectives for waste management proposed by the EU cannot be fulfilled without focusing on this waste stream. Incorrectly managed, biowaste could cause important environmental and economic threats (Pubule et al., 2015), being a source of GHG emissions and public health problems.

Figure 1.10 shows that per capita biowaste production is highly variable among European countries, being influenced by the differences in the way in which countries report municipal biowaste coming from non-household sources such as the consideration of garden waste or the waste derived from the hospitality sector. In any case, countries such as Hungary and North Macedonia generate small quantities of biowaste in comparison with other countries such as Denmark, also depending on the dietary patterns of each country.

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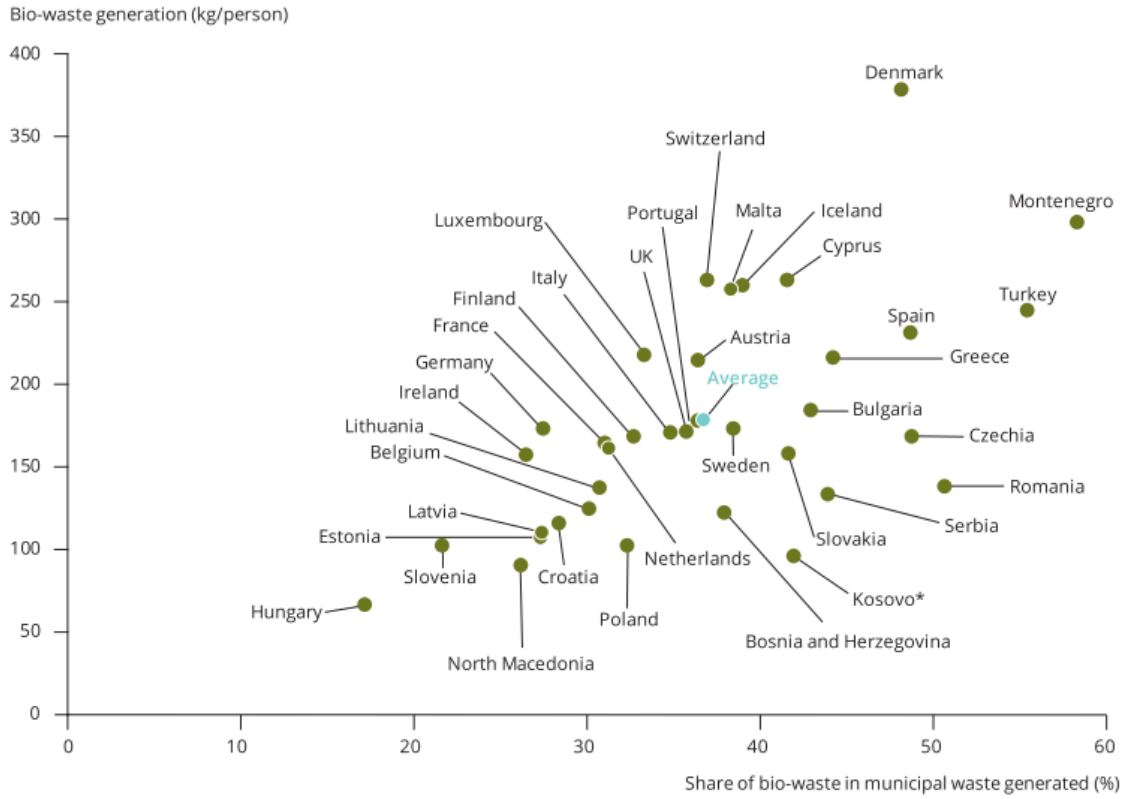

Figure 1.10. Generation of municipal biowaste per person and share of biowaste in municipal waste generated by country in 2017. Source: European Environmental Agency, (2020); authorised reproduction.

In line with circular economy principles, there are two main treatment methods for biowaste which was collected separately:

-Composting is an aerobic process which usually happens in open-air windrows or in-vessels. The organic solids are biodegraded, and a humic substance is generated which can be applied as fertiliser, growing media constituent or solid improver. The process is favoured when substrates with high moisture such as FW and structure-improving organic matter such as garden waste are mixed.

-AD is an anaerobic process which happens in closed digesters without oxygen presence and biogas is produced, which can be used to generate electricity or heat, and also upgraded into a fuel. A digestate is also obtained and can be used as an organic fertiliser. Although AD is a widely used process, wood materials cannot be correctly digested due to its high lignin content (JRC, 2015).

Municipal biowaste treatment techniques are also applicable to biowaste coming from other sources and that is the reason why municipal biowaste is sometimes treated together with other organic streams, always choosing the treatment technology which allows the greater energy and material recovery, being therefore the environmentally preferable choice. The European Commission's Joint Research Centre created a 'hierarchy' of options for biowaste treatment according to life-cycle analysis which is shown in Figure 1.11. Waste prevention and reuse is the first recommended option from an environmental point of view, being AD the second one followed by composting, given that AD implies the recovery of both materials and energy. In general, countries do not choose one specific biowaste treatment option, electing a combination of techniques, being able to target different types of biowaste from several sources.

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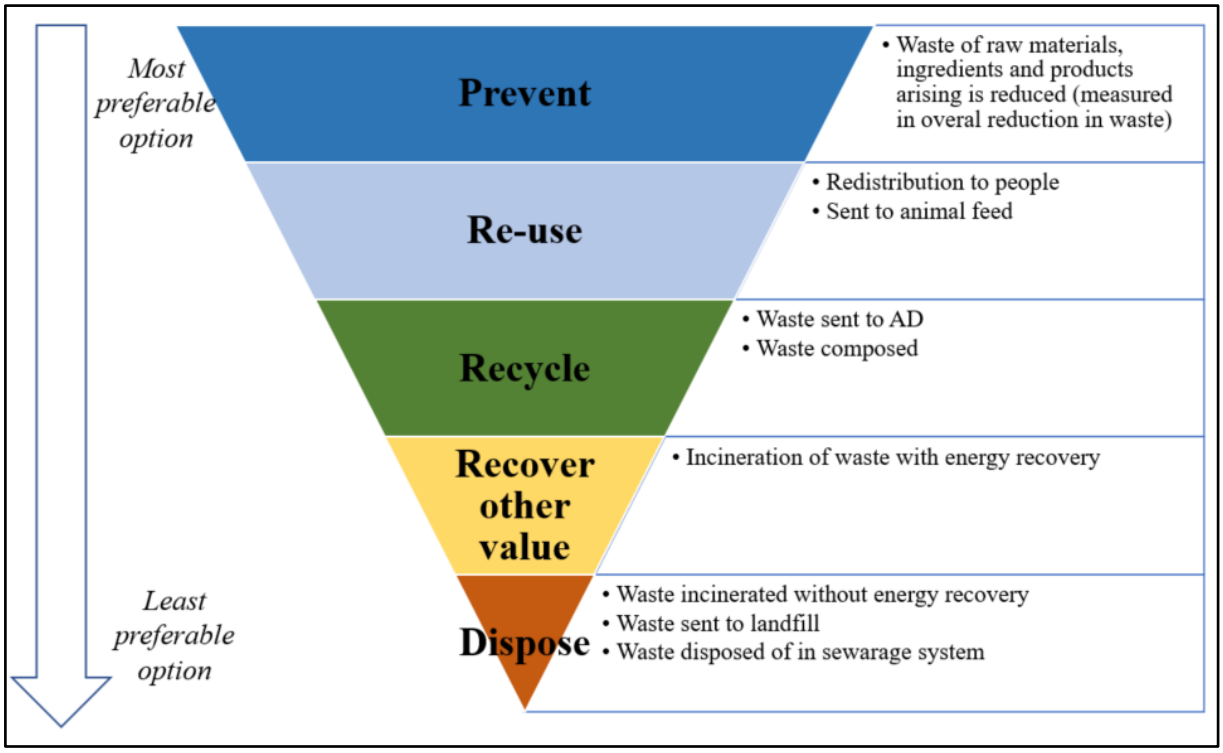

Figure 1.11. The food waste treatment hierarchy. Source: own elaboration from European Environmental Agency, (2020).

Considering that in all the experimentation carried out in this doctoral thesis no biodegradable waste from parks or gardens was used, from this moment onwards reference will always be made to FW.

1.3. CONVERTING ORGANIC WASTE INTO VOLATILE FATTY ACIDS (VFA)

The concept "carboxylate platform" named above as one of the biorefinery models refers to a process from which a mixture of carboxylates is generated from organic waste streams. Among the products derived from this conversion are carboxylic acids, a term which refers to any compound which has a carboxyl group (–COOH), being within this group the so-called volatile fatty acids (VFA), which are a mixture of a carboxylic acid and its corresponding carboxylate salt in a ratio that depends on pH. VFA can be also classified into short-chain VFA (C1 to C4) and medium-chain VFA (C5 to C8).

The mixed VFA-streams, although they could be used for some purposes, must undergo either chemical conversion to produce esters and polymers or biological conversion for producing polyhydroxyalkanoates, lactate, ethanol, electricity and bioenergy through fuel cells or medium-chain VFA, among others (Ramos-Suarez et al., 2021). Another option available would be the physical separation of the VFA from the culture broth in order to subject them to purification processes.

Figure 1.12 shows the wide variety of end uses of VFA, including building blocks, fragrances, antimicrobials, animal feedings and many more. Given the great versatility of these chemical compounds, there is great interest in directing AD towards their production instead of looking for other end products.

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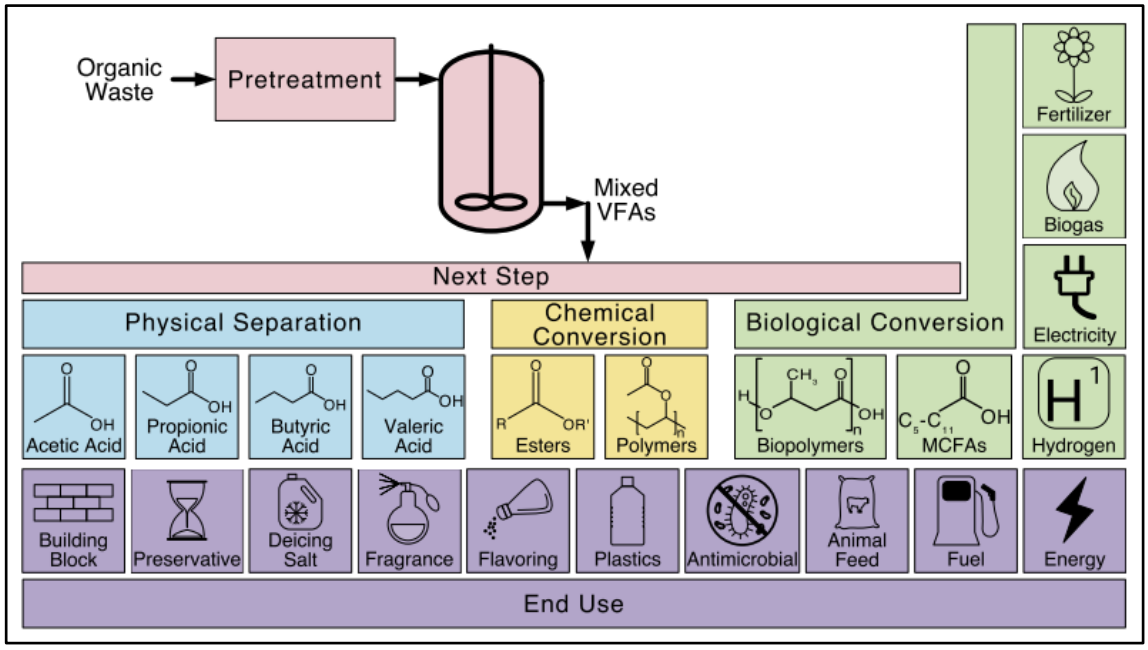

Figure 1.12. Options for using mixed VFA from organic waste fermentation. Source: Ramos-Suarez et al. (2021) under CCA 4.0 IL.

The physical separation of VFA has been the option chosen in this doctoral thesis, since a direct substitution of VFA based on petrochemical processes was the main target. Depending on the purity sought in the final product, the fermentation stream in which the VFA are found must be subjected to different purification operations. Thus, to replace VFA currently produced from fossil sources, the target purities are greater than 90-95 %. On the other hand, if the final objective is to generate products which require a lower degree of purity, it would not be necessary to apply these processes to obtain the final product. This section presents the main properties and applications of pure VFA as well as an analysis of its market.

1.3.1. Main properties and applications of VFA

VFA are a subgroup of fatty acids which have chains of less than six carbons and their chemical properties are shown in Table 1.1. It shows how when the number of carbons increases, molecular mass, density and pKa also do.

Table 1.1. Chemical properties of VFA.

Acid	Chemical formula	Molecular mass (g/mol)	Density (g/cm^3) at 20 °C	Boiling point (°C)	pKa
Acetic (HAc)	CH_3COOH	60.05	1.05	117.9	4.76
Propionic (HPr)	CH_3CH_2COOH	74.07	0.99	141.1	4.88
Isobutyric (iBut)	$(CH_3)_2CHCOOH$	88.10	0.95	154.4	4.84
Butyric (HBu)	$CH_3CH_2CH_2COOH$	88.10	0.95	163.7	4.82
Isovaleric (iVal)	$(CH_3)_2CHCH_2COOH$	102.13	0.93	176.5	4.77
Valeric (HVal)	$CH_3(CH_2)_3COOH$	102.13	0.94	186.1	4.84
Caproic (HCapr)	$CH_3(CH_2)_4COOH$	116.16	0.93	205.0	4.88

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Acetic acid or ethanoic acid is a liquid organic compound with a pungent smell and distinctive sour taste. Vinegar is around 3-9 % acetic acid by volume, being therefore a main component in the food & beverage industry. It is also used in the manufacturing process of synthetic fabrics and fibres, as well as in production of cellulose acetate for photographic film and to produce polyvinyl acetate for wood glue. As main domestic use, acetic acid is diluted to produce descaling agents and in the food industry is commonly utilised as an acidity regulator. The most important use of this acid is in the production of vinyl acetate monomer, followed by acetic anhydride and ester. It is also used as food preservative and solvent (Ramos-Suarez et al., 2021).

Propionic acid, also known as propanoic acid, is a carboxylic acid which occurs naturally. It is a liquid with an unpleasant smell like body odour. Its properties are those of the carboxylic acids because it can form amide, ester, anhydride and chloride derivatives. At concentrations between 0.1-1 % by weight, it inhibits the growth of mold and some types of bacteria. That is the reason why it is used as a preservative for human and animal food. It can be also used as an intermediate of the generation of other polymers (Atasoy et al., 2019).

Regarding butyric acid, also known as butanoic acid, it is found in milk and in butter, parmesan cheese, and as a product of anaerobic fermentation (Zigová & Šturdík, 2000). Various butyrate esters are prepared from butyric acid and they have pleasant tastes and aromas. The product finds major application as food and perfume additives owing to its supportive properties. Growing perfumes industry owing to the rising grooming and hygiene activities will propel the butyric acid market which in turn will positively affect the carboxylic acid market. As far as isobutyric acid, it finds primary applications in latex paints and other chemical intermediates such as plasticisers. It is also used to produce esters to be used as solvents wherever the desirable odours and flavours are required (Market Insights, 2017).

Valeric acid is popularly known as pentanoic acid. It is used in the synthesis of its esters. These volatile esters have a pleasant aroma, with applications in perfumes and cosmetics. Rising use of cosmetics and perfumes owing to increasing personal hygiene awareness will propel the market further. It is widely used in esters of synthetic lubricants, which are employed in refrigeration oils, aviation turbine oils, etc. and also used as a food additive to enhance flavours. As far as caproic acid, it is used in the manufacturing of its esters for artificial flavours, and hexyl derivatives such as hexylphenols. Rising flavour enhancers market owing to the changing food patterns of the global youth population will boost the caproic acid market demand in the coming few years (Market Insights, 2017).

1.3.2. Current production methods and market analysis

Table 1.2 shows some market figures of each of VFA as well as the main application and current production method of them. Acetic acid is the product with the largest global market volume and its price in Spain varied between 405-893 €/t within the period 2017-2022 (product with purity >95 %). An upward evolution of the acetic acid market is expected in the coming years. Some of the main factors that will accelerate demand is the increasing consumption of solvents (including vinyl acetate monomer) in different industries such as adhesives and sealants, paints and coatings and others. Furthermore, increasing demand for purified terephthalic acid in polyester manufacturing for textile industry will further boost market growth. The vinyl acetate monomer category in acetic acid market sales accounted for over 45 % in 2021 and is estimated to witness significant growth in years to come. Rising demand for vinyl acetate monomer in paper and paint coatings along with increasing home renovations are the key factors for category dominance.

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Table 1.2. VFA Spanish market figures, main application and production methods.

Acid	Market size (2017-2024) (kt/year)	Market price variation (2017-2022) (€/t)	Application	Production method
Acetic (HAc)	159-196	405-854	Food additive, vinegar, solvent, ester production, chemicals, vinyl acetate monomer (adhesives, dyes, polymers)	Chemical synthesis (carboxylation of methanol) and microbial fermentation (anaerobic and oxidative)
Propionic (HPr)	18-20	1,064-1,730	Animal feed supplement, pharmaceuticals, fishing bait additive, aroma additive, flavouring	Chemical synthesis (carboxylation of ethylene, ethylene hydroformulation, oxidation of hydrocarbons) and microbial fermentation
Butyric (HBut)	4-4.7	1,860-2,759	Food additive, solvent, chemical intermediate, flavouring agent	Extraction from butter, microbial fermentation and chemical synthesis (oxidation of butyraldehyde)

Table 1.2 also shows the main production methods for each of the VFA, most of which are based on fossil fuels. For example, there are three conventional acetic acid production pathways (Figure 1.13): the methanol carbonylation process, the acetaldehyde oxidation process and the hydrocarbon oxidation process. The most commonly used is the carbonylation process, also known as the Monsanto process, in which methanol and carbon monoxide react in the liquid phase in the presence of a rhodium-based catalyst at 150-200 ºC at a pressure of 30-50 bar, yielding acetic acid with 95 % selectivity and 5 % side products such as formic acid and formaldehyde. This process has evolved over time by replacing the rhodium catalyst with an iridium-based one, giving rise to the Cavita process. The second most employed process was the acetaldehyde oxidation process, where acetic acid is produced from the oxidation of ethylene using palladium and copper chloride, resulting in a high yield of acetic acid production. Finally, there is the process based on the oxidation of hydrocarbons such as butane and naphtha using cobalt acetate and chromium acetate as catalyst.

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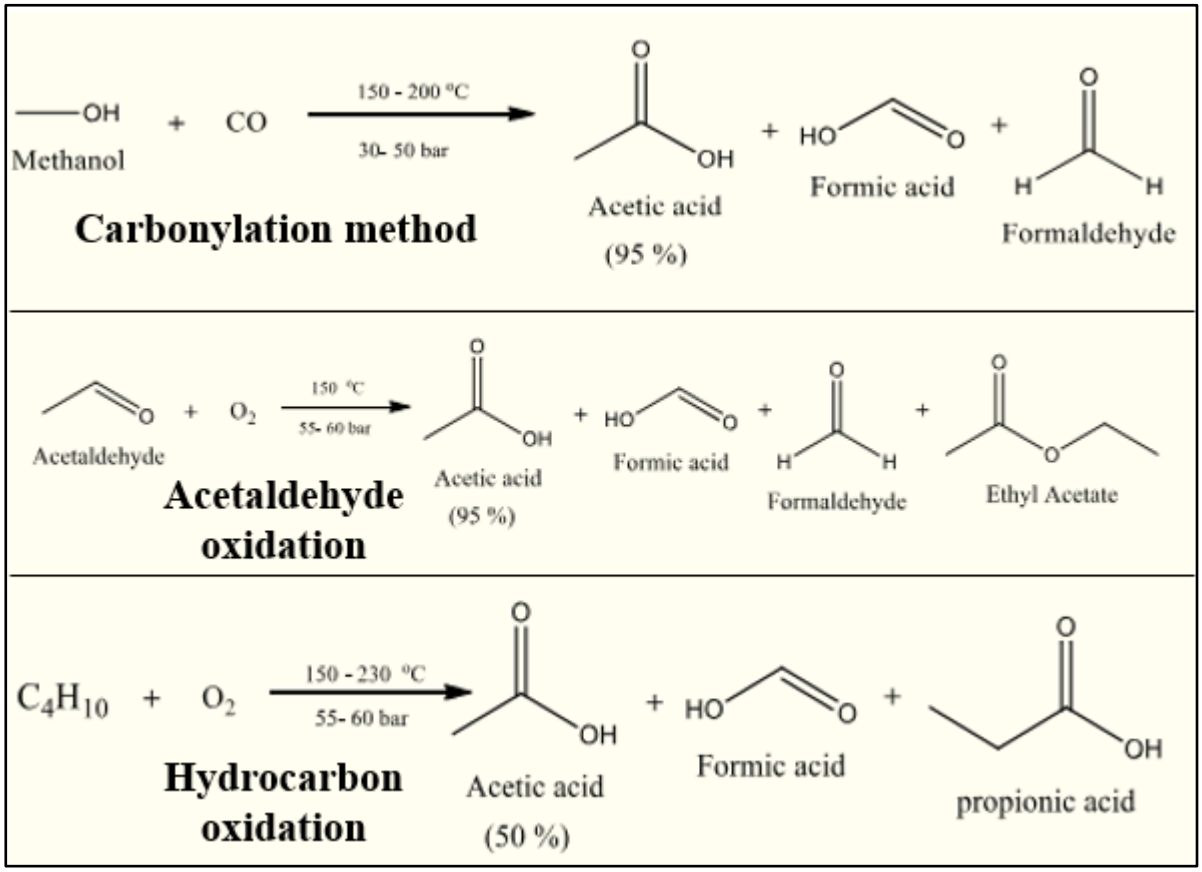

Figure 1.13. Conventional acetic acid production methods. Source: own elaboration.

As far as the production of propionic acid is concerned, one of the most famous is the so-called Reppe process, which produces a wide range of aliphatic compounds from steam, CO and ethylene. Another alternative is the Larson process, where it is produced from CO and ethanol using BF_3 as a catalyst. It is also a by-product of the Fischer-Tropsch process for the synthesis of fuel oil (Eş et al., (2017)). As for butyric acid, it is industrially obtained from the oxidation of butyraldehyde, which comes from propylene derived from crude oil by oxo synthesis (Cascone, 2008).

When studying the market volumes in some of the European countries, the general trend is that the market size of acetic is much larger than that of propionic and butyric. The total market size of France, Spain and Italy are very similar, while in Germany is slightly higher, being in United Kingdom even superior (Figure 1.14). In Spain, acetic acid had a market volume of 182.7 kilotons in 2022, followed by propionic and butyric acid with 19.8 and 4.5, respectively. They are shown only the data of the three main carboxylic acids in terms of market volume (acetic, propionic and butyric), being the rest less important.

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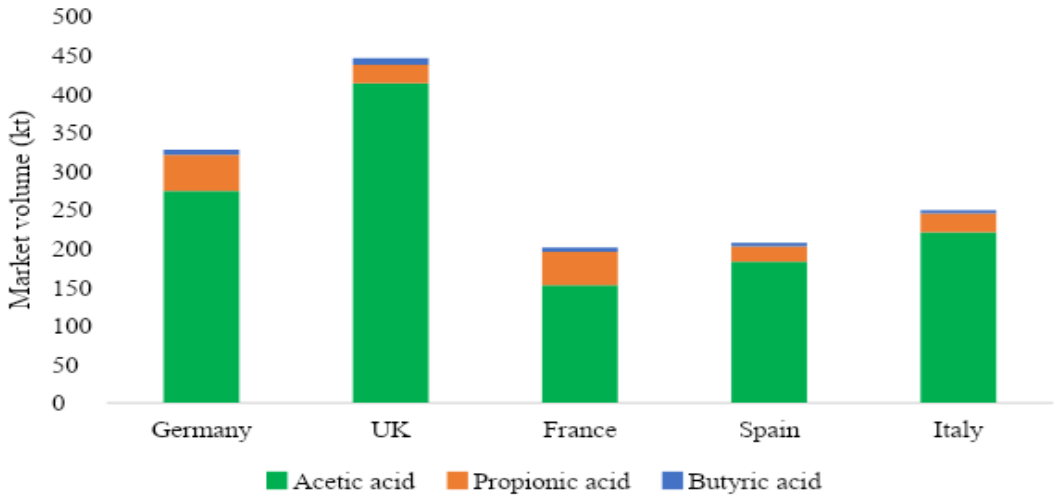

Figure 1.14. Market volume per country in Europe of the three main VFA. Source: own elaboration from data of Market Insights, (2017).

Given the existing similarity between the market volumes of the different European countries, from now on the specific data for Spain will be presented. In this country, there is a large demand for VFA to be covered, being necessary to import due to a demand higher than a supply within the nation. In fact, looking at the imports of the product, Germany is the largest supplier of monocarboxylic acids to Spain with 18 % of the total Spanish imports of carboxylic acids. China is the Spain's second supplier, followed by Singapore, Saudi Arabia and Belgium (Estacom, 2023). As it can be checked, suppliers of carboxylic acids in Spain are quite distributed between the EU and third countries. The general trend in Spain is the decrease in imports of carboxylic acids from most countries, except China, which in the last year increased its sales to Spain.

Acetic acid is the one with the biggest market volume also in Spain and it is increasing significantly, going from 159 kilotons in 2017 to 196 in 2024 (Market Insights, 2017) as Figure 1.15 depicts. The average market price of acetic acid in Spain was 893 €/t in 2022, which implied an increase of 27.5% compared to 2017.

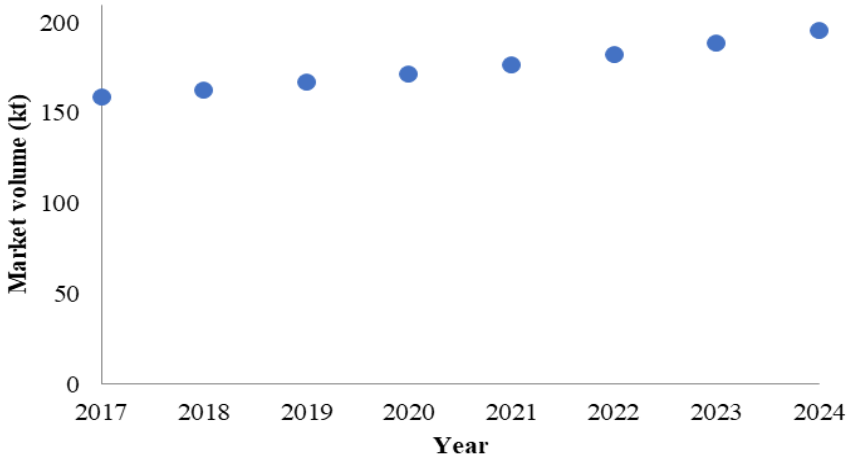

Figure 1.15. Evolution of the Spanish market volume of acetic acid. Source: own elaboration from data of Market Insights, (2017).

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The average market price of propionic acid in Spain was 1,730 €/t in 2022, which represents an increase of 38.5 % compared to 2017. The growth perspective of this market will come from the firm progression of propionic acid use in the agricultural industry and the increase in consumption of its derived products (dairy, livestock and poultry products). As a result of its application to reduce microbial growth in stored grain, hay, poultry waste and livestock water, propionic acid is widely used as an effective grain preservative. Likewise, the increasing demand for natural preservatives in ready-to-eat and canned food products is also driving market expansion.

Propionic acid is the second with the biggest market volume and it is increasing notably, going from 18 kilotons in 2017 to 20 in 2024 (Market Insights, 2017) as is shown in Figure 1.16.

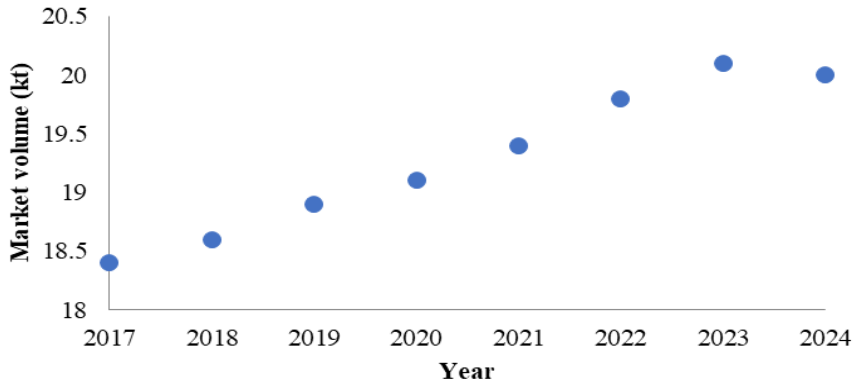

Figure 1.16. Evolution of the Spanish market volume of propionic acid. Source: own elaboration from data of Market Insights, (2017).

The total butyric acid market closed 2022 with an average price of 2,759 €/t, which represents an increase of 48.0 % compared to 2017 when the price stood at 1,860 €/t. The main drivers of expansion in the butyric acid market in the coming years are the growing demand from the food and flavour sectors. This can create a significant business opportunity in the coming years. Moreover, increased demand for packaged foods due to changing customer preferences is a key driver of the sector's expansion.

Butyric acid is the third with the biggest market volume and, although has a very small market volume compared with acetic and propionic acids, it is also growing, going from 4 kilotons in 2017 to 4.7 in 2024 (Market Insights, 2017) as Figure 1.17 shows.

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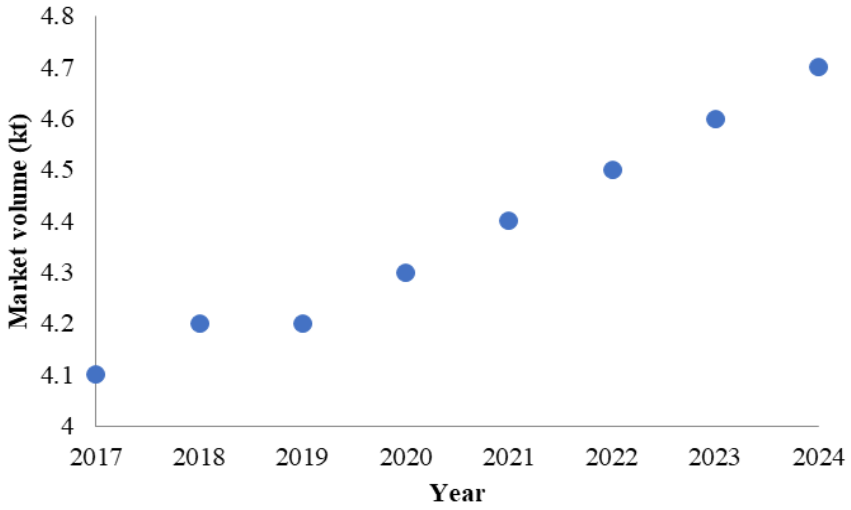

Figure 1.17. Evolution of the Spanish market volume of butyric acid. Source: own elaboration from data of Market Insights, (2017).

Acetic acid is produced in Spain, mainly in plants located in the provinces of Tarragona and Huelva (mainly Dow Chemical Ibérica and Celanese Chemicals Ibérica). Although Spain produces acetic acid, most of the product used in the country is imported from other countries, because the demand is greater than the national production capacity, especially in the food and pharmaceutical industries. Regarding imports disaggregated by autonomous communities, the main Autonomous Community in terms of product purchases is Catalonia, with total imports representing 50 % of the total, followed by Andalusia with 35 %.

Regarding propionic acid, in Spain there are several companies producing it: mainly BASF Spain and Quimipur. Despite this, a significant amount of this compound is imported. This may be due to a demand higher than production, quality (higher purity in the imported one) or production costs. The main Autonomous Community in terms of product purchases is Catalonia, having acquired a 76 % of total national imports, followed by Madrid Community with 11 %.

Butyric acid is also produced in Spain by several companies: Bioiberica, Química Sintética, Síntesis Química and Indukern; despite this, the quantity produced is not enough to satisfy the national demand for this chemical compound, so the product is also widely imported. The main Autonomous Community in terms of product purchases is also Catalonia, acquiring a 41 % of the total national imports. In second place is Aragon 31 % followed by Madrid Community with 21 % of the total.

The import data presented for the three main VFA show that in general, the demand is greater than the supply for these products, so there is a great opportunity to make up for these shortcomings by seeking their production through alternative routes like those that will be presented later.

When referring to the end user of the VFA, where Spain and the rest of the EU countries share similar trends. Figure 1.18 manifests that consumer goods is the most important end user with 41 % of the market, followed by other uses with 28 % and lubricants with 9 % (Market Insights, 2017). Other end users such as personal care products and the food and beverage market would have less interest as it becomes more difficult to introduce bioproducts generated from waste in these sectors.

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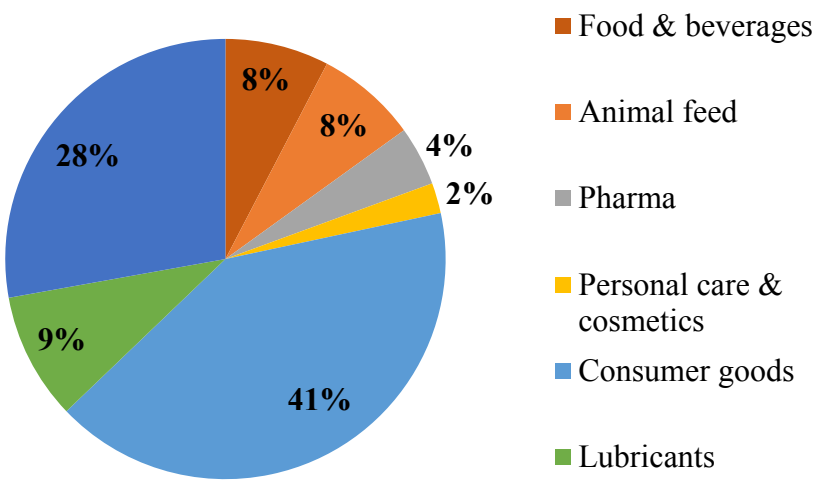

Figure 1.18. Spanish carboxylic acid market share by end-user in 2022. Source: own elaboration from data of Market Insights, (2017).

1.4. OPEN MIXED FERMENTATION TO PRODUCE VFA FROM WASTE

Nowadays, the bio-based VFA production is not viable in economic terms compared to petroleum-based production methods mainly due to two reasons: the manufacturing cost of petroleum-based products is lower because they are well optimised, apart from the lower efficiency of the bio-based ones (Atasoy et al., 2018). Nevertheless, the depletion of petroleum sources as well as the high damage on the environment such as GHG emissions, chemical requirements and waste generation makes the transition from petro-based production mandatory. Bhatia & Yang, (2017) highlighted in their work the necessity of going towards the bio-based production of VFA in relation with the terms sustainability, degradability and renewability, being crucial for the fulfilment of the Paris Agreement (2016). The production of VFA from waste implies processes which have started to be developed to make it more efficient in terms of fermentation optimisation and separation techniques.

Biological VFA fermentation can be carried out from two different perspectives: using pure cultures or mixed cultures. Pure culture utilisation implies the employment of single microbial species to produce VFA such as *Clostridium ragsdalei* or *Acetobacter okinawensis* from pure sugars or waste products. Some authors like Atasoy et al. (2019) reported that individual VFA production is higher when pure cultures are used but has the disadvantages of needing aseptic working conditions as well as high quality raw material. By applying mixed culture fermentation, the main advantage is that sterile conditions are not needed, apart from accepting a wide variety of carbon sources, not only food resources (Varghese et al., 2022). For mixed culture fermentation, an inoculum coming from the WWTP's anaerobic digesters or one treating FW can be used, but the main drawback is that the product composition is variable depending on the operational parameters which are being applied. This second alternative is the one used in this doctoral thesis, thus taking advantage of the capacity of mixed microbiology to adapt to the variability of substrates, making the scalability of the process more feasible within reasonable costs.

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1.4.1. Fermentation

1.4.1.1. Process stages and metabolic reactions

AD is a proven technology, utilised for the valorisation of waste towards biogas. The revenues which can be obtained from CH_4 production are not too high for waste management companies without subsidies, apart from the important capital expense needed due to the volume of digesters and high hydraulic retention time (HRT) required by the process to complete the metabolic reactions. Currently, considering the demand for bioproducts such as carboxylic acids, new alternatives to biogas production are opening up such as VFA production from waste streams through acidogenic fermentation. Anaerobic fermentation is part of the whole AD process and generates the VFA, apart from producing CO_2 and H_2 (Kleerebezem et al., 2015). Figure 1.19 shows the AD process steps and pathways, showing how the last stage must be inhibited in order to accumulate VFA avoiding their consumption to further produce biogas. The encouraged pathways are indicated by the thick line.

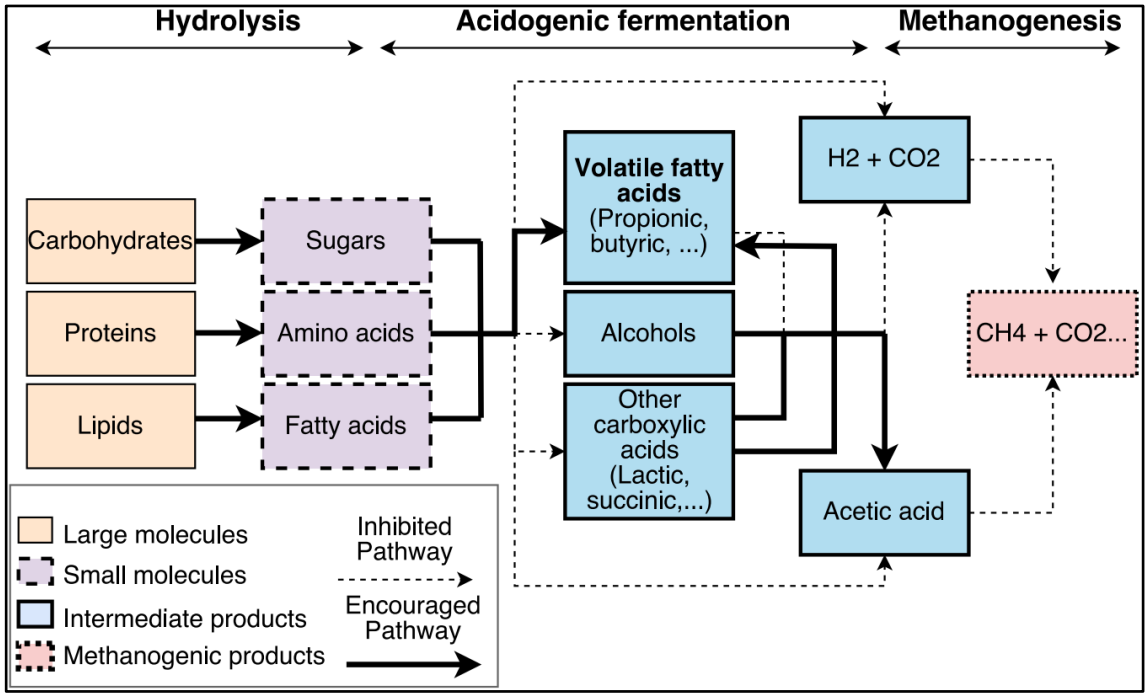

Figure 1.19. Steps of the anaerobic digestion process. Source: Ramos-Suarez et al. (2021) under CCA 4.0 IL.

In order to further understand deeply the microorganisms which are involved in each AD step, Figure 1.20 illustrates the role of each microbial community in the four phases of AD, being the VFA production an intermediate step. The AD process begins with complex materials being hydrolysed through enzymatic catalysis by hydrolytic bacteria converting carbohydrate, protein and lipid into sugars, amino acids and fatty acids. During the second stage, the acidogenesis, the monomers are transformed into VFA along with lactate, ethanol, H_2 and CO_2. Then, the syntropic fatty acid oxidising bacteria convert VFA into acetate and H_2 in the acetogenesis step and in the last stage, the H_2 and CO_2 may further be converted into biogas.

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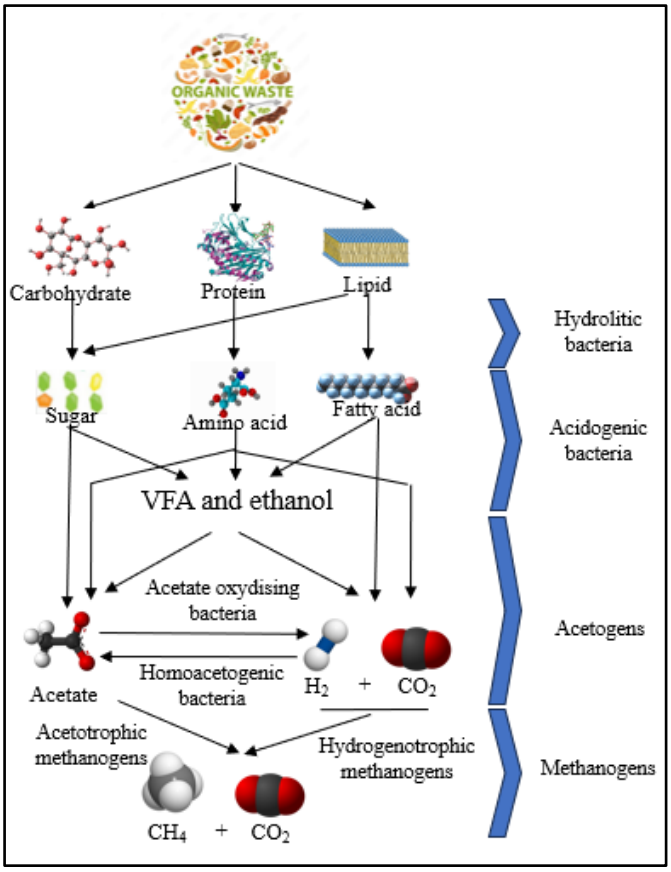

Figure 1.20. Role of several microbial communities during the four stages of anaerobic digestion. Source: own elaboration.

As it has been detailed, anaerobic fermentation has four main processes including solubilisation, hydrolysis, acidogenesis, and methanogenesis, during which both VFA and CH_4 are produced. Mainly, the microorganisms responsible for producing VFA are known as acid-forming bacteria, and include both acidogenic, acetogenic and homoacetogenic bacteria. They can incorporate organic compounds such as amino acids, monosaccharides, and long-chain fatty acids and accumulating them within their cells as intermediate products called pyruvates. Pyruvates are converted to Acetyl-CoA to produce acetate, as well as other bioproducts that can be converted to acetate by acetogens. Immediately afterwards, the methanogens can use the acetic produced as well as the H_2/CO_2 to grow (Yuan et al., 2019).

Part of the optimisation of the VFA production process consists about understanding the synergies that exist between the microbial communities involved in fermentation. Although work has already been conducted in this field, so far, the known species of acidogenic microorganisms are quite limited, with three phyla which have been most extensively studied: *Bacteroidetes*, *Proteobacteria* and *Firmicutes*, which can degrade organic compounds of various types to produce VFA. Furthermore, depending on the target acid to which the fermentation process is directed, there is one dominant involved phylum or another. As mentioned, acetogens can solubilise organic matter and hydrolyse it in order to convert macromolecules into micromolecules, subsequently converting them into VFA. The mechanism by which acetogens use soluble organic compounds as a substrate to produce acetic acid is shown in Figure 1.21.

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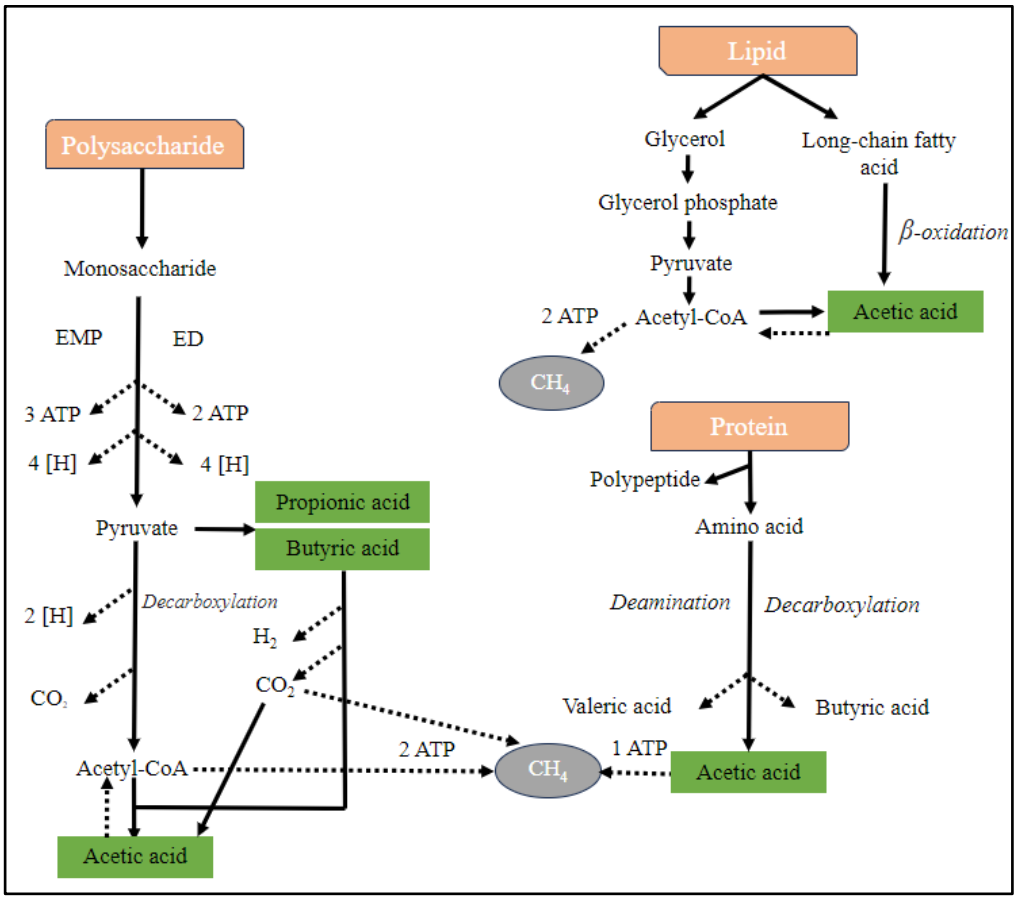

Figure 1.21. Metabolic pathways of acetogens and methanogens for VFA production. Source: own elaboration from Yuan et al. (2019).

Lipids are first hydrolysed to long chain fatty acids and glycerol. Subsequently, glycerol is converted to glycerol phosphate which gives rise to pyruvate, which is converted to small organic molecules, while long-chain fatty acids give rise to acetic and Acetyl-CoA by beta-oxidation. Proteins are first converted into polypeptides and amino acids thanks to the work of various extracellular hydrolases, some of which can be converted into microbial cell tissues, which continue to undergo decarboxylation and deamination reactions, becoming VFA. Regarding polysaccharides, once they are converted into monosaccharides, they are transformed into pyruvate through the Embden-Meyerhof route, giving rise to acetic, propionic, butyric, as well as small organic molecules with the participation of Acetyl-CoA.

Strictly anaerobic methanogens are one of the groups involved in fermentation and they use both acetic, formic and H_2 as growth substrates (Zhao et al., 2017). That is why they can be considered as competitors of acetogens. So far, 83 species of methanogens have been described and fall into three specific nutritional categories: 20 species of methylamines use methyl compounds such as methanol, methylamines, or dimethyl sulphide; 20 species are acetoclastic, since they use acetic acids to give rise to CH_4, and 61 species are hydrogenotrophic, oxidising H_2 and reducing CO_2 to produce CH_4 (Garcia et al., 2000). Only one third of the CH_4 generated comes from CO_2 reduction, while the remaining two thirds come from the methyl group of acetic acids, so there is significant competition between the accumulation of acetic acid and its transformation.

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In their process of transforming acetic acid, H_2, formic acid, carbonic acid, and methanol into CH_4, CO_2, and new cellular material, methanogens use a series of enzymes and coenzymes that can be divided into two categories depending on their functions. One is an electron carrier that includes coenzyme F420 and F430, ferredoxin, coenzyme B, cytochrome, and methophenazine (Hu et al., 2018). The other category is the C1 carrier, including mainly tetrahydropalmatine, coenzyme M, and tetrahydromethane. Acetic acid is used as a substrate for growth by species within the genera *Methanosaeta* and *Methanosarcina*; while *Methanosaeta* consumes 2 mol ATP and uses acetyl-CoA synthase to convert acetic to acetyl-CoA, *Methanosarcina* consumes 1 mol ATP and uses acetate kinase to catalyse acetic to acetyl-CoA.

It is therefore seen that acetyl-CoA is a vital intermediate in the methanogenic fermentation of acetic acid. The special route of acetate-utilising anaerobes using acetic is that the electrons used come from the oxidation of the carbonyl group of acetyl-CoA to CO_2 (Ferry, 1992); therefore, the high consumption of ATP and acetic acid to give rise to acetyl-CoA indicates that the active metabolism of methanogens is an important competitor.

Competition between acidogenic bacteria and archaea was revealed, so the inhibition of methanogenesis is not a trivial process. To achieve this inhibition, different strategies have been proposed, from physical methods such as heating to chemical inhibitors. Liu et al. (2011) made an exhaustive review of the chemical inhibitors which can be used to inhibit this stage, noting that some of them inhibited only methanogens but others were not specific and also inhibited the activity of acidogenic bacteria. Several methanogenic inhibitors have been identified such as 2-bromoethanesulfonate (BES), chloroform ($CHCl_3$), and fluoroacetate (FCH_2COONa), iodoform, etc., above all used at lab and pilot scale tests, becoming an indispensable tool for investigating and understanding the mechanisms of VFA production and consumption in the fermentation process. In particular, BES is a typical kind of specific inhibitor for acetotrophic and hydrogenotrophic methanogens which is frequently used (Hongbo et al., 2016).

It must be taken into consideration that these chemical inhibitors represent a significant cost if they are continuously added to a process, so when thinking about scaling the technology to an industrial scale, they must be discarded. There is another way to inhibit the methanogenic stage by applying certain operational parameters to the reactors, specifically playing with pH, temperature, organic loading rate (OLR), HRT and so on, as Ramos-Suarez et al. (2021) indicated in their work. For instance, alkaline fermentation could be one of the most popular strategies for inhibiting methanogenesis when working with SS and has the advantages of being low cost, easy to operate, promotes solid dissolving, etc. Other strategies to inhibit methanogenic stage could be those related to the difference between the living habits of hydrolytic bacteria and methanogens, for example, considering that methanogens have a growth cycle between 25-30 days and that of hydrolytic bacteria is much shorter. In this way, reducing sludge retention time of fermentation reactor could boost VFA accumulation and debilitate methanogens activity (Hongbo et al., 2016).

1.4.1.2. Operational parameters affecting fermentation

- ***pH***

It is one of the main parameters that have the greatest effect on the hydrolysis and acidification efficiencies of the fermentative process since it affects the activity of enzymes and changes the selective permeability of cell membranes and the toxicity of the substrates in the medium. Although anaerobic bacteria are characterised by having the capacity to adapt to a wide pH range, very acidic and alkaline environments, that is, with a pH lower than 3 and higher

than 12, are lethal for these microorganisms. It was reported that the optimal pH range to ferment sludge would be between 8-10 (Azman et al., 2015).

Alkaline fermentation is commonly favourable when it comes to improving VFA production yields, since in addition to being inhibitory for the methanogenic archaea that consume VFA, it promotes the solubilisation of carbohydrates and proteins in bacterial cells. In this way, Chen et al. (2007) saw that the hydrolysis of biological sludge improved when passing from pH 6 to 11, with VFA production being notably higher in the alkaline range than in the neutral and acid range. Yu et al. (2008) also reported that a pH close to 10 increased the contact between extracellular organic matter and enzymes, thus favouring the breakdown of the sludge matrix and allowing microorganisms to improve VFA production.

In any case, the substrate used for the fermentation process can vary the optimum pH range, so this must be considered. Apart from influencing the metabolic efficiency and therefore on the VFA production yield, it also influences the selectivity of the process, determining the fractions of the VFA obtained (Fang et al., 2020).

- ***Temperature***

Temperature is another of the parameters which affects the production of VFA and anaerobic fermentations have been carried out in temperature ranges from psychrophilic to thermophilic, passing through the mesophilic. Hydrolytic and fermentative bacteria have a high capacity for adaptation, although it is generally the higher temperature which favours the dissolution of organic substances and the formation of intermediates such as VFA (Ho and Dang, 2014).

There is considerable consensus in the scientific literature that the mesophilic temperature (35 ºC) is the most efficient in terms of VFA yield, as well as being cheaper than the thermophilic range in terms of operating costs. From a thermodynamic explanation, the initial Gibbs free energy in the biological degradation of organic matter is reduced by increasing temperature, resulting in a faster rate of digestion (Kanokwan, 2006).

The mesophilic range is somewhere in between, making it ideal for VFA production. Biomass found in thermophilic environments is usually less resilient to changes than that found in mesophilic environments, with thermophilic microorganisms being also much less resistant to changes in temperature (Luu et al., 2008). In addition, the pKa of VFA increases with increasing temperature, so their toxicity for bacteria also grows by favouring their undissociated fraction.

- ***Substrate***

Substrate composition also affects VFA production, both in terms of C/N ratio, trace elements content and so on. In this way, substrates with a high concentration of solids can cause ammonia pressure, which directly affects the microbial community, improving the oxidation reaction of acetic acid, but for example, in the case of substrates rich in proteins, propionic production may be limited (Hongbo et al., 2016). The enzymes secreted by acetogens affect this type of process, influencing the metabolic pathways of each VFA in particular.

The carbon to nitrogen relation (C/N ratio) of the substrate also influences microbial metabolism. Anaerobic bacteria need an adequate C/N ratio to grow stably. When choosing a C/N ratio, ranges between 20-30 are the most appropriate in anaerobic fermentation according to Zhang et al. (2014), but specifically, SS is characterised by having a lower C/N (around 6-9), given its high nitrogen content and low amount of biodegradable carbon. An alternative to balance this relationship is to add carbon-rich substrates through co-digestion, such as the one

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that could be done between sludge and biowaste, with the aim of improving the activity of acidogenic microorganisms and enzymes related to VFA production.

Not only the C/N ratio of the substrate is important, but other parameters also have a significant role. For example, trace elements such as iron, zinc, cobalt and nickel must be available during the fermentation process, since they activate and maintain the enzymatic activity (Thanh et al., 2016). Although there are several studies focused on the effect of the concentration of these trace elements on biogas production, their influence on VFA production was much less studied.

- ***HRT and OLR***

HRT means the average time which the substrate spends in the reactor, or what is the same, the time available for the microorganisms to contact the substrate. In general, in most cases of fermentation of both SS and biowaste, the substrate and biomass are mixed during continuous operation, causing the HRT and SRT to equalise. A correct choice of the applied HRT is important, since it may not be decisive in simple and highly soluble substrates where hydrolysis is already favoured, but it may have a great influence on complex substrates fermentation.

It is known that AD when biogas is the desired product requires HRTs greater than 20 days, but when HRTs are lower, VFA production is generally stimulated, since the growth of methanogenic archaea is prevented with this strategy. In general, it was observed that the production rate of VFA increases with HRT up to a certain point, since more protein and carbohydrates are available in their soluble form (Jankowska et al., 2017); but if the HRT value is very small, VFA production may be reduced since there is not enough time for the substrate to be fermented.

OLR is directly related to the HRT as well as to the substrate concentration and refers to the kilograms of organic substrate that are fed daily per unit volume of the digester. A higher applied OLR means higher treatment capacity and in general, VFA performance increases with the OLR within a reasonable range. But the increase of OLR through the reduction of HRT may imply a drop in hydrolytic efficiency, also leading to a drop in VFA production. Besides, this parameter has an important influence on the distribution of the VFA mix generated; if OLR is increased through either an increase in substrate concentration or a reduction in HRT, a shift from more oxidised to more reduced compounds occurs, such as propionic production from the lactate-propionic pathway (Rodríguez et al., 2006).

1.4.2. Concentration and purification

When VFA are produced, the fermentative process is just as important as the subsequent separation and concentration stage. In fact, this last stage is the bottleneck, since no matter how optimised the fermentation is, streams with high concentrations are difficult to produce. In addition, depending on the final application of the VFA, this stream must be sold as a mixture, or it must be separated between the different VFA individually. That is why before choosing a specific separation process, the use of the final product must be kept in mind. In addition, the number of unit operations necessary to reach the desired final product as well as the energy demand needed to carry out the separation must also be taken into consideration.

When recovering VFA from waste streams, fermentation must be optimised in order to start from VFA concentrations as higher as possible, but there are also other products in the broth that are not VFA, such as forms of nitrogen and phosphorus, although their low concentrations probably do not affect the recovery processes too much. Even so, there are ions such as Na^+ from the addition of NaOH to regulate the pH that can sometimes be found in

concentrations higher than 5 g/L, having an impact on the separation processes (Zhang et al., 2014).

Some of the current methods to recover VFA from waste streams are detailed below:

-Gas stripping with absorption

Stripping with gas or air is a method which is normally applied when the aim is to remove volatile organic compounds from water, causing clean air to meet contaminated water through a high surface area (Singh et al., 1992). This process was applied on a few occasions in the recovery of VFA from anaerobic fermenters, and what is done is to insufflate gas with an open tube and later subject this gas, which contains the stripped VFA, through an absorbent that acts as a recovery system; thereafter, the VFA salts formed are recovered through evaporation.

-Adsorption

The adsorption process involves the adhesion of compounds, molecules or ions contained in a liquid or a gas to a surface. To recover the VFA through this method, the fermented effluent rich in VFA is contacted with a column in which there is an adsorbent (usually ion exchange resins). The VFA in form of anion (working at basic pH for having the acids in their deprotonated form), is absorbed in the resin through the exchange between the functional anions of the resin. Of course, to make the process economically viable it is necessary to regenerate the resin column with a solvent (Atasoy et al., 2018).

-Electrodialysis

The electrodialysis process has been extensively researched as a VFA recovery technique, and it requires the VFA being in its charged form, so it could operate at pH ranges typical of fermentative processes. A series of charged membranes are placed between two electrodes and the applied current facilitates ionic transfer, thus giving rise to a chamber that contains the VFA-rich stream.

-Membrane contactor

A membrane contactor is a separation system which causes contact between a liquid phase and a gas phase with the aim of producing a mass transfer of both phases through a membrane, but without dispersion of a phase within the other. They generally have hydrophobic microfiltration membranes, but these systems do not work thanks to applied pressure like other membranes. The partial pressure differences and the concentration gradients between both sides of the membrane cause VFA to pass from the liquid phase to the gas phase through the hydrophobic membrane (Aydin et al., 2018).

Although there are also some additional separation methods such as esterification or pervaporation, their use is much more sporadic (Ramos-Suarez et al., 2021). Therefore, special emphasis will be placed on the separation technologies which were proposed in the technological development presented in this doctoral thesis; specifically, it deals with pressure-driven membrane filtration, liquid-liquid extraction (LLE) and distillation.

In particular, the technologies chosen in the process proposed in this doctoral thesis are those presented below along with the parameters affecting their efficiency.

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1.4.2.1. Pressure-driven membrane filtration

Pressure-driven membrane processes are one of the most tested separation methods for purifying VFA-rich streams. There are two types of membrane filtration, which would be dead-end and cross-flow. While in dead-end filtration the feed solution crosses the membrane surface in a vertical direction, in cross-flow the feed flows tangentially across the membrane surface giving rise to two different streams, which are the permeate and the rejection (Figure 1.22). Moreover, in the membrane industry, there is a wide variety of modules that can be hollow, flat sheet, tubular and so on.

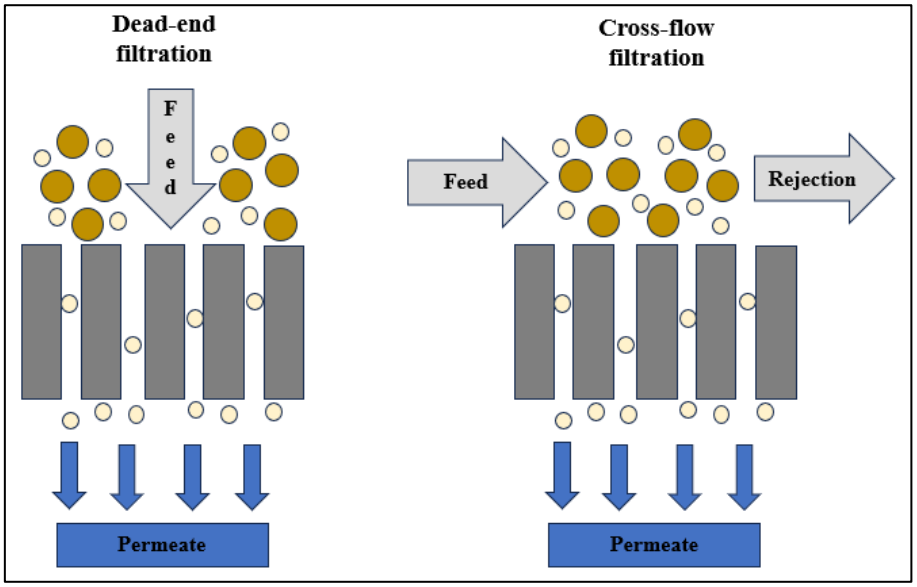

Figure 1.22. Configuration of dead-end filtration (left) and cross-flow filtration (right). Source: own elaboration.

Depending on the pore size of the membrane and the driving force, i.e., the transmembrane pressure (TMP) during operation, four groups of membrane filtration can be distinguished (Figure 1.23): microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO).

In the separation by MF, membranes with pore sizes between 0.1 and 10 µm are used, applying pressures ranging from 0.2 to 5 bar, depending on the specific type of membrane. There are different types of MF membranes depending on the material with which they were made, such as polymeric, metallic or ceramic, and they manage to retain particles larger than the pore size of the membrane, such as emulsified components, suspended particles or bacteria and protozoa. MF membranes are often of the polymeric type.

When reducing the pore size of the membranes, in this case from 0.01 to 0.1 µm and increasing the TMP applied (ranges between 1 and 10 bar), there can be found the UF membranes, which can already separate size compounds such as sugars and proteins.

As for NF membranes, they still have a smaller pore size, between 0.001 and 0.01 µm and their molecular weight cut-off (MWCO) range from 300-500 daltons (Da). That is why they can retain divalent inorganic ions as well as acids of lower molecular weight (MW) by combining different operating mechanisms, such as dielectric poles or the Donnan effect (Ernst et al., 2000). The pressures necessary to operate with these membranes are 5-20 bar.

The last type of membrane in terms of pore size is RO, with pore sizes ranging from 0.0001 to 0.001 µm, therefore requiring higher pressures to operate, which can be between 10-150 bar. In this filtration range there are no longer ceramic membranes, so organic and polymeric RO membranes are usually utilised due to their good balance cost-efficiency.

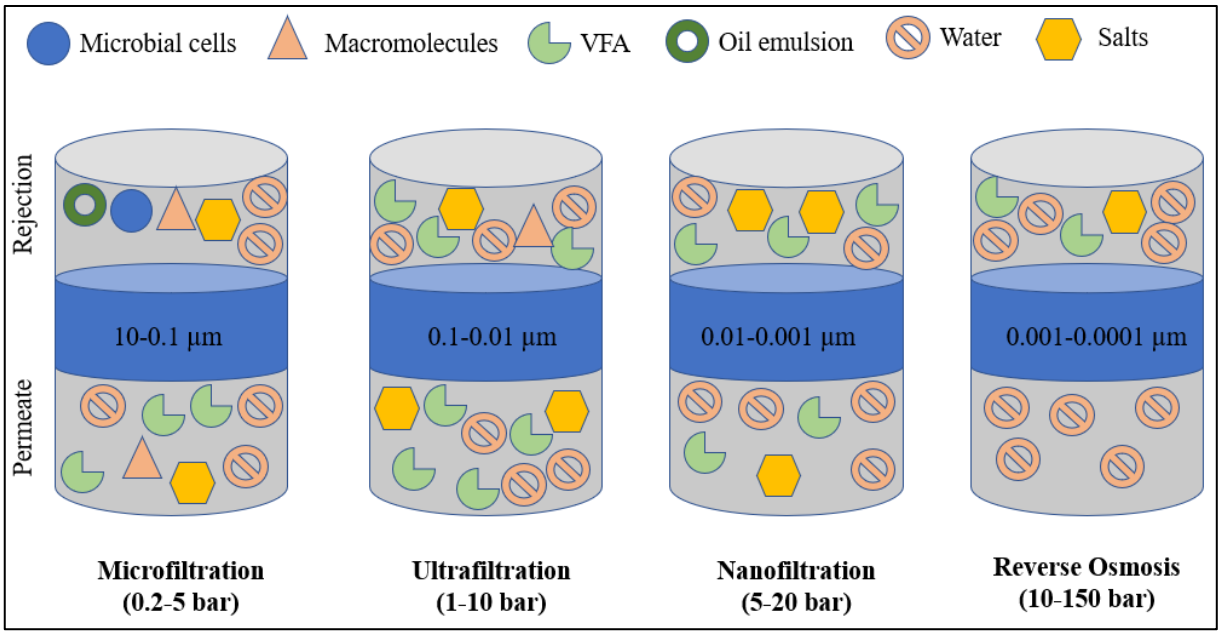

Figure 1.23. Characteristics of the different ranges of membrane filtration for VFA recovery. Source: own elaboration from Liao et al. (2018).

Although MF and UF membranes are useful for preparing fermented effluents for further concentration, their large pore size does not allow them to efficiently recover VFA. The characteristics of VFA molecules make NF/RO membranes necessary for this purpose. There are several studies in the literature that have shown retained VFA reaching values greater than 75 % and even exceeding 86 % through NF membranes (Xiong et al., 2015; Zacharof et al., 2016), although it was seen that the performance of these membranes is influenced by various factors such as filtration temperature, pH of the feed solution, feed concentration, or applied pressure. In addition, there are other mechanisms that include charge interaction, size exclusion, the Donnan effect and dielectric effects.

When referring to pressure-driven membrane processes, there are several parameters which influence the level of separation of the VFA present in the fermented effluent, and they depend both on the characteristics of the medium (pH, temperature, ionic strength and feed solution) as well as on the properties of the membrane itself (pore size and permeability, material, charge and hydrophobicity, among others). These must be considered to guarantee a stable filtration process sustained over time. The influence of each of them is presented below:

- ***pH***

The pH of the solution to be filtered is a key factor in the recovery of VFA from fermented effluents. The acidity constant (pKa) of the VFA is crucial in the success of the process, being the highest recovery percentages reported beyond their pKa values as these are converted to their dissociated forms. This was verified by different authors such as Han & Cheryan (1995), who recovered more than 75 % of acetic acid at pH 6.8 but this recovery was reduced to 10 %

when the pH was 2.7. This means that at higher pH, NF membranes become negatively charged and the solute, in this case acetic acid, was transferred to acetate, which is negatively charged, therefore electrostatic repulsion occurred causing the rejection percentages to suddenly increase. On the other hand, at low pH the solute is positively charged and is attracted to the negative membrane surface, reducing the retention percentages. In any case, there are also works such as that of Xiong et al. (2015), who reported that high percentages of butyric recovery were achieved even at an acidic pH of 3 units.

- ***Operating temperature***

Operating temperature could also be a determining factor when it comes to analysing a pressure-driven filtration process. Pervez et al. (2022) carried out a bibliographical review showing that lower percentages of VFA recovery have been obtained by increasing the temperature, since when this parameter is higher, the diffusion of the solute through the membrane surface increases. Furthermore, the polymeric structure in the active layer and the pore size of the membrane change markedly with increasing temperature, impacting VFA recovery. Thus, significant drops in the percentages of acetic acid recovery were observed by Lyu et al. (2015) and Liu et al. (2020) when increasing feeding's temperature using NF and RO membranes, respectively. The bibliographic evidence led the experimentation of this work to be carried out at room temperature (around 15 ºC).

- ***Ionic strength***

The existence of inorganic salts can alter the trends for VFA separation through membranes due to ionic forces. Although there are a variety of results, there is evidence such as that of Zacharof et al. (2016), which indicates that the addition of salts increasing the ionic strength boosts the percentages of acetic and propionic recovery, with sodium bicarbonate and sodium chloride being the ones which led to the best results. The influence of this parameter will be evaluated through experimentation if differences are detected when using a synthetic or real medium.

- ***Feeding solution***

The filtration performance of the membrane and therefore the VFA recovery percentages also depend on the initial concentration of the feed. Normally, MF and UF membranes cannot effectively retain VFA and although they do influence other compounds present in the matrix, they are not suitable for concentrating these molecules. In contrast, the passage of the feedings through the NF and RO membranes implies important changes in VFA concentration in the permeate and reject solutions; that is why, depending on the initial concentration from which the feed starts, one or the other concentrations will be reached after filtration.

Han & Cheryan (1995) prepared three solutions with different concentrations of acetic acid, specifically 1, 5 and 7.5 %, and found that they were able to recover 40 % of the acid when it was at 1 %, while only 20 % when the concentration was initially increased until 7.5 % when using a NF membrane. This could be due to the ion exchange capacity of the membrane surface. Instead, Zhou et al. (2013) presented different results when using a RO membrane. When they increased the concentration of acetic in the feed from 1 % to 5 %, the percentage recovery increased from 50 to 55 %. On the other hand, other authors also detected that there were no changes in the recovery percentages when varying the starting acetic acid concentration (Liu et al., 2020).

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- ***Pore size and permeability***

The pore size is essential when using membrane-based filtration processes since it is closely related to the characterisation of the permeate and rejection. Thus, MF membranes determine their selectivity by indicating their pore size, while UF, NF and RO membranes express it as MWCO, which can be measured as Da. MF and UF membranes have quite large pore sizes which allow the removal of large microorganisms, particles and macromolecules; on the other hand, those of NF and RO have a MWCO capable of retaining and eliminating ions and low MW compounds (Pervez et al., 2022).

Various works have reported the use of MF and UF membranes to purify the fermented effluent containing VFA, but significant selective retention of them cannot be achieved (Longo et al., 2015). Negative retention percentages have even been reported for VFA using MF and UF, which imply a higher concentration in the permeate than in rejection, which shows that pore sizes of these membranes do not satisfy the selective removal of VFA, being required smaller pore sizes like those of NF or RO membranes.

Regarding the permeability, it depends to the pore size since it indicates the rate of passage of a compound per unit area of the membrane and time. Although this can be varied depending on the construction material of the membranes, ceramic membranes are the ones that usually report higher permeability than their polymeric analogues, although the VFA recovery percentages are lower. Luiz-Santos et al. (2020) saw in their study that ceramic membranes with high permeability were suitable for fractioning compounds with higher MW, while polymeric membranes with lower permeability were better for removing substances with lower MW.

Regarding the application of NF membranes, it was found that membranes with MWCO of 200 Da can provide high percentages of acetic acid recovery compared to membranes with higher MWCO, specifically 400-1000 Da, which registered recovery percentages up to 50 % lower (Afonso, 2012). When the pore size is further reduced, RO appears, which boasts better performance compared to NF membranes when it comes to recovering VFA. In fact, Cho et al. (2012) reported that using membranes with a MWCO around 100 Da resulted in a butyric acid recovery of 78 %, falling to 62 % when recurring to 150 Da membranes, reinforcing again the importance of the pore size in VFA recovery. Therefore, there is a direct relationship between the percentage recovery of VFA and the MWCO.

- ***Membrane material***

Membrane filtration efficiency is also highly influenced by the material from which the membranes are constructed. When it comes to recovering VFA from fermented effluents, membranes could be metallic, ceramic or polymeric depending on the material used in their construction.

Metallic membranes are used in MF/UF units and are more resistant to high temperatures, as well as more resistant to fouling. On the other hand, ceramic membranes have also been used commercially in MF/UF systems since they offer more resistance to corrosion and provide higher fluxes because there is less interaction between the membrane surface and foulants. Kim et al. (2005) used ceramic MF membranes to recover more than 80 % VFA in solution and Zacharof & Lovitt (2014) used them successfully to remove large particles from the fermented effluent. In any case, both metallic and ceramic membranes have limitations when it comes to weight, modularity and price. Their cost was the main reason which led to choosing polymeric ones to conduct the experimentation, keeping in mind a future industrialisation of technology. Furthermore, the need for NF and RO pore sizes seeking to concentrate VFA reinforced the

decision, with the offer of these filtration ranges with metallic and ceramic membranes being practically non-existent.

- ***Membrane charge***

The surface charge of the membranes is also considered a key parameter to design efficient separation processes. To know the surface charge of a membrane, zeta potential values can be used, since these provide information about the electrostatic interactions produced between the membrane surface and the feeding. If, for example, the charge on the membrane surface is positive and the charge on the supply stream is negative, strong electrostatic attractions are produced, implying a low retention percentage. On the other hand, if both the surface of the membrane and the stream fed are negatively charged, repulsive forces are produced which improve the retention percentages according to Donnan's theory (Pervez et al., 2022).

While MF and UF membranes require chemical surface modifications to be charged since they are initially neutral, NF and RO membranes are synthesised with a negative charge on their surface under alkaline and neutral conditions and are positively charged under acidic conditions.

Although the membrane train must be applied to prepare the stream for subsequent treatments, the final concentration of VFA in the stream is not sufficient to meet the needs requested by some end-users, so other unit operations would have to be included later for this purpose.

1.4.2.2. Liquid-liquid extraction

LLE is the mass transfer operation in a two liquid-phase system. The components of a solution separate on contact with another immiscible or partially miscible liquid, distributing selectively between the two liquid phases. The solute is called the component which is separated (in this case VFA); the solvent is that introduced to create a new phase and separate the solute and the diluent is that from which the solute is to be separated. The phase that leaves the extraction equipment and contains most of the solvent and solute is known as extract, while the phase that contains most of the diluent is called raffinate. The economic feasibility of a LLE process depends mainly on the chosen solvent and the procedures used to recover it (Sánchez, 2016).

A solvent extraction process requires the conjunction of two main and complementary steps: the extraction stage itself, and the solvent regeneration stage, where the solvent is separated from the compounds which were generally solubilised through distillation. The extraction stage itself can be carried out discontinuously (batch) or continuously. Batch operation is usually practiced in laboratories for analytical purposes or in low-capacity production processes. For significant flows, continuous extraction is imposed.

Supposing a compound A being extracted from aqueous solution into an organic liquid, then for compound A, dissolution equilibrium can be represented by Eq. 1.1:

$$A\ (aq) \leftrightarrow A\ (org) \qquad \text{(Eq. 1.1)}$$

In the thermodynamics of LLE there is no simple theory such as that of ideal solutions for liquid-vapour equilibrium. The most common way to represent liquid-liquid equilibrium data is by an equilateral triangular diagram. Each vertex is a pure component of the mixture, while

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each side is a mixture of the two pure components located at its ends. Any point inside the triangle is a ternary mixture (Figure 1.24).

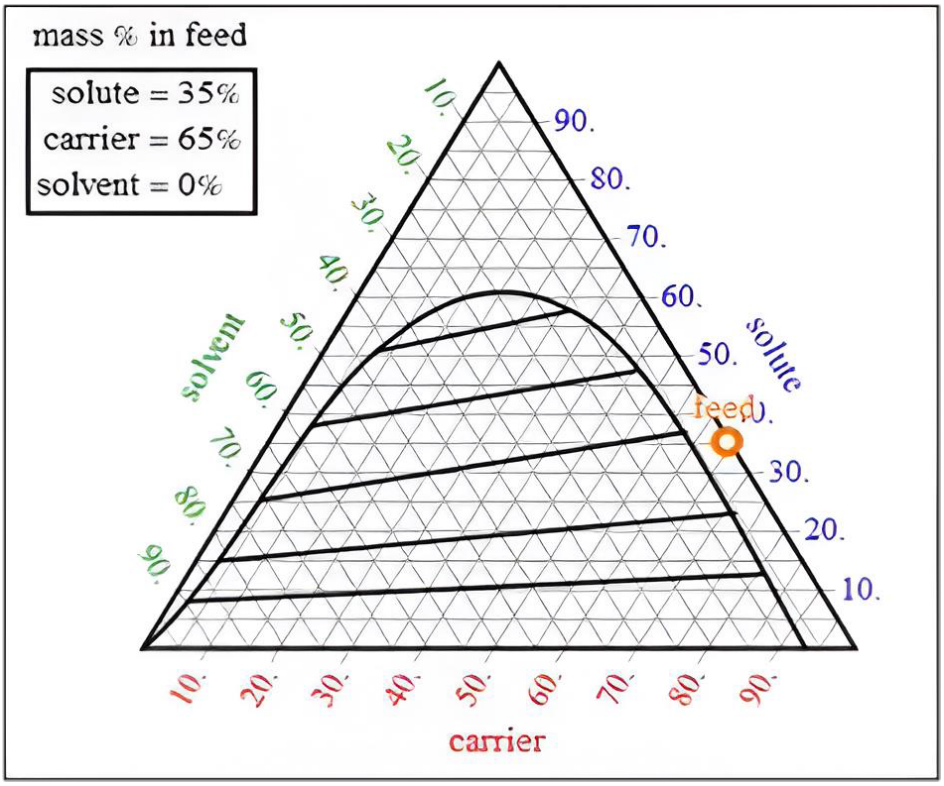

Figure 1.24. Example of an equilateral triangular diagram. Source: modified from Henley and Seader (1981).

Another important concept in LLE is the theoretical stage or equilibrium stage, which is an apparatus capable of mixing two immiscible liquids until equilibrium concentrations are reached, and then physically separating the two phases. Depending on the number of proposed stages, extraction can be carried out in different ways. One-stage extraction is nothing more than the separation of the mixture (feeding/solvent) through the distribution of the thermodynamic equilibrium into two phases: the raffinate (solvent-poor phase) and the extract (solvent-rich phase). Multi-stage extraction includes many schemes: cross-flow or co-current, simple counter-current and counter-current with reflux processes (Treybal, 1963).

In any case, this technology appears to be attractive for recovering VFA and it is common in research that the pH of the feeding must be lower than the pKa of the acids to achieve better recoveries and that selectivity increases with the number of carbons of the VFA (although it is also possible to separate acetic acid, the one with the shortest carbon chain). In addition, the higher the solvent/feeding (S/F) ratio the process is more effective although less economically viable (Ramos-Suarez et al., 2021). Instead, previous application of this unit operation, submitting the feeding stream to a pre-treatment is necessary, so in this case the separation with membranes was proposed.

Although in published works there were not many references regarding the maximum concentrations that can be reached in the solvent or the best way of separate said solvent from the VFA, the coupling of another unit process such as distillation is mandatory.

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1.4.2.3. Distillation

Distillation is a unit operation that is frequently used in the chemical industry for the separation of components (Henley and Seader, 1998). In this way, two liquid phases are separated due to the difference in volatilities between the chemical components of the mixture. The more volatile component (lower boiling point) is called distillate, while the other component (with higher boiling point) is called bottom product or residue.

In the simplest case, the distillation column separates the two-component feeding into two products: a top product (with a high fraction of the lower boiling component) and a bottom product (with a high fraction of the higher boiling component). The mixture of both liquids will have different boiling point characteristics depending on the concentration of the components present in it. Therefore, distillation processes depend on the vapour pressure characteristics of the mixture of liquids. This vapour pressure is created through the supply of heat as a separation agent. This separation method is very energy intensive, particularly when the relative volatility of the components is low; it is usually carried out in multi-tray columns, sometimes packed which makes them more efficient.

Relative volatility is a measure of the differences in volatility between two components, and thus of their boiling points. This parameter indicates how easy it is to carry out the separation of component A with respect to component B; it can be defined with Eq. 1.2. Thus, if the relative volatility between two components is equal to 1, separation by distillation would not be possible; the higher the value of α, the easier it would be to conduct the separation. For this doctoral thesis, in order to conduct the separation of the VFA with the solvent (in particular the binary separation of acetic acid/ethyl acetate), distillation was selected because of the relatively high volatility of ethyl acetate with respect to acetic acid.

$$\alpha_{AB} = \frac{y_A/x_A}{y_B/x_B} \qquad \text{(Eq. 1.2)}$$

where y_A and x_A are the mole fractions of component A in the vapour and in the liquid, respectively, being the subscript b analogous for component B.

To represent a phase diagram of a mixture with only two components (for example, a more volatile component A and a less volatile component B), there are two types of phase diagrams which can be utilised: at constant pressure (the most commonly used) and at constant temperature.

Figure 1.25A shows a general constant pressure phase diagram, while Figure 1.25B presents a specific one used in this doctoral thesis, that of the mixture acetic acid/ethyl acetate. At constant pressure, depending on the relative concentrations of each component in the liquid, various boiling temperatures are possible for the liquid mixture. The “bubble point temperature” is that at which the liquid begins to boil, while the “dew point temperature” is that at which the vapour begins to condense. The boiling of the liquid mixture takes place over a range of boiling points, as does the condensation of a vapour mixture. The higher curve of the diagram is called the dew-point curve (DPC), and the lower curve is called bubble-point curve (BPC). At each temperature, the liquid and vapour are in equilibrium.

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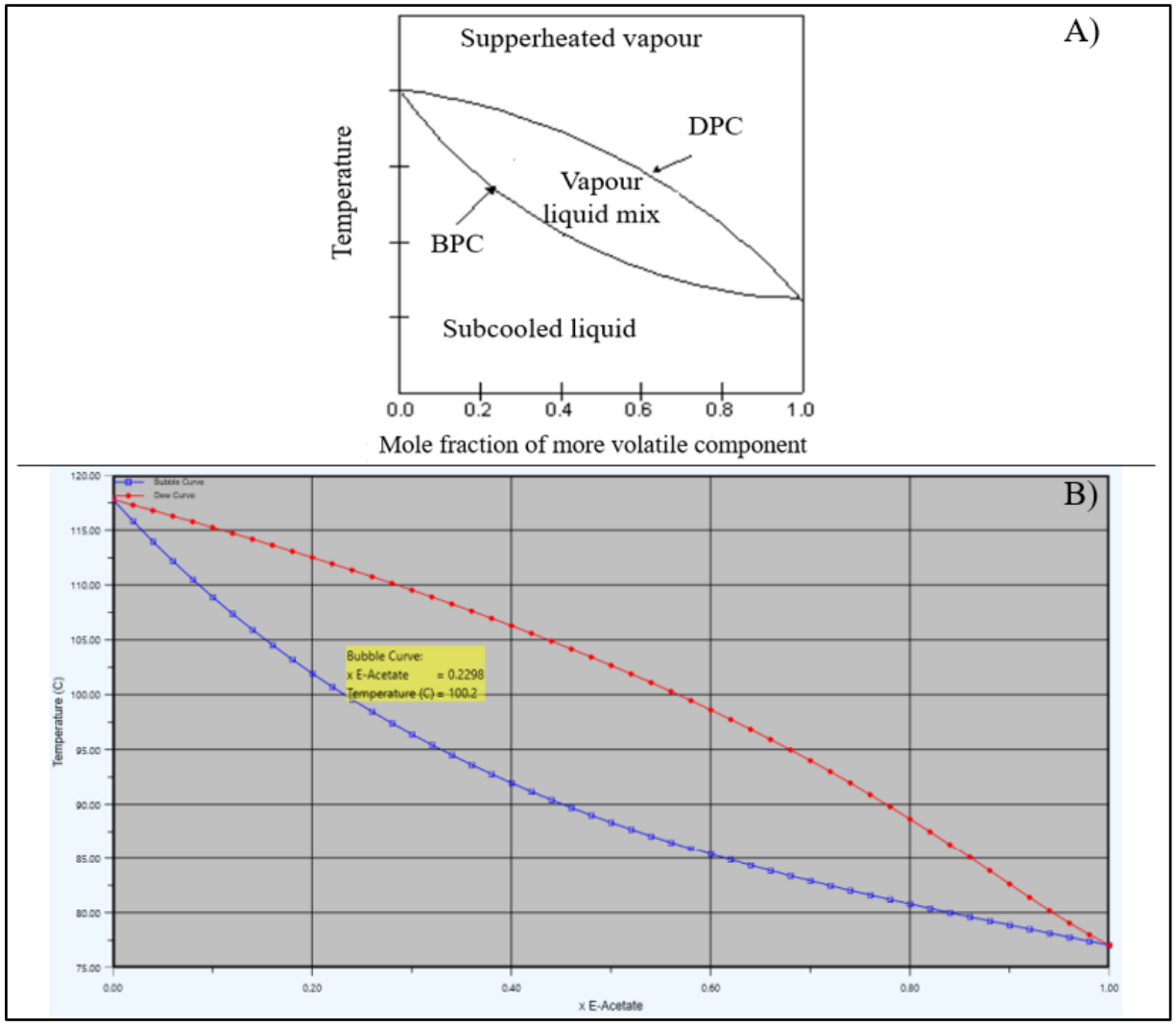

Figure 1.25. Phase diagram of binary system at constant pressure. A) General case. B) Equilibrium diagram mix ethyl acetate/acetic acid (1 atm of pressure).

Having chosen the LLE process as a unit operation for the evaluation of the technology proposed in this work, distillation is necessary as a complement to it.

1.4.3. Limitations for VFA production from waste

When it comes to marketing VFA from waste with the aim of replacing those produced through conventional methods based on fossil fuels, there are technical, economic, legal and environmental barriers which must be overcome.

1.4.3.1. Technical barriers

From a technical point of view, the production of bio-based VFA from waste is still complex for different reasons. VFA production yields and the mix produced vary greatly depending on the type and composition of the substrate used, as well as the inoculum available and the different operational parameters applied (pH, temperature, OLR, ...) as stated Vázquez-Fernández et al. (2022) in their review.

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Although it has been shown that the production of VFA from monocultures of microorganisms can improve the results of VFA yields compared to mixed culture fermentation, the scaling of this technology would imply unaffordable associated costs to maintain aseptic conditions. Therefore, more research is needed through new perspectives involving metabolic engineering techniques such as the modification, deletion, and addition of specific genes in order to expand the fermentation capacity of microbes. On the other hand, the lack of a complete understanding of these metabolic interactions, as well as the deficiency of effective tools for genetic modification, are still a barrier to VFA production (Varghese et al., 2022).

To this complexity of the fermentation process must be added the no less important purification/concentration and separation of VFA from the fermented effluent, which many authors cite as the most tangled part. Due to the low concentrations reached during the fermentation, this process is vital in order to achieve the desired final products. The presence of compounds other than VFA which must be eliminated as well as the large amount of water contained in the generated product make this separation complicated.

In any case, most of the research focuses on the one hand on fermentation and on the other hand on the recovery of the product, with little focus on the optimisation of the process from an integral point of view. To produce bio-based VFA competitively, research needs to be planned taking into account the final product and the integrated process design as in the case of this doctoral thesis.

1.4.3.2. Economic barriers

From an economic perspective, several works have shown that VFA production is more favourable compared to the production of biogas from waste streams, although it is evident that the degree of technological maturity of the solutions is very different. In any case, VFA production can give rise to a much wider range of products than the generation of renewable gases.

Moreover, recovering VFA from waste streams also adds a significant cost to the fermentation. Thus, depending on the final use of the VFA, the most economical schemes will be chosen in order to maximise recovery at minimum cost. During the course of this doctoral thesis, various approaches have been made with VFA consumer companies, which concluded that they would not be willing to pay more for acquiring a bio-based product.

Making the process more economically favourable in relation to traditional production methods becomes complicated, given that these are highly optimised. In any case, references including economic analysis are quite scarce and usually base their results only on simulations without considering experimentation, which implies great uncertainty when it comes to scaling the technology.

1.4.3.3. Legal barriers

One of the threats to this type of biorefinery for producing VFA from organic waste streams is the acceptance of the final product by users, as well as its quality certification. A change in the legal framework which allows the use of products obtained from waste is needed and this requires the involvement of public authorities and the general public to work towards acceptance. Additionally, this change is not feasible without compliance with quality standards and health regulations which can only be guaranteed through technical and scientific progress. Moreover, a change in urban waste management systems is also needed to integrate separation at source and favour recycling and, for which, public acceptance is vital.

The main legal barriers which the management of SS and biowaste must face are the following:

A) Technical criteria for the use of VFA coming from sludge and biowaste. It is needed to specify if the current criteria which apply to VFA coming from the petrochemical industry would apply also to those coming from waste. Moreover, it must be checked these bio-based VFA meet those criteria, being competitive both from technical and economical point of view.
B) Lack of uniformity in legislative development. Although European Directives regulate the waste management systems, each Member State asks for different requirements, which complicates the operation of companies internationally, therefore conditioning the creation of scale in the market. In Spain, where each Autonomous Community owns its specific legislation, this phenomenon is even more complicated.
C) Separation collection of biowaste as the only available option. An investment in improving the selective collection network will be needed in terms of education, awareness and citizen's sensibilisation tasks. Moreover, local authorities will need to be further involved in this selective collection, implementing solutions such as penalties or incentives towards good practices.
D) The complexity related to the term end-of-waste status established in the legislation. Thus, given the origin of these bioproducts, they must undergo recycling or recovery operations to achieve this status (process which can take up to 3 years), therefore meeting these criteria: they are used for specific purposes, there is a niche market for them, they fulfil the technical requirements for the specific purpose and they comply with the existing legislation and product standards; moreover, their use will not lead to adverse environmental or health impacts. In addition, product approval by the European Chemicals Agency must be obtained in parallel.

1.4.3.4. Environmental barriers

The importance of the fight against climate change and the need to leave behind non-renewable resources was revealed in this Chapter and today it is often accepted that bio-based products are better than those based on fossil fuels. There is no doubt that it seems more sustainable to use resources that are part of the planet's natural cycles, such as those that give rise to bio-based products; however, there are times when this is not the case.

Therefore, it is necessary to take a look at the entire life cycle of the product to be evaluated, in this case the VFA, including all the associated environmental impacts from the substrate collection until the final product reaches the market. Only in this way a well-informed decision can be made between bio and fossil fuel-based alternatives.

Nowadays, there are other barriers which make it difficult to identify truly more sustainable methods such as the so-called eco-labels. Thus, although some of them, such as ECO LABEL™, are governed by ISO standards certified by organisations based on environmental criteria, there are many others which are not verified and therefore only create confusion for the consumer. When marketing VFA from renewable sources, it would be also necessary to make clear the appropriate methodology for achieving the associated eco-label.

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2) OBJECTIVE, METHODOLOGY AND STRUCTURE

2.1. OBJECTIVE

The main objective of this doctoral thesis is to provide an economically viable technological solution to produce VFA from two different urban organic waste streams: SS and municipal biowaste (in particular FW). To this end, all the experimentation was designed and executed from an industrialisation approach, highlighting the operation of pilot plants that allowed the obtention of operational data needed to evaluate the techno-economic feasibility of building VFA biorefineries. These could favour the replacement of current VFA production methods through the chemical synthesis processes which depend on fossil resources.

In order to evaluate the fulfilment of the main objective of the present document, the following specific objectives were established:

- To determine the VFA yields and fermentation selectivity from SS, considering the influence of pre-treatments such as THP (Chapter 3).
- To quantify the VFA yields and fermentation selectivity from FW, also assessing the scalability of the fermentation process (Chapter 4).
- To evaluate the capability of membrane technology for VFA pre-concentration as a prior step to purification processes (Chapter 5).
- To technically and energetically compare the LLE and distillation with two different organic solvents (ethyl acetate and ethyl octanoate) through experimentation and simulations, respectively (Chapter 6).
- To establish the optimal conditions for the global VFA production process from an economic perspective (Chapter 7).
- To assess the economic viability of an industrial plant converting SS and FW into VFA in Galicia (Chapter 7).
- To identify the environmental impacts of VFA produced through the proposed process and compare them with those obtained from traditional production routes (Chapter 8).

2.2. METHODOLOGY

Considering that very different methodologies have been followed during the course of this doctoral thesis, given the variety of equipment used both at laboratory and pilot plant scale, it was considered to detail the Materials and Methods used in each Chapter. Therefore, the methodologies are always specified prior to the presentation and discussion of the results in question, which facilitates their follow-up and understanding.

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2.3. THESIS STRUCTURE

Chapter 1 presents the introduction, which contextualises the current situation of the paradigm shift necessary to combat climate change leaving behind the fossil fuels. The problem of the enormous generation of organic waste streams is also described. From the synergy of these two problems arises the concept of biorefinery, which gives place to the production of high added-value compounds such as VFA, whose properties and uses are presented. Finally, the main routes to produce VFA from waste and their concentration and purification technologies are exposed, as well as the limitations that currently exist for these to reach the market.

Chapter 2 presents the objectives of this doctoral thesis, both the main one and those more specific, as well as the contents of each of the Chapters.

Chapter 3 addresses the production of VFA from SS. Firstly, it introduces the potential benefits of applying pre-treatments to sludge to produce VFA and sanitise it. Then, results at laboratory scale are presented, both in batch and continuous mode. Subsequently, the results obtained by operating a semi-industrial pilot plant are discussed, using the information collected on a smaller scale, with the aim of seeing if the VFA yields and selectivity of the fermentation process are scalable.

Chapter 4 details the investigations carried out when using FW as a substrate instead of SS. In this case, the methodology used is the same as in Chapter 3, beginning the experimentation in batch mode on a laboratory scale, to later continue with the operation of 5-L continuous reactors, which have served to choose the optimal conditions of the pilot plant from a techno-economic point of view.

Chapter 5 presents the perspectives of applying pressure-driven membrane filtration to pre-concentrate the VFA produced in fermentation. The pilot plant in which the experiments were carried out is described, as well as the results obtained with both synthetic medium and a real fermentation supernatant.

Chapter 6 describes the unit operations chosen to produce high-purity VFA. Thus, the results obtained experimentally for the LLE are shown as well as the simulations carried out in ASPEN HYSYS for the distillation.

Chapter 7 describes a tool developed which integrates all the unit operations of the process and that allowed carrying out an economic study of the minimum viable scale as well as the construction of a VFA biorefinery in Galicia with SS and FW.

Chapter 8 assesses the environmental impacts of VFA production in order to be able to compare them with those obtained from the petrochemical industry.

Chapter 9 discusses the main results derived from this thesis and proposes the next steps necessary to get closer to the implementation of the proposed technology on an industrial scale. In addition, existing alternatives to the production of high-purity VFA are suggested.

The general conclusions of the thesis are presented in **Chapter 10**. In successive Chapters there is the list of publications derived from this work (**Chapter 11**) as well as the Disclosures and the Bibliography (**Chapters 12** and **13**).

Figure 2.1 shows the structure of the thesis graphically and makes it possible to distinguish the relationship between Chapters and the information that each of them provides to the global process integration. As it can be seen, the experimental results obtained in **Chapters 3**, **4** and **5** and **6** provide all the input information for the tool developed in **Chapter 7**.

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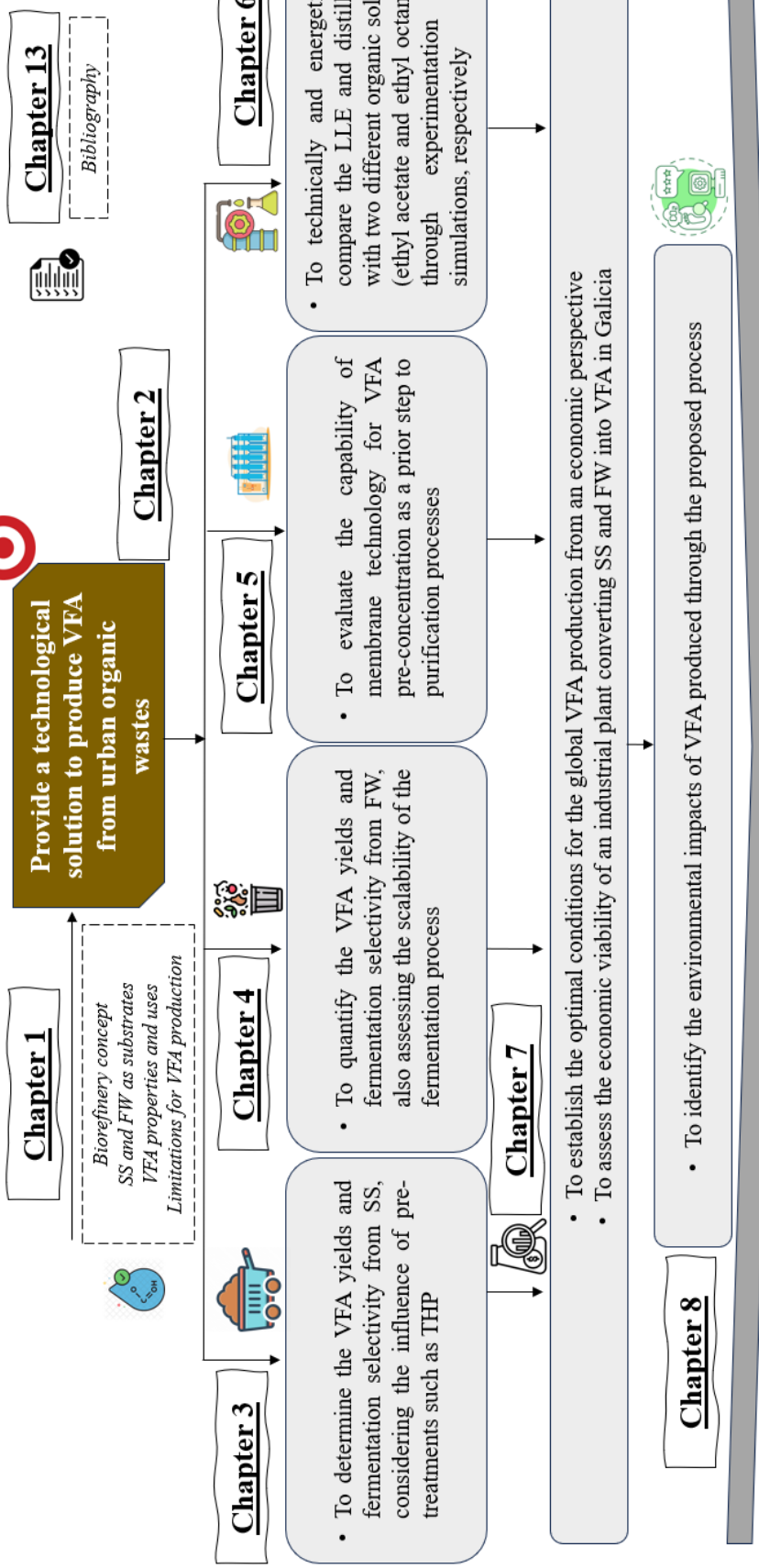

Figure 2.1. Thesis structure and connections between Chapters.

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3) PRODUCTION OF VFA FROM SEWAGE SLUDGE

*Some of the results from this Chapter have already been published as Castro-Fernandez, A.[1,2], Taboada-Santos, A.[1], Balboa, S.[2] & Lema, J. M.[2]. (2023). Thermal hydrolysis pre-treatment has no positive influence on volatile fatty acids production from sewage sludge. *Bioresource Technology*, *376* (March), 128839. https://doi.org/10.1016/j.biortech.2023.128839

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3.1. INTRODUCTION

Over the last decades, the growing population connected to sewerage, the building of WWTPs and the enhancement of current ones to comply with severe water treatment legislation led to a dramatic increase in SS production (Zheng et al., 2021), and this tendency is expected to continue in the future.

SS disposal usually accounts for around 60 % of the total operating costs of WWTPs (Maragkaki et al., 2018). As a consequence, AD has been broadly implemented; at first to decrease the amount of SS produced but then also for biogas production, a renewable energy that is normally used in the installation to warm up the digester and produce electricity, which reduces energy costs (Pramanik et al., 2019).

The first -and in many cases limiting-stage of SS AD is hydrolysis, which consists of the breakdown of particulate organic matter into smaller compounds. To boost this stage, a vast diversity of pre-treatment technologies such as ultrasonic, microwave, acid/alkali, ozone, and thermal ones have emerged with the aim not only to accelerate the digestion process but also to increasing biogas production (Zhen et al., 2017). Amongst them, thermal hydrolysis pre-treatment (THP) has evolved into one of the most popular because it also achieves: (i) odour reduction and pathogen sterilisation; (ii) improvement of sludge dewaterability and (iii) diminution of sludge viscosity (Bougrier et al., 2006). Cambi™ and Exelys™ are the two largest commercial processes among THPs, having more than 50 plants around the world and treating more than 1,000,000 tons of sludge until 2014 (Dong et al., 2016). However, some authors have also reported some disadvantages of this pre-treatment such as the dependence on centrifugal separators and polymers for sludge thickening to make the process profitable and the potential generation of recalcitrant and inhibitory compounds in the centrate (Barber, 2016; Eskicioglu et al., 2006).

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In recent years, intentions to go beyond biogas have sped up, focusing on producing bioproducts such as VFA. This interest is both economical (VFA present higher added value than biogas and wide variety of applications, for instance as preservatives in the pharmaceutical and food industry and in the production of esters, bioplastics, and bioenergy (Kleerebezem et al., 2015)) and environmental, since commercial production of VFA is mainly based on fossil resources (Chen et al., 2017).

The impact of pre-treatment technologies on SS transformation into VFA has been less studied than into biogas, but in general literature shows that THP is advantageous for the production of VFA from sludge as well. Morgan-Sagastume et al. (2011) found that the production capacity of VFA from hydrolysed sludge increased by 2-5 times in comparison with raw sludge. However, the comparison was made at different HRT, and this is one of the parameters that was reported to affect most the fermentation, together with others like pH, temperature and substrate (Yuan et al., 2019). Zhang et al. (2019) also studied the effect of THP on VFA production, getting the best results with pre-treated sludge at 35 ºC, 44.6 % higher than those reported with raw sludge.

The objective of this Chapter is to study the potential of producing VFA from SS, first at a laboratory scale, to identify the best operating conditions in terms of pH and substrate pre-treatment application or not. Subsequently, the optimal conditions were established in the pilot plant. Therefore, the discussion of the results should shed light on the fermentation process from SS.

3.2. LABORATORY SCALE

There are some works in the literature which have already studied the influence of THP on VFA production from SS, most of them at laboratory scale. For instance, Morgan-Sagastume et al. (2011) reported advantages of THP regarding VFA production. However, they established different OLR between reactors (16 vs 42 g chemical oxygen demand (COD) per litre and day), being this affected both by the different HRT applied and by the concentration of organic sludge, what makes it difficult to determine which parameter influenced the most VFA generation. In their work, Zhang et al. (2019) also studied the effect of THP on VFA production, but they did so in semi-continuous mode reactors and the batch/continuous mode of operation may be a key factor in fermentation. Wen et al. (2022) studied the microbial fermentative populations in batch mode while Iglesias-Iglesias et al. (2019) conducted the same analysis of microbial communities involved in raw and pre-treated sludge fermentation, but without including the research of VFA-related enzymes. Thus, the novelty of the experimentation at laboratory scale lies in the fact that it encompasses two modes of operation and the microbiological study in continuous reactors, going beyond through the comparison of predicted profiles of VFA-related enzymes.

The objective of these experiments was to evaluate: i) the effect of pH and THP on maximum VFA production yield and selectivity in batch mode and ii) how THP affects VFA production in continuous operation and the implication of this pre-treatment on bacterial dynamics at laboratory scale.

3.2.1. Material and methods

3.2.1.1. Sludge samples and inoculum

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Sludge samples (raw and thermally pre-treated) were picked up in a WWTP located in north-western Spain. The facility has a full-scale high-pressure THP in which a stream

composed by a mix of primary sludge (70 % COD basis) and biological sludge (30 % COD basis) is first thickened (15 % TS), heated and subsequently thermal hydrolysed by submitting it to 6 bar and 165 ºC for 30 minutes. After that, the stream is conducted to a flash tank where the temperature and pressure are suddenly diminished. The inoculum used was collected from the anaerobic digester of the same WWTP operating in mesophilic range, being therefore fed with thermally pre-treated sludge.

The physicochemical characterisation of the inoculum and substrate used for the acidification test is summarised in Table 3.1. The pH of pre-treated sludge was slightly higher than that of raw one, probably due to the breakdown of proteins, which causes an increase in ammonia and alkalinity (Barber, 2016). Total COD of raw sludge was considerably higher than those conventionally reported for raw mixed sludge (Zhu et al., 2021) because, in WWTP with THP, sludge must be thickened to make the process energetically efficient (Taboada-Santos et al., 2019). Pre-treated sludge shows lower TS, volatile solids (VS), and COD concentrations because of the dilution caused by steam condensation. As expected, a higher fraction of COD was solubilised in pre-treated sludge (15.3 %) in comparison with raw sludge (4.8 %). The ratio VS/TS (0.82 and 0.81 g/g for raw and pre-treated sludge, respectively) and tCOD/VS (around 2 g/g in both cases) are also in line with those reported by other authors for SS (Zhang et al., 2019), meaning that THP did not remove organic compounds and TS in the substrate.

Inoculum characterisation was similar to that of other anaerobic digesters treating thermally pre-treated sludge (Gong et al., 2021). An accumulation of sCOD indicates that the refractory or recalcitrant compounds formation may have been due to the occurrence of Maillard reaction (Yan et al., 2022). For this reaction to take place, there is necessary the presence of a reducing sugar and a free amino group under certain thermal situations.

Table 3.1. Characterisation of both substrates and inoculum used in the batch acidification experiments.

	Raw sludge	Pre-treated sludge	Inoculum
pH	6.12 ± 0.00	6.31 ± 0.01	8.02 ± 0.00
tCOD (g O_2/L)	200.8 ± 2.0	162.5 ± 1.1	58.6 ± 1.0
sCOD (g O_2/L)	9.6 ± 0.2	24.9 ± 0.4	5.4 ± 0.2
TS (g/L)	114.2 ± 1.1	91.0 ± 1.5	46.7 ± 0.3
VS (g/L)	94.0 ± 1.3	73.4 ± 1.4	26.5 ± 0.1
TP (g P/L)	2.7 ± 0.0	1.9 ± 0.1	2.0 ± 0.2
P-PO_4^{3-} (g/L)	0.146 ± 0.001	0.133 ± 0.002	0.094 ± 0.002

3.2.1.2. Acidification test in batch mode

A batch acidification experiment was conducted in triplicate utilising bottles of 0.5 L of working volume. The food/microorganism ratio was 2 g COD substrate/g VS of inoculum. The addition of $NaHCO_3$ provided a mineral medium and by establishing an alkalinity of 1 g $CaCO_3$/g COD substrate, an immediate fall in pH was avoided. BES salt was incorporated in order to inhibit methanogenesis (2 g/L), using distilled water to fill up the volume to 0.5 L. pH in the bottles was initially adjusted to 8 or 10 adding NaOH 50 % (w/v) and blanks at both pHs were used to study the production of VFA from the inoculum. The decision to test these pH values was because Yuan et al. (2006) conducted batch fermentation tests at pH ranging from 4 to 11, reporting that VFA production at alkaline pH was much higher than at acidic and neutral conditions due to the increase in protein degradation efficiency and the lack of VFA consumption by methanogens.

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Before being sealed, the bottles' headspace was purged with N_2 for two minutes to ensure an anaerobic environment. To carry out the experiments, a modified AMPTS II equipment from Bioprocess Control (Figure 3.1) allowing liquid sampling, was employed. The bottles were placed inside a thermostatic bath at a temperature of 37 ± 1 °C (mesophilic operation).

Figure 3.1. AMPTS II in which acidification tests were conducted.

3.2.1.3. Continuous stirred-tank reactors

Two continuous stirred-tank reactors (CSTR) with 5 L of working volume (Figure 3.2) were operated maintaining a temperature of 37 ± 1 °C using a thermostatic bath and an agitation speed of 170 rpm (IKA RW Digital 60). The reactors were firstly inoculated with 5 L of inoculum, for later withdrawing and feeding twice a day the corresponding volume in order to operate at an HRT of 10 days using the Masterflex L/S series pump. Aiming at operating at the same OLR, feedings of both reactors, raw sludge one (reactor RS) and pre-treated sludge one (reactor PS), were diluted to around 50-60 g COD/L, keeping the pH controlled at 8.5 during the complete operation period (80 days) with an OLR ranging between 4.8-6.5 g COD/(L·d) due to fluctuations in the characterisation of WWTP's streams. Samples were analysed in both the influent and the effluent twice a week.

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Figure 3.2. Continuous reactors used to perform the experimentation.

In order to compare both the VFA yield as well as the acidification yield obtained in both reactors, the t-Student test was used for independent samples of equal variance with $\alpha = 0.05$. Student's t-test is commonly utilised when two independent groups are compared, being a parametric test and assuming normality of the data and equality of variances across comparison groups. In order to check normality, it was used the IBM SPSS Statistics 28 software through the Shapiro-Wilk statistic (number of samples less than 50), being also confirmed homoscedasticity through Levene's test.

3.2.1.4. Analytical methods and calculations

The determination of TS and VS was done as indicated in Standard Methods (APHA/ AWWA/WEF, 2012). Total and soluble chemical oxygen demand (tCOD and sCOD), total ammonia nitrogen (TAN), total nitrogen (TN) phosphate ($P\text{-}PO_4^{3-}$) and total phosphorus (TP), were analysed with a Hach LCK cuvette test system. Determination of tCOD, TN, and TP was carried out with the raw sample, but to analyse sCOD, TAN, $P\text{-}PO_4^{3-}$ and VFA it was necessary to pre-treat the samples centrifuging at 6,000 rpm for 10 minutes and then filtrating through Whatman GFC filters of 0.45 µm pore size.

VFA concentration was determined through gas chromatography (AGV-DB1 method). As equipment, it was used an Agilent 6850 with a flame ionisation detector equipped with a column DB-Wax from Agilent Technologies (30 m x 0.250 mm x 0.25 µm) and with an injector at 200 °C, being N_2 the carrier gas.

The VFA yield in batch mode (expressed in g COD-VFA/g COD fed) was calculated with Eq. 3.1:

$$VFA\ yield\ (t) = \frac{\Sigma VFA\ (t) - \Sigma VFAblank\ (t)}{tCOD, i} \qquad \text{(Eq. 3.1)}$$

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where *ΣVFA(t)* represents the amount of the different VFA (as g COD) that is obtained at a fixed reaction time (t) in each bottle, *ΣVFAblank (t)* represents the amount of the different VFA (as g COD) that is obtained at a fixed reaction time (t) in the blank and *tCOD* is the amount of substrate added (g COD) for each assay.

When referring to continuous operation, VFA yield was expressed as a percentage in g COD-VFA/g COD influent, being calculated with Eq. 3.2:

$$VFA\ yield\ (t) = \frac{\Sigma VFAo\ (t) - \Sigma VFAi\ (t)}{tCODi(t)} \cdot 100 \qquad \text{(Eq. 3.2)}$$

where *ΣVFA (t)* represents the concentration of VFA (as g COD/L) measured in the inlet (i) and outlet (o) at a particular moment and *tCODi (t)* corresponds to the total COD concentration of the inlet.

The acidification yield, expressed as mg COD-VFA/g VS was calculated as Eq. 3.3:

$$Acidification\ yield\ (t) = \frac{\Sigma VFAo\ (t) - \Sigma VFAi\ (t)}{VSi\ (t)} \qquad \text{(Eq. 3.3)}$$

where *ΣVFA(t)* represents the concentration of VFA (as mg COD/L) measured in the inlet and outlet at a particular moment and *VS* is the concentration of VS in the inlet (g/L).

3.2.1.5. Analysis of the microbial community

Once reached steady operation, six samples were taken both from the inlets and outlets of the two continuous reactors (from day 21 of operation to 79).

The extraction of total genomic DNA was conducted in duplicate using Nucleospin Microbial DNA extraction kit (Macherey Nagel) and pooled together after quantification, and quality control using Nanodrop and Qubit fluorometer (Thermo Fisher Scientific Waltham, MA, USA). For Bacteria analysis, The V3-V4 hypervariable region was amplified using Bakt_805R (5' GAC TAC HVG GGT ATC TAA TCC 3') and Bakt_341F (5' CCT ACG GGN GGC WGC AG 3') (Herlemann et al., 2011). DNA metabarcoding analyses of the region were executed in an Illumina NovaSeq PE250 platform, generating paired ends reads (2 x 250) by AllGenetics & Biology SL (www.allgenetics.eu).

Bioinformatic analyses were conducted through the Microbial Genomics module workflow (version 21.1) of the CLC Genomics workbench (version 21.0.3). After filtration of unprocessed sequences to discard low-quality reads, sequences were clustered into OTUs (Operational Taxonomic Units) at 97 % cutoff for sequence similarity and grouped upon the non-redundant version Midas 4 database (https://www.midasfieldguide.org/guide). For further analysis, only the bacterial OTUs with more presence (above 1 % of the total observed OTUs) were considered.

Beta diversity was determined by Bray-Curtis distances between each pair of samples and plotted in a principal coordinate analysis (PCoA). Significance was assessed by PERMANOVA.

Alpha diversity was predicted from the rarefaction analysis utilising phylogenetic tree of OTUs obtained from the MUSCLE algorithm, with a maximum sampling depth of 36,846 reads.

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The functional pathways related to VFA metabolism were predicted using PICRUSt software (Lawrence et al., 2017), which provides functional annotations based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases using the closest relative of the OTUs whose genome has been sequenced.

3.2.2. Results and discussion

3.2.2.1. Effect of pH in batch mode

The aim of the acidification batch tests was to find out the most favourable pH for obtaining the maximum VFA yield from SS, both raw and pre-treated. In addition, it was intended to evaluate the effect of pH on the selectivity of the different assays.

pH is one of the crucial parameters influencing VFA generation (Varghese et al., 2022). It affects the efficiency of substrate conversion, the utilisation of the carbon source, and the microorganisms' growth rate, apart from having a strong impact on the VFA profile generated through mixed culture fermentation (Temudo et al., 2007).

Fang et al. (2020) compiled the results of several works and found that the pH range to obtain higher VFA yields from SS was between 8-11. Accordingly, an acidification batch test was conducted at pH 8 and 10 to determine the effect of pH on VFA production from raw and pre-treated sludge. Working in this alkaline condition, hydrolysis rate and biodegradation are enhanced due to the disintegration and solubilisation of proteins and carbohydrates of bacterial cells (Fang et al., 2020); moreover, this condition largely inhibits the methanogens' activity, which convert VFA to methane letting their accumulation.

Figure 3.3 clearly shows that pH 8 resulted more favourable in terms of VFA production than pH 10. At pH 8, the maximum VFA yields obtained were 0.41 and 0.27 g COD-VFA/g COD fed for raw and pre-treated sludge, respectively, whilst for pH 10 were 0.26 for raw sludge and 0.15 g COD-VFA/g COD fed for pre-treated one. Presti et al. (2021) stated that the optimum condition to produce VFA from SS was an initial pH adjustment to 10, but they registered variations of pH during the batch test of around 2.5 units, while in this work the addition of alkalinity avoided a pH decrease higher than 0.5 units, thus the optimal average fermentation pH being very similar in both cases. The results obtained are also in line with those reported by Feng et al. (2009), who registered the highest VFA production yields at pH 8 treating secondary sludge. The most favourable effect of pH 8 in comparison with pH 10 could be attributed to the fact that higher pH promotes the formation of hard-biodegradable organic matter (lignin-like and humic-like substances) with refractory nature which hinders the fermentative process (Ma et al., 2019).

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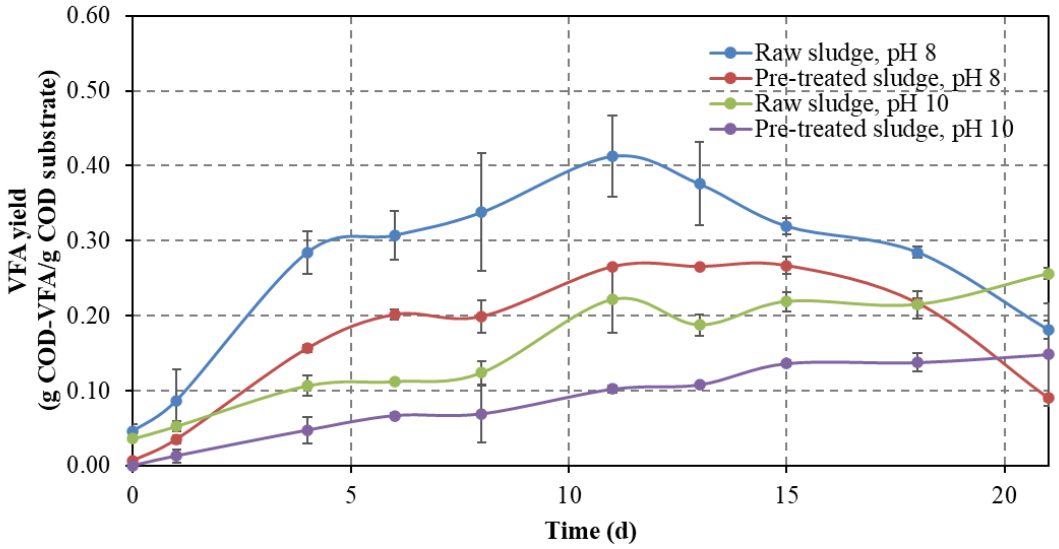

Figure 3.3. Acidification yield of raw and pre-treated sludge at both pH tested.

pH resulted crucial in VFA production yields as stated Atasoy et al. (2019), although in their case higher VFA productions were obtained at initial pH of 10 using a synthetic medium, while in this work a real sample of SS was used. Figure 3.3 shows that after reaching the maximum VFA production on day 11, in experiments at pH 8 the yield started to decrease and a small volume of methane was registered, indicating that the inhibitory effect of the BES salt was just temporary and archaea were partially active (Ward et al., 2008). Contrary, at pH 10 this decrease was not observed since this pH range is not favourable for archaea populations.

THP presented a negative influence on the production of VFA from SS, contrary to what is indicated in most of the works in the literature. A possible explanation could be the use of an inoculum not adapted to THP as it is a key factor in the fermentation's pathways (De Gioannis et al., 2013). However, in this case, the inoculum was obtained from a digester operating with pre-treated sludge and therefore well adapted to the substrate.

THP could result not only in a higher organic matter solubilisation but also in the production of hardly biodegradable structures which can be even inhibitory for biotransformation. These compounds such as melanoidin and melanin produced through the Maillard reaction could be found in the bottles and can provoke a decrease in the expected yield as they could preferentially affect fermentative microorganisms (Chandra et al., 2008).

Another hypothesis to explain the higher VFA yield obtained with raw sludge could be the generation of humic acids during THP, compounds that can reach concentrations in the range of 8-15 g/L in pre-thickened sludge digestion (Ghasimi et al., 2016) as the used in this work, causing important inhibitions on cellulose hydrolytic enzymes (Yap et al., 2018).

Moreover, Vidal-Antich et al. (2021) found that hydrolysis pre-treatments (in their case at lab-scale and a temperature of 55 ºC) did not improve fermentation yields in batch mode when using a mixture of sludge and biowaste as a substrate. The production of toxic and non-fermentable compounds during THP could be the reason for the results obtained, so strict temperature control in THP is vital to balance the enhancement in sludge solubilisation and melanoidins generation (Yan et al., 2022).

Figure 3.4 shows the distribution of the VFA mixture produced at both pH tested. Figures 3.4A and B show that there was no noticeable difference between the VFA spectrum obtained with raw sludge and the pre-treated one. With raw sludge, the presence of acetic acid on the

VFA mix was 80 % (mass basis), whereas with pre-treated sludge it accounted for 83 % (mass basis). Contrarily, the proportion of propionic was slightly higher in raw than in pre-treated sludge (15 % versus 12 %). The rest of the VFA accounted for a much lower fraction. These results are similar to those presented by Iglesias-Iglesias et al. (2019), who reported that working at the same food to microorganism ratio, the main acid was also acetic acid (accounting for 50 % of the COD of the VFA mix) followed by propionic acid. The lower proportion of acetic acid might be attributed to the fact that they worked at uncontrolled pH and acidic pH favours the production of other VFA such as butyric, while acetic is the main acid at neutral and alkaline pH (Cokgor et al., 2009).

Figures 3.4C and D also show that at pH 10 there are no major differences between the VFA mix produced with raw and pre-treated sludge, being acetic acid again the dominant VFA with around 70 % of the distribution (mass basis). These results correspond with those reported by Morgan-Sagastume et al. (2011), who stated that the VFA spectrum is highly subjected to the type of waste fermented and pH rather than on the application of pre-treatments. Contrary to results obtained at pH 8, valeric acid is the second VFA in abundance (accounting for up to 8 and 11 % of the VFA mix on mass basis with and without pre-treatment, respectively). Valeric acid production could be associated with protein fermentation through the Stickland reaction whose stoichiometry is very much affected by pH (Bevilacqua et al., 2021).

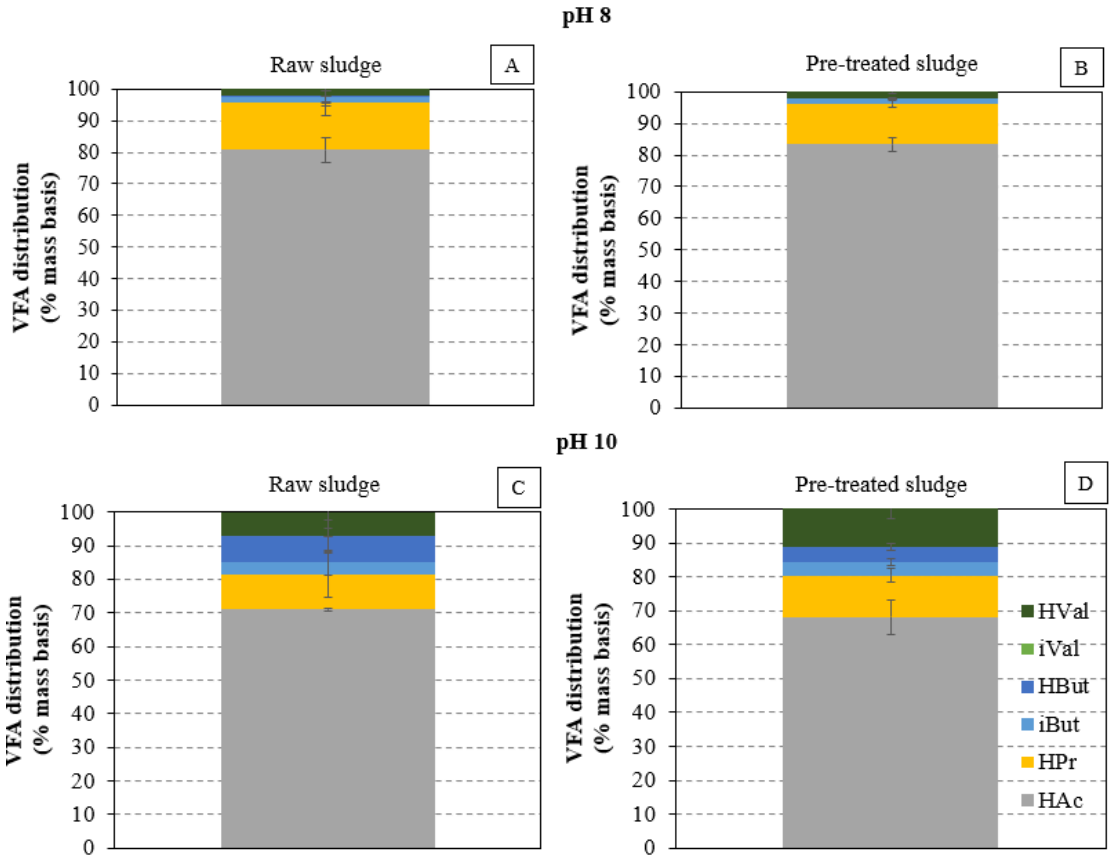

Figure 3.4. VFA distribution of the produced mix on the batch tests.

In summary, the acidification test showed that pH 8 was more favourable in terms of VFA yield and that the raw sludge obtained better results. At both pH, acetic acid was predominant in the mix, always exceeding 60 % since alkaline conditions favour its production (Fang et al.,

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2020). Moreover, the addition of the BES has not been effective working at pH 8 in the long term as a partial methanisation occurred after day eleven of the experiment (Figure 3.3).

3.2.2.2. Continuous operation with bacterial community analysis

Two CSTRs fed with raw and pre-treated sludge (Reactor RS and PS, respectively) were operated setting pH at 8.5 to ensure a high VFA production and a less favourable condition for methanogenesis. HRT was set at 10 days because it was the time of maximum VFA production yield in batch operation, apart from being the most favourable HRT in terms of VFA production according to Yuan et al. (2009). The process stability was evaluated as well as the VFA yields and selectivity. Moreover, aiming at elucidating the influence of THP on bacterial community fermentation, six samples were taken and analysed.

Table 3.2 shows the characterisation of the inlet and outlet as well as the metrics of VFA production performance. Inlets were taken from the same WWTP as those used in batch mode, although they were diluted to around 60 g COD/L, operating in an OLR interval of 4.8-6.5 g COD/(L·d), values similar to those applied by Wu et al. (2018). As it can be expected, the sCOD of the PS reactor's inlet is much higher than that of the RS reactor (corresponding with 19.9 % of tCOD versus 8.8 %) as a result of THP. TAN and $P\text{-}PO_4^{3-}$ concentration increased after THP due to the breakdown of cell material.

Table 3.2. Mean characterisation of raw and pre-treated sludge fed in the continuous operation reactors and reactors performance.

	Reactor RS	Reactor PS
Inlet characterisation		
pH	6.42 ± 0.09	6.65 ± 0.10
tCOD (g O_2/L)	48.7 ± 4.1	53.2 ± 4.3
sCOD (g O_2/L)	4.3 ± 0.7	10.6 ± 1.0
TS (g/L)	40.7 ± 1.4	42.2 ± 1.6
VS (g/L)	29.3 ± 1.1	30.6 ± 1.3
TAN (g N/L)	0.096 ± 0.042	0.183 ± 0.031
$P\text{-}PO_4^{3-}$ (g/L)	0.019 ± 0.007	0.034 ± 0.009
Outlet characterisation		
pH	8.44 ± 0.12	8.42 ± 0.10
tCOD (g O_2/L)	40.8 ± 4.0	47.6 ± 4.6
sCOD (g O_2/L)	12.4 ± 0.5	13.3 ± 1.1
TS (g/L)	38.0 ± 1.9	43.3 ± 1.1
VS (g/L)	20.7 ± 1.4	24.3 ± 1.6
TAN (g N/L)	0.582 ± 0.121	0.622 ± 0.123
$P\text{-}PO_4^{3-}$ (g/L)	0.066 ± 0.005	0.088 ± 0.010
Reactor performance		
VFA concentration (g COD/L)	8.1 ± 0.8	8.0 ± 0.9
VFA yield (% g COD-VFA/g COD substrate)	16.6 ± 0.9	15.1 ± 1.3
Acidification yield (mg COD-VFA/g VS)	294.3 ± 4.9	263.9 ± 5.3
COD-VFA/sCODout (%)	66.3 ± 2.1	60.3 ± 1.6

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Figures 3.5A and B depict the COD distribution of the inlet and outlet of both continuous reactors. The graph shows a period of operation in which the reactors are already working at a steady state after a stabilisation period of more than 30 days.

For reactor RS (Figure 3.5A), 8.1 g COD-VFA/L were obtained, which implies a VFA yield of 16.6 % g COD-VFA/g COD substrate. These results are slightly higher than those reported by Zhang et al. (2019) who also run semi-continuous reactors with an inoculum adapted to the THP process (mean VFA yield of 10 % g COD-VFA/g COD substrate). Besides, Iglesias-Iglesias et al. (2019) also ran a continuous reactor with raw sludge, reaching a similar average conversion. Finally, Yuan et al. (2009) obtained a mean VFA yield of 14.1 % g COD-VFA/g COD substrate operating a semi-continuous reactor with secondary sludge at HRT of 10 days, which is in line with this work, but the slightly lower yield could be related to the composition of waste activated sludge, containing mostly bacterial mass and having the cell lysis as the rate-limiting step.

A mean acidification yield of 294.3 mg COD-VFA/g VS was obtained in reactor RS, which is higher than that reported by Morgan-Sagastume et al. (2011) and Zhang et al. (2019) working with raw sludge in the mesophilic range (150 and 100 mg COD-VFA/g VS, respectively). In line with the acidification yield reported in this work, Da Ros et al. (2020) obtained a value of 322 mg COD/g VS operating a sequencing batch reactor fed with primary sludge. The COD-VFA/sCODout ratio was 66 %, very similar to that reported by Zhang et al. (2019), who reported a value of 71 %, showing that most of the soluble organic matter is turned into VFA.

Regarding reactor PS (Figure 3.5B), an average yield of 15.1 % g COD-VFA/g COD substrate was obtained, comparable to the one reported by Zhang et al. (2019) (14 % g COD-VFA/g COD substrate), who also worked with pre-treated sludge and an inoculum adapted to THP. Morgan-Sagastume et al. (2011) also reported a VFA yield of 15 % g COD-VFA/g COD when they fed their reactors with thermal hydrolysed sludge. The mean acidification yield in reactor PS was 263.9 mg COD-VFA/g VS, which is almost the same as that reported by Liu et al. (2019) working at a pilot scale with alkaline pre-treated SS (261.3 mg COD/g VS). For this PS reactor, the COD-VFA/sCODout ratio was 60 %, in line with that reported by Zhang et al. (2019) with pre-treated sludge (52 %).

It was detected that for both continuous reactors, between 10-15 % of the total COD fed was lost (likely as H_2), which indicates that operating pH and HRT have been effective in inhibiting methanogenic archaea (Yuan et al., 2019). Moreover, the COD-VFA/sCODout ratio was higher in the RS reactor, which reflects that soluble carbonaceous compounds released by THP have a lower biodegradability than that of raw sludge, a fact that could be related to the generation of bio-refractory compounds during Maillard reaction as it was discussed previously.

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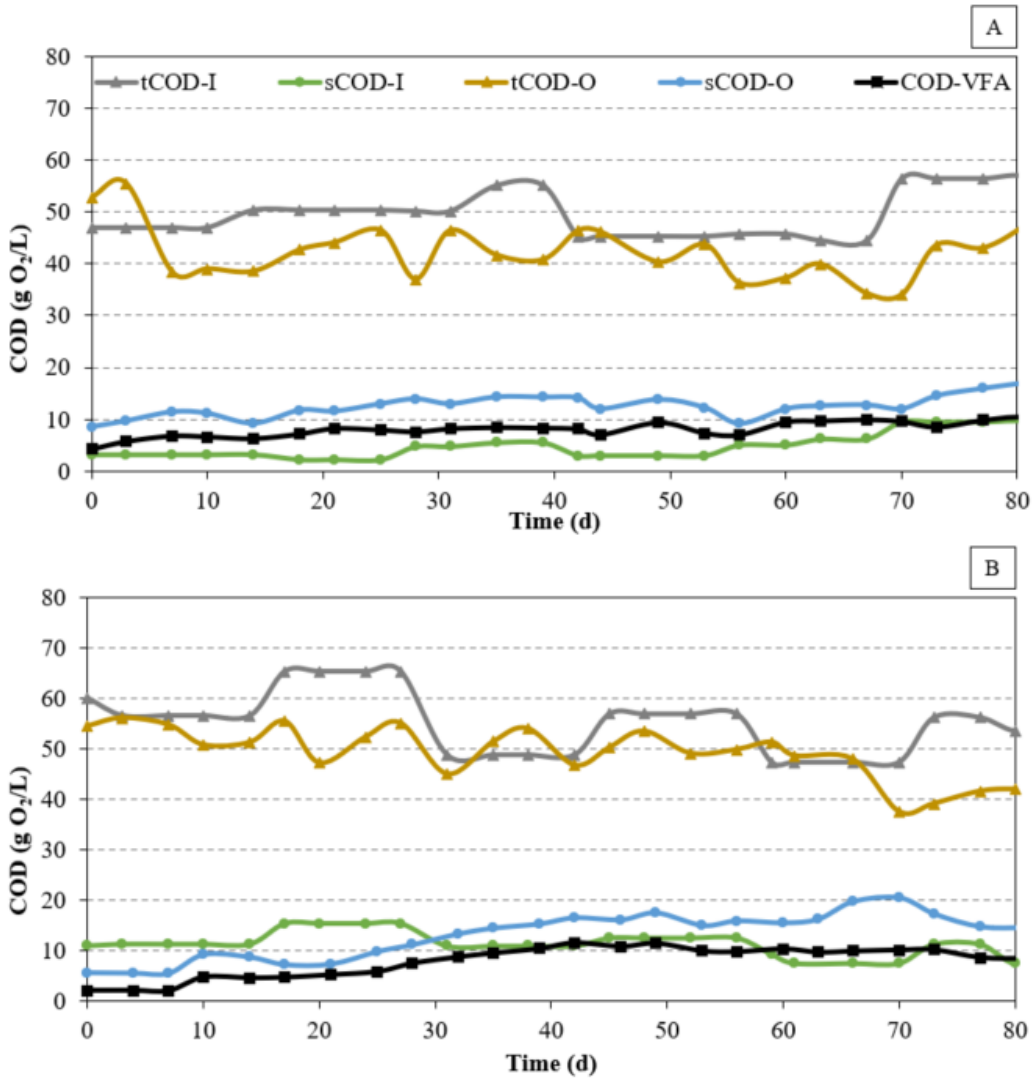

Figure 3.5. COD distribution of the inlet (I) and outlet (O). A) Reactor RS. B) Reactor PS.

Statistical analysis performed through Student's t test concluded that the difference between mean VFA yield obtained in reactor RS and PS was not significant. It was also found that there was no significant difference between the acidification yield obtained in the two reactors. Therefore, working in continuous mode, THP has any influence on VFA production from SS, unlike the results obtained in batch mode in which a negative effect was observed.

During the operation of the continuous reactors, the composition of the VFA mixture obtained was analysed (Figure 3.6) and there was no major difference between the mix obtained with raw and with pre-treated sludge; Tayou et al. (2022) also observed that THP applied before a mesophilic fermentation did not have a relevant effect on the VFA mix produced. In both cases, acetic acid was the dominant product (presence in the mix based on mass higher than 65 %), followed by propionic and other VFA. Comparable results were reported by other authors such as Iglesias-Iglesias et al. (2019) using raw sludge in a continuous reactor. However, Zhang et al. (2019) comparing the fermentation of raw and pre-treated sludge in semi-continuous mode at uncontrolled pH, saw that the proportion of VFA in their mix was practically the same with both substrates. However, the weight of acetic acid based on mass did not exceed 27 %, much lower than in this test. In their work, Ma et al. (2019) operating semi-continuous fermenters fed with secondary sludge at pH 9 (close to the operating pH of 8.5), observed that the proportion

of acetic acid was around 35 % of the mix distribution, with about the same propionic, followed by isovaleric. Yang et al. (2014), operating a CSTR with hydrolysed waste-activated sludge, observed that the acetic-acid type fermentation was predominant for almost the entire time of operation, being acetic acid the predominant acid with 51.8 % of presence in the mix.

Thus, obtained results show a proportion of acetic acid exceeding that usually seen in the literature, highlighting the influence of both the substrate used and the specific operating conditions chosen in the fermentative process (Yuan et al., 2019).

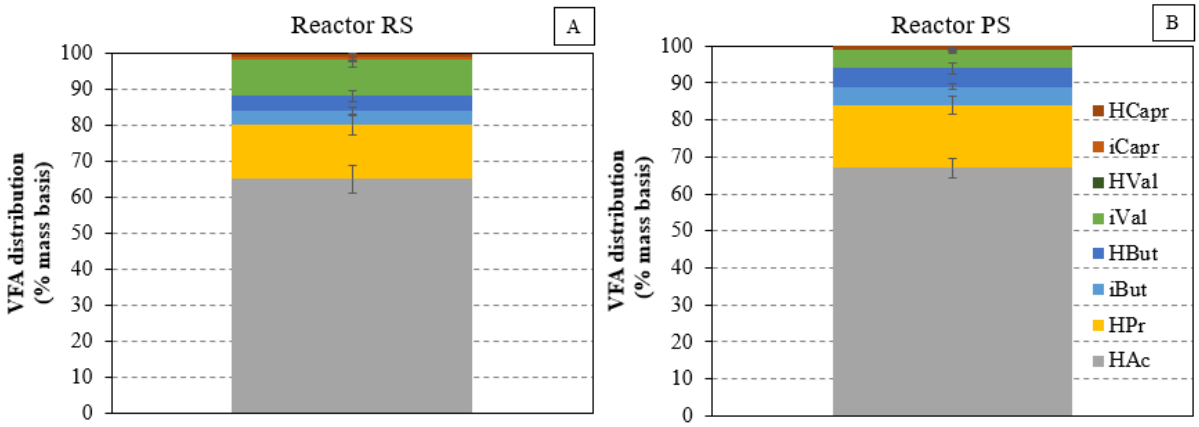

Figure 3.6. VFA distribution of the produced mix in the continuous reactors. A) Reactor fed with raw sludge (RS). B) Reactor fed with pre-treated sludge (PS).

A priori, higher VFA yields were expected when using pre-treated sludge as substrate, since the hydrolytic step was already boosted in THP. However, sometimes refractory organic compounds are produced, and they cause a lower biodegradability of organic matter, preventing the fermentation process (Bougrier et al., 2007).

It seems that the negative effect of THP observed in batch mode practically disappears in continuous operation. Indeed, batch mode assays execution could imply a higher concentration of inhibitory compounds generated from the beginning of the assay due to the high food/microorganism ratio applied. However, the particularities of operating in continuous mode favour a lower concentration of these compounds since these reactors were fed with diluted sludge, both raw and pre-treated. This is consistent with the fact that works involving refractory compound generation have been carried out in batch mode (Eskicioglu et al., 2006; Penaud et al., 2000).

A similar behaviour when comparing VFA yields in operation modes was observed by Iglesias-Iglesias et al. (2019) and Owusu-Agyeman et al. (2020), who reported that a change from a batch system to a continuous one implied a remarkable drop in VFA production (from a maximum of 45 % g COD-VFA/g CODfed in batch to around 20 % in semi-continuous mode).

Once continuous reactors were running stably, six sludge samples of inlets and outlets were taken to study the impact of THP on microbial communities.

A total of 1,894,431 reads were registered after trimming and quality filtering, ranging from 41,275 (PS-O day 21) and 110,395 from RS-I day 42 (Table 3.3). In order to obtain the rarefaction curves, a normalisation of OTUs count for individual biological replicates was done, showing that all of them reached the plateau and that 36,846 reads are the adequate sample sequencing depth to analyse the diversity.

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Table 3.3. Data summary and Alpha diversity indexes.

Sample	Reads	OTUs	Shannon	Simpson	Chao 1
PS-I_21	73,603	184	3,575	0,860	137,641
PS-O_21	41,275	286	4,834	0,940	178,599
RS-I_21	83,150	1693	6,544	0,963	477,547
RS-O_21	96,260	1462	4,915	0,872	527,162
PS-I_28	82,644	433	4,955	0,909	185,273
PS-O_28	63,196	446	5,378	0,936	235,733
RS-I_28	67,132	1896	6,774	0,967	497,321
RS-O_28	79,331	1444	5,203	0,886	534,008
PS-I_30	93,129	282	2,818	0,668	155,621
PS-O_30	54,943	408	5,144	0,937	225,591
RS-I_30	89,703	2054	6,440	0,947	498,952
RS-O_30	84,822	1413	4,792	0,846	521,331
PS-I_35	83,286	245	3,745	0,878	154,969
PS-O_35	45,637	382	4,763	0,919	240,184
RS-I_42	110,395	2378	7,027	0,974	510,827
RS-O_42	50,870	997	3,666	0,721	502,301
PS-I_73	67,321	676	4,733	0,932	408,533
PS-O_73	78,033	444	3,739	0,828	221,463
RS-I_73	103,257	1760	5,863	0,928	485,960
RS-O_73	64,553	1031	4,789	0,888	512,248
PS-I_79	42,250	315	4,283	0,896	178,375
PS-O_79	58,604	351	3,730	0,791	221,976
RS-I_79	76,083	1296	5,073	0,887	462,619
RS-O_79	69,665	1002	5,126	0,919	463,017

*Total reads indicate the number of reads after low-quality data trimming and chimera removal. OTUs are total number of OTUs identified. PS-I: Reactor PS-Inlet; PS-O: Reactor PS-Outlet; RS-I: Reactor RS-Inlet; RS-O=: Reactor RS-Outlet; numbers indicate operation days.

The beta diversity measured by Bray-Curtis index separated the samples according to the treatment (raw or pre-treated sludge) and location (inlet or outlet of reactors), as shown in Figure 3.7, and thus 4 groups were defined. Moreover, PERMANOVA analysis showed significant differences among those groups using both a phylogenetic method, unweighted UniFrac distances (Pseudo-F = 26.737; p= 0.00001) and non-phylogenetic methods, Bray Curtis method (Pseudo-F = 10.753; p= 0.00001), demonstrating that the origin of the sample has a strong influence on the microbiome.

The alpha diversity measures (Figure 3.7) suggest a specialisation of the microbial communities present in both reactors converging towards similar hydrolytic and acidogenic populations which give rise to the same VFA yields in continuous operation, independently of the pre-treatment applied to the inlet. Bacterial richness measured as the total number of OTUs was significantly higher in the reactor RS (both in the inlet and outlet) because it was fed with sludge not thermally hydrolysed. When the richness is calculated considering Shannon entropy and Simpson indexes, there was no significant difference between the outlets of both reactors; only if Chao 1 index is considered, which is focused on rare OTUs, there was a significant difference between the outlets from both reactors.

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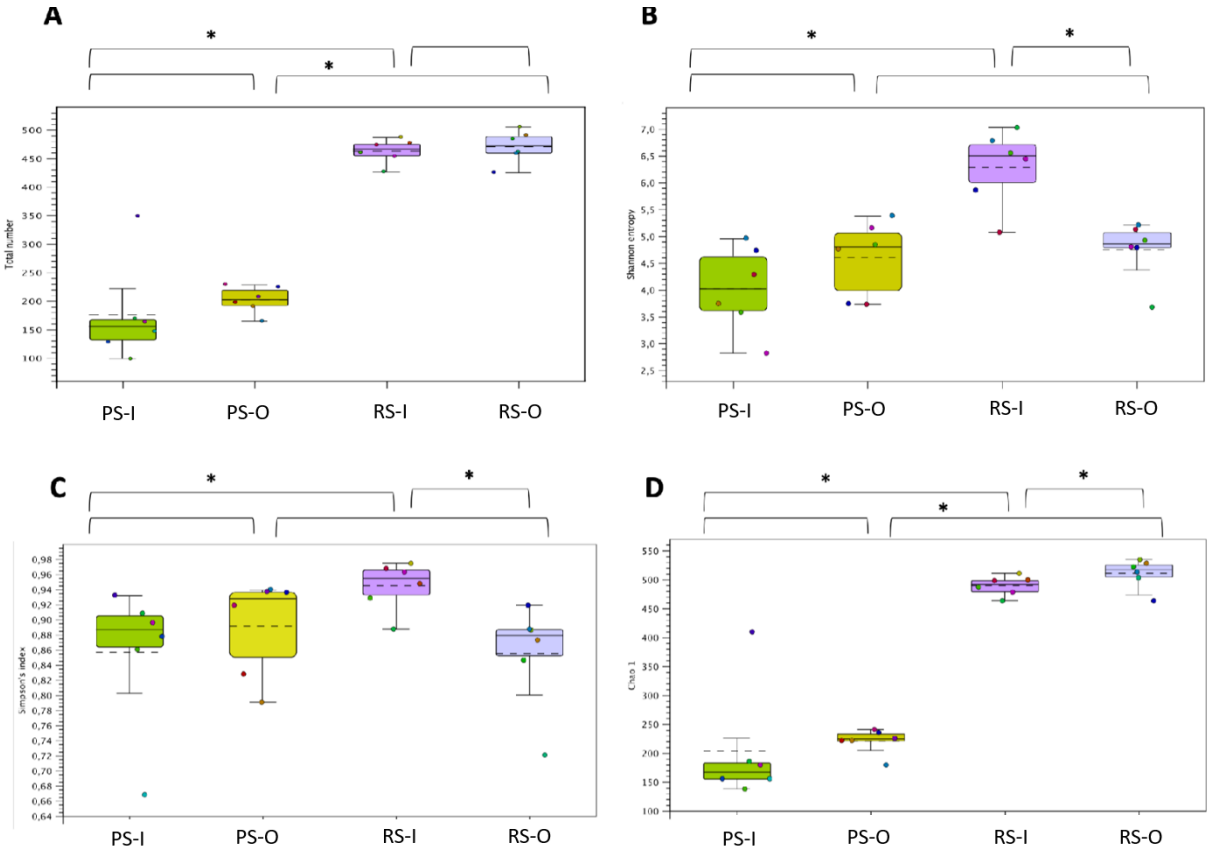

Figure 3.7. Alpha diversity statistics shown as boxplot. A) Total observed OTUs; B) Shannon entropy index; C) Simpson index; D) Chao 1 index. Statistical significance was measured by Kruskal-Wallis test. *:p>0.05.

Figure 3.8A shows the relative abundance of the main phyla detected in the six samples of reactors' outlets. The most abundant phylum in reactor RS was *Firmicutes*, whose mean relative abundance was 56.7 ± 11.6 %, followed by *Proteobacteria* (33.5 ± 9.7%) and *Actinobacteriota* (4.9 ± 1.7 %). In reactor PS, the main phylum was also Firmicutes (62.3 ± 10.9 %), followed by *Actinobacteriota* (23.2 ± 8.5 %) and *Proteobacteria* (8.5 ± 4.6 %). *Firmicutes* can secrete lipases, proteases and cellulases as well as other extracellular enzymes involved in lipid, protein and polysaccharide hydrolysis and acidogenesis stages (Chen and Chang, 2017). The difference between the bacterial populations in the two reactors could be related to the generation of certain compounds such as humic acid, which could explain the higher abundance of *Actinobacteria* in reactor PS, a group highly resilient to humic acid (Yap et al., 2018). The higher presence of *Proteobacteria* in reactor RS could be because this phylum produces hydrolytic extracellular enzymes such as polysaccharase, protease and lipase (Jang et al., 2014), which implies that fermentation of raw sludge may require a higher presence of hydrolytic populations to disintegrate particulate matters, being unnecessary for pre-treated one.

Figure 3.8B outstands that the predicted profile of VFA-related enzymes showed very similar trends in both reactors, being most routes related to acetic metabolism (blue colour), followed by enzymes of propionic routes (yellow colour), the two most abundant VFA produced as shows Figure 3.6. The pattern seen during the different days of sampling is very similar, and the following enzymes belonging to Enzyme Commission number (EC) 6 stand out: propionyl-CoA carboxylase (EC 6.4.1.3), acetate-CoA ligase (EC 6.2.1.1) and phenylacetate-CoA ligase (EC 6.2.1.30), whose common function is joining together two

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molecules by synthesising bonds with ATP's breakdown (Feng et al., 2022). This similarity reaffirms the equality both in the production yields and in the selectivity of the acidogenic process, being the same as those key players involved in the VFA metabolic pathways.

Although THP has any effect on VFA production in continuous mode, it has other benefits like the sterilisation and sanitation of the SS by eliminating most of the bacteria with presence in feedstock, affecting also to physicochemical properties of the sludge such as solubilisation and viscosity's reduction (Bougrier et al., 2006).

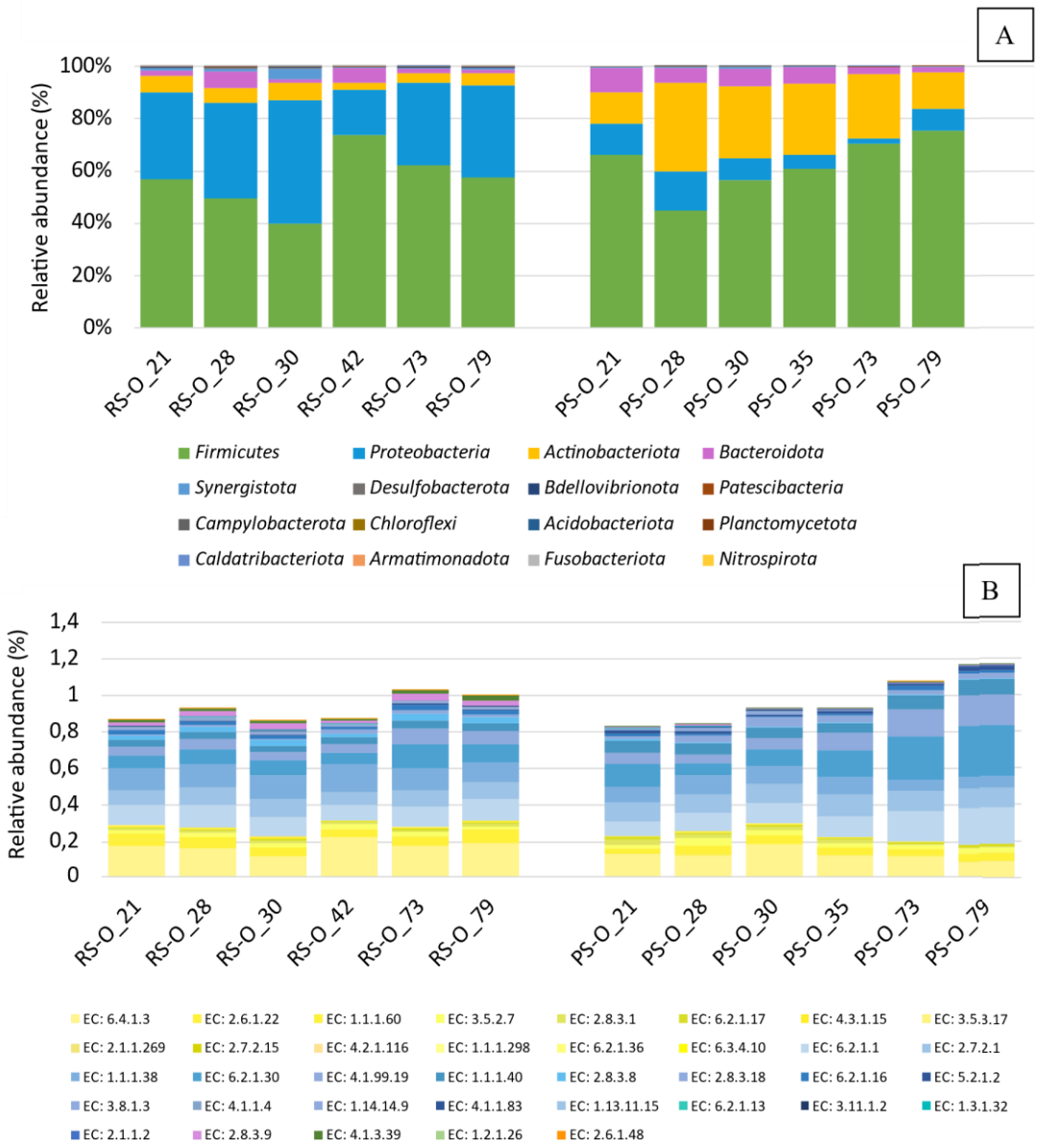

Figure 3.8. A) Bacterial community composition at phylum level considering only OTUs with total abundance higher than 1 % and B) Detailed enzymes with their EC number related to VFA metabolism. Numbers represent day of operation.

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Therefore, in batch mode, THP resulted in lower VFA yields at both tested pH, being pH 8 the most favourable in terms of VFA yield. In continuous reactors, the negative effect of THP disappeared, obtaining similar VFA yields and distribution mix independently of the pre-treatment of a fed substrate. Continuous mode microbiology analysis confirmed that both reactors converged into similar bacterial populations with *Firmicutes* phylum as the most abundant one, being the VFA-related enzymes also alike.

3.3. PILOT SCALE

Once the results of the fermentation of SS at laboratory scale were discussed, the technology was scaled up through the construction and operation of a pilot-scale prototype, operating it in the best conditions according to the information gathered at smaller scale.

There are not many references which study the production of VFA in a pilot plant, since practically all the work carried out is at laboratory scale. Adding to this the use of thermally hydrolysed sludge coming from the CAMBI^{TM} process as a substrate, the result is a work of great interest with a view to potentially scaling VFA production technology looking for industrial implementation.

3.3.1. Material and methods

3.3.1.1. Outline of the pilot plant

Figure 3.9 shows a piping and instrumentation diagram (P&ID) and a general view of the pilot plant in which the work was conducted. The first tank with a volume of 1 m^3 (cooled with a recirculating water jacket) was to homogenise the SS before being submitted to the fermentation process. This tank was filled in batch depending on the HRT chosen to operate the fermenter, but usually around one time per week to avoid substrate degradation. The fermentation process was conducted in continuous mode, being the fermenter a CSTR with a volume of 1.5 m^3, which has a concentric jacket for temperature control as well as a recirculation cell for pH control. The substrate was semi-continuously pumped from the homogenisation tank to fermenter and discharged to another tank (1 m^3 of volume), which received the effluent where the substrate was mixed with coagulants and flocculants before being submitted to solid-liquid separation process in the press filter. All the aforementioned tanks have a mechanical stirrer with speed control which led to a good homogenisation. The separation process conducted in the press filter was in batch, obtaining two streams, a solid cake, and a VFA-rich liquid stream.

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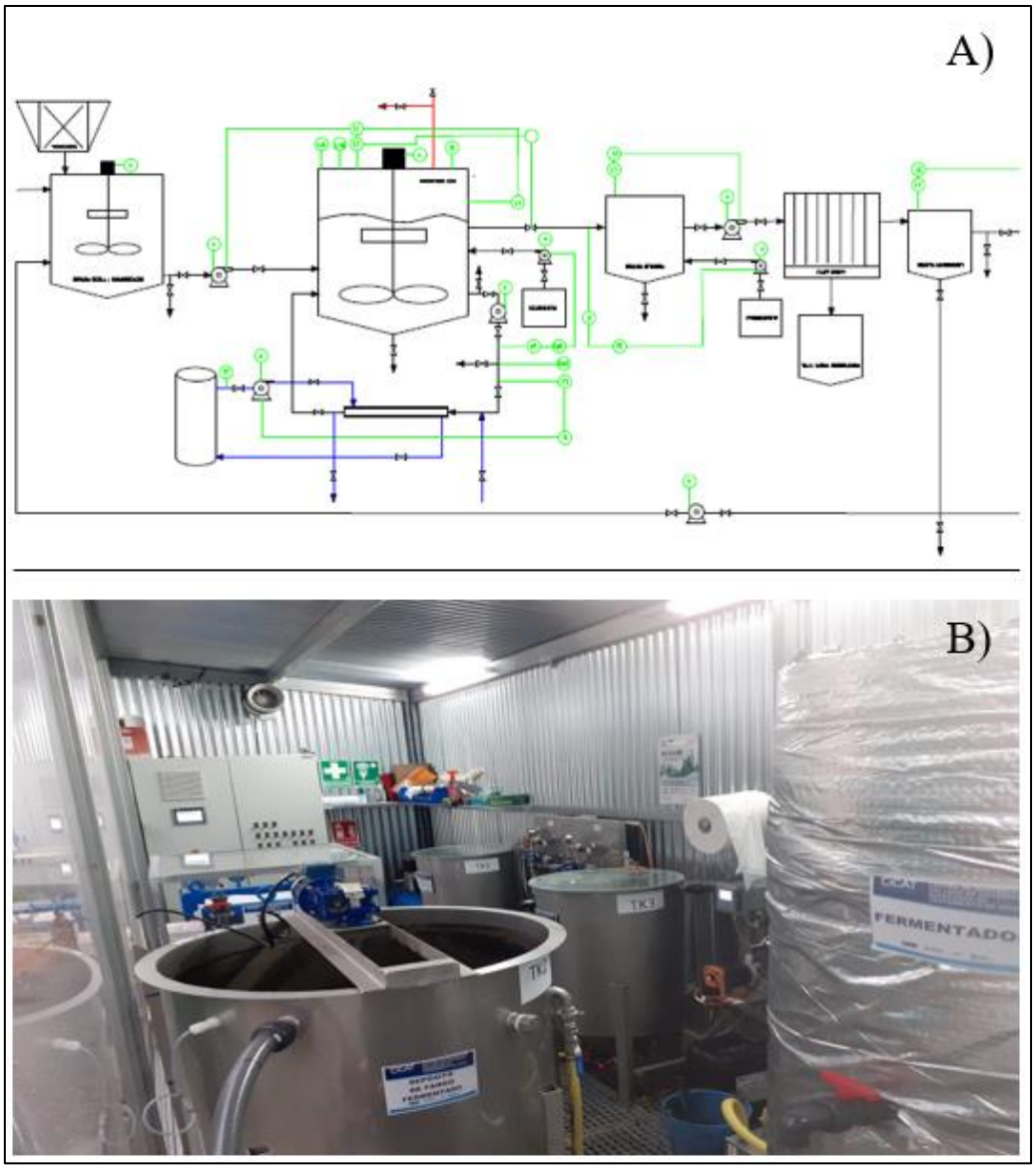

Figure 3.9. Pilot plant prototype. A) P&ID. B) Photography of the interior.

3.3.1.2. Substrate and inoculum

Considering that the pilot plant fermenter was operated as a CSTR, the laboratory-scale results have shown that THP had no influence on the VFA yields obtained. Therefore, hydrolysed sludge was used, given that it is sanitised and the digestate could be applied directly in agriculture. Therefore, the substrate used to feed the pilot plant was the same as the used for laboratory experimentation. Regarding the inoculum used, it was also the same as that used in batch and continuous mode of laboratory-scale assays.

The characterisation of the substrate (shown in Table 3.1 of 3.2.1) is similar to that reported by Liu et al. (2022) in terms of TS and VS, being in this case the relation VS/TS higher than that obtained by them (80.1 % versus 57.6 %, respectively). pH is also in line with that of other works as the used by Wandera et al. (2018), with a value of around 6.3. The hydrolysed sludge utilised to feed the pilot plant has the 15.3 % of the total COD in soluble form, which is a lower value than that reported by Wandera et al. (2018) and Gong et al. (2021), who registered values

of 35.8 and 26.8 % respectively. Morgan-Sagastume et al. (2011) stated that THP can provoke a degree of COD solubilisation of around 30 %. The nutrient concentration is also similar to that of samples of hydrolysed sludge reported by Zhang et al. (2019) and Gong et al. (2021), whose nitrogen and phosphorus concentrations are almost identical. Regarding the inoculum, adapted to THP like the one used by Gong et al. (2021), it should be noted that it has a slightly higher soluble COD than the one they characterised, which indicates that there is greater difficulty in degrading sCOD, which may be related to the mode of operation of the THP deriving in the generation of recalcitrant compounds (Zhou et al., 2018).

3.3.1.3. Operation of the anaerobic fermenter

The operation of the pilot plant was divided into two stages (Table 3.4). The first one lasted about 130 days and a feeding dilution to the hydrolysed SS of 1:2 was applied, working at an HRT of 10 days, with an average OLR of about 4.6 kg COD/(m^3·d). The second one lasted 70 days of operation, in this case with a feeding dilution of 2:3 and an HRT of 6 days in order to increase the OLR to 21.8 kg COD/(m^3·d).

Table 3.4. Average operating parameters of the fermenter at different operational stages.

	Stage 1	Stage 2
pH	8.50 ± 0.21	8.50 ± 0.19
Feeding dilution	1:2	2:3
Influent flow (L/d)	105	253
HRT (d)	10	6
OLR (kg COD/(m^3·d))	4.6 ± 1.4	21.8 ± 2.3
Operation time (d)	130	70
Temperature (ºC)	37 ± 0.5	37 ± 0.5

Both the analytical methods and the equations used have been previously defined in section 3.2.1, given that they were the same as those used on a laboratory scale.

3.3.2. Results and discussion of the fermentation process

The potential to produce VFA from hydrolysed sludge as substrate has been determined in the fermentation process, characterising the inlet and outlet streams to the fermenter twice a week. As it was indicated in Table 3.4, during the operation of the fermenter, two different stages were distinguished, each of which operated under certain conditions; during the first week of operation, the fermenter was operated without feeding dilution.

Laboratory-scale fermentation experiments have shown that pH 8.5 has turned out to be suitable for sludge fermentation, achieving yields with thermally pre-treated sludge of around 15.1 % g COD-VFA/g COD substrate, which is why this same pH was chosen also to operate the pilot plant. This pH should result adequate to avoid VFA consumption by archaea, given that in order to inhibit these methanogenic microorganisms the operating pH must be less than 6 (Steinbusch et al., 2009) or higher than 8 (Appels et al., 2013).

Figure 3.10 shows the evolution of COD of the inlet and outlet of the fermenter. Figure 3.10A corresponds to the Stage 1, in which the fermenter was operated in the conditions indicated in Table 3.4. Total COD of inlet and outlet were similar, indicating that the methanogenic stage has been successfully inhibited, thus avoiding loss of COD as CH_4/H_2. The average VFA concentration as COD was 7.34 g COD/L, achieving a maximum value of 13.1 g

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COD/L. So, the average VFA yield for Stage 1 was 12.1 % g COD-VFA/g COD of substrate or 221.0 mg COD-VFA/g VS. Liu et al. (2018) operated a full-scale plant to produce VFA from dewatered sludge, also during several stages with different operational stages, but their results are in line with those obtained in this work. They reported an average VFA yield of 261.3 mg COD-VFA/g VS in their Stage 3 (working in semi-continuous mode), in line with the 221.0 mg COD-VFA/g VS obtained in this experiment. The average VFA concentration in their fermenter was 7.5 g/L, aligned with the concentration of 5.9 g/L measured in this case.

Figure 3.10B corresponds to Stage 2. In this case, tCOD of inlet and outlet are very similar, which implies no losses of carbon in form of CH_4, but the operating conditions are not favouring the organic matter solubilisation, given that sCOD of outlet is close to the inlet one. In Stage 2, the average VFA concentration as COD was 10.1 g COD/L, achieving a maximum value of 14.2 g COD/L. So, the average VFA yield for Stage 2 was 8.1 % g COD-VFA/g COD of substrate or 172.1 mg COD-VFA/g VS, lower values than for Stage 1.

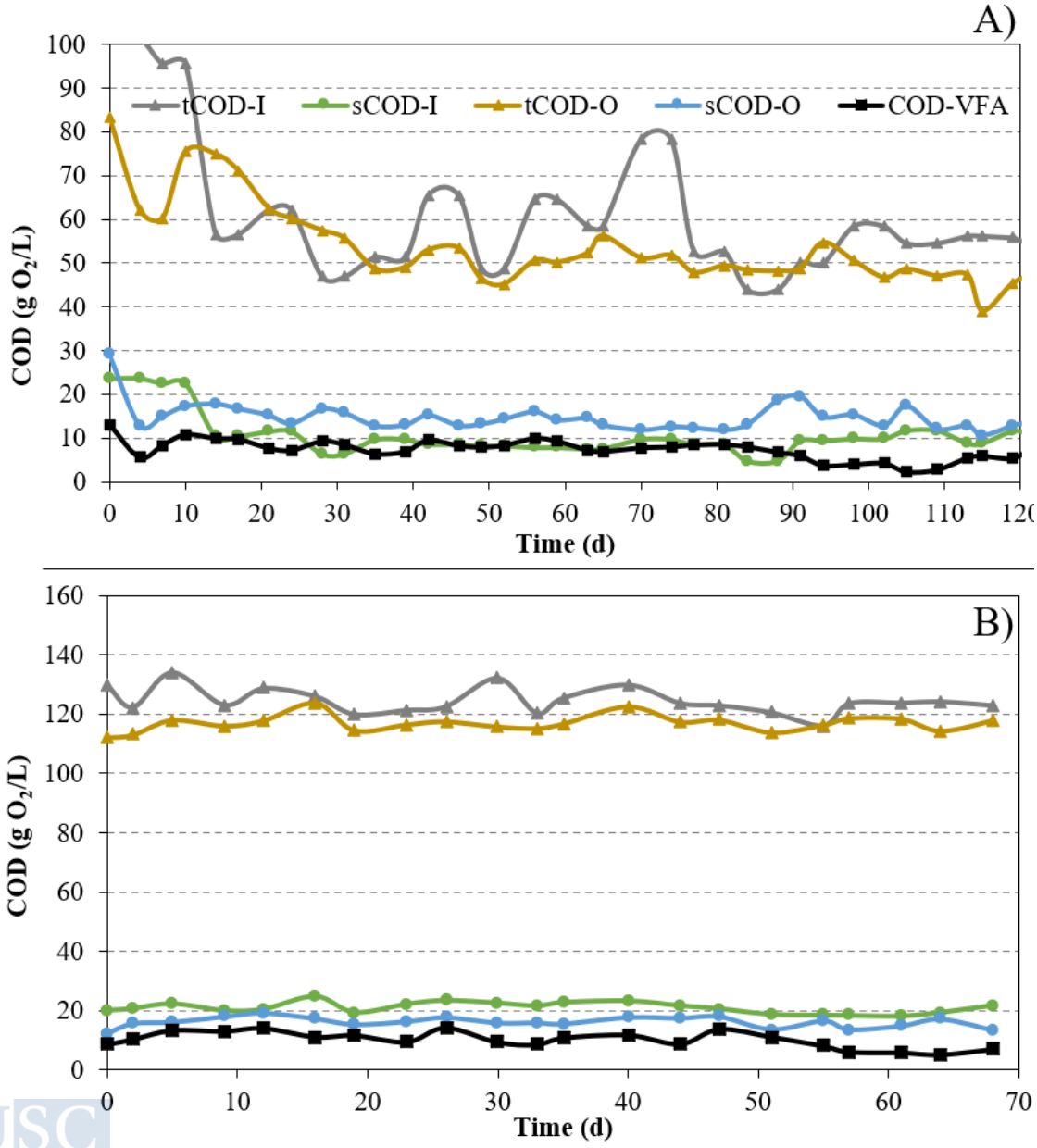

Figure 3.10. Evolution of COD in the fermentation process at pilot scale. A) Stage 1. B) Stage 2.

Figure 3.11A and B shows the variation of OLR applied to the fermenter during the Stage 1 and 2 of operation, respectively, as well as the productivity of VFA (measured as OLR-VFA in the outlet) and VFA yield. OLR is a parameter which expresses the COD, TS or VS quantity which are fed into a reactor per unit of working volume (Wainaina et al., 2019) and it was varied between stages because it is usually reported that an increase in OLR implies a maximisation of VFA production (Varghese et al., 2022).

Figure 3.11A shows that apart from day 10 of operation, the average applied OLR in Stage 1 was 5.3 kg COD/(m^3·d), being the average productivity 0.60 kg COD-VFA/(m^3·d). Da Ros et al. (2020) operated a pilot plant to produce VFA from cellulosic primary sludge, in their case using a sequencing batch fermentation reactor, applying three different OLR from 8.5 to 26.6 kg COD/(m^3·d). They obtained the best results with the intermediate OLR value (11 kg COD/(m^3·d)), being the average VFA yield 322 mg COD-VFA/g VS, thus overcoming the result obtained for Stage 1 in this fermentation process, which was 221 mg COD-VFA/g VS. Liu et al. (2018) operated a reactor that achieved a stable condition working at an OLR very similar to that applied in Stage 1 (in particular around 4.8 kg VS/(m^3·d)) and using an alkaline fermentation of SS (pH 10-11), they obtained a slightly higher VFA yield, 261.3 mg COD-VFA/g VS in comparison with the obtained in Stage 1 of the operation of this pilot plant (211 mg COD-VFA/g VS). This value of OLR resulted to be better than a higher one, applied in Stage 2, showing that VFA production increases with increased OLR within a reasonable range (Min et al., 2005), but increasing OLR through an HRT drop may imply a VFA production reduction because of the fall of hydrolysis efficiency (Fang et al., 2020).

Figure 3.11B shows the Stage 2 of the pilot plant operation, in this case applying an average OLR of 21.8 kg COD/(m^3·d), with the aim of boosting VFA production as well as benefiting from the advantages of working at a higher OLR in terms of fermenter volume with the associated energy savings and land requirements reduction. The average productivity in the Stage 2 was 1.76 COD-VFA/(m^3·d). Da Ros et al. (2020), when operated at an OLR of 26.6 kg COD/(m^3·d), similar to that applied in Stage 2, obtained an average of 154 mg COD-VFA/g VS, while in the case of this pilot plant an average value of 172.1 mg COD-VFA/g VS was obtained.

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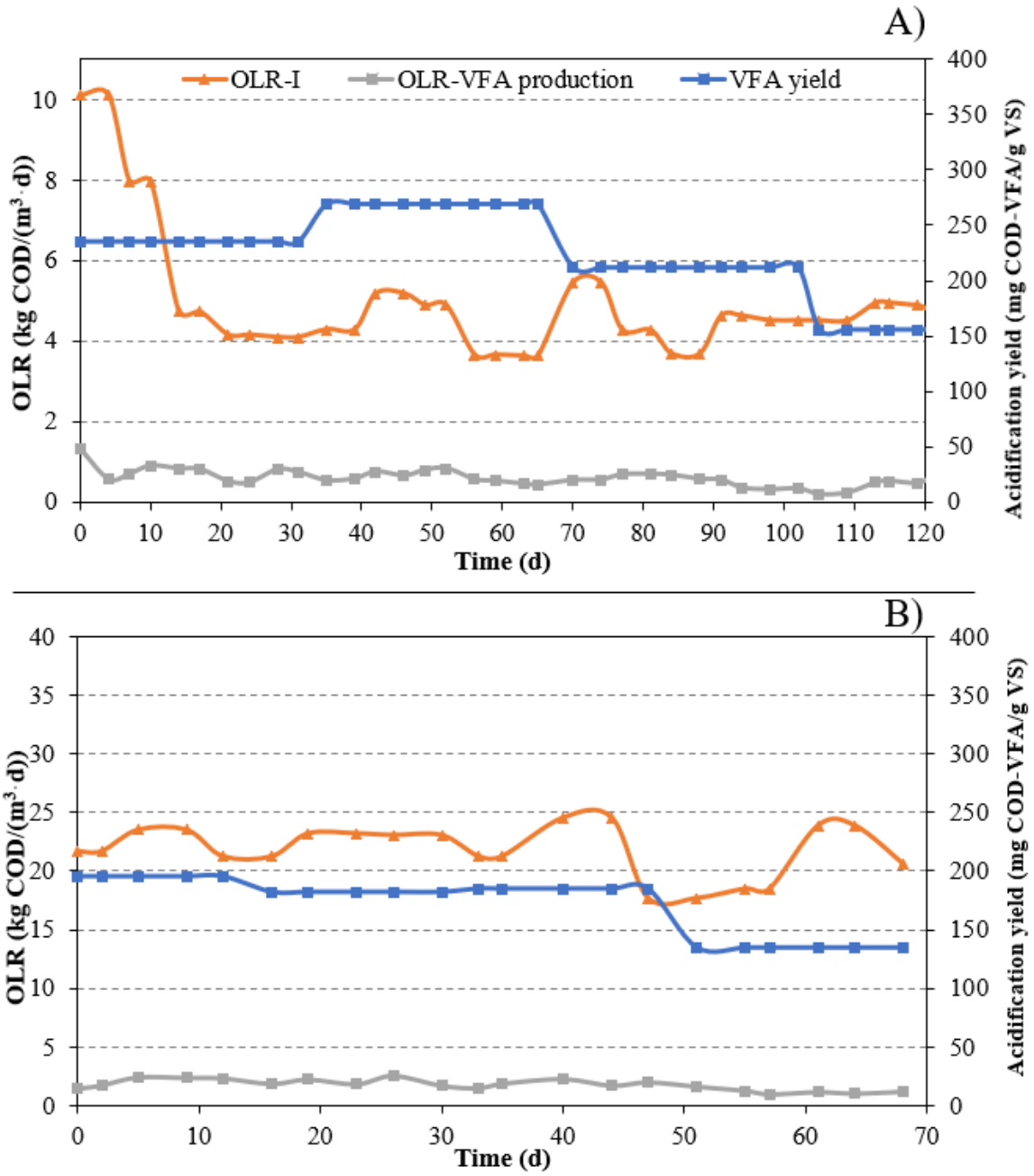

Figure 3.11. Applied OLR in the fermentation process and acidification yield. A) Stage 1. B) Stage 2.

All the information on both the effluent characterisations and the performance metrics of the fermentation process are shown in Table 3.5, both for Stage 1 and 2. It can be highlighted again that the increase in OLR applied when going from Stage 1 to 2 had a negative influence on the VFA yield and acidification yield, although considering that it implied a shorter HRT, it also led to an increase in VFA productivity.

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Table 3.5. Average and standard deviation of fermentation outlets and process performance.

	Stage 1	Stage 2
Effluent characteristics		
pH	8.64 ± 0.2	8.48 ± 0.9
tCOD (g/L)	53.7 ± 2.4	116.9 ± 3.2
sCOD (g/L)	14.8 ± 1.3	16.1 ± 1.5
TS (g/L)	48.6 ± 1.0	77.7 ± 0.8
VS (g/L)	28.9 ± 0.9	56.9 ± 1.2
TN (g/L)	3.8 ± 0.3	4.6 ± 0.5
TP (g/L)	1.3 ± 0.1	1.7 ± 0.3
TAN (g N/L)	1.24 ± 0.04	1.39 ± 0.09
$P\text{-}PO_4^{3-}$ (g/L)	0.131 ± 0.002	0.165 ± 0.005
Process performance		
VFA yield (g COD-VFA/g COD substrate)	12.1 ± 0.02	8.1 ± 0.01
Acidification yield (mg COD-VFA/g VS)	221.0 ± 5.2	172.1 ± 3.9
VFA productivity (kg COD-VFA/(m^3·d))	0.60 ± 0.03	1.76 ± 0.12

Figure 3.12A and B shows the distribution of VFA produced during the fermentation process for Stages 1 and 2, respectively. Working at different operational conditions implied a variation in VFA profile, being in both cases acetic acid the predominant VFA, comprising 77 % of the distribution mix in Stage 1 and 64 % in Stage 2 (mass basis). The second most predominant acid was propionic in both cases, but with a higher proportion in Stage 2 (18 % of the mix, mass basis). The presence of the rest of the acids was similar for the Stage 1 and 2, accounting for less than 20 % of the mix in both cases. The comparison between the two mixes obtained shows the effect of the variation of operational parameters in the VFA profile, in particular of the OLR due to the increase in feed concentration and decrease in HRT. So, increasing the OLR in Stage 2 implies a shift from more-oxidised compounds to reduced ones, such as propionate generation through lactate-propionate pathway (Li et al., 2016). Yu et al. (2002) conducted experiments in which working at mesophilic temperature, when they increased OLR from 4 to 24 kg COD/(m^3·d) (almost the same as the change from Stage 1 to 2), the propionate percentage went from 13 % to 41 % of total, being the acetate reduced its presence from 40 to 17 %. The shift from Stage 1 to 2 implied a drop in acetate presence from 77 to 64 %, while propionate increased from 9 % in Stage 1 to 18 % in Stage 2.

Liu et al. (2018), operating their pilot plant in stable conditions obtained a presence of acetic acid of 57.7 % with a fermentation carried out at pH 10-11, being the propionic presence around 7.5 % of total VFA. These results are in line with those obtained during this operation because alkaline pH usually favours acetic acid production (Temudo et al., 2007). Da Ros et al. (2020), also working at pilot scale, reported results in which propionic acid was the most abundant acid, but in their case, they operated at pH near to 5 and with a different sludge and reactor configuration.

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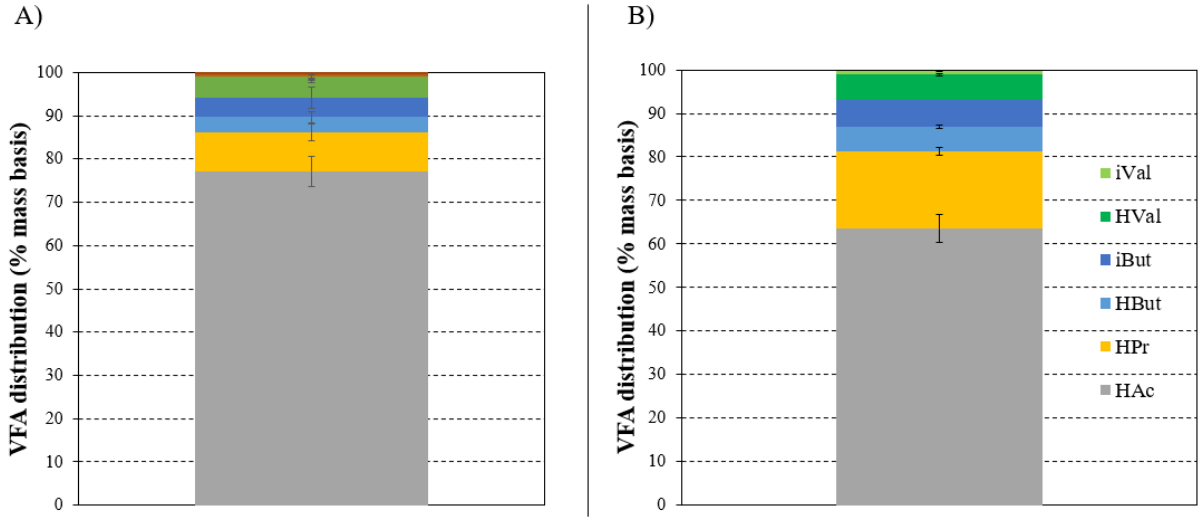

Figure 3.12. VFA distribution of the produced mix during fermentation process at pilot scale. A) Stage 1. B) Stage 2.

In summary, the pilot plant was operated with thermally pre-treated sludge, attempting to scale up VFA production technology from laboratory experiments to pilot scale. It was seen that the operation during Stage 1, at an average OLR of 4.6 g COD/(m^3·d), was more favourable in terms of VFA yield, reaching an average of 12 % g COD-VFA/g COD substrate. Under these conditions, the VFA mix produced had a greater abundance of acetic acid than when increasing the OLR by reducing the HRT during Stage 2. In any case, it was detected that skipping from lab to pilot-scale meant a slight drop in performance even operating in both cases in semi-continuous mode, going from a VFA yield of 15 % g COD-VFA/g COD substrate to 12 %. Regarding the distribution of VFA generated when comparing Stage 1 of the pilot plant with the continuous reactors (section 3.2.2.2), it remained practically identical. This indicates that scaling-up the fermentation process is technically viable and that the results obtained on a smaller scale have served to optimise the operation in the pilot prototype.

3.4. CONCLUSIONS

Raw sludge was compared with thermally pre-treated sludge at laboratory scale to observe the effect of THP on VFA production in batch and continuous operation. In batch mode, the application of the pre-treatment implied drops in the VFA yields, and it was seen that pH 8 was the one which gave the best results. On the other hand, when operating in continuous mode, no differences were seen between the VFA yields or between the distribution mix, results that were reinforced by the microbiological analysis, given that in both reactors was identified the phylum *Firmicutes* as the most abundant one.

Therefore, results showed that THP had no influence on VFA production yields on continuous operation at laboratory scale (around 15 % g COD-VFA/g COD substrate). This was the reason why, together with the benefits in terms of sludge sanitisation of the pre-treatment, the 1.5 m^3 pilot plant fermenter was fed with thermally pre-treated sludge. Under the most favourable conditions (Stage 1), an average VFA yield of 12 % g COD-VFA/g COD substrate with acetic acid as main product was achieved with a presence of about 70 % of the mixture (mass basis), very similar to that reported at laboratory scale.

During this Chapter, the scaling of the fermentation process from SS was presented, from batch bottles of 0.5 L volume to a pilot-scale fermenter operating continuously. It was

confirmed in this pilot plant that the process was robust and gave rise to notable concentrations of VFA (especially acetic acid), which must be concentrated and purified for achieving the suitable specifications for their final use.

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4) PRODUCTION OF VFA FROM FOOD WASTE

*The results from this Chapter have already been published as Castro-Fernandez, A.[1,2], Rodriguez-Hernandez, L.[3], Castro-Barros, C. M.[1], Lema, J. M.[2] & Taboada-Santos, A.[1]. (2024). Scale-up and economic assessment of volatile fatty acids production from food waste. *Biomass and Bioenergy*, *182* (March), 107112. https://doi.org/10.1016/j.biombioe.2024.107112

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4.1. INTRODUCTION

The continuous growth of the human population has resulted in the generation of large amounts of FW, reaching 59 million tons per year in the EU, more than 10 % of the total food available to consumers (UNEP, 2021). Although a few years ago the most frequent mechanisms for managing FW were incineration and landfill disposal, Directive 2008/98/EC establishes that there are other priorities in the waste treatment hierarchy.

A more sustainable alternative to manage FW is AD, a mature technology traditionally directed towards biogas production. AD comprises hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vázquez Fernández et al., 2022). Using FW as a substrate, applying an HRT between 20-30 days and an OLR in the range 1-5 g COD/(L·d) (Mao et al., 2015) could be appropriate, so all the stages of the process occur smoothly with biogas as the final product. This renewable gas is typically used as fuel for on-site steam, heat and electricity generation (Andriani et al., 2014).

As it was previously stated, an alternative approach for FW valorisation is the production of other higher added value compounds derived from fermentation, such as VFA, which accumulate when the AD process is stopped by inhibiting methanogenesis. As VFA are currently produced mainly from fossil resources, there is a major necessity to promote the change towards processes based on renewable materials such as the case of FW.

In recent decades, extensive work has been done at the laboratory scale to optimise the production of VFA from FW, studying the effect of parameters such as pH (Khatami et al., 2021), temperature (Strazzera et al., 2021), OLR (Jiang et al., 2013) and substrate composition (Alibardi and Cossu, 2016). At this scale, a usual strategy was based on the addition of some methanogenesis inhibitors such as BES salt (Yin et al., 2016). However, this solution is not economically sustainable when the process is scaled-up, and different approaches such as the

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operation at acidic or alkaline pH and/or low HRT have been used to avoid the growth of methanogenic microorganisms and accumulate VFA (Ramos-Suarez et al., 2021). Besides, the consumption of chemical reagents is frequently overlooked at lab-scale reactors (Esteban-Gutiérrez et al., 2018), but it is crucial in the economy of the process when the technology scales up (Liu et al., 2018).

The main objective of this Chapter is to optimise the production of VFA from FW at lab scale, scaling-up the best tested conditions to the pilot plant. In this way, it will be studied the potential of this substrate in the fermentation process, seeing if it is scalable and the results are maintained at larger scale.

4.2. LABORATORY SCALE

4.2.1. Material and methods

4.2.1.1. Food waste samples and inoculum

The FW sample for batch tests was provided by a supermarket chain in Galicia (Spain). The following food proportions were established in order to imitate the composition of waste generated by a European family: 50 % (w/w) fruit and vegetables, 25 % (w/w) bread, pastries and pasta and the remaining 25 % (w/w) meat and fish. These proportions of food types were proposed to give rise to a substrate representative of that generated in Southern Europe (Favoino and Giavini, 2020). Before being fed to batch acidification bottles, the substrate was crushed with a laboratory scale shredder. This sample presented an acidic pH, tCOD and solids content (Table 4.1) in line with others seen in the literature. For example, Khatami et al. (2021) presented a waste with a tCOD of 215 g O_2/L coming from a restaurant. Dogan and Demirer (2009) collected a FW residue from homes and mixed it with vegetables and fruits from a supermarket chain and also provided a characterisation with a tCOD of 241 g O_2/L and 4 g TN/kg. The sCOD is in line with that reported by other authors such as Cheah et al. (2019).

As for the inoculum used, it came from the same digester as that used when working with SS, so its characterisation has hardly been varied. This inoculum is in line with that of other anaerobic digesters fed with thermally pre-treated sludge (Castro-Fernandez et al. 2023). Zhang et al. (2019) also showed a characterisation of an inoculum from a plant with THP in its sludge line, with practically identical values of TS and VS.

Table 4.1. Characterisation of substrate and inoculum used in the batch acidification experiment (average ± standard deviation of three samples).

	FW sample	Inoculum
pH	4.72 ± 0.05	8.05 ± 0.00
tCOD (g O_2/kg)	283.4 ± 9.5	56.6 ± 1.0
sCOD (g O_2/kg)	78.7 ± 0.7*	5.2 ± 0.2
TS (g/kg)	208.6 ± 1.1	45.7 ± 0.3
VS (g/kg)	188.2 ± 1.9	26.9 ± 0.1
TN (g N/kg)	4.2 ± 0.8	2.6 ± 1.5
TAN (g N/kg)	0.12 ± 0.01*	0.34 ± 0.3

*Measured after diluting the substrate 1:10 with process water.

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With respect to the samples used in the continuous reactors, the origin was the same and those were shredded with a semi-industrial crusher and diluted with process outlet water from a WWTP to attain the desired dilution.

4.2.1.2. Acidification test in batch mode

The experimentation with FW began with the execution of an acidification test in batch mode with the objective of revealing the maximum VFA yields that could be achieved with this substrate as well as the selectivity of the fermentation.

The experiment was conducted in triplicate utilising bottles of 0.5 L of working volume. The food/microorganism ratio was 2 g COD substrate/g VS of inoculum. The addition of $NaHCO_3$ provided a mineral medium, establishing an alkalinity of 1 g $CaCO_3$/g COD substrate in order to avoid the pH fall. BES salt was added in order to inhibit methanogenesis (2 g/L), using distilled water to fill up the volume to 0.5 L. pH in the bottles was initially adjusted to 6.5, 8 or 10 adding NaOH 50 % (w/v) and blanks were used to study the production of VFA from the inoculum.

Before being sealed, the bottles' headspace was purged with N_2 for two minutes to guarantee an anaerobic environment. To conduct the experiments, the modified AMPTS II equipment from Bioprocess Control allowing liquid sampling was used. The bottles were placed inside a thermostatic bath at a temperature of 35 ± 1 °C (mesophilic operation).

4.2.1.3. Continuous stirred-tank reactors

Two CSTR with a working volume of 5 L were handled at 35 ± 1 °C with an agitation speed of 170 rpm (IKA RW Digital 60). They were semi-continuously operated by feeding twice daily using peristaltic pumps (Masterflex L/S series) to set the desired HRT. pH was continuously measured and controlled by adding NaOH 50 % (w/v). Inlet and outlet samples were analysed twice a week in terms of TS and VS, tCOD and sCOD, TN, TAN and VFA.

Different operating conditions were assayed to determine the most favourable scenario for VFA production at the pilot plant. The effect of pH (5.5, 6 and 7) and OLR (6 and 10 g COD/(L·d)) on VFA production were considered, keeping an HRT constant at 10 days but varying substrate dilution. The selection of this HRT aimed at providing sufficient time for hydrolysis and acidogenesis of the complex carbohydrates and proteins contained in a heterogeneous substrate such as FW (Lim et al., 2008). In fact, de Groof et al. (2021) reported that an HRT of 10 days was suitable for VFA production from FW, obtaining a higher concentration in the fermented effluent compared to operating periods at lower HRT.

The selection of pH range considers a balance between the expected VFA yield derived from the enhanced substrate solubilisation (Zhang et al., 2010) and the economics related to reagent consumption, which should be considered when scaling up the process.

4.2.1.4. Analytical methods and calculations

The procedure of analysis and periodicity was the same as that described in 3.2.1 for SS.

VFA performance indicators were calculated through two different parameters, VFA yield (previously detailed in the Chapter 3) and VFA production rate.

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The VFA production rate, expressed as g COD-VFA/(L·d), was calculated using Eq. 4.1:

$$VFA\ production\ rate\ (t) = \frac{\Sigma VFAo\ (t) - \Sigma VFAi\ (t)}{HRT} \qquad \text{(Eq. 4.1)}$$

where *ΣVFA(t)* represents the concentration of VFA (as g COD/L) measured in the outlet and intlet at a particular moment, and *HRT* is the fixed hydraulic retention time applied to the reactor (days).

4.2.2. Results and discussion

4.2.2.1. Batch operation

Figure 4.1 shows that the maximum VFA yields were obtained between day 7 and 9 of the test, depending on each case. It was seen that the maximum VFA yields were 0.41 and 0.39 g COD-VFA/g COD substrate for pH 8 and 10, respectively (both reached on day 7). On the other hand, the bottles at pH 6.5 obtained exactly the same maximum VFA yield that at pH 10 (0.39 g COD-VFA/g COD substrate), but two days later (on day 9 of experiment).

Jiang et al. (2013) tested the most favourable pH in batch mode to produce VFA from FW, and they saw the best results at pH 6. Khatami et al. (2021) also carried out acidification tests in batch mode, observing that the highest VFA yields were obtained on day 10 when using an inoculum not adapted to FW as in the case of the one used in this doctoral thesis; moreover, the results were slightly higher at pH 10 than at pH 5, the two pH ranges that they checked. On the other hand, Cheah et al. (2019) reported the highest VFA concentrations in their batch tests also on day 10 of the experiment, with the best results seen at pH 9, although with little difference with those at pH 7.5.

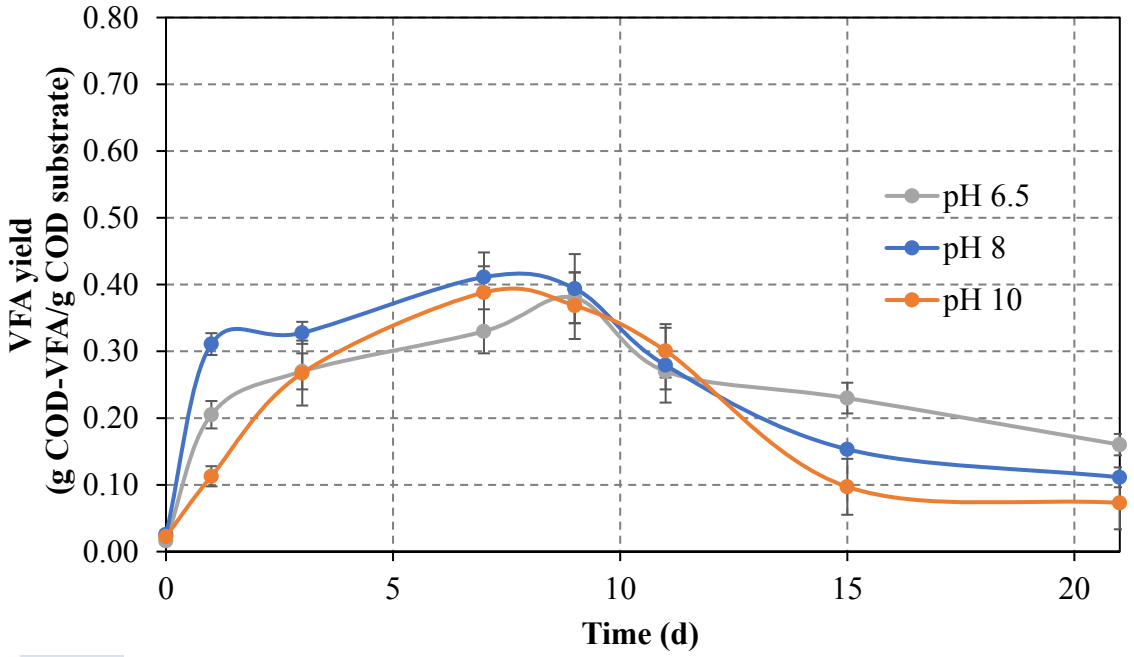

Figure 4.1. Acidification yields of food waste sample at different pH tested.

Regarding the distribution of VFA in the mix, it was observed that regardless of the pH chosen to carry out the test, acetic acid was the predominant in the mix followed by propionic and butyric. In any case, it is evident that as the pH rises to more alkaline ranges, the proportion of acetic acid becomes more relevant to the detriment of the other VFA (going from 37 % on a mass basis at pH 6.5 to 60 % at pH 10). Results in line with these were reported by Khatami et al. (2021), who observed that at pH 10, acetic acid reached an average presence of 60 %, followed by propionic with proportions of around 20 %. Furthermore, the presence of butyric in the mix at pH 6.5 is aligned with that reported by Jiang et al. (2013), given that when these authors operated in batch mode at pH 7, they obtained values around 20 %. Besides, Cheah et al. (2019) also obtained VFA distributions in line with those presented in Figure 4.2.

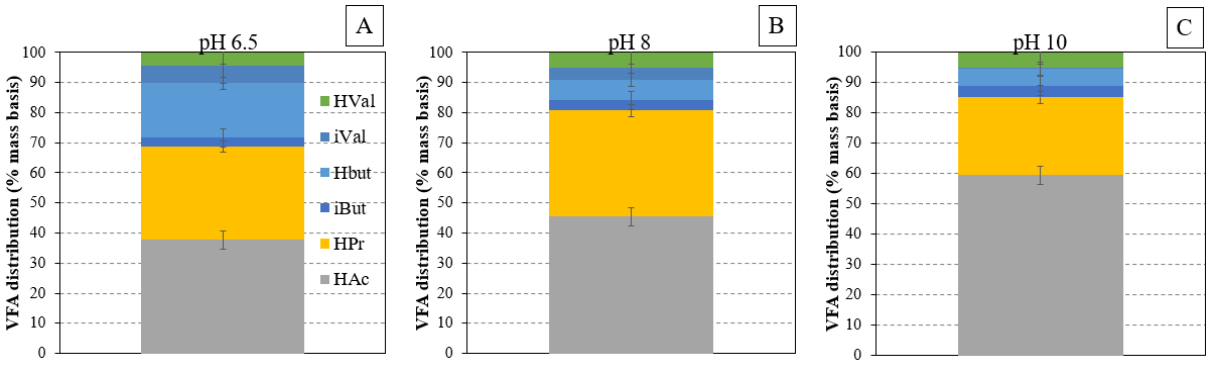

Figure 4.2. VFA mix distribution in batch tests with FW (day of maximum VFA yield). A) pH 6.5. B) pH 8. C) pH 10.

Considering that there were hardly any differences in the maximum VFA yield obtained at pH 6.5, 8 and 10, the change from operating in batch to continuous mode implied changing the pH range applied for several reasons.

It should be considered that the pH value remains decreasing due to the accumulation of VFA itself, and these conditions can inhibit acidogenic bacteria, so it is always necessary to have an automatic pH control system to keep it in the sought value. Although there are studies that have shown that alkaline conditions efficiently improve the transition from particulate to soluble matter (Li et al., 2014), FW usually has a pH between 3 and 6, so the adjustment to neutral or alkaline range requires the addition of large amounts of chemicals. This cost must be considered in the industrialisation of the technology, which is why the fermentation process under acidic conditions has already been proposed by several authors such as Yu et al. (2021) or Jin et al. (2019). Thus, it is more economically reasonable to control the pH to weakly acidic or neutral conditions in continuous mode.

4.2.2.2. Continuous stirred-tank reactors

The CSTRs were operated during 80 days at three pH values (5.5, 6 and 7) and OLR (6 and 10 g COD/(L·d)), to assess their influence on VFA production and selectivity, keeping constant other parameters such as temperature (35 ºC) and HRT (10 days). The different OLRs were achieved through different dilutions to raw FW. All of these operating parameters are known to seriously affect FW fermentation's performance, as Vázquez Fernández et al. (2022) reported.

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Although Jiang et al. (2013) proved an improvement in the hydrolytic stage at thermophilic conditions, they reported that VFA yield was not substantially affected when reducing temperature until the mesophilic range. When comparing psychrophilic range (15 ºC) against mesophilic one, Wei and Guo (2018) reported lower accumulation of VFA at lower temperature. Therefore, a mesophilic regime was chosen considering the balance between VFA yield and economic savings.

Table 4.2 shows the physicochemical characterisation of both inlets and outlets of the lab-scale reactors in the different scenarios. The inlet characterisation shows how the FW, after being diluted, slightly increases their sCOD/tCOD, which indicated that the dilution with water produced a solubilisation of particulate material.

After fermentation, sCOD/tCOD ratio largely increased, exceeding 50 % in all cases. This result indicated that the hydrolytic stage was not limiting, being the applied HRT adequate to operate with this substrate.

Outlet characterisation shows how when pH approaches neutrality, there were fewer soluble compounds other than VFA. The obtained results agree with Lim et al. (2008), who operated semi-continuous reactors under the same conditions. In general, they also reported COD-VFA/sCOD ratios between 40-70 %, reaching average VFA concentrations during the optimal operating conditions close to 40 g COD/L, in line with scenario 6 of Table 4.2. Jiang et al. (2013) obtained maximum concentrations between 17 and 37 g/L, resulting very similar to those measured at pH 6 and 7 in this work.

Table 4.2. Mean characterisation of the different scenarios inlets and outlets applied to 5-L continuous reactors.

Scenario	1	2	3	4	5	6
pH	5.5 ± 0.2	5.5 ± 0.1	6.0 ± 0.1	6.0 ± 0.2	7.0 ± 0.3	7.0 ± 0.2
OLR (g COD/(L·d))	5.6 ± 0.5	9.1 ± 0.9	6.4 ± 0.3	9.8 ± 0.8	6.2 ± 0.5	10.7 ± 1.1
Inlet characterisation						
tCOD (g O_2/L)	55.6 ± 4.1	90.7 ± 6.3	64.8 ± 4.3	98.1 ± 6.9	62.2 ± 4.0	106.6 ± 7.5
sCOD/tCOD (%)	34.5	39.5	30.1	39.3	30.9	35.9
TS (g/L)	27.2 ± 2.4	47.3 ± 4.6	40.9 ± 3.6	49.2 ± 3.9	39.2 ± 2.6	61.1 ± 4.6
VS (g/L)	25.3 ± 2.1	44.8 ± 2.9	36.9 ± 2.3	47.6 ± 3.3	35.1 ± 4.3	57.2 ± 5.1
Outlet characterisation						
tCOD (g O_2/L)	44.8 ± 4.0	80.9 ± 4.6	60.4 ± 4.5	81.5 ± 3.6	60.6 ± 5.6	100.9 ± 6.6
sCOD/tCOD (%)	54.7	51.8	58.6	57.9	55.9	63.0
Total VFA (g COD/L)	9.6 ± 1.8	15.0 ± 0.9	18.5 ± 2.9	28.6 ± 3.4	22.0 ± 3.1	39.0 ± 5.9
COD-VFA/sCOD (%)	39.0 ± 6.1	35.8 ± 7.6	52.7 ± 6.5	60.6 ± 8.6	61.8 ± 7.4	71.5 ± 9.6

- ***VFA yield and production rate***

Figure 4.3 shows the VFA yield and the VFA production rate obtained in all the tested scenarios. pH played a crucial role in VFA yield and production rates since it affects the balance between acidogenic and methanogenic microorganisms (Varghese et al., 2022). When pH shifts from 5.5 to 7 (orange and grey bars of Figure 4.3, respectively), both VFA yield and production rates increase for both OLRs tested, which indicates that neutral pH was the most favourable

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one to maximise the conversion of organic matter into VFA. However, it is relevant to consider the costs associated with the use of chemicals needed for pH control.

Although the best experimental results were obtained at pH 7, there are references from other authors indicating that more acidic values are also favourable for anaerobic fermentation of FW. Strazzera et al. (2021) reported a VFA yield of 33 % g COD-VFA/g COD substrate at pH 7, slightly lower than that obtained in this work for both OLRs. Also, Lim et al. (2008) reported that VFA yield increased progressively from pH 5 to 6, while Jiang et al. (2013) obtained lower VFA yields at uncontrolled pH compared with experiments conducted at pH 6 and 7. Moreover, Zhang et al. (2010) conducted experiments to ferment kitchen waste, concluding that pH 7 was the best in VFA yield compared to pH 5, 9 and 11. Contrary, Jiang et al. (2013) found that a better acidification yield was obtained at pH 6 (442 mg COD-VFA/g VS) in comparison with pH 5 and 7, notably lower than that obtained in this work at the same pH (581 and 600 mg COD-VFA/g VS for OLR 6 and 10 g COD/(L·d), respectively).

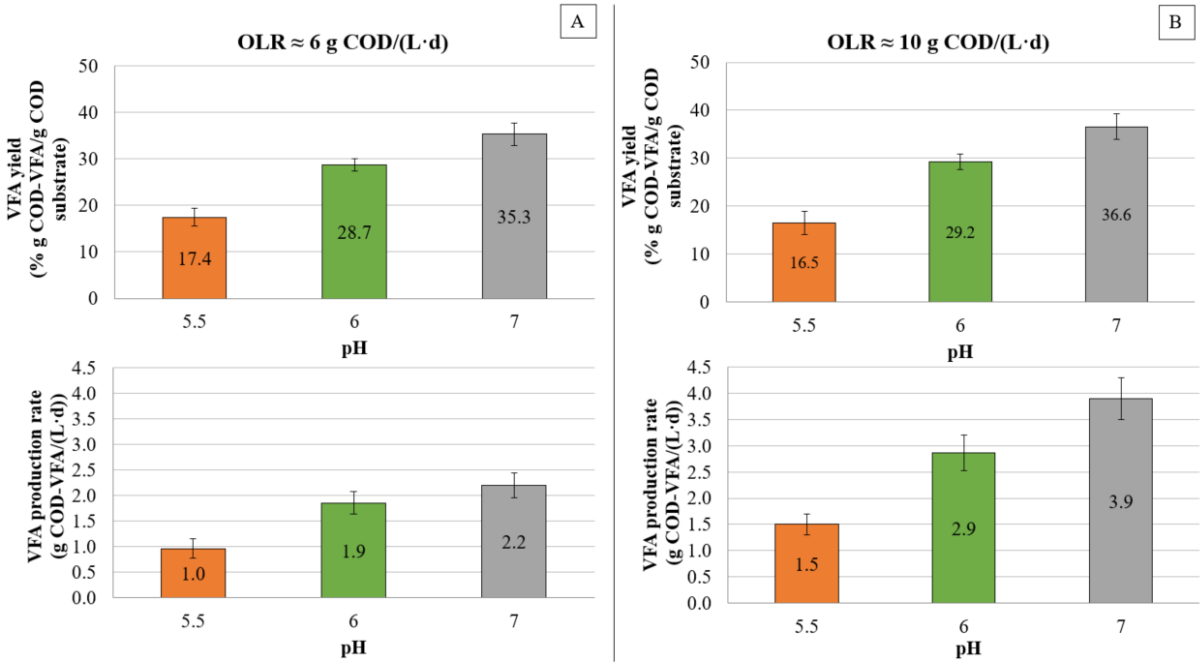

Figure 4.3. VFA yield and production rate of 5-L continuous reactors in the scenarios tested. A) OLR 6 g COD/(L·d). B) OLR 10 g COD/(L·d).

On the contrary, VFA yield was not affected by increasing OLR from 6 to 10 g COD/(L·d). At an OLR of 10 g COD/(L·d), a VFA production rate of 3.9 g COD-VFA/(L·d) was registered, which is slightly higher than that reported by Possente et al. (2022), who obtained 3.5 g COD-VFA/(L·d) when applying an OLR of 7 g COD/(L·d). The best results achieved in this work in terms of VFA production rate were also slightly higher than the one reported by Lim et al. (2008) (3.7 g COD-VFA/(L·d)), and the one presented by Garcia et al. (2018) (3.3 g COD-VFA/(L·d)).

- ***VFA selectivity***

Figure 4.4 shows the selectivity of the fermentation process from FW in the different scenarios tested (mass basis). The results operating on continuous mode at pH 6 and 7 (scenarios 3 to 6) are aligned with those of VFA distribution in batch experiments executed at pH 6.5

(Figure 4.2A), so it can be stated that the mode of operation did not have much influence on the VFA spectrum.

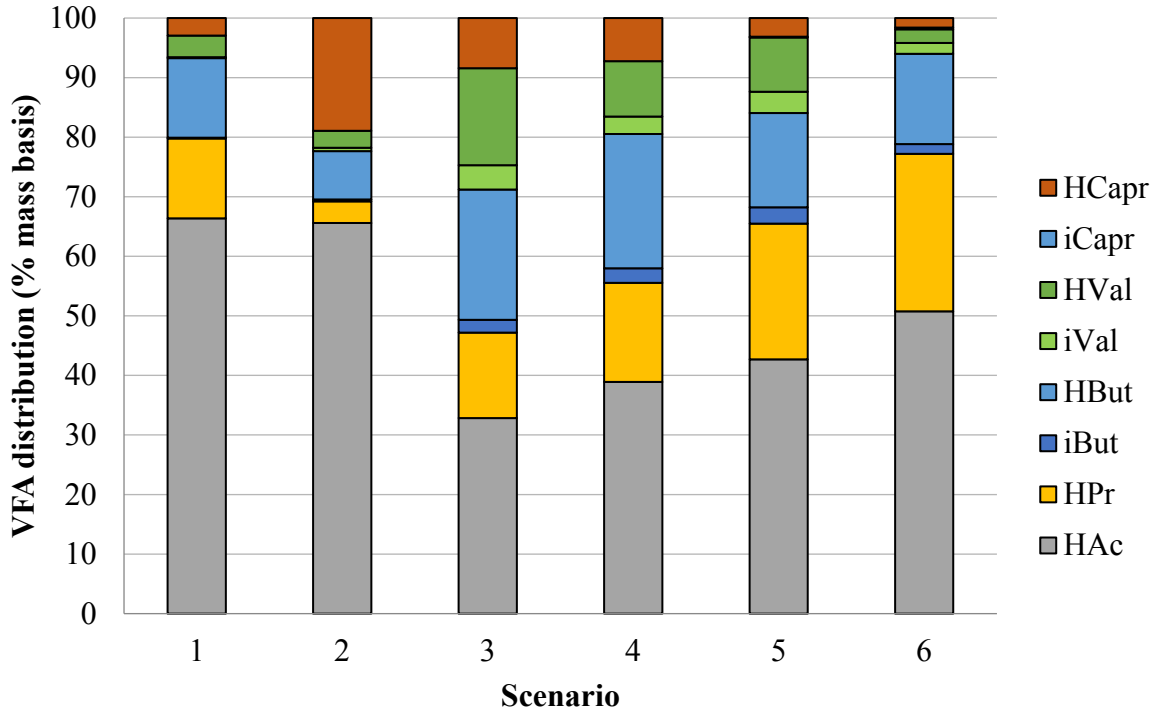

Figure 4.4. VFA distribution mix obtained in each scenario tested.

Figure 4.5 depicts the amount of VFA obtained from 1 t of raw FW in order to quantify the total product generation from substrate regardless of the applied dilution, taking into consideration the selectivity of the process. It can be verified that pH affected VFA yields and significantly influenced selectivity, an essential feature regarding the significant differences in the market price and applications of each organic acid. At pH 5.5, acetic acid was the most abundant VFA, representing around 50 % of total production. However, at higher pH, its proportion decreased in detriment of other VFA such as propionic and butyric. These results agree with those of Jiang et al. (2013) and Wei and Guo (2018), who obtained at pH 5.5 more than 50 % of the VFA mix as acetic acid and at higher pH, propionic and butyric increased their proportion until reaching 20 % and 30 %, respectively. Feng et al. (2018) also observed that at pH near 5, acetic acid was predominant in the mix, but at pH 6, valeric acid became more relevant, approaching values higher than 25 %, similar to that obtained in this work at pH 6 and OLR 6 g COD/(L·d).

Figure 4.5 also depicts a noticeable amount of caproic acid in scenario 2 (almost 20 % of total presence) operating at a higher OLR but at pH 5.5. Operating at pH 6 (scenarios 3 and 4), it also appears but in lower proportions (8 and 7 % of the total, respectively), almost disappearing at pH 7. Caproic acid could be generated from chain elongation processes, which are favoured by a pH within the range 5.25-5.5 (Ge et al., 2015).

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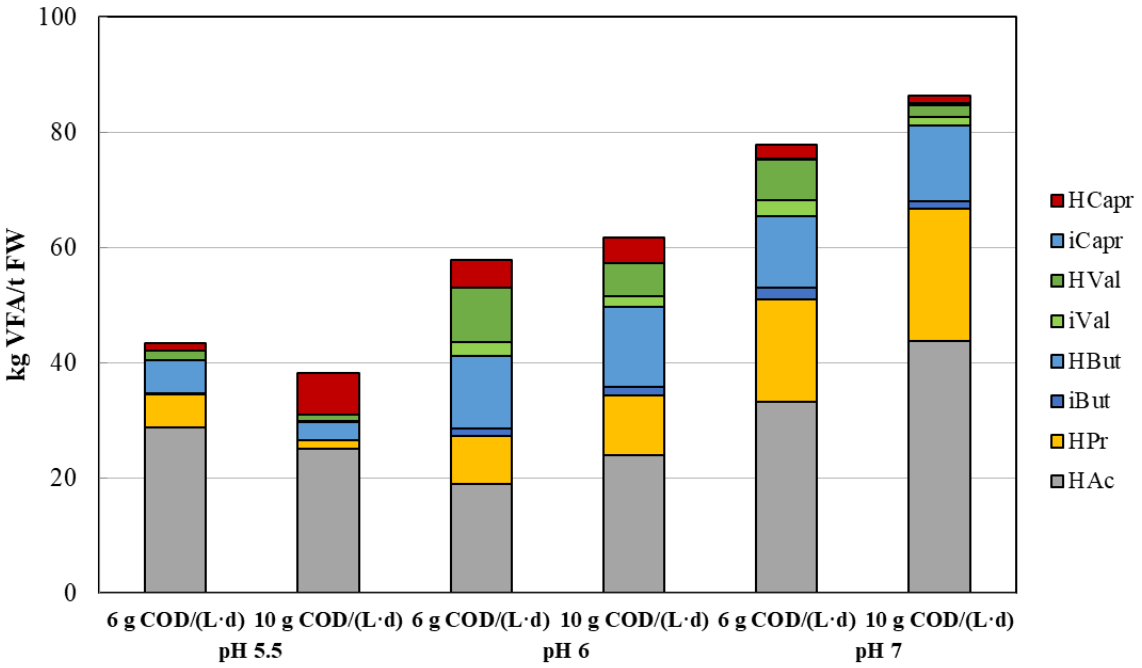

Figure 4.5. Selectivity of VFA production obtained per t of food waste in each scenario.

In general, no significant changes were observed in the distribution of the VFA obtained at the same pH when applying a different OLR, except for scenario 2. Besides, no remarkable differences were observed between the spectrum of VFA obtained when comparing scenarios operating at the same pH. Jiang et al. (2013) did not report significant changes either in the VFA mix produced from FW when various OLR ranges were applied. Conversely, they observed significant differences when varying pH, reporting an acetic fraction of 60 % at pH 5, dropping to 34 % at pH 7, results similar to those shown in Figure 4.4.

4.3. PILOT SCALE

4.3.1. Material and methods

4.3.1.1. Physicochemical characterisation of substrate and inoculum

FW was collected from expired products and food scraps from a supermarket. These residues were shredded with a semi-industrial crusher and diluted with process outlet water from a WWTP to attain the desired dilution (mean composition shown in Table 4.3). The chemical characterisation matches quite well with those reported by Hansen et al. (2007) and Khatami et al. (2021).

FW presented an approximate dryness of 20 %, in line with that reported in other works, such as that of Garcia et al. (2018), who used a mixture of synthetic FW representative of the Mediterranean countries, reporting a dryness of 26 %. VS/TS was approximately 0.9, similar to that reported by Alibardi and Cossu (2016), who presented the chemical characterisation of eight FW mixtures, all with VS/TS exceeding 0.9. The large presence of meat and fish in FW makes it a high-protein substrate. Its protein content could be estimated at around 40 g/kg using the TN x 6.25 formula proposed by Mariotti et al. (2008).

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Considering its high dryness, after crushing and mixing, FW was diluted 1:10, resulting in a tCOD and sCOD of 279 g O_2/kg and 81.7 g O_2/kg, respectively, indicating significant solubilisation of compounds. Its pH (around 4.9) indicates that it is partially acidified, even considering the short collection time (no more than 3 days after disposal in containers), a value similar to the one found by Wang et al. (2019) for this type of substrate, between 3 and 5.

Table 4.3. Characterisation of collected food waste (mean of 50 samples).

	FW sample
pH*	4.93 ± 0.90
tCOD (g O_2/kg)	279.4 ± 13.2
sCOD (g O_2/kg)*	81.7 ± 9.9
TS (g/kg)	201.6 ± 14.1
VS (g/kg)	178.2 ± 11.3
TN (g/kg)	7.2 ± 0.9
TAN (g N/kg)*	0.42 ± 0.05

*Measured after diluting the substrate 1:10 with process water.

4.3.1.2. Pilot plant description

Figure 4.6 shows a simplified flow diagram of the semi-industrial scale pilot plant (the same as the one shown in Figure 3.9, but the crusher originally installed was too small and a semi-industrial one was acquired). FW was fed to the semi-industrial crusher (model ENG SA2) once per week and then diluted with process water (around 17 % and 43 % of FW/H_2O in w/w for OLR 6 and 10 g COD/(L·d), respectively). It was stored in tank TK0 (1 m^3), which was agitated (150 rpm) and maintained at 4 ºC thanks to a thermostatic bath. The fermenter (TK1) had a useful volume of 1.5 m^3 and its temperature was controlled at 35 ± 1 ºC (by circulating hot water through an external heat jacket). pH was controlled at 7 by the addition of NaOH 50 % (w/v). The fermented FW was discharged into TK2 (1 m^3), in which the effluent was mixed with coagulant (0.11 kg calcium oxide/t FW at 150 rpm for 3 min) and flocculant (0.004 kg PAM/t FW at 150 rpm for 2 min and 50 rpm for 5 min). Finally, a press filter separates the solid-liquid into two streams: a solid cake (around 30 % dryness) and a VFA-rich liquid stream.

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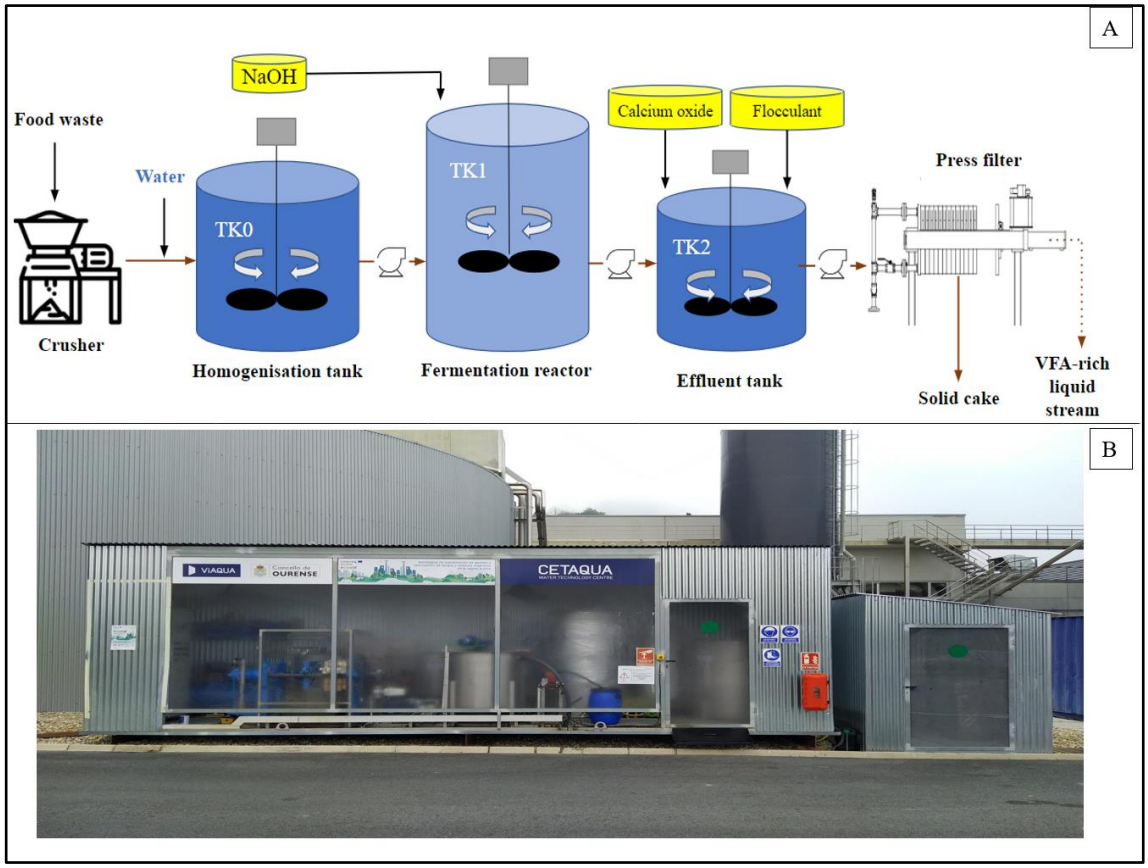

Figure 4.6. Layout of the semi-industrial scale pilot plant to produce VFA from food waste. A) Simplified block diagram. B) Panoramic view of the pilot plant.

4.3.2. Results and discussion of pilot plant operation

The semi-industrial scale pilot plant was operated for 170 days at a mesophilic range and pH 7. Apart from giving rise to better VFA performance indicators, pH 7 implies a more varied spectrum of VFA, with lower presence of acetic acid and a greater abundance of VFA of longer-chain with elevated market value.

When scaling-up the technology, chemical consumption also acquires vital importance. Operation at pH 7 implied highest NaOH consumption (5.0 and 4.2 kg NaOH/t FW for OLR 6 and 10 g COD/(L·d), respectively) in comparison with that at pH 6 (3.0 and 2.2 kg NaOH/t FW for scenarios 3 and 4) and pH 5.5 (1.50 and 1.40 kg NaOH/t FW for scenarios 1 and 2, respectively), but this slight difference was not enough to change the chosen pH. In fact, operation at pH 7 instead of pH 5.5 would imply an extra cost of 2.8 €/t FW and 2.3 €/t FW for OLR 6 and 10 g COD/(L·d), respectively (NaOH price of 800 €/t (Wei et al., 2013)), but VFA production would be reduced by almost half going from 80 kg VFA/t FW at pH 7 to 40 kg VFA/t FW at pH 5.5.

Moreover, the higher VFA concentration in the outlet implies a reduction of cost related to purification, which might entail using a membrane train containing MF and/or UF membranes for cleaning the stream, followed by a NF or RO one to concentrate VFA (Zacharof et al., 2013). Zhou et al. (2013) reported that the recovery percentage of acetic acid from the VFA-rich liquid stream with RO was favoured when going from an initial concentration of 10 to 50 g/L,

implying both energy savings and less membrane replacement. In addition, other separation processes are also influenced by initial concentration such as LLE, in which the S/F ratio is a key parameter, allowing cost savings by starting with a smaller feeding volume, this is, from a more concentrated stream in VFA.

Figure 4.7A depicts the COD distribution of the inlet and outlet of the pilot plant fermenter, considering two periods, the first one until day 80, in which an OLR of 6 g COD/(L·d) was applied to achieve a gradual biomass acclimation (Iglesias-Iglesias et al. (2019)), and the second one from day 80 onwards, increasing the OLR up to near 10 g COD/(L·d). During most of the operation there was no significant differences between tCOD in the inlet and outlet, suggesting that organic matter was not removed as biogas. In this sense, the differences observed (e.g., days 80-110) were expected to be related to the change in inlet's COD, since the HRT applied (10 days) is too high to observe immediate changes in the outlet and the observation of the effect is delayed.

For the first period, 18.5 g COD-VFA/L were obtained, implying a VFA yield of 35.0 ± 3.4 % g COD-VFA/g COD substrate, comparable to the lab-scale reactor working in the same conditions. These results are slightly higher than those reported by Pramanik et al. (2020), who ran a single-stage semi-pilot scale reactor, obtaining a VFA yield of around 27 % g COD-VFA/g COD substrate. Besides, Micolucci et al. (2020) operated a two-phase anaerobic system consisting of a 200-L acidogenesis reactor coupled with a 600-L methanogenic reactor for the production of VFA and other compounds from FW, obtaining an average VFA yield of 32 % g COD-VFA/g COD fed, in line with the results of this work.

Regarding the second period, 37.6 g COD-VFA/L were obtained, which implies a VFA yield of 37.9 ± 4.5 % g COD-VFA/g COD substrate, which according to a Student's t-test statistical analysis was not significantly different from that attained in the first period. A similar COD-VFA/sCOD ratio was also obtained for both periods, 69 % in the first and 67 % for the second period, in line with other works like that of Jiang et al. (2013). Therefore, the OLR did not affect neither VFA yield nor COD-VFA/sCOD ratio at pilot scale.

Concerning VFA production rate, an average of 1.8 g COD-VFA/(L·d) was registered during the first period of operation, while for the second it was 3.4 g COD-VFA/(L·d), both slightly lower than those obtained in the lab-scale reactors (2.2 and 3.9 g COD-VFA/(L·d), respectively). These minor differences may be due to the lower control over the operational parameters on a larger scale.

Figure 4.7B depicts the composition of the VFA mixture obtained in the pilot plant. No major differences were seen between both experimental periods, showing that the OLR did not affect the VFA selectivity obtained at the pilot scale either, as it was previously reported for lab-scale reactors. The VFA mix produced consisted of approximately 50 % acetic acid (mass basis), followed by propionic and butyric acids with approximately 20 % each and a minor proportion of iso-butyric, n-valeric and iso-valeric acids, which may be expected under neutral pH conditions (Agler et al., 2011). Garcia-Aguirre et al. (2017) reported a similar distribution of VFA when using an organic fraction of municipal solid waste as substrate at a slightly acidic pH. On the contrary, Jiang et al. (2013) or Strazzera et al. (2021) measured a higher proportion of butyric acid when operating at neutral pH in the mesophilic range (more than 40 % in mass basis). Most probably, differences with other works can be related to substrate composition, which highly affects the selectivity of the VFA fermentation (Vázquez-Fernández et al., 2022).

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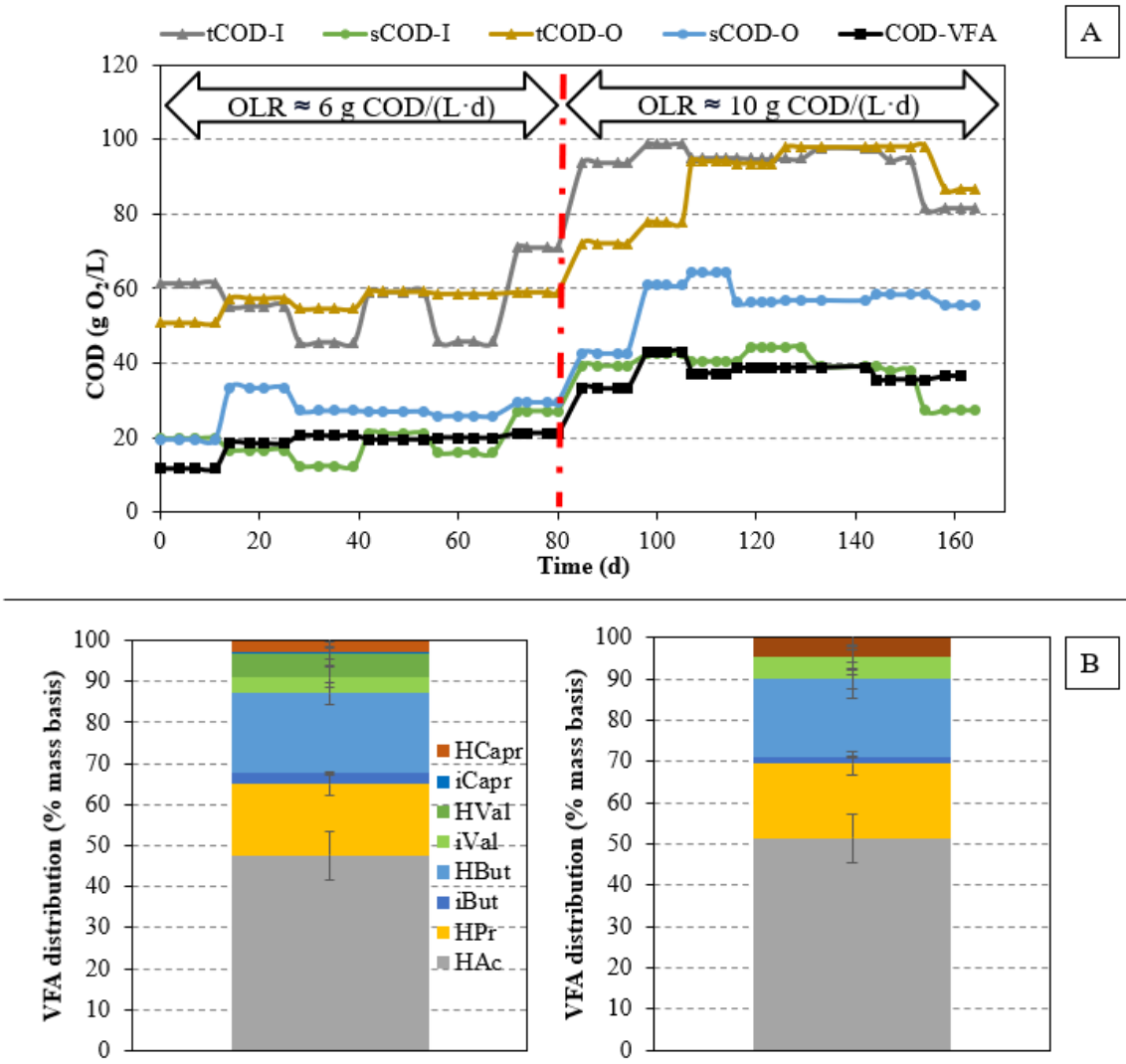

Figure 4.7. VFA production at pilot scale in the two scenarios tested. A) COD distribution of the inlet (I) and outlet (O). B) Distribution of the mix working at OLR 6 g COD/(L·d) (left) and OLR 10 g COD/(L·d) (right).

4.4. CONCLUSIONS

Results shown in this Chapter demonstrated the great potential of FW to produce VFA, obtaining better results than experimentation with SS presented in Chapter 3. The scalability of the fermentation process when going from laboratory scale (both in batch mode and with 5-L continuous reactors) to pilot scale was assessed. As a substrate, food leftovers from a supermarket chain were collected and mixed in appropriate proportions achieving a FW sample representative of those generated in European households (maintaining practically constant proportions for all the experimentation).

The batch mode acidification test showed that the maximum VFA yields were 41 % g COD-VFA/g COD substrate at pH 8 and 10, and slightly lower at pH 6.5 (39 % g COD-VFA/g COD substrate). This small difference in VFA production performance implies a quest to avoid

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the significant chemical expense when working in continuous mode, so to operate the 5-L reactors it was selected a slightly acidic and neutral pH range.

The operation of these continuous reactors allowed to validate the optimal pH in terms of VFA yields and productivity, which has been neutral, taking it therefore to the pilot plant. Furthermore, it was found that the OLR applied had no influence on the VFA yields obtained.

In general, the scaling up of the VFA production from FW was satisfactory. The results obtained from the pilot plant operation were comparable to those of the lab-scale reactors, which gives a certain reliability concerning a possible technology industrialisation. The average VFA yield resulted in approximately 35-38 % g COD-VFA/g COD substrate regardless of the OLR applied. Regarding VFA productivity, the highest OLR implied a higher value of VFA production rate, specifically 3.4 g COD-VFA/(L·d). As for the VFA mix obtained, acetic acid was the predominant one with practically a 50 % presence in mass basis, followed by propionic and butyric acid, with approximately 20 %. Moreover, 70 % of the sCOD in the outlet were VFA.

In this way, it can be concluded that the VFA yields have hardly fallen when going from batch to continuous operation, nor has it barely fallen in the transition from a volume of 5 L to 1.5 m^3. Therefore, this mode of operation in the pilot plant turned out to be steady, allowing the generation of a fermented effluent with total VFA concentrations above 30 g VFA/L, with a high potential to be further treated through other unit operations after fermentation.

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5) MEMBRANE TECHNOLOGY FOR VFA CONCENTRATION

5.1. INTRODUCTION

In Chapters 3 and 4, it was found that VFA concentrations after fermentation were between 8-35 g VFA/L. Thus, although VFA are present in the effluents obtained from the fermentation process, this stream is an unpurified mixture, containing salts, microorganisms, proteins, lipids, etc. which severely prevent the future application of VFA if not removed. The high-water content of these effluents makes the VFA so dilute that their potential uses are severely restricted.

Among all existing technologies for VFA purification, pressure-driven membrane technologies are frequently used to purify and concentrate them from blending solutions, as they have several benefits such as scalability, relatively low energy demand and minimal phase change during the separation process. The membrane train is ideal for integration before other unit operations in order to pre-treat the effluent, adapting it for the subsequent stages. In this doctoral thesis, it was proposed just after fermentation and before LLE and distillation.

This Chapter presents the pilot plant in which the membrane experimentation was conducted, as well as the method of calculating the factors defining membrane performance, given that there is considerable variability in the definition of these terms depending on the source consulted. Moreover, the results obtained with synthetic medium are discussed, which served to establish the optimal operating conditions to work with a real matrix.

The objective of this Chapter is to determine the optimal operating conditions in the pressure-based membrane train to maximise the final VFA concentration obtained. In addition, it also aims to collect operational data of interest for the techno-economic study of the global process such as reagent consumption or variation of the permeate flux over time.

5.2. MATERIALS AND METHODS

5.2.1. Pilot plant description

Figure 5.1 shows the P&ID of the membrane train used for experimentation. First, there is a filtration system made up of three cylindrical filters placed in series whose filtration range corresponds to a MF (they are placed successively, and their pore size are 50, 20 and 1 μm, respectively); this system allows the sample to be conditioned before being fed to the rest of the membranes when real medium is used.

The membrane train is composed of three vessels to place three membranes: UF membrane, NF membrane and one RO membrane. Figure 5.1 depicts as before each membrane vessel, there is a tank where the feeding to that membrane is stored. These tanks have level probes, and to feed the membrane itself there are two pumps prior to each tank, a low pressure one (Grundfos

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CM1) which raises the pressure to around 2 bar and a high-pressure pump (Danfoss APP 25), which elevates the pressure until the desired point. The pilot plant also has a membrane cleaning system; it is composed of a peristaltic pump that drives the desired chemicals and/or water to carry out cleaning from the storage tanks through a circuit.

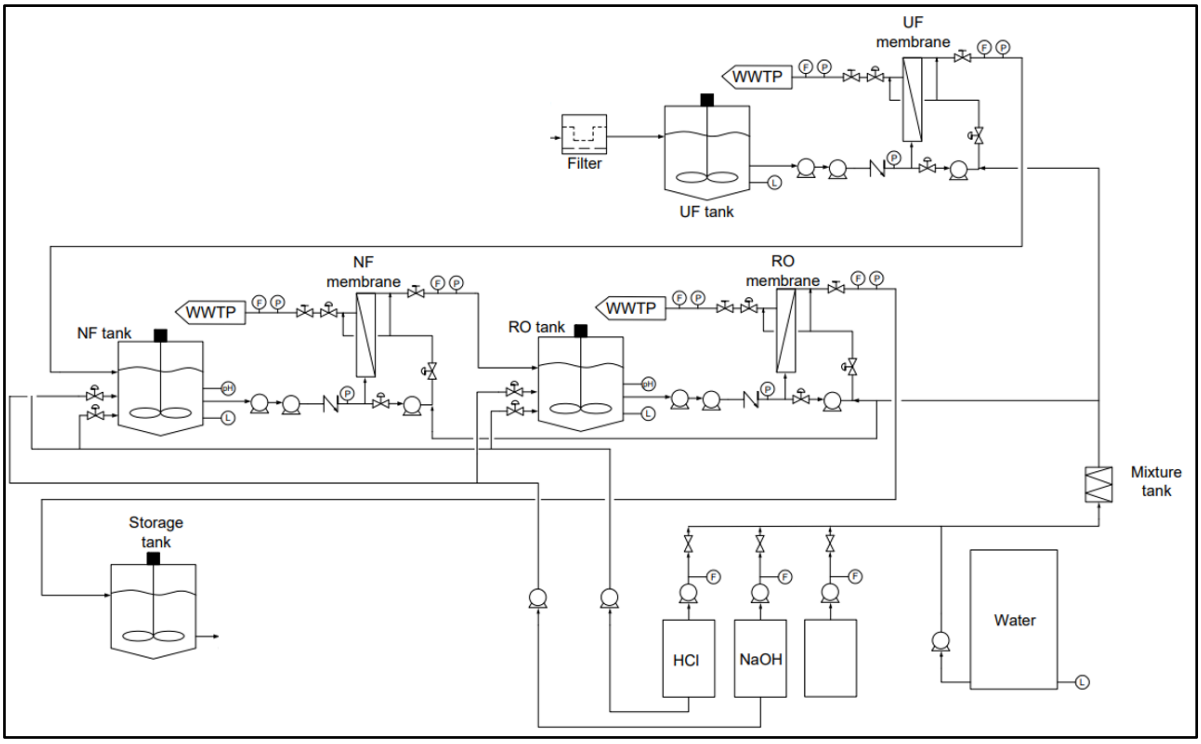

Figure 5.1. P&ID of the membrane train for membrane experimentation.

Figure 5.2 shows an internal image of the pilot plant in which the experiments were carried out. The image on the left shows the MF cartridge system in the foreground, while the image on the right shows the membrane vessel, together with the pumps as well as a pressure sustaining valve used to keep a constant pressure during the operation.

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Figure 5.2. Internal view of the membrane train used on a pilot scale.

The technical characteristics of the membranes used are shown in Table 5.1. They were all of the polymeric type, with an outer wrap made of fiberglass, except for the RO VEOLIA AG2540TM, whose outer wrap was tape. It is observed that as the filtration range varies from UF to RO, the maximum operating TMP also does.

Table 5.1. Technical characteristics of the membranes used.

Filtration range and model	Average permeate flow (m^3/d)	Minimum $MgSO_4$ rejection (%)	Active area (m^2)	Outer wrap	Typical operating flux ($L/(m^2 \cdot h)$)	Maximum operating TMP (bar)
UF VEOLIA GE2540F30	2.8	-	2.6	Fiberglass	8-34	27.6
NF VEOLIA DK2540F30	2.0	98.0	2.6	Fiberglass	8 -34	41.3
RO VEOLIA AG2540TM	2.8	99.5	2.5	Tape	15-35	41.3
RO DOW FILMTEC SW302540	2.6	99.4	2.5	Fiberglass	26-37	55.0

5.2.2. Calculations

One of the vital parameters in membrane filtration processes is the applied TMP, calculated through Eq. 5.1:

$$TMP = \frac{(P_f + P_r)}{2} - P_p \qquad \text{(Eq. 5.1)}$$

where *Pf* is the pressure of the feeding stream, *Pr* is the pressure of the rejection stream and *Pp* is the pressure of the permeate stream.

One of the important factors to define when working with membranes is the so-called "Recovery rate", defined by Eq. 5.2:

$$Recovery\ rate\ (\%) = \frac{Q_p}{Q_f} \cdot 100 \qquad \text{(Eq. 5.2)}$$

where *Qp is* the permeate flow rate and *Qf* is the feeding flow rate.

When the aim is to purify and concentrate a VFA-rich stream, the function of the NF membrane and the RO membrane could not be the same. In the first of them, the aim was to clean the sample without losing VFA in the rejection stream, so the parameter "Mass flow VFA permeate" was evaluated with Eq. 5.3:

$$Mass\ flow\ VFA\ permeate\ (\%) = \frac{(Q_p \cdot C_p)}{(Q_f \cdot C_f)} \cdot 100 \qquad \text{(Eq. 5.3)}$$

where *Qp is* the permeate flow rate, *Cp* is the VFA concentration in the permeate and *Qf* and *Cf* are the analogue in the feeding, respectively.

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It is also important to determine the so-called "Permeate flux", calculated through Eq. 5.4:

$$Permeate\ flux = \left(\frac{Q_f}{A_m}\right) = \left(\frac{\frac{dv}{dt}}{A_m}\right) \quad \text{(Eq. 5.4)}$$

being *Am* the surface of the membrane. When the units of *Qf* and *Am* are in L/h and m^2, respectively, the permeate flux would be expressed in $L/(m^2 \cdot h)$, which in the jargon of membrane filtration is known as litres of permeate per square metre of membrane area per hour (LMH).

On the other hand, the purpose of the RO membrane could be different, to achieve maximum recovery and concentration factors. To calculate these, both the term "Recovery" and the "Concentration factor" were evaluated, indicated in the Eq. 5.5 and 5.6:

$$Recovery\ (\%) = \frac{(Q_r \cdot C_r)}{(Q_f \cdot C_f)} \cdot 100 \quad \text{(Eq. 5.5)}$$

being *Qr* the rejection flow rate and *Cr* the VFA concentration in the rejection and the denominator factors being those already defined above in Eq. 5.3:

$$Concentration\ factor = \frac{C_r}{C_f} \quad \text{(Eq. 5.6)}$$

being *Cr* and *Cf* the VFA concentrations in the rejection and feeding, respectively.

Regarding the removal of nutrients in the membranes, they were calculated as shown in Eq. 5.7 and 5.8. In the case of NF, the removal of nutrients was considered as their reduction in the permeate stream, since it was the stream of interest in the proposed train; while in the case of the RO, the reduction of these in the rejection stream was considered, as this may be the stream with the highest concentration of VFA.

$$NF\ nutrient\ removal\ efficiency\ (\%) = \frac{(Q_f \cdot C_f) - (Q_p \cdot C_p)}{(Q_f \cdot C_f)} \cdot 100 \quad \text{(Eq. 5.7)}$$

being *Qf* and *Cf* the flow and concentrations of $N\text{-}NH_4^+$ or $P\text{-}PO_4^{3-}$ in the feeding, respectively, and *Qp* and *Cp* the analogous in the permeate.

$$RO\ nutrient\ removal\ efficiency\ (\%) = \frac{(Q_f \cdot C_f) - (Q_r \cdot C_r)}{(Q_f \cdot C_f)} \cdot 100 \quad \text{(Eq. 5.8)}$$

being *Qf* and *Cf* the flow and concentrations of $N\text{-}NH_4^+$ or $P\text{-}PO_4^{3-}$ in the feeding, respectively, and *Qr* and *Cr* the analogous in the rejection.

5.2.3. Synthetic medium

A synthetic VFA solution with a concentration of 15 g/L (10 g/L acetic, 3 g/L propionic and 2 g/L butyric, all from Sigma Aldrich > 99.7 % purity) was prepared, with the aim of

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imitating an outlet stream of the fermenter operating with SS as a substrate (Table 3.5). These feedings were adjusted to the desired pH range by adding NaOH (50 % w/v) and were fed at room temperature (around 15 ºC). Furthermore, with synthetic medium, a recovery rate of 80 % was applied, i. e., 80 % of the fed flow was caused to go through the permeate and the remaining 20 % through the rejection stream.

Operating with this synthetic medium, membrane cleanings were carried out between experiments. The procedure was the following: cleaning with drinking water for 10 min, cleaning with HCl 3 % (v/v) for 15 min, cleaning with drinking water for 10 min, cleaning with NaOH 3 % (w/v) for 15 min and cleaning with drinking water for 10 min. Between experiments, the membranes were stored in a 1 % (v/v) solution of sodium metabisulfite (Sigma Aldrich) to prevent unwanted microorganisms' growth.

The experiments with nutrient addition were conducted using NH_4Cl and KH_2PO_4 at 99.9 % purity from Sigma Aldrich.

5.2.4. Real effluent

Once the experiments with synthetic medium were executed, the information obtained was applied to conduct the experimentation with real medium, in this case effluent from fermentation at pilot scale using SS as a substrate, whose initial characterisation after solid-liquid separation is shown in Table 5.2. As it was done with synthetic medium, a hydraulic recovery of 80 % was applied in order to benefit from all the experimental information acquired so far. In addition, HCl 37 % (v/v) was added (at a dose of 0.003 g/L) to lower the pH to the desired value, in this case 3. A balance between performance and permeate fluxes was sought, apart from being the most satisfactory pH range for subsequent unit operations.

Table 5.2. Physicochemical characterisation of the liquid fraction of press filter (analysed in triplicate).

	VFA-rich liquid stream
pH	3.2 ± 0.00
tCOD (g O_2/L)	55.1 ± 3.0
sCOD (g O_2/L)	55.1 ± 3.0
N-NH_4^+ (g/L)	1.49 ± 0.24
P-PO_4^{3-} (g/L)	0.186 ± 0.004
Total VFA (g/L)	9.0 ± 0.2
Acetic acid (g/L)	4.9 ± 0.1
Propionic acid (g/L)	2.3 ± 0.1
Butyric acid (g/L)	0.8 ± 0.0
Isobutyric acid (g/L)	0.4 ± 0.0
Isovaleric acid (g/L)	0.2 ± 0.1
Isocaproic acid (g/L)	0.4 ± 0.0

The procedure for cleaning the membranes in this case was the same as for the synthetic medium, but it was decided to perform cleanings when the permeate flux decreased by 20 % with respect to the starting permeate flux for all types of membranes.

Specifically, experimentation with real medium coming from SS fermentation was accomplished with the following membranes: UF VEOLIA GE2540F30, NF VEOLIA DK2540F30 and RO VEOLIA AG2540TM.

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5.3. RESULTS AND DISCUSSION

5.3.1. Synthetic medium

-PHASE 1: Initial screening. Objective: study the effect of pH in the feeding (3, 5 and 7) and applied TMP (15, 20 and 25 bar) on NF VEOLIA DK2540F30 membrane and RO VEOLIA AG2540TM membrane (recovery rate applied of 80 % according to Eq. 5.2).

-VEOLIA DK2540F30 NF Membrane

Figure 5.3 shows that the mass flow VFA permeate was greater than 70 % in all scenarios, regardless of the applied TMP and pH, so there was no significant loss of VFA in NF rejection. It was also noted that this parameter increased slightly as the pH decreased.

Unlike what other authors such as Zacharof and Lovitt (2014) or Pervez et al. (2022) reported, the VFA pass the NF membrane and end-up in the permeate stream. This difference in results may be due to several reasons, including the type of membrane used (different MWCO), the type of stream treated (they used real effluent) or the different operating conditions (pH and TMP). In any case, there are also authors such as Domingos et al. (2022) who gave account of VFA flow going into the NF permeate, exceeding values of 50 % using synthetic effluents, even so, lower than those reported in Figure 5.3.

In any case, the fact that the "Mass flow VFA permeate" was so high makes sense considering the MWCO of membrane DK2540F30, since it is about 300 Da. It should be noted that the MW of VFA ranges from 66 to 88 Da for acetic to butyric, respectively (Table 5.3), so it is expected that at least in their dissociated form, VFA pass the NF membrane through the permeate.

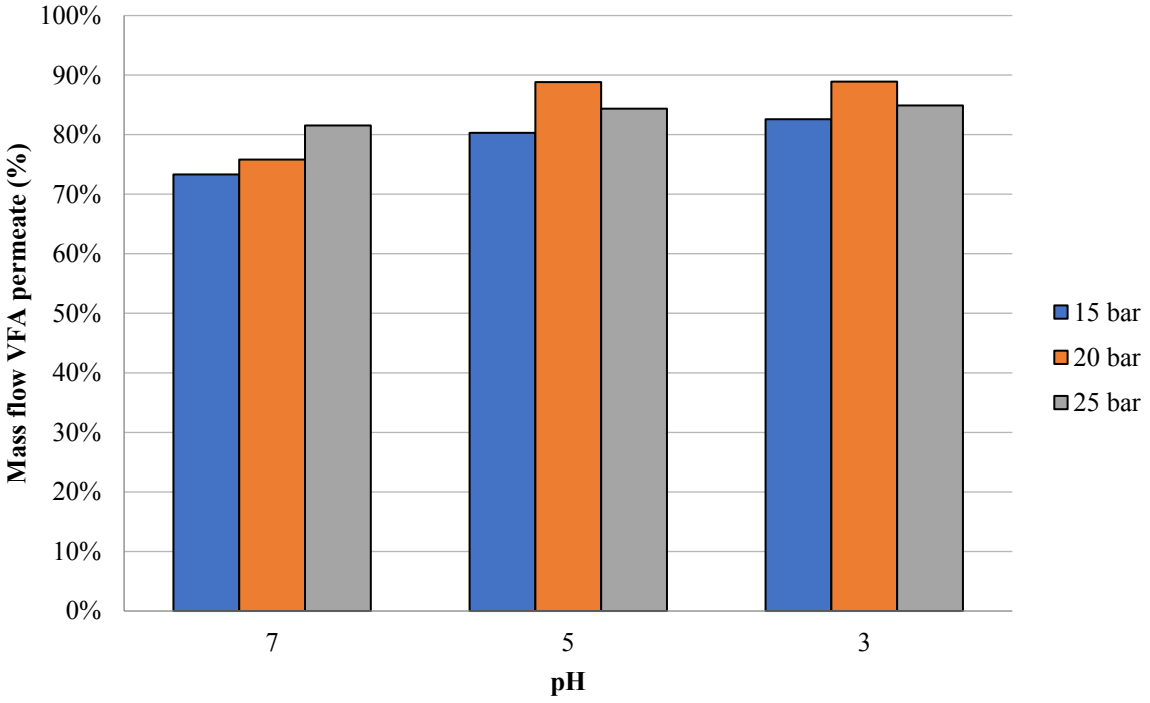

Figure 5.3. Effect of pH and TMP on mass flow VFA permeate for membrane NF DK2540F30.

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Figure 5.4 shows the influence of TMP and pH on permeate fluxes. In general, permeate fluxes increase as the TMP raises, although for pH 7 and 5 no rise was observed when going from a TMP of 15 to 20 bar. On the other hand, pH has a much more relevant impact on the permeate flux, with pH 3 being the most appropriate when a high permeate flux is sought. This behaviour was reported by different authors in their works (Al-Amoudi and Lovitt, 2007 or Al-Amoudi et al., 2008), who saw that acidic conditions favoured the permeate flux through the membranes, which can be described by their neutralising behaviour. When the pH decreases, the surface charge of the membrane moves close to the isoelectric point, resulting in that flux augmentation.

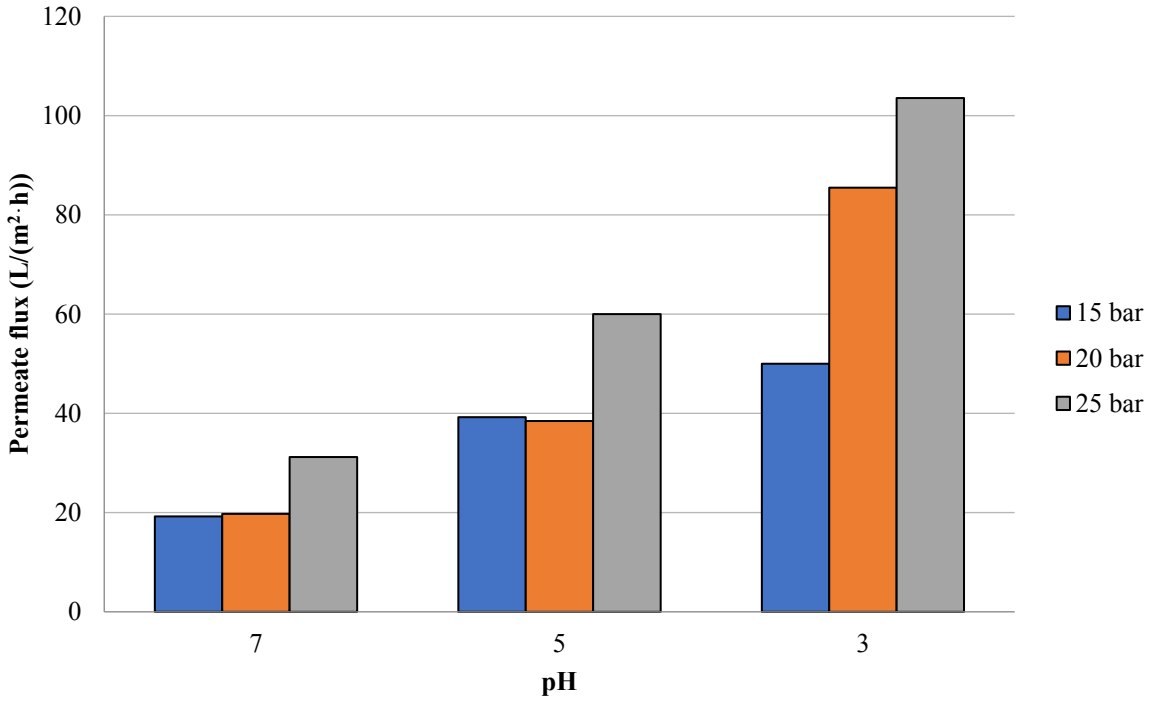

Figure 5.4. Effect of pH and TMP on permeate flux for membrane NF DK2540F30.

-VEOLIA AG2540TM RO membrane

Figure 5.5 shows that the neutral pH range is the one with the highest percentage of recovery achieved, exceeding 50 % by applying a TMP of 25 bar. Except for pH 3, it was observed that the higher the TMP applied, the higher the recovery percentage. It could also be stated that as the pH rises, recovery also increases. This behaviour was also demonstrated by Domingos et al. (2022); when the pH increased, the degree of dissociation of the VFA was higher and therefore so was the concentration of deprotonated acid (negatively charged); this makes them more easily rejected by the membrane due to electrostatic interactions. In fact, such membranes have a selective layer formed by a polyamide film supported on a layer of polysulfone, which typically acquires a negative surface charge at pH values above 4 (Hagmeyer and Gimbel, 1998).

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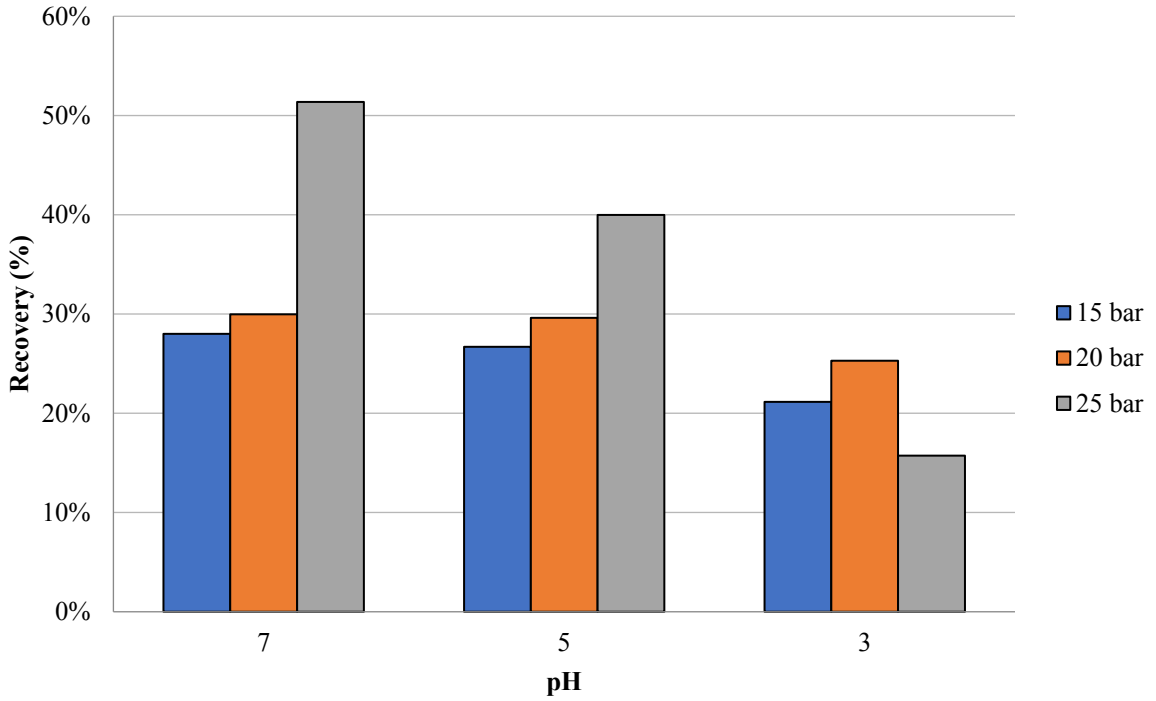

Figure 5.5. Effect of pH and TMP on recovery for membrane RO AG2540TM.

Figure 5.6 manifests how pH affects the recovery of each type of VFA. It was observed that for any of the pH studied, butyric reached higher recovery values than propionic and acetic. This could be related to the size of the molecules. Table 5.3 shows that the molecular mass of acetic, as well as its volume and length are markedly lower than that of propionic and butyric, which influences the degree to which they behave in the RO membrane. Domingos et al. (2022) observed something similar; when they operated their RO membranes, they saw that longer chain-VFA such as valeric and caproic recovered noticeably better than shorter-chain VFA.

Table 5.3. Estimation of molecular size of VFA and water.

	MW (g/mol)	Density (g/cm^3)	Volume (cm^3)	L (m)
Acetic	66.1	1.05	1.0E-22	4.7E+09
Propionic	74.1	0.99	1.2E-22	5.0E-10
Butyric	88.1	0.95	1.5E-22	5.4E-10
Water	18.0	1.00	3.0E-23	3.1E-10

The results shown in Figure 5.6 revealed that butyric acid was better retained in RO AG2540 TM than propionic and acetic and fully coincide with those reported by Zhu et al. (2020) when they used a NF membrane. With a feeding concentration of 30 g/L composed by proportions of VFA very similar to those selected for these experiments at pH 8, they reported acetic recoveries close to 20 % (Figure 5.6 for pH 7 shows that around 35 %), of propionic about 45 % (in Figure 5.6 37 % at that pH) and of butyric also at 60 %. This result is strange given that the behaviour reported in these experiments with a RO membrane were achieved by other authors in NF range. In any case, this could be due to the different types of membranes

used, since sometimes the ranges to consider a membrane as NF or RO are wide as far as MWCO is concerned, apart from many other factors which could play an important role such as electrostatic interactions and so on.

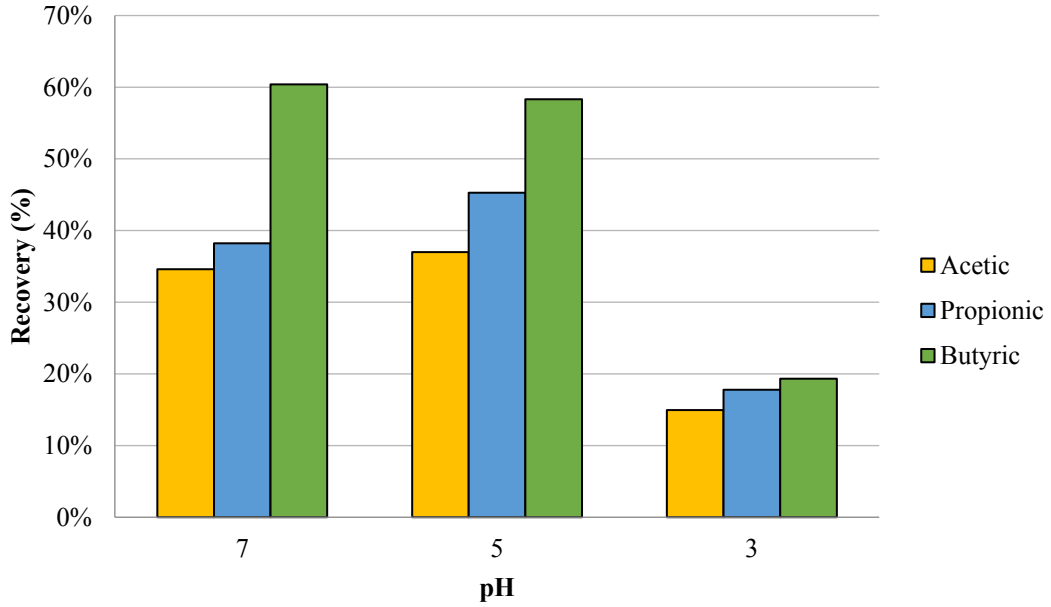

Figure 5.6. Effect of pH on recovery of different VFA for RO membrane AG2540TM (TMP=25 bar).

Figure 5.7 showed that the concentration factor is not greatly affected by the operating pH; there is also no clear trend of how pH influences. The concentration factor of 2.0 obtained at a TMP of 25 bar stands out. In comparison with the work of Domingos et al. (2022), it has been manifested that the concentration factors obtained are quite low, given that they reported values close to 3. The difference could be related to the mode of operation, since they use a total recirculation mode causing the feeding to be continuously recirculated through the membrane.

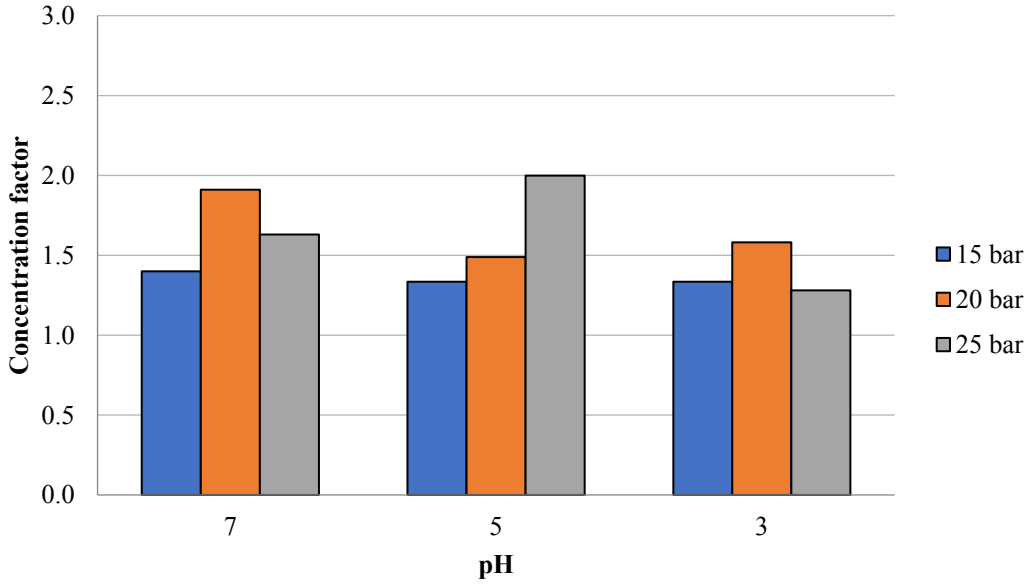

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Figure 5.7. Effect of pH and TMP on concentration factor for RO membrane AG2540TM.

Figure 5.8 illustrates that the concentration factor is always higher for the butyric and propionic than for the acetic, since the molecular size has an important influence as also seen in the recovery. This phenomenon was widely reported and studied in works such those of Pervez et al. (2022) and Van der Bruggen et al. (2002).

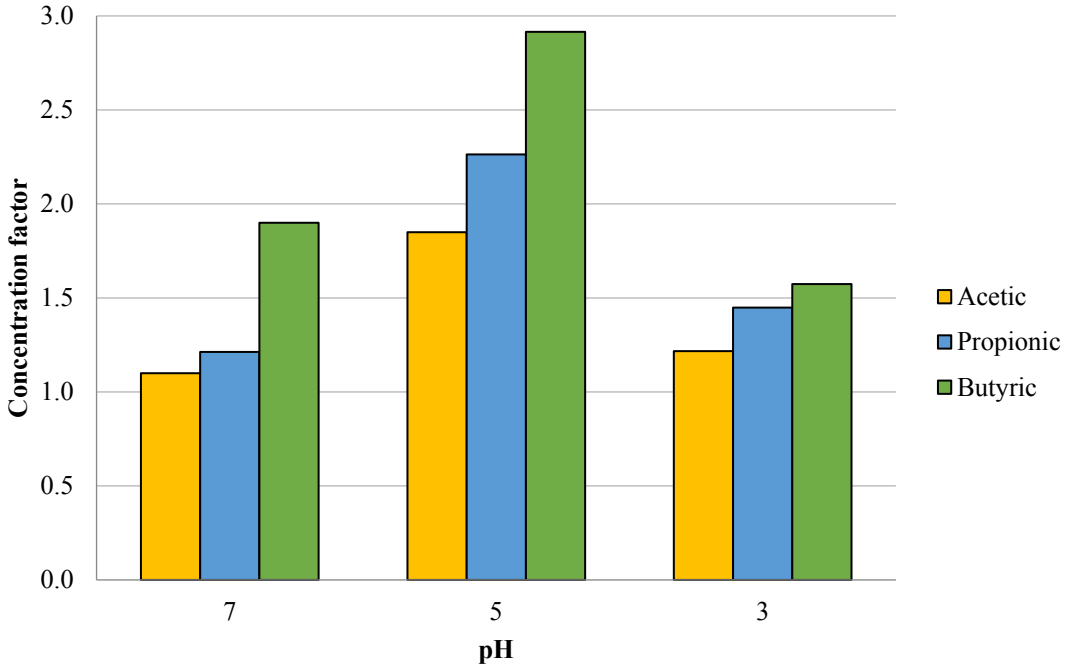

Figure 5.8. Effect of pH on concentration factor of different VFA for membrane RO AG2540TM (TMP=25 bar).

Figure 5.9 shows that with the RO membrane, as the TMP increases so does the permeate flux; what stands out is that at neutral pH the treatment capacity is greatly reduced, with values of permeate flux less than 2 L/(m^2·h) for all applied TMP. Domingos et al. (2022) operated their RO membrane in total recirculation mode with the VFA feeding concentration increasing over time and they reached values of permeate flux in a range of 10 to 20 L/(m^2·h) applying a TMP of 30 bar. Figure 5.9 shows an average of 11 L/(m^2·h) operating at 25 bar, and noting that the higher the TMP, the higher this parameter, even an upper permeate flux would be expected if the applied TMP continued to increase.

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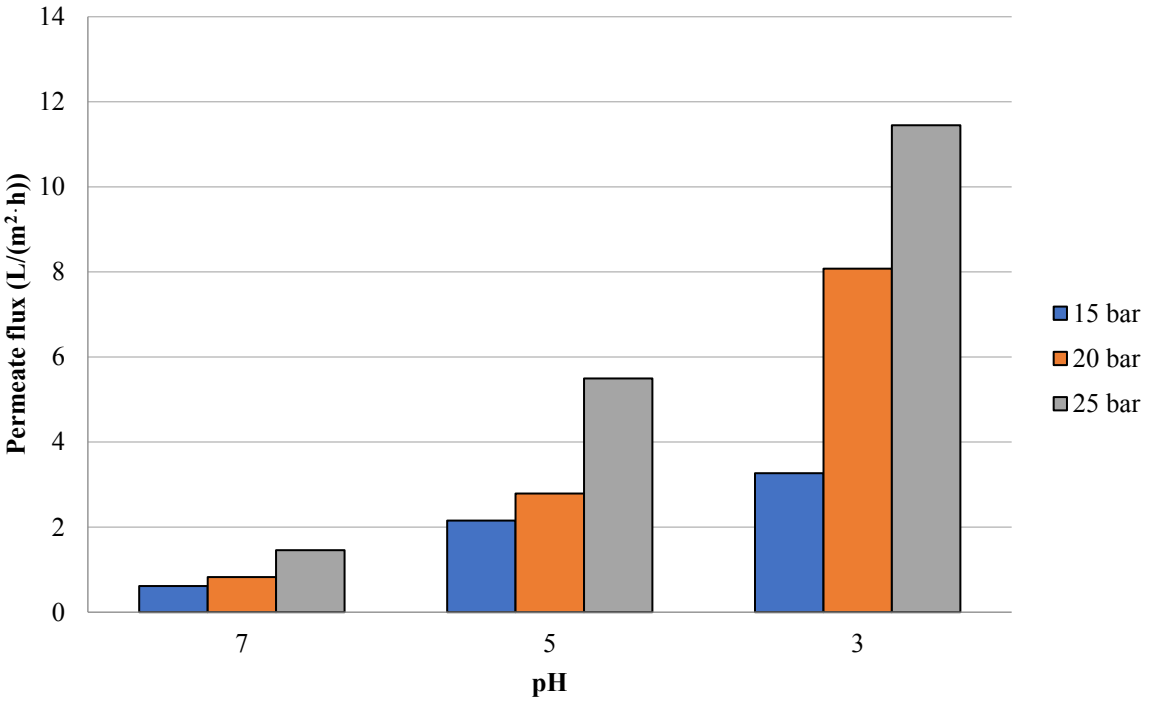

Figure 5.9. Effect of pH and TMP on permeate flux for membrane RO AG2540TM.

-PHASE 2: Check a different RO membrane behaviour. Objective: to observe if the results obtained with an RO membrane were reproducible with another membrane model (recovery rate applied: 80 %).

In order to check whether a RO membrane change would impact the results obtained previously, experiments were performed with another membrane model, the RO DOW FILMTEC SW302540. Figure 5.10 shows the recovery obtained by applying a TMP of 30 bar (this membrane allows a wider operating range), and it was seen that the results were in the same range as those obtained with the RO membrane VEOLIA AG2540TM (Figure 5.5), being the recovery increased with the rise of pH.

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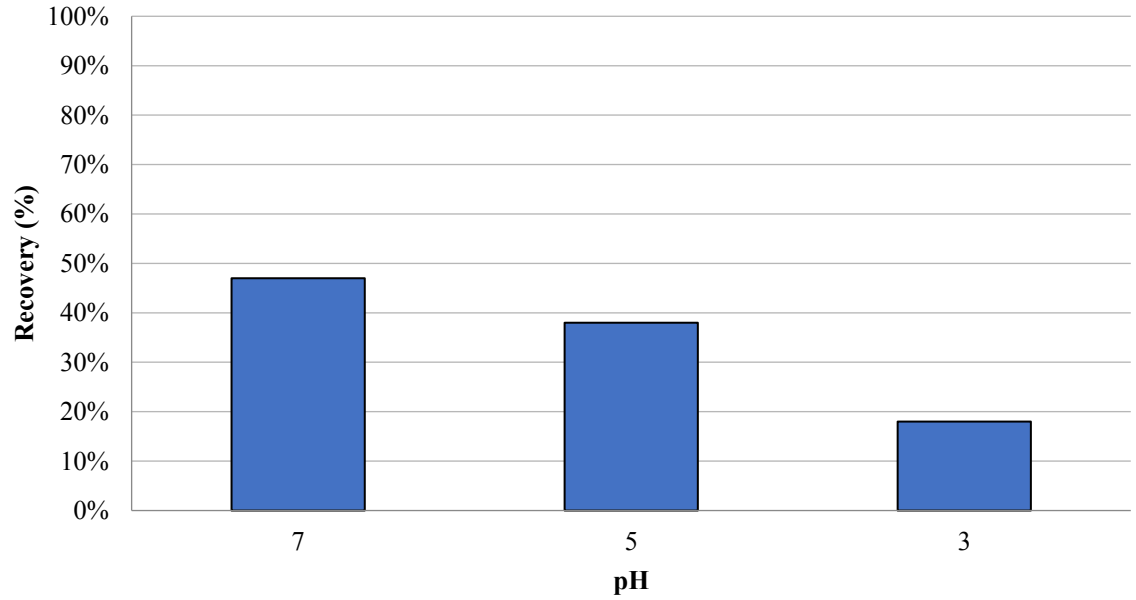

Figure 5.10. Effect of pH on recovery for RO membrane DOW FILMTEC SW302540 (TMP=30 bar).

Figure 5.11 shows the concentration factor, which in this case was 20-48 % higher (variable depending on pH) than that obtained by applying a TMP of 25 bar to the RO membrane VEOLIA AG2540TM (Figure 5.7). The different results could be related to the material from which the membrane was constructed, which in this case is different.

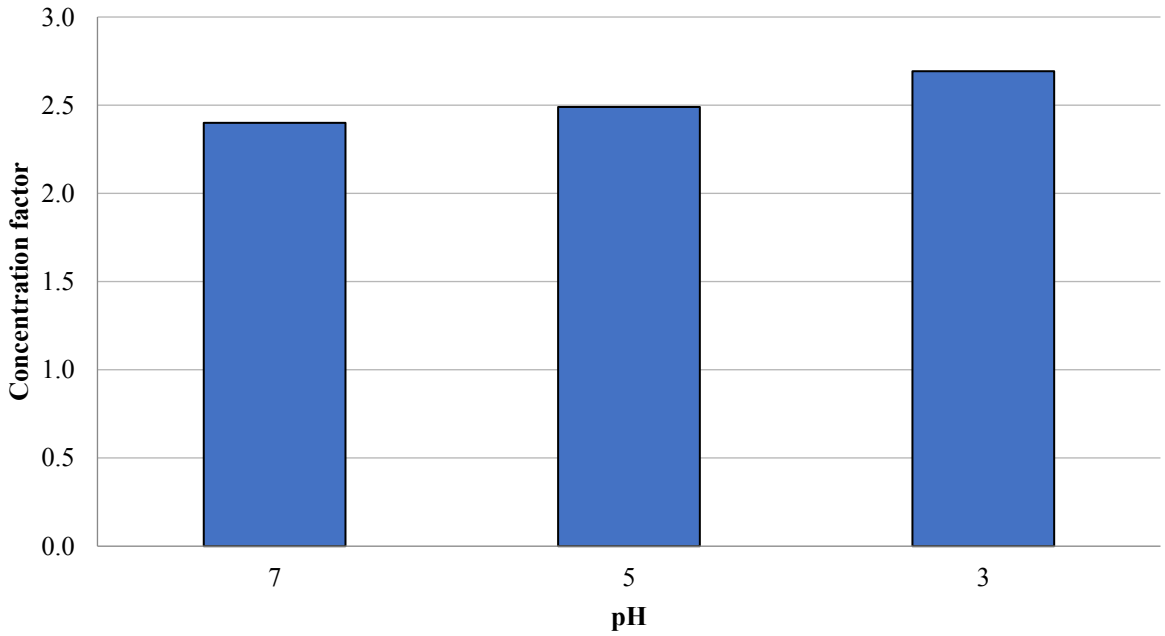

Figure 5.11. Effect of pH on concentration factor for RO membrane DOW FILMTEC SW302540 (TMP=30 bar).

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Figure 5.12 shows the variation of the permeate flux with pH, in this case applying a TMP of 30 bar (TMP more favourable according to the results shown above); the results seen are in line with those presented in Figure 5.9 with the membrane of RO AG2540TM operating at 25 bar.

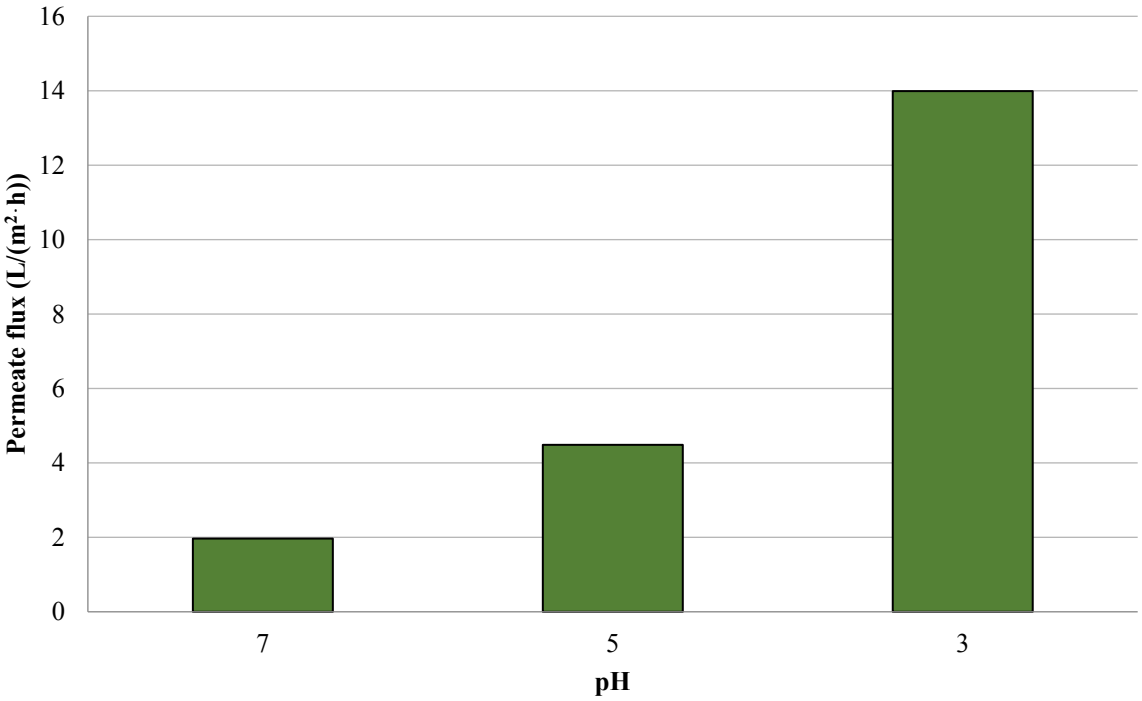

Figure 5.12. Effect of pH on permeate flux for RO membrane DOW FILMTEC SW302540 (TMP=30 bar).

-PHASE 3: Destination of the VFA and nutrients in a membrane treatment train (NF VEOLIA DK2540F30 + RO DOW FILMTEC SW302540). Objective: to determine the fate of NH_4^+ and PO_4^{3-} in the membranes and if their presence influence the fate of VFA. These nutrient concentrations (1.5 g N-NH_4^+/L and 0.2 g P-PO_4^{3-}/L) were selected to simulate the effluent of SS fermentation depicted in Table 3.5 (recovery rate applied: 80 %).

Table 5.4 shows the parameter variation obtained in the membrane train in which the NF permeate was fed to the RO membrane, as shown graphically in Figure 5.13.

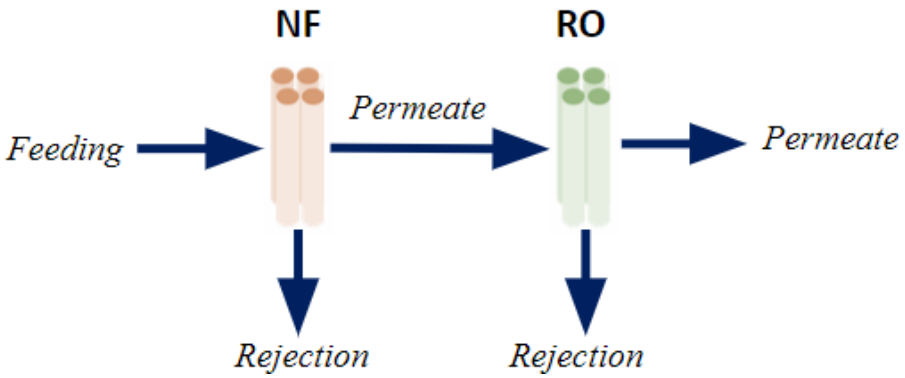

Figure 5.13. Layout of the membrane train used (NF VEOLIA DK2540F30 + RO DOW FILMTEC SW302540).

It was observed that the addition of nutrients did not have a great influence on the behaviour of the VFA in the membrane train, with the mass flow VFA permeate, recovery and concentration parameters being very similar regardless of their addition or not. It was seen that the results obtained are in line with those observed when the behaviour of the membranes was studied individually. In any case, this experimental phase has allowed the validation that the membrane train behaved the same regardless of the existence of NH_4^+ and PO_4^{3-} in the feeding.

Table 5.4. Parameters obtained with membrane train (NF VEOLIA DK2540F30 at TMP 20 bar + RO DOW FILMTEC SW302540 at TMP 30 bar).

pH 3		
	Without nutrients addition	With nutrients addition
Mass flow VFA permeate in NF (%)	91	88
Recovery in RO (%)	17	22
Concentration factor (NF+RO)	2.7	2.6
pH 5		
	Without nutrients addition	With nutrients addition
Mass flow VFA permeate in NF (%)	74	83
Recovery in RO (%)	44	35
Concentration factor (NF+RO)	1.6	1.7
pH 7		
	Without nutrients addition	With nutrients addition
Mass flow VFA permeate in NF (%)	79	72
Recovery in RO (%)	47	49
Concentration factor (NF+RO)	2.3	2.5

Regarding the removal of nutrients in the membranes, they are shown in Figure 5.14 and 5.15. Figure 5.14 depicts how the percentage of nutrient removal efficiency (Eq. 5.7) in the NF permeate was low, always under 30 % for NH_4^+ and 40 % for PO_4^{3-}. It was observed that the PO_4^{3-} was eliminated better, since it is a larger molecule, and that as the pH increased, the elimination percentages also did so (except for the PO_4^{3-}, whose removal seemed to remain stable when passing from pH 5 to 7). Anari et al. (2023) tested different NF membranes to remove nutrients and found that, working at pH 7 and TMP of 5 bar, the removal percentages were also lower for N than for P; in particular, reported similar NH_4^+ removal values with NF membranes of the same typology, ranging from 10 % to 24 % when starting from a concentration of 1 g $N\text{-}NH_4^+$/L, while in this case 22 % was obtained at the same pH. On the other hand, these authors reported higher P removals than those shown in Figure 5.14, ranging from 60 % to 75 % with a feeding concentration slightly lower than 0.2 g $P\text{-}PO_4^{3-}$/L, which could influence the results.

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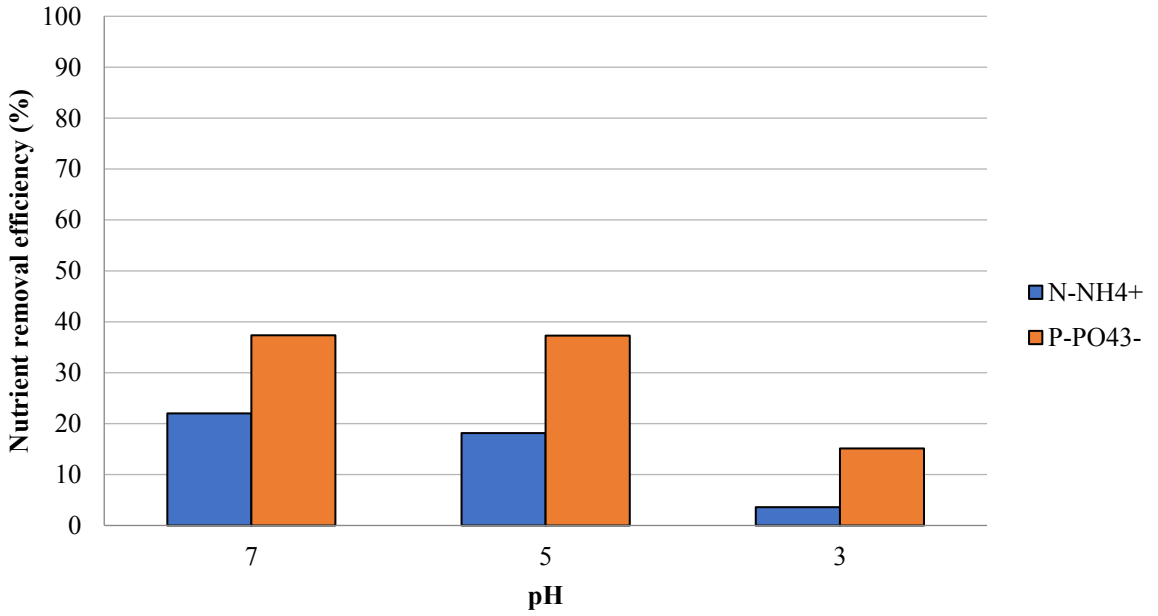

Figure 5.14. NF removal efficiency in permeate for membrane NF VEOLIA 2540F30.

Figure 5.15 shows the removal of nutrients in the RO membrane. It was observed that the nutrient removal percentages are high (>70 % for NH_4^+ and 80 % for PO_4^{3-}). As it occurred in the NF membrane, P was removed better, and the removal increased as the pH of the feeding did. When Anari et al. (2023) used RO membranes to remove nutrients, they observed that at pH 7 the removal percentages were 94 % for N and 98 % for P, respectively, which clearly coincides with those obtained in this work (Figure 5.15). Other authors such as van Voorthuizen et al. (2005) have also reported removal percentages of N and P for real anaerobic effluents at pH 7 around 90 and 98 %, respectively.

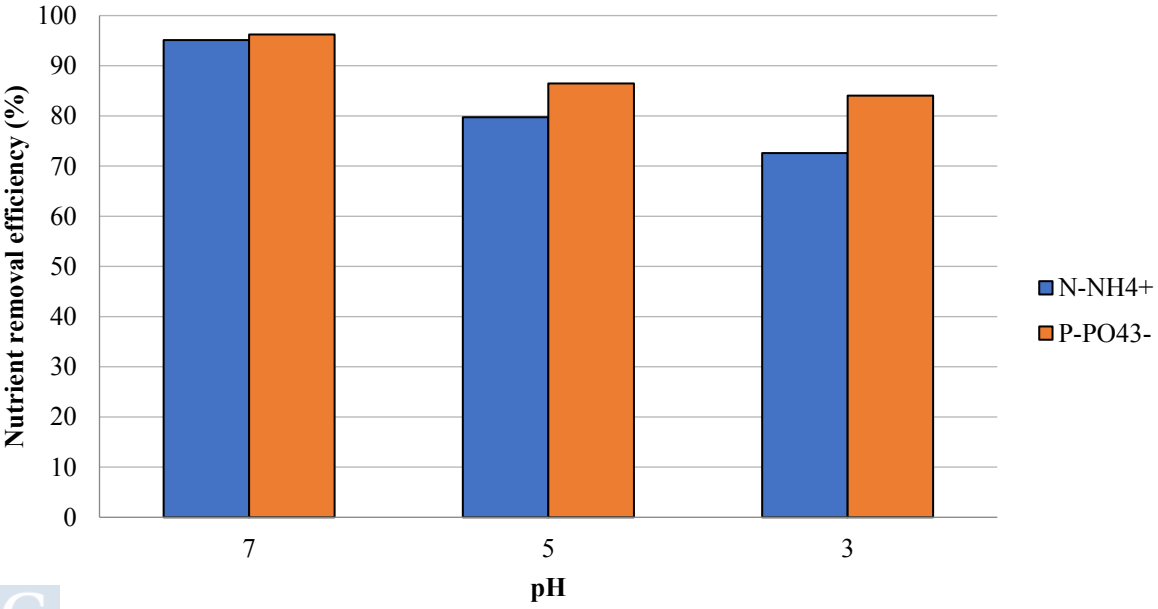

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Figure 5.15. RO removal efficiency in rejection for membrane RO DOW FILMTEC SW302540.

-PHASE 4: Maximise the two parameters studied for the RO membranes (recovery and concentration factor) by carrying out a double pass through the VEOLIA AG2540TM RO membrane. Objective: achieve the highest possible VFA concentration (recovery rate applied: 80 %).

Figure 5.16 illustrates the variation of these parameters with pH. Recovery step 1 and 2 refer to said parameter in the first pass through the RO membrane and in the second one, respectively, in which the rejection stream obtained in step 1 was fed again to the membrane. In this way, it was seen that the recovery in step 2 was always lower than in the first one for all the pHs studied. Global recovery in the membrane after the two steps is also shown. As for the concentration factor, it barely varies with pH, being between 2.5-2.7 for the three pHs tested. In any case, it is in line with those reported by other authors. For example, Zacharof and Lovitt (2014) reported concentration factors between 1.8-2.5 and Ge et al. (2023) reached concentration factors close to 4 under similar conditions. When focusing on the final VFA concentration obtained in the rejection of step 2, it was seen that at pH 5 and 7 practically the same concentration was obtained (around 40 g VFA/L), while at pH 3 almost 50 g/L.

Regarding the final VFA concentration reached, it was observed that from a feeding of around 15 g VFA/L, it has been possible to reach 50 g/L at pH 3; Domingos et al. (2022) used a membrane very similar to the RO AG2540TM and also found that it was suitable for concentrating broths rich in VFA up to values greater than 45 g/L, with figures of permeate flux varying from 5 to 30 L/(m^2·h).

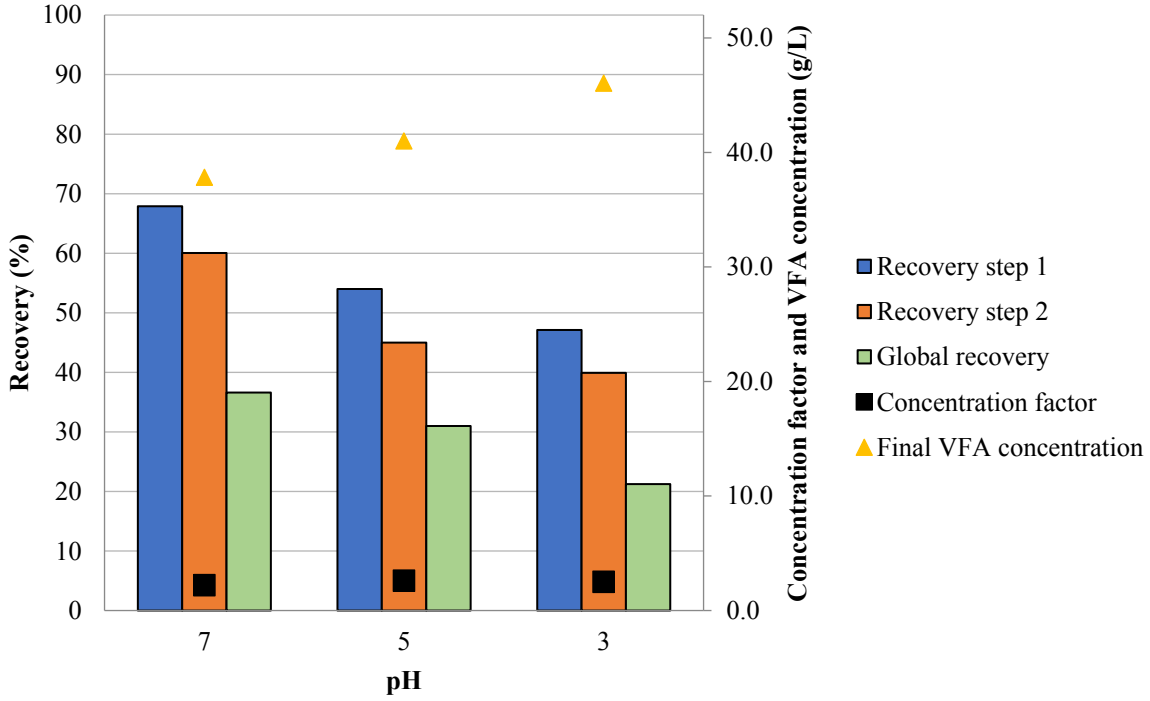

Figure 5.16. Recovery, concentration factor and final VFA concentration obtained with double passage through RO AG2540TM.

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Figure 5.17 illustrates the variation of the permeate flux between the two steps through RO. It was observed how in the second step the permeate flow dropped drastically compared to the first one, because it starts from an already concentrated sample. The same behaviour was observed by Domingos et al. (2022). They reported that, operating in full recirculation mode, permeate flux decreased with time as higher VFA concentrations in the feeding led to higher osmotic pressure effects and therefore induced lower effective TMP. In any case, it was seen that as the operating pH increases, the permeate flow greatly decreases, to the point that permeate fluxes at pH 7 and 5 are not adequate when what is sought is to achieve a reasonable treatment capacity.

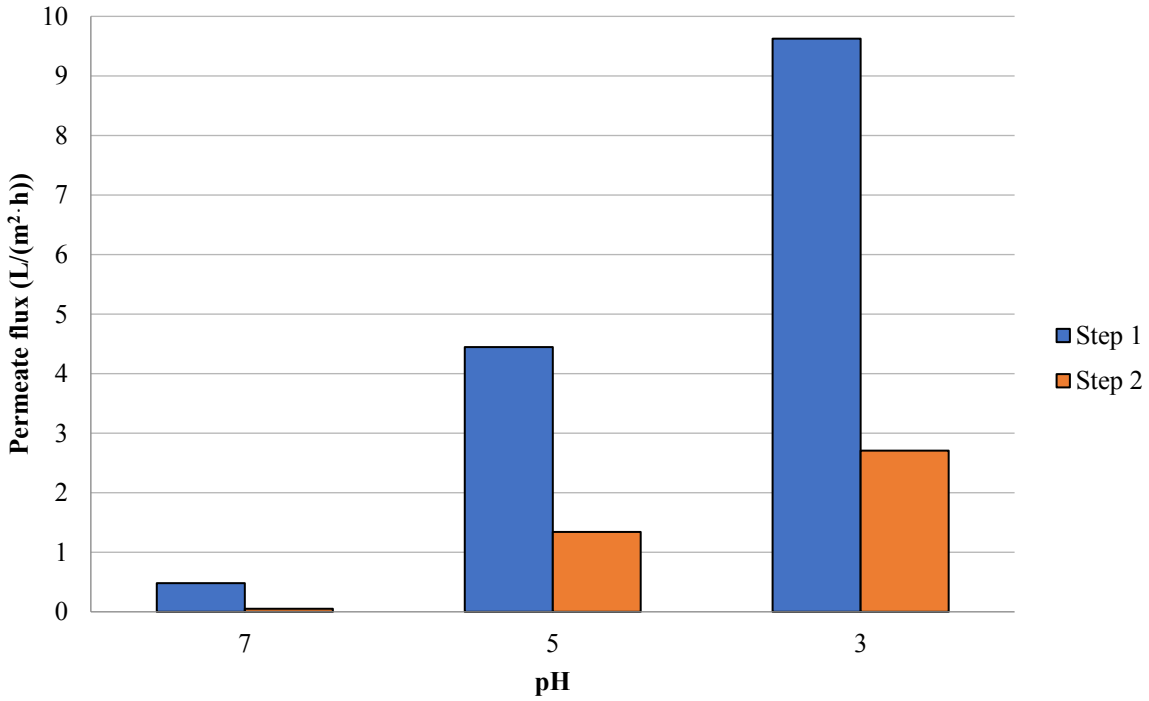

Figure 5.17. Variation of permeate flux in the two-step (double passage) experiments for RO AG2540TM.

5.3.2. Real effluent

Figure 5.18 shows the filtration process scheme treating the fermentation supernatant coming from the fermentation of SS whose physicochemical characterisation was shown in Table 5.2. Firstly, this effluent was filtered through the cylindrical MF eliminating impurities and suspended solids as proposed by other authors such as Zacharof and Lovitt (2014). Subsequently, almost the entire flow rate permeated through the UF membrane (continuously recirculating the rejection to the feed tank applying a TMP of 18 bar). The UF permeate was fed to the NF membrane (TMP of 20 bar), following the same procedure (the inclined bar between treated volumes indicates that not all the volume retained in the tank was passed through the membrane, being fed the value after the bar). Subsequently, the permeate from the NF was fed to the RO (recirculating the rejection until there was 15 L of final volume at 25 bar), obtaining in the end 35 L of permeate.

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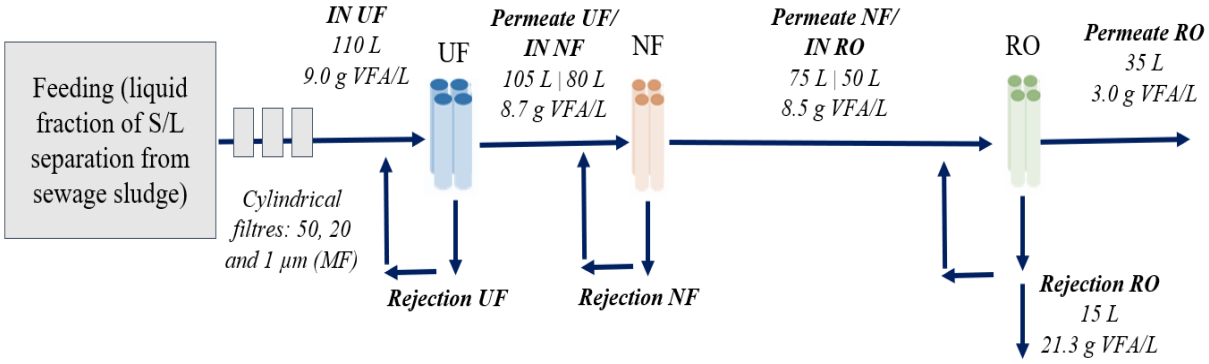

Figure 5.18. Membrane filtration of VFA-rich supernatant from sewage sludge. TMP UF: 18 bar; NF: 20 bar; RO: 25 bar. Hydraulic recovery: 80 %.

The characteristics of the rejection RO stream, which was enriched in VFA after the membrane filtration process are shown in Table 5.5. After passing the fermented effluent through the membrane train, a stream with a concentration of 21.3 g VFA/L was obtained, of which approximately 40 % by mass was acetic, 22 % was propionic, 17 % was butyric, being the remaining 21 % isobutyric, isovaleric and isocaproic acids. Thus, the VFA partitioning was somewhat influenced by the membrane action, since the initial effluent (Table 5.2) showed a mix sharing of 54 % acetic, 25 % propionic and 8 % butyric (by mass), this indicating that longer-chain VFA are better retained in RO processes than the shorter-chain ones.

Table 5.5. Physicochemical characterisation of the RO rejection (analysed in triplicate).

	RO rejection
pH	3.1 ± 0.00
tCOD (g O_2/L)	34.1 ± 2.1
sCOD (g O_2/L)	34.1 ± 0.5
N-NH_4^+ (g/L)	ND*
P-PO_4^{3-} (g/L)	ND*
Total VFA (g/L)	21.3 ± 0.2
Acetic acid (g/L)	8.5 ± 0.2
Propionic acid (g/L)	4.6 ± 0.1
Butyric acid (g/L)	3.6 ± 0.1
Isobutyric acid (g/L)	1.8 ± 0.0
Isovaleric acid (g/L)	0.9 ± 0.1
Isocaproic acid (g/L)	1.9 ± 0.2

*ND: no detected.

Table 5.6 shows the performance indicators corresponding to each membrane. The indicators point out that VFA permeate both the UF and NF membranes, as with the synthetic medium. On the other hand, the recovery of the RO as well as the concentration factor were much higher than those reported with synthetic medium for the membrane RO VEOLIA AG2540TM (Figure 5.5 and 5.7). This different behaviour could be related to the ionic strength of the medium, whose physicochemical composition is quite different from that of the synthetic medium, thus containing other compounds that could have an influence on the electrostatic interactions as discussed in the introductory part of this document. In fact, Zacharof et al. (2016) reported that the addition of salts to the feeding significantly boosted VFA recovery values.

Table 5.6. Parameters obtained with membrane filtration of the sewage sludge fermentation supernatant.

Parameter	Value
Mass flow VFA permeate in UF (%)	92.3
Mass flow VFA permeate in NF (%)	91.6
Recovery in RO (%)	75.2
Concentration factor (RO)	2.5

Figure 5.19 shows the permeate flux drop in the UF membrane over time; at minute 100, a cleaning (in the case a hydraulic one, using 20 L tap water/m^2 of membrane) was carried out since a decrease of 20 % was recorded with respect to the initial permeability value. After this cleaning, the permeate flux increased again until almost the initial value of 16 L/(m^2·h), proving that the hydraulic cleaning was effective. The permeate flux values recorded in this experiment would be aligned with others reported also with real effluents. Jänisch et al. (2019) tested different UF membranes for treating the liquid fraction from the hydrolysate of a biogas plant before subjecting it to NF and RO membranes and they reported permeate fluxes between 15-20 L/(m^2·h), which remained stable between 120 and 300 min of operation.

On the other hand, Pervez et al. (2022) reported in their experiments to purify VFA from FW that, using the Microdyn Nadir UH050 P membrane at a TMP of 5 bar and a feeding pH of 4, they obtained stable permeate fluxes of about 18 L/(m^2·h) for 160 min. Therefore, it can be affirmed that the results saw in this test are similar to those reported in other bibliographic works, with permeate fluxes between 12-16 L/(m^2·h), also showing the capacity of hydraulic cleaning to clear out the membrane recovering its initial treatment capacity.

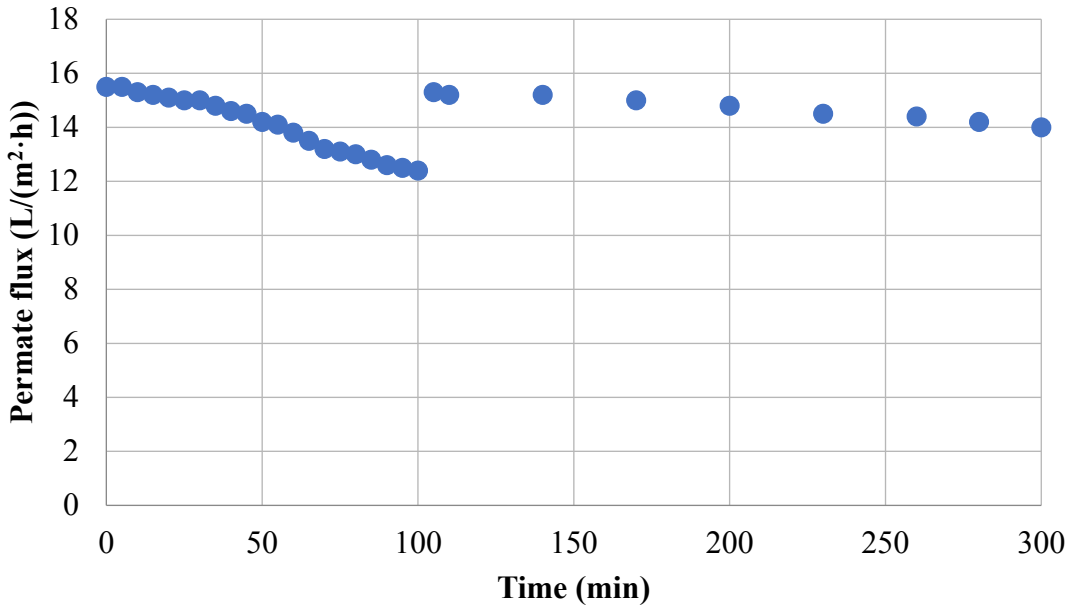

Figure 5.19. Permeate flux variation over time for UF VEOLIA GE2540F30 (TMP=18 bar).

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Figure 5.20 presents the variation of the permeate flux during NF applying a TMP of 20 bar. The permeate flux decreased from 18 to just under 16 L/(m^2·h) after 300 minutes of the experiment, which indicates that this membrane has a lower need for cleaning than the UF one. The results shown are in full agreement with others reported in the literature, such as those of Zacharof & Lovitt (2014). These authors treated effluents from anaerobic digesters of agricultural waste and they obtained permeabilities during NF filtration at pH 4 (close to the pH of this experiment feeding) within the range 12-25 L/(m^2·h), depending on the model of membrane used.

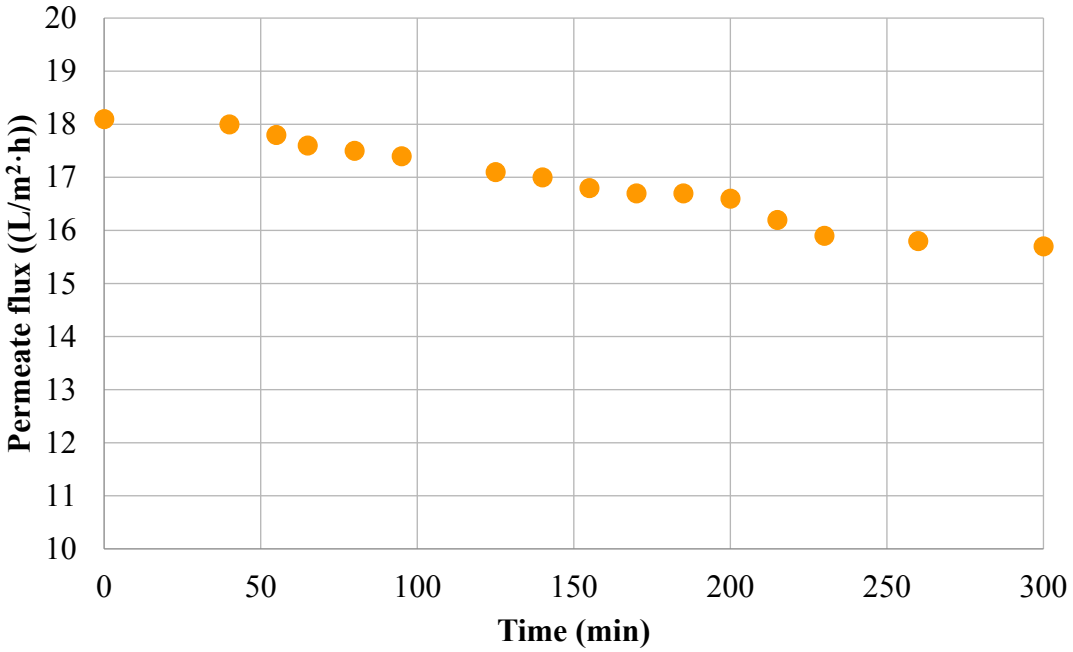

Figure 5.20. Permeate flux variation over time for NF VEOLIA DK2540F30 (TMP=20 bar).

Figure 5.21 exhibits the variation of the permeate flux of the RO AG2540TM membrane, operating at a TMP of 25 bar. The permeate flux started from a value of 16 L/(m^2·h), and at minute 150 it dropped by 20 %, so a cleaning was carried out, but in case a chemical one instead of hydraulic (10 L HCl and NaOH solution/m^2 membrane), by means of which the initial value of permeate flux was recovered. In general, the particularities of RO membranes mean that hydraulic cleaning is not enough to recover the initial permeate fluxes (Jafari et al., 2020), so both a low pH cleaning solution for cleaning mineral scale and an alkaline pH cleaning solution, generally used for cleaning biological matter, are required. Nguyen et al. (2015) treated a real effluent, in this case from the fermentation of lignocellulosic hydrolysates, and for their GE Osmonics SG membrane operating at 30 bar, they reported a value of 18 L/(m^2·h), totally aligned with those shown in Figure 5.21.

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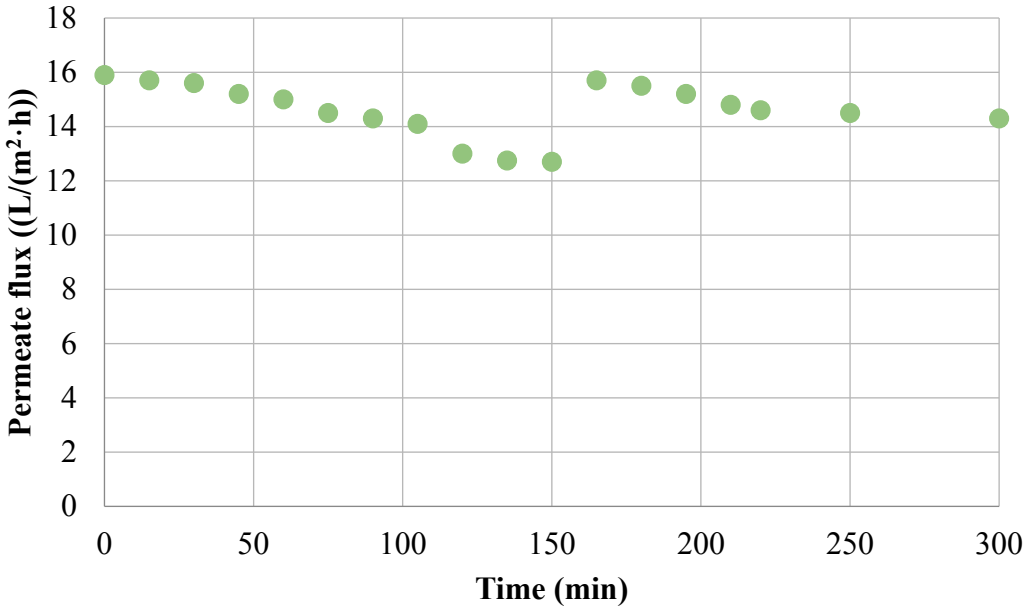

Figure 5.21. Permeate flux variation over time for RO AG2540TM (P=25 bar).

One of the biggest disadvantages of membrane technology is the occurrence of fouling (Van der Bruggen at al., (2002)). In this way, the permeate fluxes obtained using pure water or synthetic mediums are usually higher than those measured with real medium. As it was validated in the conducted experiments, the flux decay can be within reasonable percentages, but over time a total drop could occur due to the adsorption of organic material on the membrane surface.

VFA are recovered from complex streams containing impurities which need to be removed or carefully controlled to reduce membrane contamination and fouling. This results in a major operational challenge which limits performance and process efficiency (Aghapour et al., (2020)). Hydraulic and chemical cleaning are common solutions to remove impurities from the membrane surface and mitigate their detrimental effects, at the cost of some additional expenses as well as increased chemical and energy requirements. In any case, what it is clear is that pre-treatment systems for the fermented effluent are necessary to prevent fouling in the subsequent membranes. Thus, both MF and UF systems alleviate the potential for fouling and are easy to integrate within conventional VFA recovery systems.

In general, both the choice of cleaning products to use in each case to avoid membrane fouling, as well as the frequency of such cleaning, is a subject which needs to be studied in depth since the experiments performed with real effluent lasted 300 min.

Considering the importance of knowing the frequency of cleaning as well as the amount of chemicals needed to achieve robustness and non-stop operation of the membrane train, it would be necessary to continue with the experimentation by carrying out experiments of longer duration. In addition, there is a significant lack of bibliographic data reporting information from longer experiments from which valuable information could be obtained.

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5.4. CONCLUSIONS

Considering that the fermented effluents can reach maximum concentrations of 30 g VFA/L depending on the substrate used as well as the operating conditions, it is necessary to subject them to a concentration/purification step. Pressure-driven membranes were presented as a good alternative, both for cleaning the stream of those compounds which are not VFA as well as to increase their concentration before being subjected to subsequent unit operations.

In this first screening with synthetic medium, it was seen how VFA passed through the membrane NF VEOLIA DK2540F30, being the VFA mass flow in permeate around 80 % regardless of the pH and TMP applied. It was also validated that more acidic pHs favoured permeate fluxes as opposed to alkaline ones, both for NF and RO membranes. Moreover, the recovery of longer-chain VFA in membrane RO VEOLIA AG2540TM such as butyric was superior to that for shorter-chain ones, such as acetic and propionic, and the concentration factor was hardly influenced by the pH of feeding. Additionally, a different RO membrane made of another material was tested and the results were in line with those of VEOLIA's membrane, reaffirming its robustness.

Furthermore, it was observed that the addition of nutrients had no influence on the behaviour of the membranes; besides, removal percentages of PO_4^{3-} were higher than those of NH_4^+ due to its larger size. The latest tests with synthetic medium had the objective of maximising both the recovery and the concentration factor in the membrane AG2540TM RO, seeing that with a double step procedure, final concentrations around 50 g VFA/L were achieved at pH 3.

Once the initial screening with synthetic medium was conducted, the experimentation with real effluent allowed a comparison of the permeate fluxes with both feedings, which were significantly reduced when treating the supernatant coming from SS fermentation. Considering that the duration of the experiments with real effluent was 300 min, it was possible to validate that the proposed cleaning methods were effective in returning the initial treatment capacity to the membranes by executing the cleanings when permeate flux dropped 20 %. In any case, it would be necessary to extend the operating time of the membranes to be able to accurately quantify the water flow rates (hydraulic cleaning), as well as the chemical products necessary and their frequency of use to wash the membranes. Therefore, future work would be needed to continue optimising this unit.

By all means, although the mode of operation followed in the experiment with real medium meant that the final VFA concentration in the RO rejection was not maximised, it was demonstrated that there are alternatives which would give rise to concentrations of 50 g VFA/L (double pass through RO), so all the LLE experimentation was carried out starting from that initial concentration, accessible by applying this other operating mode.

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6) PURIFICATION PROCESSES

6.1. INTRODUCTION

Once the supernatant from the fermentation process was subjected to membrane treatment, it was found that concentrations of a maximum of 50 g VFA/L could be achieved. This indicates that in this stream there is a large amount of water that must be removed from the process to make it economically viable, as well as to achieve a final product with purities in accordance with what end users request. Therefore, LLE emerged as a technology in which, by bringing a solvent into contact with the feeding, this solvent, which has the ability to dissolve VFA efficiently and selectively, would allow the removal of these large quantities of water at relatively low operating costs.

When using a LLE process, the choice of solvent could be decisive. In this case, two different solvents have been experimentally tested, one with a high boiling point (ethyl octanoate) and the other with a low boiling point (ethyl acetate), with the benefits and disadvantages associated with each of them.

The LLE needs to be coupled to a consecutive distillation process in order to, on the one hand, separate the VFA stream from the solvent used and, on the other hand, separate the individual VFA in order to achieve specific uses involving a higher market value, with the mixtures having a much more limited use and therefore much lower market prices.

6.2. LIQUID-LIQUID EXTRACTION

6.2.1. Parameters of influence

The consideration of the carboxylic acid content in the aqueous solution is crucial when choosing a separation technique. For example, Drake et al. (1985) stated that when acetic acid in aqueous medium has a concentration above 50 % (w/w), distillation should be applied. On the other hand, when the concentration is below this 50 % (w/w), LLE could be proposed as the most favourable option. Considering that fermentation broths consist mainly of water and small amounts of carboxylic acids, these compounds must be targeted before the water which accompanies them. Thus, affinity-based separation techniques such as LLE use agents which target VFA to separate them selectively.

When referring to a LLE process there are several parameters which must be considered. Henley and Seader (1981) stated that the most key parameters are the type of feeding and its characteristics (composition, temperature and pressure), the dimensions and mode of operation of the column, the S/F ratio (which relates the feeding flow to the solvent fed to the column), the number of equilibrium stages and the type of solvent to be used.

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In this doctoral thesis, experiments were executed under two approaches, but always proposing multi-stage systems. Firstly, batch mode assays were carried out on a laboratory scale, with the aim of accomplishing a first approximation in relation to the feasibility of recovering VFA with organic solvents. Subsequently, a continuous column was operated in a counter-flow regime in an attempt to scale up the technology to a semi-industrial pilot plant.

For experiments in batch mode on a laboratory scale, the scheme shown in Figure 6.1A was proposed, in which the feeding (F) was mixed with the solvent (S1), giving rise to an extract (E1) and a raffinate (R1), which was combined again with new solvent (S2) and so on. Instead, for larger scale operation, the counter-flow regime shown in Figure 6.1B was followed, in which the solvent enters the stage furthest from the feed, so that the two phases advance in opposite directions.

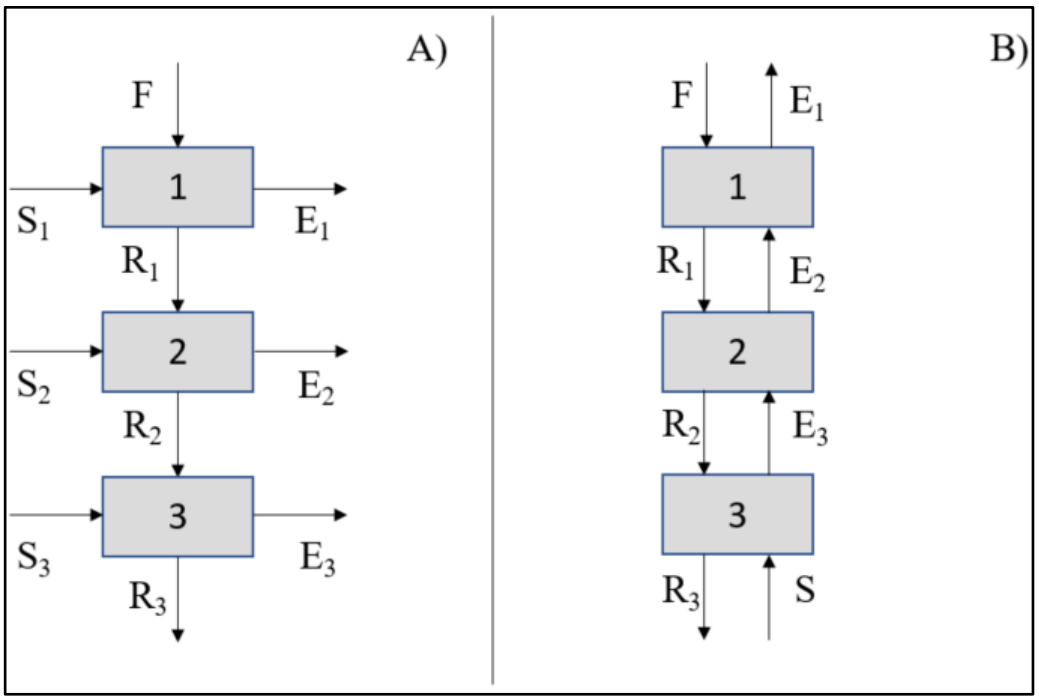

Figure 6.1. Liquid-liquid extraction applied schemes. A) Batch mode. B) Continuous mode (counter-flow).

The S/F ratio is closely linked to both final recovery and operating costs. A higher S/F ratio usually means a higher recovery of the product to be extracted from the feeding towards the solvent. On the other hand, a higher S/F ratio also implies a higher amount of solvent used and thinking about a future distillation to separate the solvent from the extracting agent, implies a higher flow to be distilled. It is for this reason that for each process, considering the needs presented by each user, a different S/F ratio must be selected which optimises those needs, both technically and economically.

When choosing a solvent for the LLE process, the following characteristics must be taken into consideration: 1) it must have a high extraction capacity of the desired species; 2) it must have adequate selectivity, considering that diluted solute concentrations generally provide higher separation factors; 3) it must have low solubility in the feed diluent, which implies a small loss of solvent in the raffinate; 4) it should have easy regeneration to be separated from the solute in the extract stream as well as recovered from the raffinate stream; 5) in addition, its physical properties should be appropriate (density, viscosity, surface tension, ...). Relatively

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cheap solvents must also be chosen, and preferably non-toxic or corrosive ones (Sánchez, 2016).

The solvent can be a mixture or a pure component. If the feeding is an aqueous solution as in the case of recovering VFA, an organic solvent is used; if the feeding is organic, the solvent is usually water. Moreover, the solvents used can be divided into physical and chemical ones. The former do not strongly interact with carboxylic acids and as a result do not form complexes with them. These are esters, ketones and alcohols (King, 1991). In contrast, chemical solvents do induce strong chemical interactions with VFA and form complexes; they could be organophosphorus compounds or amines and they are usually dissolved in a diluent as they have high viscosity. Considering that the intermolecular forces established by physical solvents are weaker, their regeneration is generally easier, even though it requires enough energy. In addition, these physical solvents are usually cheaper. Thus, both the nature of the carboxylic acids which want to be extracted and the chosen solvent determine the choice of solvent regeneration method (Reyhanitash et al., 2018).

Whether a solvent is a physical or a chemical one does not depend on its boiling point. Facing a regeneration of the solvent by means of the distillation of the extract obtained in the LLE, the boiling point of the solvent with respect mainly to that of the VFA which must be separated is a key parameter. If the solvent has a lower boiling point than the carboxylic acid it would be called a low-boiling solvent, while a higher boiling point implies working with a high-boiling solvent, and in both cases a different approach to regenerating the carboxylic acid would be required.

6.2.2. Laboratory scale

6.2.2.1. Materials, methods and calculations

With the aim of facing the LLE process and beginning to evaluate the effect of the different parameters which affect it in order to apply the knowledge for the design of the LLE column used in the pilot plant (counter-flow scheme), the first tests were conducted in batch using a separatory funnel. It is a conical or pear-shaped container with a volume of 0.25 L which contains a ground glass stopper at the top and a stopcock connecting the container with a capped outlet tube which ends in a narrowing. Considering that this discontinuous operation aims to see the effect of the different parameters affecting the LLE, it was decided to carry out the experiments with a synthetic medium, specifically with a mixture of tap water with different concentrations of VFA, being this another of the tested parameters.

Experimentation was performed between the aqueous solution (in this case a mixture of VFA + water) and the water-immiscible organic solvent (ethyl acetate or ethyl octanoate) with the aid of the extraction funnel. The relative position of the two layers (upper and lower) depends on the ratio of densities; considering that both solvents have a lower density than water (Table 6.1), when mixing, the solvent always remained at the top.

As solvents, those mentioned above, ethyl octanoate and ethyl acetate, were used. Nowadays, there are several industries already using ethyl acetate, which seems to be a viable option as a low-boiling solvent. The alternative to this is using a heavy solvent with a much lower water solubility such as ethyl octanoate. Table 6.1 shows a comparison between both solvents that are key to the process and to the decision of which one to choose. The main differences are linked to their boiling point, their solubility in water and their cost, being the price of ethyl octanoate much higher than that of acetate.

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Table 6.1. Physicochemical characteristics of the solvents used.

Physicochemical properties	Ethyl acetate	Ethyl octanoate
Molecular formula	$C_4H_8O_2$	$C_{10}H_{20}O_2$
MW (g/mol)	88.1	172.3
Water solubility (g/L at 20 °C)	87.12	0.07
Boiling point (°C at 1 atm)	77.1	208.0
Density (kg/m^3 at 20 °C)	900	870
Cost (€/t)*	1,000	10,000

*Source: Chemanalyst, 2024

The organic solvents were used for the extraction after adjusting the pH in the feeding to a desired value with HCl 37 % (v/v) or NaOH 50 % (w/v) testing 3 pH ranges: pH 3 (which is below the pKa of the VFA, and therefore they are in their protonated form), pH 5 (partially dissociated) and pH 7 (in their deprotonated form), with the aim of finding the most suitable one to recover VFA. For conducting the experiments meeting the S/F sought in kg/kg, the corresponding amounts of the aqueous phase and solvent were added to the 0.25 L total volume of the extraction funnel, before being stirred at room temperature for 2 minutes. In this equilibrium condition, the mixture settled for 1 minute, to later separate it by gravity. Once this was done, 2 mL of the aqueous part were withdrawn to carry out the VFA analysis on this matrix (through gas chromatography as indicated in section 3.2.1.4), and the organic phase (rich in VFA) was removed. The aqueous or raffinate phase was mixed again with the appropriate amount of the organic solvent to continue simulating the number of stages in batch, repeating the steps until reaching the number of desired stages. It should be considered that when measuring VFA concentration through gas chromatography, interference could happen given the overlapping of the compounds' peaks. Therefore, the calculation of the recovery percentage (*Ri*) in each stage was calculated as detailed in Eq. 6.1:

$$R_i\ (\%) = \frac{(C_{i-1} - C_i)}{C_0} \cdot 100\ +\ R_{i-1} \qquad \text{(Eq. 6.1)}$$

where *Ci* is the VFA concentration in the raffinate in g/L, *Co* is the VFA concentration in the feeding in g/L and *Ri* is the VFA recovery percentage in stage *i*.

6.2.2.2. Results and discussion

- ***Ethyl octanoate***

Figure 6.2 shows that the pH at which the LLE was carried out had an important effect on butyric acid recovery. Results show that the acid must be in its protonated form (pH 3) to obtain higher recovery percentages. In general, carboxylic acids are most effectively extracted at low pH values, lower than their pKa, where they are present in their dissociated form (Malmary et al., 2001). This is the reason why from now on all, the results shown are at pH 3, discarding the other ranges given the great differences that exist in recovery percentages. Although there was an increase in the recovery percentage as the number of stages increased, after 2 stages there was no great upgrowth even if more stages were applied to the extraction assay.

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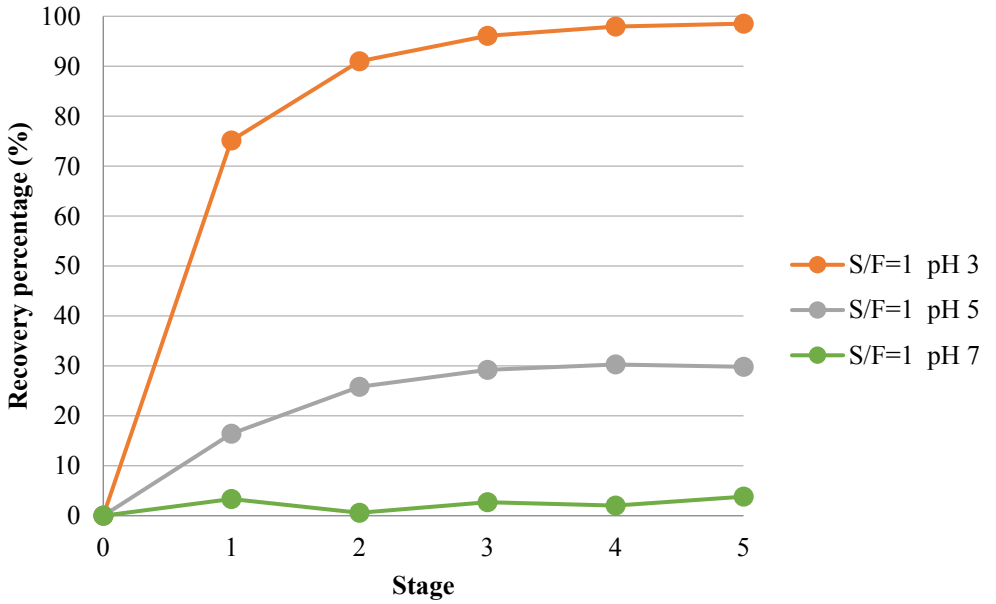

Figure 6.2. Effect of pH on butyric acid (50 g/L) recovery using ethyl octanoate with S/F of 1 kg/kg.

Almost all the experiments were conducted using an initial concentration of VFA in the feeding of 50 g/L because it could be the target output concentration of the membrane train. In any case, Figure 6.3 indicates that the initial concentration of VFA in the feeding had no influence on the recovery percentages. Although for stage 1 the one with best recovery was that starting from 100 g butyric/L, when the number of stages increased, the recovery percentages evolved practically in the same way. Therefore, the initial concentration of VFA did not influence the recovery efficiency.

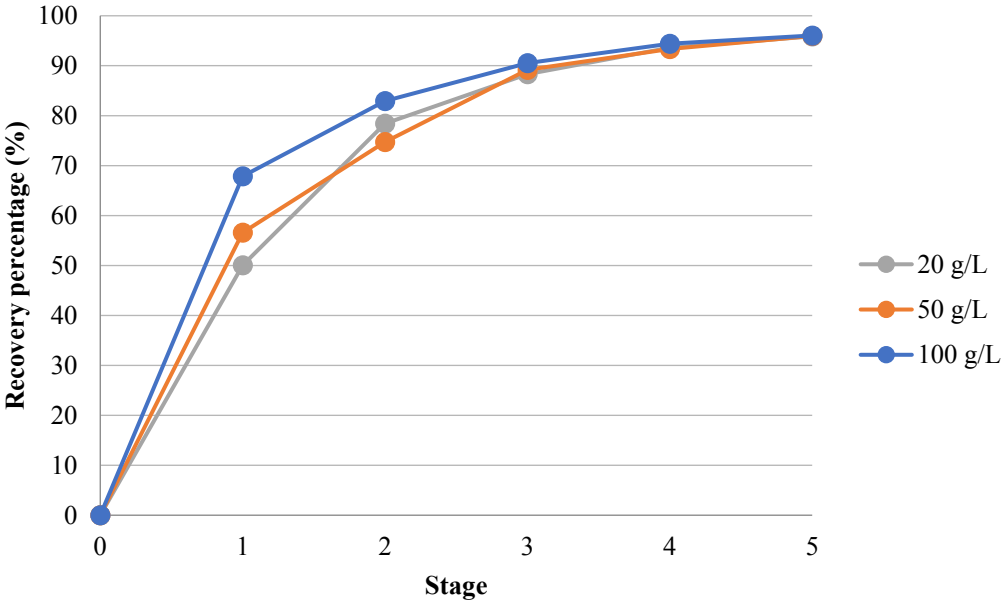

Figure 6.3. Effect of initial concentration of butyric acid on recovery using ethyl octanoate with S/F of 1 kg/kg.

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Figures 6.4 to 6.6 show the influence of the S/F on the recovery percentages of the different acids at pH 3. Using butyric acid (Figure 6.4), although it was seen that mainly in stages 1 and 2 a higher S/F implied a higher recovery percentage, from stage 3 onwards the differences practically disappeared. This VFA was recovered better than acetic and propionic.

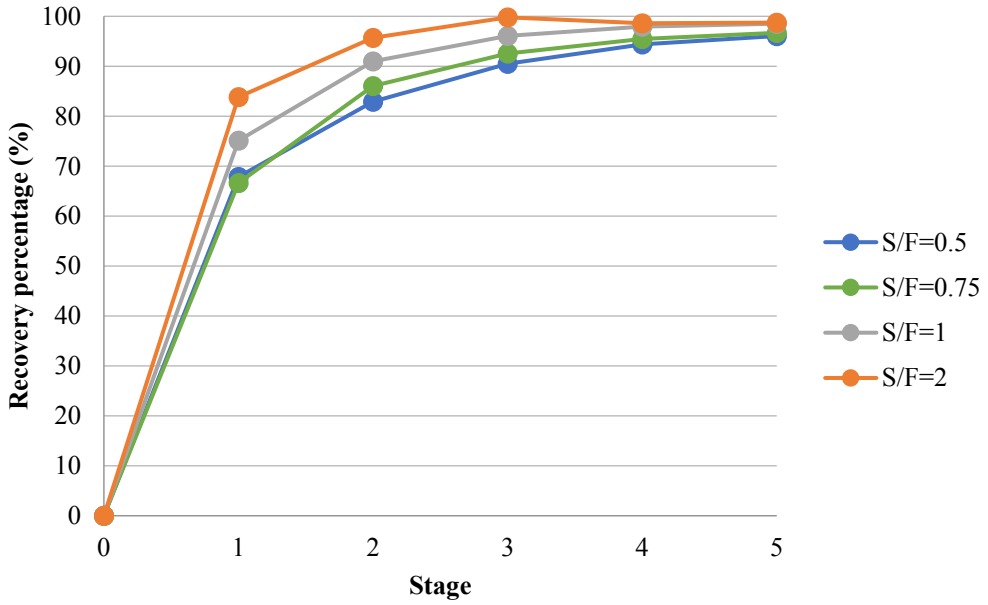

Figure 6.4. Effect of S/F on butyric acid (50 g/L) recovery using ethyl octanoate.

Figure 6.5 shows the propionic recovery percentages at pH 3. The higher the S/F applied, the higher the recovery percentage obtained. In any case, it was observed that at the same S/F, the recovery of propionic acid is higher than that of acetic.

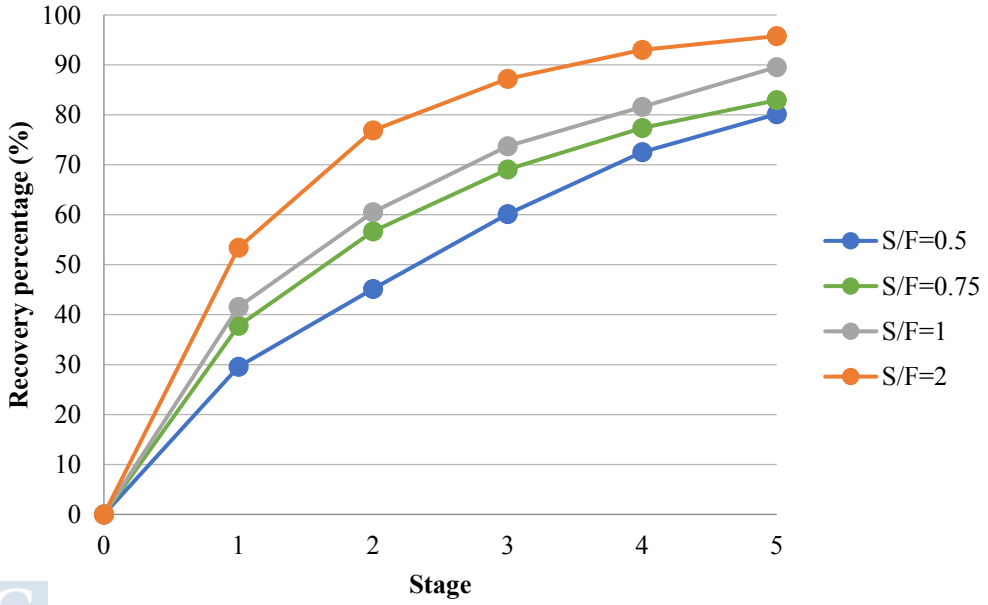

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Figure 6.5. Effect of S/F on propionic acid (50 g/L) recovery using ethyl octanoate.

Using acetic acid (Figure 6.6), as it was expected, the S/F ratios of 0.5 and 0.75 were the ones which obtained the worst results for any number of stages consulted. The recovery percentages were slightly lower than those seen by Alkaya et al. (2009) with feeding at pH 2.5.

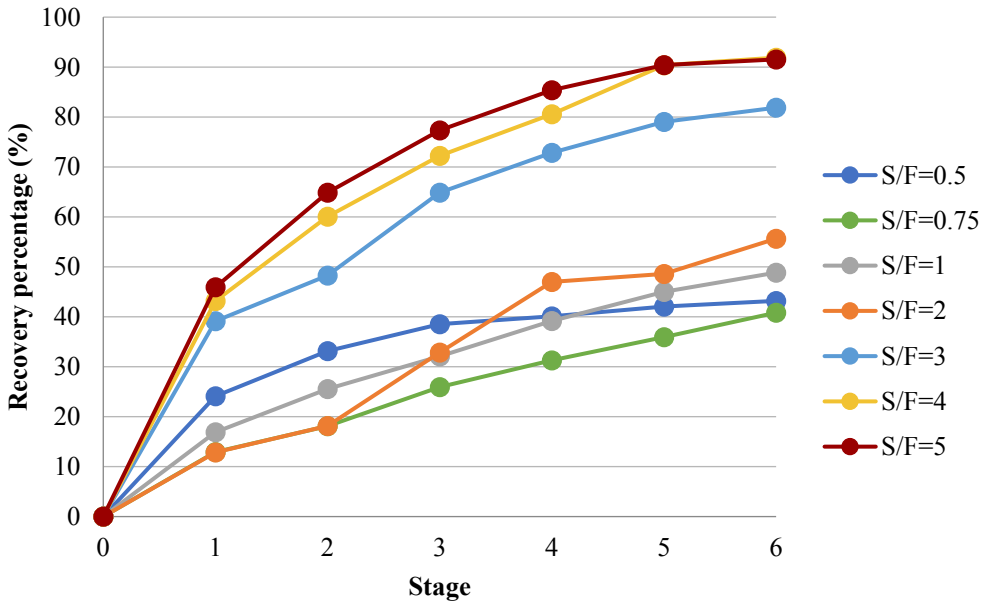

Figure 6.6. Effect of S/F on acetic acid (50 g/L) recovery using ethyl octanoate.

Results show that the higher the S/F ratio, the recovery percentage increased, specifically in the first stages. Contrary, when a large number of stages is reached, this difference becomes smaller. In any case, it is common for a higher S/F ratio to make VFA recovery more efficient, as indicated by Xing et al. (2023). Either way, a balance must be sought between the spent solvent and the recovery percentage, since the high cost associated with the acquisition of solvents can make it worth going to lower recovery percentages and therefore lower S/F ratios.

The results showed that when comparing the recovery percentages of the different acids at the same S/F ratio, those obtained with butyric are higher than those obtained for propionic and acetic acids. Figure 6.7 shows the differences in the recovery percentages of the main three VFA in the same conditions (at pH 3 and S/F ratio of 0.5 kg/kg). Again, it can be stated that as VFA have more carbons in their chain, therefore increasing their MW, the recovery is better. Thus, in stage 6, the recovery percentage of butyric was 97 %, while those of propionic and acetic were 85 % and 43 %, respectively. The fact that longer-chain VFA obtained better recovery percentages was commonly informed in the literature, ranging from 43 % with acetic to 98 % with butyric when using kerosene as solvent (Alkaya et al., 2009).

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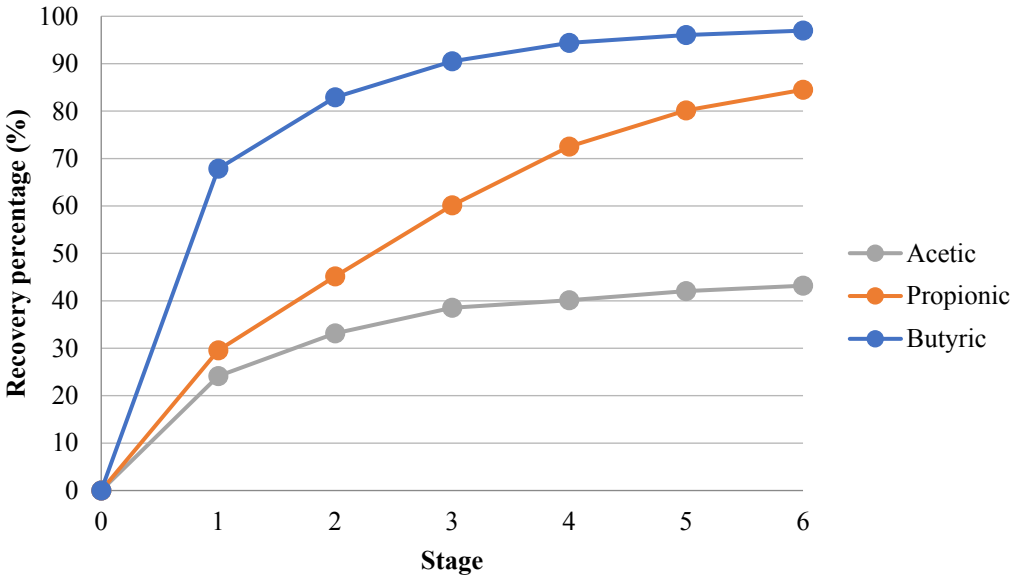

Figure 6.7. Effect of type of acid (50 g/L) on recovery percentage at pH 3 and S/F of 0.5 kg/kg using ethyl octanoate.

- ***Ethyl acetate***

As it was previously specified, the behaviour of another organic solvent, ethyl acetate, was also tested in order to establish the most favourable one.

Figure 6.8 reveals the effect of S/F ratio on the recovery of butyric acid. It can be seen that in stage 1 there was a slight difference in recovery, being the S/F ratio of 0.75 the most appropriate, but as the number of stages increased, this difference became almost non-existent and the recovery percentages exceeded 95 %.

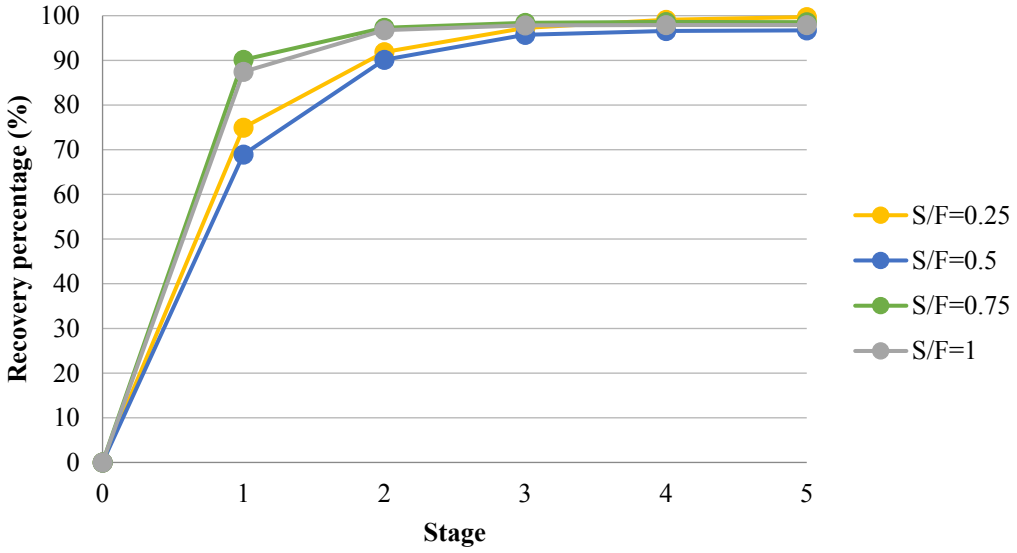

Figure 6.8. Effect of S/F on butyric acid (50 g/L) recovery using ethyl acetate.

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Figure 6.9 shows the recovery efficiency of propionic acid at different S/F ratios. It is strange that in stage 1 the lowest recovery was calculated with the highest S/F ratio, since normally a higher amount of solvent implies a better recovery as it was depicted before. This fact might be attributed to an analytical error.

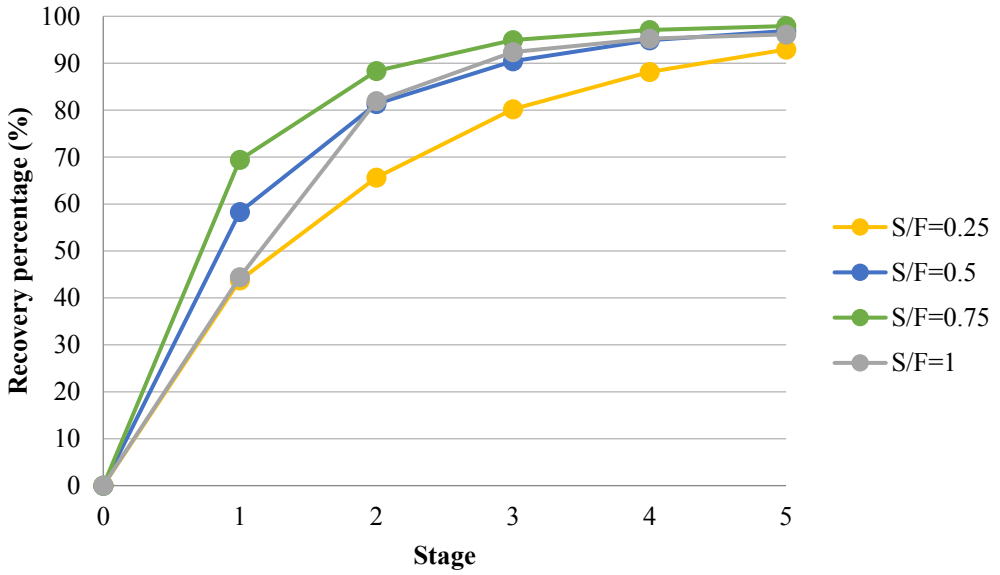

Figure 6.9. Effect of S/F on propionic acid (50 g/L) recovery using ethyl acetate.

Figure 6.10 manifests the effect of S/F on the recovery of acetic acid. The recovery percentage increased when S/F ratio did, achieving recovery efficiencies between 78 and 90 % after 6 stages regardless the S/F ratio applied. In any case, these were notably lower than those for propionic acid for all S/F ratios and number of stages tested.

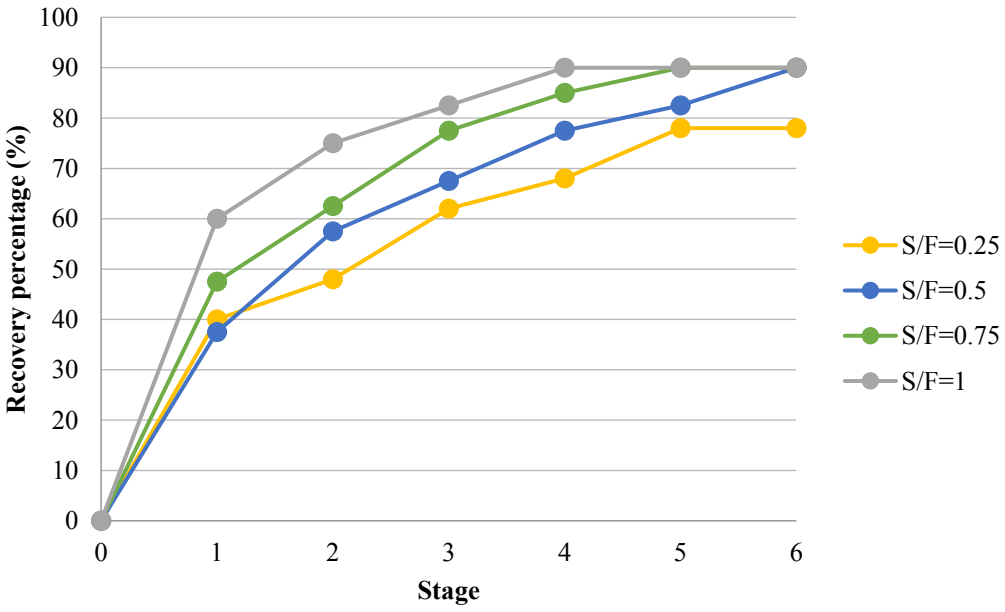

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Figure 6.10. Effect of S/F on acetic acid (50 g/L) recovery using ethyl acetate.

In all the experiments shown so far, the aim was to observe how the different parameters influencing VFA recovery affected each of the acids individually, i.e., in most cases an initial concentration of 50 g VFA/L was used in the feeding, but with the presence of only one VFA. In contrast, in the fermentation process, a mix of several acids was generated. Therefore, it is necessary to check if there would be any influence of the rest of the VFA on the recovery percentages. Figure 6.11 shows that the recovery percentages were practically the same regardless of whether the feeding contained the three VFA in question together at a total concentration of 50 g/L (Figure 6.11A, B and C for S/F 0.5, 0.75 and 1 kg/kg, respectively) or whether it only contained the target feeding concentration provided by only one, butyric, propionic or acetic (results shown so far in Figures 6.8, 6.9 and 6.10). This comparison was carried out at the three cited S/F, and it was observed that in any of the cases the presence of the rest of the VFA in the feeding had influence on the recovery percentages in batch mode.

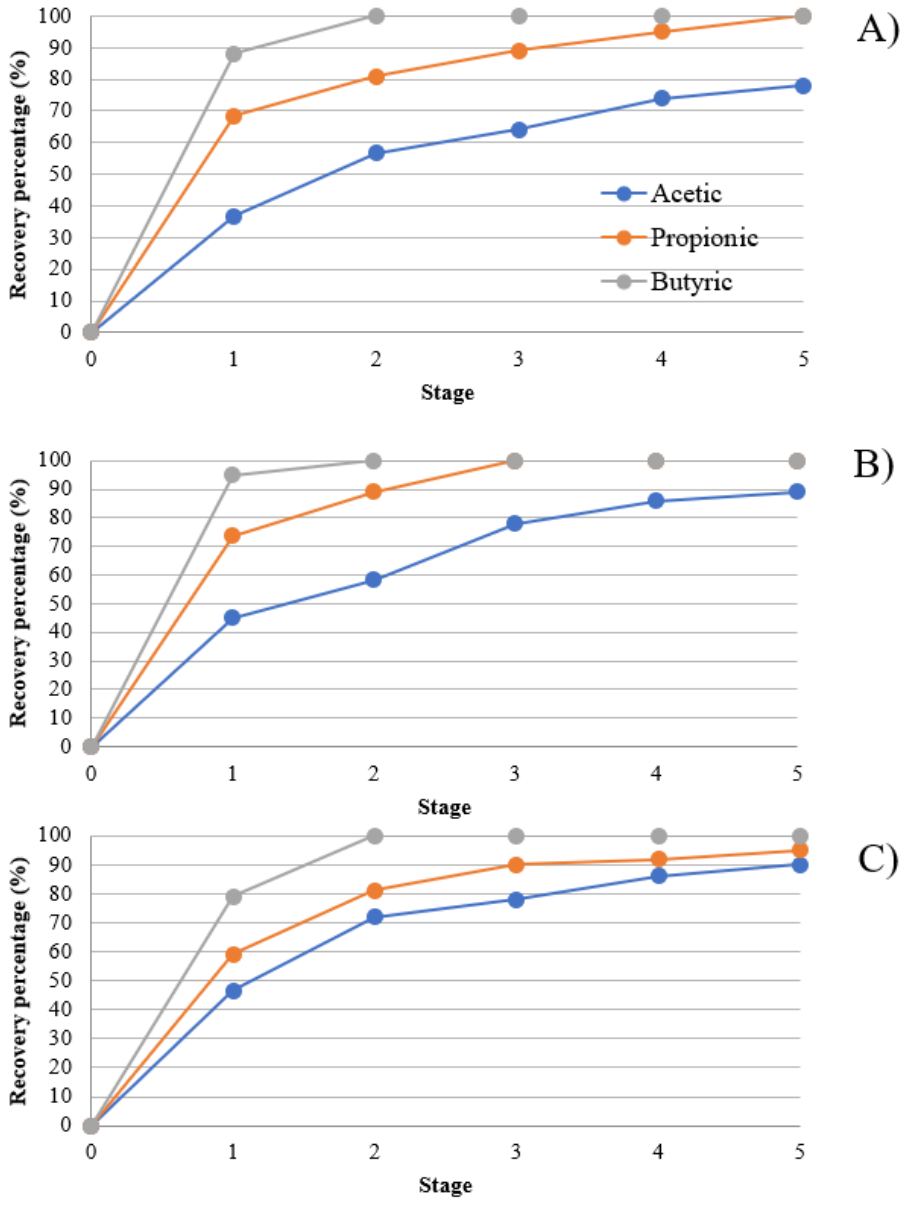

Figure 6.11. Recovery percentages achieved with all VFA in the feeding (50 g/L) at different S/F (kg/kg); A) S/F 0.5; B) S/F 0.75; C) S/F 1.

- ***Solvent comparison***

The different physicochemical properties of the two solvents may have an influence on their behaviour; in fact, when comparing them in the recovery of acetic acid, it was seen that regardless of the number of stages, ethyl acetate obtained much higher recovery percentages than ethyl octanoate (Figure 6.12), being 90 % versus 49 %, respectively.

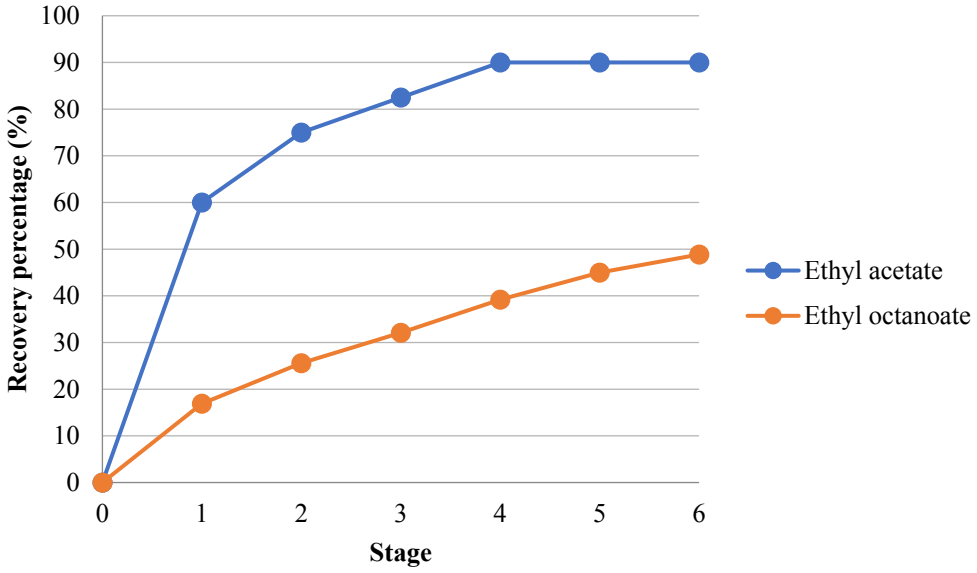

Figure 6.12. Effect of solvent on acetic acid (50 g/L) recovery at S/F 1 kg/kg.

Figure 6.13 illustrates the solvent effect on propionic acid recovery at S/F 1. It was observed that except in the first stage, ethyl acetate also obtained better recovery percentages than ethyl octanoate, although in stage 5 they were very similar. In any case, the recovery with both solvents exceeded 90 % in both cases.

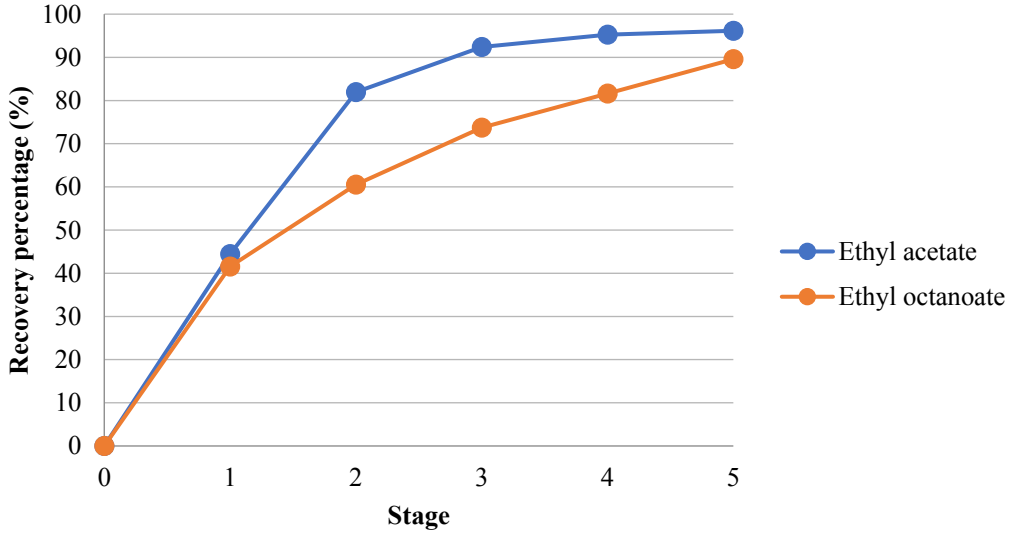

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Figure 6.13. Effect of solvent on propionic acid (50 g/L) recovery at S/F 1 kg/kg.

On the other hand, Figure 6.14 shows that when comparing the two solvents to recover butyric acid, unlike what it was seen with acetic and propionic acid, no great differences were observed, recovering 70 % in stage 1 and increasing to over 90 % with three stages for both solvents.

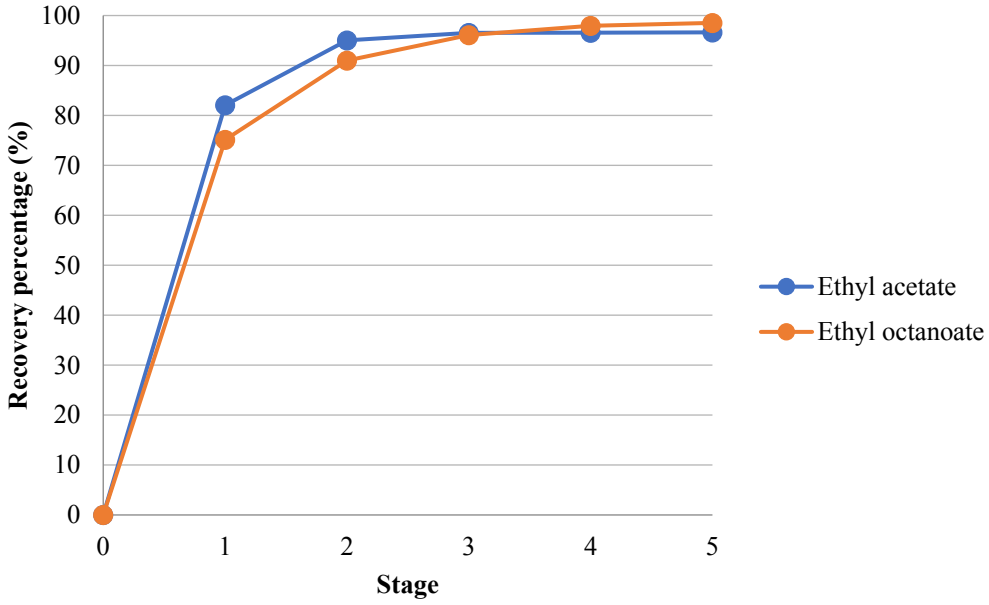

Figure 6.14. Effect of solvent on butyric acid (50 g/L) recovery at S/F 1 kg/kg.

Based solely on the ability of the solvent to recover the VFA present in the aqueous phase, ethyl acetate seems to be the best due to its higher recovery percentages in batch mode. In any case, these LLE tests were a first step to study the effect of main parameters affecting recovery and for helping in the information acquisition for designing the pilot scale column, so it must be checked that the same behaviour would be reported in the pilot plant.

6.2.3. Pilot scale

6.2.3.1. Description and design of the extraction column

The information obtained in the batch tests was useful to select the optimum pH for operation, as well as to know the variation in the percentage of VFA recovery in each stage. With this information, the feasibility of using said solvents to recover VFA in continuous mode was validated. Therefore, pilot-scale experimentation was carried out once the laboratory-scale experiment was completed, trying to assess only the best operating conditions obtained from the laboratory-scale experience.

When designing a LLE column, the most critical column dimensions are height and diameter. The height is directly related to the number of stages that the column requires. This number of stages is determined by desexed recovery, surface tension, viscosity and difference in densities. A common procedure is to obtain the number of theoretical stages at the laboratory and extrapolate that result to a commercial scale. An increase in the number of stages implies a higher product recovery, but also a higher equipment cost. For this reason, a balance must be sought between recovery and cost.

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The LLE column of the pilot plant was designed and built according to the calculations carried out by Quintana Álvarez (2023) for using ethyl acetate given the good results obtained in batch mode. In order to estimate the height (H), it was calculated by multiplying the number of equivalent stages desired (set as 6 given the outstanding results in batch mode at said number of stages) by the value of 0.5 (Henley and Seader (1981)), therefore obtaining a useful H of 3 m. Regarding column diameter (D), Eq. 6.2 was used:

$$D = \left(\frac{4 \cdot \frac{Q}{(U_D + U_C)_f}}{\pi} \right)^{0.5} \qquad \text{(Eq. 6.2)}$$

where Q is the total flow entering the column (m^3/h) and $(U_D+U_C)_f$ is the value for the corrected velocity ratio, being the ascending velocity, U_D, and the descending velocity, U_C, which takes a value equal to the S/F ratio entering the column.

For calculating this corrected velocity ratio, Figure 6.15 allows this value to be related to the same value corrected for the obstruction of neighbouring drops, $(U_D+U_C)_f$ divided by the characteristic upward speed of a single drop (u_o).

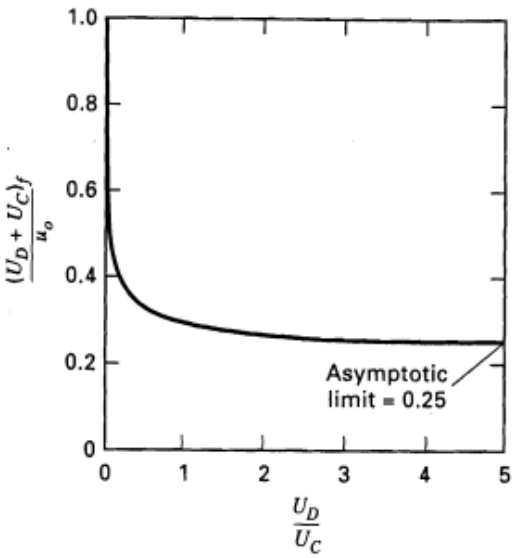

Figure 6.15. Effect of phase relationship on extractor capacity. Source: Henley and Seader (1981).

The upward speed of a single drop (u_o) can be calculated from Eq. 6.3:

$$u_0 = \frac{0{,}01 \cdot \Delta\rho \cdot \sigma}{\mu_c \cdot \rho} \qquad \text{(Eq. 6.3)}$$

where $\Delta\rho$ is the difference between the output densities at the bottom and top of the column (kg/m^3), σ is the surface tension between both liquids (N·m), μc is the dynamic viscosity of the stream leaving the bottom of the column (Pa·h) and ρ is the density of the stream leaving the bottom (kg/m^3). From this value of u_o and the relationship shown in Figure 6.15, a value for the corrected velocity ratio, $(U_D+U_C)_f$ can be obtained.

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Figure 6.16 illustrates the design scheme of the LLE column, presenting both the column and the different sample points and inlets and outlets of the same. This LLE column has 4 peristaltic pumps (Seko Kronos 50), two for the input of feeding and solvent and two for the output of extract and raffinate. The four streams include a flowmeter (IFM SM8000) to perform flow measurements. In addition, the solvent outlet and inlet streams also incorporate a flow controller that, through a frequency inverter, acts on the pumps, allowing the level inside the column to be regulated through a level sensor or through the input S/F ratio.

The useful height of the column was completely filled using glass Raschig rings (8 mm diameter), since according to Leibson and Beckman (1953), the rule shown in Eq. 6.4 in order to avoid channeling. Specifically, when using this filling, the rule is met (it would be 8.75), so it would be suitable for this purpose.

$$\frac{D_{column}}{D_{packing}} > 8 \qquad \text{(Eq. 6.4)}$$

where D_{column} is the diameter of the LLE column and $D_{packing}$ the diameter of Raschig rings.

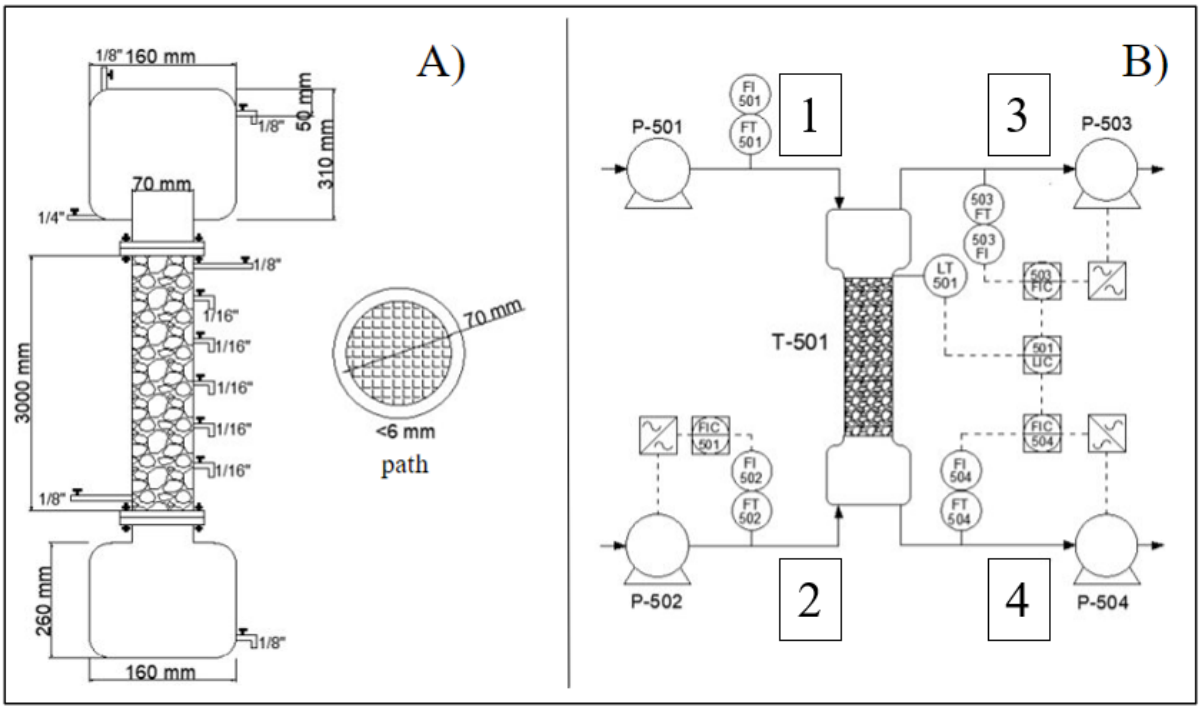

Figure 6.16. Pilot plant liquid-liquid extraction column. A) Extractor drawing. B) Instrumentation diagram (equipment notation corresponds to section 5 of the whole pilot plant) with numbered streams.

Figure 6.17 shows images of the interior of the pilot plant in which the LLE column is located, as well as its associated equipment such as the pumps ant tanks.

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Figure 6.17. Images of liquid-liquid extraction column and the associated equipment.

Although the original design of the LLE column was shown in Figure 6.17, once the results obtained were analysed and compared with those reported in batch mode, it was decided to make some modifications to it in order to optimise the recovery percentages in the pilot plant. Therefore, some improvements for the LLE column were proposed: the first one through the modification of its packing and, on the second one by installing redistributors inside (which were not contemplated in its initial design).

The proposed filling change involved replacing the 8 mm diameter Raschig rings with others of smaller diameter, specifically 6 mm. In this way, the porosity inside the LLE column would be lower, the specific surface area would be higher and consequently the mass transfer coefficient (K_L) was also increased.

Regarding the installation of redistributors in the column, the construction of those at the inlet and outlet of the column was proposed to ensure quality distribution of the drops (Figure 6.18A). Furthermore, in the central part, it was necessary to install another redistributor to avoid the preferred paths through the walls (Figure 6.18B).

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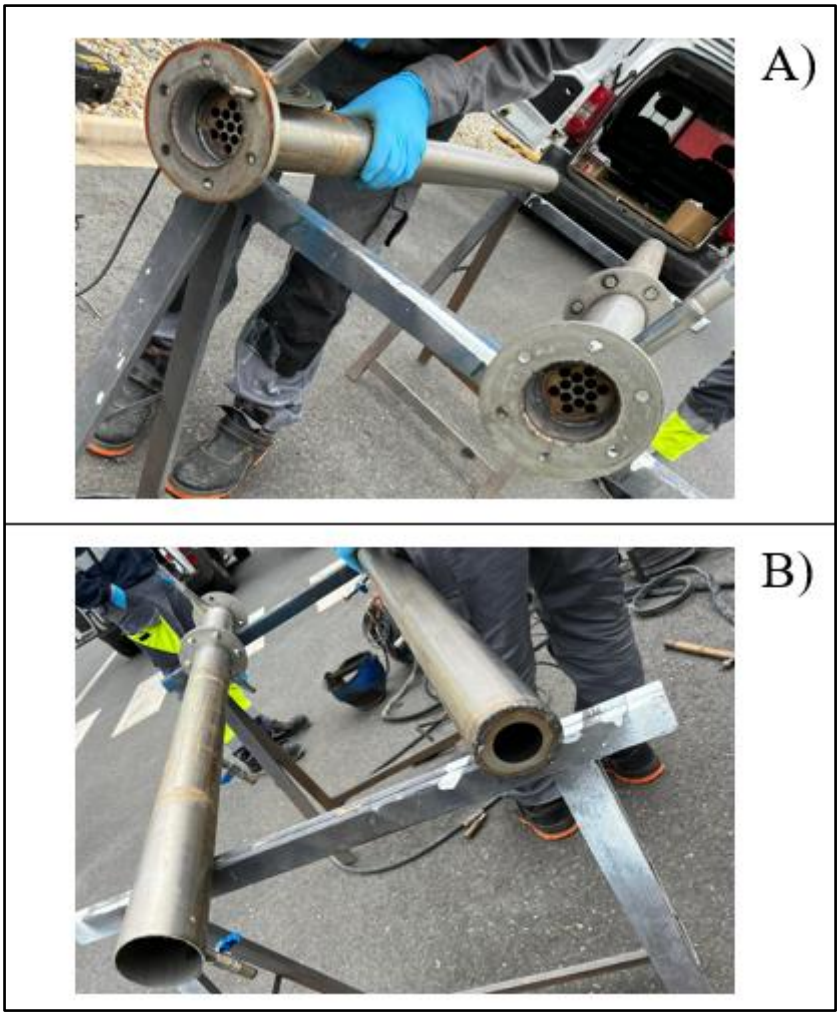

Figure 6.18. Improvements made to the liquid-liquid extraction column through redistributors' installation. A) At inlet and outlet. B) In central part.

For the calculation of the VFA recovery percentage (R_i), Eq. 6.5 was utilised:

$$Recovery\ percentage_i\ (\%) = \frac{(W_{i,1} - W_{i,4})}{W_{i,1}} \cdot 100 \qquad \text{(Eq. 6.5)}$$

where Wi,n is the mass flow of acid i in stream n, in kg/h, the number of each stream being specified in Figure 6.16B.

To carry out the LEE experiments on a pilot scale using synthetic medium, a VFA-rich feeding with tap water was prepared with the following concentrations: 20 g acetic/L, 10 g propionic/L and 20 g butyric/L from Sigma Aldrich > 99.7 % purity (which would mimic the RO rejection in the membrane train at 50 g VFA/L coming from the fermentation of FW). In total, the appropriate volume was prepared so that the experiments lasted around 4 h, assuming a column design flow rate of 40 L/h (adjusting the pump flow rates to operate at the desired S/F, always maintaining the feeding flow constant (1 in Figure 6.16B)).

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6.2.3.2. Results and discussion

Just as the results were shown for the LLE experiments in batch mode, the results obtained in the continuous column in counter-flow mode at pilot scale are presented below in order to evaluate the recovery percentages when scaling this unit operation.

- ***Ethyl octanoate***

Figure 6.19 shows that using ethyl octanoate as solvent, regardless of the S/F applied, the percentage of acetic recovery was around 25 % once steady state was attained. This result was achieved in batch mode in stage 2, reaching 50 % recovery in stage 6 (Figure 6.6). Taking into account that the column has a height of 3 m and that the equivalent height of each theoretical stage is 0.5 m, such a recovery percentage obtained in batch mode should also be achieved in the pilot plant, but the results were much lower.

Regarding propionic acid, it was seen that although in this case in continuous mode the application of a higher S/F implied a better recovery, the results were also much lower than those obtained at lab scale. In this way, for an S/F of 0.5 in continuous, 36 % was reached while in batch in stage 2 this value was already exceeded. For S/F 1, in continuous mode 60 % recovery was reached while in batch said recovery was attained in stage 2. When applying a S/F of 2, 72 % was recovered in the column, while in batch mode said result was also achieved in stage 2.

As for butyric acid, 50 % recovery was obtained continuously for S/F 0.5, 72 % for S/F 1 and 96 % for S/F 2, but in batch these results were already attained in the stage 2. Therefore, for all VFA, a considerably lower recovery efficiency was obtained in comparison with the expected one considering batch experiments.

In general, it can be stated that, as it happened in batch mode, longer-chain VFA were recovered better than shorter-chain ones, and that, except for acetic acid (where hardly any differences were seen), a higher S/F implied better recovery.

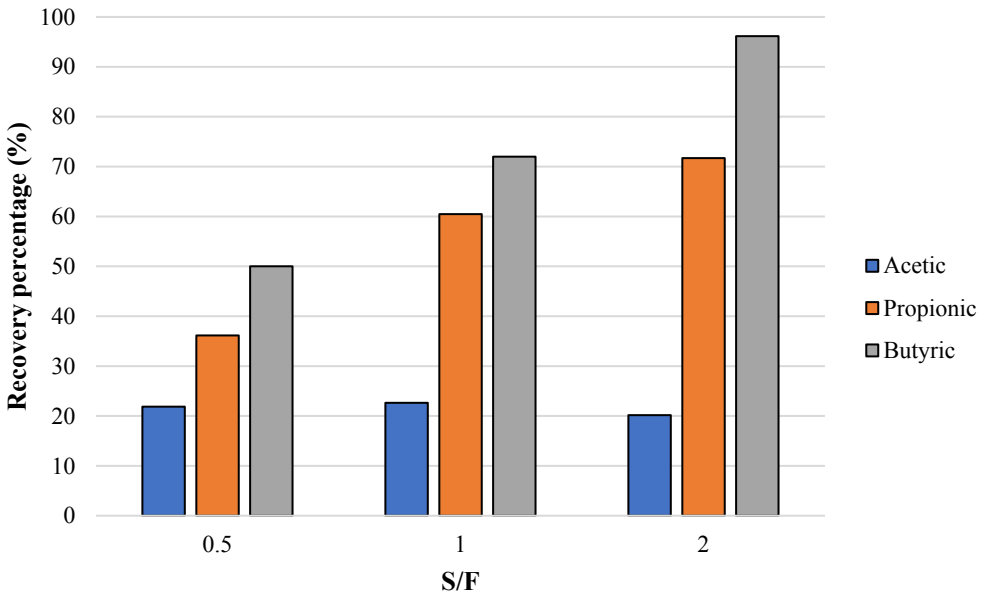

Figure 6.19. Effect of S/F on VFA recovery percentages using ethyl octanoate.

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- ***Ethyl acetate***

Figure 6.20 shows the effect of S/F on the recovery percentage of the three VFA studied, but in this case using ethyl acetate as solvent. It was seen that in continuous mode, for S/F 0.5, the recovery of acetic acid was 39 %, while in batch mode in stage 2 it already exceeded 45 %. For S/F 0.75, the acetic recovery was 60 %, a value that was reached in batch mode also in stage 2. When applying S/F 1, a recovery of 71 % was obtained, which was attained in the stage 3 in batch mode. As for the recoveries of propionic and butyric, in continuous, regardless of the S/F, a total recovery was achieved, which in this case does align with the results in batch.

In view of these results, with both solvents this drop in recovery percentages has been detected when switching to continuous mode, which must be considered when carrying out the global integration of the pure VFA production process.

With ethyl acetate as solvent, seeing that the recovery percentages were notably higher, three S/F were also tested, but the highest was 1 instead of 2, since of course the higher S/F imply greater expenses for having to use a larger amount of solvent.

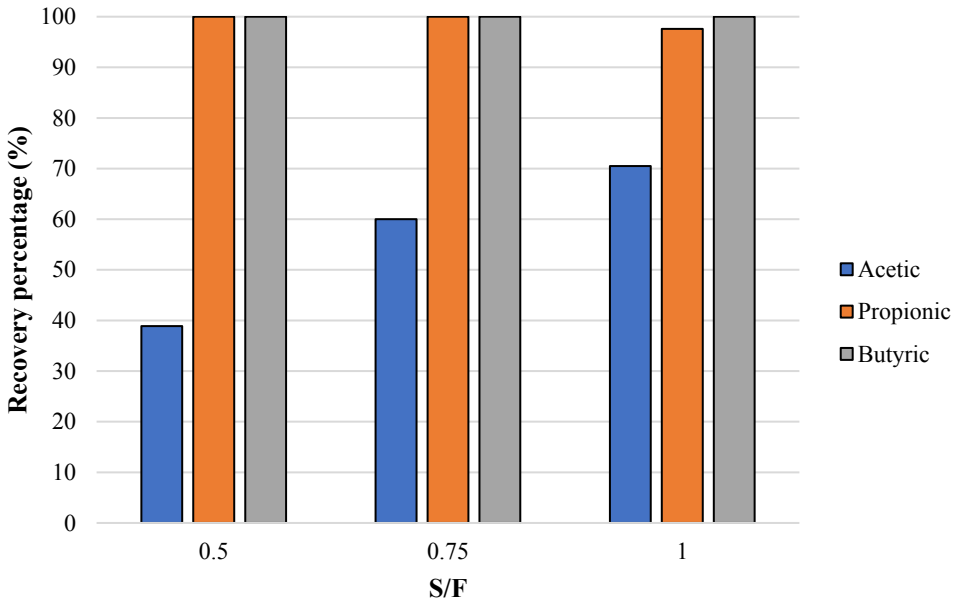

Figure 6.20. Effect of S/F on VFA recovery percentages using ethyl acetate (before liquid-liquid extraction column modifications).

Figure 6.21 shows the recovery percentages with ethyl acetate after the modifications carried out on the column. As specified above, these were implemented in order to see that the results could be improved by observing those obtained in batch mode. Thus, it was seen that with both S/F ratios, 0.75 and 1, the recovery of the three VFA was total.

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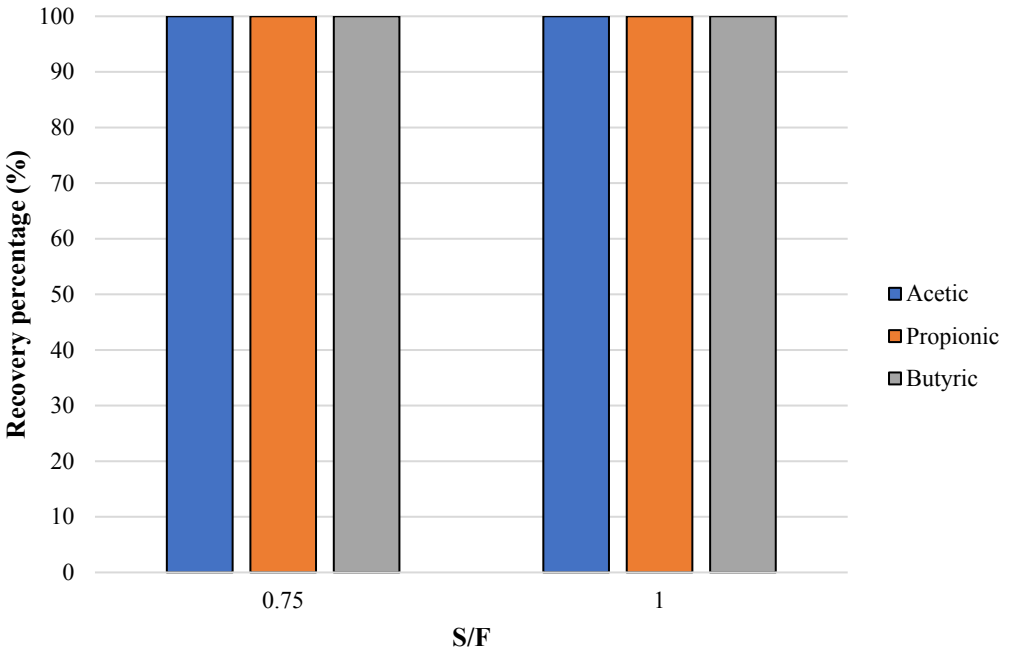

Figure 6.21. Effect of S/F on VFA recovery percentages using ethyl acetate (after liquid-liquid extraction column modifications).

- ***Solvent comparison***

It is important to establish the comparison in order to select the most appropriate one from a technical perspective to conduct an integration of the entire VFA production process in case of selecting the counter-flow mode of operation. This comparison was carried out with the results with ethyl acetate obtained before the column modifications (Figure 6.20), as it would be unfair to consider the total recovery percentages when the experimentation conducted with ethyl octanoate was executed before the LLE column modifications.

Figure 6.22A indicates that at S/F 0.5 kg/kg, the recovery of acetic acid was twice with ethyl acetate in comparison with ethyl octanoate (22 % versus almost 40 %). For propionic and butyric, the differences were also very important, achieving a total recovery of said acids with ethyl acetate.

Figure 6.22B showed that when recovering acetic at an S/F of 1, while ethyl octanoate only reached 23 %, acetate achieved a recovery of 70 %. Similar differences were observed with propionic acid. The use of ethyl acetate implied a total recovery, while with octanoate was reached only a 60 %. When the VFA intended to be recovered was butyric acid, the use of ethyl acetate also implied a total recovery, however, using octanoate limited the percentage achieved to 73 %.

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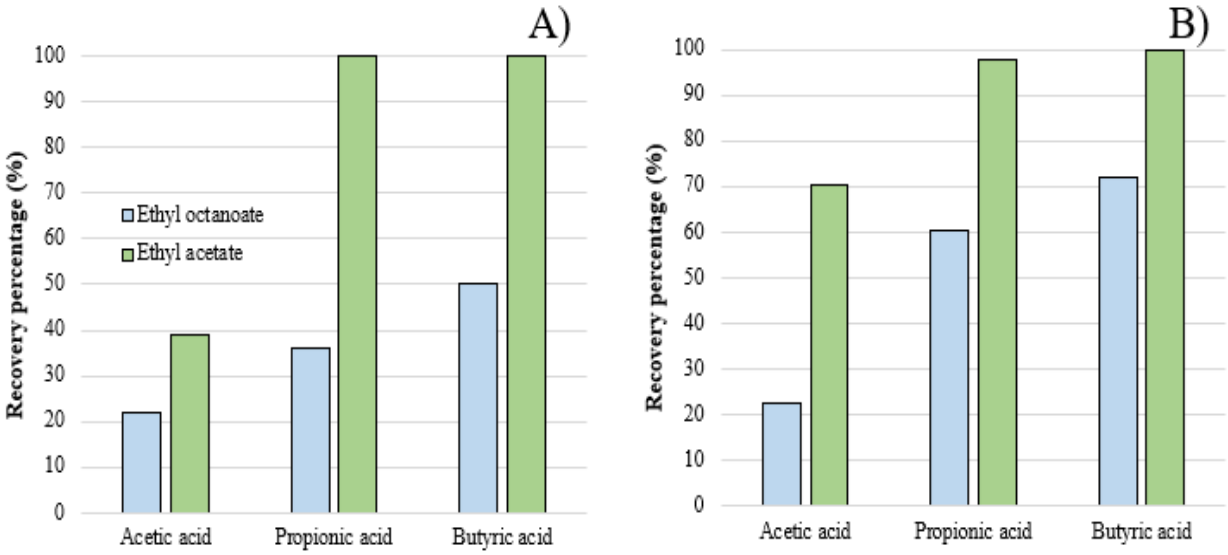

Figure 6.22. Comparison of VFA recovery with two solvents. A) S/F 0.5 kg/kg. B) S/F 1 kg/kg.

Ethyl acetate turned out to be the one that showed the best recovery percentages, with the differences with ethyl octanoate being considerable.

In any case, considering that both solvents have such different physicochemical properties such as their solubility or boiling point (Table 6.1), a selection based only on the recovery percentages in LLE would not be appropriate. Therefore, the information related to the distillation of VFA is presented next, establishing the differences in said unit operation depending on the solvent used. Moreover, in Chapter 7 an integration study of the LLE with the appropriate distillations will be shown, analysing from an economic point of view which of the two solvents tested gives rise to the most favourable monetary results to scale up the process.

6.3. DISTILLATION OF VFA

6.3.1. Simulations

Unlike what happened with the other downstream unit operations which were tested experimentally (membrane train and LLE process), the distillation data had to be acquired through simulations due to the lack of a pilot plant. In any case, it should be noted that to execute those simulations it has always been considered that the distillation was closely linked to the LLE, therefore both unit operations were contemplated as a whole. In this way, the feeding streams shown in Table 6.2 in mass fraction (selectivity in line with that reported for the output of the membrane train operating with SS (Table 5.5) and for the effluent of FW fermentation (Table 4.2)), would have to be subjected to the LLE, each VFA being recovered according to the results shown in Figures 6.19 and 6.20 of the pilot plant with each of the solvents. These feedings were configured in ambient conditions (25 ºC and 1 atm).

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Table 6.2. Composition of feedings in ASPEN simulations (mass fraction).

	SS	FW
Water	0.950	0.950
Acetic acid	0.030	0.020
Propionic acid	0.010	0.010
Butyric acid	0.010	0.020

The simulations of the VFA distillation process were performed in the chemical process simulator ASPEN HYSYS in its version 12.1. They were executed with two selected solvents (ethyl octanoate and ethyl acetate). Considering that ethyl octanoate was not found within the simulator's component library, octanoic acid was selected instead of ethyl octanoate due to similar properties (Table 6.3).

Table 6.3. Comparison between ethyl octanoate and octanoic acid. Source: National Institute of Standards and Technology (2023) and Chemeo (2023).

	Ethyl octanoate	Octanoic acid
Cp (J/(K·mole))	330	310
Δvap H° (kJ/mole)	60	79
Δfus H° (kJ/mole)	24.4	21.3
Boiling temperature (°C)	211	236

A continuous distillation in several stages was considered. This consists of a column containing an equivalent number of theoretical stages (N) organised in a cascade of two sections (Figure 6.23A); a condenser in which the overhead vapor leaving the stage above is condensed to give rise to a liquid distillate product (D) and a liquid reflux which is returned to the upper stage; a reboiler in which the liquid from the lower stage is vaporised to give rise to a bottom product (B) and a vapour that is returned to the lower stage; the accumulator is generally a container through which the condensed vapour is collected. The heat exchanger (not represented in Figure 6.23) is where the hot bottom stream is used to heat the feeding before entering the column. The feeding stream (F) enters the column in the feed stage, which contains more volatile components (called light key component (LKC)) and fewer less volatile components (called heavy key ones (HKC)). In the feed stage, this can be liquid, vapour or a mixture of liquid and vapour. The section above the feed where the steam is washed with reflux to remove or absorb the HKC is called the rectification section, while the section below the feed in which the liquid is stripped of LKC by the rising steam is called the stripping section. In order to simulate the distillation columns in ASPEN HYSYS, it was selected the *Distillation Column* equipment which performs a simulation that allows obtaining more information than the *Shortcut Column equipment*. The configuration of the distillation columns is shown in Figure 6.23B, including the total condenser and the partial boiler.

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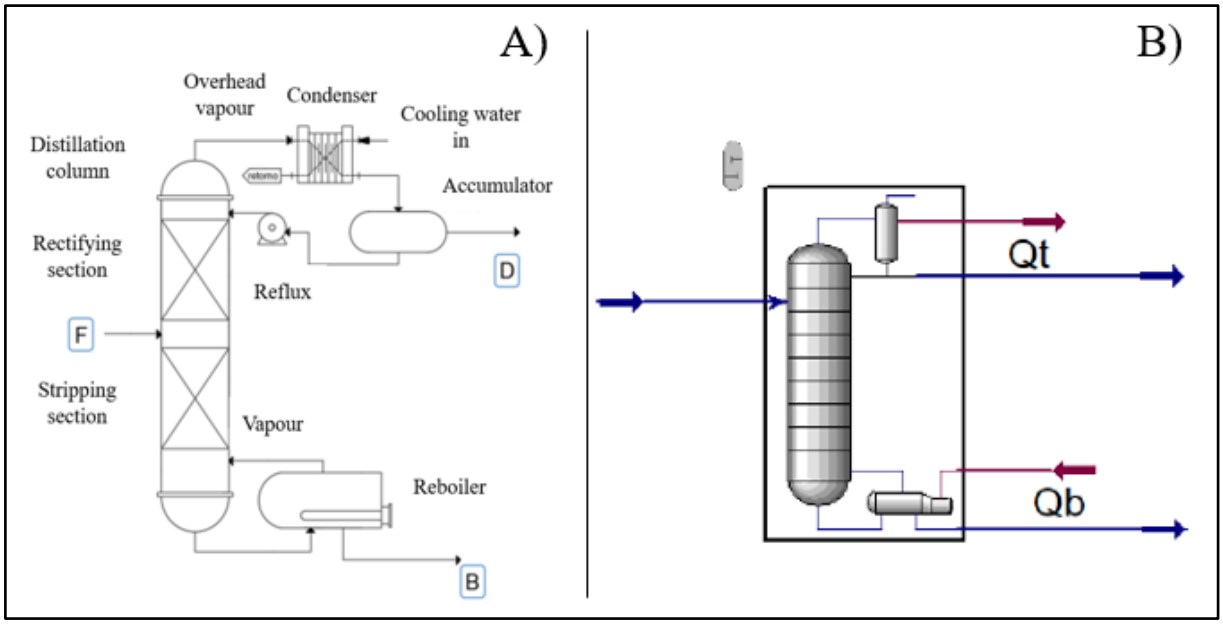

Figure 6.23. Scheme of the continuous distillation column. A) Detail of the different components. B) Drawing in ASPEN HYSYS.

When separation in distillation processes is accomplished, it is necessary to consider the key compounds of each column, that is, the elements with the closest boiling points between them that will mark the separation during the process. The LKC will be the component with the highest boiling point exiting the column through the heads section (top of the column), while the HKC will be that with the lowest boiling point exiting the tails section (bottom of the column). To choose these components it is necessary to know the boiling points of each one, which are shown in Table 6.4.

Table 6.4. Boiling points of all compounds at atmospheric pressure.

Compound	Boiling point (ºC)
Ethyl acetate	77
Ethyl octanoate	211
Water	100
Acetic acid	118
Propionic acid	141
Butyric acid	164

As it was previously discussed, the choice about which is the most suitable solvent is not easy, given that there are important differences between them that were previously commented, which imply noticeable changes in the global process. In fact, solvent solubility is the most important point since it causes a significant loss of solvent in the raffinate stream of the LLE column. In the case of ethyl octanoate, due to the low solubility, hardly any solvent is lost and there is no need to treat that outlet stream. However, in the case of ethyl acetate, there is a significant loss of solvent which must be recovered within an extra distillation column. As a result, the differences between both processes are shown in Figure 6.24A and B. In Figure 6.24A, the process using ethyl acetate is shown with the presence of the "Distillation 2" for solvent recovery from raffinate, column of which the option with octanoate dispenses.

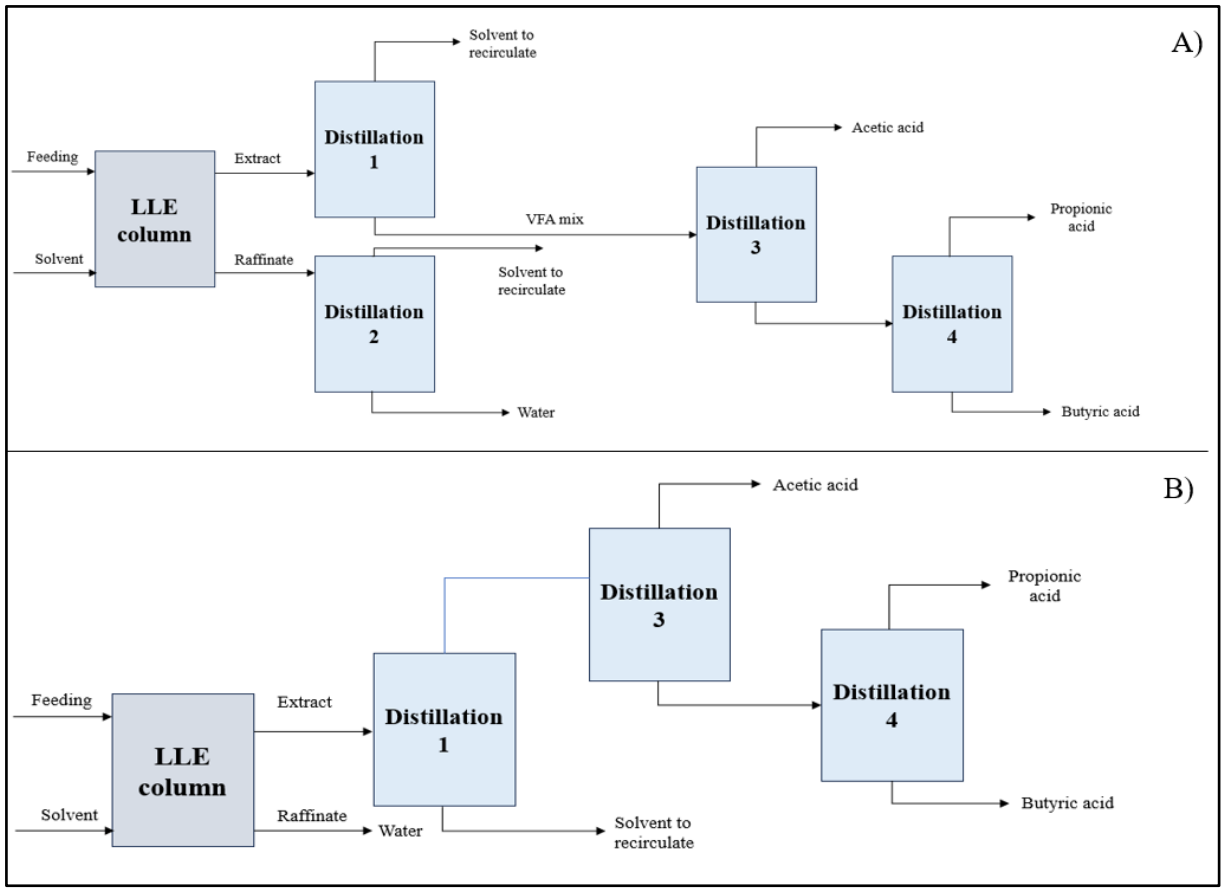

Figure 6.24. Differences in liquid-liquid extraction and distillation with both solvents. A) Ethyl acetate. B) Ethyl octanoate.

In the column called Distillation 1, the separation of the solvent and the VFA takes place, being the inlet to said column the extract of the LLE. In the *Monitor* section, the reflux ratio values of the column and the percentage of solvent recovery by heads were set. The simulation was run for a number of 13 stages, and the S/F tested in the pilot plant in the LLE tests were simulated (0.5, 1 and 2 for ethyl octanoate and 0.5, 0.75 and 1 for ethyl acetate). The LKC was acetic acid, so the data to be determined thanks to the simulations were: the recovery percentage of acetic acid, the heat flow in the boiler and condenser, as well as the solvent mass fraction in the VFA mixture that would be removed by tails in the column, directly related to the final purity of the product.

In the column called Distillation 3, acetic acid was separated by heads and a mixture of propionic and butyric acid by tails. The feeding to this column was the tails stream from Distillation 1. The values set in *Monitor* were the recovery of acetic acid by heads (LKC) and the recovery of propionic acid by tails (HKC), seeking to obtain the heat flows in the boiler and condenser.

In Distillation 4, the separation of propionic and butyric acid was carried out. In this case, the feeding to the column was the tails stream from Distillation 3. The values set were the recovery of butyric acid by tails and that of propionic acid by heads, HKC and LKC, respectively. As in the case of Distillation 3, the parameters to be obtained were the heat flow in the condenser and boiler.

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When ethyl acetate is used as a solvent, it is necessary to introduce an extra column, Distillation 2, to which the raffinate of the LLE was fed to recover part of the lost solvent given its high solubility in water. Through the simulation, the aim was to calculate the heat flow of the boiler and the condenser for the different feeding values, setting the reflux ratio of the column and the purity of the solvent that is recovered by heads to be recirculated to the process.

In the case of using ethyl octanoate as solvent, the configuration was different. The Distillation 1 was again the column that separated the solvent from the VFA, with the feeding to this column also being the extract from the LEE. In this column the LKC was butyric acid while the HKC would be ethyl octanoate. To carry out the simulation, in the *Monitor* section, butyric acid recovery values in tails and a solvent recovery value in heads were set. Simulations were carried out with 12 stages and three S/F ratios (0.5, 1 and 2). The values which were collected from the simulator were the condenser and boiler heat flow, the amount of butyric acid lost by tails and the mass fraction of solvent in the outlet stream by heads, linked to the final purity of the acids. Due to the low solubility of ethyl octanoate in water, it was not necessary to include Distillation 2 for solvent recovery in the process since the loss in the aqueous stream is minimal. Distillation 3 and 4 were simulated in the same way as in the case of ethyl acetate, setting the same parameters and seeking to obtain the same data.

6.3.2. Results and discussion

6.3.2.1. Ethyl octanoate

When simulating the column Distillation 1 with ethyl octanoate, the key components were the butyric acid and the solvent, allowing the VFA to be separated by heads and the solvent by tails. In this unit, the solvent and traces of water were separated from the rest of VFA. Figure 6.25 shows the calorific energy per m^3 fed to LLE needed to supply and withdraw in the boiler and in the condenser of Distillation 1 (considering the feeding composition corresponding to FW in Table 6.2). In the case of the boiler, it can be seen how the energy requirement of the same was related in the form practically linear with the S/F ratio, since the higher this ratio, the more solvent flow is necessary to evaporate. On the contrary, the S/F ratio had a much smaller influence on the energy to be removed in the condenser since the VFA flow obtained by the heads stream is partially influenced by the S/F ratio considering that only the recovery of acetic is affected, while that of propionic and butyric is not.

In addition, Figure 6.25 clearly indicates that the energy supplied in the boiler was always greater than the energy flow that was removed from the condenser, therefore, the distillation will always require an external supply of energy, since neither the total energy withdrawn is usable. Considering that a solvent recovery of 99.9 % was set, there was a noticeable difference between the heads and tails values since the difference between the reflux ratio and the *boilup ratio* can be significant (in fact, by looking at those values in the simulator a reflux ratio of 1 was obtained while the *boilup ratio* value was 2.5, which would explain this difference). In any case, the butyric losses by tails would not be greater than 2 % in mass. In summary, a higher S/F ratio applied in the LLE would increase the energy needs of the downstream units by increasing the flow rate fed to them.

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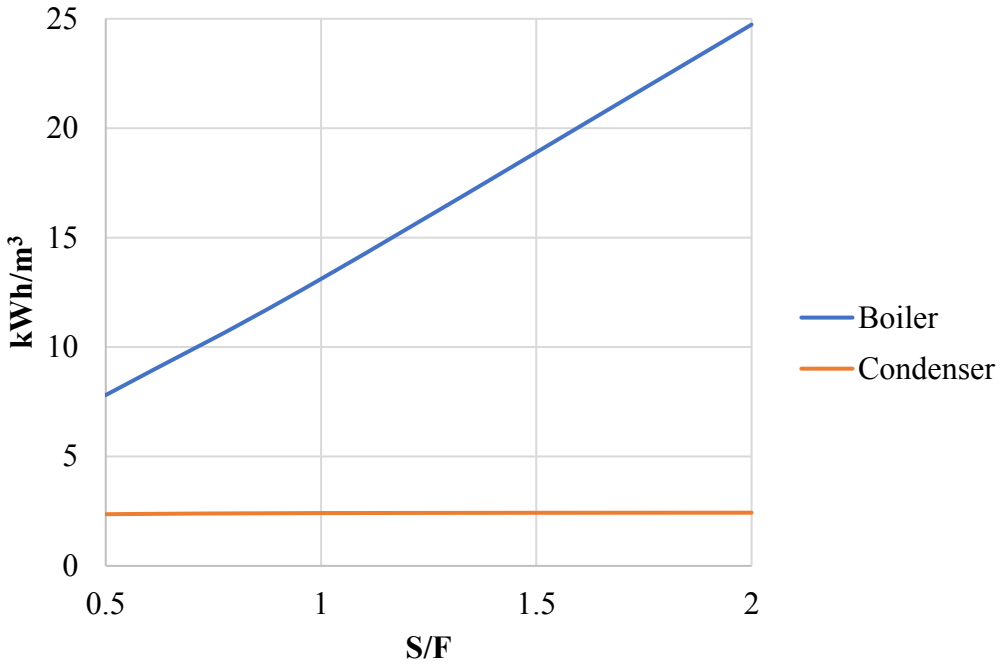

Figure 6.25. Calorific energy flows to be supplied in the boiler and to be removed in the condenser of Distillation 1 with ethyl octanoate (considering food waste fermentation selectivity).

6.3.2.2. Ethyl acetate

When operating with ethyl acetate, this solvent would come out of the heads of Distillation 1 accompanied by traces of water, but it should be noted that between these two compounds there is an azeotropic mixture. This can be defined as a mixture of two or more compounds which boils at a constant temperature and behaves as it were made up of a single component, so when it boils, its vapour phase will have the same composition as its liquid one. In this way, it presents deviations from Raoult's Law resulting in the impossibility of separating said compounds by simple distillation (Henley and Seeder, 1988).

When the simulations were carried out in ASPEN HYSYS, the existence of the azeotrope was confirmed with an operating temperature of 30 ºC (Figure 6.26). In this case, the consequence of this azeotrope was that since the amount of water is minimal and it is to the right of the azeotrope, the LKC will not be water, but ethyl acetate. Between ethyl acetate and acetic acid there was not azeotropic complexity (Figure 1.25), which makes the separation easier.

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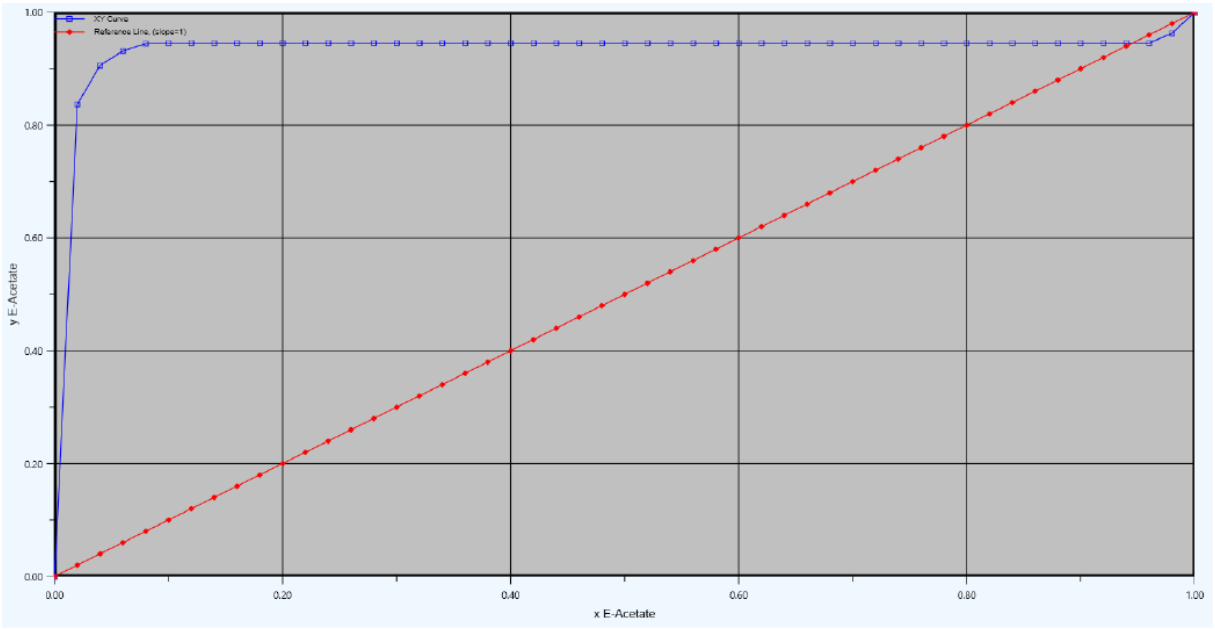

Figure 6.26. Azeotrope of the binary system ethyl acetate + water in ASPEN HYSYS.

The simulation was in 13 stages, since it was seen that the recovery was practically total. The Figure 6.27 shows that by varying the S/F ratio of the LLE, different percentages of acetic acid recovery were obtained. The S/F ratio was not decisive since the recovery depends on the volatilities of the key components and not on the volume fed to the column. In addition, the Figure 6.27 also shows on the right axis the amount of ethyl acetate that was lost in the tails stream (mass fraction) and that would be classified as an impurity when separating the VFA to be sold. As it is evident, the higher the S/F, the more ethyl acetate would also be lost, but it was confirmed that this value would be always lower than 3 %, so it would result as an acceptable limit for commercialising the VFA.

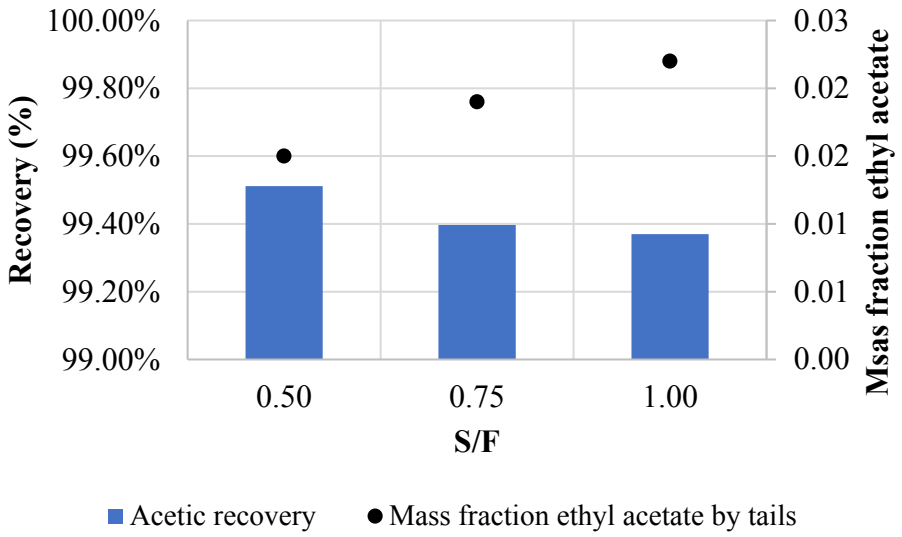

Figure 6.27. Acetic acid recovery and mass fraction of ethyl acetate in tails stream of Distillation 1.

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In the same way as it was carried out with ethyl octanoate, it was also possible to obtain the energy requirements in the boiler and in the condenser of the Distillation 1 (Figure 6.28). The trend lines of both energy flows are almost the same, noting that the energy expenditure was influenced by the S/F ratio, given that it is directly proportional to the amount of feeding that would reach the column.

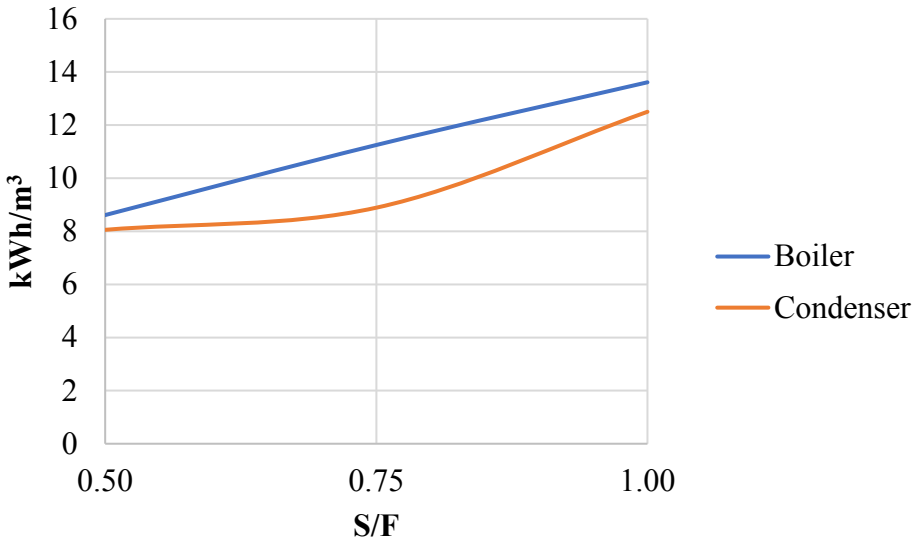

Figure 6.28. Calorific energy flows to be supplied in the boiler and to be removed in the condenser of Distillation 1 with ethyl acetate (considering food waste fermentation selectivity).

6.3.2.3. Differences between solvents

Figures 6.29 and 6.30 show a comparison between the different mass and energy balances derived from the use of one solvent or another, with the aim of clearly seeing the different streams and product destinations. For both scenarios, the same S/F ratio was applied (S/F 1 kg/kg), as well as the same recovery percentages in the LLE (70 % of acetic, 98 % of propionic and 100 % of butyric, although this does not happen as it was seen in LLE results in the pilot plant). What is evident in Figure 6.29 is how the flow rate of ethyl acetate that was lost in the raffinate of the LLE column was much higher than when octanoate was used (Figure 6.30). This is because ethyl acetate has a much higher solubility in water (87 g/L at 20 ºC). This flow rate does not vary almost with the S/F ratio applied in LLE since it is only related to the water present in the feeding and not to the amount of solvent used. Seeing in Figure 6.29 that the flow of acetate lost in raffinate was much higher than the VFA stream flow, the need arises to add the additional stage (Distillation 2). The simulations carried out considered a 100 % recovery of the solvent even if there were water impurities, but the fact of returning the solvent to the column where it would be mixed again with an aqueous feeding reduces the importance of these impurities.

As the last part of the process, the Distillation 3 and 4 were simulated, where acetic, propionic, butyric acid and very low proportions of solvent were separated. The energy consumptions were different depending on the solvent used, because the VFA mixture stream had a different temperature at the entrance to the Distillation 3 (the same as at the exit from the Distillation 1). In fact, it can be stated that energy consumption in the process with ethyl acetate is greater than in the case of ethyl octanoate, regardless of the S/F ratio selected.

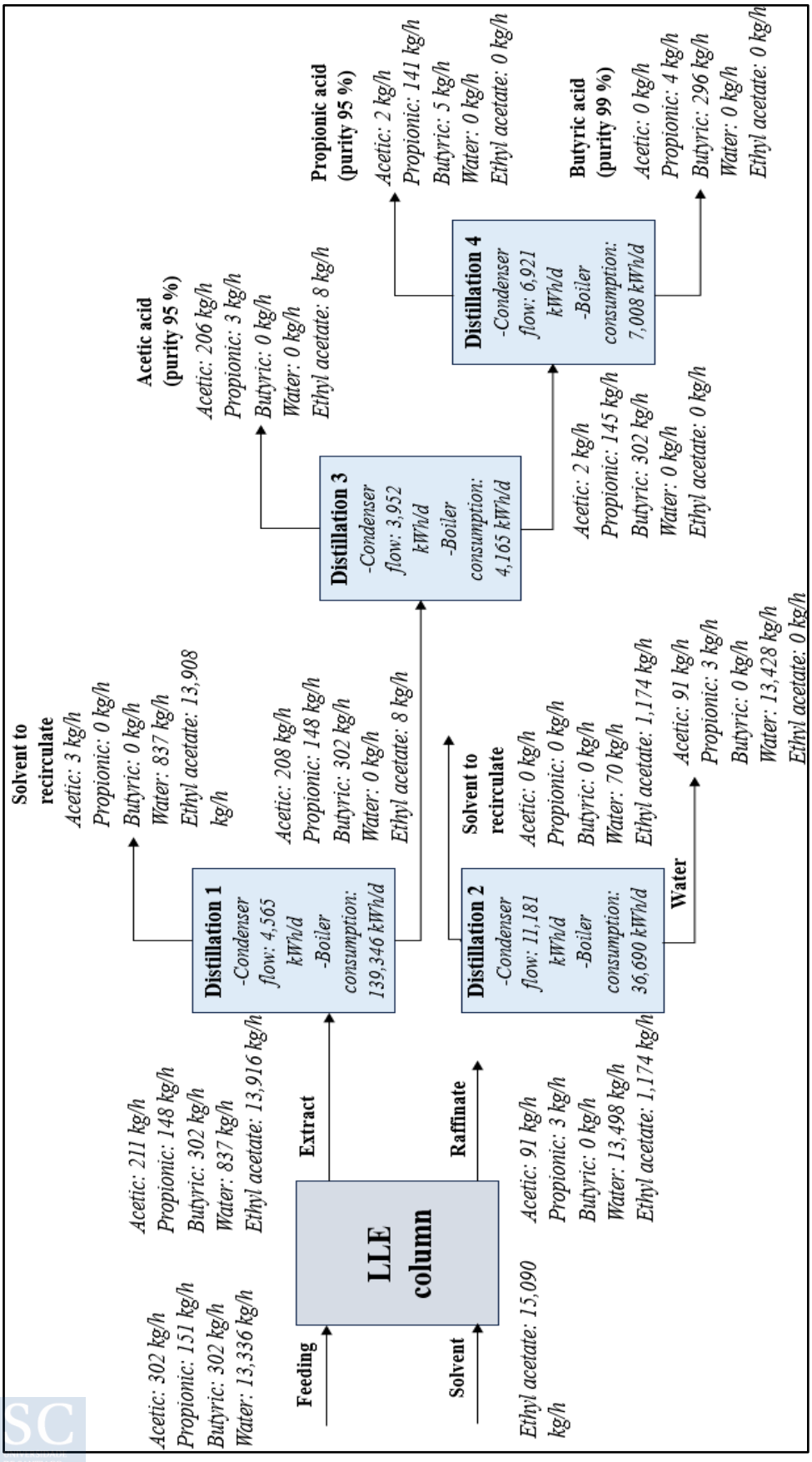

Figure 6.29. Mass and energy balances of liquid-liquid extraction + distillation using ethyl acetate (S/F 1 kg/kg) for a specific scenario (food waste fermentation selectivity and feeding flow of 15 m^3/h).

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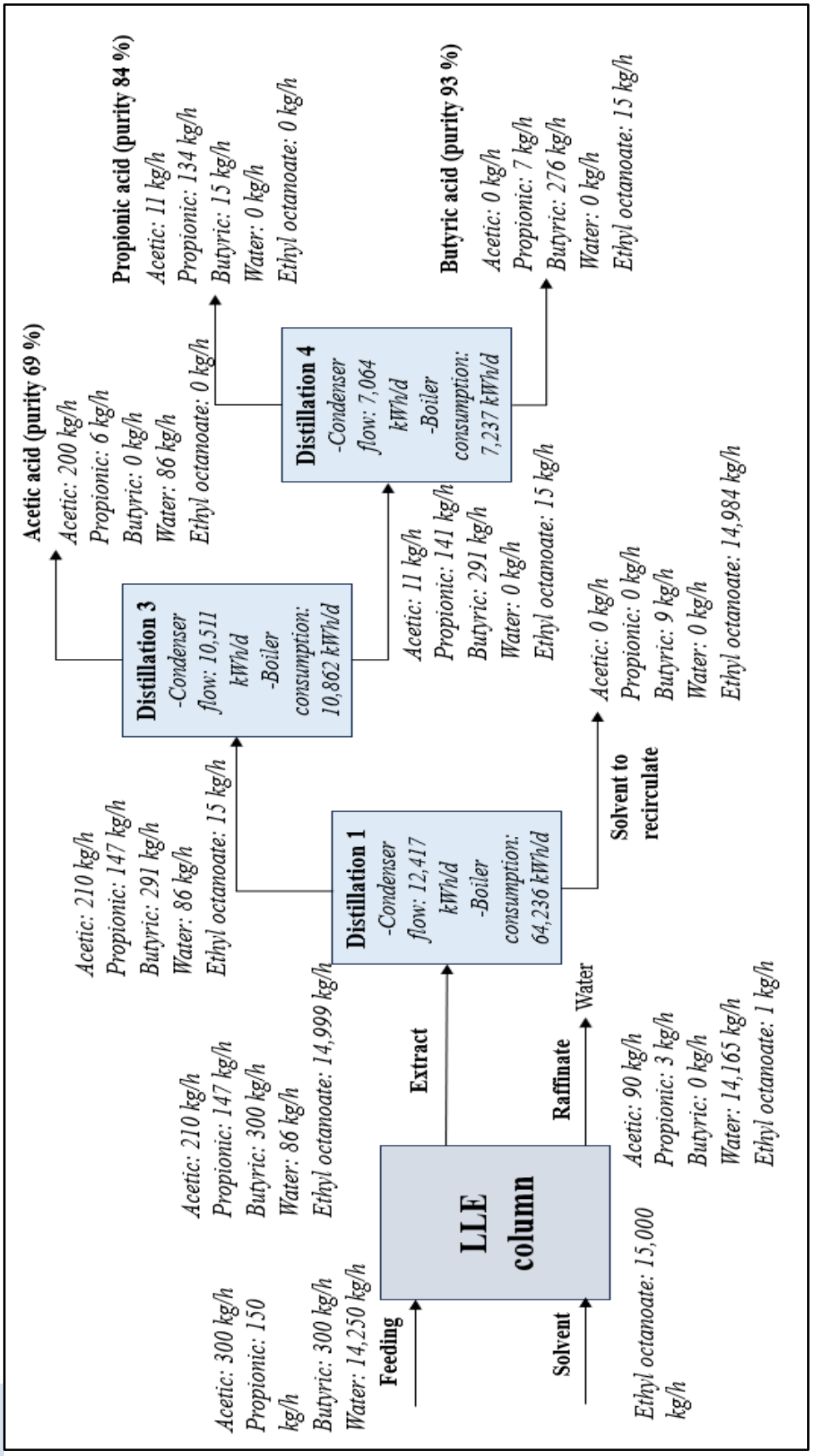

Figure 6.30. Mass and energy balances of liquid-liquid extraction + distillation using ethyl octanoate (S/F 1 kg/kg) for a specific scenario (food waste fermentation selectivity and feeding flow of 15 m^3/h).

With the objective of determining the energy consumption of each distillation column regardless of the flow they handle, a correlation was proposed between the calorific energy consumption of the columns and the feeding flow to them. Thus, for the different distillations, the correlations to be used with ethyl acetate were detailed in Eq. 6.6, 6.7 and 6.8 and 6.9:

$$Consumption_{Distillation\ 1}(ethyl\ acetate) = 9.04 \cdot Q_{Distillation\ 1} \quad \text{(Eq. 6.6)}$$

$$Consumption_{Distillation\ 2}(ethyl\ acetate) = 2.48 \cdot Q_{Distillation\ 2} \quad \text{(Eq. 6.7)}$$

$$Consumption_{Distillation\ 3}(ethyl\ acetate) = 6.25 \cdot Q_{Distillation\ 3} \quad \text{(Eq. 6.8)}$$

$$Consumption_{Distillation\ 4}(ethyl\ acetate) = 15.61 \cdot Q_{Distillation\ 4} \quad \text{(Eq. 6.9)}$$

where $Consumption_{Distillationn}$ are the consumption taken by all the units (kWh/d) and $Q_{Distillationn}$ are the flows (kg/h).

Regarding ethyl octanoate, the methodology was the same, therefore being able to know the consumption of each of the distillations through the establishment of the correlations shown in Eq. 6.10, 6.11 and 6.12:

$$Consumption_{Distillation\ 1}(ethyl\ octanoate) = 4.08 \cdot Q_{Distillation\ 1} \quad \text{(Eq. 6.10)}$$

$$Consumption_{Distillation\ 3}(ethyl\ octanoate) = 14.50 \cdot Q_{Distillation\ 3} \quad \text{(Eq. 6.11)}$$

$$Consumption_{Distillation\ 4}(ethyl\ octanoate) = 15.80 \cdot Q_{Distillation\ 4} \quad \text{(Eq. 6.12)}$$

6.4. CONCLUSIONS

Laboratory scale tests determined that for the LLE, pH 3 was the most appropriate in terms of recovery percentages. The initial concentration of VFA in the feeding had no influence on the recovery efficiency, which increased when the number of extraction stages did. Furthermore, it was observed that the presence of other VFA in the feeding had no influence on the results, being practically identical as when the experiments were executed with a single VFA in that stream.

At pilot scale (counter-flow mode), it was verified that compared to the batch tests, for the same number of theoretical stages, the recovery percentages were lower with both solvents tested (ethyl acetate and ethyl octanoate). This could be due to an incorrect calculation of the theoretical stage or to the occurrence of preferential flows inside the column. In fact, the results of the experimentation conducted after making the modifications to the column showed a great improvement in the recovery percentages achieved, reaching 100 % for all the VFA, in line with those reported in batch mode.

Comparing both solvents at pilot-scale before modifications' implementation, when applying the same S/F, the recovery percentages obtained with ethyl acetate were notably higher than with ethyl octanoate. With ethyl acetate, for S/F 1 kg/kg, the recovery of acetic was 70 %, propionic 98 % and butyric 100 %, while when the S/F was lowered to 0.5 the only difference was the recovery of acetic acid, which dropped up to 40 %. In any case, it was validated that the column improvements optimised the LLE process.

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Distillation simulations with ASPEN HYSYS revealed the need to incorporate Distillation 2 into the process when choosing ethyl acetate as solvent, so that the energy demands are higher when selecting this solvent. But more importantly, even though simulations were performed for a specific scenario to compare both solvents, they allow establishing a series of correlations through which the calorific consumptions of distillation columns were calculated based on the flow rate they received. These are of great help in Chapter 7 when it comes to estimating operational expenses (OPEX) of the whole VFA production process.

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7) GLOBAL PROCESS INTEGRATION

7.1. INTRODUCTION

In previous Chapters, both the fermentation and purification stages to produce high-purity VFA from SS and FW were presented. When seeking to obtain a final product, both steps must be combined, without losing sight of the fact that production from waste must be competitive from an economic point of view. Therefore, it is necessary to optimise the process from a global perspective, since the optimum of each unit may not be the optimum of the global process.

Figure 7.1 presents the simplified technological train of the technology developed within the framework of this doctoral thesis for the production of high-purity VFA from urban waste streams, being the same regardless of the substrate used, SS or FW. Firstly, the substrate (either pre-treated or not in the case of SS and crushed and diluted in the case of FW) is anaerobically fermented to produce and accumulate VFA. Subsequently, the fermented effluent undergoes a solid-liquid separation to obtain the solid cake as well as the liquid fraction in which the VFA are present as soluble compounds. The solid fraction obtained is subjected to an AD process to generate heat and electricity through the combustion of the biogas produced in a combined heat and power (CHP) engine and reduce the final amount of solids to be disposed. Regarding the liquid fraction, the VFA can be in concentrations around 1-3 % and purities higher than 90 % are required in the chemical industry. Therefore, the fermented effluent is pre-concentrated applying membrane technology and purified through LLE and distillation by separating the different VFA individually.

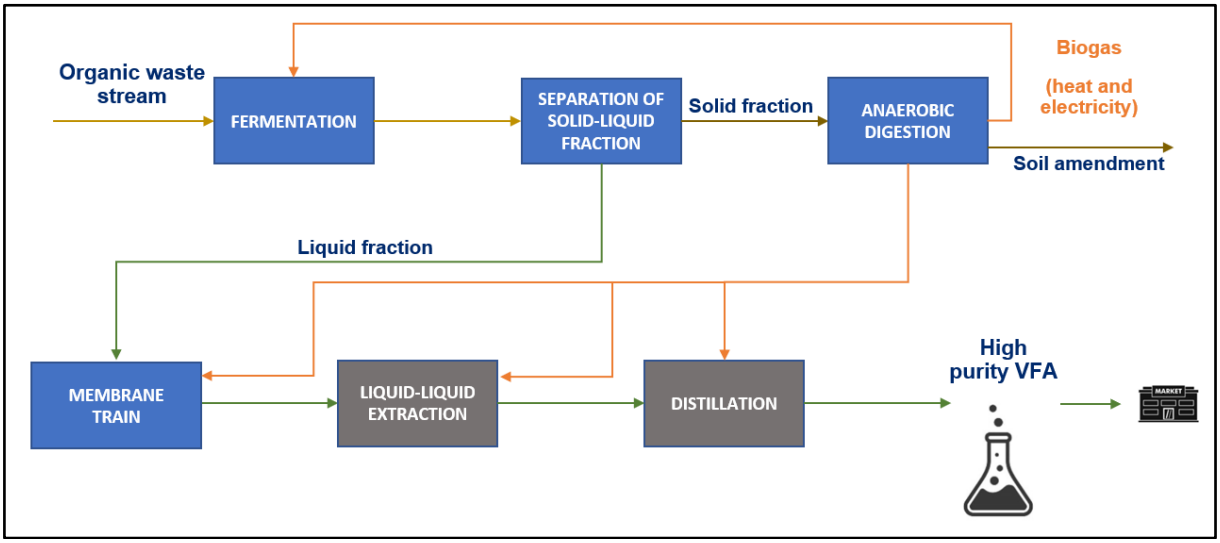

Figure 7.1. Simplified technological train to produce high-purity VFA from organic waste streams.

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The objective of this Chapter is to integrate all the stages of the process, as well as to optimise its operating conditions in order to study the economic feasibility and the industrial viability of the VFA production technology.

7.2. METHODOLOGY: PROCESS SIMULATION AND OPTIMISATION TOOL

The proposed process is shown more in detail in the block diagram of Figure 7.2 (in this case using ethyl acetate as solvent). In order to determine the production potential of VFA from the chosen substrates, a tool was developed in a Microsoft Excel spreadsheet which includes all the mass and energy balances of the global process, integrating the experimental data obtained in the fermentation and in the purification steps with some coming from scientific sources.

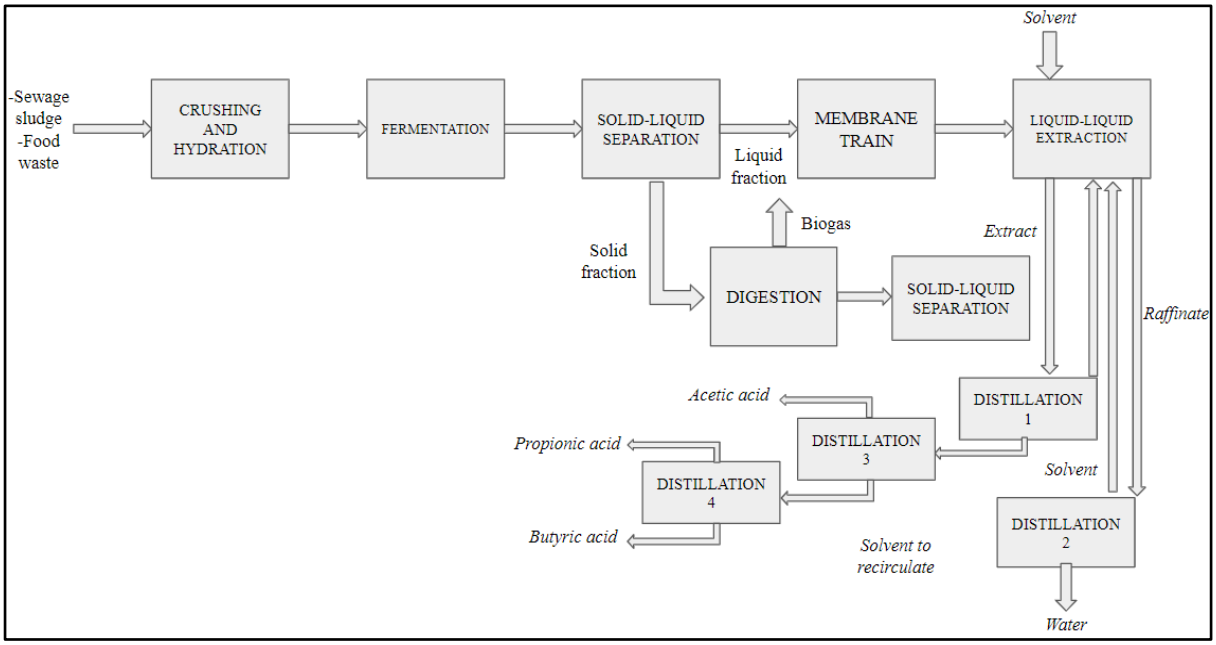

Figure 7.2. Block diagram of the technological train proposed for VFA production and purification (with ethyl acetate).

It was assumed that both SS or FW fed into the process present 20 % TS, respectively. Both substrates are diluted and hydrated up to 7 % TS before being fed to the mesophilic fermenter. Additionally, FW needs to be previously crushed. Subsequently, the fermented effluent is separated into its solid and liquid fractions in a centrifugation process by adding a coagulant and a flocculant. The liquid fraction containing the diluted VFA is subjected to UF and NF to remove soluble impurities such as proteins, sugars, etc. After this, the VFA-rich stream is pre-concentrated in the rejection of the RO membrane. For membrane cleaning, the clean-in-place (CIP) method is used with its related reagent consumption.

After membrane treatment, the pre-concentrated VFA solution is subjected to LLE and distillation processes to remove water and recover a mix of high-purity VFA. This mix is fed to successive distillation processes to separate individual fractions of VFA ready to commercialise.

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Regarding the solid fraction obtained after centrifugation, it is fed to a mesophilic anaerobic digester together with the rejection of UF and NF membranes and RO permeate to produce biogas. This biogas is at first sent to a boiler to cover thermal needs of the fermentation and distillation processes. Biogas in excess is sent to a CHP engine to produce the electricity necessary for the overall process. Finally, another solid-liquid separation is necessary in order to obtain the final digested sludge which has to be managed.

7.2.1. Substrate availability and fermentation/digestion assumptions

Different city sizes were selected to carry out the techno-economic study of VFA biorefineries. To calculate the amount of substrate available in each case, they were considered 70 g of TS per capita per day of SS and 90 g of TS per capita per day of FW (Battista et al., (2020)).

In the case of FW collection, different authors have determined that with optimised and effective selective collection systems, the maximum achievable amount of FW which could be picked up in urban environments results around 85 % (Jain et al., 2019). This percentage was based on the collection rate achieved in Milano (Italy), one of the first cities to implement separate household food collection, but it must be noted that not all countries still have a well-implemented food scrap collection strategy, so an average of 25 % could be quite realistic today (a large part of FW is still inappropriate or deposited in the wrong container).

The VFA yields considered for the study were 12 % g COD-VFA/g COD for SS and 37 % g COD-VFA/g COD for FW, obtained from pilot plant operation at pH 8.5 and pH 7, respectively (Table 3.5 and 4.2). Regarding VFA production selectivity, it results crucial on the process economic evaluation because of the different sale price of the VFA. This spectrum differs notably between SS and FW, being 80 % acetic acid, 10 % propionic and 10 % butyric acid for SS and 40 % acetic, 20 % propionic and 40 % butyric for FW (mass basis).

Methane production from the solid cake obtained after the solid-liquid separation (35 % TS) was determined through a biomethane potential (BMP) test. This was conducted at 37 ºC in bottles with a useful volume of 0.5 L, following the protocol described by Holliger et al. (2016). The inoculum (coming from a WWTP's anaerobic digester) was degassed for 1 day in order to eliminate residual organic matter. A ratio of 2 g VS inoculum/g VS substrate was used. Three blanks were included with inoculum, without addition of substrate. The bottles were purged with N_2 to ensure anaerobic conditions and incubated under gentle agitation (75 rpm), adjusting the initial pH between 7.2 and 7.8. Methane production during the experiment was continuously monitored, using a volumetric method (AMPTS II equipment, Bioprocess Control). The trial was stopped when the production of methane for three consecutive days was less than 0.5 % of the accumulated production (on day 23 for SS and 19 for FW), following the criterion proposed by Holliger et al. (2016). All tests were performed in triplicate.

Figure 7.3 shows the results of the BMP tests. For SS, a final value of 184 L(N) CH_4/kg VS was obtained while for the FW the value was higher, specifically 447 L(N) CH_4/kg VS. These figures were considered for the techno-economic study.

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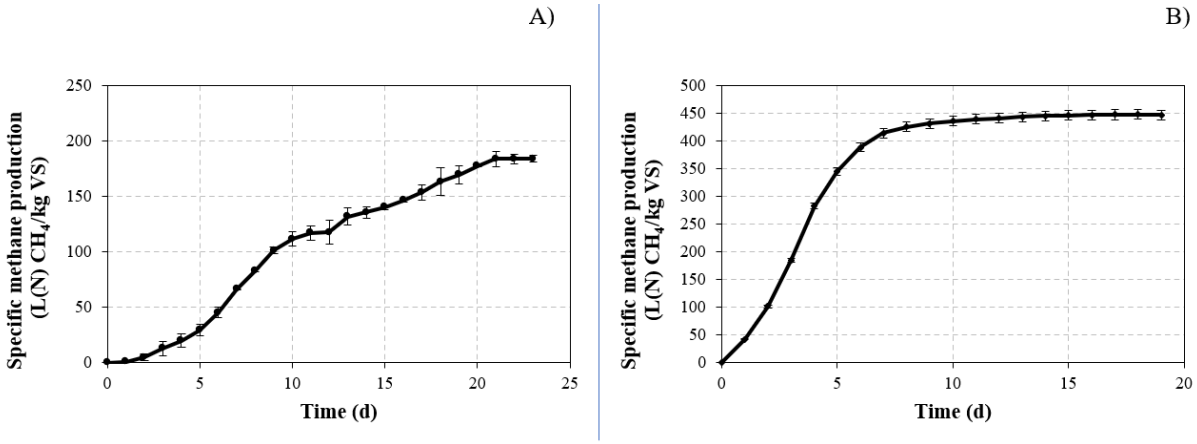

Figure 7.3. Specific methane production of the solid cake after solid-liquid separation. A) Sewage sludge. B) Food waste.

7.2.2. Energy balances

All data obtained experimentally from the overall process (VFA yields and selectivity of the fermentation, consumption of chemicals for pH control and separation of solid-liquid fractions, data from membrane treatment, recovery percentages in the LLE, etc.) were combined with the distillation process simulations.

Once the mass balances and energy demand for the whole process were established, biogas production was calculated. The thermal energy generated in the boiler was quantified through Eq. 7.1:

$$E_{c,cal} = Q_{biogas} \cdot \%_{boiler} \cdot LCV \cdot \eta_{boiler} \cdot \eta_{heat\ exchanger} \quad \text{(Eq. 7.1)}$$

where $E_{c,cal}$ is the thermal energy generated in the boiler (MWh/d), Q_{biogas} is the biogas flow (Nm^3/d), $\%_{boiler}$ is the biogas percentage sent to the boiler, LCV is the lower calorific value of biogas (0.006 MWh/Nm^3) and η_{boiler} as well as $\eta_{heat\ exchanger}$ are their calorific yields (assumed as 0.9 for both).

The thermal energy generated in the CHP engine and transmitted to a fluid through an exchanger was calculated from Eq. 7.2:

$$E_{c,CHP} = Q_{biogas} \cdot (1 - \%_{boiler}) \cdot LCV \cdot \eta_{heat\ exchanger} \cdot \eta_{CHP} \quad \text{(Eq. 7.2)}$$

where $E_{c,CHP}$ is the thermal energy generated in the CHP engine and η_{CHP} is the yield of the motogenerator (assumed as 0.35).

The total generated thermal energy ($E_{c,}$) was calculated with Eq. 7.3:

$$E_c = Q_{biogas} \cdot LCV \cdot \eta_{heat\ exchanger} \cdot [(1 - \%_{boiler}) \cdot \eta_{CHP} + \%_{boiler} \cdot \eta_{boiler}] \quad \text{(Eq. 7.3)}$$

The electrical energy generated in CHP engine was calculated with Eq. 7.4:

$$E_{e,CHP} = Q_{biogas} \cdot LCV \cdot \eta_{CHPe} \quad \text{(Eq. 7.4)}$$

where η_{CHPe} is the yield of CHP engine for electricity production (assumed as 0.35).

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7.2.3. Operational expenditures

The tool developed, in addition to calculating the VFA flows produced as well as the energy needs of the equipment, includes a module which allows obtaining the OPEX corresponding to VFA biorefineries treating the waste flows of a given equivalent population.

In order to calculate OPEX, a list of inputs and outputs to the VFA production biorefinery was created. The cost of staff was estimated through the number of employees needed and their salaries (Turton et al., 2009). The necessary energy was divided into necessary thermal demand and electrical one. As it was previously specified, the first is required for heating the fermenter and the digester, as well as for the distillation columns, and the second one for pumping and equipment operation. Once these thermal needs were covered through the energy produced by the boiler, the excess biogas would be used to feed the CHP engine to cover the electricity demand; in case of surplus electricity, it would be sold.

The chemical expenses consider NaOH used to control fermentation pH; HCl to adjust pH before membrane treatment; chemicals needed for coagulation/flocculation during the solid-liquid separations (obtained experimentally in pilot plant's operation) as well as solvent losses. Chemical doses and their respective prices for each substrate are indicated in Table 7.1.

In order to calculate the chemicals needed for membranes, both hydraulic and chemical cleanings (with NaOH and HCl, both prepared at 1 % (v/v)) were considered. The experimental assays presented in Chapter 5 indicated that around 10 L/m^2 of membrane and a frequency of 1 cleaning/day were needed (except for UF membrane, which additionally required hydraulic cleaning due to its greater fouling, in this case 20 L/m^2 with a frequency of 6 times/day).

Moreover, the transport of wastes to the facility, the distribution of the final product, the costs of renovation and maintenance and other indirect costs were also contemplated. Table 7.1 indicates in detail how each of the OPEX of the process was determined.

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Table 7.1. Assumptions made for the calculation of operational expenditures.

ITEM	Assumption	Source
Staff	For treatment capacity between 0-400,000 inhabitants: 1 plant manager, 1 commercial and 4 operators; from 400,000-1,500,000: 1 plant manager, 1 controller, 1 sales manager, 2 commercials, 2 administratives and 6 operators; from 1,500,000-3,000,000: 1 director, 2 sales managers, 2 controllers, 2 production leaders, 2 commercials, 4 administratives and 10 operators	Estimation of staff and salaries based on Turton et al. (2009)
Energy	0.021 MWh/m^3 for fermenter/digester heating (10 % of heat losses). Distillation heat energy based on ASPEN HYSYS simulations (calculations through the correlations shown in Chapter 6). Crusher consumption: 20 kWh/t; membrane consumptions: 2 kWh/m^3 (UF) and 3 kWh/m^3 (NF and RO). S-L separation: 20 kWh/t TS. Pumping: 0.45 kWh/m^3. Electricity for LLE and distillation: 0.50 kWh/m^3. Agitation fermenter/digester: 3 W/m^3 digester	Schultz et al. (2023) and Metcalf & Eddy (2014)
Chemicals (coagulation/flocculation and dehydration)	Coagulant dose (after fermentation): 2,500 ppm and 500 ppm (1 %) for SS and FW, respectively (2,500 €/t). Flocculant dose (after fermentation): 1,500 and 400 ppm for SS and FW, respectively (2,500 €/t). Coagulant dose (after digestion): 12.7 kg/t DM, both for SS and FW	Chemicals doses used in pilot plant operation with both substrates. Prices provided by suppliers
Solvent	Ethyl acetate: 1,000 €/t Ethyl octanoate: 10,000 €/t	Webpage of Chemanalyst (2024)
Chemicals pH control and membrane cleaning	Fermentation pH: 8.5 for SS and 7 for FW (consumption of 0.003 t NaOH/m^3 SS and 0.002 t NaOH/m^3 FW). 800 €/t NaOH (50 % w/v) and 1,000 €/t HCl (37 % v/v). For membranes, cleaning frequency of 6 hydraulic cleaning/d and 1 chemical cleaning/d (UF) and 1 chemical cleaning/d (NF and RO)	Chemical consumption based on experimentation and supplier purchase price for costs. Estimation based on experimentation for membrane cleaning
Transport raw material	0.06 €/(km·t); 0.1 €/t; 140 km of mean distance	Reasonable figures assumed
Waste management	15 €/t	Average management cost in Galicia (Spain)
Renovation and maintenance	5 % of CAPEX except for buildings cost	Movahed, (2010)
Other indirect costs	10 % of the total OPEX	Turton et al. (2009)

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7.2.4. Determination of optimum operating conditions

The developed tool optimises the global process through the identification of the most favourable operating conditions for the purification stage. This optimisation involves finding on the one hand, the most economically advantageous solvent (ethyl octanoate or ethyl acetate) and, on the other hand, the optimal S/F ratio. It should be noted that this optimum S/F ratio may differ depending on the substrate due to the different selectivity of the fermentation process.

In order to economically compare the overall process with both solvents and their associated S/F ratios, the tool calculates the "profit margin" of the installation for year 1, obtained through Eq. 7.5, which subtracts the earnings of the plant minus the costs (without considering those OPEX items which are invariant when changing the solvent and/or the S/F ratio). With the aim of conducting the comparison, the amount of SS and FW generated by 300,000 inhabitants was selected (medium-sized city), considering the recovery percentages for LLE depicted in Figures 6.19 and 6.20.

$$Profit\ margin = (Earnings - Costs) = (VFA + Energy + Solvent) \quad \text{(Eq. 7.5)}$$

where *Profit margin* is expressed in €, being *VFA* always earnings for VFA selling and *Solvent* a cost derived from the purchase of the required amount of solvent. As for the term *Energy*, it could be positive or negative depending on whether electricity would be sold to the grid or whether heat and electrical energy need to be acquired.

Table 7.4 depicts VFA sale price and Table 7.1 the solvent cost. The term energy is more complex given that the process requires both heat and electrical energy. Specifically, the heat energy required for both heating the fermenters and the digesters, as well as the different distillations columns was quantified (assumptions shown in Table 7.1). Thus, this heat demand was attempted to be supplied by biogas burned in the boiler from the valorisation of the solid fraction after solid-liquid separation (if even so the heat demand was not supplied, heat energy would be purchased at a price of 46.7 €/MWh corresponding to the cost of natural gas). Regarding electrical energy, the amount needed for pumping, membranes, purification processes, agitation and returns must be purchased, although if the heating needs were met with the boiler, the remaining biogas would be fed to the CHP engine to reduce said electrical demand (if there was still excess, it would be sold at the price shown in Table 7.4 generating an extra income).

7.2.5. Capital expenditure

The tool also provides the capital expenditure (CAPEX) associated with the facilities. In order to calculate it, a method of factored estimation was applied, existing the possibility of including uncertainties of up to 30 %. The main pieces of equipment necessary for the construction of the plant were searched first in the literature (Turton et al., 2009), to later use the Eq. 7.6 proposed by Smith et al. (2005) aiming to scale the equipment costs to the amount of substrate sought in each case:

$$Cost_a = Cost_b \cdot \left(\frac{Flow_a}{Flow_b}\right)^n \quad \text{(Eq. 7.6)}$$

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where $Cost_a$ refers to the equipment to treat a given $Flow_a$ and ^{n} is a scaling exponent which can be found in the chemical industry between 0.5 and 0.8, although in this work a factor of 0.7 was applied, the most frequently used.

Table 7.2 details the selected references to calculate the CAPEX of each of the units which would be part of the VFA biorefinery. It should be noted that for the cost of the equipment, the bare module cost was considered, which was multiplied by a factor of 1.5 in order to include the following additional costs: previous actions and land movement, treatment of headworks return, deodorisation, urbanisation and networks, auxiliary equipment and services, electrical installation and control building and workshop. Turton et al. (2009) and Morales-Vera et al. (2020) applied the same procedure in their works. It should be noted that all costs to build a completely new facility were included except land acquisition.

Table 7.2. Assumptions made for the calculation of equipment and capital expenditure.

ITEM	Assumption	Source
Reception/pre-treatment/crushing (FW)	1 M€ for treating 414,000 t/y	Estimation based on a private project (confidential)
Fermentation/digestion	244,000 € for a CSTR of 2,550 m^3 (HRT of fermentation: 10 days; HRT of digestion: 25 days)	Nwobi, (2013)
Gasometer (5,000 m^3)	110,000 € for a unit of 1,200 m^3	Estimation based on market prices
Biogas boiler	65,000 € for a thermal power of 1000 kW	Website of Cerney boilers
CHP engine	530,000 € for a CHP unit of 600 kW	Website of Altare engines
Fermentation and digestion sludge dehydration	150,000 € for a dehydrator of 10 m^3/h	Technical Memo 8 (2019)
Membranes (UF/NF/RO)	1,000 €/(m^3·d) for membranes of 40 LMH. Permeability of 12 LMH (UF), 16 LMH (NF) and 14 LMH (RO) according to Figures 5.19, 5.20 and 5.21. Membrane price of 40 €/m^2	Guo et al. (2014), Yirmibesoglu, (2016) and Movahed, (2010)
LLE column	Estimation made according to equipment casing mass (application of factorial method)	Quintana-Álvarez (2023)
Distillation columns	Estimation made according to equipment casing mass (application of factorial method)	Quintana-Álvarez (2023)
Stainless steel solvent tank (20 m^3)	5,000 € for a 20 m^3-tank	Website of Usalab (2024)
Commissioning	10 % of the equipment	Bonk et al. (2015)

CAPEX of LLE column was evaluated following the procedure of the factorial method, which is in a high degree of development and is currently the most applied according to Sinnot and Towler, (2007). Therefore, Eq. 7.7 was used in order to follow this procedure:

$$C_e = a + b \cdot S^n \qquad \text{(Eq. 7.7)}$$

where C_e is the equipment cost (€), *a, b* and *n* are factors which depend on the evaluated equipment and *S* is supported equipment design parameter, whose units vary depending on the variable, being a differential design variable for each equipment unit.

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Equipment cost should be corrected according to operation conditions as indicated in Eq. 7.8:

$$C_e = (a + b \cdot S^n) \cdot f_m \cdot f_p \cdot f_t \quad \text{(Eq. 7.8)}$$

where f_m is the correction factor depending on the material used, f_p is the correction factor depending on the operating pressure and f_t is the correction factor depending on the operating temperature. All these correction factors are indicated in the work of Sinnot and Towler, (2007).

As it was defined in Table 7.2, the CAPEX of the LLE and distillation columns were calculated from the mass of the equipment casing through Eq. 7.9:

$$m_{case} = \pi \cdot D \cdot H \cdot t \cdot \rho \quad \text{(Eq. 7.9)}$$

where m_{case} is the mass of the case (kg), D is the diameter of the column (m) (Eq. 6.2 of the previous Chapter for LLE column), H is the column height (number of equivalent stages multiplied by 0.5), t is the case thickness (assumed as 0.007 m given the operating pressure), and ρ is the steel density (7,700 kg/m^3).

Regarding the calculation of CAPEX of the distillation columns, Eq. 7.9 was also used, being the height of each theoretical stage also 0.5. In this case, the diameter calculation was obtained through Eq. 7.10:

$$D_c = \sqrt{\frac{4 \cdot A_C}{\pi}} \quad \text{(Eq. 7.10)}$$

where D_c is the distillation column diameter (the same for enrichment and depletion sections) and A_c is the total section of the column (m^2), calculated with Eq. 7.11:

$$A_c = \frac{A_n}{(1 - \%_{downspout})} \quad \text{(Eq. 7.11)}$$

where A_n is the net area of column section (m^2) calculated with Eq. 7.12 and $\%_{downspout}$ is estimated as 12 % of the A_n:

$$A_n = \frac{Q_{vmax}}{u_n} \quad \text{(Eq. 7.12)}$$

where Q_{vmax} is the steam flow rate which passes through each section of the column, heads and tails (m^3/s), being calculated from Eq. 7.13 and u_n is the net vapour velocity (Eq. 7.14).

$$Q_{vmax} = \frac{V_W}{\rho_V} \quad \text{(Eq. 7.13)}$$

where is the vapour mass flow (kg/s) and ρ_V is vapour density (kg/m^3).

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$$u_n = u_f \cdot \%_{flooding} \quad \text{(Eq. 7.14)}$$

where u_f is the flooding velocity based on net cross-sectional area of column (m/s) calculated with Eq. 7.15:

$$u_f = K_1 \cdot \sqrt{\frac{\rho_L - \rho_V}{\rho_V}} \quad \text{(Eq. 7.15)}$$

where K_1 is constant obtained from a correlation between vapor-liquid factor (F_{LV} calculated with Eq. 7.16) and the spacing between plates. This correlation to obtain K_1 can be found in Smith et al. (2005).

$$F_{LV} = \frac{L_W}{V_W} \cdot \sqrt{\frac{\rho_V}{\rho_L}} \quad \text{(Eq. 7.16)}$$

where F_{LV} is the vapour-liquid circulation factor (kg), L_W is the liquid mass flow (kg/s), V_W is the vapour mass flow (kg/s), ρ_L is liquid density (kg/m^3) and ρ_V is vapour density (kg/m^3). The values of these densities for each distillation column (Figure 6.24) are listed in Table 7.3.

Table 7.3. Density values in the different distillation columns. Source: ASPEN HYSYS simulations.

	Ethyl acetate		Ethyl octanoate	
	ρL (kg/m^3)	ρv (kg/m^3)	ρL (kg/m^3)	ρv (kg/m^3)
Distillation 1 heads	807	4.72	842	1.38
Distillation 1 tails	816	1.94	796	3.33
Distillation 2 heads	845	2.59	-	-
Distillation 2 tails	948	0.59	-	-
Distillation 3 heads	938	1.88	941	1.89
Distillation 3 tails	741	2.26	739	2.28
Distillation 4 heads	844	2.18	841	2.19
Distillation 4 tails	677	2.48	677	2.48

Furthermore, to the cost of the LLE column itself it must added the cost of the packing (calculating its volume through Eq. 7.17) and to the distillation columns the cost of the plates (calculated from Eq. 7.8), knowing that their diameter would be the same as that of the column.

$$V = \frac{D^2}{4} \cdot \pi \cdot H \quad \text{(Eq. 7.17)}$$

where V is packing volume (m^3).

The cost of all the equipment shown in the Table 7.2 was already updated considering the date of consultation of the sources, except that of LLE and distillation in which bibliography based on historical data was used for CAPEX calculation. For this reason, a cost update was conducted through the variation of indices published annually by *Chemical Engineering Plant Cost Index (CEPCI)*. Thus, updated costs were calculated through Eq. 7.18:

$$C_{e,2023} = \frac{I_{ce,2023}}{I_{ce,2006}} \cdot I_{ce,2006} \quad \text{(Eq. 7.18)}$$

where $C_{ce,2023}$ is the cost in year 2023 and $I_{ce,2006}$ is the update index in year 2006, whose values are 478.60 and 768.55 according to *CEPCI*, 2023.

7.2.6. Economic evaluation

The most suitable economic indicators for evaluate the profitability of this type of projects are the net present value (NPV), the internal rate of return (IRR) and the payback. These were calculated for the proposed VFA production biorefineries corresponding to each specific population and for each one of the substrates studied, SS and FW.

NPV is a key economic indicator which defines if an investment would be profitable or not and it was proposed as appropriate for evaluating this type of projects as Faaij et al. (1997) or Bastidas-Oyanedel and Schmidt (2018) highlighted in their works. NPV, when is equal to 0, means that the investment would not generate losses or profits, while the higher it is above 0, the greater the profitability of the project. For the NPV calculation, Eq. 7.19 was used:

$$NPV = -CAPEX + (FCF_1)/(1+i)^1 + \ldots + (FCF_{20})(1+i)^{20} \quad \text{(Eq. 7.19)}$$

where *CAPEX* calculation's methodology was defined before (Table 7.2), *FCF* is the free cash flow for a specific year which in this case is equal to the Earnings Before Interest, Taxes, Depreciation and Amortisation (*EBITDA*) (Eq. 7.20) and *i* is the discount rate (assumed as 10 %).

$$EBITDA = E_{VFA} + E_{elec} + E_{wm} - OPEX - amort \quad \text{(Eq. 7.20)}$$

where E_{VFA} are the earnings for VFA selling (€/year), E_{elec} are the earning in case of electricity selling (€/year), E_{wm} are the earnings for waste management of substrates (€/year), *OPEX* are the total operational cost of the facility and *amort* is the cost of amortisation in twenty years (CAPEX/20), being this reasonable when referring to medium to large chemical production plants (Turton et al., 2009).

The economic indicator NPV served as a reference to define the so-called minimum viable scale in view of the treatment capacity for which the VFA biorefinery would be viable. It should be noted that in section 7.2.4, OPEX items were used to calculate the profit margin, but at no time did CAPEX come into play. Instead, the minimum viable scale is determined by this parameter since the economic parameter NPV was used to define said scale as Eq. 7.19 showed.

IRR, being an economic indicator closely related to NPV was calculated with Eq. 7.21:

$$IRR = (FCF_1)/(1+i)^1 + \ldots + (FCF_{20})(1+i)^{20} = 0 \quad \text{(Eq. 7.21)}$$

where *FCF* is the free cash flow for a specific year, coinciding with *EBITDA*.

Regarding the calculation of payback or return time of the investment, it was obtained with the following formula (Eq. 7.22):

$$Payback = (Last\ year\ with\ negative\ FCF) + (|\ Last\ negative\ FCF|/FCF\ next\ year) \quad \text{(Eq. 7.22)}$$

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For conducting the economic evaluation, some financial parameters were considered, which are shown in Table 7.4, all very similar to those used by Veluswamy et al. (2021) and Bastidas-Oyanedel and Schmidt (2018) in their works where they compared the production of VFA against biogas. In addition, once the most favourable conditions for purification were selected following the methodology detailed in 7.2.4, the VFA recovery percentages achieved in the modified LLE column were assumed (Figure 6.21) for being the highest.

Table 7.4. Summary of financial parameters used for economic evaluation.

	Parameter	Value
1	Type of financing	French system
2	Payment frequency	Yearly
3	Nominal interest rate	4.5 %
4	Project life	20 years
5	General revenue and cost variance	2 % yearly
6	Tax	25 %
7	Discount rate	10 %
8	Amortisation	CAPEX/Project life
10	Electricity price	150 €/MWh
11	Acetic acid selling price	1,500 €/t*
12	Propionic acid selling price	2,000 €/t*
13	Butyric acid selling price	2,800 €/t*
14	Waste management fee (entry to the process)	20 €/t
15	Final waste management fee	15 €/t

*VFA selling prices: International Trade Centre (2022).

7.3. RESULTS AND DISCUSSION

7.3.1. Solvent selection and optimum conditions identification

Figure 7.4 shows the comparison between solvents for SS and clearly manifests how the profit margin with ethyl acetate is higher to that obtained with ethyl octanoate regardless of the S/F ratio chosen. This is because the recovery percentages in the LLE reported with this solvent were superior (Figure 6.22), which is reflected in a much higher VFA production revenue. With ethyl octanoate (Figure 7.4A), the variation of the profit margin with the S/F is not very significant, since as the S/F increases so does the income from VFA sale slightly, but at the same time the energy and solvent expenditure also rise. In any case, the optimal S/F with ethyl octanoate would be 2 kg/kg, with a profit margin of 193,000 € compared to the 180,000 € obtained with S/F 1 kg/kg.

Figure 7.4B, analogue of 7.4A but with ethyl acetate as solvent, shows how the expenditure on solvent is insignificant when it comes to representing it in comparison with the other items. As the applied S/F ratio increased, so did the energy expenditure, given that the solvent flows increase throughout the process, requiring pumping and distillation to obtain the individualised VFA with the energy costs which this entails. On the other hand, the income related to the VFA sale did not increase when going from S/F 0.75 to 1, since the recovery percentages were very

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similar. That is why, when SS was used as a substrate, the S/F ratio 0.75 kg/kg has turned out to be optimal, given that it is the one that would imply the greatest profit margin (818,035 €).

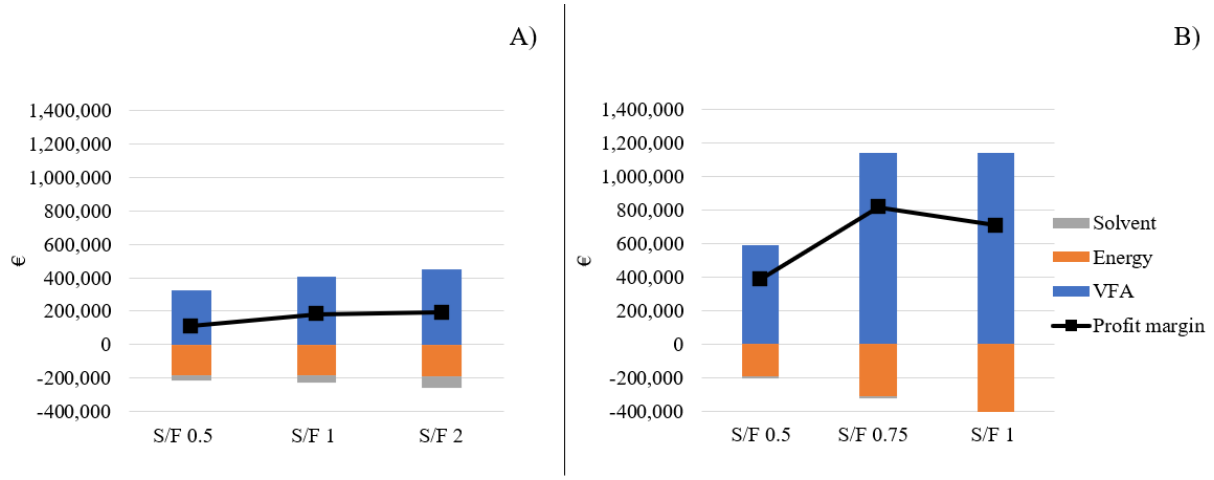

Figure 7.4. Variation of the profit margin with S/F for sewage sludge. A) Ethyl octanoate. B) Ethyl acetate.

Figure 7.5 shows how the profit margin with FW as substrate was higher than with SS, above all with ethyl octanoate, with profit margins between 700,000 € in comparison with those close to 200,000 € with SS. With FW, the process was not only able to supply its own heat and electricity needs, but also selling it to the grid, providing an extra income (orange bar). On this occasion, as with SS, choosing ethyl acetate involved higher profit margins than ethyl octanoate. Figure 7.5A indicates that the highest profit margin with octanoate was obtained with S/F 2 kg/kg (795,000 €), while with ethyl acetate (Figure 7.5B) the most favourable S/F was 0.75 kg/kg (987,000 €), although closely followed by S/F 1 kg/kg (985,500 €). In any case, it was validated that with both wastes the optimum was obtained with the same solvent (ethyl acetate) and the same S/F (0.75 kg/kg).

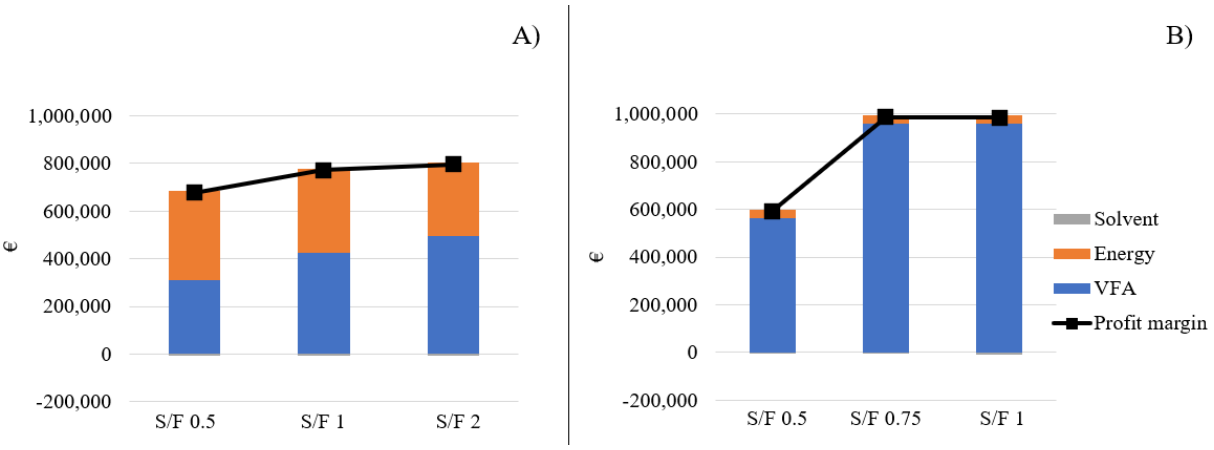

Figure 7.5. Variation of the profit margin with S/F for food waste. A) Ethyl octanoate. B) Ethyl acetate.

In view of the results of this section, from now on all the results presented will be with ethyl acetate once its greater suitability was demonstrated both from a purely technical and economic point of view.

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7.3.2. Operational expenses calculation

Figure 7.6 shows the variation of VFA production with plants treating the SS and FW generated by a specific population. Moreover, it represents the variation of OPEX with the number of inhabitants. As it can be seen, for the same number of inhabitants, the production of VFA from SS is higher than with FW due to the availability of substrate. For example, for a biorefinery treating the waste generated by 1,000,000 inhabitants, 2,336 t VFA/year would be produced with SS and 1,663 t VFA/year with FW. In contrast, the OPEX for SS would be 11.2 M€/year, while for FW it would be around 2.2 M€/year. This means that although the VFA production from SS is almost double that from FW for the same number of inhabitants, the OPEX is 5 times higher, which will be reflected in the economic indicators.

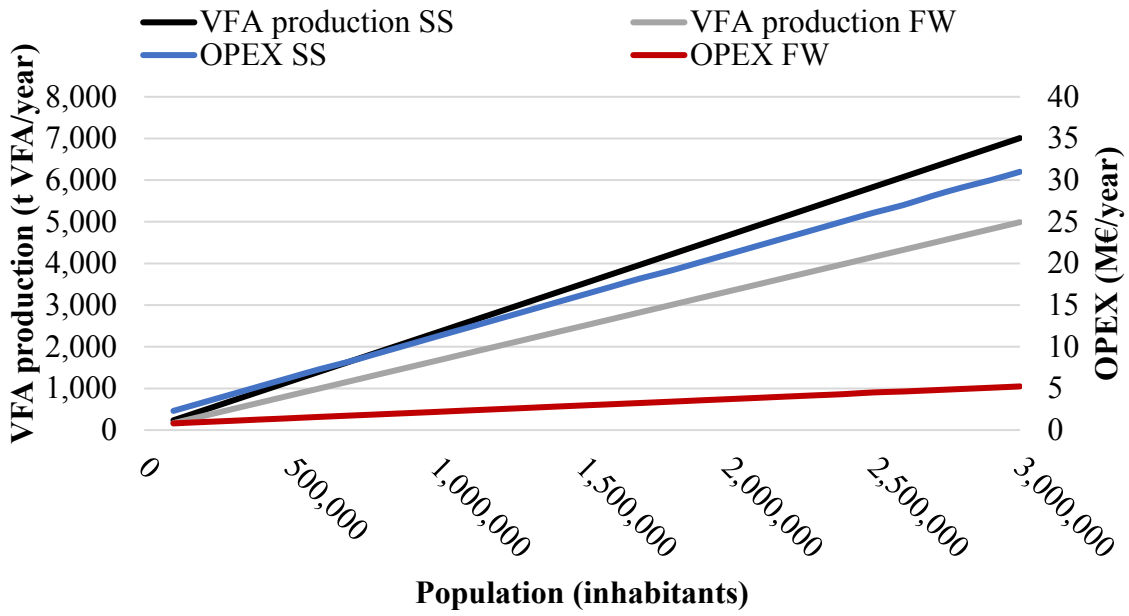

Figure 7.6. Variation of VFA production and operational expenditures with population for both substrates.

Regarding the OPEX distribution (Figure 7.7), it was observed that the expenditure on chemicals for cleaning membranes is the most important, accounting for 28 % of the total. Behind, it is the expense related to transporting the substrate to the plant, with 20 %, followed by the cost of chemicals for pH control in fermentation, with 10 % (total cost in chemicals rises to more than 50 % of the distribution including those for digestion). Staff expenditure also accounts for 10 % of the distribution. It is worth noting that the contribution of some OPEX items such as energy may vary with the scale (Figures 7.7, 7.8, 7.10 and 7.11 correspond to a plant treating the waste of 2,700,000 inhabitants).

When comparing this OPEX distribution sharing with other works such as that of Veluswamy et al. (2021), some differences could be identified, especially in the cost of chemicals (the membrane train operation is an important bottleneck in this process). Thus, both these authors and Biehl and Inman (2010) allocate around 15 % to maintenance, while in this case accounted for 6 %, and they allocate 16 % to chemicals, while the cost reported in Figure 7.7 is much higher. Regarding energy expenditure, in this case it is only 6 % given that the

installation of a CHP allows the generation of electrical energy, when this is not contemplated in their work (Biehl and Inman (2010) reported a 34 % in energy expenditure). Therefore, even though there are differences with the OPEX reported by other authors due to the particularities of each of the scenarios studied, in general they are quite similar, which provides veracity to the resulting economic indicators.

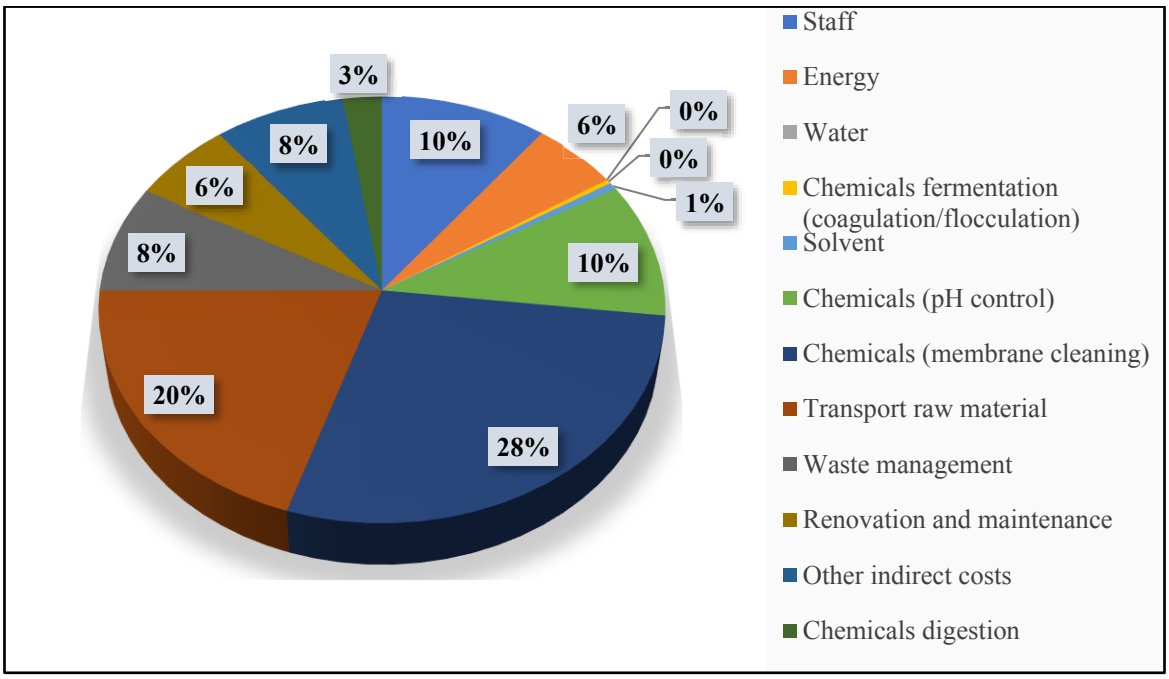

Figure 7.7. Operational expenditures distribution for a VFA biorefinery treating sewage sludge.

Figure 7.8 shows the OPEX distribution of a VFA biorefinery treating FW. Again, the most relevant item was the expenditure on chemicals, which includes both those for pH control (16 %) and membrane cleanings (namely HCl and NaOH) with 31 % of the sharing (total cost of chemicals including those of digestion accounts for 50 % of the total OPEX). Renovation and maintenance costs are also important, exceeding 21 %. After that, staff costs and waste management both account for 10 %, with the remaining ones being less important. This graph also shows the need to reduce either the number of cleanings or the ratio of cleaning solution volume/membrane surface with the aim of slashing this OPEX.

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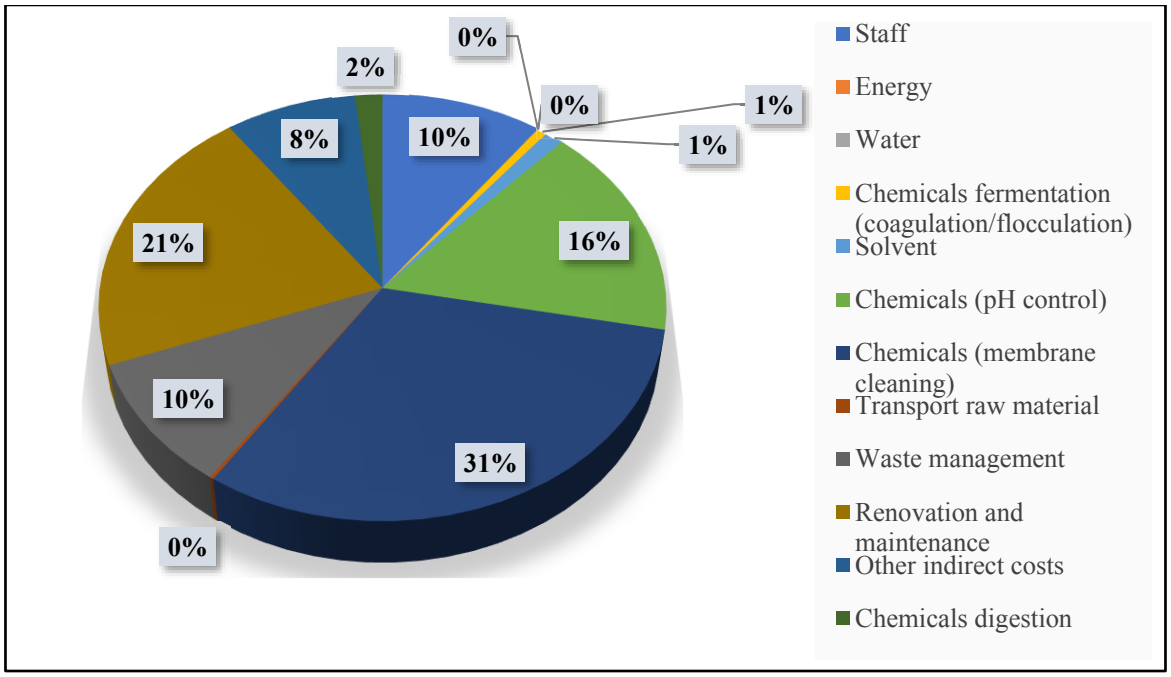

Figure 7.8. Operational expenditures distribution for a VFA biorefinery treating food waste (25 % selective collection).

7.3.3. Capital expenditure estimation

Analogous to what was represented in Figure 7.6 with OPEX, Figure 7.9 represents the variation of CAPEX with the number of inhabitants. It can be observed that a larger inhabitant's number implied a greater availability of substrate, and therefore, a greater production of VFA, with CAPEX being increased at their own rate. Thus, a plant to convert the SS generated by a city of 500,000 inhabitants into VFA would produce around 1,168 t VFA/year and would have a CAPEX of 12.7 M€. Instead, a plant treating the SS of 1,000,000 inhabitants would produce around 2,336 t VFA/year and would have a CAPEX of 21.4 M€. When looking at the data for FW, the same is true. Again, the effect of economy of scale on the variation of CAPEX was manifested. Thus, for example, for a plant treating the FW generated by a population of 500,000 inhabitants, a CAPEX of around 5.3 M€ would be necessary, whereas if the treatment capacity is doubled to 1,000,000 inhabitants, the CAPEX would increase to around 8.2 M€, but in no case double the initial value.

Figure 7.9 also indicates that the VFA production of the same number of inhabitants is higher with SS than with FW. This is because the production per capita of each of the substrates is similar, but the collection rate of FW considered is only 25 %. On the other hand, the CAPEX required for the plant treating FW is also much lower, which will significantly influence the calculation of the economic indicators.

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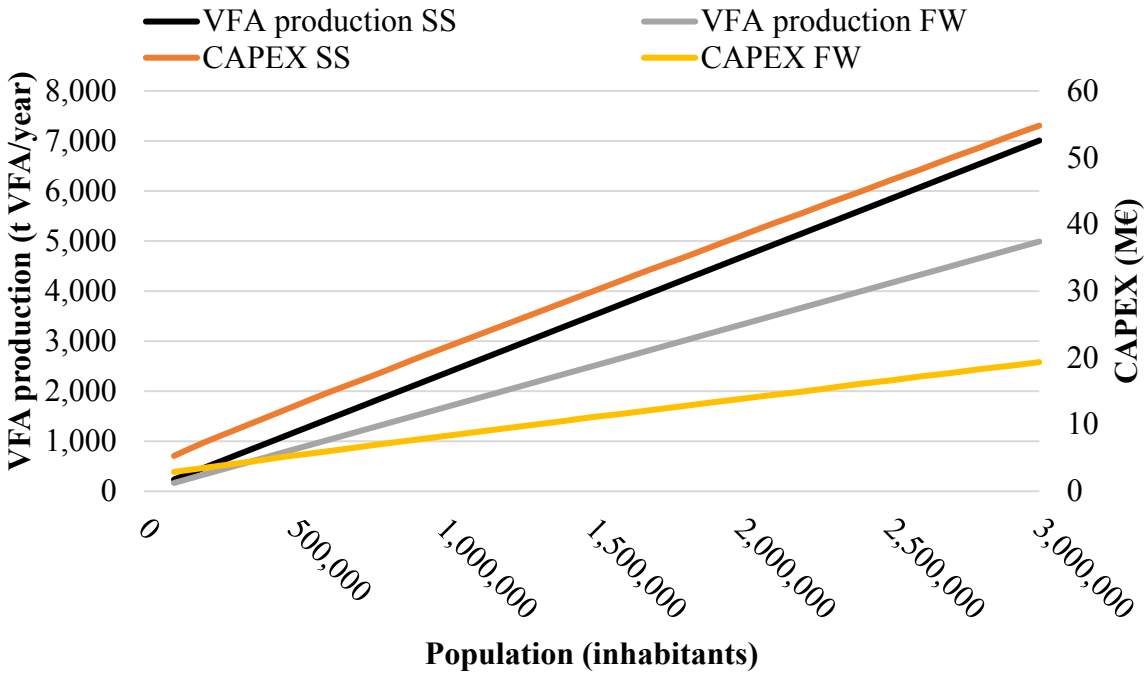

Figure 7.9. Variation of VFA production and capital expenditure with population for both substrates.

The distribution of CAPEX is independent of the scale of the installation and is presented in detail in Figure 7.10. It is observed that the most important cost is the membrane equipment, accounting for 47 % of the total, followed by the construction of the fermentation units with 16 %. Subsequently, the plant commissioning would entail 11 % followed by the digestion infrastructure with 9 %. The rest of the costs are much lower.

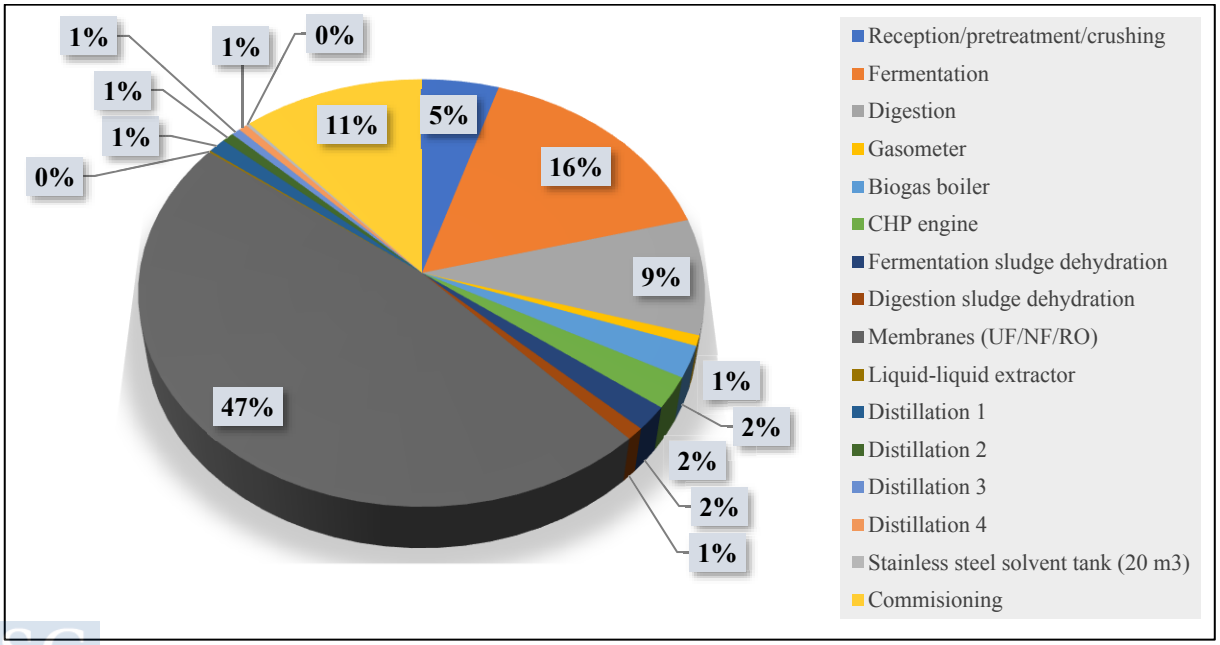

Figure 7.10. Capital expenditure distribution for a VFA biorefinery treating sewage sludge.

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Figure 7.11 shows that the distribution of CAPEX for a plant treating FW is very similar to the one reported for SS in Figure 7.10. Thus, the membrane cost represents around 43 % of the CAPEX, since considering the necessary filtration surface given the permeate fluxes obtained, the associated expenditure would be very high. The construction of the digesters would be in second place with 12 % of the CAPEX, followed by the cost of fermenter and commissioning (both with 10 %). The most noticeable difference with the CAPEX of that treating SS lies in the importance of the reception/pre-treatment/crushing stage, which in this case accounts for 10 %, double than with SS given the costs related to the crusher's acquisition. In any case, it is striking that membranes are the most important CAPEX, which once again highlights the influence of the low permeate fluxes obtained with the tested real effluent.

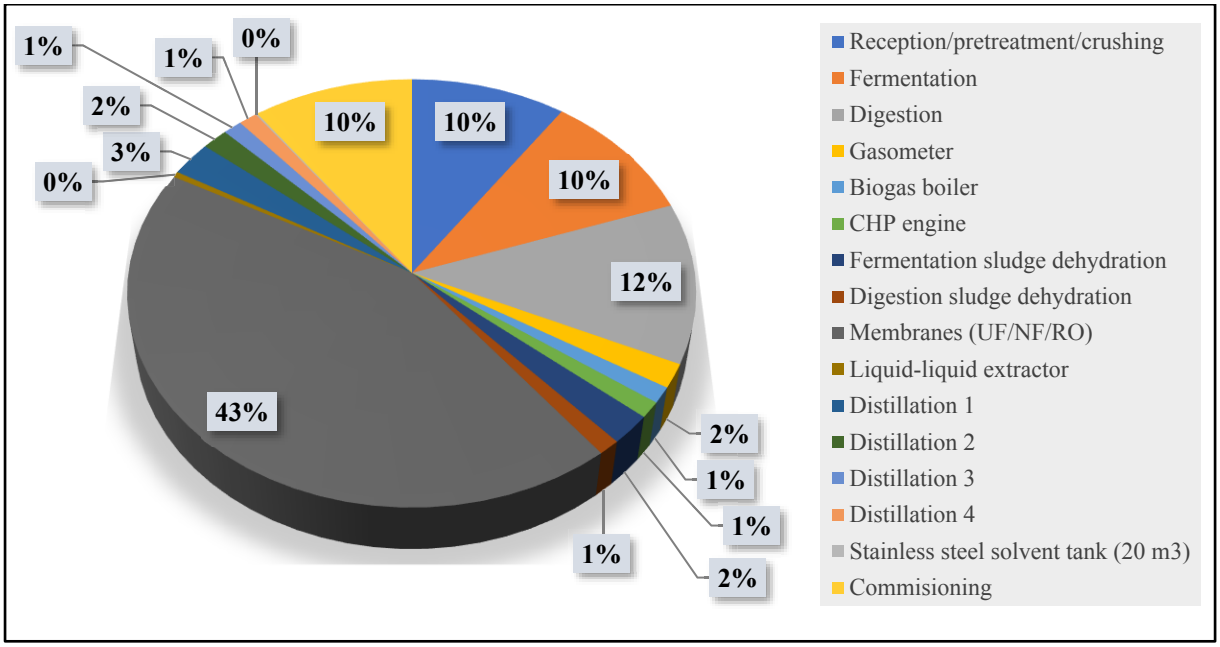

Figure 7.11. Capital expenditure distribution for a VFA biorefinery treating food waste (25 % selective collection).

7.3.4. Economic viability assessment: VFA biorefinery in Galicia

Figure 7.12 shows the variation of the payback and the NPV for the construction of a VFA biorefinery with the generation of SS from cities with different number of inhabitants. Thus, the minimum viable scale would be an installation treating the SS generated by a population of 871,500 inhabitants, given that this is the value for which the NPV=0 and for which the investment would be recovered in a payback of 20 years. As the number of inhabitants increases, so does the SS generated, therefore the payback decreases. The investment would be recovered in 10 years for the SS generated by a population of 2,000,000 inhabitants, which is quite ambitious for a project of this magnitude. Furthermore, it is observed that when the number of inhabitants is less than that of the minimum viable scale, the NPV begin to acquire negative values. Figure 7.12 also shows where the Galicia's VFA biorefinery from SS would be located, data which will be analysed in detail below.

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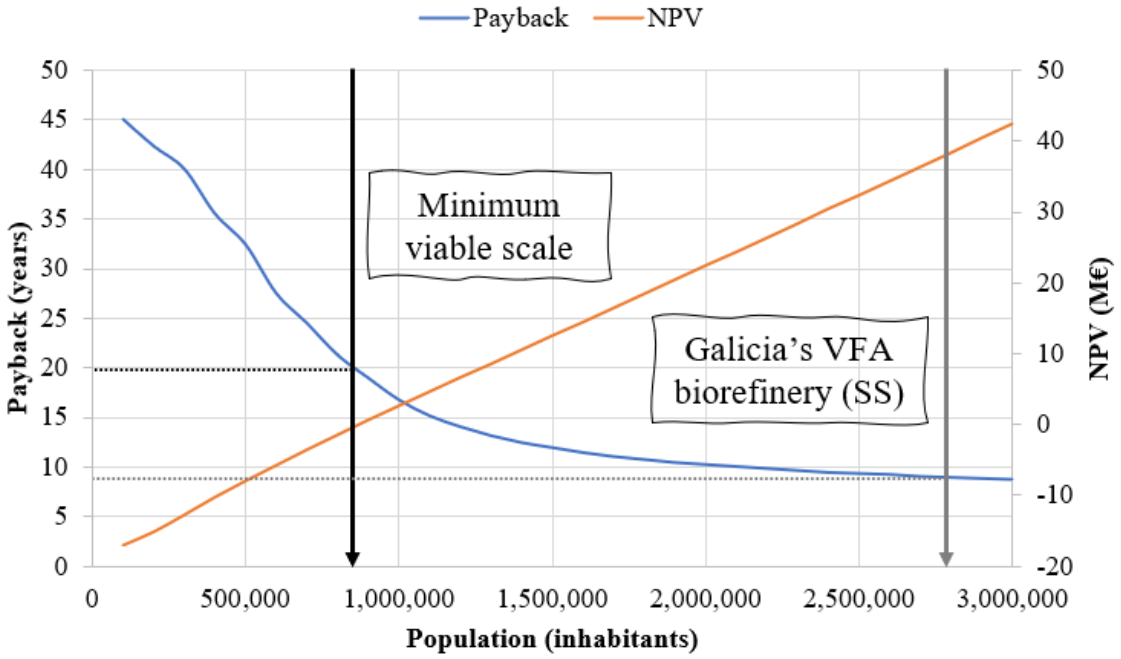

Figure 7.12. Economic indicators evolution with population for sewage sludge and for Galicia's biorefinery.

Figure 7.13 indicates that the minimum viable scale for a biorefinery treating FW would be that corresponding to the generation of 438,000 inhabitants, so a lower number would be needed to recover the investment in 20 years than in the case of total collection of the generated FW (110,000 inhabitants). In any case, considering the information provided by different research projects such as H2020 Waste2Road or INTERREG Sudoe Ecoval, this scenario would be much more objective. The economic indicators studied are also shown for Galicia's VFA biorefinery from FW.

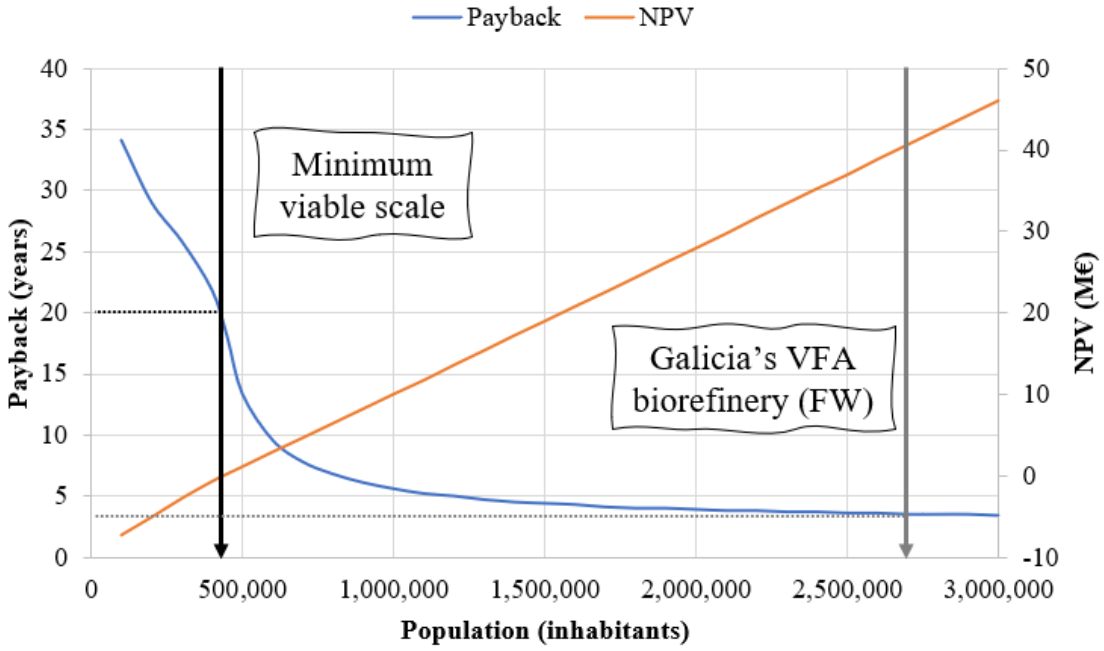

Figure 7.13. Economic indicators evolution with population for food waste (25 % selective collection) and for Galicia's biorefinery.

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These results showed how the minimum viable scale using FW is much lower than using SS (438,000 versus 871,500 inhabitants), mainly due to the differences concerning the VFA yields and fermentation selectivity, which economically favour the installations treating FW.

In order to evaluate in detail the potential construction of a biorefinery to produce VFA located in Galicia (Spain), the CAPEX, OPEX and economic indicators obtained with both substrates are presented. To do this, using the production per capita of each of the urban waste flows detailed in section 7.2.1, it was calculated that there would be an availability of 68,958 t DM/year of SS and 22,174 t DM/year of FW (25 % selective collection), considering a population of 2,700,000 inhabitants in 2021 according to the National Institute of Statistics.

Table 7.5 shows that the VFA biorefinery treating SS would produce a flow of 6,308 t VFA/year (4,811 t of acetic, 696 t of propionic and 604 t of butyric), having a CAPEX of close to 50 M€ and an OPEX around 28 M€/year. This installation would have a NPV of 36.4 M€ and a payback of around 9.1 years (Figure 7.12), being its incomes around 31.2 M€/year (34 % coming from VFA selling and 66 % from SS management fee).

Regarding the VFA biorefinery from FW in Galicia (data also shown in Table 7.5), it would involve a lower treatment capacity and consequently also lower CAPEX and OPEX (17.7 M€ and 4.8 M€/year, respectively). In this case, both NPV and payback would be more favourable than for SS (Figure 7.13), being the NPV higher (40.6 M€) and a payback lower (3.7 years). Regarding total incomes, they were around 11.5 M€/year (77 % coming from VFA selling, 20 % from FW management fee and 3 % from electricity sale).

Table 7.5. Characteristics of centralised VFA biorefinery in Galicia with both substrates.

Plant capacity	SS	FW
Treatment capacity (t DM/year)	68,958	22,174
VFA production (t/year)	6,308	4,491
Acetic acid production (t/year)	4,811	2,278
Propionic acid production (t/year)	696	1,207
Butyric acid production (t/year)	604	1,006
Economic indicators		
CAPEX (M €)	49.8	17.7
OPEX (M €)	28.1	4.8
Total incomes (M€/year)	31.2	11.5
Incomes VFA selling (M€/year)	10.7	8.9
Incomes waste management (M€/year)	20.5	2.3
Incomes electricity selling (M€/year)	-	0.3
NPV (M €)	36.4	40.6
IRR (%)	18.0	36.0
Payback (years)	9.1	3.7

Bastidas-Oyanedel and Schmidt (2018) conducted a techno economic analysis of a VFA biorefinery treating 18,250 t DM FW/year (slightly lower treatment capacity than that of Galicia's one) and they reported a CAPEX of 13.6 M€. Furthermore, these authors reported an OPEX of 373 €/t VS of FW for the dark fermentation scenario, while in the VFA biorefinery proposed for Galicia a value of 323 €/t VS of FW was calculated, results completely aligned. In addition, they outlined profits of around 44 €/t FW, while in the Galicia's biorefinery it would be 60 €/t FW. The fact that the economic indicators obtained are similar to those of other authors

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allows to provide robustness to the results obtained in this doctoral thesis, given that the techno economic analysis was nourished by all the experimental results achieved.

It is important to note that although the economic indicators are very attractive, it must be considered that economy of scale plays a transcendental role as demonstrated. The proposed VFA biorefinery in Galicia would require the availability of the entire generation of each of the wastes, which would be unlikely given the other valorisation alternatives currently available.

7.4. CONCLUSIONS

The economic evaluation showed that the best solvent for the process was ethyl acetate, since the profit margins were much higher. With this solvent, it was also defined that for both substrates, S/F 0.75 kg/kg was the one which derived to higher profit margins, being those 818,035 € in the case of SS and close to 1,000,000 € with FW (for a plant treating the waste produced by 300,000 inhabitants).

OPEX distribution indicated that chemical expenses related to pH control and membrane cleaning reagents were very important, making it clear that the membrane train is the bottleneck of the overall process, which obviously needs further optimisation to improve its efficiency or replacement by another technology. The CAPEX evaluation also manifested that membrane equipment resulted in very relevant costs (exceeding 40 % of the total).

The minimum viable scale for a VFA biorefinery treating SS was 871,500 inhabitants with a CAPEX of 19 M€ and an OPEX of 9.9 M€/year. With FW, considering a realistic scenario, that is, with 25 % selective collection of the total substrate generated, it was seen that a population of 438,000 inhabitants would be necessary. This highlighted the importance of promoting citizen awareness and biowaste collection systems in order to increase the ratio of available substrate.

The tool was also used to evaluate the economic viability of building a centralised VFA biorefinery in Galicia (Spain), treating all the waste generated in the Autonomous Community. When receiving SS, it would treat a flow of 68,958 t DM/year, generating 6,308 t VFA/year, with a CAPEX close to 50 M€ and OPEX around 28 M€/year, being the payback 9.1 years. When the VFA biorefinery in Galicia is proposed for treating FW, the economic indicators were more favourable, being the payback of 3.7 years. This VFA biorefinery would produce 4,491 t VFA/year and would mean a CAPEX and OPEX of 17.7 M€ and 4.8 M€/year, respectively.

Overall, it was shown that the production of VFA is positioned as a bioprocess not only viable from a technical viewpoint, but also economically. Despite the uncertainty associated with the calculation methods (especially CAPEX), satisfactory economic indicators were estimated, with reasonable paybacks when it comes to installations of this magnitude. Furthermore, the results underlined the importance of optimising the membrane technology since it implies the need for a large filtration surface (reflected in the CAPEX distribution) and a high expenditure of chemicals for cleaning tasks.

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8) ENVIRONMENTAL EVALUATION

*The results from this Chapter are in process of publication as Castro-Fernandez, A.[1], Estévez, S.[1], Lema, J. M.[1], Taboada-Santos, A.[2], Feijoo, G.[1], Moreira, M. T.[1]. Envisioning the environmental profile of volatile fatty acids production from sewage sludge and food waste.

[1]CRETUS Institute, Department of Chemical Engineering, School of Engineering, Universidade de Santiago de Compostela, E-15782, Santiago de Compostela, Spain

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8.1. INTRODUCTION

In Chapter 1, the circular economy paradigm has been presented, given that in view of the increasing generation of organic waste streams, it is necessary to valorise them as potential feedstocks to produce energy and consumer goods. The importance of industry in GHG emissions was also presented, so it is necessary to develop new greener processes such as those proposed in this doctoral thesis. Atasoy et al. (2018) have identified VFA production as a key challenge to be addressed in the chemical industry.

Conventional industrial production routes for the three main VFA, acetic, propionic and butyric, were also presented in Chapter 1. Considering that they are based on petrochemical processes, a priori their carbon footprint may be higher than their generation from SS or FW. In any case, considering that conventional production methods are highly optimised, it is necessary to carry out an exhaustive study to see if this is actually true.

In general, bio-based products have lower environmental impacts than petrochemical-based ones. In terms of GHG emissions, Zuiderveen et al. (2023) stated that in general, those coming from renewable sources had significantly lower emissions. Specifically, 80 of the 98 products analysed showed a lower carbon footprint (on average 45 %). But these authors also stated that this is not always the case and sometimes the worse efficiency of certain bio-based processes leads to higher GHG emissions. This is why the environmental impacts of each specific product must be studied individually.

Although fermentation of waste streams to produce VFA is a widely studied process, authors such as Elginoz et al. (2020) and Gálvez et al. (2021) agree that Life Cycle Assessment (LCA) of these systems are still scarce, especially if the aim is to achieve a final high-purity VFA. The differentiating aspect is that while other works use data based entirely on simulations and literature, in this case the integral VFA production process presented in Chapter 7 was evaluated, based almost completely on experimental work.

Once the possibilities from the economic viewpoint of converting SS and FW to VFA have been validated, such technological development must be accompanied by an assessment of the environmental aspects. Therefore, the objectives of this Chapter are: (1) to detect the hotspots of the process from an environmental perspective and (2) to compare the carbon footprint of bio-based VFA with those coming from petrochemical processes.

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8.2. MATERIALS AND METHODS

8.2.1. Process description

To carry out the environmental evaluation, the VFA biorefineries for Galicia were selected for both substrates, whose treatment capacity was detailed in Table 7.5. In order to better evaluate the environmental performance of the facilities, the integral VFA process was subdivided into five subsections (Figure 8.1): pre-treatment and hydration (SS1), fermentation (SS2), solid-liquid separation and VFA pre-concentration (SS3), VFA purification (SS4) and solid fraction digestion (SS5).

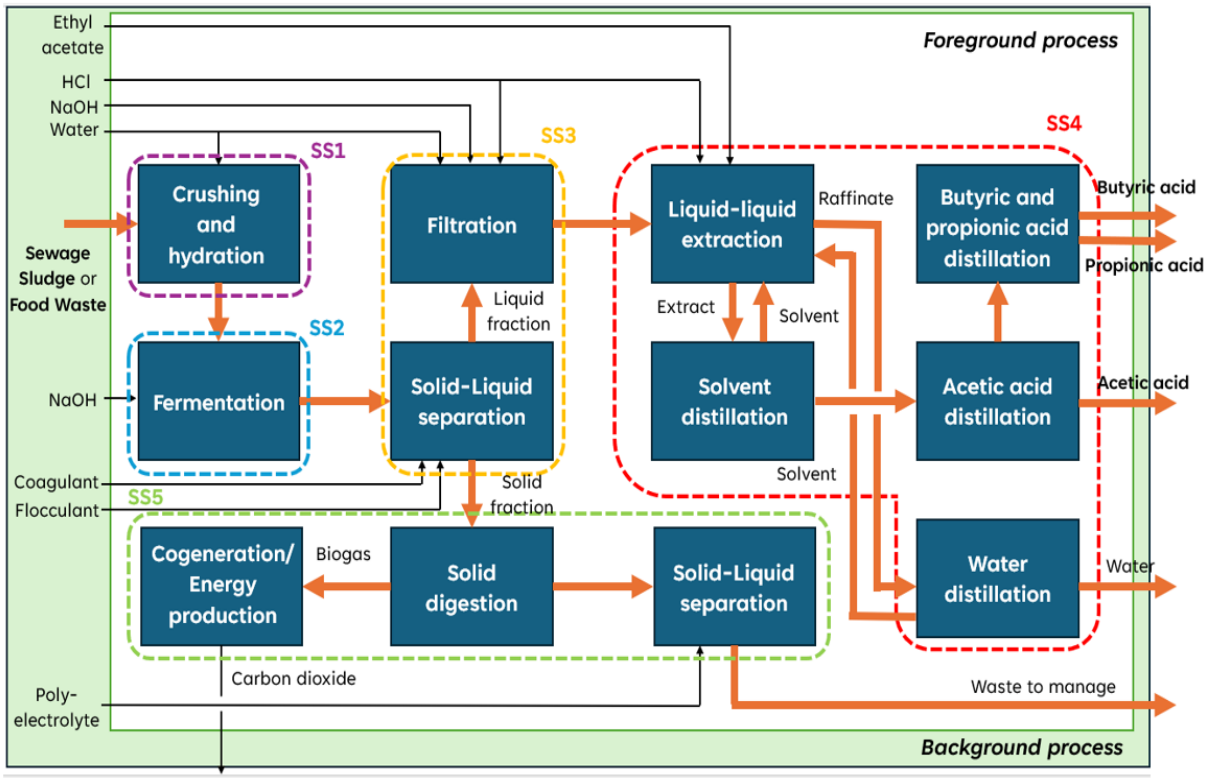

Figure 8.1. Subsections of VFA biorefinery defined for environmental evaluation.

8.2.2. Life Cycle Assessment

The LCA is the methodology used to unveil the environmental profile of the valorisation of SS and FW for the production of high-purity acetic, propionic and butyric acids. The analysis was performed considering the guidelines provided in the ISO 14040 and 14044 standards with a product-based approach (ISO, 2006). In this regard, the outcomes of this research may be compared with the literature addressing waste fermentation as technology for bio-based VFA production as well as with other traditional fossil-based technologies currently implemented. Therefore, a systematic literature review was performed in order to define the methodological aspects such as the selection of the method, the functional unit and the impact categories.

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8.2.2.1. Definition of system boundaries and functional unit

This study provides a *Cradle-to-gate* analysis with system boundaries subdivided into foreground and background processes (as previously shown in Figure 8.1). As in any other LCA, the foreground system accounts for the direct emissions produced during the operation and/or construction of processes. Both SS and FW scenarios have only emitted CO_2, which comes from the valorisation of the biogas into electricity and heat. Although SS and FW can be considered as renewable materials, the emissions were not assumed as biogenic (methodological guidelines provided by the IPPC (2019) indicate that WWTPs also treat organic materials coming from industrial activities working with chemicals of fossil origin).

The technical boundaries were limited to the operational phase given that primary data came from pilot plants operation, but not from the construction phase (whose scale-up is not direct and involves the use of different materials). The geographical system boundaries were restricted to Europe, although exceptions were made for NaOH and ethyl acetate production. This is because the Ecoinvent database V3.8, from where the background processes were taken, does not yet have characterisation factors for these processes at European level. Therefore, the impact from these two processes at global scale were assumed to be equivalent to those of a similar process facility located in Europe. Transport of the chemicals consumed was already considered inside the selected processes from the Ecoinvent database. However, the impacts from the SS or FW transport were accounted separately. Apart from transport, no other impacts were allocated to the waste to be valorised inside the biorefinery scenarios. A cut-off was applied for these possible feedstocks. If considering the impacts from the cradle, the SS and the FW should have the respective impacts from the facilities from where they were collected.

Although the proposed VFA biorefinery is multifunctional given that it can valorise waste and produce marketable products and therefore a process-based approach could also be considered, it was considered a product-based one (the ultimate purpose of the construction of the facilities was VFA production). Therefore, the functional unit is 1 t of VFA produced.

8.2.2.2. Life Cycle Inventory

The Life Cycle Inventory (LCI) has been collected under an attributional and bottom-up approach (for the foreground process). As Table 8.1 depicts, the data were classified into three groups: inputs from the Technosphere for the human-made chemicals and energy consumed by the facility, outputs to the Technosphere for the products manufactured and residues and outputs to the nature for direct emissions. This table contains the information for the selected functional unit and for the two scenarios compared (SS and FW biorefineries). Both primary and secondary data were used for the creation of the LCI as consequence of the scale-up for the foreground process and the use of background data from the Ecoinvent V3.8 database.

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Table 8.1. Life cycle inventory for sewage sludge and food waste. Functional unit: 1 t of VFA produced.

Inputs from the Technosphere	SS scenario	FW scenario	Unit
Generic			
Electricity for pumping	89.44	44.20	kWh
Transport of substrate	8,585.90	3,389.67	t·km
SS1: Pre-treatment and hydration			
Electricity for grinding	0.00	479.76	kWh
Water for hydration	76.20	41.34	m^3
SS2: Fermentation			
NaOH in fermenter	937.43	319.84	kg
Heating in fermenter	830.11	0.00	kWh
Electricity for stirring in fermenter	112.49	28.79	kWh
SS3: Solid-liquid separation and VFA pre-concentration			
Coagulant in solid-liquid separation	3.91	4.00	kg
Flocculant in solid-liquid separation	2.34	3.20	kg
Electricity solid-liquid separation	312.05	167.63	kWh
Water for cleaning in UF	18.53	10.05	m^3
NaOH for cleaning in UF	98.68	53.54	kg
HCl for cleaning in UF	0.09	0.04	kg
Electricity in UF	0.25	0.14	kWh
Water for cleaning in NF	2.93	1.59	m^3
NaOH for cleaning in NF	93.74	50.86	kg
HCl for cleaning in NF	0.08	0.04	kg
Electricity in NF	0.36	0.13	kWh
Water for cleaning in RO	2.79	1.51	m^3
NaOH for cleaning in RO	41.81	22.69	kg
HCl for cleaning in RO	51.57	27.98	kg
Electricity in RO	0.45	0.19	kWh
SS4: VFA purification			
HCl before LLE	138.86	75.34	kg
Ethyl acetate in LLE	22.67	12.18	kg
Heating in distillation 1	1.53	0.00	kWh
Heating in distillation 2	0.59	0.00	kWh
Heating in distillation 3	0.08	0.00	kWh
Heating in distillation 4	0.02	0.00	kWh
Electricity LLE and distillation	66.82	2.56	kWh
SS5: Solid fraction digestion			
Water for hydration before AD	46.29	15.00	m^3
Polyelectrolyte dosage for solid-liquid separation	82.99	65.95	kg
Heating in AD	471.84	0.00	kWh
Electricity for stirring in AD	127.88	37.99	kWh
Outputs to the Technosphere	**SS scenario**	**FW scenario**	**Unit**
Products			
Acetic acid	790.73	542.36	kg

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Propionic acid	25.64	233.70	kg
Butyric acid	101.52	229.28	kg
Residues			
Solid cake from AD digestate	25.91	7.54	kg
Wastewater returns	62.90	18.84	m^3
Membrane cleaning water	28.66	15.55	m^3
Outputs to nature	**SS scenario**	**FW scenario**	**Unit**
Emissions to air			
CO_2 from biogas burning units	5244.54	4261.40	kg

8.2.2.3. Life Cycle Impact Assessment: selection of method and categories

The translation of the LCI into environmental impacts was done with the support of the software SimaPro 9.3.0.2. The program uses the characterisation, normalisation or weighting factors from Life Cycle Impact Assessment (LCIA) methods for such purpose. A middle point of the environmental chain was considered under analysis to reflect the changes caused by emissions and, thus, an impact of the MidPoint study was provided. Among the possible LCIA methods found in SimaPro (CML, Cumulative energy Demand, Ecological Scarcity, Environmental Footprint, IMPACT World, ReCiPe, TRACI and USEtox), Environmental footprint V3.0 was selected. Multiple were the reasons behind its selection: the method is not superseded, the characterisation factors are provided at European level, it is not a commonly employed but recommended by the EU (as indicated in Commission Recommendation 2021/2279) and it has categories aligned with the goals of the study. These impact categories were selected among those of the method based on the goals of the study and the feasibility of comparison with literature: freshwater and marine eutrophication (FE and ME), climate change (CC), mineral resource scarcity (MRS), fossil resource scarcity (FRS), ozone depletion (OD), acidification (AC) and water consumption (WC).

8.3. RESULTS AND DISCUSSION

8.3.1. Analysis of inventory

Table 8.1 compiled the relevant inputs and outputs of the two compared scenarios, SS and FW. For a clearer description, the results achieved would be discussed considering the performance of the installations in terms of energy and chemicals.

When it comes to energy, the SS scenario reports 62.7 % higher demand than the FW scenario per t of VFA produced. This is because for the VFA biorefinery in Galicia using FW as substrate, the calorific needs of the plant would be fully supplied thanks to the valorisation of the biogas produced in the AD process (Table 8.1 shows that both the calorific energy for heating the fermenter and digester as well as the distillations take on a value of 0). Therefore, the amount of electricity which would have to be purchased in this scenario is much lower than in the SS scenario, making it a much more energy-efficient process. Moreover, energy distribution inside the installation is different for both scenarios. With SS, energy is mainly used in the biological processes (49 % for SS2 or fermentation) followed by solid fraction digestion or SS5 with 31 %. However, in FW scenario, energy is destined to the pre-treatment (67 %) due to the need of crushing as well as to the solid-liquid separation (23 %).

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Although both processes are almost identical in terms of infrastructure, their dissimilarity in terms of operation is related to the composition of the substrates used as raw materials. These differences result in a much higher VFA yield for FW, as well as different selectivity in the fermentation process. In addition, the solid cake obtained after fermentation has a much higher potential to produce biogas than the one obtained from SS (Figure 7.3). Therefore, the onsite valorisation of biogas as heat has been proven to be a good strategy for decreasing external demand and thus reducing the relative consumption of heating in biological processes, above all for the FW scenario. The decrease of heating needs in the FW scenario compensates for the extra demand of electricity in the grinding resulting in a better environmental profile.

Regarding chemicals, the use of NaOH for the pH control in the fermenter and membrane cleaning activities accounts for around 70-80 % in mass (depending on the scenario). Once again, the influence of the consumption of reagents in the membrane stage on the overall process is evident, in line with CAPEX and OPEX distribution graphs presented in Chapter 7. Moreover, SS scenario report a higher demand for chemicals (46 % and 18 % for HCl and polyelectrolyte, respectively), which is even more accentuated for NaOH consumption (61 % bigger). This is because SS and FW scenarios were operated at pH 8.5 and 7, respectively.

8.3.2. Comparison between scenarios and hotspot study

The problem with the standalone analysis of a LCI is that the indirect impacts from the production of the different types of energy and chemicals are not accounted for. Thus, the most polluting scenario may not necessarily be the one consuming the largest amount of energy and/or chemicals. Therefore, the scenarios were also compared after the LCI data characterisation in the LCIA stage. In line with what was advocated in section 8.3.1 on the suitability of the FW scenario in terms of energy and NaOH consumption, the LCA results reinforced this. Thus, Figure 8.2 indicates that the VFA biorefinery in Galicia using FW is by far the most environmentally friendly alternative for all impact categories (in some of them such as AC or ME, the contribution is even less than half).

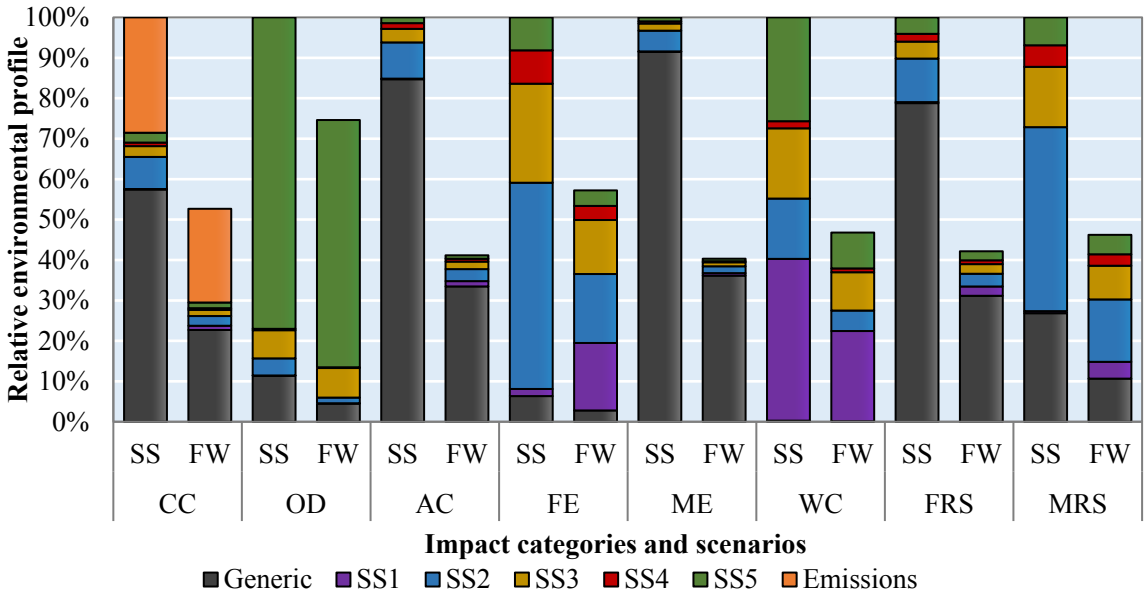

Figure 8.2. Comparative and contribution relative environmental profile per facility subsection of sewage sludge and food waste scenarios.

Seven items can be identified in Figure 8.2 to clarify which subsection of the facility, emissions or generic aspects (pumping and feedstock transport) can be highlighted as critical and thus, point them out for future facility enhancement. When analysing the scenario with SS, "Generic" seems to be the hotspot of the profile since it is concerning in half of the categories (around 57 % for CC, 85 % for AC, 92 % for ME and 79 % for FRS). Inside "Generic category", transport is important in practically all impact categories as shown in Figure 8.3, especially in CA, ME and FRS where exceeds 70 % of the relative environmental contribution.

In addition to the item "Generic", SS2, which encompasses the SS fermentation process, is also of significant importance, being FE and MRS the most affected categories. Regarding this SS2, NaOH has an impact between 82 % (FRS) and 99 % (WC). SS1 and SS5 are also remarkable for WC and OD, respectively.

As it can be distinguished in Figure 8.3, the facility is therefore defined by chemical demand and not by energy. This goes some way to demonstrating the relevance of the energy optimisation which was achieved in the system, so that heating of the fermenters and digesters or agitation is no longer an environmental concern. Instead, these results highlight once again the need for optimisation of chemical reagents consumption, which has a major environmental influence in addition to the economic costs. Because there is no need for grinding in SS scenario, SS1 was only characterised by the consumption of water for hydration. In SS5, the polyelectrolyte consumption was the reason behind the score of OD.

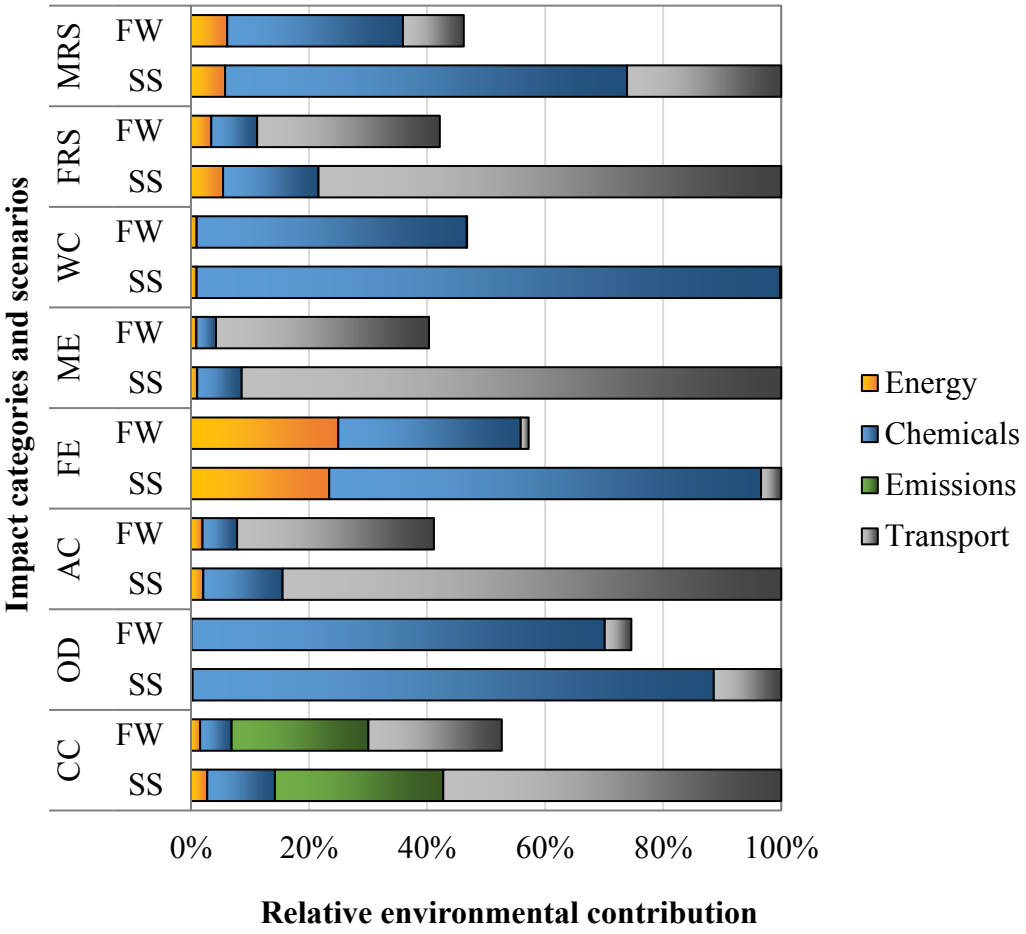

Figure 8.3. Comparison of sewage sludge and food waste scenarios regarding environmental profile classified as energy, chemicals, emissions and transport.

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Figure 8.3 depicts that SS transport to the facility is a key factor in ensuring a sustainable treatment since it could represent up to 12 times bigger impact than the operation of the facility (for the category ME). Other important numbers can be cited for CC (2.3 times higher), AC (6.4 times) and FRS (4.6 times). In fact, SS transport from WWTPs of generation to the biorefinery is leading the environmental profile in 4 of the 8 categories under study. It is therefore worth discussing the choice of the site of the installation before it is built, as no changes can be made once it is chosen.

For the other 4 categories in which SS transport is not the predominant item (OD, FE, WC and MRS), the major impact comes from chemicals consumption. Direct emissions, such as CO_2 from biogas valorisation, have negligible impact in all categories except for CC (28.5 %). Compared to transport and chemicals, the contribution of energy is low (around 0.2 %-5.8 % for OD and MRS) but for higher for FE (23.4 %). Electricity demand is the major concern in all categories except OD on which heating has a representativeness of 69.3 %. The solid-liquid separation is the activity of the facility with the biggest impact in terms of electricity demand because its remarkable consumption (20 kWh/t MS). These results differ from those obtained in the analysis of the inventory of section 3.1, on which the energy in the SS2 was the main area of interest. This is because the environmental impact of the production of 1 kWh of European electricity is higher than that to produce the same amount of industrial heat. That is, 0.37 kg CO_2/kWh of electricity are generated in comparison to 0.18 kg CO_2/kWh for heat. Therefore, the profile of SS is mainly characterised by the use of energy in the biological processes (49 % for SS2 or fermentation and 31 % for SS5 or solid fraction digestion).

Since SS and FW scenarios share the same facility infrastructure, with only the small dissimilarities already highlighted, the environmental outcomes of the FW scenario were in line with those already provided for SS. Therefore, it is important to focus only on aspects which vary from the SS scenario. When performing the study per facility subsection (Figure 8.2), the ranking of the more relevant items inside each of the categories remains the same. The only deviation can be found for CC since the share of the "Generic" item in the scenario FW decreased from 57 % to 43 %. Instead, the direct emission of CO_2 from the burning and valorisation of the biogas from SS5 became the dominant impact (this was caused by the higher biogas production). The use of biogas compensates for the heating needs both in the fermenter, in AD reactors as well as in distillation columns. Moreover, the results of Figure 8.3 indicate that for FW scenario there is a considerable reduction in the relevancy of the chemicals in FE (from 73 % to 54 %), whose reason was the lower NaOH consumption which involves FW fermentation in comparison with SS one (Table 8.1 of the LCI).

8.3.3. Carbon footprint comparison

With the aim of conducting a comparison of the results achieved in this research with others from the literature, the environmental impact outcomes were re-estimated and expressed in other product-based functional units used in publications found with a systematic literature review (some of them are summarised in Table 8.2). However, one of the biggest problems when comparing LCA results is the large diversity of technical and methodological (i.e., system boundaries) assumptions taken by the LCA practitioner.

Taking as an example the production of acetic acid from SS, the CC impact of the treatment facility is 23.25 kg CO_2 eq./kg acetic acid produced. Nevertheless, there is multifunctionality, which implies that other two VFA are also manufactured (propionic and butyric acid). Because of this, the standalone production of acetic acid results in 20.03 kg CO_2 eq./kg acetic acid produced considering a mass allocation. This outcome becomes even lower if no transport were

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considered: 8.56 kg CO_2 eq./kg acetic acid produced. This is a value in line with that reported by Elginoz et al. (2020), who aimed to produce acetic acid from dairy wastewater but focused only on fermentation without considering purification, getting a value of 6.03 kg CO_2 eq./kg acetic acid. Regarding FW scenario without transport (these authors do not include it in their study either), this impact would be reduced until 5.50 kg CO_2 eq./kg acetic acid, being therefore lower than that of Elginoz et al. (2020). If the comparison is made against traditional acetic acid production methods, the carbon footprints of both scenarios without transport are much higher than the 1.50 kg CO_2 eq./acetic acid reported by Medrano et al. (2019) for the methanol carbonylation method (the main production route at present, being depicted in Figure 1.13). They are also notably higher than the 2.50 kg CO_2 eq./kg acetic acid reported by Budsberg et al. (2020) using also ethyl acetate as solvent, more than double in the case of FW without transport and almost 4 times higher in the case of SS (the differences including the transport of substrate to the facility would be even higher). In contrast, the results of this evaluation are in line with those reported by Petrescu et al. (2022), since they reported a range between 4.41-10.95 kg CO_2 eq./kg acetic acid, although these authors focus on acetic acid concentration from using isopropyl acetate and isopropanol mixture by thermally coupled process or by double effect distillation, thus using different routes than the traditional ones, being therefore less efficient.

When comparing the carbon footprint related to the production of propionic acid obtained in this work without taking into consideration the transport (8.56 and 5.50 kg CO_2 eq./kg propionic acid for SS and FW scenarios, respectively) with that reported by Dahiya et al. (2020), they are clearly much higher values. This is because, in addition to utilising data from a laboratory-scale fermentation using synthetic wastewater, they did not consider at all the separation of VFA from the fermented effluent, which results in such a little carbon footprint (0.86 kg CO_2 eq./kg propionic acid). On the other hand, when comparing these numbers with the 4.40 kg CO_2 eq./kg propionic acid (CarbonCloud, 2024) based on the traditional petrochemical route, it is seen that in this case the scenario with FW reported a higher but close value; for SS scenario it would be practically double.

Regarding butyric acid, no references were found about petro-based production methods, so it has not been possible to compare it with these. In contrast, Suazo et al. (2024) presented values between 0.327-0.727 kg CO_2 eq./kg butyric when producing it from wheat straw, highlighting the great environmental performance of its process compared to that proposed by Camara-Salim et al. (2021), with a value of 6.89 kg CO_2 eq./kg butyric, fully aligned with those reported in this work without considering transport (8.56 and 5.50 kg CO_2 eq./kg butyric acid for SS and FW scenarios, respectively). In this way, VFA biorefineries from these substrates would have impacts on the CC category similar to those of other bioprocesses.

It should be noted that several authors such as Dahiya et al. (2020) and Elginoz et al. (2020) approached their studies considering only the fermentation without including VFA purification. If only the impacts of SS1 (pre-treatment) and SS2 (fermentation) were considered for the proposed biorefineries, the production of acetic acid from SS (no transport) would be 1.61 kg CO_2 eq./kg acetic acid. This means that the production of VFA could be competitive enough against those coming from petrochemistry if the purification stages improved their current environmental performance. When focusing on FW scenario, CC result including these two stages would be 0.61 kg CO_2 eq./kg acetic acid produced. This indicates that the environmental viability of the VFA biorefinery is not only technologically dependent, but it also relies on the availability of feedstock and the distance from where it is supplied.

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Table 8.2. Comparison of the results of this research with literature for the climate change category.

Type of impact approach	Unit	SS	FW	SS (no transport)	FW (no transport)	Petro-based literature	Bio-based literature
VFA production							
Impact of acetic acid production/kg VFA	kg CO_2 eq.	15.84	5.22	6.77	2.98	-	0.186 (Gracia et al. 2024)
Impact of propionic acid production/kg VFA	kg CO_2 eq.	0.51	2.25	0.22	1.28		
Impact of butyric acid production/kg VFA	kg CO_2 eq.	2.03	2.21	0.87	1.26		
Total, impact/kg VFA	kg CO_2 eq.	18.39	9.68	7.86	5.53		
Acetic acid production							
Impact of acetic acid production/kg acetic acid	kg CO_2 eq.	20.03	9.63	8.56	5.50	1.50 (Medrano et al., 2019); 2.50 (Budsberg et al., 2020) and 4.41-10.95 (Petrescu et al. 2022)	6.03 (Elginoz et al., 2020)[1]
Impact of propionic acid production/kg acetic acid	kg CO_2 eq.	0.65	4.15	0.28	2.37		
Impact of butyric acid production/kg acetic acid	kg CO_2 eq.	2.57	4.07	1.10	2.32		
Total, impact/kg acetic acid	kg CO_2 eq.	23.25	17.85	9.94	10.19		
Propionic acid production							
Impact of acetic acid production/kg propionic acid	kg CO_2 eq.	617.72	22.35	264.06	12.76	4.40 kg CO_2 eq. (CarbonCloud, 2024)	0.86 (Dahiya et al., 2020)[2]
Impact of propionic acid production/kg propionic acid	kg CO_2 eq.	20.03	9.63	8.56	5.50		
Impact of butyric acid production/kg propionic acid	kg CO_2 eq.	79.31	9.45	33.90	5.39		
Total, impact/kg propionic acid	kg CO_2 eq.	717.06	41.44	306.52	23.65		
Butyric acid production							
Impact of acetic acid production/kg butyric acid	kg CO_2 eq.	156.03	22.78	66.70	13.01	-	0.327-0.727 (Suazo et al. 2024)[3] and 6.89-8.23 (Camara-Salim et al., 2021)[3]
Impact of propionic acid production/kg butyric acid	kg CO_2 eq.	5.06	9.82	2.16	5.60		
Impact of butyric acid production/kg butyric acid	kg CO_2 eq.	20.03	9.63	8.56	5.50		
Total, impact/kg butyric acid	kg CO_2 eq.	181.12	42.23	77.42	24.11		

Notes: [1]Dairy wastewater; [2]Synthetic wastewater; [3]Wheat straw.

8.4. CONCLUSIONS

Taking into account that the economic indicators of the VFA biorefineries proposed for the Autonomous Community of Galicia turned out to be interesting, it was necessary to evaluate the hotspots of the process from an environmental point of view as well as try to compare the bioproducts generated with their analogues produced from traditional routes.

The conducted LCA allowed the comparison of the VFA biorefinery treating SS with the one which would treat FW in Galicia, showing that for all the impact categories studied, the scenario with FW was more environmentally favourable, largely due to the greater suitability of the substrate for produce VFA as discussed in previous Chapters. Thus, the energy optimisation achieved in this scenario, as well as the lower associated chemical consumption (mainly NaOH), tipped the balance towards this alternative. In addition, the results also highlighted the great importance of the transport of substrates to the biorefineries in environmental impacts, since it was the item which had the greatest influence in most of the impact categories studied for both substrates. In this way, it would be necessary to establish a suitable place to locate the facilities within the territory to seek to minimise transport contribution.

The comparison of the carbon footprint of the VFA obtained in the process with SS and FW with those obtained from other waste streams, as well as those produced through traditional means, was complicated by the different system boundaries assumptions considered by other authors. In any case, it was observed that although the impact in the CC category could be similar to that reported in some works assessing other bio-based processes, when comparing them with traditional routes, the carbon footprint of the proposed process (even without including the transport of the substrates to the facilities) is superior. On the other hand, it was observed that these figures decreased drastically if the purification of the VFA was not considered, so it is in the optimisation of this stage where efforts should be focused in order to align the impact with that of petro-based VFA consumed nowadays.

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9) GENERAL DISCUSSION

9.1. MAIN OUTCOMES OF THE THESIS AND RESEARCH GAPS

The main objective of this doctoral thesis was fulfilled, since a technology to produce VFA from SS and FW was satisfactorily scaled-up, reaching a relevant degree of technological maturity. This technology was not only validated from a technical point of view, but the economic results related to its industrialisation were also evaluated.

This work responded to some of the bottlenecks involved in the production of VFA from organic waste streams. Below are the main **outcomes** of the thesis related to the initially stated objectives and the gaps which still exist regarding the technology to achieve its implementation at an industrial level. Consequently, the reflections which answer to each of the specific objectives proposed are:

- **To determine the VFA yields and fermentation selectivity from SS, considering the influence of pre-treatments such as THP.**

The potential of SS to produce VFA was evaluated in this doctoral thesis. Specifically, the operation of the pilot plant was conducted with hydrolysed sludge since continuous operation in 5-L reactors demonstrated that THP had no influence on the VFA yield or selectivity, but this provides a clear advantage such as the sludge sanitation. Although the average yields were slightly lower in the pilot plant, it can be stated that they would be between 12-15 % g COD-VFA/g COD substrate applying the most favourable operating conditions (pH 8.5, HRT 10 days and OLR of around 4.6-6 kg COD/(m^3·d)). It was seen that operating with SS, acetic acid was the main one (with variable proportions between 64-78 % on mass basis), followed by propionic acid (between 10-15 %) and the rest of the VFA.

Regarding the influence of THP on VFA production, the results indicated that in batch mode, this pre-treatment implied lower VFA yields than when using raw sludge. Conversely, the change of operation to continuous mode in the 5-L reactors made this effect disappear, thus showing that it had no influence on either the VFA yields or the selectivity of the process. Therefore, the mode of operation did vary the results obtained. In terms of scale, it was seen that when moving from the 5-L reactors operating continuously to the 1.5-m^3 pilot scale fermenter, the average VFA yield fell slightly from 15 to approximately 12 % g COD-VFA/g COD substrate in Stage 1, although this was surely due to less control over the process derived from the scaling of the technology.

- **To quantify the VFA yields and fermentation selectivity from FW, also assessing the scalability of the fermentation process.**

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The results seen at laboratory scale 5-L reactors with FW validated that, after screening several pHs and OLR applied, pH 7 and an OLR of around 10 g COD/(L·d) were the conditions that resulted in a higher VFA yield and rate. Although operating at this neutral pH involved greater addition of NaOH than in other conditions, the notable difference in the results led to these same operating conditions being chosen for the pilot plant fermenter. At this scale, VFA yields between 35-38 % g COD-VFA/g COD substrate were reported with an acetic and butyric proportions of around 40 %, followed by propionic with approximately 20 % (mass basis).

Therefore, it could be stated that scaling the process from lab to pilot scale hardly influenced the results, with the continuous mode being stable and capable of generating an effluent with a high concentration of VFA, which was around 40 g COD-VFA/L.

- **To evaluate the capability of membrane technology for VFA pre-concentration as a prior step to purification processes.**

The proposed membrane train consisted of an UF membrane, followed by NF and RO ones. Experimentation with real medium (supernatant coming from SS fermentation) showed that a concentration factor of 2.5 was achieved through RO, reaching a final concentration of 21.3 g VFA/L. Furthermore, although validated with synthetic medium, it was seen that a double passage through RO allowed recovery to be increased even further and concentrations close to 50 g VFA/L were measured.

In view of its ability to purify and pre-concentrate the sample before LLE process, membrane technology would be an interesting technology. In any case, it should be noted that the claim of suitability of this unit operation is based on its purely technical capacity to pre-treat the sample; if its associated costs are considered in an overall perception of the process, the low permeate fluxes mean that the operation should be significantly optimised or, failing that, to look for alternative technologies.

- **To technically and energetically compare the LLE and distillation with two different organic solvents (ethyl acetate and ethyl octanoate) through experimentation and simulations, respectively.**

The experiments conducted in the LLE column validated that ethyl acetate was clearly the solvent which obtained the highest recovery efficiencies applying the same S/F in both cases. With this solvent, the recovery percentages for propionic and butyric were above 99 %, being acetic acid's recoveries lower, still reaching values of 70 % at S/F 1 kg/kg. Furthermore, once the modifications to improve the column were made, a total recovery of acetic acid was also achieved.

Simulations run in ASPEN HYSYS showed that separating individual VFA was technically possible. Although the process flow diagram varies when using one or another solvent given the need for an additional distillation column with ethyl acetate, high-purity VFA streams (greater than 90 %) could be achieved, although with this solvent the energy demands are also higher.

- **To establish the optimal conditions for the global VFA production process from an economic perspective.**

Experimentation related to the fermentation stage revealed the suitability of alkaline pH for SS and neutral pH for FW, always with the balance between yield and reagent consumption in mind. Instead, it is in the purification step where greater doubts arose. In this way, to choose the most suitable solvent for scaling up the process, an economic comparison between ethyl

acetate and octanoate was conducted. The results showed that, regardless of the substrate chosen, ethyl acetate was the most profitable one since the recovery percentages seen in the LLE column highly influenced the results. Regarding the optimal S/F, it was seen that for both SS and FW, operating with ethyl acetate, S/F 0.75 kg/kg was the optimum.

- **To assess the economic viability of an industrial plant converting SS and FW into VFA in Galicia.**

To define the minimum viable scale of a VFA biorefinery, the capacity of the plant must be contemplated. Thus, considering SS, the amount of waste generated by an equivalent population of 871,500 inhabitants would be required in order to produce around 680 t VFA/year and recover the investment in the proposed period of 20 years. In the case of having FW, considering an availability of 25 % of the total generated, the waste equivalent to 438,000 inhabitants would be necessary.

The Autonomous Community of Galicia has a population of around 2,700,000 inhabitants, so it clearly exceeds the minimum viable scale for both substrates. So, when receiving SS, the plant would produce 6,308 t VFA/year, with a CAPEX close to 50 M€ and OPEX around 28 M€/year, being the payback 9 years. If it would be treating FW as a substrate, the economic indicators would be more favourable than for SS, obtaining a payback of 3.7 years. This VFA biorefinery would produce 4,491 t VFA/year with an associated CAPEX and OPEX of 17.7 M€ and 4.8 M€/year, respectively.

- **To identify the environmental impacts of VFA produced through the proposed process and compare them with those obtained from traditional production routes.**

The LCA carried out allowed to determine that the environmental impacts derived from VFA production are lower when FW is used as substrate. The transportation of waste to the biorefinery turned out to be the item which affected most, although the consumption of chemicals (mainly NaOH) also played an important role. When comparing the carbon footprint of the proposed process with that reported for producing VFA through traditional petrochemical routes, it was seen that although it is a process based on renewable feedstocks, there is still important optimisation needed in order to be aligned with the reported values, being notably higher even without considering the transport of the substrates to the facilities.

From the work carried out, some **gaps** in knowledge emerge which should be explored further. In this way, the next steps to take on the path towards the industrialisation of the technology developed in this doctoral thesis would be:

- **Optimisation of purification/concentration with membranes.**

Although membranes turned out to be a technology with potential to purify and pre-concentrate the VFA-rich stream after fermentation, they imply great weight in the OPEX of the overall process. Although an experiment was carried out with real medium which allowed obtaining permeate fluxes in line with the few reported in the literature with fermentation effluents, the cleaning method plays a crucial role both in the consumption of chemicals and in the evolution of the permeate flux over time.

In future work it would be good to lengthen the execution time of the experiments to establish the daily number of cleanings, the consumption of water and chemicals, and if really after cleanings it is possible to return to permeate flux values very close to the initial ones. Furthermore, it would be desired to determine the durability of the membranes and their

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replacement rate. With all these data, the tool developed in Chapter 7 would be much more precise, and the CAPEX and OPEX related to the membranes could be refined in order to reduce uncertainty in the figures.

- **Validation of LLE process with real medium.**

Once it was proven that the modifications implemented in the LLE column were beneficial, notably increasing the recovery percentages, it would be necessary to carry out the experiments with the modified column also with ethyl octanoate. Besides, it would be of interest to validate the results obtained with synthetic medium with those coming from the fermentation of SS and FW.

- **Experimental validation of distillation process.**

At the time of writing this document, a pilot distillation plant was under construction, which could not be operated within the framework of this doctoral thesis (Figure 9.1). Therefore, although the simulations performed are more adequate to estimate the heat needs of an industrial level, an experimental validation of the separation between the solvent and the VFA stream, as well as of the separation of the acids from each other, would be necessary.

Figure 9.1. Distillation column under construction.

- **Assessment of SS and FW co-fermentation.**

In this doctoral thesis, the potential to produce VFA from SS and FW separately was studied, and since these waste streams proved to be feasible substrates for this purpose, a next step would be to test the possible synergies and improvements derived from a co-fermentation of both. In this way, the VFA yield could be increased, and a different selectivity could be achieved depending on the proportions of each substrate used. In addition, a scenario would

arise in which the acquisition of substrates in large quantities would be simpler, and it would not be necessary to treat the production of all city dwellers for this purpose.

- **Study of alternatives to improve the environmental performance of the proposed VFA biorefineries in Galicia.**

In view of the fact that the LCA results manifested the enormous influence of substrate transport in most of the impact categories studied, it would be interesting to conduct a study to define the ideal location for the facilities within the Autonomous Community of Galicia. Likewise, given the great importance of the use of chemicals in the process (and in particular of NaOH), it would be of great value to perform a sensitivity analysis to determine whether there are other alternative bases which would minimise environmental impacts.

- **Address existing legal barriers to the commercialisation of bio-based VFA.**

In order to achieve the commercialisation of these products, in Chapter 1 different barriers that they face were defined, among which the legislative ones stand out. That is why solutions must be proposed, including:

i) Clear detail whether it is necessary to develop specific regulatory or technical criteria which specify the conditions for commercialising VFA from SS and biowaste or would apply the same legislation for those produced through petrochemical methods.

ii) Harmonisation of regulatory requirements at EU, national and regional level to ensure that VFA or any other bioproduct obtained waste streams can be placed on the market on an equal footing.

iii) Reinforcement of the communication of the benefits of organic fraction's selective collection, which is the only possible alternative for this biorefinery approach, regardless of the need to comply with legal requirements.

iv) Simplification of administrative processes associated with end-of-waste status through a case-by-case assessment, thus giving rise every day to more product references achieving it. This would promote motivation to create circular models.

9.2. ALTERNATIVES TO THE PRODUCTION OF HIGH-PURITY VFA

9.2.1. Biomethane versus VFA production?

Many authors reported the interest of directing AD towards VFA from waste streams instead of biogas or biomethane, because of their higher added value. During this doctoral thesis, the technical and economic feasibility of producing these bioproducts was demonstrated, but it would be of interest to compare it with biomethane production in economic terms.

Although the great advantage in producing VFA is already evident, it must be considered the problematic energy situation which is being experienced in Europe. In this context, the opportunity of using the vast gas infrastructure favours biogas upgrading to biomethane by removing CO_2 and other undesired compounds such as water, H_2S, NH_3 and siloxanes (Soreanu et al., 2008). In 2020, biomethane production in Europe was almost 3 billion cubic metres (with 900 working plants), which is expected to continue growing (Sulewski, 2023).

To carry out the economic comparison between the production of VFA and biomethane, a compilation of the costs that would imply the operation of each of the bioprocesses was conducted, as well as the potential incomes they would generate, both from SS and FW.

Concerning chemical costs, AD naturally regulates pH between 6.5-7.5 thanks to the carbonic acid/bicarbonate system when biomethane is the target. Instead, for VFA production,

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consumption was determined experimentally in the pilot plant described in Chapters 3 and 4. Dewatering costs were also determined thanks to the operation of the pilot plant, being comparable for both bioprocesses since it is necessary to dehydrate the fermented effluent.

OPEX related to the purification/concentration step were calculated through the Excel tool, being 12.2 €/t SS and 40.9 €/t FW, respectively. For SS no references were found in the literature, but using FW as substrate, Bonk et al. (2015) indicated that VFA separation technologies can be performed on a range of around 13 €/m^3 effluent (considering the ratio 0.32 m^3 FW/m^3 effluent operating the pilot plant at an OLR of 10 g COD/(L·d), therefore being 40 €/t FW). In addition, Bastidas-Oyanedel and Schmidt (2018) also reported a purification cost to obtain acetic and butyric acid of 34 €/t FW, figures which fully coincide with the calculation conducted in this doctoral thesis. Regarding biogas upgrading in order to be injected into the gas grid (95 % CH_4 by volume), a cost of 0.15 €/Nm^3 for water scrubbing technology was adopted (Muñoz et al., 2015).

Concerning income, for the case of biomethane production, the values obtained in BMP tests shown in Figure 9.2 were considered (137 Nm^3 CH_4/t COD substrate for SS and 280 Nm^3 CH_4/t COD substrate for FW), as they are aligned with the values reported by Taboada-Santos et al. (2019) for pre-treated sludge and with the bibliographic references of Jabeen et al. (2015) and Agyeman and Tan (2014), who operated pilot plants from FW. This BMP test was conducted at 37 ºC using the AMPTS II (Bioprocess Control) following the protocol described by Holliger et al. (2016). The test was performed in triplicate applying a ratio of 2 g SV inoculum/g SV substrate.

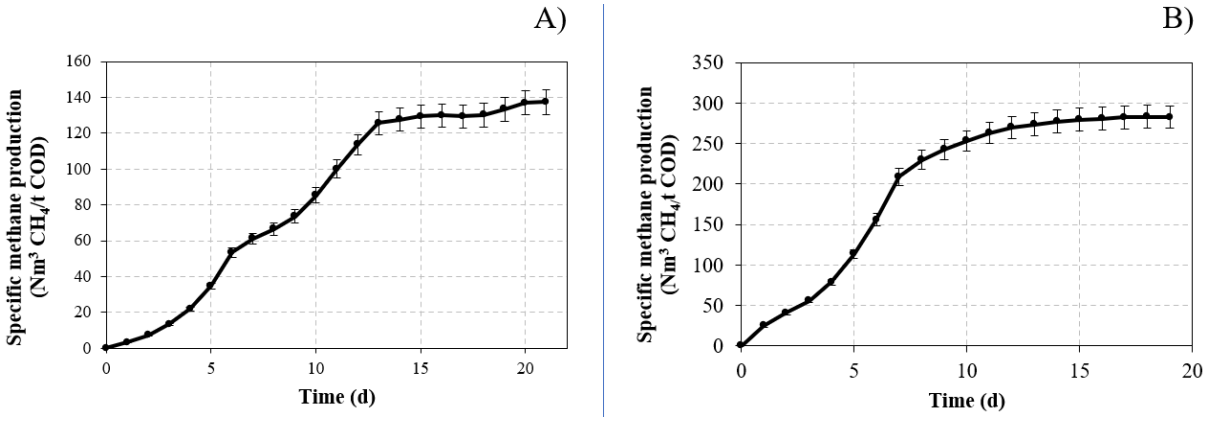

Figure 9.2. Specific methane production from BMP test from substrates considered. A) Sewage sludge. B) Food waste.

A 0.5 €/Nm^3 value was assumed for biomethane's price, considering that it was sold without subsidy at the mean price of natural gas for large users in Europe in 2022 (0.037 €/kWh according to Eurostat Report, 2023) and its lower heating value is 10.78 kWh/Nm^3 (Carvill, 1993). As VFA's price, it was assumed 1,700 €/t VFA for SS case (selectivity of the process implies generation mainly of acetic acid) and 2,100 €/t VFA for FW, which gives rise to a bigger amount of propionic and butyric with higher market value.

Tables 9.1 and 9.2 present the comparative of the most relevant costs and expected incomes for VFA and biomethane production from SS and for FW. Identical treatment capacity of the plant was considered, so in the case of biomethane production, a bigger reactor than to produce VFA is needed considering the different HRT applied (25 and 10 days, respectively), so the construction cost (not included in this comparative) would be higher.

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Table 9.1 shows that the costs of producing biomethane from hydrolysed sludge result around 2.8 €/t SS, lower than for VFA production (16.4 €/t SS). The income from VFA selling is also greater than that obtained for biomethane, 4.8 and 17.0 €/t. The result is that the net profit of producing VFA is notably lower than producing biomethane (0.6 versus 2.0 €/t SS) and the CAPEX would result also higher due to the more complex technologies needed.

Table 9.1. Economic comparison of biomethane production versus VFA production from sewage sludge.

	Biomethane production	VFA production
Cost of chemicals for pH control (€/t SS)[a]	0.0	2.8
Cost of dewatering (€/t SS)[b]	0.09	0.09
Cost of VFA purification/concentration (€/t SS)	0.0	12.2
Cost of biogas upgrading (€/t SS)	1.4	0.0
Cost of digester heating (€/t SS)[c]	1.3	1.3
Total cost (€/t SS)	**2.8**	**16.4**
Production yield (Nm^3 biomethane or kg COD-VFA/t COD influent)	137	120
Amount of biomethane produced (Nm^3/t SS)	9.6	-
Amount of VFA produced (t VFA/t SS)	-	0.010
Price of products (€/Nm^3 biomethane and €/t VFA)	0.5	1,700
Income (€/t SS)	**4.8**	**17.0**
NET PROFIT (€/t SS)	**2.0**	**0.6**

[a]NaOH cost of 0.8 €/kg (Wei et al., 2013). [b]Dewatering chemicals: dose of 2.5 kg/t SS for coagulant and 1.5 kg/t SS for flocculant (2,500 €/t). [c]Natural gas price considered (0.09 €/kWh).

Table 9.2 shows how the costs of producing VFA from FW are much higher than those of producing biomethane (51.2 versus 19.6 €/t FW). Therefore, unlike with SS, the net profit of VFA production is 10 times higher than biomethane production.

Table 9.2. Economic comparison of biomethane production versus VFA production from food waste.

	Biomethane production	VFA production
Cost of chemicals for pH control (€/t FW)[a]	0.0	2.4
Cost of dewatering (€/t FW)[b]	0.07	0.07
Cost of VFA purification/concentration (€/t FW)	0.0	40.9
Cost of biogas upgrading (€/t FW)	11.7	0.0
Cost of digester heating (€/t FW)[c]	7.8	7.8
Total cost (€/t FW)	**19.6**	**51.2**
Production yield (Nm^3 biomethane or kg COD-VFA/t COD influent)	280	370
Amount of biomethane produced (Nm^3/t FW)	78.2	-
Amount of VFA produced (t VFA/t FW)	-	0.074
Price of products (€/Nm^3 biomethane and €/t VFA)	0.5	2,100
Income (€/t FW)	**39.1**	**155.4**
NET PROFIT (€/t FW)	**11.7**	**104.2**

[a]NaOH cost of 0.8 €/kg (Wei et al., 2013). [b]Dewatering chemicals: 0.11 kg calcium oxide/t FW (520 €/t) and 0.004 kg PAM/t FW (3,300 €/t). [c]Natural gas price considered (0.09 €/kWh).

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The price of the obtained product plays a crucial role in the comparison, being the value of biomethane certainly lower than that of VFA, although this could vary depending on the specific country given the financing mechanisms established in order to support the development of bioenergy projects. The production of biomethane in the current market situation cannot compete due to the availability of natural gas and its low price. However, the urgent need to cut CO_2 emissions and the decarbonisation of the global economy could provoke a biomethane impulse boosted by support systems such as subsidies and tax incentives (Sulewski et al., 2023).

From SS, the net profit of VFA production is higher for biomethane production than for VFA, while with FW it is the other way around. In any case, the results obtained with FW fully coincide with those reported by Bastidas-Oyanedel and Schmidt (2018), who reported how acetic and butyric acid production from FW was 16 times more profitable than methane injection into the grid. Other authors such as Pfaltzgraff et al. (2013) also came to the same conclusion.

This discussion between VFA and biomethane production reflects that depending on the type of substrate, it might be more cost-effective to go towards one product or the other. Thus, when SS is available as a substrate, it was found that biomethane resulted in a higher net profit. This is due to the high purification/concentration costs involved, the VFA yields not being very high, and the selectivity of the process implies a higher proportion of acetic in the mix, with a much lower market price than other VFA.

On the other hand, when FW is available, VFA production clearly implies higher net profits than going towards biomethane. It should be noticed that this comparison only considered OPEX and did not contemplate the CAPEX associated with each of the processes or their amortisation. In fact, the results could change significantly if equipment were considered, given that as shown by Bastidas-Oyanedel and Schmidt (2018), the associated CAPEX for a plant producing biomethane from FW and selling it to the grid was estimated at 2 M€, while the plant for conducting dark fermentation to produce purified acetic and butyric acid was almost 15 M€.

9.2.2. VFA-based disinfectants

Since the purity required for VFA in the chemical industry must be very high, the search for alternative end products could be proposed, such as the production of VFA-based cleaners, requiring much lower concentrations for this purpose. As it has already been reported, VFA have characteristics that give them the ability to eliminate pathogens, which may include fungi, bacteria and viruses (Ryssel et al., 2009).

The demand for multi-surface cleaning products has been boosted in recent years in a very notable way due to several factors such as increasing awareness of environmental protection and health risks associated with traditional disinfectants, which often contain harsh chemicals harmful to humans and the ecosystem. In fact, there are ecological certification and labelling programs promoted by governments and regulatory bodies which help the promotion of biologically-based cleaning agents by ensuring that they meet specific criteria.

Thus, seeing the potential that the production of these VFA-based cleaners would have, it would be necessary to study the viability of producing them. To do this, different issues should be clarified such as:

i) Check if the output stream of the membrane train would be adequate for its production without being subjected to additional operations.

ii) Establish the minimum concentration of VFA in the product to achieve the virucidal and/or bactericidal capacity established in the regulations.

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iii) Elucidate which VFA provides that cleaning capacity, therefore defining the substrate and operating conditions which favour the production of the specific VFA.

In fact, there is already a company that is marketing all-purpose cleaner base rich in acetic acid produced from biosolids (Renew®), whose composition specifies a concentration of acetic acid and other organic acids < 5% by volume, in addition to other ingredients such as surfactants, and "recycled water". This is a clear example of alternatives to the individualised VFA separation, although such a different approach still fails to provide high-purity VFA with their respective uses which could replace petrochemical-based ones.

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10) GENERAL CONCLUSIONS

The general conclusions drawn from this doctoral thesis are:

- The production of high-purity VFA from SS and FW emerges as a technically feasible alternative to traditional petrochemical routes, since it is a way to valorise these organic wastes generated in urban environments.
- The fermentation of SS attained VFA yields noticeably lower than those from FW, being the process selectivity also different. While the alkaline fermentation of SS produces mainly acetic acid, the neutral pH fermentation of FW gives rise to a mix in which propionic and butyric acids have a greater presence.
- In general, there is consistency in the results seen for laboratory-scale fermentation with those reported in the pilot plant, which gives robustness for the process industrialisation.
- Membrane technology is useful to increase the concentration of VFA up to around 50 g/L, although the permeate fluxes reported with real medium are much lower than those specified in the membrane technical sheets. This highlights the disadvantages of using membranes that were not initially designed to treat matrices of this type, making this unit operation the bottleneck of the overall process also from a monetary perspective.
- VFA recovery percentages obtained on LLE at pilot scale with both ethyl acetate and ethyl octanoate were initially lower than those reported at laboratory scale, but they aligned after the column modifications.
- The distillation units necessary to produce high-purity VFA with ethyl acetate involve greater energy consumption than with ethyl octanoate, since its higher solubility makes an additional column necessary to recover the solvent lost in raffinate. Even so, ethyl acetate turned out to be the best solvent when considered the global process economy, with 0.75 being the S/F which resulted in the greatest profit margin.
- Large plant scales are needed in order to amortise the required CAPEX over a period of 20 years. For SS, a facility treating the generation of 871,500 equivalent inhabitants is required. In contrast, the minimum viable scale when treating FW would be 438,000 inhabitants.
- The LCA showed that the hotspots of the process from an environmental perspective were the transport of the substrates to the facilities and the chemical consumption. In addition, the comparison between the carbon footprint obtained for produced VFA turned out to be significantly higher than those reported for petrochemical processes. Therefore, the overall process needs to be further optimised to reduce these impacts.
- The elevated purification costs may mean that other alternatives such as the production of biomethane from certain substrates could be positioned as a more economically favourable choice.
- Other options such as the production of VFA-based disinfectants might be a more advantageous target product given its versatility and lower production complexity.

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11) LIST OF PUBLICATIONS

Thermal hydrolysis pre-treatment has no positive influence on volatile fatty acids production from sewage sludge

Bioresource Technology. *376* (March), 128839. https://doi.org/10.1016/j.biortech.2023.128839

Specific contribution to the publication:

Investigation, conceptualisation, visualisation, writing-original draft, software.

Journal quality indices:

The journal where Chapter 3 was published currently presents a journal impact factor of 11.4, a Journal Citation Indicator of 1.79 (2022 Journal Citation Reports) and this position in the following category: quartile 1 (Q1) in Biotechnology & Applied Microbiology (SJR 2022) calculated by Journal Citation Reports:

https://jcr.clarivate.com/jcr-jp/journal-profile?journal=BIORESOURCE%20TECHNOL&year=2022&fromPage=%2Fjcr%2Fbrowse-journals

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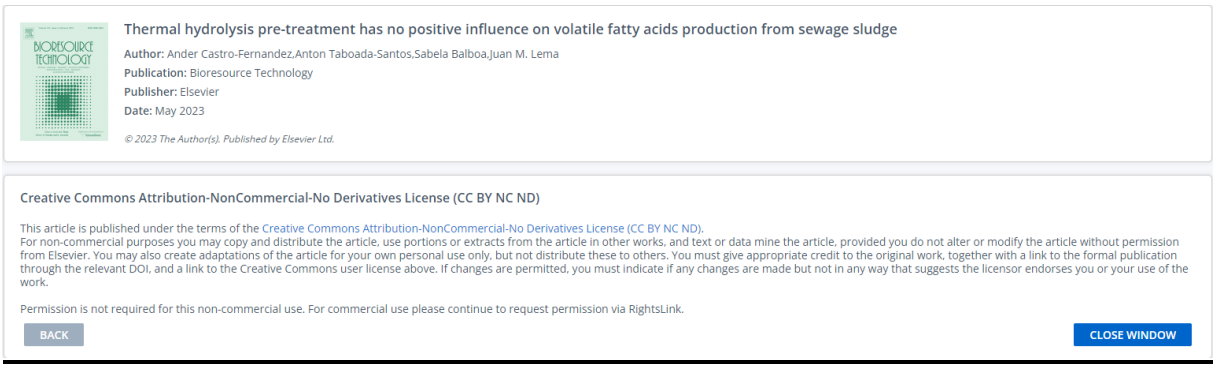

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Scale-up and economic assessment of the production of volatile fatty acids from food waste

Biomass and Bioenergy. *182* (March), 107112. https://www.sciencedirect.com/science/article/pii/S0961953424000655#appsec1

Specific contribution to the publication:

Investigation, conceptualisation, visualisation, writing-original draft, software.

Journal quality indices:

The journal where Chapter 4 was published currently presents a journal impact factor of 6.0, a Journal Citation Indicator of 0.98 (2022 Journal Citation Reports) and this position in the following category: quartile 1 (Q1) in Biotechnology & Applied Microbiology (SJR 2022) calculated by Journal Citation Reports:

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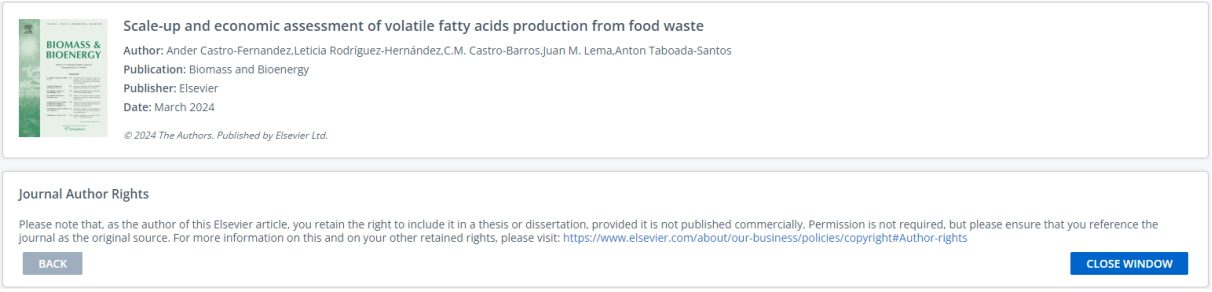

12) DISCLOSURES: CONFLICT OF INTEREST, USE OF IMAGES AND PUBLISHED CONTENT

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In a context of population growth, so does the generation of urban organic streams such as sewage sludge and biowaste. These can be subjected to fermentation processes to produce volatile fatty acids, chemical compounds highly demanded in the industry which are currently originated from fossil fuels. This work addresses the scaling up of the proposed technology to provide high-purity volatile fatty acids, optimising unit operations from a global perspective. Thus, experimentation started at laboratory scale to reach semi-industrial pilot scale, evaluating both the fermentation as well as the purification stages. All the information gathered experimentally allowed to economically evaluate the feasibility of building biorefineries for this purpose, therefore approaching industrial implementation.