UNIVERSIDAD DE SANTIAGO DE COMPOSTELA
Departamento de Ingeniería Química

Modelling, Optimisation and Control of Anaerobic Co-digestion Processes

Memoria presentada por
Santiago García Gen

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Informan:

Que la memoria titulada "Modelling, optimisation and control of anaerobic co-digestion processes" que presenta Don Santiago García Gen para optar al grado de Doctor en Ingeniería Química, Programa de Doctorado en Ingeniería Química y Ambiental, ha sido realizada bajo nuestra dirección en el Departamento de Ingeniería Química de la Universidad de Santiago de Compostela.

Y para que así conste, firman el presente informe en Santiago de Compostela, a mayo de 2015.

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Resumen

La digestión anaerobia es un proceso biológico que transcurre espontáneamente en la naturaleza, y que transforma la materia orgánica en metano y dióxido de carbono (biogás) por la acción de un conjunto de microorganismos anaerobios que catalizan las reacciones de transformación. Su rendimiento y producción de metano varía ampliamente dependiendo del tipo de sustrato orgánico y las condiciones ambientales. La instalación de plantas de biogás permite mejorar los rendimientos de transformación de la materia orgánica de residuos a metano cuando éstas se operan bajo determinadas condiciones. El objetivo de estas plantas es doble: por un lado, recuperar de energía en forma de biogás y, por otro, conseguir un efluente líquido (digerido) más estabilizado, es decir, con un menor contenido en materia orgánica que el residuo de entrada a la planta. De esta manera, la digestión anaerobia se presenta como un proceso que permite aprovechar los recursos energéticos y renovables de los residuos orgánicos y, al mismo tiempo, evitar la emisión incontrolada de metano a la atmósfera, que se podría producir durante la descomposición natural de la materia orgánica del residuo.

En las últimas décadas, el interés por los procesos de digestión anaerobia se ha ido incrementando desde la aplicación inicial del proceso para la estabilización de lodos de depuradoras en plantas de tratamiento de aguas residuales hasta el tratamiento de la fracción orgánica de residuos sólidos urbanos y para el tratamiento de residuos agro-industriales. Más recientemente, el concepto de co-digestión anaerobia (digestión simultánea de dos o más residuos) está despertando un mayor interés, debido a las sinergias que se pueden lograr al combinar diferentes tipos de residuos ya que las características físico-químicas de la mezcla resultante pueden mejorar las características de los sustratos individuales, y por tanto, permiten obtener mayores rendimientos de operación. Sin embargo, la elección de la mezcla de sustratos en sistemas de co-digestión que permitan una operación estable y optimizada no es trivial y requiere un conocimiento detallado del proceso y experiencia en operación con digestores anaerobios. Esta Tesis pretende contribuir a la modelización, optimización y control de procesos de co-digestión anaerobia con el objetivo de mejorar el rendimiento de operación de los co-digestores anaerobios.

El Capítulo 1 presenta una revisión bibliográfica de los principales aspectos de digestión anaerobia y co-digestión. Describe los fundamentos del proceso y explica con detalle los mecanismos de reacción. Además, realiza una revisión de los residuos que se utilizan como sustrato principal y co-sustrato en el proceso, analiza las diferentes configuraciones de operación y reactores que se utilizan en la actualidad, así como los

principales parámetros físico-químicos que se consideran para la monitorización y control. En otro apartado del capítulo, se realiza una revisión bibliográfica de los aspectos relacionados con la modelización del proceso, centrándose sobre todo en la descripción del modelo denominado *Anaerobic Digestion Model No. 1* (ADM1). Desde su publicación en el año 2002, ADM1 ha sido utilizado como modelo estándar sobre el cual se han desarrollado nuevas extensiones del modelo, que permiten describir mejor los proceso biológicos y físico-químicos que tienen lugar durante la digestión, además de incluir nuevos sustratos que permiten aplicar el modelo a residuos de diferente naturaleza. Por último, se presentan las principales estrategias que se han desarrollado para el control de digestores anaerobios operando en régimen de mono-digestión anaerobia (controladores PID, controladores basados en lógica difusa, redes neuronales…) y se constata la falta de propuestas para co-digestión anaerobia.

El Capítulo 2 describe con detalle la planta piloto en la que se llevan a cabo las pruebas experimentales de los Capítulos 3, 5 y 6. La planta consta de un reactor híbrido UASB-AF (Upflow Anaerobic Sludge Blanket – Anaerobic Filter) de 1 m^3 de volumen útil equipado con la instrumentación clásica de los digestores anaerobios (sensores de pH, temperatura y caudalímetro de gas) y con analizadores avanzados para la monitorización de alcalinidad, ácidos grasos volátiles, composición de biogás y carbono orgánico total. Además, la planta dispone de un sistema de adquisición de datos que permite registrar y almacenar los datos de proceso de la planta.

En el Capítulo 3 se desarrolla un procedimiento para la incorporación de sustratos solubles fermentables en un modelo ADM1 modificado. Las reacciones de fermentación de sustratos tales como el etanol o glicerina, no incluidos originalmente en ADM1, se implementan como reacciones en equivalentes de fermentación de glucosa. Considerando que la acidogénesis es la etapa más rápida del proceso, no se requiere una descripción muy rigurosa de la estequiometría de los sustratos (etanol, glicerina, lactato…) y productos de reacción (ácidos grasos volátiles: butirato, propionato y acetato) en las reacciones de fermentación. Se asume que, en sistemas metanogénicos, los diferentes ácidos intermedios productos de la reacción son rápidamente convertidos a acetato, dióxido de carbono e hidrógeno. El modelo, implementado en una plataforma MATLAB/Simulink, permite la simulación de los experimentos de co-digestión que se describen en los Capítulos 3 y 5, que utilizan co-sustratos que contienen etanol y glicerina, respectivamente. En el capítulo 3 se muestran los resultados experimentales y de simulación de un sistema de co-digestión, operado en

modo continuo, que trata purín porcino y vino diluido. A partir de los resultados obtenidos, se concluye que: (1) la simplificación que se propone para modelar la fermentación de sustratos solubles como una reacción equivalente de glucosa, resulta ser adecuada para sistemas metanogénicos, considerando que la cinética de formación de ácidos grasos volátiles (acidogénesis) es mucho más rápida que la etapa de formación de metano (metanogénesis); (2) este método generalizado permite ampliar la aplicación del modelo ADM1 a otros sustratos no incluidos inicialmente en el modelo; (3) el modelo ha sido validado en un experimento a largo plazo de co-digestión en continuo, siendo los resultados muy satisfactorios tanto en estado estacionario como en régimen dinámico. El modelo resulta de gran utilidad para predecir el comportamiento y rendimiento de un proceso de co-digestión en una planta real; (4) la simulación del proceso de co-digestión utilizando los parámetros cinéticos del modelo ADM1 original permite una buena predicción de los resultados experimentales, a excepción del $K_{I,NH3}$, la constante de inhibición descrita en la etapa *metanogénesis acetoclástica* debido a la presencia de amoníaco, cuyo valor tiene que ser calibrado para obtener un buen ajuste de los resultados.

El tratamiento de residuos sólidos mediante digestión o co-digestión anaerobia resulta atractivo ya que ofrece un mayor contenido de materia orgánica y, por tanto, un mayor potencial de recuperación de energía. El Capítulo 4 presenta un modelo novedoso para la descripción de las etapas de desintegración e hidrólisis de sustratos sólidos complejos (entendiéndose por sólidos complejos, aquéllos que están formados por aglomeraciones de proteínas, lípidos, carbohidratos e inertes). Este aspecto resulta de interés ya que la etapa de desintegración-hidrólisis es la más lenta de todo el proceso de digestión de residuos sólidos. El modelo propuesto considera que un residuo sólido está formado por dos fracciones sólidas, una rápidamente biodegradable y otra lentamente biodegradable, las dos con idéntica composición. A partir de esta idea, se desarrolla un modelo que considera la desintegración por separado de estas dos fracciones con el objetivo de describir con más precisión la degradación de los sustratos sólidos. El modelo se incorpora al modelo de co-digestión presentado en el Capítulo 3, para simular experimentos de digestión y co-digestión anaerobia de sólidos. El Capítulo 4 presenta resultados experimentales y de simulación a escala laboratorio de (i) sistemas de digestión anaerobia (operados en modo discontinuo o *batch*) de residuos de frutas y verduras, subproductos de plantas de refino de aceites vegetales y residuos de pescado, y (ii) un sistema de co-digestión (operación en modo pseudo-

continuo) de un conjunto de residuos de frutas y verduras. Es importante destacar que para poder calcular los parámetros cinéticos con precisión se utilizaron datos de ensayos experimentales en reactores batch de 6 L para los que se había seguido un procedimiento optimizado, caracterizado por: (a) realizar una serie de 6 a 8 ensayos batch consecutivos con el mismo sustrato hasta obtener una biomasa perfectamente aclimatada al residuo y evitar cualquier fase de retardo en la degradación del sustrato; (b) utilizar una relación sustrato/biomasa baja (se emplea un ratio de 0.08 en vez del valor típico de 0,5 - 1,0 utilizado en los ensayos de biodegradabilidad) que permite obtener tiempos de reacción cortos (inferiores a una semana) y por tanto, permite mantener la biomasa con una buena actividad; y (c) disponer de un sistema de medida muy preciso que permite medir y registrar los datos de volumen de gas producido cada 2 minutos y así obtener una curva de biodegradabilidad muy exacta. El protocolo de experimentación y los ensayos en reactores fueron desarrollados por investigadores del *Laboratoire de Biotechnologie de l'Environnement*, INRA-LBE, de Narbonne, Francia. A partir de los resultados obtenidos se concluye que: (1) el modelo propuesto para las etapas de desintegración e hidrólisis es capaz de describir con precisión procesos de digestión y co-digestión anaerobia de residuos sólidos en modo discontinuo y pseudo-continuo; (2) la aplicación de una etapa de desintegración propia para las fracciones rápidamente y lentamente biodegradables del sólido complejo permite estimar parámetros cinéticos de desintegración e hidrólisis que son válidos para un conjunto grande de residuos de la misma familia (residuos de frutas y verduras); (3) la fracción rápidamente biodegradable de un residuo se determina experimentalmente a partir del ensayo de biodegradabilidad. Por tanto, se considera una característica inherente del sustrato y no un parámetro estimado mediante calibración con el modelo; y por último, (4) los parámetros cinéticos calibrados con los ensayos batch de cada residuo permiten predecir el comportamiento de un sistema pseudo-continuo de co-digestión anaerobia a largo plazo.

Los sistemas de co-digestión anaerobia pueden mejorar el rendimiento de las plantas de biogás, en términos de mayor productividad de metano y mayor estabilidad de la operación, si se aprovechan las sinergias que se pueden establecer entre los diferentes residuos. Sin embargo, no todas las combinaciones de sustratos son viables o apropiadas para procesos de co-digestión. En el Capítulo 5 se presenta un método de optimización basado en programación lineal que determina la mejor mezcla de alimentación para sistemas de co-digestión (se determina el caudal de cada sustrato y

el tiempo de residencia hidráulico del sistema, TRH), capaz de maximizar la recuperación de energía a cada velocidad de carga orgánica (VCO) aplicada. Las mezclas de alimentación que se calculan con este método de optimización están sujetas a un conjunto de restricciones físico-químicas, que se introducen a partir del conocimiento heurístico del proceso de digestión anaerobia. El método define una función objetivo que se pretender maximizar, productividad de metano, y calcula la mezcla que maximiza la función objetivo teniendo en cuenta las características físico-químicas y las curvas de biodegradabilidad de los sustratos. Este método de optimización se implementó en MATLAB/Simulink y, gracias a la arquitectura que permite Simulink, se puede conectar el bloque que contiene el método de optimización propuesto (bloque denominado *Blender*) con el bloque que contiene el modelo de co-digestión anaerobia descrito en el Capítulo 3 (bloque denominado *Digester*). Los parámetros de salida del bloque *Blender* (caudales de los sustratos de la mezcla y TRH del sistema) son los parámetros de entrada del bloque *Digester* que simula el proceso de digestión. El producto que se obtiene es una herramienta para simular sistemas de co-digestión anaerobia que procesa mezclas óptimas, y que puede ser útil para predecir y evaluar la viabilidad de las mezclas de sistemas reales. En este capítulo se describe un experimento de co-digestión anaerobia a escala piloto y operado en continuo que procesa mezclas optimizadas de purín porcino, residuo de biodiésel (que contiene principalmente glicerina) y gelatina, a diferentes VCO y proporciones de mezclas. A partir de los resultados obtenidos en este capítulo se concluye que: (1) el método de optimización basado en programación lineal es una herramienta útil para calcular mezclas óptimas de residuos que permite maximizar la productividad de metano de los procesos de co-digestión anaerobia; (2) para el cálculo de las mezclas óptimas sólo se necesita la información típica que está disponible en la mayoría de las plantas de tratamiento, esto es, la composición y características de los sustratos y los ensayos de biodegradabilidad; por tanto, el método puede aplicarse de manera inmediata a plantas existentes; (3) las características físico-químicas que se imponen como restricciones lineales en el método de optimización están definidas en base al conocimiento heurístico y experiencia del proceso. Sin embargo, los límites que se imponen pueden modificarse a lo largo de la operación para conseguir así mayores producciones de metano; (4) el TRH del sistema y las proporciones en las que se combinan los diferentes sustratos obtenidos mediante programación lineal pueden variar dependiendo de la VCO aplicada. Por tanto, cuando en un sistema de co-digestión se modifica la VCO hay que volver a ejecutar el método de optimización para calcular la

nueva mezcla óptima; (5) el método de optimización se validó en un experimento en continuo a diferentes condiciones de VCO en estado dinámico; y (6) la simulación del experimento real de co-digestión con el modelo ADM1 modificado permite validar una vez más el método generalizado de incorporación de sustratos fermentables descrito en el Capítulo 3, al predecir con gran exactitud el comportamiento de un sistema de co-digestión que incorpora glicerina como compuesto mayoritario en términos de contenido en materia orgánica.

Para poder controlar un proceso de co-digestión anaerobia que opera en modo continuo, a diferentes condiciones de VCO y con diferentes mezclas de alimentación, es necesario establecer un método de diagnosis y control adecuados que permita identificar el grado de estabilidad del sistema. El método de diagnosis debe ser capaz de: (i) detectar las perturbaciones que se producen durante la operación que evite una desestabilización irreversible del proceso; (ii) detectar la oportunidad de incrementar la capacidad de carga orgánica del sistema cuando el proceso está estable, de manera que se puedan obtener mayores producciones de biogás. Por otro lado, la acción de control debe ser capaz de (a) revertir las desestabilizaciones que se produzcan y alcanzar un nuevo punto de operación estable para el proceso; (b) seleccionar las nuevas condiciones que permitan mejorar la productividad en biogás; (c) mantener una calidad de digestato preestablecida.

En este sentido, el Capítulo 6 presenta una estrategia de control novedosa para procesos de co-digestión anaerobia que pretende abordar estos aspectos. La estrategia de control propuesta funciona en lazo cerrado con la siguiente secuencia: (a) se determina una mezcla óptima a partir del método de optimización descrito en el Capítulo 5; (b) se opera el digestor con la mezcla óptima durante un determinado tiempo; (c) se diagnostica y cuantifica el grado de estabilidad de la operación al comparar dos indicadores físico-químicos del proceso con dos valores de referencia que establecen el límite de estabilidad; (d) se calcula un indicador final de control que modifica los límites de las restricciones lineales definidas en el método de optimización de mezclas; (e) se calcula una nueva mezcla para el sistema y se ejecuta el lazo desde (a) hasta (e) durante toda la operación. Se establecen los parámetros de *ratio de alcalinidades* y *producción de metano* como los indicadores de proceso para estimar, respectivamente, la estabilidad del sistema frente a la acidificación del digestor y la capacidad de producción de metano remanente del sistema. En función del resultado de la diagnosis, la acción de control modifica el valor de la restricción limitante del

conjunto de restricciones lineales (aquélla que al alterar su valor permite obtener el mayor incremento/disminución del valor de la función objetivo). La acción de control permite alcanzar nuevos puntos de operación cuando el método de optimización calcula una alimentación sujeta a nuevas restricciones, obteniéndose una mezcla y un TRH diferentes. Como resultado, se obtiene una operación controlada en la que cada cierto tiempo se evalúa el desempeño y estabilidad del digestor cuando procesa una determinada mezcla óptima (en este caso, se ejecuta el lazo de control cada ¼ TRH). Cuando el sistema esté estable (el valor *ratio de alcalinidades* es inferior a un valor de referencia) el controlador incrementa la VCO en el siguiente ciclo de control, permitiendo así obtener mayores producciones de metano; cuando el sistema se muestre inestable, se limita la VCO en el siguiente ciclo de control con el fin de favorecer la recuperación del sistema respecto a una acidificación transitoria. En el Capítulo 6 se describe un experimento de co-digestión operado en modo continuo y sujeto a la estrategia de control. Durante la operación se alimentan al reactor mezclas binarias o ternarias de purín porcino, residuo de biodiésel y gelatina (calculada mediante el método de optimización) durante una operación de 7 meses trabajando a diferentes velocidades de carga orgánica. A partir de los resultados obtenidos, se concluye que: (1) la estrategia de control se valida como una herramienta útil para operar sistemas de co-digestión anaerobia en continuo que procesa diferentes mezclas de residuos a diferentes VCO; (2) los parámetros *ratio de alcalinidades* y *producción de metano* se comportan como indicadores clave de la estabilidad de la operación y grado de conversión de la materia orgánica a metano; estos indicadores son muy sensibles a cambios producidos en las condiciones de operación y composición de las mezclas de alimentación; (3) la acción de control, al modificar los límites de las restricciones lineales aplicadas al cálculo de las mezclas, permite alcanzar nuevos puntos óptimos de operación que mejoran el rendimiento de los procesos de co-digestión en términos de estabilidad y recuperación de energía; y finalmente (4) la estrategia de control se demuestra útil para lograr dos objetivos: maximizar la producción de metano cuando el sistema está estable y recuperar el sistema cuando está desestabilizado por una acidificación transitoria, siempre manteniendo una calidad del digestato compatible con su uso posterior.

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Summary

Anaerobic digestion (AD) is a biological process that occurs spontaneously in nature. However, its performance and methane yield varies widely depending on the type of organic matter and the surrounding environmental conditions to which the substrates are exposed. The installation and operation of anaerobic digesters under controlled operating conditions can enhance the efficiency of AD process treating different sorts of organic substrates. The aims of these facilities include: to achieve energy recovery in the gas stream (biogas), and to obtain a more stabilised liquid effluent (digestate). In the last decades, the interest in anaerobic digestion has grown to treat organic wastes of different types such as sewage sludge generated in wastewater treatment plants, organic fraction of municipal solid wastes and agro-industrial residues. More recently, the interest in the simultaneous digestion of different organic wastes, known as anaerobic co-digestion (AcoD), has emerged based on the potential synergistic effects of some types of residues. The resulting blend of substrates can be enhanced taking advantage of the different compositions and physicochemical characteristics of the individual co-substrates. The determination of the adequate blend of substrates in AcoD that leads to a stable operation is not trivial. It requires knowledge on the process and expertise on the operation since AD involves a complex reaction pathway to convert complex substrates into biogas. This thesis contributes to the modelling, optimisation and control of AcoD processes aiming at improving the performance of anaerobic co-digesters.

The fundamentals of anaerobic digestion and co-digestion are introduced in Chapter 1. In addition, a review of the main substrates used in anaerobic co-digestion, the typical reactor configurations and the key physicochemical parameters used for monitor and control are presented. A general background on AD modelling is also presented, mainly focussed on the description and applications of the Anaerobic Digestion Model No. 1 (ADM1). Since its publication, ADM1 has become the standard model to simulate AD processes, on top of which many extensions have been developed. Regarding control and optimisation of AcoD systems, a list of control strategies are reviewed and classified in different categories (PID controllers, fuzzy logic controllers, neural networks…).

Chapter 2 gives a detailed description of the pilot plant used to carry out the experiments of this thesis. It consists of a hybrid Upflow Anaerobic Sludge Blanket (UASB) and Anaerobic Filter (AF) highly instrumented. The pilot plant is not only equipped with conventional sensors for pH, temperature or gas flow, but also with non-conventional advanced sensors and analysers to monitor on-line variables such as

volatile fatty acids (VFA), alkalinity, biogas composition or total organic carbon. The experiments described in Chapters 3, 5 and 6 have been performed entirely in this plant.

A generalised modelling approach to implement diverse soluble fermentable substrates into an ADM1-based model is developed and validated in Chapter 3. The fermentation reactions of substrates such as ethanol or glycerol, not included in the original ADM1, are implemented as glucose equivalent fermentation reactions. Assuming acidogenesis as the fastest step in anaerobic digestion, an accurate description of the stoichiometry in the fermentation of soluble substrates (ethanol, glycerol, lactate…) into VFA products (butyrate, propionate and acetate) is not required in detail as long as the mass and electron balances remain accurate since all these intermediate acids are quickly converted to acetate, hydrogen and carbon dioxide in methanogenic systems. The model is implemented in MATLAB/Simulink and allowed simulating the AcoD experiments carried out in Chapter 3 and Chapter 5, treating an ethanol-containing co-substrate (diluted wine) and a glycerol-containing co-substrate (biodiesel by-product), respectively.

The treatment of solid wastes with anaerobic digestion or co-digestion is attractive due to their high organic content and potential energy recovery. In the case of treating particulate substrates, the disintegration-hydrolysis stage is considered the slowest step of the process. Chapter 4 presents a novel modelling approach of the disintegration and hydrolysis stages of complex particulate substrates. Solid substrates are assumed to contain a readily- and a slowly-biodegradable fraction. The modelling approach considers a decoupled disintegration of these two fractions to better describe the degradation of solid wastes. This approach is validated with the ADM1-based model by simulating batch assays of numerous fruit and vegetable wastes (FVW) and a continuous AcoD experiment treating a set of FVW.

Anaerobic co-digestion can improve the performance of biogas plants in terms of higher methane productivities and more stabilised operations taking advantage of the synergisms between different co-substrates. However, not all combinations of substrates are feasible or appropriate for AcoD processes. Chapter 5 formulates an optimisation method based on linear programming to calculate the best feeding (substrate flows and hydraulic retention time, HRT) of co-digestion systems, capable of maximising the energy recovery at each organic loading rate (OLR) applied. The resulting blend of substrates is subjected to a set of physicochemical constraints,

defined based on the heuristic knowledge of AD processes. This optimisation method is validated at pilot scale treating different blends of three substrates (pig manure, glycerine and gelatine) at different OLR.

Finally, Chapter 6 introduces a novel control strategy for anaerobic co-digestion. An optimum blend obtained by linear programming is fed in a continuous AcoD experiment, and after a period of time a diagnosis system assesses the performance of the process. Alkalinity ratio and methane production are defined as key diagnosis indicators to estimate the stability of the process against acidification and the extent of energy recovery, respectively. Based on the diagnosis outcome, the control action modifies the boundaries of the restrictions applied in the calculation of the optimum feedings. This control action leads to the reach of a new optimum point when the optimisation method is again evaluated, obtaining a new blend of substrates and HRT for the next period of operation. As a result, the strategy works as a closed-loop controller that optimises the feeding and diagnoses its performance over time. When the system is stable (low alkalinity ratio values are obtained) new calculated feedings are inputted to increase the methane production rate (normally at higher OLR); and when the system is unstable, lower OLR are applied to favour the system to recover from acidification or to prevent from failure.

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Chapter 1. Introduction

Contents

Abstract

This chapter provides background for the research developed in this thesis. Anaerobic co-digestion is introduced with a brief explanation of the reaction pathway. General aspects of the mathematical modelling in anaerobic digestion are presented together with a review of the state of the art. A review of control strategies applied in anaerobic digestion is also introduced. Finally, an outline of the contents of this thesis is presented.

1.1. Anaerobic co-digestion

Anaerobic digestion is a biological process converting the organic matter into biogas through a complex reaction pathway catalysed by different groups of microorganisms. It has been traditionally associated with the treatment of agro industrial waste streams and of sewage sludge from aerobic wastewater treatment plants. The term "co-digestion" stands for the digestion of two or more substrates simultaneously in the same reactor. The possibility of treating blends of multiple organic substrates under co-digestion has recently extended the applicability of anaerobic digestion to substrates not originally conceived for mono-digestion.

The performance of the overall process is much dependent on the type and the composition of the material to be digested (Murto et al., 2004). The digestion of single residues presents some drawbacks concerning their own properties; for instance, (a) low energy recovery can be achieved from substrates as sewage sludge, characterized by having low organic matter, (b) cattle manures involve both low organic contents and high nitrogen concentrations, which may inhibit methanogenesis, (c) seasonal residues as crops and agro-industrial wastes may be deficient in nitrogen content, and (d) residues with high protein and lipid contents as slaughterhouse wastes can be potential inhibitors of the methanogenic activity. Most of these problems can be overcome by the addition of co-substrates and treated under anaerobic co-digestion (Mata-Álvarez et al., 2014).

Co-digestion is preferably used for improving yields of anaerobic digestion of solid organic wastes. The potential benefits of co-digestion include: dilution of toxic compounds, increased load of biodegradable organic matter, improved balance of nutrients and higher biogas yields. Co-digestion also provides nutrients in excess (Hartmann and Ahring, 2005), which can accelerate biodegradation of solid wastes through bio-stimulation. Additionally, digestion rate and stabilization are increased (Sosnowski et al., 2003; Lo et al., 2010). A digestate of higher quality can be obtained (Jingura and Matengaifa; 2009) with potential uses as fertiliser. Feeds to bioreactor are stabilised by improving the carbon to nitrogen (C/N) ratio (Cuetos et al., 2008) and adjusting the lipid content and nitrogen content (Castillo et al., 2006). Finally, the use of a co-substrate can also help to achieve the required moisture of the feeding (Mata-Álvarez et al., 2000).

Thanks to the complementary characteristics of different residues, co-digestion can not only increase biogas production (Mata-Alvarez et al., 2011), but also achieve other environmental, technological and economic advantages: a more efficient use of equipment and cost-sharing by processing multiple waste streams in a single facility (Alatriste-Mondragón et al., 2006), or lower greenhouse gas emissions and climate change impact in comparison to composting or anaerobic mono-digestion (Krupp et al., 2005). However, co-digestion still presents some drawbacks associated to: the high-cost transfer of substrates from the generation point to the anaerobic plant, the risk of spreading toxic substances originated from the industrial or municipal waste, deal with different regulatory and management regimes depending on the nature of the residue or include pre-treatment of substrates for hygienisation requirements (Astals et al., 2011; Iacovidou et al., 2012; Braun and Wellinger, 2003).

The key aspects to improve the performance of the co-digestion process and enhance the energy recovery involve the appropriate selection of co-substrates, the application of pre-treatments to raw wastes to improve their characteristics and the choice of a suitable digester design (Shah et al., 2015).

1.1.1. Main substrates and co-substrates

The different type of substrates used for co-digestion include organic fraction of municipal solid wastes (OFMSW), sewage sludge (SS), waste oils and animal fats, energy crops, agricultural wastes and manures (Appels et al., 2011; Mata-Álvarez et al., 2011).

Mata-Álvarez et al. (2014) has recently reviewed the trend of the main substrates and co-substrates used in co-digestion in the last years (Figure 1.1). Manures (mainly pig manure and cow manure) and sewage sludge (SS) appear to be the main substrates used. Regarding the co-substrates used with manures, agro-industrial waste were the most applied co-substrate, followed by OFMSW, crude glycerol, cheese whey, and olive mill waste. On the other hand, sewage sludge, traditionally co-digested with OFMSW, has been recently highly reported as co-digested with fats, oils and greases (FOG), allowing a 30–80% increase in digester gas production in two full scale wastewater biosolids anaerobic digesters (Long et al., 2011).

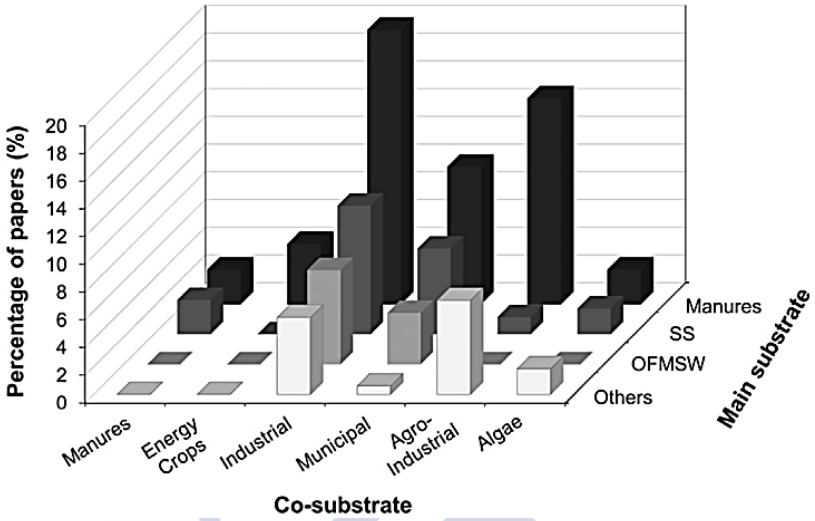

Figure 1.1. Main substrates and co-substrates used in co-digestion according to the papers published between 2010-2013 (Mata-Álvarez et al., 2014).

Other co-substrates such as fruit and vegetable waste (FVW), slaughterhouse wastes and algae have also been reported. Manure has been identified as the best co-substrate for fat-containing wastes to reduce the lipid concentration in the digester (Appels et al., 2011). According to Angelidaki and Ellegaard (2003) the suitability of manure as co-substrate is based on: a) the moisture content in manure acts as solvent for dryer types of wastes, solving problems of pumping and mechanical treatment of solid wastes, b) the buffering capacity contained in manure protects the process against acidification and c) the high nutrient content of manure favours optimal bacterial growth.

1.1.2. Pre-treatments

The main effects of pre-treatments on different substrates can be identified as a) reduction of particle-size, b) solubilisation, c) biodegradability enhancement, d) formation of refractory compounds, and e) loss of organic material (Carlsson et al., 2012). These processes include biological pre-treatment (largely thermal phased anaerobic digestion and enzyme hydrolysis), thermal hydrolysis, mechanical treatment (such as ultrasound, high pressure and lysis), chemical treatment (mainly ozonation and also acid, hydrogen peroxide and alkali treatment) and microwave pre-treatment (Appels et al., 2008; Carrère et al., 2010; Lee et al., 2014).

1.1.3. Digester design

Depending on the characteristics of the substrates and the operation conditions (mainly organic loading rate, OLR, and hydraulic retention time, HRT), different digester designs are utilised. Anaerobic digestion can be carried out in a one-stage or two-stage configuration, using different reactor types and operated in continuous or batch systems (Shah et al., 2015; Nasir et al., 2012).

In a one-stage digestion all the biological reactions occur in one reactor, while in two-stage configuration the overall digestion is achieved in different reactors. In a two-stage process, the hydrolysis and acidogenesis steps take place in the first digester, whereas acetogenesis and methanogenesis in the second reactor. The one-stage system is the most popular at industrial scale due to its simplicity of operation, reduced costs and lesser technical problem (Shah et al., 2015). If classified based on the solid content, dry digesters treat wastes having a 20-40% dry matter content and wet digesters those with dry matter up to 20%, the continuously stirred tank reactor (CSTR) being the most commonly used configuration of wet digesters.

Other types of reactors were developed and adapted to allow higher reaction rate per unit volume of digester (Nasir et al., 2012). Anaerobic filters (AF), upflow anaerobic sludge blanket (UASB), anaerobic packed-bed and fluidized-bed reactors are utilized as high-rate digesters both at laboratory and industrial scale, the UASB being the most popular in the recent years. AF and UASB digesters achieve high solid retention time (SRT) via attachment of biomass to high density carriers or formation of granules in these digesters, respectively (Shah et al., 2015).

Regarding the operation mode, the different reactor designs comprise batch reactors, continuous reactors (one–stage and two–stage), anaerobic sequencing batch reactors (ASBR) and plug flow reactors (PFR). The continuous system uses mechanical agitation or biogas recirculation to homogenise the content of the digester The PFR is an unmixed system where waste flows semi–continuously as a plug through a horizontal reactor (Nasir et al., 2012). The examples of continuous digesters include PFR, CSTR, AF and UASB. According to Shah et al. (2015), most of the industrial scale plants currently operating in Europe are different continuous one-stage digesters.

1.2. Degradation reaction pathways in anaerobic digestion

The biological reaction steps in anaerobic digestion include hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gujer and Zehnder, 1983). The Figure 1.2 shows a scheme of the reaction pathway of AD process (Madsen et al., 2011).

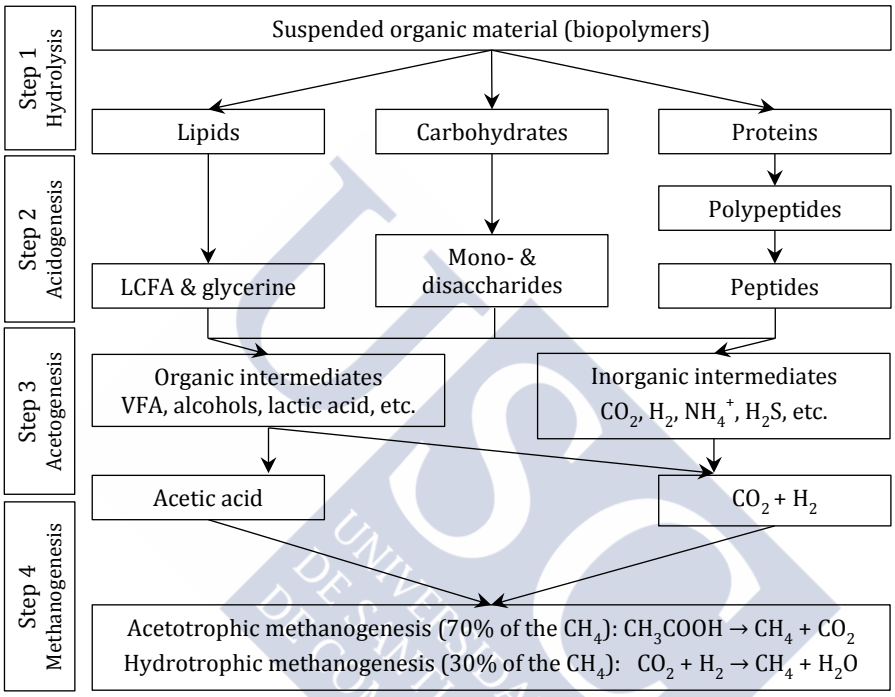

Figure 1.2. Biological degradation reaction pathway in anaerobic digestion (adapted from Madsen et al., 2011).

Hydrolytic bacteria bring about initial degradation of complex biopolymers such as carbohydrates, proteins and lipids into sugars, amino acids and long chain fatty acids. The hydrolysed compounds are further degraded by acidogenic bacteria to volatile fatty acids (VFA) together with ammonia, carbon dioxide, hydrogen sulphide and other by-products. Then, VFA are converted to produce mainly acetate, hydrogen and carbon dioxide. This conversion is controlled to a large extent by the partial pressure of hydrogen. Hydrogen-producing acetogenic bacteria grow only in syntrophic association with hydrogen-consuming methanogenic archaea (Appels et al., 2008;

Gupta et al., 2012). Finally, methanogenesis produces methane by means of two groups of degraders: acetoclastic methanogens converting acetate into methane and carbon dioxide and hydrogenotrophic methanogens using hydrogen as electron donor and carbon dioxide as acceptor to produce methane.

1.2.1. Main physicochemical parameters affecting AD

Parameters like pH, temperature, carbon source, nitrogen content, carbon to nitrogen ratio (C/N), or solids and hydraulic retention time, can affect the performance of the overall process (Appels et al., 2008; Khalid et al., 2011; Gupta et al., 2012).

Each group of microbial degraders has a different optimum pH range. Methanogenesis occurs efficiently at pH 6.5–8.2 (Lee et al. 2009), while hydrolysis and acidogenesis at pH 5.5 and 6.5, respectively (Kim et al., 2003). For the overall process, pH ranges within 6.5–7.5 are considered favourable for anaerobic digestion (Ward et al., 2008; Liu et al., 2008). During the operation, buffering capacity must be maintained for a stable digestion (Appels et al., 2008).

The temperature of the process influences the growth rate and metabolism of microorganisms and therefore the population dynamics (Appels et al., 2008). Low temperatures decrease microbial growth, substrate utilization rates and biogas production (Kim et al., 2006). In anaerobic digestion, two main ranges of temperature are used: mesophilic conditions (20–45 °C) and thermophilic conditions (50–65 °C) (Gupta et al., 2012). The operation in the mesophilic range is more stable and involves lower energy costs; however, operation under thermophilic conditions typically allows for faster degradation rate of organic waste, higher gas production rate and larger pathogen destruction (Khalid et al. 2011).

The composition of the biogas and the methane yields achieved depend on the characteristics of the main substrate. Theoretical gas yields vary with the average degree of reduction of the main substrate, which is different in carbohydrates, proteins and lipids. Lipids provide the highest biogas yield, but require longer retention time due to their poor bioavailability. On the contrary, carbohydrates and proteins show much faster conversion rates though lower gas yields (Weiland, 2010).

Nitrogen is a required nutrient for biomass growth. Proteins are converted to ammonium and then microbes assimilate ammonium for the production of new cell mass. However, depending on the concentration of ammonia and the operation pH, free

ammonia may lead to the inhibition of methanogenesis (Khalid et al., 2011). The relationship between the amount of carbon and nitrogen in a substrate is given by the C/N ratio. This parameter plays an important role in the process since an unbalanced nutrient content is considered an important limiting factor in the digestion of organic wastes. High C/N ratios can lead to exhaust the necessary N source for biomass growth whereas lower C/N ratios lead to the accumulation of ammonia (Divya et al., 2015). For a stable operation, the optimum C/N ratio falls between 20 and 70 gCOD/g N (Mata-Álvarez et al., 2011).

The solids retention time (SRT) stands for the average time the solids remain in the digester, whereas the hydraulic retention time (HRT) is the average time the liquid sludge stay in the digester. The SRT is a fundamental operating parameter for all anaerobic processes. A decrease in the SRT decreases the extent of the reactions involving solid substrates while at the same time possibly washing out specific slow growing microbial biomass such as methanogens. During the operation, a fraction of the biomass is constantly removed together with the digestate, thus implying that the cell growth must at least compensate the cell removal to ensure steady state and avoid process failure (Appels et al. 2008).

1.2.2. Inhibitions

There is a large variety of inhibitory compounds that may become the primary cause of digester failure when they are present in significant concentrations in wastes. Both inorganic and organic compounds can cause the process upset. Inorganics such as ammonia, sulphide, light metal ions (Na^{+}, K^{+}, Mg^{+2}, Ca^{+2} and Al^{+3}), heavy metals as well as molecular hydrogen must remain within certain concentration ranges to prevent process inhibition. In addition, numerous organic compounds have been reported as toxicants to anaerobic digestion at certain concentrations. These include chlorophenols, halogenated aliphatics, N-substituted aromatics, long chain fatty acids, volatile fatty acids, lignin and lignin related compounds (Chen et al., 2008; Appels et al, 2008). The accumulation of these substances may lead to reactor failure, after a reduction in biogas production and/or biogas methane content. The mechanism of inhibition of each toxicant may affect different microbes of the anaerobic process in different ways. For instance, high concentrations of free ammonia or sodium ions inhibits methanogenesis, sulphide is toxic to various groups of microorganisms. Heavy metals (Cu, Pb or Zn) can cause toxicity by disrupting the enzyme structure and function (Appels et al., 2008). High salt content causes bacterial cells to dehydrate due

to osmotic pressure; the toxicity of salts was found to be predominantly determined by the cations of salts (Chen et al., 2008). VFA are key intermediates in the process and, their free protonated forms, can inhibit methanogenesis when in high concentrations. Although acetic acid is usually present in higher concentration than any other fatty acids, propionic and butyric acids are more inhibitory to methanogens (Weiland, 2010). The mechanism of LCFA toxicity is believed to be caused by adsorption onto the microbial surface, leading to interference with the transport of substrates and products from or to the cell (Palatsi et al., 2009).

1.3. Modelling of anaerobic digestion/co-digestion

The mathematical modelling of anaerobic digestion was motivated by the need for efficient operation of anaerobic systems in the early 1970s (Donoso-Bravo et al., 2011; Rodríguez, 2006). Initially very simple models were developed because of the limited knowledge of the complexity of anaerobic digestion. The first approaches focused on describing the limiting step of the process that controls the overall rate, where hydrolysis and methanogenesis were identified as the rate-limiting steps (Eastman and Ferguson, 1981). A second generation of models considered VFA concentration as the key parameter, including acidogenesis and acetogenesis separately (Hill, 1982). A third generation incorporated additional processes, species, kinetics and inhibitions from microbiological studies (Donoso-Bravo et al., 2011).

At present, the Anaerobic Digestion Model No. 1 (ADM1), developed by the IWA Task Group of Modelling (Batstone et al., 2002), has been widely used as the reference model for further model developments and validation studies on anaerobic digestion and co-digestion. ADM1 has become available in Matlab and Simulink, but also in specific water related simulation software, such as WEST, BioWin and Aquasim (Lauwers et al., 2013).

1.3.1. The ADM1 as reference AD model

The ADM1 is a general model that incorporates a description of the most important reaction and transport processes of anaerobic digestion: disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis steps. It comprises a large number of simultaneous and sequential biochemical and physicochemical reactions. Extra-cellular enzymes are assumed to catalyse biochemical reactions involving biologically-available organic substrates (hydrolysis step). All extra-cellular ADM1 biochemical reactions are

assumed to follow empirically-based first order rate law kinetics, and all intra-cellular ADM1 biochemical reactions (acidogenesis, acetogenesis and methanogenesis) are assumed to follow Monod-type substrate uptake kinetics. Substrate uptake reaction rates are considered proportional to the biomass growth rate and biomass concentration (Kythreotou et al., 2014).

Figure 1.3. presents an overview of the substrates and conversion processes described by the ADM1 model.

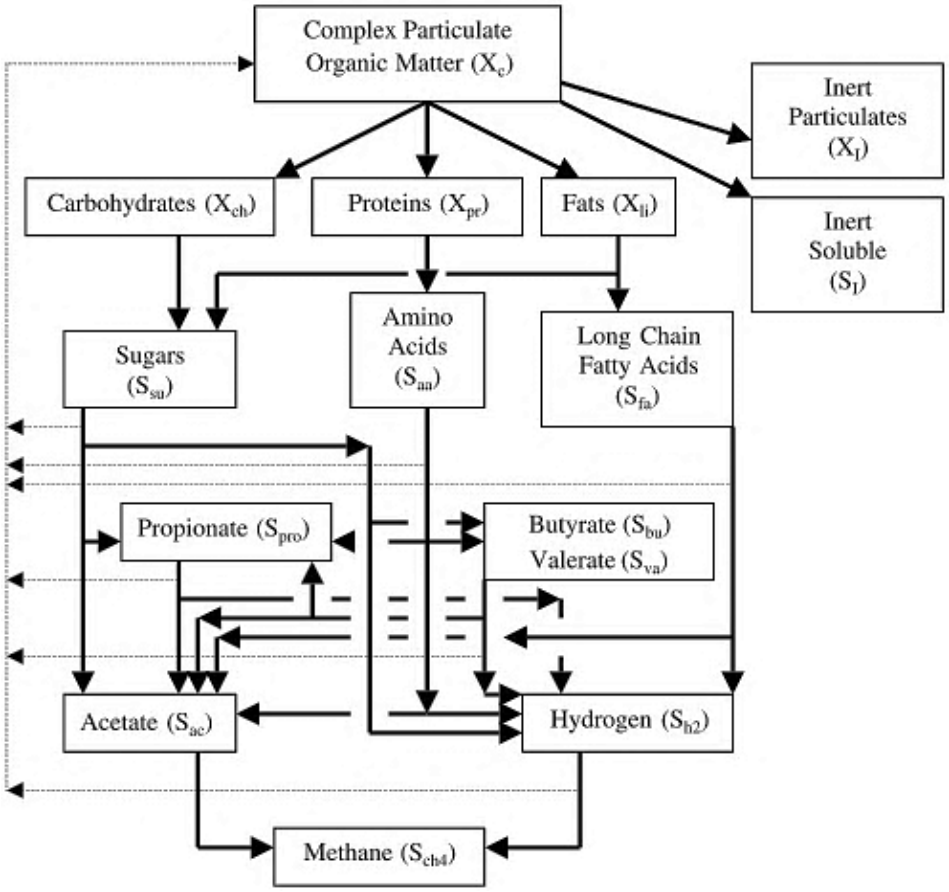

Figure 1.3. Overview of processes and variables described by the ADM1 (Parker, 2005).

The model describes the complex particulate substrates as disintegrated into inerts, carbohydrates, proteins and lipids. Then, the disintegrated products are hydrolysed to sugars, amino acids and long chain fatty acids (LCFA), respectively. Carbohydrates and proteins are fermented to produce VFA (acidogenesis stage) and molecular hydrogen. LCFA produce acetate and molecular hydrogen via β-oxidation. VFA (propionate, butyrate and valerate) are converted to acetate and molecular hydrogen in the acetogenesis stage. Finally, methane is produced by both uptake of acetate (acetoclastic methanogenesis) and reduction of carbon dioxide by molecular hydrogen (hydrogenotrophic methanogenesis) (Parker, 2005). Inorganic carbon and nitrogen, i.e. CO_2, HCO_3^-, NH_3, NH_4^+, act as source-sink terms and effectively close the mass balance for C and N (Lauwers et al., 2013).

The model describes the dynamics of 24 components, 12 soluble (S_i) and 12 particulate (X_i), through 19 biochemical reaction processes (Batstone et al., 2002). These include: 4 processes for particulate matter degradation, 8 for soluble matter degradation and 7 processes for biomass concentration. In addition, the model also considers 6 acid/base equilibrium reactions and 3 liquid-gas transfer reactions (for CH_4, CO_2 and H_2). Finally, the biochemical reactions include inhibition due to pH, lack of inorganic nitrogen, presence of H_2 (in the degradation of fatty acids, butyric/valeric acids, propionic acid) or presence of NH_3 (in the degradation of acetate).

To avoid additional complexity the ADM1 in its original version excludes specific processes (for instance, sulphate reduction or precipitation) and components deemed necessary only in certain applications. Specific limitations of the ADM1 have been highlighted (Batstone et al., 2006): (i) the regulation of VFA products from glucose under a fixed stoichiometry is inaccurate since it is an area still under research (Gonzalez-Cabaleiro et al., 2015), (ii) a large degree of uncertainty remains in some parameter values. Other weak points of the model were addressed by Kleerebezem and van Loosdrecht (2006). Particularly, they claim that a COD-based description of the reaction stoichiometry is not convenient for the case of AD, where the calculation of inorganic carbon production is important for the calculation of biogas composition and pH; secondly, that the widely adopted use of a constant value for the SRT lead to obtain non-realistic values of total biomass concentration when simulating high-rate anaerobic bioreactors with ADM1; and finally, as ADM1 contains no restrictions for thermodynamic boundaries, the anaerobic oxidation of valerate, butyrate and propionate is predicted to occur by ADM1 at ΔG-values bigger than 0.

Recently, Batstone and Rodríguez (2015) have addressed the disadvantages of defining the complex particulate *state* (X_c) as it was originally conceived in ADM1 for primary and activated sludges. The *state* X_c does not allow variation in the nitrogen and energy content or in its degradability over time, as these are fixed in the X_c variable. Secondly, it results in a two-step hydrolysis process, since both X_c and subsequent particulate substrates are subject to hydrolysis, where carbohydrate, protein, and lipid hydrolysis rates are set artificially high to avoid excessively slow kinetics. Instead of defining X_c state, the authors suggest skipping disintegration stage and treating inerts, carbohydrates, proteins, and lipids as the primary input point.

1.3.2. ADM1-based model developments

The original goals of the ADM1 are: (a) to increase model application for full-scale plant design, operation and optimisation, (b) to provide a tool for further development on process optimisation and control in full-scale plants, (c) to become a reference model for further development and validation studies to in comparable and compatible output terms, and (d) to facilitate the technology transfer from research to industry (Batstone et al., 2006). Although the ADM1 was originally developed to describe anaerobic digestion of waste activated sludge from WWTP, the ADM1 has been extensively used on different substrates and applied to co-digestion since its publication in 2002. For instance, it has been applied to simulate AD processes treating cattle manure and energy crops (Lübken et al., 2007), organic fraction of municipal solid wastes, OFMSW, and sewage sludge (Esposito et al., 2008; Derbal et al., 2009), mixtures of manures and vegetable wastes (Galí et al., 2009), combinations of solid wastes (Zaher et al., 2009), and mono-digestion of complex substrates such as grass, maize, green weed silage, and industrial glycerine (Biernacki et al., 2013), or microalgae (Mairet et al., 2011).

The original ADM1 structure has the advantage that it serves as a standard for further modifications which lead to the improvement of the model. Most modifications are dedicated to specific substrates, although some are of interest when describing non methanogenic fermentations (Penumathsa et al., 2008), or the thermodynamic dependence of the fermentation stoichiometry (Rodríguez et al., 2006). In addition, considerable effort has been put into the modelling of solid wastes, often focused on hydrolysis kinetics (Ramirez et al., 2009; Mottet et al., 2013). In general, two premises are considered when using ADM1 for co-digestion: (1) the model component for complex substrates cannot be used as an inflow fraction; substrate characterisation

should be inputted in terms of carbohydrates, proteins and lipids (Batstone and Rodríguez, 2015) and (2) the disintegration/hydrolysis step is generally considered the rate-limiting step during the degradation of solid wastes (Mata-Álvarez et al., 2011).

An overview of extensions and adaptations of ADM1 was reviewed by Lauwers et al. (2013). These include: precipitation of $CaCO_3$; reduction of sulphate to H_2S by oxidising ionised VFA and hydrogen oxidising biomass; reduction of nitrate to nitrite, nitric oxide, nitrous oxide and nitrogen by propionate and butyrate/valerate degraders; expressions for the hydrogen inhibition of acetogenesis and hydrogen consumption in methanogenesis; kinetics for hydrolysis; lactate and ethanol as intermediates; inhibition of sodium on acetoclastic methanogens, etc.

Finally, as Mata-Álvarez et al. (2014) stated in a recent review on anaerobic co-digestion, more efforts should be made to improve ADM1 so that it can predict the viability of different substrates under co-digestion, determine their dosage rates and assess their interactions from the performance of the process.

1.4. Control in anaerobic digestion

1.4.1. Objectives of AD control

In the context of a waste management framework legislation such as that in the European Union, in which landfilling of organic waste is prohibited or limited, it becomes of interest the utilisation of already existing biogas plants of waste treatment for the recovery of energy. By treating suitable co-substrates together with municipal sludge under co-digestion the extra capacity of exisiting digesters would be put to good use to enhance the biogas production (Murto et al., 2004). Normally however digesters in waste treatment plants are usually poorly monitored and, as a result, they are typically run at lower conservative OLR values to avoid overload. In this context a number of different control strategies have been developed aiming at improving the performance of these digesters (Holubar et al., 2002; Steyer et al., 2006; Méndez-Acosta et al., 2010; García-Diéguez et al., 2011). Such control strategies are often aimed at ensuring stable operation and at maximising the biogas production (Lauwers et al., 2013).

Anaerobic digesters are nonlinear and highly dynamic systems, fact that becomes more evident when they are operated close to its maximum capacity. As a consequence, linear time-invariant controllers seem not to be adequate for AD and more complex control techniques are required to bring the process towards a maximum biogas production while ensuring stable operation at higher OLR values (Olsson, 2012). This, together with the fact that no universal control algorithms has been developed that is capable of handling all possible disturbances occurring in AD, it is recommended that control laws are also combined with advanced diagnosis of the process performance (Lardon et al., 2004). Three issues have been recognised as critical in AD monitoring (Steyer et al., 2006): an efficient rejection of process disturbances, an adequate handling of non linearities and the management of explicit uncertainties. In general, the use of a specific control strategy implies data and model availability as well as knowledge of the process.

1.4.2. Key variables in AD control

Traditionally the stability of operation in AD is normally assessed through different physicochemical parameters, though other aspects as the nature of the influent or the reactor configuration can also be critical to the overall performance of the process (Steyer et al., 2006). The most typically measured variables in AD are pH, alkalinity, biogas flow rate and composition and VFA concentration. However, for control purposes, there are different approaches about what variable or combination of variables would be the best indicators of digester performance and possible failure (Castellano et al., 2007; Olsson, 2012). The sensitivity of the different physicochemical parameters can vary widely when a digester is exposed to the same disturbance. With regard to this, Boe et al. (2010) carried out a study in which controlled disturbances were applied to an AD experiment in order to find the best state indicators of stability; variables such as biogas production, pH, VFA, dissolved hydrogen, methane and hydrogen content in biogas were assessed against different organic overloads. The combination of acetate, propionate and biogas production was considered the best combination to monitor the process effectively. In other study, Castellano et al. (2007) determined the minimum number of monitored variables that are capable of identifying different process states (normal operation, hydraulic overload, organic overload or complete destabilisation of the process) using a technique called factorial discriminate analysis (FDA). The study concluded that using only 1 out of 7 the following binary combinations of variables (%CH_4-%H_2; %CH_4-H_2 flow rate; %H_2-gas

flow rate; $\%H_2$-CH_4 flow rate; $\%H_2$-H_2 flow rate; gas flow rate-H_2 flow rate; and CH_4 flow rate-H_2 flow rate) together with the basic instrumentation of a plant (that monitors feed flow rate, effluent pH, influent and effluent temperature) the process states can be clearly identified.

1.4.3. Sensors and models for control

Another concern in AD control is the accurate monitoring of the process. Limited monitoring has been traditionally the norm in conventional designs, where the fate of the process relied on the expertise of the plant operator. Introducing reliable monitoring and control technology would allow for AD plants to be operated closer to their effective capacity limit (Madsen et al., 2011). Characteristics such as COD or total organic carbon (TOC) can be monitored in industrial reactors with spectroscopy-based sensors, VFA with gas chromatography or by titration (Bernard et al., 2005), and dry matter content with microwaves (Madsen et al., 2011). In addition, near infrared spectroscopy has been used to monitor total ammonia nitrogen (Raju et al., 2012).

Together with advanced monitoring, the existing dynamic models of AD processes can be used for control purposes. However, as on-line monitoring is still mostly reduced to pH, soluble COD, alkalinity, total VFA and biogas composition (Lauwers et al., 2013), model-based controllers are mostly less sophisticated than the ADM1 model introduced in the previous section. One of the most effective models was developed by Bernard et al. (2001). It consists of a dynamic model that considers the acidogenesis and methanogenesis steps described using four state variables (organic substrate, VFA, acidogens and methanogens). A number of model-based controllers have been developed based on this model (Lauwers et al., 2013).

A non-linear process like AD requires a non-linear control strategy. Ward et al. (2008) suggested the decision on the most suitable control strategy based on different cases: a) if a reliable model is available it should be used but not in a wide range of operating conditions, b) if a large amount of data are available but not a reliable model and limited knowledge on the process, then the control strategy can be designed based on artificial neural networks, c) if there are no data or model available but comprehensive expertise on the process, a fuzzy logic controller is proposed as a good alternative.

1.4.4. Types of controllers

A number of control approaches have been tested and validated by using different strategies namely: feedback controllers (of P, PI or PID type), fuzzy logic and neural control approaches; feedback/feedforward control structures and model-based predictive controllers (Olsson, 2012; Méndez-Acosta et al., 2005). In addition, control systems can also be classified as robust/adaptive controllers, interval-based controllers or advanced controllers.

PID controllers

PID controllers have been used in AD for stabilising alkalinity and pH levels by controlling the addition of bicarbonate (Marsili-Libelli and Beni, 1996). A control algorithm based on a PI structure was developed by von Sachs et al. (2003) using VFA and methane production as input variables and volumetric feed rate as control variable; another PI controller considered the effluent COD as input variable and dilution rate as control variable (Alvarez-Ramirez et al., 2002). Other strategy used a linearizing feedback control by means of a robust PI^2 controller (Aguilar et al., 2011), where the dilution rate is used as control input to regulate the substrate concentration in the reactor.

Advanced controllers

Some controllers were conceived to maximise the methane yield in AD process, such as the advanced control by disturbance monitoring (Steyer et al., 1999). There, the control system takes into account the response of only two parameters, biogas output flow rate and pH, in order to decide if the load can be increased. The main advantage of this controller relies on its capability to automatically adapt the input flow rate to changes in the influent concentration. Rodríguez et al. (2006b) developed a hydrogen-based variable-gain controller, where the dilution rate is modified according to the values of hydrogen concentration in the biogas and methane productivity. In addition, a cascade control strategy was validated by García-Diéguez et al. (2011) based on the effluent VFA concentration and the methane flow rate. The control system sets a reference signal for the methane flow rate and determines the feed flow rate for adjusting the organic load applied to the reactor.

Model-based controllers

For control purposes, simpler models than ADM1 are usually taken. The mathematical behaviour of sophisticated models can be complex and derivation of an automatic controller becomes a very tedious task (Steyer et al., 2006). Simple models as the one developed by Bernard et al. (2001) have been used in many non-linear based control approaches. For instance, Antonelli et al. (2003) made use of this model to design a bounded output feedback control law. The dilution rate is selected as the manipulated variable and the output methane gas flow rate as the controlled variable. A similar approach of output feedback controller was studied by Mailleret et al. (2003), who developed a robust regulation of AD systems using methane flow rate as process variable and dilution rate as manipulated variable to regulate total organic substrates in the digester. Based on the same model, Mendez-Acosta et al. (2005) developed a linear control approach with a feedforward/feedback structure to regulate the substrate COD in vinasse treatment. Dimitrova and Krastanov (2012) aimed at stabilising the nonlinear dynamic AD model before mentioned. A nonlinear adaptive feedback was proposed, which stabilised the dynamics asymptotically towards the maximum methane production, depending on the biochemical oxygen demand (BOD). The methane production was enhanced via extremum seeking algorithm. This optimisation algorithm has been extensively used to improve the productivity of CSTR. Usually, the extremum seeking approach is used to iteratively adjust the dilution rate to lead the process to a set point, where an optimal value of the output is achieved. The same authors, Dimitrova and Krastanov (2014), proposed a simple approach for global asymptotic stabilization of the AD model (Bernard et al., 2001) without using any feedback control strategy, adjusting the dilution rate and maximizing the biogas flow rate. The latter was achieved by using a numerical extremum seeking algorithm.

Simeonov and Queinnec (2006) designed an algorithm to linearize the control of AD systems by adding acetate as control action. It was assumed that the addition of stimulating substances in appropriate concentrations can stabilise the process and increase biogas flow rate. This strategy aimed at regulating the biogas flow in the case of variations of the inlet composition. Savoglidis et al. (2013) developed an output feedback control law for a chemostat (using a model-based controller) aiming at maintaining constant the yield between methane production rate and feeding rate in a chemostat. For two-stage AD processes, Aguilar-Garnica et al. (2009) proposed a multivariable nonlinear controller to regulate VFA in the first reactor and effluent COD

in the second reactor (both fixed bed bioreactors), by manipulating their respective recycle flow rates.

Other designs considered a control strategy based on multiple inputs and multiple outputs. For instance, Méndez-Acosta et al. (2010) addressed the stability of the process with a robust regulator (derived from an AD model) of both VFA and total alkalinity. The feedback controller manipulates the feed flow rate and regulates the VFA concentration. In addition, an alkali solution is added to the digester to maintain the total alkalinity at the set-point. Haugen et al. (2013a) analysed a feedback control system (under both on/off and PI structures) using methane flow as control variable and feed flow rate as manipulated variable using a modified Hill model (Haugen et al., 2013b). Lara-Cisneros et al. (2015) designed a controller to regulate the VFA concentration while maximising the methane production, using the dilution rate as manipulated variable.

Fuzzy logic and expert systems

Fuzzy logic is a problem-solving tool that can achieve a definite conclusion from imprecise information, allowing intermediate values rather than simple yes/no evaluations. They are empirically based, using experience on the process rather than understanding the fundamentals of the process. The ability to assess vague information makes fuzzy logic interesting for anaerobic digestion (Ward et al., 2008). The core of the fuzzy logic resides on the definition of membership functions that link the extent of the output (or control action) to the range of values of the different variables.

Puñal et al. (2002) developed an expert system for monitoring and diagnosis of anaerobic wastewater treatment plants, based on IF-THEN rules, using gas flow, methane and carbon monoxide composition in biogas, feed flow, recycling flow, pH and reactor temperature as variables of the control system.

To avoid overloads in AD processes Murnleiter et al (2002) developed a fuzzy logic control. The system was designed to handle significant fluctuations in the substrate concentration as well as in the volumetric loading rate. Variables such as hydrogen concentration, methane concentration, gas production rate, pH and level of the buffer tank were used as input variables for the fuzzy logic structure. Carrasco et al. (2004) developed a diagnosis system based on fuzzy logic for identification of acidification states in an AD process. The diagnosis system uses the expertise of plant operators to estimate the acidification state through the information of several variables measured

on-line. The variables biogas flow rate, methane and carbon monoxide contents in biogas, feeding flow rate and pH were selected for the diagnosis system.

Neural networks

Artificial neural networks are designed from interconnected processing elements, forming a structure that gathers the relationships between the main variables of a process. The first layer of processing elements receives an input (for instance, a sensor signal) and sends an output to the next layer. This may continue through a cascade of layers until outputs are obtained (Ward et al., 2008). During the process, the different processing elements (or nodes) are continuously weighed, in a process called training period, until the network is able to reproduce the relationship between inputs and output. The training period usually considers a wide variety of operating conditions to cover all eventualities of normal operation. Neural networks have been used in anaerobic digestion systems to describe trace gases (Strik et al., 2005), controlling the addition of bicarbonate (Guwy et al., 1997) and control methane or biogas production (Holubar et al., 2002; AbuQdais et al., 2010).

Other studies made used of both neural networks and fuzzy logic systems simultaneously. For instance, Waewsak et al. (2010) developed a neural-fuzzy control structure for the control of biogas production in an AD reactor. The neural network estimated the value of variables such as pH, alkalinity and total volatile fatty acids. Then, this information is used as input data for the fuzzy logic system to calculate the influent feed flow rate.

1.4.5. Control of co-digestion. Optimisation of blends of substrates

The control strategies presented in former sections were developed for mono-digestion systems. The control of co-digestion processes can be addressed following the same strategies utilised for AD processes; however, in the case of co-digestion it is crucial to characterise comprehensively the co-substrates and to choose adequately the blend of substrates to be treated. Unintentional organic overloading, accidental addition of toxic substrates and other process interruptions may occur frequently if this is not conducted properly. Lack of raw material quality control is believed to be one of the main limitations for effective raw material handling (Madsen et al., 2011).

Ashekuzzaman and Poulsen (2011) investigated the methane yield obtained from co-digestion process compared to the yields obtained from single digestion of substrates.

Bench-scale and full-scale assays were carried out for both mono-digestion and co-digestion (using cow dung as main substrate) to identify the relationship between methane yield and substrate composition. The results showed that using co-substrates improved the methane yield much more than expected from digestion of individual substrates. In addition, process stability (based on fluctuations of biogas production, pH and ammonium content) was improved with co-digestion.

(Wang et al., 2012a) carried out experiment to demonstrate the benefits of co-digestion versus anaerobic mono-digestion. Different ratios of distiller grains (DG) and food wastes (FW) were examined and concluded that high ratios of DG/FW improved the performance of the process compared to mono-digestion. The synergies of the co-substrates lower the propionate/acetate ratio and VFA/alkalinity ratio.

Alvarez et al. (2010) developed a methodology based on linear programming to determine the best combination of substrates capable of achieving an optimised biodegradation potential under co-digestion. For that purpose, the linear programming optimisation method defined restrictions on several characteristics of the mixture, establishing minimum and maximum boundaries to characteristics such as NH_4^+, lipids or C/N ratio. The methodology was validated in batch experiments using three types of waste with different characteristics (pig manure, fish waste and biodiesel waste).

As C/N ratio is one of the key parameter in AD process, an optimum C/N ratio can lead to obtain higher methane yields. Wang et al. (2012b) observed that the digestion of multiple substrates (dairy manure, chicken manure and wheat straw) achieved superior performance in terms of methane potential than the digestion of individual substrates. In addition, they concluded that C/N ratios of 25 or 30 improved the performance enabling a stable pH and a low concentration of total ammonium. The same author in a further study made used of statistical methods to optimise the feeding composition of co-digestion systems (Wang et al., 2013). The methods called *simplex-centroid mixture design* and *central composite design* were evaluated with methane potential as the response variable. Each co-substrate served as an independent variable together with the C/N ratio of the blend.

1.5. Outline of this thesis

This thesis intends to contribute to the field of anaerobic co-digestion, tackling the objective of improving the overall process performance from different perspectives including modelling, optimisation and control of continuous co-digestion operations.

The study described in this thesis deals with the development and validation of an integrated methodology for simulation, optimisation and control of anaerobic co-digestion. Motivations and some background have been presented in Chapter 1, in which the control of co-digestion processes and the optimisation of blends of substrates are highlighted as challenging topics of interest in the recent years.

Chapter 2 introduces the pilot plant where the co-digestion experiments were carried out to validate the proposed co-digestion model (Chapter 3), the optimisation method of co-digestion feedings (Chapter 5) and the control strategy (Chapter 6) developed as part of this thesis.

The increasing interest in anaerobic co-digestion for higher energy recovery makes its modelling and simulation of enormous importance in order to predict biogas yields and feasibility of substrates blends under co-digestion. In Chapter 3 a generalised modelling approach for anaerobic co-digestion of fermentable substrates is developed. It introduces a methodology to implement new soluble substrates (ethanol, glycerol, lactic acid) into ADM1, not originally described in the model, in order to extend its application to numerous diverse substrates.

Chapter 4 assesses the description of the disintegration and hydrolysis steps of the ADM1-based model, developed in Chapter 2, during the degradation of solid wastes (food and vegetable wastes). Decoupled disintegration kinetics for readily and slowly biodegradable fractions of solid wastes is considered, and disintegration and hydrolysis remain as independent steps. The experiments of this study were carried out at laboratory scale.

In Chapter 5 an optimisation method to calculate blends of substrates as feeding of co-digestion processes is presented. The method is based on linear programming and aims at calculating blends of substrates to achieve the highest methane productivity at each OLR applied. The solutions achieved are also subjected to a set of physicochemical constraints that are applied during the optimisation.

Once an optimised feeding is calculated by linear programming, an analysis of the process stability should be carried out in order to check the feasibility of the different blends of substrate from medium to long-term operations. A control strategy for co-digestion systems is presented in Chapter 6. A diagnosis of process stability is developed based on the measures of alkalinity ratio and methane flow rate. The objective of the diagnosis is to assess the viability of the optimised feedings in maintaining the stability of the system. Based on the diagnosis outcome, the control action modifies the restrictions applied in the optimisation method in order to calculate a new optimum blends for subsequent periods of operation.

Finally, the last section compiles the general conclusions of the thesis.

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Chapter 2. Pilot Plant

Contents

Abstract

This chapter provides a description of the main facility used for the experiments carried out in this thesis, an anaerobic co-digestion pilot plant. It describes the characteristics of the reactor and a detailed account of the instrumentation installed in the plant. In addition, it gives a brief explanation of the information system used to acquire, save and monitor the process data in a PC. Experiments of chapters 3, 5 and 6 were performed in this pilot plant.

2.1. Description

The pilot plant consists on a USBF reactor, a hybrid Upflow Anaerobic Sludge Blanket (UASB) and Anaerobic Filter (AF), with a total liquid volume of 1 m^3 (Figure 2.1). Approximately, 75% of the liquid volume corresponds to the UASB zone, and 25% to the anaerobic filter (AF). The gas is confined in a headspace of around 0.4 m^3.

Figure 2.1. Anaerobic co-digestion pilot plant located in the School of Engineering (USC).

Four ports are located at different heights on the UASB zone of the reactor to enable liquid and biomass sampling. The AF zone is filled with PVC rings (50 mm of diameter by 50 mm of length). These rings are randomly distributed (void fraction of 96%) and supported on a grid above the UASB zone. The reactor headspace is connected to a water seal (2 metre high) (Ruiz, 2005).

The reactor, made of stainless steel, has a cylindrical geometry with an internal diameter of 30 in (around 0.75 m). The feeding enters the digester from the bottom, through a ring with 3 lateral input ports and 1 centred port in the base of the reactor. Both UASB and AF compartments are arranged with double wall to guarantee thermal isolation in the reaction zone (liquid space), whereas the top of reactor is coated with glass wool.

2.1.1. Liquid line

The digester can be fed with up to three lines simultaneously using a system of three membrane dosing pumps (of different sizes and flow ranges). They work independently and are selected for each co-substrate according to its flow. A static mixer blends the different co-substrates flows and, immediately after, a side tube sends a feeding sample to an on-line analyser of total organic carbon (TOC) and total inorganic carbon (TIC). Before entering the digester, the feeding is mixed with the recycling stream before returning to the reactor. The inlet temperature to the digester is monitored using a Pt-100 probe.

The recycling flow is conceived to: (1) ensure an appropriate mixing within the reactor and (2) regulate the temperature in the system (37 °C). For the later purpose, the recycling line passes across a double-pipe heat exchanger (made of stainless steel) and the heating water, driven by a centrifugal pump, passes through the carcass. An electrical heater provides the required energy for the heating water.

A membrane pump propels the recycling stream, at a flow monitored by an electromagnetic flow-meter 7ME251 (Siemens). A filter is placed before the pump to retain any possible solids with a diameter bigger than 1 mm coming out the reactor. The high recycling flow rate of 320 L/h ensures a good mixing simultaneously keeping a moderate superficial velocity (approx. 0.7 m/h) to prevent biomass from washout. A pH meter is located in the recycling line as the pH in the recycling line is assumed to be the same that in the reactor.

The digestate leaves the reactor by gravity. A level switch CLS 200 (Pointek) sets the on/off signal of a pneumatic valve aiming at maintaining a constant liquid level in the reactor. A settler of 2 L is placed just after the valve to remove solids. Subsequently, a filtration system takes the digestate from the settler, by means of a peristaltic pump, and filters the liquid with a ceramic microfiltration membrane MF80 (Atech Innovations) with a pore size of 0.80 μm. The non-filtered liquid returns to the settler and the filtered effluent is sent to two on-line analysers to determine: (i) dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) and (ii) VFA and both partial and total alkalinity. In addition, the non-filtered digestate can be also analysed in terms of total organic carbon.

Note: Although the TOC analyser is connected to the pilot plant, this was not used in any of the experiments of this Thesis. Instead, off-line analysis of COD of both influent and effluent were regularly taken.

2.1.2. Gas line

Biogas comes out from the top of the reactor and heads into two consecutive condensation traps to retain the water that can accompany the biogas. Then, the gas flow is split into two streams. One of them is connected to a methane and carbon monoxide analyser Ultramat 22P (Siemens) and in parallel to an analyser AO2020 (ABB) for methane, carbon dioxide and hydrogen sulphide quantification. Both gas analysers can work simultaneously or alone and make non-invasive measures of the biogas composition. The outlet gas streams of both analysers return to the headspace of the reactor.

The second gas stream is connected to a mass flow meter 5860 (Brooks) to monitor biogas flow rate and subsequently connected to a hydrogen analyser Sensotox 420 (Sensotrans) to measure hydrogen concentration. As this analyser makes use of an invasive method of measurement (electrochemical sensor) the outlet gas from the hydrogen analyser is vented to the atmosphere.

Finally, a pressure transmitter 7MF1563 (Siemens) determines the headspace pressure within the range of 0-1.6 atm. A good calibration between pressure in the headspace and the gas flow at constant liquid level was validated in previous studies (Molina, 2007).

2.1.3. Information line

The sensors laid out in the plant send standard analogue signal of 4-20 mA. Some of these signals are transmitted to displays (Ditel series Cosmos Alpha-P) and placed in a control panel, and later sent to PLCs (Siemens series S7-200). The majority of signals are sent directly to the PLCs. The PLCs convert the analogue signals into digital signals of 2 bytes (a PLC number from 1 to 32767) that are sent to a computer where the SCADA system is implemented (Rodríguez, 2006).

2.2. On-line analysers

Typical variables such as temperature, pH and gas flow are monitored with conventional on-line sensors in both liquid and gas lines. More advanced sensors and analysers are in place to measure on-line gas composition, TOC, VFA and alkalinity (García, 2010).

2.2.1. Liquid phase

Alkalinity and VFA analyser

The analyser AnaSense® (Applitek) monitors alkalinity of the filtered effluent on-line (Molina et al., 2009). The titrimetric analyser is equipped with a pH sensor and it makes use of recorded titration curves to calculate bicarbonate, partial and total alkalinity (PA and TA) and VFA concentrations by using a methodology developed by LBE-INRIA (Bernard et al., 2005). The complete analysis requires 5 to 10 min and three-hour frequency was selected.

2.2.2. Gas phase

Methane and carbon monoxide analyser

The infrared gas analyser Ultramat 22P (Siemens) has been used to measure continuously CH_4 and CO concentrations in the gas stream. The measurement is based on the principle of non-dispersive infrared absorption. An infrared (IR) source emits radiation that passes through the biogas sample and an IR detector receives the radiation not absorbed by the sample. The differences between the radiations emitted and absorbed are proportional to the methane or carbon monoxide concentrations. The measure principle is non-invasive. The analyser is provided of two chambers, one for each gas (CH_4 and CO), and measures their concentrations in the following ranges: methane content between 0-100% (% vol.) and carbon monoxide between 0-300 ppm. The calibration procedure is carried with nitrogen gas and a certified bottle of CH_4 and CO to correct the zero point and the slope of the correlation concentration *vs.* signal.

Methane, carbon dioxide and sulphide analyser

The gas analyser Advance Optima AO2020 (ABB) monitors continuously the composition of CH_4, CO_2 and H_2S in the effluent gas. It is provided of two modules: (i) Uras26, an IR analyser module for the determination of CH_4 and CO_2; and (ii) Limas11, and UV photometer analyser module for H_2S quantification. The measurement

principle of the Uras26 module is based on the non-dispersive infrared absorption in the wavelength range of 2.5–8 μm. Measurement ranges between 0-50% for CO_2 and 0-100% for CH_4 (% vol.). The measurement principle of the Limas11 module is based on a gas filter correlation or wavelength comparison in ultraviolet and visible spectrum range between 200-600 nm. Concentrations of 0-1% of H_2S can be detected in this unit.

The analyser is also provided of a SCC-F module, a simple gas feed unit connected to the gas analyser with a required inlet flow rate of 20-100 L/h. Calibration has been carried out periodically with nitrogen gas and a certified mixture of CH_4, CO_2 and H_2S.

Hydrogen analyser

Hydrogen concentration in the gas stream is determined with an electrochemical sensor Sensotox 420 (Sensotrans). The measurement principle is based on the oxidation of the hydrogen in a cell that generates an electrochemical potential proportional to its concentration. The analyser measures concentrations in the ranges of 0-2000 ppm. The calibration is carried out using N_2 gas to correct the zero point and a certified mixture N_2:H_2 for adjusting the slope of the correlation concentration *vs.* signal. As the presence of other gases such as hydrogen sulphide can affect the lifespan of the cell, an absorption column Purafil® Chemisorbant Media (Purafil Inc.) is placed before the analyser to preserve the cell from damage. The column is filled with potassium permanganate on alumina that must be changed when exhausted.

2.3. Data acquisition system

A data acquisition programme, named Acquirer, was previously developed in Visual Basic 6.0 (Rodríguez, 2006) to monitor all the signals from the pilot plant. The PLCs manage the signals coming from the installed sensors and the Acquirer programme receives the data from the PLC S7-200 (Siemens) by means of a serial port RS-232. In general, sensor signals are registered every 5 s, filtered using a moving average window of 15 min and finally saved in a MS Access database. However, signals obtained in particular periods of time require a specific filtering procedure (e.g. TOC/TIC, VFA, bicarbonate, TA, PA). H_2 concentration in the gas phase requires a special filtering procedure to distinguish between the values of actual hydrogen concentration and those from electrochemical cell saturation at the beginning of each measurement. A PLC code establishes the appropriate value of H_2 concentration.

Figure 2.2 depicts the data acquisition system architecture from pilot plant to the computer.

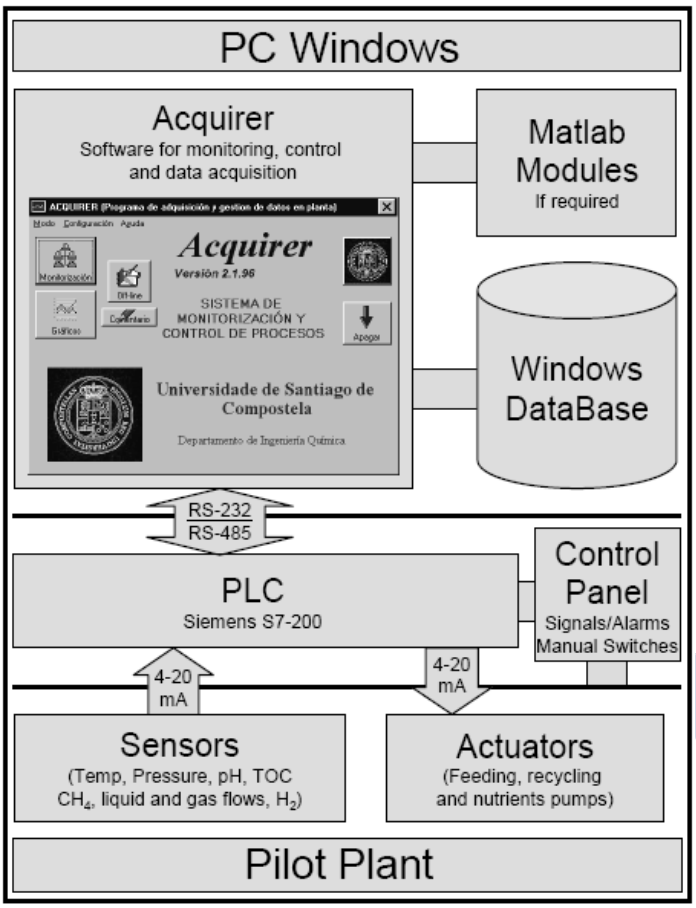

Figure 2.2. Architecture of the information system in the pilot plant (Rodríguez, 2006).

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Chapter 3. Generalised modelling approach for anaerobic co-digestion of fermentable substrates

Contents

Abstract

A general methodology to implement fermentable soluble substrates in the IWA Anaerobic Digestion Model No. 1 (ADM1) that extends its application to anaerobic co-digestion of multiple substrates is presented. The approach considers the fermentation of new soluble substrates, not originally described in ADM1, as channelled through mass- and electron-balanced sugar fermentation equivalent reactions, and that fermentable substrates are degraded by a generic group of fermenters instead of the original ADM1 sugar fermenters. Therefore, no additional microbial group state is required. An additional term that modifies the ADM1 sugar fermentation kinetics equation was included to account for the competition among multiple substrates to be degraded by a particular biomass group. The model was validated at pilot scale treating a blend of pig manure (soluble fraction), wine and gelatine at mesophilic conditions. Only the ADM1 acetoclastic ammonia inhibition parameter was calibrated to obtain consistent model prediction of gas and liquid composition.

3.1. Introduction

Anaerobic digestion (AD) has been traditionally associated with the treatment of agro industrial waste streams and of sewage sludge from aerobic wastewater treatment plants. The possibility of using blends of multiple substrates (co-digestion) has recently extended the applicability of anaerobic digestion, since it provides a number of environmental, technological and economic advantages. Anaerobic co-digestion can increase methane production depending on the operating conditions and the co-substrates used (Alvarez et al., 2010). This is achieved through synergies between blended substrates that complement each other in terms of C/N ratio, COD, dilution of inhibitors, alkalinity, dry matter, etc. (Hartmann et al., 2003).

Many organic wastes, which often cause a problem of disposal and at the same time represent potential energy sources, were successfully treated by Anaerobic co-Digestion (AcoD); for example, the by-product containing glycerol generated in a biodiesel producing plant, the whey containing lactose generated in the cheese factories, or the vinasse wastewaters containing ethanol from wine distillery (Astals et al., 2012; Comino et al., 2012; Riaño et al., 2011). Achieving the correct blend of wastes in AcoD that leads to a stable operation is not trivial. It requires knowledge on the process since it involves a complex network of reactions to convert complex substrates into biogas. In this sense, models can early evaluate the viability of a particular blend of wastes treated in an AcoD system.

A few years ago, part of the research on AD started to focus on modelling in order to describe its mechanisms accurately (Angelidaki et al., 1993, Donoso-Bravo et al., 2011). The IWA Anaerobic Digestion Model No.1 (ADM1) (Batstone et al., 2002) is a structured model that describes the main processes involved in AD to convert complex organic substrates into biogas: disintegration, hydrolysis, acidogenesis (or fermentation), acetogenesis and methanogenesis. The model defines state variables to describe the behaviour of soluble and particulate components along the reaction path and includes 7 groups of bacterial degraders, classified by their functions: degraders of sugars, amino acids, LCFA, valerate and butyrate, propionate, acetate and hydrogen. All organic species and molecular hydrogen are described in terms of COD, whereas inorganic carbon or inorganic nitrogen species are described in molar basis.

In recent years, there has been an increasing interest on AcoD modelling (Mata-Álvarez et al., 2011). In spite of the existing literature about AcoD modelling, it seems to lack

generalised methodologies to implement soluble substrates into ADM1. Some authors incorporate fermentative reactions of soluble substrates such as phenol, ethanol, glycerol, or lactic acid in the ADM1 following their own methodologies (Fezzani and Ben Cheikh, 2009; Rajinikanth et al., 2008; Galí et al., 2009; Hidaka et al., 2010); however, different pathways to degrade ethanol, glycerol or lactate can be found in the literature. According to Schink et al. (1985) ethanol can be degraded to organic acids through different pathways: butyrate formation, simultaneous acetate and propionate formation or acetate as sole acid, and concluded that ethanol was not exclusively metabolised via acetate. The same was observed by Seeliger et al. (2002) where ethanol could be degraded via acetate plus propionate. Glycerol degradation has been implemented in ADM1 through carbohydrates too (Galí et al., 2009; Biernacki et al., 2013), with default ADM1 catabolic products of sugars fermentation. According to Sørensen et al. (1991), lactate degradation pathways included: acetate formation; formation of acetate, hydrogen and carbon dioxide; formation of propionate, acetate and carbon dioxide; or fermentation to acetate, propionate and hydrogen. However, batch experiments conducted by Sørensen et al. (1991) encountered acetate as the major intermediate produced during batch assays. In general, the fermentation via propionate and acetate seems to be the most common pathways in the literature (Antonopoulou et al., 2012; Skiadas et al., 2000; Seeliger et al., 2002). Nevertheless, different acetate to propionate ratios together with different hydrogen pressures were found depending on the bacteria used according to experiments conducted by Seeliger et al. (2002). Therefore, the key issue is to find the catabolic yields (stoichiometry) of the organic acids (acetate, propionate and butyrate) from these fermentable substrates.

The purpose of this work is to present a generalised methodology to easily incorporate fermentable soluble substrates into ADM1 and to extend the model for AcoD application. The proposed model was implemented in an Excel-Matlab/Simulink platform (Rodríguez et al., 2009) and the experimental validation study was conducted at pilot scale, in a highly instrumented hybrid Upflow Anaerobic Sludge Blanket - Anaerobic Filter reactor (UASB-AF reactor) treating a blend of three substrates (namely wine, gelatine and pig manure) in continuous operation under mesophilic conditions.

3.2. Materials and Methods

3.2.1. ADM1-based AcoD Model

In order to simulate co-digestion processes, the ADM1-based model was implemented on Excel-MATLAB/Simulink platform (Rodríguez et al., 2009) and adapted to run both batch and continuous operations. The model calculates the blend flow and the composition of the influent to the digester. The ordinary differential equations of all states were coded and implemented using Matlab and integrated with the ODE113 solver.

To validate the model, simulations were conducted under the same operating conditions as those in a pilot plant treating a blend of three soluble substrates at mesophilic conditions. All ADM1 parameters remained at their default values except the acetoclastic ammonia inhibition parameter, $K_{I,NH3,}$ as sole calibrated parameter to fit experimental results.

3.2.2. Experimental set-up for continuous experiment

A continuous AcoD experiment was conducted in a fully instrumented pilot plant consisting of a hybrid UASB-AF reactor of 1 m^3 of liquid volume (Ruiz, 2005). The high recycling flow applied to the reactor guaranteed quasi-complete mixed reactor behaviour in the liquid phase. The on-line measurement devices connected to the plant include pH meter (Siemens, SIPAN pH/ORP 7MA 1010), gas flow meter (Brooks®, 5860E), continuous CH_4, CO_2, H_2S analyser (ABB, AO2020) and a hydrogen gas analyser (Sensotrans, Sensotox 420). A data acquisition programme developed in Visual Basic allowed the data acquisition and monitoring of the pilot plant. PLCs (Siemens, series S7-200) managed the signals coming from the different sensors and analysers connected to the pilot plant (Molina et al., 2007). Figure 3.1 shows a schematic of the experimental set-up. The model simulated the same system in terms of process layout with a reactor modelled as perfectly mixed with biomass retention to mimic the UASB–AF as it is conventionally done.

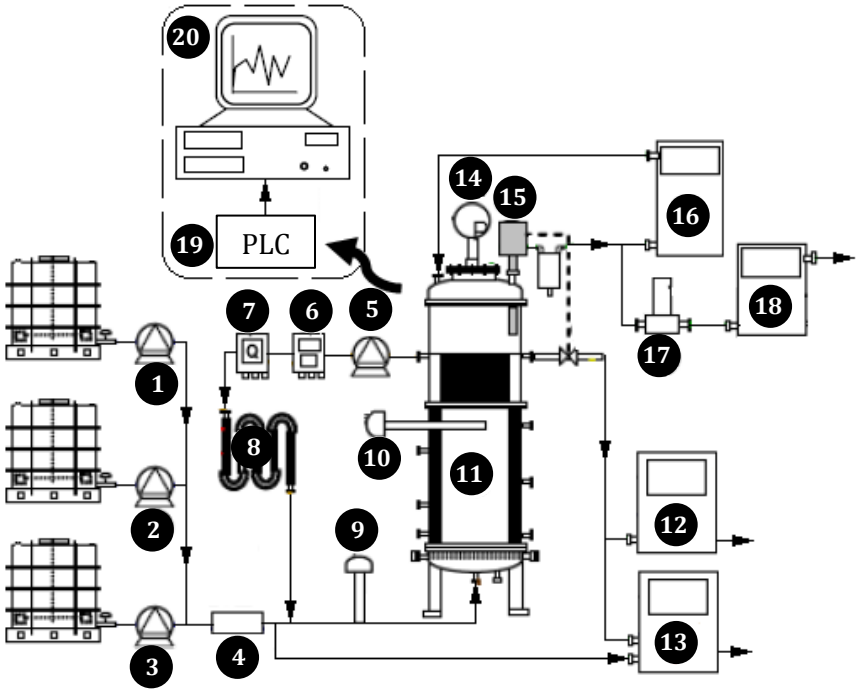

Figure 3.1. Layout of the pilot plant: (1), (2), (3) feeding pumps of pig manure, gelatine and wine. (4) Static mixer. (5) Recirculation pump. (6) Flow meter. (7) Effluent pH meter. (8) Heat exchanger. (9) Influent temperature probe. (10) Reactor temperature probe. (11) UASB-AF reactor. (12) On-line alkalinity and VFA analyser (not used). (13) Total organic carbon analyser (not used). (14) Pressure probe. (15) Level switch. (16) CH_4 and CO_2 analyser. (17) Gas flow meter. (18) Hydrogen gas analyser. (19) Rack of PLCs, (20) PC provided with a data acquisition system to monitor the process (adapted from Ruiz (2005)).

The reactor was inoculated with sludge from an industrial internal circulator reactor in a brewery factory and operated for 5 months treating a blend of three soluble substrates (soluble fraction of pig manure, wine and gelatine) at OLR of 2.5 g COD/L·d, HRT of 11 days and 13 g VSS/L under mesophilic conditions. The characteristics of the different substrates are summarised in Table 3.1. In addition, water was added to the feeding (37.2 % feed volume) to simulate a vinasse stream from wine, reduce nitrogen

and sulphate contents of the blend and achieve an influent alkalinity around 3 $gCaCO_3/L$.

Table 3.1. Characteristics of wine, gelatine, soluble fraction of pig manure as well as bend influent to the reactor.

Parameter	Wine	Gelatine	Pig manure	Influent
% volume in feed	6.2	11.8	44.8	100.0
Liquid fraction (%)	100	100	99	99.6
pH	3.3	5.5	7.8	6.7
Density (kg/L)	0.98	1.04	0.96	1.0
TS (g/L)	0	0	9.90	4.4
VS (g/L)	0	0	5.42	4.3
CODt (g/L)	174	101	10.1	27.1
CODs (g/L)	174	100	4.6	27.0
TKN (g N/L)	0.12	12.9	3.7	3.2
NH_4^+ (g N/L)	0.02	0	3.7	1.7
TA (g $CaCO_3$/L)	0	1.8	6.2	3.0
Chloride (g/L)	0.02	0	0.85	0.4
Sulphate (g/L)	1.94	4.10	0.03	0.6
Proteins (g/L)	0	83.6	0	9.8
Lipids (g/L)	0	0	0	0
Carbohydrates (g/L)	0	0	9.49[a]	4.3
Ethanol (g/L)	83.38	0	0	5.1
Biodegradability (%)	100	77.7	79.3	81.0

[a] This concentration comprises both carbohydrates and VFA contents and amended to this value to meet CODt.

Biogas flow, biogas composition (CH_4, CO_2, H_2S, H_2), pH, total and partial alkalinity were monitored online and saved to database. In addition, offline values of NH_4^+, COD, alkalinity, volatile fatty acids (VFA) or pH were regularly measured to monitor the process.

3.2.3. Analytical methods

Characteristics such as pH, COD, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), total

alkalinity (TA) and partial alkalinity (PA) were performed following the Standard Methods (APHA, 1998). Ammonium was measured with phenate method (APHA, 1998) with a spectrophotometer (Shimadzu UV-1603, UV–Visible) at 640 nm. VFA (acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric) were analysed by gas chromatography (HP, 5890A) with a Flame Ionisation Detector (Molina et al., 2008). The anions chloride (Cl^-) and sulphate (SO_4^{2-}) were determined by ion chromatography (IC) with an Advanced Compact IC system (861, Metrohm). Ethanol concentration was measured from COD of wine (assuming that wine never gets vinegary). Lipids were measured with Soxhlet method (APHA, 1998). Protein concentration was estimated multiplying organic nitrogen (TKN minus $NH4^+$) by 6.25. Carbohydrates were estimated as the remaining fraction of VS (Galí et al., 2009).

3.3. Results and Discussion

3.3.1. ADM1 modification for new soluble substrates

In order to make ADM1 appropriate for anaerobic co-digestion, a number of modifications were implemented: (i) any complex substrate included in the model was defined by its content in proteins, lipids, carbohydrates and refractory fraction (determined from the non-biodegradable fractions in experimental BMP assays); (ii) additional states and fermentation reactions were added for those soluble fermentable substrates that cannot be allocated in existing ADM1 states; (iii) new inert states were included in order to handle the non-biodegradable fractions of complex substrates; (iv) a new state was created for dead biomass, X_d, that eventually hydrolyses to sugars, amino acids, fatty acids and soluble inerts where their yields on dead biomass (f_{su_xd}, f_{aa_xd}, f_{fa_xd}, f_{si_xd}) are those considered in ADM1 for the disintegration of particulate composite, X_c, except f_{si_xd} that lumped the yields of both non-biodegradable particulate state, X_i and non-biodegradable soluble state, S_i; (v) all state variables and ADM1 parameters are expressed in molar basis to avoid mass balances inconsistencies or dual basis COD-based and molar-based for organic and inorganic species (Kleerebezem and van Loosdrecht, 2006).

Figure 3.2 shows the main modifications (grey blocks) with respect to original ADM1 (Batstone et al., 2002), where the degradation of new soluble fermentable substrates (such as ethanol, glycerol or lactate) are channelled through glucose equivalent

reactions, and inactive biomass (X_d) is lumped disintegrated and hydrolysed to sugars, amino acids and LCFA, and differentiated from complex particulate waste (X_c).

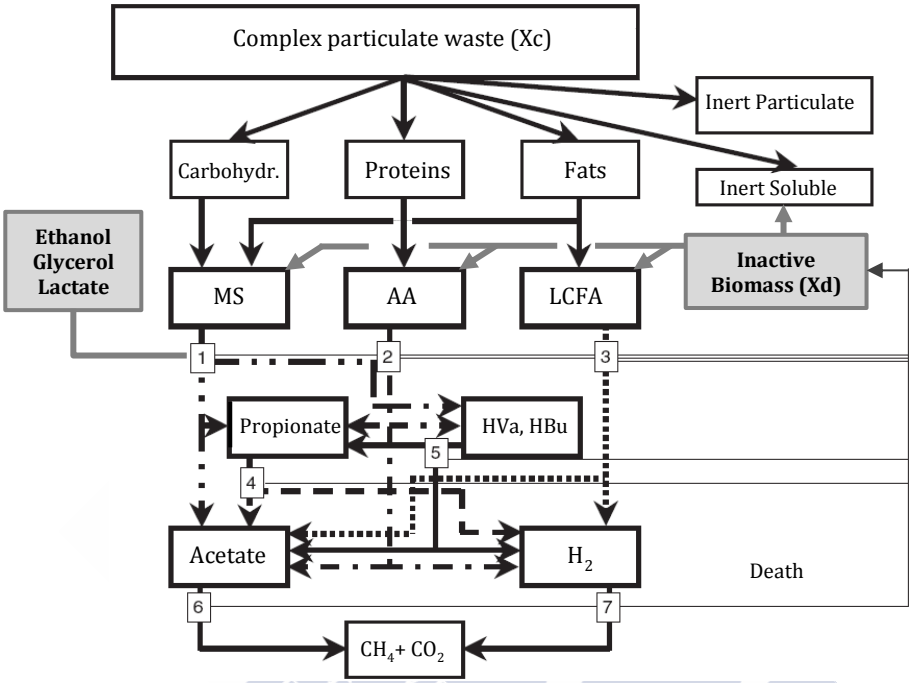

Figure 3.2. The anaerobic model as implemented including biochemical processes (1) Acidogenesis from sugars (monosaccharide, MS), ethanol, glycerol and lactate, (2) Acidogenesis from amino acids (AA), (3) Acetogenesis from LCFA, (4) Acetogenesis from propionate, (5) Acetogenesis from butyrate and valerate, (6) Aceticlastic methanogenesis, and (7) Hydrogenotrophic methanogenesis (adapted from Batstone et al., (2002)).

The above modifications with respect to original ADM1 are needed to implement the fermentation of new soluble fermentable substrates (ethanol, glycerol and lactate) not originally included in ADM1 and to differentiate between particulate organic waste (X_c) and inactive biomass (named in this model as X_d).

The proposed ADM1-based model integrated ethanol, glycerol and lactate fermentation implemented through glucose equivalent reactions. The stoichiometries of these new

reactions (catabolic yields) were derived from the stoichiometry of glucose fermentation following a generalised methodology. Thus, no other catabolic yields values are required as stoichiometric coefficients, avoidingthe increased of the number of parameters and, , consequently, the complexity and uncertainty of the model. Only default ADM1 catabolic yields of glucose are needed to calculate the stoichiometry of these new fermentations.

The sugar oxidisers (X_{su}), originally defined in ADM1 are redefined as fermenters (X_{fer})responsible for the fermentation of ethanol, glycerol, lactate and sugars as well as other possible fermentable substrates. The uptake kinetic rates incorporate a competitive term to account for the multiple fermentable substrates. Under this approach, no additional microbial groups were added to the model as only one group is defined to degrade all soluble fermentable substrates.

Stoichiometry of generic soluble fermentation reactions

In this work, the AcoD of a mixture of pig manure (soluble fraction), gelatine and wine was modelled as a selected example of complementary substrates. Pig manure and gelatine were defined by their content in carbohydrates, proteins or lipids, whereas wine (containing mainly ethanol) could not be assigned to any existing state. For that purpose, a new state variable (S_{et}) as well as its corresponding uptake reaction stoichiometry was included in the Petersen matrix integrated in the generalised methodology.

Considering acidogenesis the fastest stage in anaerobic digestion, an accurate description of the stoichiometry in the fermentation of soluble substrates (such us ethanol, glycerol or lactate) into VFA products (butyrate, propionate and acetate) is not required in detail since all these intermediate acids are quickly converted to acetate,hydrogen and CO_2 in methanogenic systems. In this system, mass and electron balances are the elements that must be very accurately described instead. In most cases, the impact of this simplification on the prediction of effluent COD and gas flow and composition is negligible, while only small differences would appear in the prediction of pH under overloading conditions, as long as methanogenesis is not largely inhibited (Rodríguez et al., 2006).

Under these premises, the catabolic ADM1 stoichiometry coefficients *(f)* of acidogenesis of any soluble fermentable substrate can be in principle expressed in terms of equivalent glucose fermentation. Thus, each catabolic yield is function of the

standard ADM1 sugar fermentation yields. ADM1 sugars fermenting biomass (X_{su}) is substituted by one single group of generic fermentative biomass (X_{fer}) capable of degrading all soluble fermentable substrates. The proposed generalised methodology can be applied not only to ethanol but also to other easily fermentable soluble substrates such as glycerol or lactic acid as explained in Table 3.2.

Table 3.2. Stoichiometric calculations for generalised implementation of fermentable substrates. Example for ethanol, glycerol and lactic acid.

Procedure: 1) find the stoichiometric ratio among soluble compounds and glucose to obtain the catabolic products and yields as glucose equivalents and 2) amend substrate COD with H_2.

ETHANOL

$$C_2H_6O + H_2O \rightarrow \tfrac{1}{3}\, C_6H_{12}O_6 + 2\, H_2$$

$$(C_6H_{12}O_6 \rightarrow fbu\_su\, Bu + fpro\_su\, Pro + fac\_su\, Ac) \cdot \tfrac{1}{3}$$

$$C_2H_6O + H_2O \rightarrow \underbrace{\tfrac{1}{3}\, \boldsymbol{fbu\_su}}\, Bu + \underbrace{\tfrac{1}{3}\, \boldsymbol{fpro\_su}}\, Pro + \underbrace{\tfrac{1}{3}\, \boldsymbol{fac\_su}}\, Ac + 2\, \boldsymbol{H_2}$$

$$C_2H_6O + H_2O \rightarrow \quad \boldsymbol{fbu\_et}\, Bu + \quad \boldsymbol{fpro\_et}\, Pro + \quad \boldsymbol{fac\_et}\, Ac \quad + 2\, \boldsymbol{H_2}$$

GLYCEROL

$$C_3H_8O_3 \rightarrow 0.5\, C_6H_{12}O_6 + H_2$$

$$(C_6H_{12}O_6 \rightarrow fbu\_su\, Bu + fpro\_su\, Pro + fac\_su\, Ac) \cdot 0.5$$

$$C_3H_8O_3 \rightarrow \underbrace{\boldsymbol{0.5\, fbu\_su}}\, Bu + \underbrace{\boldsymbol{0.5\, fpro\_su}}\, Pro + \underbrace{\boldsymbol{0.5\, fac\_su}}\, Ac + \boldsymbol{H_2}$$

$$C_3H_8O_3 \rightarrow \quad \boldsymbol{fbu\_gly}\, Bu + \quad \boldsymbol{fpro\_gly}\, Pro \quad + \quad \boldsymbol{fac\_gly}\, Ac \quad + \quad \boldsymbol{H_2}$$

LACTIC ACID

$$C_3H_6O_3 \rightarrow 0.5\, C_6H_{12}O_6$$

$$(C_6H_{12}O_6 \rightarrow fbu\_su\, Bu + fpro\_su\, Pro + fac\_su\, Ac) \cdot 0.5$$

$$C_3H_6O_3 \rightarrow \underbrace{\boldsymbol{0.5\, fbu\_su}}\, Bu + \underbrace{\boldsymbol{0.5\, fpro\_su}}\, Pro + \underbrace{\boldsymbol{0.5\, fac\_su}}\, Ac$$

$$C_3H_6O_3 \rightarrow \quad \boldsymbol{fbu\_lac}\, Bu + \quad \boldsymbol{fpro\_lac}\, Pro \quad + \quad \boldsymbol{fac\_lac}\, Ac$$

Firstly, the stoichiometric conversion from ethanol to glucose (1:3) and glycerol or lactic acid to glucose (1:2) are considered; then, the same metabolic reaction in ADM1 for sugars (glucose) is used to channel the fermentation of these new substrates and to obtain their corresponding catabolic yields of butyrate, propionate and acetate. Finally, the fermentation reactions are amended with hydrogen to meet mass and electron balances (COD). The growth of X_{fer} fermenters varies depending on how much of each substrate (sugars, ethanol, glycerol or lactate) is degraded since the biomass yields on

each one are different. Therefore, biomass yields on ethanol, glycerol or lactate (Y_{et}, Y_{gly}, Y_{lac},)should be calculated. Kleerebezem and van Loosdrecht (2010) formulated a generalised method, Thermodynamic Electron Equivalent (TEEM) method to estimate the biomass yield on any substrate based on thermodynamic and energy Gibbs dissipation per mol biomass formed. The TEEM method is recommended to estimate the biomass yield of X_{fer} on ethanol, glycerol and lactate.

Fermentation kinetics: uptake reactions of fermentable substrates

The uptake reaction rate expressions of fermentable soluble substrates (eq. 1) were also modified to account for the competition of the generic biomass fermenters, X_{fer}, for multiple substrates. A competitive term (eq. 2) must be incorporated to the rate expressions to account for the fraction of each fermentable substrate in the system (analogous to ADM1 for the uptake of valeric and butyric acids degraded by the same group of microorganisms, X_{c4}).

$$\text{Uptake rates:} \quad \rho_j = k_{m,i} \cdot \frac{S_j}{K_S + S_j} \cdot X_{fer} \cdot \frac{S_j}{S_j + \sum_{i \neq j} S_i} \cdot I_2 \qquad (1)$$

$$\text{Competitive term} = \frac{S_j}{S_j + \sum_{i \neq j} S_i} \qquad (2)$$

where i, j = sugars, ethanol, lactate, etc…

The kinetic parameters values for ethanol, Monod maximum specific uptake rate ($k_{m,et}$) and half saturation value ($K_{s,et}$) were taken from Batstone et al. (2004), k_{m_et} = 3 kg COD/kg COD·d and K_{s_et} = 0.5 kg COD/m^3, expressed in molar units. Table 3.3 depicts all the processes considered in the model where differences with respect to original ADM1 are highlighted.

Table 3.3. Biochemical processes and their kinetic expressions considered in the model. New processes are highlighted; the rest remain as in ADM1 (uptake of sugars is not considered a new process but modified with respect to original ADM1) (Batstone et al., 2002).

Process name	**Process Rate (mol/L·d)**
Disintegration of particulate waste (X_c)	$k_{dis} \cdot X_c$
Hydrolysis of inactive biomass (X_d)	$\boldsymbol{k_{hyd,\_Xd} \cdot X_d}$
Hydrolysis of carbohydrates	$k_{hyd,ch} \cdot X_{ch}$

(Continue in next page)

(Continued from last page)

Process name	Process Rate (mol/L·d)
Hydrolysis of proteins	$k_{hyd,pr} \cdot X_{pr}$
Hydrolysis of lipids	$k_{hyd,li} \cdot X_{li}$
Uptake of sugars	$\boldsymbol{k_{m,su} \cdot \frac{S_{su}}{K_{s\_su} + S_{su}} \cdot X_{fer} \cdot \frac{S_{su}}{S_{su} + S_{et} + S_{gly} + S_{lac}} \cdot I_1}$
Uptake of ethanol	$\boldsymbol{k_{m,et} \cdot \frac{S_{et}}{K_{s\_et} + S_{et}} \cdot X_{fer} \cdot \frac{S_{et}}{S_{su} + S_{et} + S_{gly} + S_{lac}} \cdot I_1}$
Uptake of glycerol	$\boldsymbol{k_{m,gly} \cdot \frac{S_{gly}}{K_{s\_gly} + S_{gly}} \cdot X_{fer} \cdot \frac{S_{gly}}{S_{su} + S_{et} + S_{gly} + S_{lac}} \cdot I_1}$
Uptake of lactate	$\boldsymbol{k_{m,lac} \cdot \frac{S_{lac}}{K_{s\_lac} + S_{lac}} \cdot X_{fer} \cdot \frac{S_{lac}}{S_{su} + S_{et} + S_{gly} + S_{lac}} \cdot I_1}$
Uptake of amino acids	$k_{m,aa} \cdot \frac{S_{aa}}{K_{s\_aa} + S_{aa}} \cdot X_{aa} \cdot I_1$
Uptake of LCFA	$k_{m,fa} \cdot \frac{S_{fa}}{K_{s\_fa} + S_{fa}} \cdot X_{fa} \cdot I_2$
Uptake of valerate	$k_{m,c4} \cdot \frac{S_{va}}{K_{s\_va} + S_{va}} \cdot X_{c4} \cdot \frac{S_{va}}{S_{bu} + S_{va}} \cdot I_2$
Uptake of butyrate	$k_{m,c4} \cdot \frac{S_{bu}}{K_{s\_bu} + S_{bu}} \cdot X_{c4} \cdot \frac{S_{bu}}{S_{bu} + S_{va}} \cdot I_2$
Uptake of propionate	$k_{m,pro} \cdot \frac{S_{pro}}{K_{Spro} + S_{pro}} \cdot X_{pro} \cdot I_2$
Uptake of acetate	$k_{m,ac} \cdot \frac{S_{ac}}{K_{Ssac} + S_{ac}} \cdot X_{ac} \cdot I_3$
Uptake of hydrogen	$k_{m,h2} \cdot \frac{S_{h2}}{K_{s\_h2} + S_{h2}} \cdot X_{h2} \cdot I_1$
Decay of X_{fer}	$k_{dec\_Xfer} \cdot X_{fer}$
Decay of X_{aa}	$k_{dec\_Xaa} \cdot X_{aa}$
Decay of X_{fa}	$k_{dec\_Xfa} \cdot X_{fa}$
Decay of X_{c4}	$k_{dec\_Xc4} \cdot X_{c4}$
Decay of X_{pro}	$k_{dec\_Xpro} \cdot X_{pro}$
Decay of X_{ac}	$k_{dec\_Xac} \cdot X_{ac}$
Decay of X_{h2}	$k_{dec\_Xh2} \cdot X_{h2}$

3.3.2. Experimental results

An important objective of this work was to validate the ADM1-based AcoD model proposed and described above. For that purpose, experiments were conducted at pilot scale. The selected co-substrates (wine, gelatine and soluble fraction of pig manure) present different characteristics that may enhance the blend to be treated by co-digestion and, consequently, facilitates the stability and efficiency of the operation. Wine contributes with a high COD content and easily biodegradable organic matter, gelatine with nutrients (nitrogen content) and high COD content; and pig manure, provides the necessary alkalinity to the blend.

The feed composition, HRT, and OLR remained constant during the operation except for a short period when gelatine was changed to NH_4Cl solution (to keep the nitrogen content; and influent COD content was amended with wine in substitution to gelatine) or for a short period (one week, days 95 to 102) when feed flow and OLR was reduced on purpose due to absence of the operator in the pilot plant.

Figure 3.3 shows the feeding strategy applied. Even though the measured influent CODt and calculated OLR from the experiment varied slightly along the operation (Figure 3.3.b), the influent CODt and OLR considered in the model were kept constant and set to 27 g COD/L (16.7 % pig manure, 43.7 % gelatine and 39.6 % wine, % COD) and 2.5 g COD/L·d, respectively.

During the five-month operation, although the characteristics of wine and gelatine remained constant, those of the pig manure were slightly changing over the time, mainly affecting alkalinity and COD content. However, the flow of all substrates was kept constant. This explained why the alkalinity of the system varied continuously over the time, which is due to the variability of the pig manure alkalinity, ranging between 2 and 7 g $CaCO_3$/L (0.04 and 0.14 Eq/L, respectively).

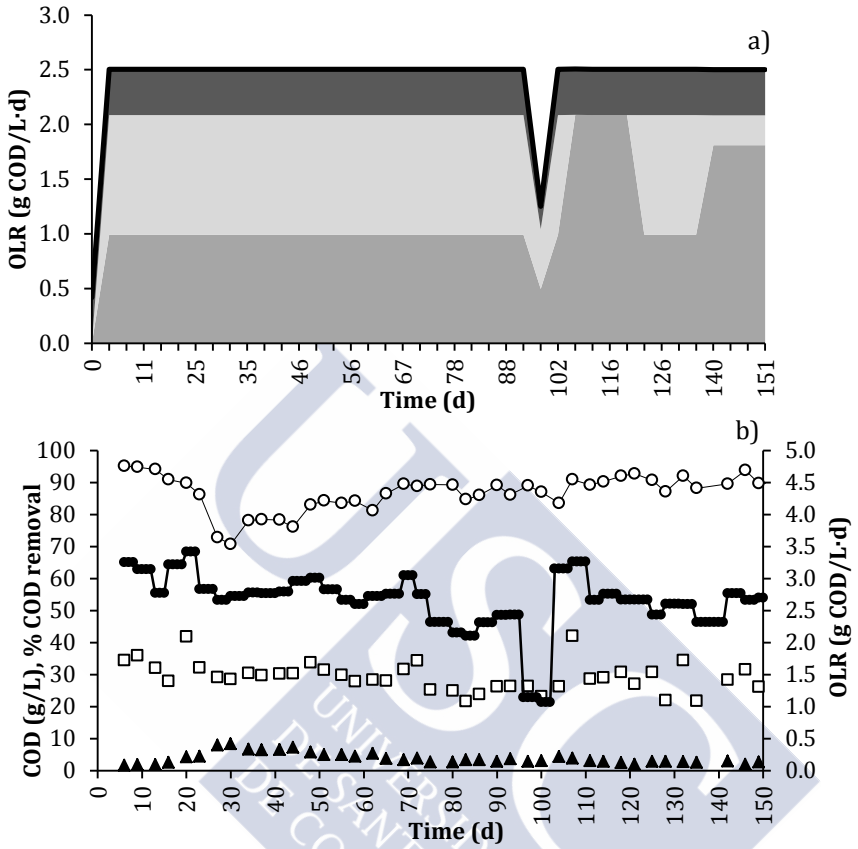

Figure 3.3. Feeding at HRT= 11 d. a) Simulation: (▬) Pig manure, (▬) Wine, (▬) Gelatine, (-) OLR. b) Experimental results: (●) OLR, (□) Influent CODt, (▲) Effluent CODt, (○) % COD removal.

3.3.3. Calibration/validation of ADM1-based AcoD model

In order to estimate the original microbial biomass composition, a long simulation, applying the conditions of the digester (fed with wine and pig manure at an OLR of 10 g COD/L·d and HRT of 2 days), was conducted before starting the experiment. From that simulation, the ratios between the different biomass groups were obtained and used to estimate their concentrations in the digester.

The new parameters added to the model are shown in Table 3.4 (expressed in molar units). The rest of the parameters used in the model remained as set originally in ADM1 (all in molar units) except for the acetoclastic inhibition by ammonia ($K_{I,NH3}$) that was modified from 1.8 to 0.6 mM_{NH3} to fit the experimental data.

Table 3.4. Parameters added to ADM1 required for the degradation of ethanol.

Parameter	Present Model	Units
$k_{m,\,et}$	0.0438	$molS_{et}/CmolX_{fer}\cdot h$
$K_{s,\,et}$	0.0052	$molS_{et}/L$
Y_{et}	0.07	$molX_{fer}/CmolS_{et}$
$k_{hyd,\,Xd}$	0.25	d^{-1}
$f_{su,xd}$	0.035	$molS_{su}/molX_d$
$f_{aa,xd}$	0.202	$molS_{aa}/molX_d$
$f_{fa,xd}$	0.185	$molS_{fa}/molX_d$
$f_{si,xd}$	0.353	$molS_i/molX_d$
k_La	24.2	d^{-1}
SRT	200	d

Parameters of Table 3.4 can remain constant even if other substrates are treated. Parameters regarding to dead biomass: fractionation into sugars, amino acids and fatty acids and inerts are constants and equal to the ADM1 considered fractionation of inactive biomass into carbohydrates, proteins and lipids and inerts. A lumped disintegration and hydrolysis stage was considered in this model for inactive biomass. Original ADM1 suggests different values for disintegration and hydrolysis of carbohydrates, proteins and lipids at mesophilic conditions (k_{dis} = 0.4 d^{-1}, k_{hyd_CH} = 0.25 d^{-1}, k_{hyd_PR} = 0.20 d^{-1}, k_{hyd_LI} = 0.10 d^{-1}) but subjected to a variability within a factor of 100% (Batstone et al., 2002). Based on these values for disintegration and hydrolysis and the variability indicated, as only one kinetic coefficient for the lumped disintegration and hydrolysis of dead biomass is required for the present model, an in-between value of 0.25 d^{-1} (and equal to the coefficient for hydrolysis of carbohydrates) was selected.

The biomass yield on ethanol was estimated using TEEM method (Kleerebezem and van Loosdrecht, 2010) and kept constant. The same method could be used to calculate biomass yields of other substrates such as glycerol or lactate. The monod-kinetic

parameters, k_m and K_s, must however be determined for each specific substrate (ethanol, glycerol or lactate). In this case, k_m and K_s for ethanol uptake rate were taken from Batstone et al. (2004). Only parameters such as k_La or SRT can vary as they are largely depend on reactor configuration.

SRT was set in a value which allowed obtaining a simulated final biomass concentration similar to that determined experimentally (11 g/L). Mass transfer coefficient k_La was calculated from the following expression that correlates k_La with biogas flow ($k_La = 23.726 \cdot F_{biogas}^{0.804}$; where $[k_La] = d^{-1}$ and biogas flow, $[F_{biogas}] = m^3/d$), which has been previously obtained in the same pilot plant (Garcia, 2010).

Figure 3.4 depicts both the experimental and simulated results for gas and liquid phases for the entire operation. Gas flow rate and biogas composition predicted with the model are consistent with experimental results (Figures 3.4a, 3.4b, 3.4c). The simulated hydrogen gas revealed consistent results most of the time except in two transient periods, between days 105 to 125 and 140 to 150 (that coincided with a feed change where most of the organic content was wine), when peaks of hydrogen pressure up to 35-50 ppm were predicted by the model (Figure 3.4c). The thermodynamics of syntrophic acetogenesis and hydrogen-utilising methanogenesis reactions requires low hydrogen concentrations to maintain the oxidation reactions of organic acids thermodynamically feasible. Only a narrow range of hydrogen concentration makes this possible simultaneously to methanogenesis (Batstone et al., 2002). Harper and Pohland (1986) found the methanogenic niche around 10 and 100 ppm of hydrogen partial pressure. In the experiment carried out in the pilot plant, the hydrogen pressure remained at very low values (around 20 ppm) and within the range of the methanogenic niche. As acetic acid was the major intermediate during the operation and the hydrogen pressure remained at low values, a good syntrophic relationship between acetogenic bacteria and hydrogenothrophic methanogens was maintained. Different reasons can explain the model predictions of these peaks of hydrogen: (1) the differential equations solver showed a high degree of stiffness on hydrogen gas predictions due to the fact that hydrogen is simultaneously produced and consumed at high fluxes causing many fluctuations in hydrogen values, (2) the ethanol fermentation was modelled via glucose equivalents with a specific stoichiometry for hydrogen production (Table 3.2).

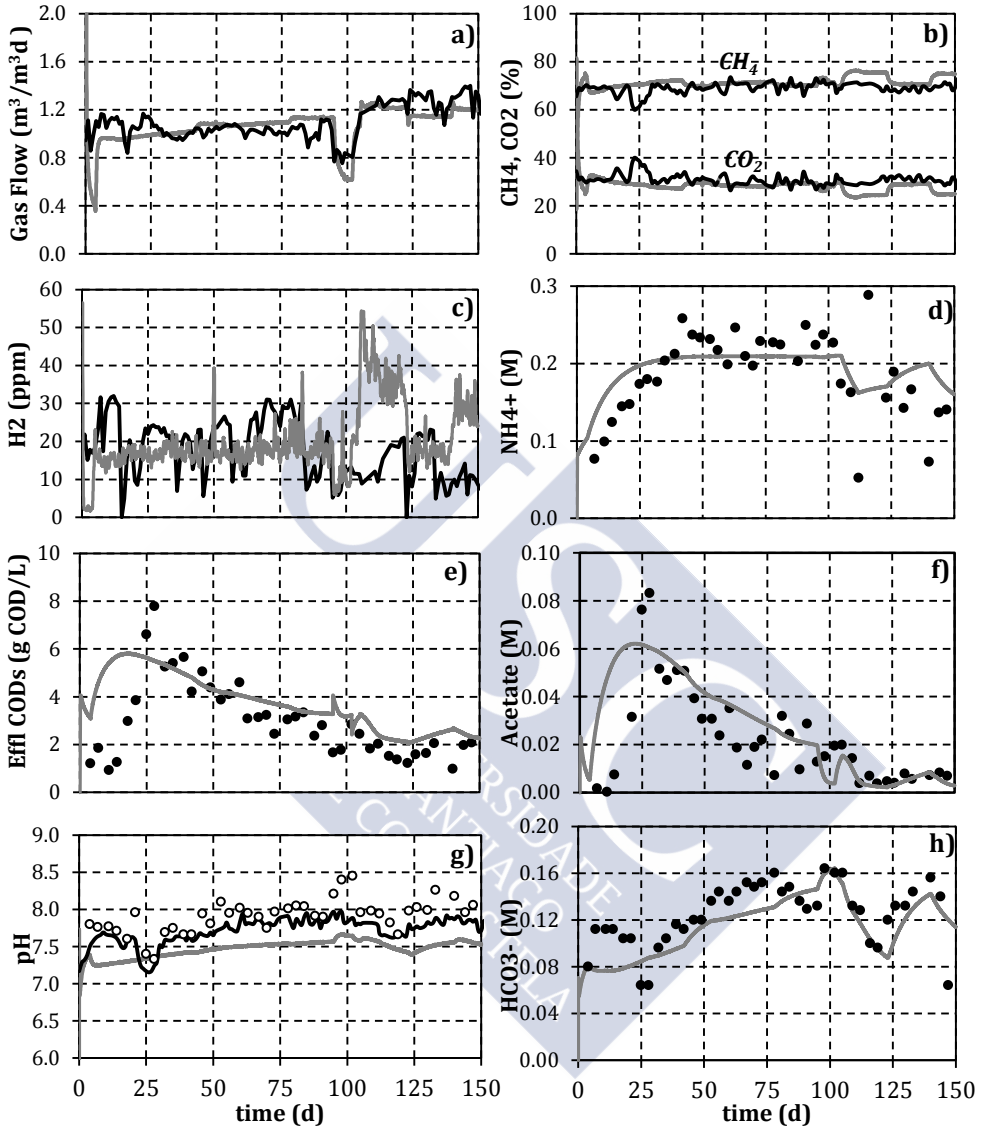

Figure 3.4. Simulated and experimental results; a) biogas production, b) biogas composition, c) H_2 (gas), d) NH_4^+, e) CODs, f) acetate, g) pH, h) bicarbonate; (▬) Simulated results; (▬) Experimental results (on-line); (●), (○) Experimental results (off-line)).

In those transition periods where most of the organic load was wine, a sudden over production of hydrogen might have been predicted due to the specific stoichiometry not being fully accurate. Despite this possible limitation, in general, predicted hydrogen pressure achieved acceptable values during the entire operation, even with no calibration applied to the hydrogen stoichiometry from the ethanol fermentation. Similar or lower predicted hydrogen values with respect to other studies were obtained (Pauss and Guiot, 1993; Lübken et al., 2007). The predicted hydrogen concentrations are in the range for methanogenic systems and despite the differences at certain time intervals, simulated hydrogen gas fit to a large extend experimental results.

Ammonium content achieved values up to 0.20-0.25 M (around 4 g NH_4^+/L) in the initial stages of the operation (Figure 3.4d). The high value of inorganic nitrogen was assumed to cause the acetoclastic methanogens inhibition and the accumulation of acetic acid as encountered in the initial 25 days of operation (Figure 3.4f). Although, the free ammonia, NH_3, is considered the main cause of inhibition, inhibitory concentrations are often expressed in terms of total ammonia nitrogen (TAN) that stands for the two nitrogen inorganic species NH_4^+ and NH_3. Inhibitory TAN concentrations ranging from 1.7 to 14 g/L have been reported depending on the substrate, inoculum and environmental conditions (Chen et al., 2008). In the initial stages, it is supposed that biomass was not adapted to high nitrogen concentrations since in the previous nine-month period the digester treated a blend of pig manure and wine involving a much less nitrogen content. As long as the microorganisms were adapted to such nitrogen concentration, acclimated methanogens succeeded at obtaining a stable digestion (where the accumulation of VFA decreased over time) with NH_4^+ concentration exceeding 4 g/L.

Most of CODt content of the effluent was soluble and most of CODs, acetic acid (Figures 3.4e, 3.4f). Simulated acetic acid showed an earlier accumulation in comparison to experimental results in the initial stages. This is attributed to either the model not being able to predict the change in the methanogenic activity due to NH_3 inhibition, or non-described hydraulic effects. Acetic acid acclimation was initially tested in the model through biomass adaptation by selecting different initial concentrations of acetoclastic methanogens (X_{ac}), but the prediction of the acetic peak was poor. By combining initial X_{ac} concentration and a calibration of the ammonia inhibition parameter, $K_{I,NH3}$, a much better fit was obtained. $K_{I,NH3}$, was calibrated to 0.6 mM and

estimated initial acetoclastic methanogens concentration was reduced ten folds in order to observe the experimental peak of acetic concentration. Although the model was not able to reproduce the exact same peak of acetic, the trend over the time was similar to the experimental values and simulated results were considered acceptable. Parker (2005) indicated that overestimation or underestimation of simulated VFA with ADM1 depended on the digester configuration and operating conditions. Figures 3.4g and 3.4h depict pH and bicarbonate, respectively. Experimental pH remained stable and around 7.5-8.0. The model appeared to underestimate measured pH possibly due to the presence of an unidentified buffer, a weak base (such as $CaCO_3$) that was not accounted for in the model (Batstone et al., 2004).

Bicarbonate fluctuated over the time as the alkalinity of pig manure did. Different alkalinity contents for pig manure were assumed to simulate the experiment. Both experimental pH and bicarbonate fell in day 25 due to the accumulation of acetic acid and then recovered to their normal levels when the peak of acetic acid disappeared. On the other hand, the simulated pH and bicarbonate showed a very slight drop at the beginning of the operation (around day 5) when the simulated acetic started to accumulate.

The experimental acetate accumulation was more severe than the simulated, which explains why the experimental pH fell more abruptly. The simulation results with ethanol-rich substrate resulted satisfactory with a minimum parameter calibration needed. As acidogenesis is the fastest stage in anaerobic digestion, modelling the fermentation of any soluble substrate as a sugars equivalent fermentation is an acceptable approximation provided mass and electron balances are consistently set up. This is independent from how exact the intermediate catabolic fermentation products yields are (i.e. butyrate, propionate, acetate), since all species are converted quickly to acetate, CO_2 and H_2 (Rodríguez et al., 2006). In fact, the differences encountered between the gas flow, gas composition or effluent COD predicted with the model and those obtained experimentally are negligible or acceptable to a large extent. This evidence is shown in the case of ethanol degradation via very different pathways. Batstone et al. (2004) successfully validated the ethanol fermentation via acetate and hydrogen, and so did this proposed generalised methodology via glucose equivalent reaction (where acetate was observed as the major intermediate during the process). Although more experiments should be carried out to validate the applicability of this methodology to other fermentable substrates (glycerol or lactate) besides ethanol, it

was assumed this generalised methodology will work well for glycerol as other models succeeded at considering glycerol fermentation via carbohydrates with ADM1 (Galí et al., 2009) and for lactate based on the results leading to acetate production (Sørensen et al., 1991). Acid formation from fermentation is a complex process that involves many products and bacterial groups depending on the operating conditions and microbial population. ADM1 considers 7 types of microbes and defines for them constant yields of products and intermediates. This provides acceptable results when evaluating methanogenic systems; however, in the case of non methanogenic conditions, good reliable models are still not available to predict organic acid produced in e.g. short solid retention times or low pH (Rodríguez et al., 2006; Hidaka et al. 2010).

The methodology presented here facilitates the incorporation of new soluble fermentable substrates (such as ethanol, lactate or glycerol) with a simple fermentation mechanism and extends the applicability of ADM1 to treat multiple substrates in both AD and AcoD processes. However, this methodology can only be applied to easily fermentable substrates, but not to substrates considered toxic or very slowly biodegradable such us phenol where inhibitory effects can occur (Hernandez and Edyvean, 2008).

It is worth noting that the set of ADM1 parameters were kept at their default values during this model validation. Only the inhibition constant of the acetoclastic methanogens due to free ammonia ($K_{I,NH3}$) was calibrated. The good simulation results obtained suggests that ADM1 can be a powerful tool to simulate real AD or AcoD process treating a variety of substrates. In addition to the model prediction capabilities for AcoD processes of multiple substrates, a reliable dynamic model of these systems becomes a powerful tool to develop and test dynamic control strategies and optimization that could include testing different blends of substrates seeking a global optimisation of the process performance in terms of biogas production and system stability.

3.4. Conclusions

The generalised approach to implement new soluble fermentable substrates into ADM1 was successfully validated in pilot plant. It facilitates the incorporation of new substrates and extends ADM1 applicability to co-digestion systems. The default set of

ADM1 parameters values can describe accurately the co-digestion of fermentable substrates. In this work, only an adjusted value for the acetoclastic ammonia inhibition was required to describe VFA dynamics. The results obtained suggest that the methodology proposed can be used as AcoD dynamic predictor model as well as for the development of control and optimisation strategies in AcoD.

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Chapter 4. Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes

Contents

Abstract

A methodology to estimate disintegration and hydrolysis kinetic parameters of solid wastes and validate an ADM1-based anaerobic co-digestion model is presented. Kinetic parameters of the model were calibrated from batch reactor experiments treating individually fruit and vegetable wastes (among other residues) following a new protocol for batch tests. In addition, decoupled disintegration kinetics for readily and slowly biodegradable fractions of solid wastes was considered. Calibrated parameters from batch assays of individual substrates were used to validate the model for a semi-continuous co-digestion operation treating simultaneously 5 fruit and vegetable wastes. The semi-continuous experiment was carried out in a lab-scale CSTR reactor for 15 weeks at organic loading rate ranging between 2.0 and 4.7 gVS/L·d. The model (built in MATLAB/Simulink) fit to a large extent the experimental results in both batch and semi-continuous mode and served as a powerful tool to simulate the digestion or co-digestion of solid wastes.

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4.1. Introduction

The high production and disposal of organic solid wastes and their uncontrolled decomposition generates a large-scale environ- mental pollution. Particularly, fruit and vegetable wastes (FVW) that are highly biodegradable constitute a source of methane emission, odours and air contamination in municipal landfills (Garcia- Peña et al., 2011). In order to reduce and stabilise the volume of organic wastes, anaerobic digestion has become an alternative technology against incineration or composting and has been proved as a well establish process for the treatment of many types of organic wastes, solids or liquids (Bouallagui et al., 2009). Among biological treatments, anaerobic digestion or co-digestion is the most cost-effective, due to the high energy recovery as biogas and its limited environmental impact (Mata-Alvarez et al., 2000), and can be applied to FVW in order to raise the value of this kind of wastes.

Anaerobic Digestion (AD) consists on the biological degradation of organic matter by the action of a microbial consortium that converts organic substrates into methane and carbon dioxide through a complex reaction path. The mechanism involves a series of parallel and sequential stages: hydrolysis, acidogenesis, acetogenesis/ dehydrogenation, and methanation, each one catalysed by a particular group of microorganisms (Gupta et al., 2012).

Recently, the interest is growing on anaerobic co-digestion (AcoD). The term co-digestion stands for the simultaneous digestion of two or more organic substrates, and has become more attractive than single-substrate AD due to several advantages such as the improvement of the balance of nutrients and ratio C/N, dilution of toxics and inhibitors or achievement of higher biogas yield and methane production (Khalid et al., 2011; Mata-Alvarez et al., 2011). The co-digestion benefits from the synergism of the different characteristics of the co-substrates (Hartmann et al., 2003). In 2002, the IWA task group for mathematical modelling developed the Anaerobic Digestion Model No. 1, ADM1 (Batstone et al., 2002), that serves as a reference for on-going models on anaerobic digestion and co-digestion. ADM1 is a structured model that describes the main processes involved in AD to convert complex organic substrates into biogas: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. ADM1 was used as a standard to build AcoD models and majority of papers dealing with AcoD modelling appeared in 2007–2009 (Fezzani and Cheikh, 2008; Lübken et al., 2007; Zaher et al., 2009).

An ADM1-based AcoD model has been recently developed (García-Gen et al., 2013) and successfully applied to soluble substrates in continuous operation. In order to simulate the digestion of solid wastes, an accurate description of the disintegration and hydrolysis should be achieved since these steps are considered the rate-limiting stages of the overall kinetics (Pavlostathis and Giraldo-Gomez, 1991). The mechanism of the disintegration and hydrolysis is one of the limitations in the current AD modelling paradigm. The disintegration stage proposed in ADM1 is a source of constant questioning about its realism in representing what happens in the initial stages of anaerobic digestion. In many cases, such as solid wastes in which disintegration is the rate-limiting step, this drawback becomes an obstacle for successful simulations. As the main objective of this study is to estimate kinetic parameters from model calibration, a new disintegration and hydrolysis modelling approach is proposed to improve the simulation and fitting between model results and experimental results. The review of numerous papers dealing with disintegration and hydrolysis kinetics of solid wastes shows that different kinetic coefficients have been obtained from the classical BMP tests depending on the experimental conditions, inoculum, substrate/ inoculum ratio or lumped effect of disintegration and hydrolysis (Vavilin et al., 2008). As FVW have a complex organic matter structure, it can be assumed that the particulate organic matter is initially only a conglomerate of carbohydrates, proteins and lipids that is firstly disintegrated into its constituents and then these particulate compounds (carbohydrates, proteins and lipids) are hydrolysed to soluble forms. Fractionation of the organic matter into readily and slowly biodegradable fractions has been successfully applied to describe the bio-accessibility of particulate organic matter (Mottet et al., 2013), and can be used to estimate the kinetic coefficients of disintegration and hydrolysis stages of particulate complex substrates.

This work presents a novel and optimised procedure for biodegradability test (carried out in batch reactors rather than BMP tests) to better estimate kinetic parameters from batch experiments of individual substrates.

The aim of the present study is to estimate, within the ADM1 model framework using a proposed mechanism for disintegration/hydrolysis based on the fractionation of the particulate organic matter (Mottet et al., 2013), the disintegration and hydrolysis kinetic coefficients of a large number of substrates (mainly FVW) from experimental batch assays carried out with a new methodology for biodegradability tests. Finally, the co-digestion model is validated in semi-continuous operation at an AcoD lab-scale

reactor treating a blend of five FVW constituting a strong validation of all the procedures proposed.

4.2. Materials and Methods

4.2.1. Substrates

The main substrates of this study were fruit and vegetable wastes (FVW) in both batch and semi-continuous operation. As FVW were mainly made of carbohydrates with low lipid or protein content, some other batch assays were carried out with rich lipid content (vegetable oil wastes) and rich protein content (fish waste) in order to calibrate the hydrolysis kinetic parameters of both lipids and proteins.

For batch experiments, 15 substrates have been used: 3 fruits (peach, mango, and banana), 6 vegetable (cabbage, potato, carrot, lettuce, tomato, and cauliflower), 5 solid residues from the refining of vegetable oil taken from different streams of the refining process (100% fatty wastes named as Dc1 and Dc2 (deodorizing condensates), WE (used winterisation earth), Ts1and Ts2 (tank sediments) and 1 fish waste (pollock, a variety of cod). The substrates were reduced to approximately 1 cm size in a Blik BB 230 crusher equipped with stainless steel rotating blades, mixed thoroughly and stored at -20 °C. For semi-continuous experiments, thoroughly equal quantities of apple, banana, carrot, potato and lettuce were treated under co-digestion. (Note: none batch test was carried out to apple; thus, its kinetic coefficients and readily fraction were taken from mango having a similar composition (Table 4.1)).

Table 4.1. Fruit and vegetable average composition taken from *www.aprifel.com.*

FVW	Mass (g)	Fibres (g)	Carbohydrates (g)	Proteins (g)	Lipids (g)	Water (g)
Tomato	98.9	1.20	2.80	0.80	0.10	94.0
Potato	100.2	2.10	19.00	2.00	0.10	77.0
Cauliflower	99.4	2.50	3.50	2.40	0.00	91.0
Peach	98.8	2.00	9.00	0.70	0.10	87.0
Cabbage	97.0	3.40	2.80	2.80	0.00	88.0
Banana	99.0	2.00	20.50	1.20	0.30	75.0
Mango	99.9	1.90	14.30	0.60	0.10	83.0
Lettuce	98.8	1.50	1.30	1.20	0.30	94.5
Carrot	99.8	3.00	6.70	0.80	0.30	89.0
Apple	99.6	2.10	12.6	0.30	0.30	84.3

4.2.2. Batch reactors

The experiments were carried out in four double-walled glass reactors of 6-L effective volume, maintained at 35 °C by a regulated water bath. Mixing in the reactors was done by a system of magnetic stirring. The biogas production was measured on-line every 2 min by Milligascounter MGC-1 flow meters (Ritter gas meters) fitted with a 4–20 mA output. The ‘‘Modular SPC’’ software developed at the INRA laboratory was used to log gas output. The reactors were seeded at a volatile suspended solids concentration (VSS) of around 13 gVSS/L with anaerobic sludge taken from an industrial-scale anaerobic UASB reactor treating the effluents from a sugar refinery. After seeding and before starting the addition of the waste, the reactors were fed 5 times with 5mL of ethanol as sole carbon and energy source to check the activity of the sludge (Torrijos et al., 2013). All substrates mentioned before were treated in these reactors in a series of 6–8 consecutive batches at a concentration of 1 gVS/L per batch. Batch reactor experiments where preferred to BMP assays for three main reasons: (i) carrying out several consecutive batches facilitates the obtaining of an acclimated biomass to substrate under investigation and avoid any lag phase, (ii) the substrate/bio- mass ratio is low (S_0/X_0 ratio of 0.08 instead of 0.5–1 in conventional BMP) and hence the quantity of substrate added is low, allowing brief reaction times (less than a week) which permits to maintain a biomass with a good activity, (iii) the accuracy of the measurements is very high with an acquisition of the volume of biogas produced every 2 min with an increment each 3mL of biogas, which correspond to the degradation of less than 1 mg COD/L in the 6 L reactor.

4.2.3. Semi-continuous reactor

The semi-continuous AcoD experiment was carried out in a double-walled stainless steel reactor maintained at 35 °C by a regulated water bath. The total volume of the reactor was 15 L with an effective sludge weight of 10 kg. Feeding and draining were carried out through an opening in the top part of the reactor. The reactor was equipped with a paddle-shaped stirrer powered by a 1 HP motor. Mixing times were programmed through a process controller. For this experiment, mixing time was fixed at 5 min/h. The reactor was inoculated with 10 kg of anaerobic sludge from the same origin that that used in the batch assays. As the reactor is made of stainless steel, measuring the volume inside the reactor is difficult so, the reactor was placed on a weighing scale and the total weight of the reactor set-up was measured once a week and sludge withdrawal was accordingly adjusted to maintain the weight of the reactor

sludge constant at 10 kg (density of the sludge was approximately 1 kg/L, so the reactor treated 10 L of residue). The volume of biogas produced was recorded as described in the previous paragraph. The reactor was fed with a blend of 5 FVW: lettuce, apple, potato, banana and carrot with a total and volatile solids content of 12.7% and 11.5%, respectively. The mix was treated for 15 weeks at 3 different organic loading rates (OLR): 2.0 gVS/L·d (first 10 weeks), 3.8 gVS/L·d (weeks 12–14) and 4.7 gVS/L·d (week 15) with hydraulic retention times (HRT) of 80, 45, and 36 days, respectively. The reactor was mostly fed 5 times a week but occasionally, 4 times a week.

4.2.4. ADM1-based AcoD model

The ADM1-based AcoD model (García-Gen et al., 2013) was used to simulate the batch assays of all substrates and the semi- continuous AcoD experiment. The model, previously validated for soluble substrates, was modified to include a better description of the disintegration–hydrolysis kinetics (considered as the rate- limiting step of the overall kinetics for solid wastes). The model was used to estimate the disintegration–hydrolysis kinetic parameters from batch assays to then use these calibrated coefficients to validate the model with the semi-continuous AcoD experiment. The model is implemented in an Excel–MATLAB/Simulink platform (Rodríguez et al., 2009), and adapted to run both batch and continuous operations. The model is written in molar units with default ADM1 parameters values, except for disintegration or hydrolysis kinetic coefficients that had to be calibrated. The ordinary differential equations of all states were coded and implemented using MATLAB and integrated with the ODE113 solver.

4.2.5. Analytical methods

Biogas composition (CH_4, H_2, O_2, N_2, CO_2) was measured using a gas chromatograph (Shimadzu GC-8A) connected to a C-R8A integrator and equipped with a CTRI Alltech column. The carrier gas was argon at 2.8 bars. The temperatures were 30 °C for the oven and 100 °C for the injector and the detector. The detection of gaseous compounds was done using a thermal conductivity detector and the intensity of current was 80 mA. The volume of injected biogas was 1 mL. Volatile fatty acid (VFA) concentration was measured using a gas chromatograph (GC-8000, Fisons Instruments) equipped with a flame ionisation detector and an automatic sampler (AS 800, Fisons instruments). The column used was a semi-capillary Econocap FFAP (Alltech) column 15m long, 0.53 cm

diameter with 1.2 µm Phase ECTM 1000 film. The temperature of the spitless injector was 250 °C; the temperature of the detector was 275 °C. The temperature increased from 80 °C to 120 °C in 3 min. The carrier gas was nitrogen (25 kPa). The volume of sample injected was 1 µL.

LCFA were measured by chromatography using GC-FID. The column used was a CP-Select CB for FAME fused silica WCOT (VAR- IAN), 50 m length, 0.25 mm internal diameter, film thick 0.25 µm. Oven temperature was set to 185 °C for 40 min, then 15 °C/min until 250 °C and 250 °C for 10.7 min. Injection was performed by split 1:100, 1 µL, at 250 °C for 55 min. Detection was done by FID at 250 °C and the gas phase consisted of helium at 1.2 mL/min flow rate. Samples were prepared according to the following procedure: initial Tert-Butyl Methyl Ether (TBME) solubilisation after sulphuric acid hydrolysis; filtration (0.45 µm) of TBME extracts; and extract transesterification using 0.5M Tri Methyl Sulfonium Hydroxide (TMSH) in ethanol at the proportion of 160 µL extract for 40 µL TMSH.

Other parameters such as pH, COD, TS, VS, VSS or alkalinity were measured following Standard Methods (APHA, 1998).

4.3. Results and Discussion

The disintegration–hydrolysis kinetic parameters were calibrated with the ADM1-based AcoD model, running batch experiments for each single substrate to obtain its particular parameters. Eventually, the model was validated in semi-continuous operation by simulating a semi-continuous AcoD experiment treating simultaneously a mix of five FVW, with the parameters calibrated from batch assays.

4.3.1. ADM1 model modifications. Disintegration reactions

The anaerobic digestion of solid wastes often implies disintegration/hydrolysis as the rate limiting step of the overall kinetic. Batch assays of solid wastes typically showed two different stages for the overall kinetics: in the initial steps, the overall rate was fast (as readily biodegradable organic matter was being converted into biogas) and after a certain time, the rate slowed down (as all readily organic matter had been converted to biogas, and only slowly biodegradable matter remained). Based on this observation, the particulate complex substrates (defined and calculated as the linear combination of protein, lipid, carbohydrate and fibre contents) could be expressed (eq. 1) as the

addition of two fractions (readily and slowly biodegradable fractions of a complex substrate).

$$\text{Complex substrate} = X_C = X_{C,READILY} + X_{C,SLOWLY} = f_R \cdot X_C + (1 - f_R) \cdot X_C \quad (1)$$

where f_R is the readily biodegradable fraction of the particulate substrate, X_C is the complex substrate and $X_{C,READILY}$, $X_{C,SLOWLY}$ are the readily and slowly biodegradable content of the complex substrate.

A disintegration kinetic mechanism for particulate substrates is proposed to be used in the ADM1-based AcoD model, based on the same idea of fractionation described by Mottet et al. (2013). The disintegration reaction was split into two fractions, readily- and slowly-biodegradable fractions, both with first order kinetics (eq. 2).

$$-\frac{dX_C}{dt} = -\left[\frac{dX_{C,READILY}}{dt} + \frac{dX_{C,SLOWLY}}{dt}\right] = k_{dis\_XcR} \cdot X_{C,READILY} + k_{dis\_XcS} \cdot X_{C,SLOWLY} \quad (2)$$

where k_{dis_XcR} is the disintegration kinetic of the readily biodegradable fraction, k_{dis_XcS} is the disintegration kinetic of the slowly bio- degradable fraction; and X_C, $X_{C,READILY}$, $X_{C,SLOWLY}$ are the total, readily and slowly particulate substrate.

The model was modified to incorporate new states for the readily and slowly biodegradable fractions (X_{cR}, X_{cS}) of the complex substrates (X_c). As fruit and vegetable wastes always involve carbohydrates and fibres (the latter mainly made up of hemicellulose and other slowly biodegradable carbohydrates), new states were added to distinguish between fast biodegradable carbohydrates (X_{fch}) and slowly biodegradable carbohydrates (X_{sch}). These two carbohydrates will be then hydrolysed to sugars (S_{su}) with different kinetics parameters. Finally, as a fraction of the fibres can be recalcitrant, a state for non-biodegradable fraction of fibres, X_i, was also considered.

Figure 4.1 depicts a scheme for the disintegration and hydrolysis stages of any solid substrate as it is conceived in the model. The complex substrate, Xc, was defined by fractions X_{cR} and X_{cS}, which then both disintegrates into lipids (X_{li}), proteins (X_{pr}), carbohydrates (X_{fch}, X_{sch}) and non-biodegradable fibres (X_i), to finally be both further hydrolysed from carbohydrates to sugars (S_{su}), proteins to amino acids (S_{aa}), and lipids to fatty acids (S_{fa}). The rest of the biochemical processes involved in the model and their parameters remained as default ADM1 values (see Table 4.2).

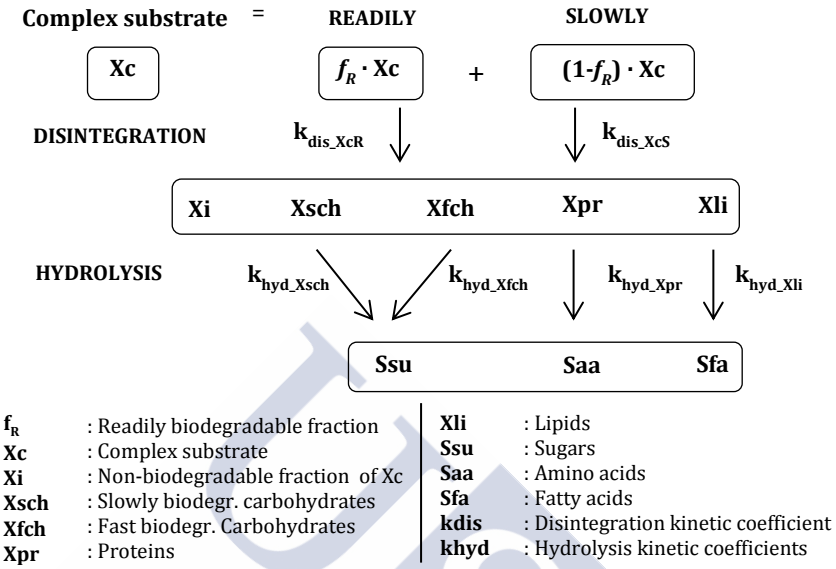

Figure 4.1. Disintegration and hydrolysis mechanism for solid substrates proposed in our ADM1-based AcoD model.

Table 4.2. Biochemical processes and kinetics of the model. New processes are highlighted; the rest remained as in ADM1 (Batstone et al., 2002).

Process name	Process Rate (mol/L·d)
Disintegration of readily biodegradable particulate complex substrate (X_{cR})	$k_{dis\_XcR} \cdot f_R \cdot X_c$
Disintegration of slowly biodegradable particulate complex substrate (X_{cS})	$k_{dis\_XcS} \cdot (1 - f_R) \cdot X_c$
Hydrolysis of inactive biomass (X_d)	$k_{hyd\_Xd} \cdot X_d$
Hydrolysis of fast biodegradable carbohydrates	$k_{hyd\_Xfch} \cdot X_{fch}$
Hydrolysis of slowly biodegradable carbohydrates	$k_{hyd\_Xsch} \cdot X_{sch}$
Hydrolysis of proteins	$k_{hyd\_Xpr} \cdot X_{pr}$
Hydrolysis of lipids	$k_{hyd\_Xli} \cdot X_{li}$

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Process name	Process Rate (mol/L·d)
Uptake of sugars	$k_{m,su} \cdot \frac{S_{su}}{K_{s\_su} + S_{su}} \cdot X_{su} \cdot I_1$
Uptake of amino acids	$k_{m,aa} \cdot \frac{S_{aa}}{K_{s\_aa} + S_{aa}} \cdot X_{aa} \cdot I_1$
Uptake of LCFA	$k_{m,fa} \cdot \frac{S_{fa}}{K_{s\_fa} + S_{fa}} \cdot X_{fa} \cdot I_2$
Uptake of valerate	$k_{m,c4} \cdot \frac{S_{va}}{K_{s\_va} + S_{va}} \cdot X_{c4} \cdot \frac{S_{va}}{S_{bu} + S_{va}} \cdot I_2$
Uptake of butyrate	$k_{m,c4} \cdot \frac{S_{bu}}{K_{s\_bu} + S_{bu}} \cdot X_{c4} \cdot \frac{S_{bu}}{S_{bu} + S_{va}} \cdot I_2$
Uptake of propionate	$k_{m,pro} \cdot \frac{S_{pro}}{K_{s\_pro} + S_{pro}} \cdot X_{pro} \cdot I_2$
Uptake of acetate	$k_{m,ac} \cdot \frac{S_{ac}}{K_{s\_ac} + S_{ac}} \cdot X_{ac} \cdot I_3$
Uptake of hydrogen	$k_{m,h2} \cdot \frac{S_{h2}}{K_{s\_h2} + S_{h2}} \cdot X_{h2} \cdot I_1$
Decay of X_{su}	$k_{dec\_Xsu} \cdot X_{su}$
Decay of X_{aa}	$k_{dec\_Xaa} \cdot X_{aa}$
Decay of X_{fa}	$k_{dec\_Xfa} \cdot X_{fa}$
Decay of X_{c4}	$k_{dec\_Xc4} \cdot X_{c4}$
Decay of X_{pro}	$k_{dec\_Xpro} \cdot X_{pro}$
Decay of X_{ac}	$k_{dec\_Xac} \cdot X_{ac}$
Decay of X_{h2}	$k_{dec\_Xh2} \cdot X_{h2}$

4.3.2. Fractionation into readily and slowly biodegradable fractions

The readily fraction f_R of each substrate was estimated from the experimental cumulative biogas production from each individual substrate batch assay (Figure 4.2a). Applying derivatives to the cumulative methane curve, after removal of the endogenous respiration, provides the biogas production rate with time (Figure 4.2b). The area below this curve is proportional to the total quantity of COD removed during the batch. The calculated derivative curve (Figure 4.2b) represented the biogas flow and showed two clearly distinguishable areas: an initial stage where the biogas production rate was high, and a final stage where the biogas production rate was much lower. The existence of different biogas productions rates suggested the presence of

readily- and slowly-biodegradable organic matter. Readily- and slowly-biodegradable fractions could be graphically determined, assuming disintegration as the rate- limiting stage of the AD.

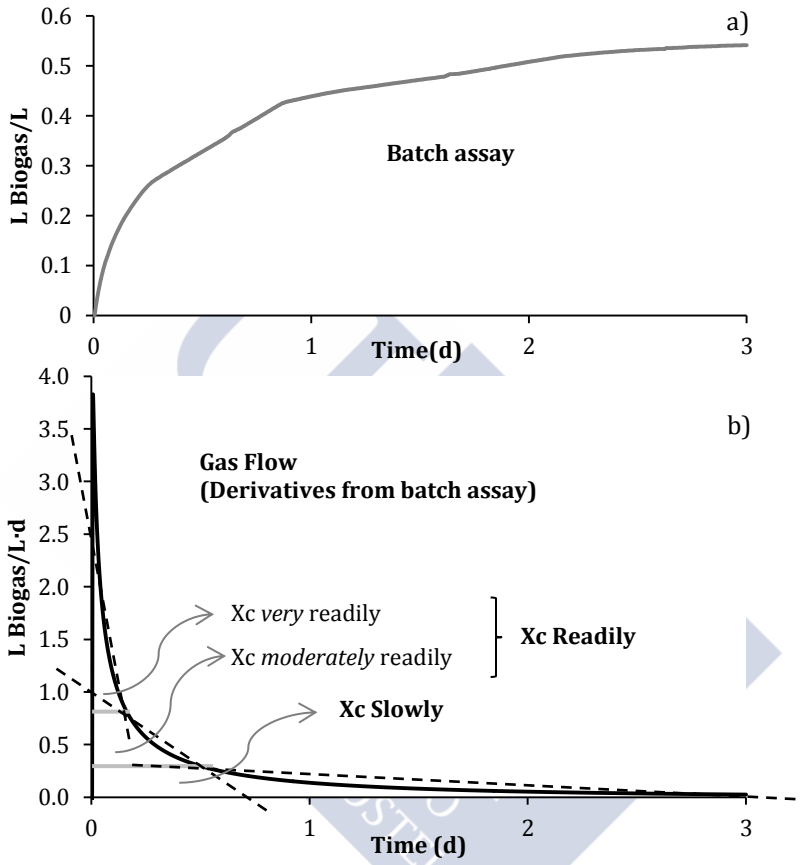

Figure 4.2. Experimental batch assay of mango. (a) Cumulative biogas production per reactor volume. (b) Gas production rate (computed from cumulative derivative).

Even though there is no rule to split the area into readily and slowly biodegradable fractions, it was assumed, based on the derivative curves, that biogas production rate higher than 0.75 L biogas/L·d stood for very readily biodegradable fraction, rates between 0.25 and 0.75 L biogas/L·d for moderately readily biodegradable fraction and

rates lower than 0.25 L biogas/L·d for slowly biodegradable fraction. These thresholds of biogas production rates were taken by convention after studying the derivatives curves of all wastes and applied to all substrates. Finally, both very and moderately readily fractions were merged into a single readily biodegradable fraction. Figure 4.2b depicts the derivative curve for mango, where the areas for readily and slowly biodegradable fractions could be differentiated at 0.25 (or 0.02 L biogas/g VSS·d, since all experiments were carried out with a biomass content of 13 g VSS/L).

4.3.3. FVW composition and calculation of recalcitrant fraction of fibre

TS and VS concentrations were measured for each waste as they are used to assess the quantities of waste to add in the batch reactors. The details of the composition of each fruit and vegetable used for modelling purposes were not measured experimentally. The average composition of FVW was obtained from APRIFEL, 2012 (www.aprifel.com). FVW are mainly made up of carbohydrates, proteins, lipids and fibres (Table 4.1). The average composition of pollock waste was obtained from Huss (1995). Pollock composition is similar to cod; it contains, on average, for 100 g of total mass: 19 g of proteins, 0.5 g of lipids and 80.5 g of water.

Fibres include hemicellulose and other non-biodegradable polymers of carbohydrates. Parts of these fibres were considered in the model as slowly biodegradable carbohydrates (X_{sch}) and the rest as non-biodegradable (X_i). The non-biodegradable fraction was calculated from the simulation of the batch experiment. All species were expressed in molar units to meet the model unit basis. Lipids and proteins species were the same as those considered in ADM1, carbohydrates were defined as a polymer of glucose, and fibres as hemicelluloses. In the case of solid fatty wastes from vegetable oil refining process, a complete characterisation of LCFA was carried out and an average LCFA composition was calculated for the model. LCFA of these fatty wastes are mainly made of C18:1, C18:2, C18:0 and C16:0 (Table 4.3).

Table 4.3. LCFA characterisation of fatty wastes. (Highlighted in bold the most abundant fatty acids of each waste).

		(Dc1)	**(Dc2)**	**(WE)**	**(Ts1)**	**(Ts2)**
LCFA	No. C	%	%	%	%	%
Caproic	6:0	0	0	0	0	0
Caprylic	8:0	0	0	0	0	0
Capric	10:0	0.1	0	0	0	0
Lauric	12:0	0.5	0	0	0	0
Myristic	14:0	1.3	0.2	0.1	0	0
	15:0	0.1	0	0	0	0
Palmitic	**16:0**	**44.8**	**5.1**	**6.7**	**6.9**	**4.7**
	16:1	0.2	0	0.1	0.3	0.1
Margaric	17:0	0	0.1	0.1	0	0.1
	17:1	0	0	0	0	0
Stearic	**18:0**	**4.3**	**9.8**	**4.1**	**1.5**	**3.0**
Oleic	**18:1**	**37.8**	**31.2**	**26.2**	**57.3**	**79.9**
Linoleic	**18:2**	**10.1**	**34**	**60.3**	**24.7**	**9.3**
	18:3	0	0.5	0.1	8.5	0.1
Arachidic	20:0	0.1	0.3	0.3	0	0.4
	20:1	0.3	0.2	0.2	0.7	0.3
Behenic	22:0	0.1	1.7	0.8	0	1.1
	22:1	0.1	0	0	0	0
Lignoceric	24:0	0.1	0.5	0.3	0	0.4
	24:1	0	0	0	0	0
Non-identified		0.1	16.4	0.7	0.1	0.6

To assess the recalcitrant fibre fraction, different recalcitrant contents were considered together with high kinetic values for dis- integration and hydrolysis until the simulated final biogas volume met the experimental value (Figure 4.3a). Once the non-biodegradable fraction was adjusted, the kinetic parameters for disintegration and hydrolysis were calibrated to fit experimental batches (Figure 4.3b).

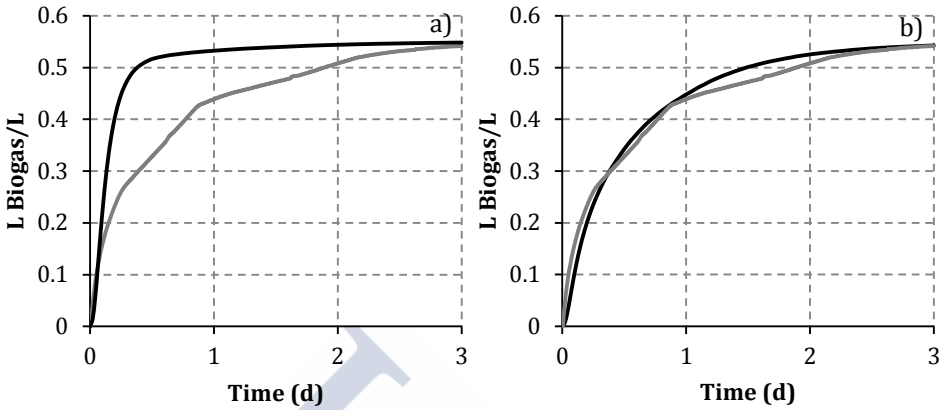

Figure 4.3. Experimental and simulated batch experiment of mango, (a) using overestimated disintegration and hydrolysis parameters to adjust the slowly and non-biodegradable content of fibres and to ensure the final accumulated biogas volume is achieved, b) after kinetic parameter calibration. (▬) Simulated results; (▬) Experimental results.

4.3.4. Fractionation and kinetic parameters estimation with ADM1-based model

Experimental and simulation results from batch assays

The disintegration and hydrolysis kinetic parameters of each residue were determined from the last batch of a series of 6–8 successive batches applied in the same reactor. This strategy was followed to ensure the biomass was adapted to that particular residue to eventually determine the actual disintegration and hydrolysis kinetics. The proposed ADM1-based AcoD model was used to obtain these kinetic parameters. A series of 6 batches for each residue was simulated to obtain the last batch for kinetic parameters estimation, as it was conceived experimentally. Before starting the simulations, the initial concentrations of the biomass groups were determined by running a long simulation (250 days) with each residue, to establish the initial biomass conditions of the reactor in the model. The hydrolysis kinetic parameters of fast biodegradable carbohydrates, proteins and lipids (k_{hyd_Xfch}, k_{hyd_Xpr}, k_{hyd_Xli}, respectively) were set to the default values that ADM1 suggested for solid wastes at mesophilic conditions (Batstone et al., 2002). Only the hydrolysis parameter of lipids was modified by calibrating batch assays of several fatty wastes streams from a vegetable oil refining

process, and a new hydrolysis parameter for slowly biodegradable carbohydrates (k_{hyd_Xsch}) was calibrated from FVW batch assays (see Table 4.4).

Table 4.4. Disintegration and hydrolysis kinetic parameters applied in the model (k_{hyd_Xfch} and k_{hyd_Xpr} taken from Batstone et al. (2002); the rest of the parameters, calibrated with the batch experiments).

Substrate	k_{dis_XcS} (d^{-1})	k_{dis_XcR} (d^{-1})	k_{hyd_Xsch} (d^{-1})	k_{hyd_Xfch} (d^{-1})	k_{hyd_Xpr} (d^{-1})	k_{hyd_Xli} (d^{-1})
FVW	1.7	60	1.0	10	10	1.2
Fish waste	1.4					

Simulated and experimental batches of all FVW, pollock and fatty wastes are presented in Figure 4.4. The model was able to fit the experimental batches, except for potato, where the model overestimated the biogas accumulation in the initial stage of the batch. Consequently, the hypothesis that disintegration controlled the overall reaction might be not true for potato. The simulated batch results fitted to a large extent the experimental results in terms of biogas accumulation curve, $\%CH_4$ and gas yield. Methane yield for FVW was in the range of values (around 300–400 mL/gVS) found in the literature (Bouallagui et al., 2005). Although simulated $\%CH_4$ results seemed to reach lower values than those in experimental assays, simulated results were considered acceptable taking into consideration that the characterisation of each waste was taken on average from literature and that experimental $\%CH_4$ was measured only once (on the total volume of biogas produced and stored in a bag) at the end of each week.

Readily- and slowly-biodegradable fractionation

Table 4.5 shows the readily fraction values of all substrates determined with the derivatives curves of the cumulative biogas production from the experimental batch assays. This table also presents the non-biodegradable fraction of fibres (inerts) calibrated from the batch assays, and the comparison between experimental and simulated results for $\%CH_4$, gas yield and methane yield. The f_R calculated with the experimental curves were a good approximation to fit experimental and simulated results, according to the similar results obtained in Figure 4.4 (split in Figures 4.4a and 4.4b) for most of the substrates. The fractionation of readily- and slowly-biodegradable organic matter and the consideration of disintegration as the rate-limiting step of the overall kinetics resulted as a good approach for solid FVW and fish waste.

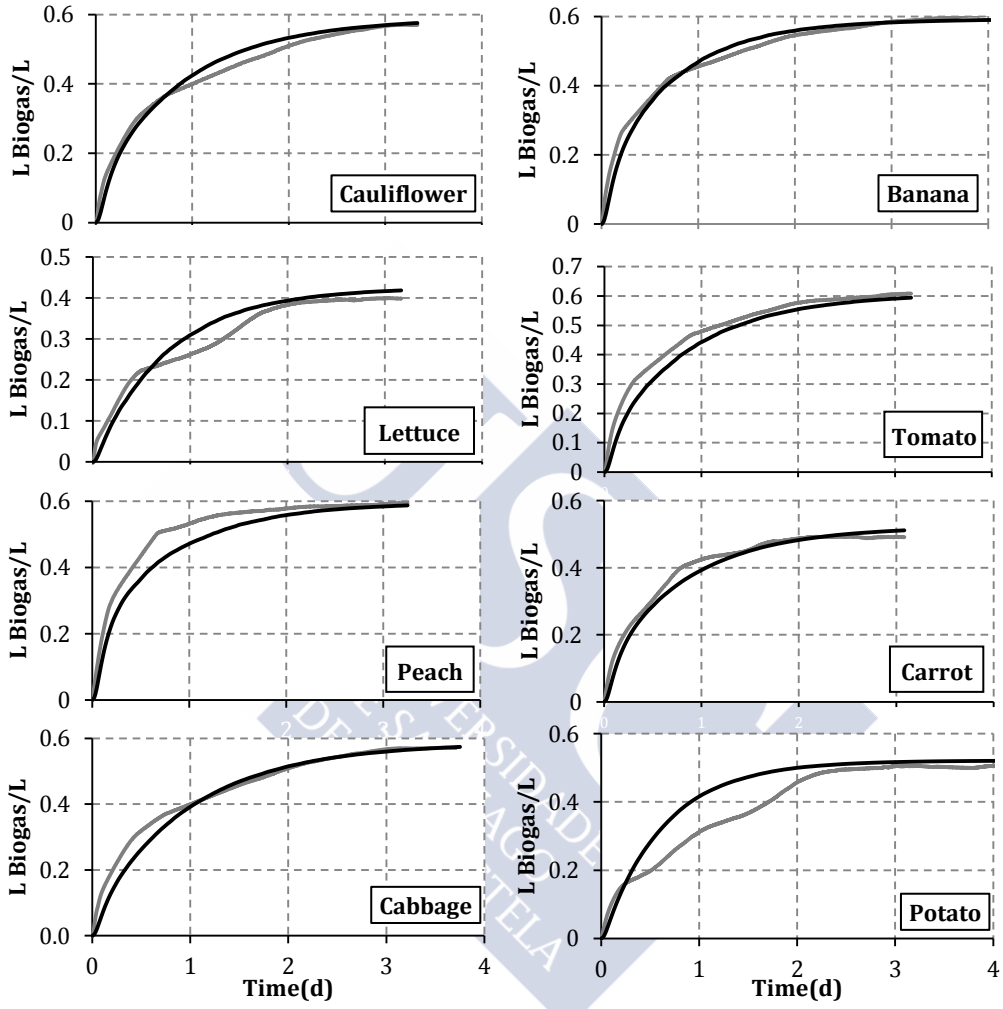

Figure 4.4.a. Simulated and experimental batch results for FVW. (▬) Simulated results; (▬) Experimental results.

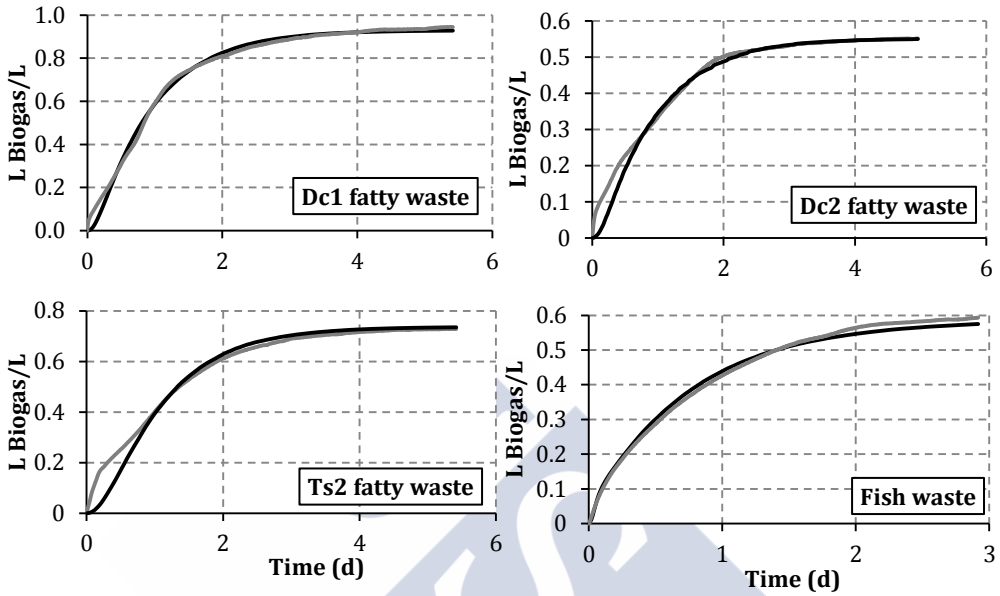

Figure 4.4.b. Simulated and experimental batch results for fatty wastes (Dc1, Dc2 and Ts2) and the fish waste. (▬) Simulated results; (▬) Experimental results.

Table 4.5. Batch experiments. Comparison between experimental and simulation results on % CH_4, gas yield (Gas Y) and methane yield (CH_4 Y).

Substrate	f_R	Inerts[b] (%)	%CH_4 Exp	%CH_4 Sim	Error (%)	Gas Y Exp	Gas Y Sim	Error (%)	CH_4 Y Exp	CH_4 Y Sim	Error (%)
Cauliflower	0.37	10	64.8	59.6	8.0	570	571	0.2	369	340	7.8
Banana	0.39	0	NA	57.4	-	619	593	4.3	-	340	-
Lettuce	0.22	100	66.0	64.6	2.1	399	419	5.0	263	270	2.8
Tomato	0.41	5	63.7	59.0	7.4	608	594	2.3	387	350	9.5
Peach	0.52	5	65.8	57.3	12.9	594	587	1.3	391	337	13.9
Carrot	0.41	55	65.0	58.5	10.0	491	512	4.1	319	299	6.3
Cabbage	0.32	10	60.4	59.9	0.8	575	574	0.1	347	344	0.9
Potato	0.19	100	63.5	57.9	8.8	507	520	2.6	322	301	6.4
Mango	0.41	60	55.2	57.2	3.6	541	543	0.2	299	310	3.8
Dc1[a]		29	69.4	73.9	6.5	944	928	1.8	655	686	4.6
Dc2[a]		57	72.7	72.8	0.1	551	550	0.3	401	400	0.2
WE[a]		23	68.3	73.9	8.2	996	1001	0.5	680	740	8.8
Ts1[a]		34	66.2	74.0	11.8	862	855	0.9	571	632	10.8
Ts2[a]		43	66.3	73.9	11.5	728	735	0.9	483	543	12.6
Fish waste	0.29	0	72.0	71.6	0.6	593	576	3.0	427	412	3.5

[a] Dc1, Dc2, WE, Ts1 and Ts2 stand for different fatty wastes.

[b] Inerts describes the non-biodegradable fraction of fibres in FVW, and the non-biodegradable fraction of lipids in the wastes Dc1, Dc2, WE, Ts1, Ts2.

Units: [Gas Yield] = mL gas/gVS ; [CH_4 Yield] = mL CH_4/gVS.

Disintegration and hydrolysis kinetic parameters

The calibration process to estimate the kinetic parameters followed the next procedure: (1) calibrate the hydrolysis kinetic parameter of lipids (k_{hyd_Xli}) from the experimental batch tests of lipid wastes (almost 100% lipid content) as sole estimated parameter; (2) calibrate the hydrolysis kinetic coefficient of proteins (k_{hyd_Xpr}) from the batch test of fish waste (protein content com- prises 97% of its organic content) as sole estimated parameter and use k_{hyd_Xli} already calibrated with lipid waste to hydrolyse the lipid content (3% of organic content) of the fish waste. In this case, the default k_{hyd_Xpr} value of ADM1 became a good estimation; (3) the hydrolysis kinetic parameter of fast hydrolysed carbohydrates (k_{hyd_Xfch}) was set equal to the default ADM1 value for carbohydrates; (4) the hydrolysis kinetic parameter of slowly hydrolysed carbohydrates (k_{hyd_Xsch}) was set to a constant value for all FVW batch assays; (5)

disintegration kinetic parameter of readily biodegradable fraction (k_{dis_XcR}) was estimated and set to a constant value for all FVW batch assays; (6) disintegration kinetic parameter of slowly biodegradable fraction (k_{dis_XcS}) was estimated and set to a constant value for each particular substrate. This coefficient was particular for each substrate and supposed that the dis- integration of the slowly biodegradable fraction was the rate- limiting of the overall process. In this sense, as it was expected, fish waste and FVW had different values of k_{dis_XcS}. The estimated kinetic parameters mentioned in (4), (5) and (6) were tested simultaneously, as there was no manner to estimate them individually. The disintegration kinetic parameters for both readily and slowly biodegradable fractions were calibrated in the model with widely different values. The estimated kinetic parameter for the slowly fraction was much lower than the hydrolysis kinetic values for carbohydrates or proteins, being this disintegration the rate- limiting kinetics of process. If the disintegration rate is lower than hydrolysis of carbohydrates, proteins and lipids, the influence of hydrolysis rates may be neglected and the lumped disintegration and hydrolysis effect can be explained only by disintegration (Vavilin et al., 2008). On average, 65% of the organic matter of FVW was slowly biodegradable, disintegration of the slowly fraction was the rate-limiting stage of the overall process for FVW, as well as for fish waste, where 87% of the organic content was slowly biodegradable.

Vavilin et al. (2008) reviewed the hydrolysis kinetics coefficients for carbohydrates, proteins and lipids among other complex substrates encountered in the literature and stated that the different values obtained can be explained by different experimental conditions, different hydrolytic biomass to substrate ratios and the lumped effect of disintegration and hydrolysis. Kinetic coefficients for disintegration–hydrolysis of carbohydrates and proteins are in the range of 0.5–2.0 d^{-1} and 0.25–0.8 d^{-1}, respectively, and lipids between 0.1 and 0.76 d^{-1} (Garcia-Heras, 2003; Masse et al., 2002; Shimizu et al., 1993). In this work, the kinetic parameter value for slowly biodegradable fraction of FVW was calibrated to 1.7 d^{-1}. Taking into account that the lumped effect of disintegration and hydrolysis can be explained by the disintegration and that most of the organic content of the FVW are carbohydrates, it is assumed that the kinetic parameter estimation was in agreement with the reference values. In the case of FVW, a 35% of the organic matter was considered as readily biodegradable; therefore, the overall effect of both disintegrations would result in a higher value than those in the literature.

In the case of fish waste (containing mainly proteins) and fatty wastes, the combined disintegration and hydrolysis kinetics would be given by the calibrated disintegration and hydrolysis kinetics coefficients of 1.4 d^{-1} and 1.2 d^{-1}, respectively, which were again higher than those reviewed in the literature. Finally, a new hydrolysis kinetic coefficient for slowly biodegradable carbohydrates (Xsch) was estimated at 1.0 d^{-1} and validated for all FVW. These higher estimated kinetic coefficients with respect to those found in the literature were considered as realistic values since: (i) the biomass-substrate ratio applied to the system was very high (the experiments were carried out with 13 g VSS/L of inoculum, and 1 g VS/L of waste), (ii) only the last batch of a series of batches was taken for kinetic parameters estimation when bio- mass was adapted to the substrate, (iii) the inoculum coming from an UASB reactor treating wastewater from sugar industry had a high specific activity.

4.3.5. Validation in semi-continuous AcoD

In order to validate the ADM1-based AcoD model for solid wastes, with the assumptions made for decoupled disintegration for readily- and slowly-biodegradable fractions, and to validate the calibrated kinetic parameters determined from batch assays (and fractions readily-, slowly-biodegradable graphically calculated from batch tests), a semi-continuous co-digestion experiment treating simultaneously 5 FVW (banana, apple, lettuce, carrot and potato) and fed 5 times a week (4 times occasionally) was carried out during 15 weeks. All calibrated parameters from batch assays were used and the rest of ADM1 parameters remained at their default values. Each week of operation was simulated separately but taking the last conditions of one week as the initial conditions for the next one. The reason to simulate the weeks separately was due to the experimental operation, where every week a bag collected the gas produced during the week to measure the average biogas composition and then replaced with a new bag for the next week, initialising the gas counter to zero. Figures 4.5 and 4.6 and Table 4.6 depict a comparison between the experimental and simulated results.

Figures 4.5 and 4.6 demonstrate that the model was able to predict to a large extent the experimental results in terms of biogas production, volatile solids content, pH and alkalinity in a semi-continuous co-digestion operation. In addition, VFA concentration in both experiment and simulation were negligible and the biogas composition around 55% of CH_4.

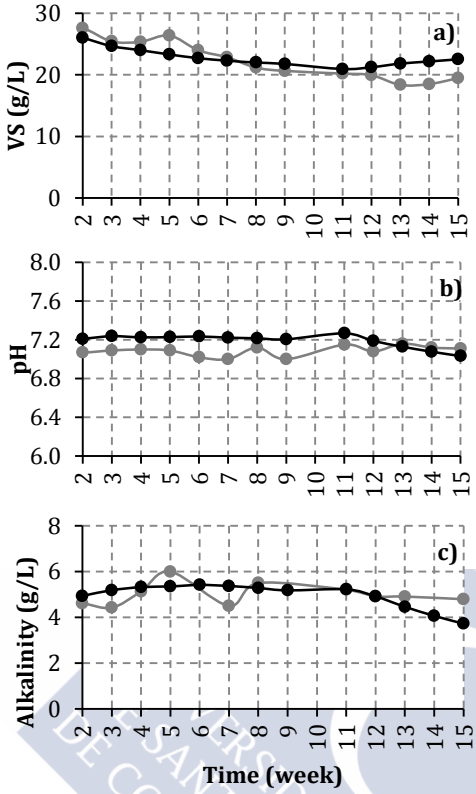

Figure 4.5. Weekly average values for simulated and experimental results for semi-continuous co-digestion experiment: at OLR of 2.0 g VS/L d (weeks 2 to 10); 3.8 g VS/L·d (weeks 12 to 14) and 4.7 g VS/L·d (week 15). a) Volatile solids; b) pH; c) Alkalinity. ( ) Simulated results; ( ) Experimental results.

Table 4.6. Semi-continuous AcoD experiment. Comparison between experimental and simulation results on %CH_4, biogas yield (Gas Y) and methane yield (CH_4 Y).

WeeK	Feed (gVS/d)	No. feeds	%CH_4 Exp	%CH_4 Sim	Error (%)	Gas Y Exp	Gas Y Sim	Error (%)	CH_4 Y Exp	CH_4 Y Sim	Error (%)
2	20.0	5	55.71	54.98	1.43	787	785	0.25	438	432	1.56
3	20.0	5	58.75	55.18	6.20	787	771	2.03	462	425	7.99
4	20.0	4	58.50	54.73	6.38	744	744	0.00	435	407	6.44
5	20.0	5	55.00	54.22	1.36	739	741	0.27	406	402	1.15
6	20.0	4	55.00	54.53	1.04	750	746	0.53	413	406	1.54
7	20.0	5	55.00	53.96	2.02	716	718	0.28	394	387	1.62
8	20.0	5	54.87	53.78	1.87	703	713	1.42	386	383	0.59
9	20.0	5	54.33	53.73	1.15	707	707	0.00	384	380	1.09
10	20.0	5	55.00	53.56	0.18	701	702	0.14	386	376	2.48
11	-	-	55.00	58.48	1.78	-	-	-	-	-	-
12	37.9	4	52.33	54.05	3.27	739	667	9.74	387	361	6.77
13	37.9	5	56.71	53.32	5.91	729	669	8.23	413	357	13.73
14	37.9	5	59.38	53.25	10.32	734	672	8.45	436	358	17.90
15	47.3	4	59.38	53.39	10.04	703	668	4.98	417	357	14.57

Units: [Gas Yield] = mL gas/gVS ; [CH_4 Yield] = mL CH_4/gVS.

Figures 4.6a and 4.6b show that the simulated biogas accumulation had the same behaviour than the experimental biogas. The OLR was 2.0 g VS/L·d for the first 10 weeks (weeks 3, 5 and 9, were not plot- ted but the same good fit was achieved) and week 11 remained as idle time where no feed was loaded to the reactor. When OLR changed to 3.8 g VS/L·d (week 12–14) or 4.7 g VS/L·d (week 15), the model was not able to accurately simulate the last periods of each corresponding week and under predicted the final biogas accumulation, above all in weeks 13 and 14. These differences on simulated and experimental biogas accumulation can be explained with two assumptions: (1) part of the fibres (hemicelluloses and other slowly biodegradable carbohydrates) calibrated in the batch assays as non-biodegradable fraction, X_i, might have been degraded in the long-term, reducing the volatile solids content in the reactor. This would be corroborated in Figure 4.5a, where the model overestimated the volatile solids con- centration that included the non-biodegradable fraction. If a higher proportion of fibres were considered in the model as slowly biodegradable instead of recalcitrant, more organic matter would have been degraded, volatile solid content would have decreased and consequently more biogas could have been produced; (2) the experimental composition fed to the system could have slightly changed in the last

weeks (13, 14 and 15) in respect to the first eleven weeks, and the simulated average feed composition taken from APRIFEL, 2012 (www.aprifel.com) would have been not representative of the real composition. This idea is supported by the fact that the total solids of the feed measured experimentally changed from 12.26% in the first eleven weeks to 13.35% in the last 3 weeks and that the experimental methane yield of week 13, 14 and 15 was significantly higher than that of the end of the first period at OLR of 2 g VS/L·d. However, due to the good results obtained in the first eleven weeks, and the small differences found in the last weeks of operation, simulated results were considered acceptable to a large extent to validate the model in semi-continuous mode.

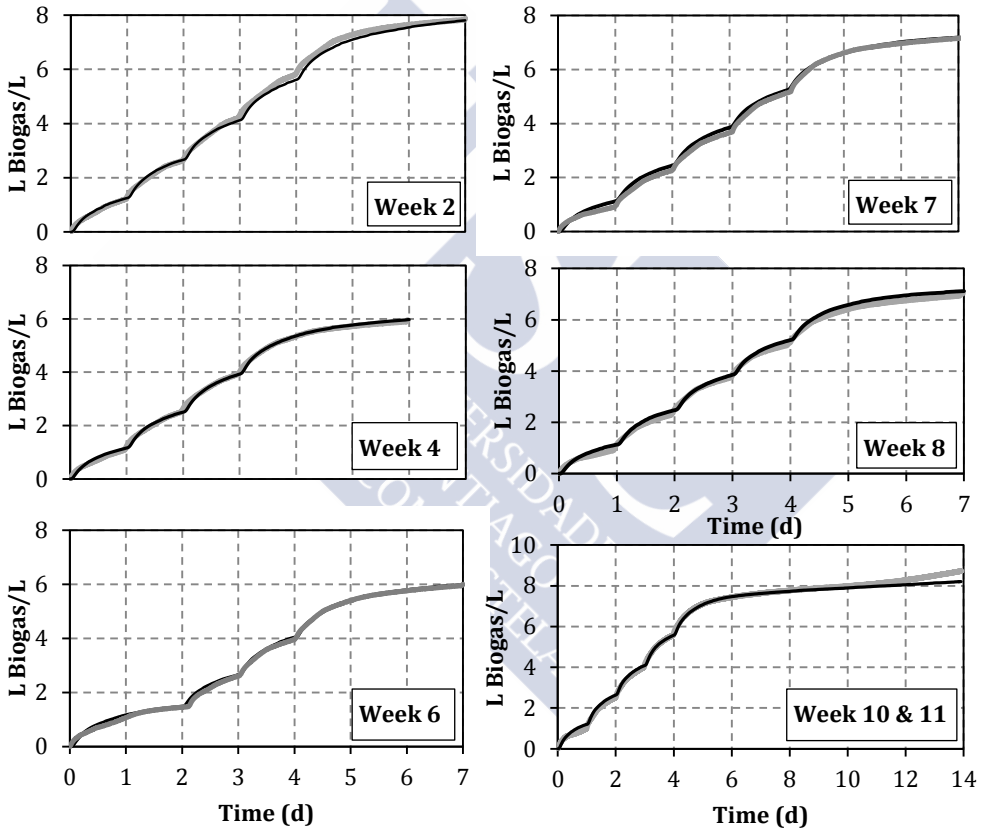

Figure 4.6.a. Simulated and experimental biogas accumulation in weeks 2, 4, 6, 7, 8, 10 & 11, for the semi-continuous co-digestion experiment. (▬) Simulated results; (▬) Experimental results.

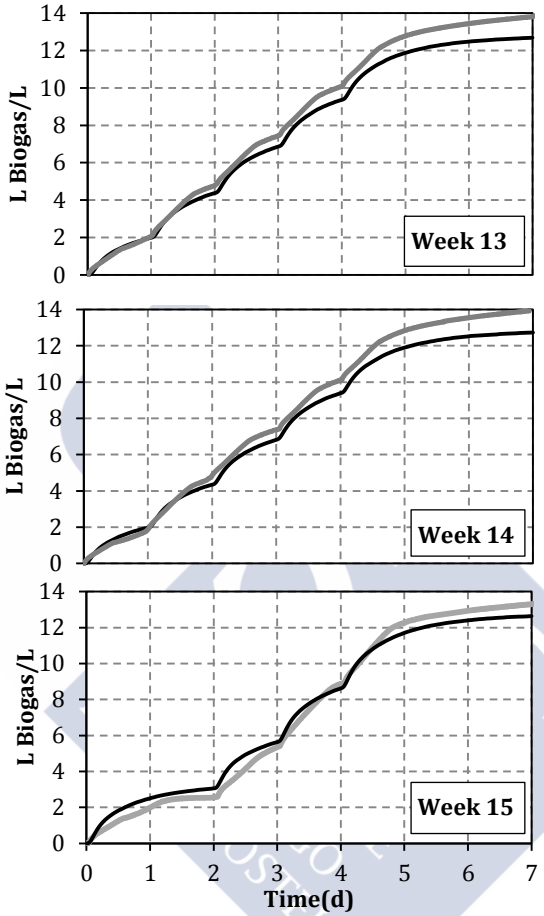

Figure 4.6.b. Simulated and experimental biogas accumulation in weeks 13,14 & 15, for the semi-continuous co-digestion experiment. (▬) Simulated results; (▬) Experimental results.

No parameter was modified for the semi-continuous AcoD process and all parameters remained at their calibrated values from batch assays. The kinetic parameters coefficients for the disintegration of all residues, the fractions readily and slowly biodegradable as well as the non-biodegradable fraction of fibres were found

accurately determined from batch experiments. The assumption of decoupled disintegration for readily and slowly biodegradable fractions was successfully tested in both batch and semi-continuous experiments and introduced a novelty to estimate kinetic coefficients and classify the fractions of any solid waste based on their kinetics, whenever disintegration is the rate-limiting stage of the overall kinetics. In addition, all kinetics parameters and fractions were determined from experimental batch assays with no further calibration requirements.

The ADM1-based AcoD model (García-Gen et al., 2013) was successfully validated in both batch and semi-continuous mode for solid wastes and it was demonstrated as a consistent tool to simulate co-digestion processes. The model will be used in further experiments to evaluate the feasibility of different blends by co- digestion, predict the biogas production when mixing readily and slowly biodegradable solid wastes, and develop control strategies to optimise the blends and enhance the performance of digesters under co-digestion.

4.4. Conclusions

The fractionated disintegration kinetics proposed for solid substrates provided adequate description of batch experiments. The parameters obtained from single substrate batches provided good simulation results in semi-continuous co-digestion of multiple substrates. The novel protocol of experimental batches is demonstrated as an appropriate strategy for the estimation of kinetic parameters and a method to incorporate kinetic information from batch tests into an AcoD model. The ADM1-based AcoD model was validated as a reliable tool to simulate co-digestion processes of solid wastes. The results suggest that the model will be useful in future works to test the feasibility of different mixes of residues and to develop control strategies to optimise the blends in order to enhance the performance of the digesters.

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Chapter 5. Optimisation of substrate blends in anaerobic co-digestion using adaptive linear programming

Contents

Abstract

Anaerobic co-digestion of multiple substrates has the potential to enhance biogas productivity by making use of the complementary characteristics of different substrates. A blending strategy based on a linear programming optimisation method is proposed aiming at maximising COD conversion into methane, but simultaneously maintaining a digestate and biogas quality. The method incorporates experimental and heuristic information to define the objective function and the linear restrictions. The active constraints are continuously adapted (by relaxing the restriction boundaries) such that further optimisations in terms of methane productivity can be achieved. The feasibility of the blends calculated with this methodology was previously tested and accurately predicted with an ADM1-based co-digestion model. This was validated in a continuously operated pilot plant, treating for several months different mixtures of glycerine, gelatine and pig manure at organic loading rates from 1.50 to 4.93 gCOD/L·d and hydraulic retention times between 32 – 40 days at mesophilic conditions.

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5.1. Introduction

Anaerobic co-digestion (AcoD) stands for the simultaneous digestion of two or more substrates and its benefits rely on the enhanced performance of the process compared to anaerobic digestion (AD) due to potential synergies among the co-substrates. Thanks to their complementary characteristics, co-digestion can increase biogas production (Mata-Alvarez et al., 2011), and achieve other environmental, technological and economic advantages: a more efficient use of equipment and cost-sharing by processing multiple waste streams in a single facility (Alatriste-Mondragón et al., 2006), or lower greenhouse gas emissions and climate change impact in comparison to composting or anaerobic mono-digestion (Krupp et al., 2005).

As anaerobic digestion involves complex biological pathways, the efficiency of the overall process can be affected by different factors such as composition of substrates, temperature, pH, moisture, carbon to nitrogen ratio (C/N), organic loading rate (OLR) or microbial consortia (Khalid et al., 2011). Different studies suggest thresholds for the key parameters of AD in order to guarantee the stability of the operation, for instance, in terms of C/N ratio (Burton and Turner, 2003; Bouallagui et al., 2009), lipid concentration (Neves et al., 2009; Palatsi et al., 2009), moisture (Mata-Alvarez et al., 2000), alkalinity (Cuetos et al., 2008), salinity (Jard et al., 2012), volatile fatty acids (VFA) concentration (Ahring et al., 1995; Nielsen et al., 2007) or sulphide in biogas (Peu et al., 2012).

Selecting the blend of substrates leading to a stable AcoD operation is not trivial as it requires knowledge and expertise on the process. The proportions of the substrates should be adequately balanced to ensure the key parameters of AD are within the ranges for stable operations. According to this, different optimisation methods can be found in the literature trying to achieve optimum blends. The conventional method consists of lab-scale batch assays with different proportions of co-substrates to evaluate the digestibility and methane potential of the different mixtures (Alatriste-Mondragón et al., 2006). Other optimisation methods include: (i) neural networks to increase the biogas production of full-scale digesters (Abu Qdais et al., 2010; Thorin et al., 2012), (ii) response surface methodologies to optimise feeding composition and C/N ratio (Wang et al., 2012), (iii) simplex-centroid mixture design and central composite design to optimise the feeding with higher methane potential (Wang et al., 2013), and (iv) linear programming approaches to obtain the optimum blend of co-substrates that maximises methane production (Alvarez et al., 2010).

Any optimum blends obtained with the different methods should be validated in continuous experiments, to confirm the long term feasibility of those mixtures. In this sense, and considering that continuous experiments are very time-consuming, models appear as a very useful tool to assess promptly the viability of different blends in continuous AcoD operations. The IWA Anaerobic Digestion Model No.1 (ADM1) (Batstone et al., 2002), which describes the main processes involved in anaerobic digestion (disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis), has been widely used as standard model for AD systems and also adapted to simulate continuous AcoD processes (García-Gen et al., 2013; Mata-Alvarez et al., 2014).

The main purpose of this work is to develop an optimisation method based on linear programming for the feeding of AcoD systems in order to obtain higher methane productivities, achieve higher COD removal efficiencies, meet the required biogas quality and ensure the stability of the operation.

The optimum blends calculated with the proposed methodology were initially tested with the ADM1-based AcoD model (García-Gen et al., 2013) and then validated with a continuous AcoD experiment performed at pilot scale, treating different blends of three substrates (glycerine waste, gelatine and pig manure) at different organic loading rates (OLR) and hydraulic retention times (HRT) at mesophilic conditions.

5.2. Materials and Methods

5.2.1. Linear programming optimisation method

To set up a linear programming problem, an objective function and a set of linear restrictions should be defined. In this study, both objective function and restrictions are calculated based on the physicochemical characteristics and the biochemical methane potential (BMP) of each substrate. The objective function is the methane production expected in a continuous AcoD system treating a mixture of substrates and the linear restrictions include the typical characteristics of AD systems (defined based on heuristic knowledge). Finally, the set of equations and the values of the restrictions can be adapted to each particular case (e.g. end use of the biogas, or characteristics of the soil where the digestate is applied). The methodology not only solves the proportions of the substrates in the blend (Alvarez et al., 2010) but also provides the HRT, a key operational parameter for continuous systems.

The method was implemented in MATLAB and makes use of two default functions, '*linprog'* to calculate the blend of substrates maximising the objective function at each HRT applied, and '*fminbnd*' to find the best HRT that optimises the methane productivity. Moreover, the linear programming optimisation informs about the restrictions that are actively limiting and that could be modified to move the operation towards a new potential optimum with a higher methane production. '*Linprog'* function returns the values of the Lagrange multipliers related to each restriction (different from 0 when they are active) that can be used to estimate what constraint mostly limits the value of the objective function. For instance, for a system with two active restrictions, the gradient of the objective function can be written as $\nabla f=\lambda\nabla g+\mu\nabla h$, where vector f stands for the objective function; vectors g and h, refer to the active restrictions; and λ, μ are the Lagrange multipliers related to each restriction. In the proposed optimisation method, the information of these multipliers will be used to assess the importance of each restriction in obtaining a new value of the objective function. The constraint with a higher Lagrange multiplier will be considered the most limiting restriction.

Objective function

The assembly of the objective function is presented in Figure 5.1. Experimental information from BMP assays is used together with substrate COD content to define the objective function, the methane production, expressed in OLR units (gCOD/L·d). The HRT of the system is calculated from the BMP tests of all substrates. This approach considers that the expected methanation of each individual substrate treated in a continuously-operated reactor working at a particular HRT would be similar to the methanation obtained in a BMP assay at a time equal to the HRT applied.

The method calculates the optimum blend and the value of the objective function at each time point of the batch tests with the '*linprog*' function. Then, '*fminbnd*' finds the time (HRT) at which the highest value of the objective function is obtained.

Therefore, the objective methane production depends on the volumetric fraction of each substrate in the blend (xi), on their total COD contents (CODt) and the percentages of methanation (pMet) from the BMP tests of all substrates at a time equal to the selected HRT. Finally, in order to express the productivity of methane in OLR units (gCOD/L·d), the equation is divided by the HRT, which it is the same value for all the substrates, so that the equation remains linear.

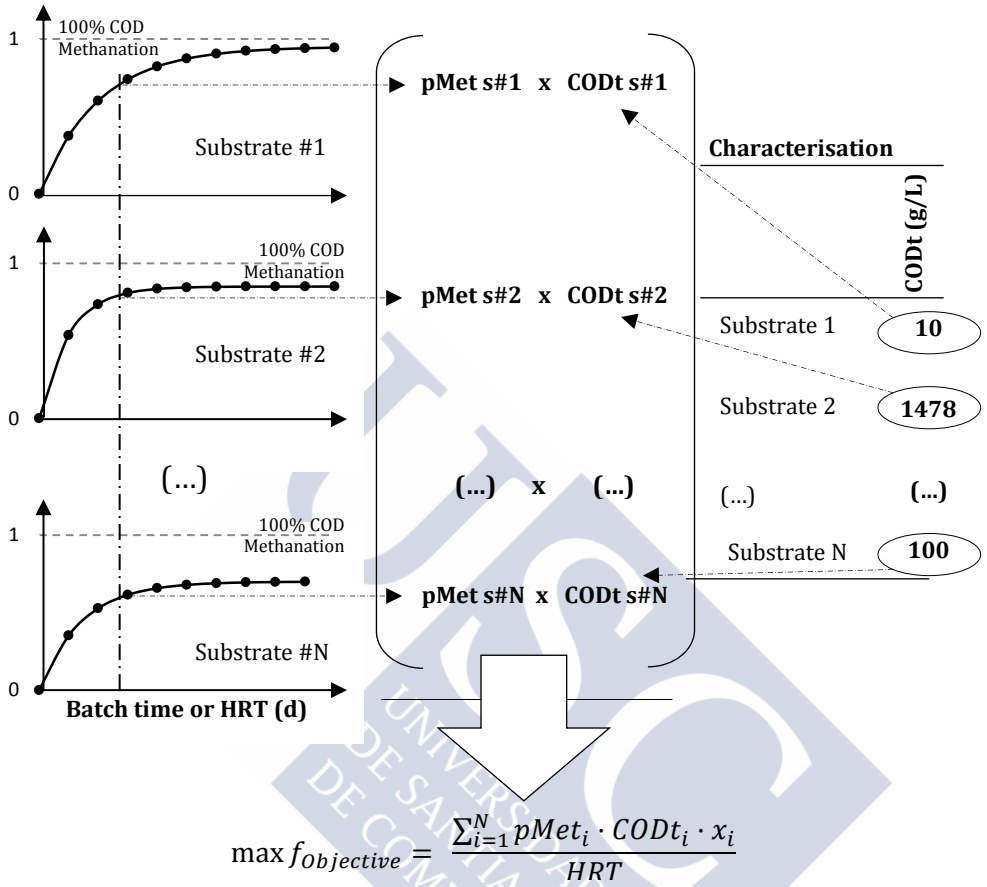

Figure 5.1. The objective function is evaluated for all HRT values and sets of substrate fractions in the blend (xi, %vol.). The sum of the terms is returned resulting from multiplication of the fraction of each substrate in the blend times its CODt (from the characterisation table) and times the percentage of expected methanogenic conversion (pMet) for the given HRT (taken from experimental batch BMP studies). The units of the objective function are $gCODCH_4/L{\cdot}d$.

Linear restrictions

The set of linear restrictions is established based on the knowledge of the AD process and are defined based on typically available substrate characteristics. As AcoD systems can be performed at different operating conditions (OLR, temperature), at different

stages (start-up, dynamic or steady-state operations), treating a wide variety of substrates or pursuing different objectives (end uses of the biogas and digestate), the set of restrictions applied to the linear programming problem should be appropriately selected according to the case.

Particularly in this study, maximum and minimum values for the following parameters were defined: (i) organic loading rate (OLR); (ii) Total Kjeldahl Nitrogen (TKN); (iii) moisture or liquid fraction; (iv) lipid content; (v) total alkalinity; salinity as (vi) Na^+ concentration and (vii) K^+ concentration; (viii) H_2S content in biogas; (ix) effluent COD content. Table 5.1 shows the intervals of all restrictions considered at the startup and they can be modified along the operation.

Table 5.1. Initial set of restrictions proposed in the linear programming optimisation method.

Linear restriction	Minimum	Maximum
OLR (g COD/L·d)	0	1.5
TKN (g N/L)	0.2	4.0
Liquid fraction (kg liquid/kg wet)	0.85	1.00
Lipids (g/L)	0	10
Alkalinity (g $CaCO_3$/L)	2	8
Na^+ (g/L)	0	3
K^+ (g/L)	0	3
Biogas quality (ppm H_2S)	0	10,000
Digestate quality (g COD/L)	0	6

The maximum OLR might appear as somehow restrictive but it was selected in accordance with the typical values used in the start-up stages of AD operations. The maximum TKN allowed in the blend is 4 g-N/L (Chen et al., 2008) in order to prevent inhibitory concentration of ammonia. With regard to liquid fraction restriction, a high liquid fraction was required to operate this particular pilot plant not designed to operate under dry digestion conditions. The maximum lipid concentration was set based on literature references (Neves et al., 2009; Palatsi et al., 2009) and a recommended alkalinity around 5 g/L was reported by Cuetos et al. (2008). Salt

concentrations in terms of Na^+ and K^+ should be between the ranges of 3-20 g/L and 0.25-12 g/L, respectively, to avoid inhibitions (Jard et al., 2012).

The OLR is calculated from the total COD and the HRT of the system, and the digestate quality from the total biodegradable COD of each substrate (equations 1 and 2, respectively):

$$OLR = CODt / HRT \quad \text{(expressed in gCOD/L·d)} \quad (1)$$

$$\text{Digestate quality} = CODt \cdot (pBiod - pMet) \quad \text{(expressed in gCOD/L)} \quad (2)$$

where pBiod is the maximum percentage of methanation (or % biodegradability) obtained in the BMP test; and pMet is the percentage of methanation achieved at the time point equal to the HRT applied to the system. Digestate quality was limited to 6 gCOD/L of biodegradable organic matter to ensure a maximum VFA around 3 or 4 gVFA/L, values that were found inhibitory (Nielsen et al., 2007) and that could limit the use of the digestate for agronomic purposes.

The quality of biogas was defined in terms of the maximum sulphide content (expressed in gH_2S/L) assuming that the S content characterised as SO_4^{-2} is fully converted into H_2S and then completely removed in the gas stream:

$$(x_1 \cdot [H_2S]_1 + x_2 \cdot [H_2S]_2 + x_3 \cdot [H_2S]_3) \cdot F_{feed} = F_{gas} \cdot [H_2S]_{gas} \quad (3)$$

where $F_{feed} = V/HRT$ is the inlet flow in L/d; F_{gas} is the biogas flow expected at the OLR used in the operation and roughly calculated as $F_{gas} = OLR \cdot V \cdot (0.38/0.70)$,where V is the digester volume (L), 0.38 stands for the ratio L CH_4/gCOD stoichiometrically obtained from complete oxidation of methane and 0.70 refers to the typical average methane composition (70%) in the biogas (expressed as %/100); x_i is the volumetric fraction of each substrate in the blend; and implicitly, the term $[H_2S] \cdot (F_{feed} / F_{gas})$ represents the biogas quality associated with each substrate. A maximum hydrogen sulphide content (up to1% vol.) in biogas was imposed to ensure further uses of biogas in electricity generators, although higher values could be applied; for instance, the direct use in microturbines can accept sulphide concentration up to 7% H_2S (Kolanowski, 2004).

The restrictions of gas and of digestate quality should be checked in the effluent streams along the operation; however, if the characterisation and BMP test of the substrates are accurate, in advance estimations of these characteristics can be obtained ahead of the operation.

5.2.2. ADM1-based AcoD Model

A continuous AcoD experiment treating different blends of substrates, calculated with the linear programming optimisation method, was simulated with the ADM1-based AcoD model developed by García-Gen et al. (2013) and built in MATLAB/Simulink. The model proposes a generalised methodology to incorporate new soluble fermentable substrates such as ethanol, glycerol or methanol, not originally included in ADM1, through glucose equivalent reactions and degraded by a generic group of fermenters (X_{fer}) instead of the original ADM1 sugar degraders. An additional term to modify the fermentation kinetics equations was included to account for the competition among multiple substrates to be degraded by the generic biomass group (X_{fer}). Following the ADM1 nomenclature (Batstone et al., 2002), the fermentation kinetics of each fermentable substrate is written as follows (equations 4 and 5):

$$\text{Uptake rate:} \quad \rho_j = k_{m,i} \cdot \frac{S_j}{K_S + S_j} \cdot X_{fer} \cdot \frac{S_j}{S_j + \sum_{i \neq j} S_i} \cdot I_2 \tag{4}$$

$$\text{Competitive term} = \frac{S_j}{S_j + \sum_{i \neq j} S_i} \tag{5}$$

where i, j = sugars, ethanol, glycerol, methanol, etc…

All ADM1 parameters were set at their default values and the kinetic parameters values for ethanol, glycerol and methanol were taken from literature. The kinetic parameters of ethanol, Monod maximum specific uptake rate (k_m) and half saturation value (K_s), were taken from Batstone et al. (2004), $k_{m,et}$ = 3 kgCOD/kgCOD·d and $K_{s,et}$ = 0.5 kgCOD/m^3; methanol kinetic parameters from Bhatti et al. (1996), $k_{m,met}$ = 3.0 gCOD/gVSS·d and $K_{s,met}$ = 1.03 kgCOD/m^3; and glycerol parameters from Fountoulakis et al. (2010), $k_{m,gly}$ = 0.149 kgCOD/kgCOD·h and $K_{s,gly}$ = 0.276 kgCOD/m^3 (and expressed in the model units, in molar basis). Biomass yields of X_{fer} on ethanol, glycerol and methanol (Y_{et}, Y_{gly}, Y_{met}) were calculated with the generalised Thermodynamic Electron Equivalent (TEEM) method described by Kleerebezem and van Loosdrecht (2010).

5.2.3. Experimental set-up for continuous experiment

The AcoD experiment was performed in a highly instrumented pilot plant consisting of a hybrid Upflow Anaerobic Sludge Blanket - Anaerobic Filter reactor (UASB-AF) of 1 m^3 of liquid volume (Ruiz, 2005). The reactor behaves as a quasi-complete mixed reactor

due to the high recycling flow applied to the system. The facility includes on-line measurement devices connected to the plant such as pH meter (Siemens, SIPAN pH/ORP 7MA 1010), gas flow meter (Brooks®, 5860E), continuous CH_4, CO_2, H_2S analyser (ABB, AO2020) and a hydrogen gas analyser (Sensotrans, Sensotox 420). In addition, a data acquisition programme developed in Visual Basic allowed for the monitoring and recording of the signals from the on-line sensors and analysers to the computer through a rack of PLCs (Siemens, series S7-200). In addition to the on-line measurements, offline analysis of NH_4^+, COD, alkalinity, VFA or pH were regularly taken to monitor the process. The characteristics of the different substrates treated in the pilot plant are summarised in Table 5.2.

Table 5.2. Characteristics of glycerine (from biodiesel residue), gelatine and soluble fraction of pig manure.

Parameter	Glycerine	Gelatine	Pig manure
Liquid fraction (%)	100	100	99
pH	10.4	6.5	7.8
Density (kg/L)	1.21	1.04	0.96
TS (g/L)	0	0	9.90
VS (g/L)	0	0	5.42
CODt (g/L)	1478	101	10.1
CODs (g/L)	1478	100	4.6
TKN (g N/L)	0	12.9	3.7
NH_4^+ (g N/L)	0	0	3.7
TA (g $CaCO_3$/L)	38.4	1.8	6.2
Na^+ (g/L)	1.63	1.06	0.17
K^+ (g/L)	18.88	0	0.51
Sulphate (g/L)	0.52	4.10	0.03
Proteins (g/L)	0	83.6	0
Lipids (g/L)	44.8	0	0
Carbohydrates (g/L)	450	0	9.49[a]
Glycerol (g/L)	474.8	0	0
Methanol (g/L)	193.9	0	0
Biodegradability (%)	90.6	77.7	79.3

[a] This concentration comprises both carbohydrates and VFA contents and amended to this value to meet CODt.

Before applying the optimisation method, the experiment started with the following operating conditions: OLR of 1.5 gCOD/L d, HRT of 20 days and initial biomass concentration in the reactor of 9.5 gVSS/L, treating a blend of wine wastewater and pig manure (84.5% of wine wastewater and 15.5% pig manure, % of COD; 53.9% and 46.1% in % volume, respectively) with a total alkalinity of 3 $gCaCO_3/L$. These operating conditions remained constant during 128 days (around 6 times the HRT) until a clear steady state was achieved. From day 129 till the end of the operation (day 250), the subsequent feedings (composed of glycerine waste, gelatine and pig manure) were calculated with the proposed optimisation method.

5.2.4. Analytical methods

Characteristics such as pH, COD, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), total alkalinity (TA) and partial alkalinity (PA) were performed following standard methods (APHA, 1998). Ammonium was measured with phenate method (APHA, 1998) with a spectrophotometer (Shimadzu UV-1603, UV–Visible) at 640 nm. VFA (acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric acids) were analysed by gas chromatography (HP 5890A), equipped with a flame ionisation detector connected to an automatic injector HP 7673A, using a glass column filled with Chromosorb WAW (mesh 100/120) impregnated with NPGA 25% and H_3PO_4 2%. The temperatures for injector, column and detector were 105 °C, 260 °C and 280 °C, respectively. Nitrogen was used as carrier gas and it was saturated with formic acid before the injector with a flow rate of 30 mL/min. Air and hydrogen were used as auxiliary gases with flow rates of 400 mL/min and 30 mL/min, respectively. Pivalic acid was used as external standard.

Sulphate (SO_4^{-2}) and cations Na^+ and K^+ were determined by ion chromatography with an Advanced Compact IC system (861, Metrohm) equipped with a CO_2 suppressor (MCS 853, Metrohm) and a sample processor (AG 838, Metrohm). SO_4^{-2} was determined following ASTM D4327-03 method (ASTM, 2003), using a Metrosep A column (250 x 4.0 mm) and a mobile phase (buffer) with 3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, and cations Na^+ , K^+ were determined following ASTM D6919-03 method (ASTM, 2003), using a Metrosep C3 column (250 x 4.0 mm) and nitric acid 3.5 mM as mobile phase. Glycerol concentration was determined by high performance liquid chromatography (Agilent HPLC 1100), using an Aminex HPX-87H column (300 x 7.8 mm) (Bio-Rad Laboratories, Richmond, CA), equipped with a pre-column Micro-Guard Cation H (30 x 4.6 mm) and coupled to an IR detector, using sulphuric acid (5 mM) as eluent

(Castellari et al., 2001). Methanol was determined by gas chromatography (Agilent GC 6850), equipped with a flame ionisation detector, using a DB-WAX column (30 m x 0.25 mm x 0.25 μm) (Agilent Technologies Inc.), using N_2 as gas carrier (Pereira et al., 1999). Ethanol concentration was measured from soluble COD of synthetic wine wastewater (prepared as diluted wine) assuming that the COD content stands for the ethanol concentration. Lipids were measured with Soxhlet method (APHA, 1998). Protein concentration was estimated by multiplying organic nitrogen (TKN minus NH_4^+) by 6.25 (Dintzis et al., 1988).

5.3. Results and Discussion

5.3.1. Experimental results

After the first 128 days of operation, the reactor achieved a steady state condition treating a blend of wine wastewater and pig manure at 1.5 gCOD/L·d and HRT of 20 days. Then, from day 129 till the end of the operation (day 250), the feedings and HRT were calculated by means of linear programming. The proposed optimisation method was successfully validated in the pilot plant for 120 days at different operating conditions.

Along the experiment, the OLR increased from 1.50 to 4.93 gCOD/L·d, and HRT ranging between 32 and 40 days. Figure 5.2 illustrates the different operating conditions applied to the digester, in which for every period of time of ¼ HRT, a new blend and HRT were calculated.

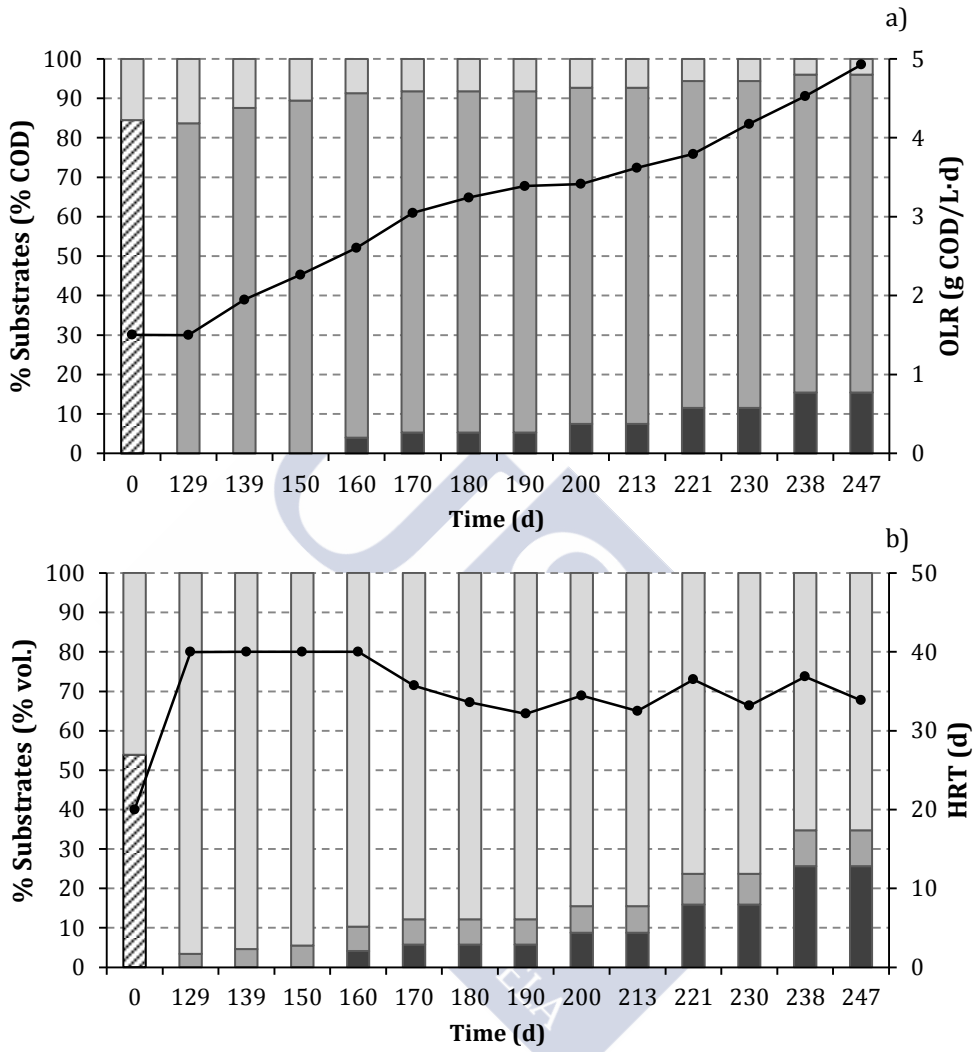

Figure 5.2. Operating conditions and blends of substrates applied to the pilot plant during the entire operation. a) OLR and blend of substrates in % COD, b) HRT and blend of substrates in % vol. (▬) Gelatine, (▬) Glycerine, (▬) Pig manure, (▨) Wine wastewater, (–•–) OLR and HRT.

The limits of the OLR restriction (initially set to 1.5 to start the operation) were modified during the operation based on the active restrictions provided by the linear programming solution. The active linear restrictions found along the operation have always been the OLR and TKN. By modifying the boundaries of these two restrictions, the optimisation method was able to calculate a new blend and operation conditions (OLR and HRT) for the next period, achieving a new optimum operation point. In this study, even though more than one restriction can be active, only the most limiting (that with higher Lagrange multiplier) was modified per period. Modifying the most active restriction implied obtaining higher values of the objective function and therefore higher methane productivities. The new limits of the restrictions increased between 5 to 30% of the span (higher limit minus lower limit of the restriction) and most of the time around 20% of the span. Table 5.3 summarises the main operating conditions of each blend derived from the optimisation method.

Table 5.3. Operating conditions of each blend of substrates calculated with the proposed optimisation method. The limiting restrictions and the increase factor of the maximum boundary of the restriction in the next period are shown.

Blend	Day	OLR	HRT	COD	C/N ratio	C/S ratio	Limiting restriction	Increase factor of restriction
1	129	1.50	40.0	60	16.7	1295	OLR	0.30
2	139	1.95	40.0	78	22.0	1494	OLR	0.16
3	150	2.26	40.0	91	25.8	1608	OLR	0.15
4	160	2.60	40.0	104	27.0	458	OLR	0.17
5	170	3.05	35.7	109	27.2	370	OLR	0.06
6	180	3.24	33.6	109	27.2	370	OLR	0.05
7	190	3.39	32.1	109	27.2	370	TKN	0.07
8	200	3.41	34.4	118	27.6	282	OLR	0.11
9	213	3.62	32.5	118	27.6	282	TKN	0.15
10	221	3.79	36.5	139	28.4	194	OLR	0.19
11	230	4.17	33.2	139	28.4	194	TKN	0.18
12	238	4.53	36.9	167	29.2	149	OLR	0.18
13	247	4.93	33.9	167	29.2	149	TKN	0.18

Units: [OLR] = gCOD/L·d; [HRT] = d; [COD] = gCOD/L; [C/N] = gCOD/gN; [C/S] = gCOD/gSO_4^{-2}

The C/N ratio of each blend fell within the optimum range (20 to 70) considered for AD process (Burton and Turner, 2003). Carbon to sulphur ratio (C/S) measured as COD/SO_4^{-2} ratio remained very high, far from the ratios of 3 to 5.6 where sulphate reducing bacteria (SRB) compete against acetogens, or far from 1.7 to 2.7 where SRB compete with acetoclastic methanogens (Chen et al., 2008).

The experimental results showed that COD removal efficiencies around 90% were achieved (%COD removal = (influent CODt – effluent CODt) ·100/ influent CODt) during the operation together with a high stability of the operation, where no VFA accumulation was observed. Figure 5.3 shows the experimental results of methane flow and OLR (both expressed in gCOD/L·d) as well as the influent and effluent CODt, where most of the COD content was converted into methane.

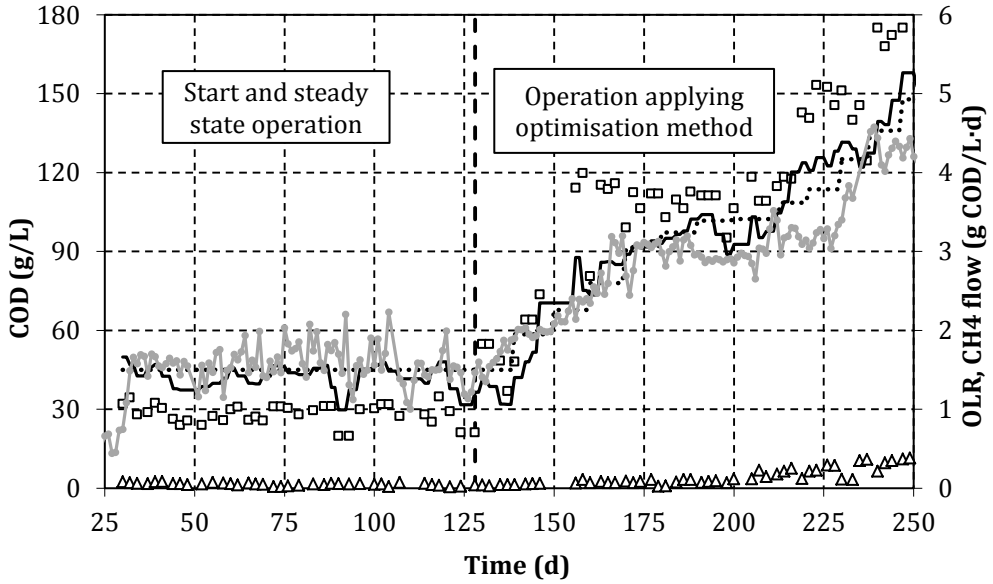

Figure 5.3. Experimental results for the entire operation. (□) Influent CODt, (△) Effluent CODt, (⋯) Theoretical OLR, (⎯) Experimental OLR, (–●–) CH_4 Flow.

The assumption of calculating HRT from BMP tests resulted in a good approximation. More conservative values of methanation are usually obtained in BMP tests compared to the higher yields achieved in continuous experiments due to adaptation and population changes within the microbial population (Jard et al., 2012).

In this study, the batch assays used to calculate the HRT were carried out with a feed to microorganism ratio (F/M) of 1 gCOD/gVSS. In the case of the continuous reactor, the calculation of F/M ratio is not straightforward. Using round calculations for the first blend (containing 60 gCOD/L), where 25 L/d were fed in the reactor of 1000 L (assuming it contains 15 g VSS/L), the F/M ratio would be 0.1 gCOD/gVSS the first day of feeding, so that, the system would take at least 10 days to achieve the F/M ratio equal to 1 in case no biodegradation takes place. Since the batch assays of all substrates showed no lag phase and close to 50% of methanation of the slowest biodegradable substrate was achieved in the first 10 days, the approximation of using the HRT from the BMP tests was considered a good estimation.

However, as influent CODt increases, the F/M ratio in the reactor tends to increase away from 1 gCOD/gVSS. Therefore, the F/M ratio applied to the system becomes an important parameter when estimating the HRT. Although methane yields are independent from the biomass concentration used in the BMP assay, the percentage of methanation at each time point would be affected. The kinetics to achieve the same methane yield depend on F/M ratio, and different curves of BMP assays can be obtained. The core of the optimisation method relies on the adaptation of the microorganisms to the operating conditions, so that the curves of the BMP assays are expected to change over time as microorganism are adapted, and once adapted the BMP tests could be updated for further optimisations at other operating conditions.

Finally, the set of linear restrictions applied in this study were based on inequalities (the parameters of the blend must be higher than and lower than the boundaries of the restrictions), regardless of the availability or scarcity of any substrate of the blend. If it were mandatory to treat a certain volume of a particular residue, a new equality restriction should be considered when solving the linear programming, (for example, fixing the proportion of pig manure in the blend to a certain value).

5.3.2. Predictions with ADM1-based AcoD model

The experiment has been previously simulated with the ADM1-based AcoD model. The model integrates the fermentation of ethanol, glycerol or methanol as sugar equivalent reactions and it has been formerly validated for ethanol (García-Gen et al., 2013). In this study, that approximation was also validated for the case of glycerol and methanol.

The parameters required by the model are shown in Table 5.4 (expressed in molar units), and the rest of the parameters remained as originally set in ADM1 (all in molar

units). Parameters such as k_La or SRT can vary as they are largely dependent on reactor configurations. SRT was set to 200 d and mass transfer coefficient k_La to 24.2 d^{-1}, the same values that a previous study with the same pilot plant (García-Gen et al., 2013).

Table 5.4. Added parameters in ADM1 required for the degradation of ethanol, glycerol and methanol.

Parameter	Present Model	Units
$k_{m,\,et}$	0.0438	$molS_{et}/CmolX_{fer}{\cdot}h$
$K_{s,\,et}$	0.0052	$molS_{et}/L$
Y_{et}	0.07	$molX_{fer}/CmolS_{et}$
$k_{m,\,gly}$	0.0447	$molS_{gly}/CmolX_{fer}{\cdot}h$
$K_{s,\,gly}$	0.0025	$molS_{gly}/L$
Y_{gly}	0.11	$molX_{fer}/CmolS_{gly}$
$k_{m,\,met}$	0.0641	$molS_{met}/CmolX_{fer}{\cdot}h$
$K_{s,\,met}$	0.0215	$molS_{met}/L$
Y_{met}	0.13	$molX_{fer}/CmolS_{met}$

The model accurately predicted the experimental results in the two stages of the operation: the first 128 days, with the blend of pig manure and wine wastewater, and the second part, from day 129 to 250, treating the optimised blends of glycerol, gelatine and pig manure (Figure 5.4). Simulated characteristics such as gas flow, gas composition, pH, effluent CODt and CODs, VFA and NH_4^+ matched to a great extent the results obtained with the pilot plant. In day 129, in the transition between the steady state operation (treating pig manure and wine wastewater) and the first optimum blend (pig manure, gelatine and glycerine), the physicochemical characteristics of the system remained stable except the biogas composition, which changed drastically due to the important substrates shift of the feeding. The complete biodegradation of a substrate involves different stoichiometries of CH_4 and CO_2, and this explains the change on the biogas composition.

Based on these results, the model was considered validated since these characteristics are the typical parameters used in the literature to validate ADM1-based models (Mata-Alvarez et al., 2011). The model was able to simulate accurately both the steady state and dynamic operation of different blends at different operating conditions. It turned

out to be a very useful tool to test the viability of different blends of substrates treated under co-digestion and can be used as a feasible forecaster for AcoD systems.

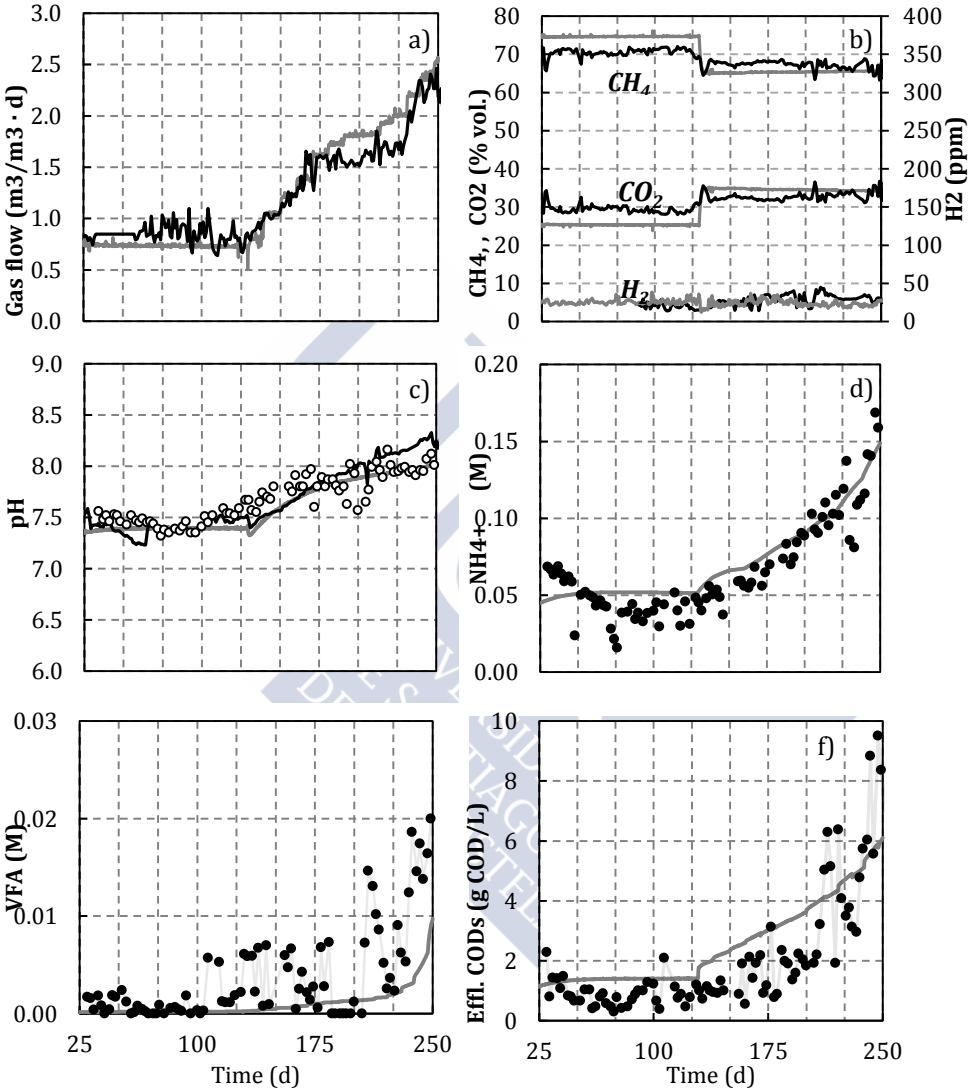

Figure 5.4. Simulated and experimental results; a) biogas production, b) biogas composition (CH_4, CO_2, H_2), c) pH, d) NH_4^+, e) VFA, f) Effluent CODs; (▬) Simulated results;(▬) Experimental results (on-line); (●), (○) Experimental results (off-line).

Finally, thanks to the modularity of MATLAB/Simulink and the good results obtained with both the optimisation method and the co-digestion model, two blocks can be built and assembled in Simulink to firstly calculate the optimum blends as feedings of the digester (first block containing the proposed linear programming optimisation method) and then evaluate that feeding blend in the reactor (second block containing the ADM1-based AcoD model). In future studies and for control purposes, a third module to assess the stability of the operation against overloads or acidification (diagnosis block) might be connected to the previous ones and feedback that information to calculate a new blend, forming a close-loop control strategy for co-digestion systems.

5.4. Conclusions

The proposed linear programming optimisation method to calculate blends of substrates as feeding of co-digestion systems appears as a useful tool for the optimisation of co-digestion processes. It represents an improvement since the feedings are optimised to maximise the methane production. The method uses typically available experimental and heuristic information, and it can be directly applicable in existing co-digestion plants. It was validated in a continuous experiment at pilot scale and the dynamic operation was accurately predicted with the ADM1-based AcoD model. This makes the model an important forecaster to early assess the feasibility of different substrates blends under co-digestion.

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Chapter 6. Control strategy for maximum anaerobic co-digestion performance

Contents

Abstract

A control strategy for optimising the performance of anaerobic co-digestion in terms of methane productivity, digestate quality and process stability is presented. A linear programming approach is adopted to calculate the feeding of multiple substrates for maximum methane productivity, subjected to restrictions based on experimental and heuristic knowledge. Process stability is quantitatively assessed by an empirical diagnosis function comparing alkalinity ratio measurements against reference values (outputs between (-1,1]). A second empirical diagnosis function is defined to compare methane flow rate measurements against a reference value of maximum capacity (outputs between (0,1]). A variable-gain control function (outputs between (-1,1]), derived from the diagnosis functions, is defined to determine the quantitative change applied to the most active constraint of the substrate blend optimisation problem leading to a new set-point of feeding substrates blend. The control strategy works in a closed-loop architecture under which the process performance for each blend of substrates is continuously assessed. The system was successfully validated in a 1 m^3 hybrid Upflow Anaerobic Sludge Blanket – Anaerobic Filter (UASB-AF) reactor, treating blends of substrates (gelatine, glycerine and pig manure supernatant) at OLR values between 0.71-6.33 gCOD/L d over a period of 210 days at mesophilic conditions.

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6.1. Introduction

One of the main advantages of anaerobic co-digestion (AcoD) in comparison to conventional single substrate anaerobic digestion (AD) is the potential higher energy recovery. According to Mata-Alvarez et al. (2014), much effort has been made over the last years in AcoD to: (i) maximise biogas production, (ii) utilise new residues as co-substrates and (iii) increase revenues by application of digestate as fertiliser.

A number of key process parameters have been suggested as diagnosis indicator of process stability: pH and biogas output flow rate (Steyer et al., 1999); H_2-gas and methane flow rate (Rodríguez et al., 2006); pH, alkalinity and total volatile acids (Waewsak et al., 2010); concentration of volatile fatty acids (VFAs) in the effluent and methane flow rate (García-Diéguez et al., 2011). Boe et al. (2010) compared the behaviour of different process indicators (biogas flow, pH, VFA, dissolved hydrogen, methane content in biogas) under organic and hydraulic overloads and concluded that VFA was the most effective indicator.

Different control strategies such as PID controllers, fuzzy logic or artificial neural networks have been used in anaerobic digestion in order to maintain good performance of the process (Ward et al., 2008). For instance, Puñal et al. (2002) developed and expert system based on IF-THEN rules to cope with hydraulic or organic overloads. Méndez-Acosta et al. (2010) proposed a robust control scheme to regulate both the VFA concentration and total alkalinity (TA) in order to improve the operational stability of continuous AD processes. Aguilar-Garnica et al. (2009) developed a multi-variable control scheme in a two-stage AD process to maintain the effluent COD and VFA concentrations at predetermined set-points. Nevertheless, most of those control strategies were designed for mono-digestion where the control action was based on modifying the HRT or OLR but not the characteristics of the substrates.

In addition to control strategies, several optimisation methods were applied to improve the performance of AD or AcoD systems. Yetilmezsoy (2012) developed a multi-objective optimisation method to obtain the best conditions for methane production rate, effluent substrate concentration and net operating cost of an AD process treating poultry manure wastewater; Wang et al. (2012) used response surface methodologies to optimise feeding composition and C/N ratio; and Alvarez et al. (2010) utilised a linear programming optimisation method to optimise the blend of substrates treated in AcoD systems in terms of methane production. The application of

control strategies, optimisation methods and advanced process analytical technologies (using spectroscopy, chromatography, and electro-chemical measurements together with multivariate data analysis) can bring AD process monitoring and control to a higher level of effectiveness (Madsen et al., 2011).

In order to make full use of the potential of anaerobic co-digestion, it is necessary to define the substrate blends that lead to co-digestion optimal performance in terms of methane productivity and digestate quality. However, the actual control systems neither deal with feedings of multiple substrates nor integrate their compositions into an optimisation method to maximise the methane production rate with anaerobic co-digestion.

The objective of this study is to develop a control strategy to maximise the performance of AcoD processes, capable of maximising methane production while maintaining the stability of the operation.

6.2. Materials and Methods

6.2.1. Control strategy for anaerobic co-digestion

An overview of the proposed control strategy is presented in Figure 6.1. A linear programming optimisation method calculates a feasible HRT and substrate blend for maximum methane productivity and subjected to a set of linear restrictions (García-Gen et al., 2014). These conditions are maintained in the digester during a specific period of time during which the diagnosis of the process stability is conducted. Subsequently, based on diagnostic indicators, namely alkalinity ratio and methane flow rate, the linear restriction boundaries are modified towards more or less conservative scenarios.

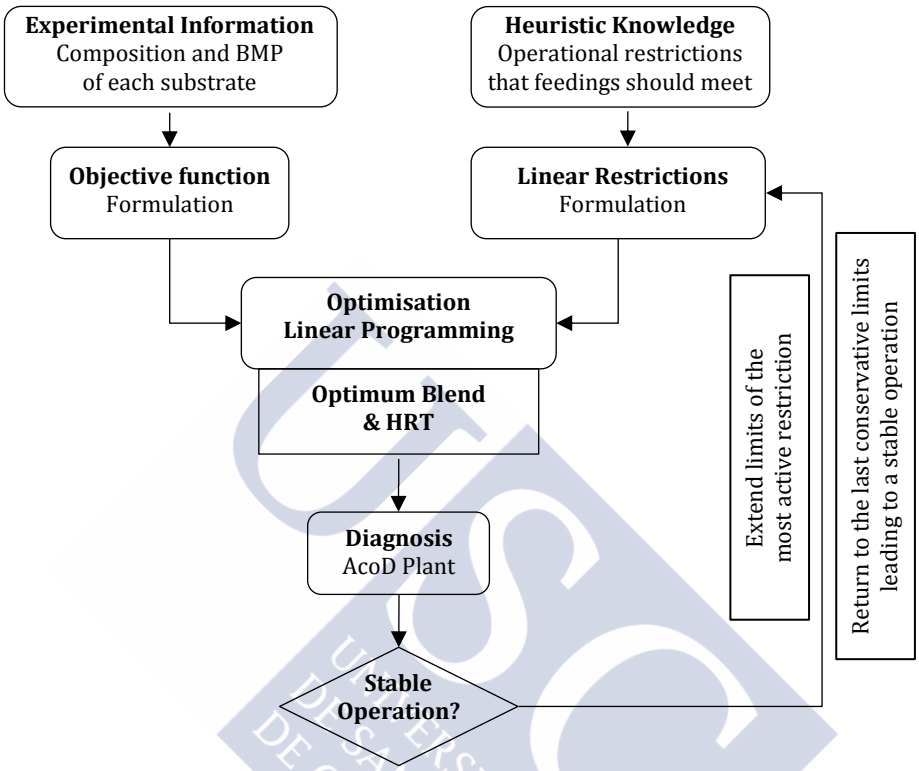

Figure 6.1. Overview of the optimum control strategy for anaerobic co-digestion of multiple substrates: linear programming optimisation of substrates blend, process performance diagnosis and feedback into the operational restrictions.

The architecture of the control strategy comprises four blocks, executed as a loop and connected to the digester: (1) Substrate Blender, (2) Filter, (3) Diagnosis and (4) Controller (Figure 6.2). A detailed description of each module is provided below.

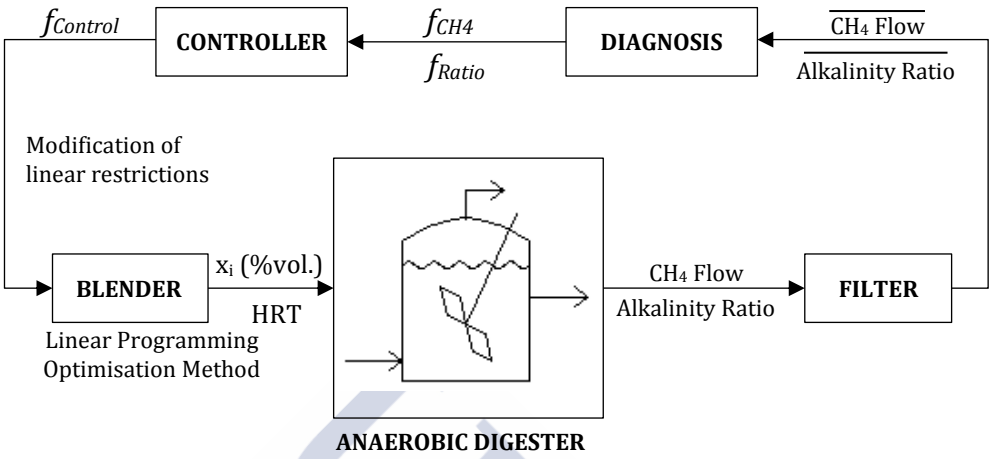

Figure 6.2. Proposed closed-loop control system architecture for anaerobic co-digestion.

Substrate Blender block. Linear programming optimisation

The *Blender* module contains a linear programming method developed by García-Gen et al. (2014). It conducts simultaneously an optimisation of both the substrates blend and HRT such that the objective function, which is the methane production rate, is maximised. The optimisation includes linear restrictions heuristically derived from typical characteristics of AD systems based on knowledge. The optimisation method, in addition to returning optimum performance variables, provides information about the current actively limiting restrictions allowing for these to be directly addressed to progress the system towards a new optimum.

The expression of the objective function is presented in Figure 6.3. Experimental information from biochemical methane potential (BMP) assays is used together with substrate COD content to define the objective function (expressed in OLR units, gCOD/L·d).

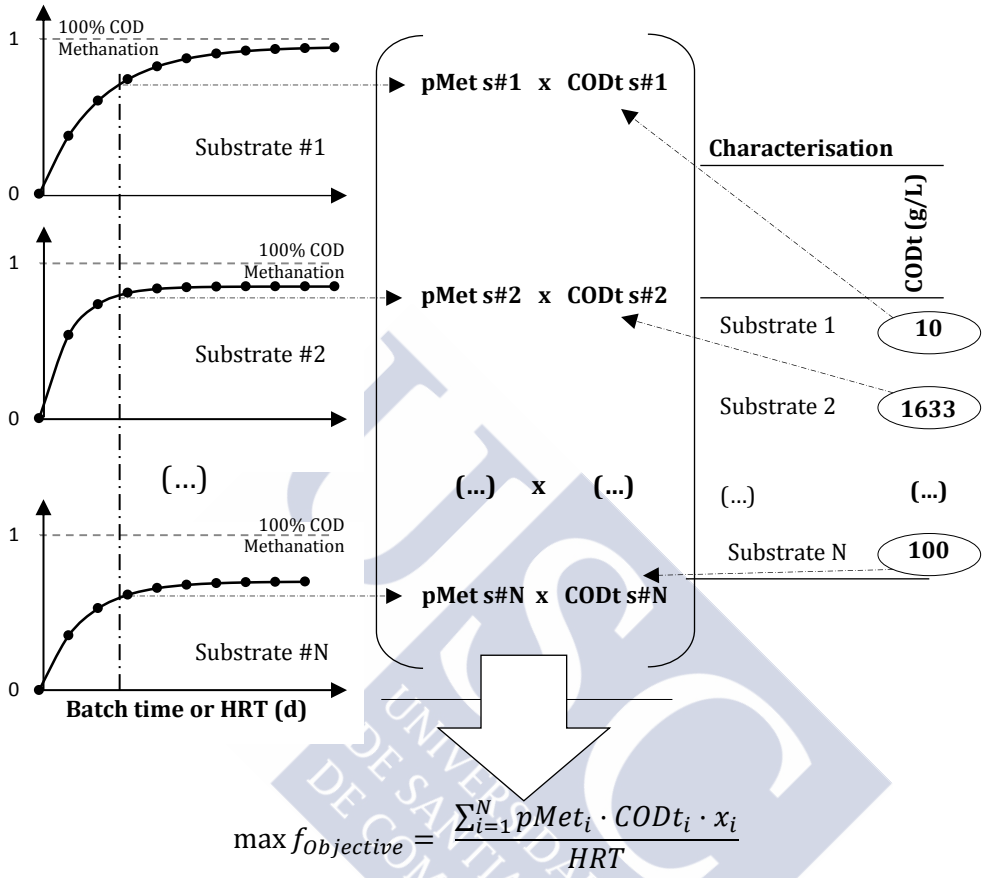

Figure 6.3. The objective function is evaluated for all HRT values and sets of substrate fractions in the blend (x_i, %vol.). The sum of the terms resulting from multiplying the fraction of each substrate in the blend times its CODt and times the percentage of expected methanogenic conversion (pMet) is returned for a given HRT (taken from experimental batch BMP studies). The units of the objective function are $gCOD_{CH4}/L \cdot d$.

Where the objective function depends on the volumetric fraction of each substrate in the blend (x_i), its COD content (CODt) and the percentage of methanation (pMet) estimated from the BMP tests at a time assumed equivalent to the selected HRT. In order to obtain the productivity of methane in OLR units (gCOD/L·d), the equation is divided by the applied HRT to the reactor (the same value for all substrates).

The set of linear restrictions is established based on heuristic knowledge and expertise on AD process operation and they are defined based on typically available substrate characteristics. Maximum and minimum values for the following variables are defined from practical values for the AD process (García-Gen et al., 2014): (i) organic loading rate (OLR); (ii) Total Kjeldahl Nitrogen (TKN); (iii) moisture or liquid fraction; (iv) lipid content; (v) total alkalinity; salinity as (vi) Na^+ concentration and (vii) K^+ concentration; (viii) H_2S content in biogas; (ix) effluent COD content. Table 6.1 shows the intervals of all restrictions that the blend feed must meet at the start of the operation. Some of these limits values are modified along the operation throughout the controller operation.

Table 6.1. Initial set of restrictions proposed in the linear programming optimisation method.

Linear restriction	Minimum	Maximum
OLR (g COD/L·d)	0	2.5
TKN (g N/L)	0.2	4.0
Liquid fraction (kg liquid/kg wet)	0.85	1.00
Lipids (g/L)	0	10
Alkalinity (g $CaCO_3$/L)	2	8
Na^+ (g/L)	0	3
K^+ (g/L)	0	3
Biogas quality (ppm H_2S)	0	10,000
Digestate quality (g COD/L)	0	6

Filter block

The *Filter* module calculates the average values of the physicochemical parameters monitored in the reactor and used as diagnosis indicators, alkalinity ratio (*Ratio*) and methane flow (F_{CH4}), during a specific period of time. The filtered values are calculated through equations (1) and (2):

$$\overline{Ratio} = \frac{\int IA\,dt \Big/ \int dt}{\int TA\,dt \Big/ \int dt} \cong \frac{\int Ratio(t)\,dt}{\int dt} \quad (1)$$

$$\overline{F_{CH4}} = \frac{\int F_{CH4}(t)\,dt}{\int dt} \quad (2)$$

where $\overline{Ratio}$ is the average alkalinity ratio, $\overline{F_{CH4}}$ is the average methane flow, IA is the intermediate alkalinity and TA is the total alkalinity. The integration time is equal to ¼ HRT, the period of time when the controller is executed. (Note: in this study $\overline{Ratio}$ calculated from both the average of IA and TA was equal to the average of the *Ratio(t)* values in at least two decimals).

Diagnosis block

The *Diagnosis* module informs about the stability of the process and methane production performance through the alkalinity ratio and methane flow parameters. The average values of these two indicators, $\overline{Ratio}$ and $\overline{F_{CH4}}$, are compared against reference values (or set points) considered as thresholds of process stability, $Ratio^*$ and F^*_{CH4}. Alkalinity ratio (Ratio = IA/TA) is defined as the ratio between alkalinity due to VFA (known as intermediate alkalinity, IA) and total alkalinity (TA) due to both bicarbonate (partial alkalinity, PA) and VFA. This term was defined by Ripley et al. (1986) as an indicator of acidification and suggests that values below 0.3 - 0.4 ensure the stability of the process against VFA accumulation. We should consider, however, that this rank may be different in co-digestion operations. The average methane flow is used as an indicator of maximum methane production capacity of the system. Eventually, the diagnosis module returns two diagnosis factors based on these indicators: stability factor (f_{Ratio}) and factor of remaining methanogenic potential (f_{CH4}).

The stability factor (f_{Ratio}) is calculated using an empirical correlation (Rodríguez et al., 2006) based on the average alkalinity ratio, $\overline{Ratio}$, with respect to a set-point $Ratio^*$ of 0.4, set as a stability boundary of the system. The returned value, between (-1, 1], informs about instability when negative, or stability when positive. The stability factor f_{Ratio} is calculated with equation (3) and its function is shown in Figure 6.4.

$$f_{Ratio} = \begin{cases} \left(1 - \frac{\overline{Ratio}}{Ratio*}\right)^{\frac{1}{m}} \; if \;\; \overline{Ratio} \leq Ratio* \\ \left(\frac{Ratio*}{\overline{Ratio}}\right)^{n} - 1 \; if \;\; \overline{Ratio} > Ratio* \end{cases} \quad (3)$$

where parameters m = 2 and n = 5 were conveniently selected to smooth the shape of the function f_{Ratio}.

The remaining methanogenic potential factor (f_{CH4}) is calculated using an empirical correlation based on the filtered methane production, $\overline{F_{CH4}}$, respect to a reference specific methane flow rate of 15 m³ CH_4/m³·d set as the maximum capacity of the system, F^*_{CH4} (the maximum OLR considered in this system was 40 kg COD/m³·d, and assuming a yield of 0.38 m³ CH_4/kgCOD, this leads to a maximum methane flow rate of 15 m³ CH_4/m³·d). The value is between (0, 1], and serves to regulate the extent at which organic load can be increased, avoiding large increases in high stable conditions when values of methane production are already close to the estimated maximum system capacity (Rodríguez et al., 2006). The remaining methanogenic potential factor (f_{CH4}) is calculated with equation (4) and its function is shown in Figure 6.4.

$$f_{CH4} = \frac{\alpha \cdot F^*_{CH4}}{\overline{F_{CH4}} + \alpha \cdot F^*_{CH4}} \quad (4)$$

where α = 0.1 was conveniently selected to smooth the shape of the function f_{CH4}.

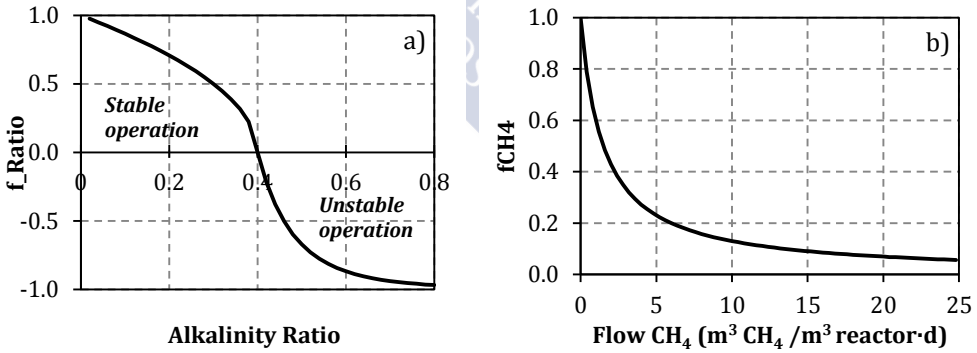

Figure 6.4. Empirical function of the stability factor. $Ratio^*$ = 0.4, m = 2 and n = 5. b) Empirical function of methanogenic potential factor. $f_{CH4}{}^*$= 15 m³CH_4/m³·d and α = 0.1.

Controller block

The *Controller* module calculates at the end of each period (¼ HRT) the control indicator, $f_{Control}$, as the product of f_{Ratio} and f_{CH4} when the system is stable (so that f_{Ratio} achieves a positive value), or equal to f_{Ratio} when systems becomes unstable and f_{Ratio} is negative (equation 5). The value of $f_{Control}$ falls between (-1, 1] and determines the extent at which the control action modifies the operational restrictions of the linear programming problem (*Blender* module).

$$f_{Control} = \begin{cases} f_{Ratio} \cdot f_{CH4} & if \quad f_{Ratio} > 0 \\ f_{Ratio} & if \quad f_{Ratio} \leq 0 \end{cases} \tag{5}$$

$f_{Control}$ modifies the boundary of the most active restriction following equation 6, where the new limit of the restriction takes into account the actual value of the limit, the range of the restriction (difference between higher and lower limits) and the control indicator $f_{Control}$.

$$Limit_{NEW} = Limit_{ACTUAL} + f_{Control} \cdot (Limit_{HIGHER} - Limit_{LOWER}) \tag{6}$$

When the system is stable ($f_{Control}$ positive), the control promotes the use of feedings with higher methane production potential. By relaxing the limits of the restrictions, the *Blender* output achieves a higher value of the objective function; consequently, higher OLR are applied and higher methane productions are obtained. Under destabilisation episodes ($f_{Control}$ negative) the control promotes the use of feeding with lower methane production in order to prevent the system from a possible acidification. By constraining the limits of the restrictions, the *Blender* output leads to lower values of objective function methane productivity, and consequently, lower methane productions are obtained.

6.2.2. Experimental set-up for continuous experiment

The AcoD experiment was carried out in a hybrid Upflow Anaerobic Sludge Blanket - Anaerobic Filter reactor (UASB-AF) of 1 m^3 of liquid volume (Ruiz, 2005). The reactor behaved as a Continuous Stirred Tank Reactor (CSTR) due to the sufficient recycling flow applied to the system. The facility is equipped with on-line measurement devices such as pH meter (Siemens, SIPAN pH/ORP 7MA 1010), gas flow meter (Brooks®, 5860E), biogas composition (CH_4, CO_2, H_2S) analyser (ABB, AO2020), hydrogen gas analyser (Sensotrans, Sensotox 420) and an on-line automatic analyser for VFA,

bicarbonate and alkalinity (Anasense®, Molina et al., 2009). Finally, a data acquisition programme developed in Visual Basic allowed monitoring and saving the signals from the on-line sensors and analysers to the computer through a rack of PLCs (Siemens, series S7-200). In addition to on-line measures, off-line analysis of NH_4^+, COD, alkalinity, VFA or pH were regularly taken to monitor the process. The characteristics of the three substrates treated in the pilot plant are summarised in Table 6.2.

Table 6.2. Characteristics of the co-substrates used during the pilot-scale AcoD operation: glycerine (from biodiesel residue), gelatine and soluble fraction of pig manure.

Parameter	Glycerine	Gelatine	Pig manure
Liquid fraction (%)	100	100	99
pH	9.8	6.5	7.8
Density (kg/L)	1.20	1.04	0.96
TS (g/L)	0	0	9.90
VS (g/L)	0	0	5.42
CODt (g/L)	1633	101	10.1
CODs (g/L)	1633	100	4.6
TKN (g N/L)	0	12.9	1.3
NH_4^+ (g N/L)	0	0	1.3
TA (g $CaCO_3$/L)	41.3	1.8	6.2
Na^+ (g/L)	18.07	1.06	0.17
K^+ (g/L)	0	0	0.51
Sulphate (g/L)	0	4.10	0.03
Proteins (g/L)	0	83.6	0
Lipids (g/L)	44.5	0	0
Carbohydrates (g/L)	310	0	9.49[a]
Ethanol (g/L)	0	0	0
Glycerol (g/L)	512.6	0	0
Methanol (g/L)	366.5	0	0
Biodegradability[b] (%)	91.0	77.7	79.3

[a]This concentration comprises both carbohydrates and VFA contents and amended to this value to meet CODt. [b]Biodegradability assays were performed with BMP tests (described in the supplementary material, section 6.6).

6.2.3. Analytical methods

Characterisation of pH, COD, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), total alkalinity (TA) and partial alkalinity (PA) were performed following standard methods (APHA, 1998). Ammonium was measured with phenate method (APHA, 1998) with a spectrophotometer (Shimadzu UV-1603, UV–Visible) at 640 nm. VFA (acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric acids) were analysed by gas chromatography (HP 5890A), equipped with a flame ionisation detector (García-Gen et al., 2014). SO_4^{-2} was determined by ion chromatography following ASTM D4327-03 method (ASTM, 2003) and cations Na^+, K^+ were determined by ion chromatography following ASTM D6919-03 method (ASTM, 2003). Glycerol, methanol and ethanol were determined by HPLC, GC, and from soluble COD, respectively, as indicated in García-Gen et al. (2014). Lipids were measured with Soxhlet method (APHA, 1998). Protein concentration was estimated by multiplying organic nitrogen (TKN minus NH_4^+) by 6.25 (Dintzis et al., 1988).

6.3. Results and Discussion

6.3.1. Control strategy results

The proposed control strategy was validated at pilot scale for 210 days at different operating conditions and blends of substrates. The two objectives of the control system were accomplished: (i) to maximise the methane production over time by calculating and applying the optimum blend of substrates and (ii) to maintain the stability of the process by recovering the system from organic overloads. The three substrates (gelatine, glycerine and pig manure) were continuously fed in different proportions and organic loads according to the control strategy. Along the operation, the OLR applied to the system increased or decreased between 0.71 and 6.33 gCOD/L·d based on the diagnosis outcome. The control algorithm was typically executed every 10 days (¼ HRT), or 15 days in the last month of operation. In total 16 cycles of control were covered during the entire operation. Figure 6.5a depicts the different operating conditions and blends of substrates applied to the digester.

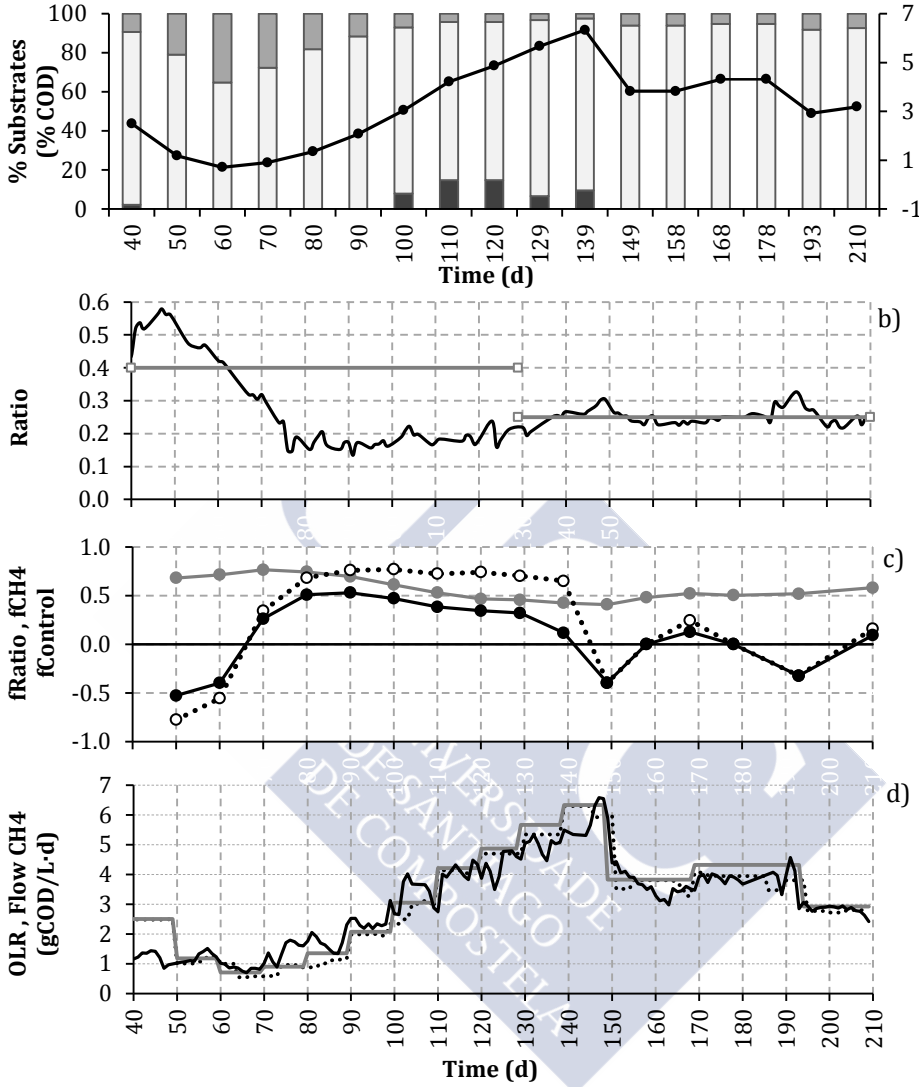

Figure 6.5. a) OLR and blends of substrates (in %COD) applied to the pilot plant during the entire operation; (▬) Gelatine, (▬) Pig manure, (▬) Glycerine, (-●-) OLR. b) Experimental alkalinity ratio; (——) Ratio, (——) Ratio set-point. c) Experimental diagnosis and control indicators; (-○-) f_{Ratio}, (-●-) f_{CH4}, (-●-) $f_{Control}$. d) Experimental results of methane flow and theoretical OLR (both expressed in gCOD/L·d) as well as the experimental OLR (calculated from influent CODt and HRT as OLR = CODt/HRT); (——) Theoretical OLR, (...) Experimental OLR, (——) CH_4 flow rate.

Figure 6.5b shows the experimental alkalinity ratio (diagnosis indicator) along the operation. The alkalinity ratio turned out to be an effective predictor of instabilities. It favoured the relaxation of restrictions to increase methane productivity when the system was stable, and constrained the restrictions to recover the system from unstable operation. Initially, a *Ratio* set-point of 0.40 was considered according to references (Ripley et al., 1986) and maintained during the first 130 days. This value is very convenient for systems operating at total alkalinity values around typical 2-4 $gCaCO_3/L$, but in co-digestion operations, much higher values of TA can be reached, depending on the characteristics of the co-substrates. In our case, values higher than 7 $gCaCO_3/L$ (TA=140 meq/L) were attained and, accordingly, from day 130 till the end of operation, the set-point was modified to 0.25, this leading to VFA concentrations lower than 3 g/L (IA=50 meq/L of acetate), a value considered as a threshold to avoid methanogenic toxicity (Ahring et al., 1995). Therefore, although alkalinity ratio was the diagnosis indicator, the *Ratio* set-point was modified based on the maximum IA expected in the process, fixed at 50 meq/L of acetate. This approach can be developed in the future to automatically update the *Ratio* set-point based on TA measures of the process.

Based on both, the stability factor (f_{Ratio}) and the remaining methanogenic potential factor (f_{CH4}), the most active constraint of the linear restrictions in each case was modified through the control indicator $f_{Control}$ (Figure 6.5c). The most frequent limiting restriction encountered along the operation was the OLR. Whenever the alkalinity ratio surpassed the set-point selected in the *Diagnosis* module, the stability factor and $f_{Control}$ become negative. Both positive and negative values of $f_{Control}$ continuously relaxed or tightened the maximum boundary of the OLR (active restriction). Consequently, the system increased or reduced the maximum OLR of the operation.

The experimental results show that COD removal efficiencies around 90% were achieved (*%COD removal = (influent CODt – effluent CODt) ·100/ influent CODt*) during the entire operation. Figure 6.5d shows the experimental results of methane flow and theoretical OLR (both expressed in gCOD/L·d), as well as the experimental OLR (calculated from influent CODt and HRT as OLR = CODt/HRT). In addition, the execution of the control loop every ¼HRT did prove as a suitable time interval to optimise the operation and maintain stability.

6.3.2. Experimental results

Based on the diagnosis and the control actions, different feedings and operating conditions were applied to the pilot plant. The *Blender* module calculated the optimum feedings and HRT during the entire operation. Table 6.3 summarises the main operating conditions of each blend calculated by linear programming.

Table 6.3. Substrate blending and operating conditions as calculated by the linear programming optimisation (blender module). The method identifies also the limiting restriction for the control factor ($f_{Control}$) to modify the boundary of the restriction in the next period.

Blend	Days	OLR	HRT	COD	C/N ratio	C/S ratio	Limiting restriction	$f_{Control}$
1	[40,50)	2.50	40.0	100.0	66.7	691.5	OLR	-0.5283[a]
2	[50,60)	1.18	40.0	47.2	36.6	1151.7	OLR	-0.3970[a]
3	[60,70)	0.71	40.0	28.4	21.8	803.0	OLR	0.2621
4	[70,80)	0.90	40.0	35.9	27.6	954.1	OLR	0.5085
5	[80,90)	1.35	40.0	54.2	42.2	1258.7	OLR	0.5296
6	[90,100)	2.07	40.0	82.8	65.7	1608.2	OLR	0.4713
7	[100,110)	3.05	40.0	121.9	52.0	270.2	OLR	0.3835
8	[110,120)	4.21	39.1	164.6	41.2	156.7	OLR	0.3446
9	[120,129)	4.87	33.8	164.7	41.2	156.7	Alkalinity	0.3208
10	[129,139)	5.67	40.0	226.7	78.8	325.8	OLR	0.1173
11	[139,149)	6.33	40.0	253.3	64.9	237.1	OLR	-0.3957
12	[149,158)	3.83	40.0	153.1	127.2	2117.7	OLR	0
13	[158,168)	3.83	40.0	153.1	127.2	2117.7	OLR	0.1276
14	[168,178)	4.32	40.0	172.6	145.3	2211.2	OLR	0
15	[178,193)	4.32	40.0	172.6	145.3	2211.2	OLR	-0.3236
16	[193,210)	2.92	40.0	116.7	94.7	1897.2	OLR	0.0918
17[b]	[210,220)	3.19	40.0	127.5	104.1	1969.9	OLR	-

[a] Control factors of first two blends were calculated as $f_{Control} = f_{Ratio} \cdot f_{CH4}$ even though $f_{Ratio} < 0$. The rest of the operation factors were calculated following the procedure explained in section *Controller block*. [b] These would have been the operating conditions from day 210 on, if the experiment had continued.

Units: [OLR] = gCOD/L·d; [HRT] = d; [COD] = gCOD/L; [C/N] = gCOD/gN; [C/S] = gCOD/gSO_4^{-2}

Along the operation, both OLR and blend of substrates changed after the control action was applied. However, the HRT remained steady at 40 days. This is explained by the fact that the main substrate treated in the plant was the pig manure (ranging between 62-98%, %vol.; most of the times over 90%), where gelatine and glycerine contributed with small volume as co-substrates. According to this, whenever the co-substrates involve much higher COD content than the main substrate, the control strategy arises as a useful tool to convert existing AD plants into AcoD systems (for instance, digesters of wastewater treatment plants, already sized to treat active sludge at a certain HRT).

The C/N ratio of each blend fell within the optimum range of 20-70 for AD process (Mata-Álvarez et al., 2011) during most part of the operation (first 150 days). From day 150 till the end of operation, the influent C/N ratio ranged between 95 and 145. These high values of C/N were not considered a setback since NH_4^+ concentration in the reactor was high (1.5 to 3 g NH_4^+/L). In addition, the carbon to sulphur ratio (C/S) measured as COD/SO_4^{-2} ratio remained high and far from the ratios of 3 to 5.6 where sulphate reducing bacteria (SRB) are known to compete with acetogens, or far from 1.7 to 2.7 where SRB compete with acetoclastic methanogens (Chen et al., 2008).

Experimental results such as biogas flow, biogas composition, pH, alkalinity, VFA, effluent CODs and NH_4^+ are shown in Figure 6.6. Biogas flow increased and decreased as OLR did with an average methane composition around 65% (Figure 6.6a, 6.6b). Hydrogen gas ranged between 20-40 ppm (Figure 6.6b) and showed no sensitivity to over or underload. pH maintained a constant value of 8.0 due to high alkaline content of glycerine and was no sensitive to acidification (Figure 6.6c). NH_4^+ concentration increased from 1 to 3 g/L as gelatine was fed to the system and reduced when it was limited (Figure 6.6h). Regarding effluent CODs (Figure 6.6g), digestate values of 10-14 gCOD/L were obtained from which only 6 gCOD/L or lower were due to VFA (biodegradable matter); the remaining fraction although not identified was thought to be recalcitrant. With these numbers restriction of digestate quality was also met during the entire operation.

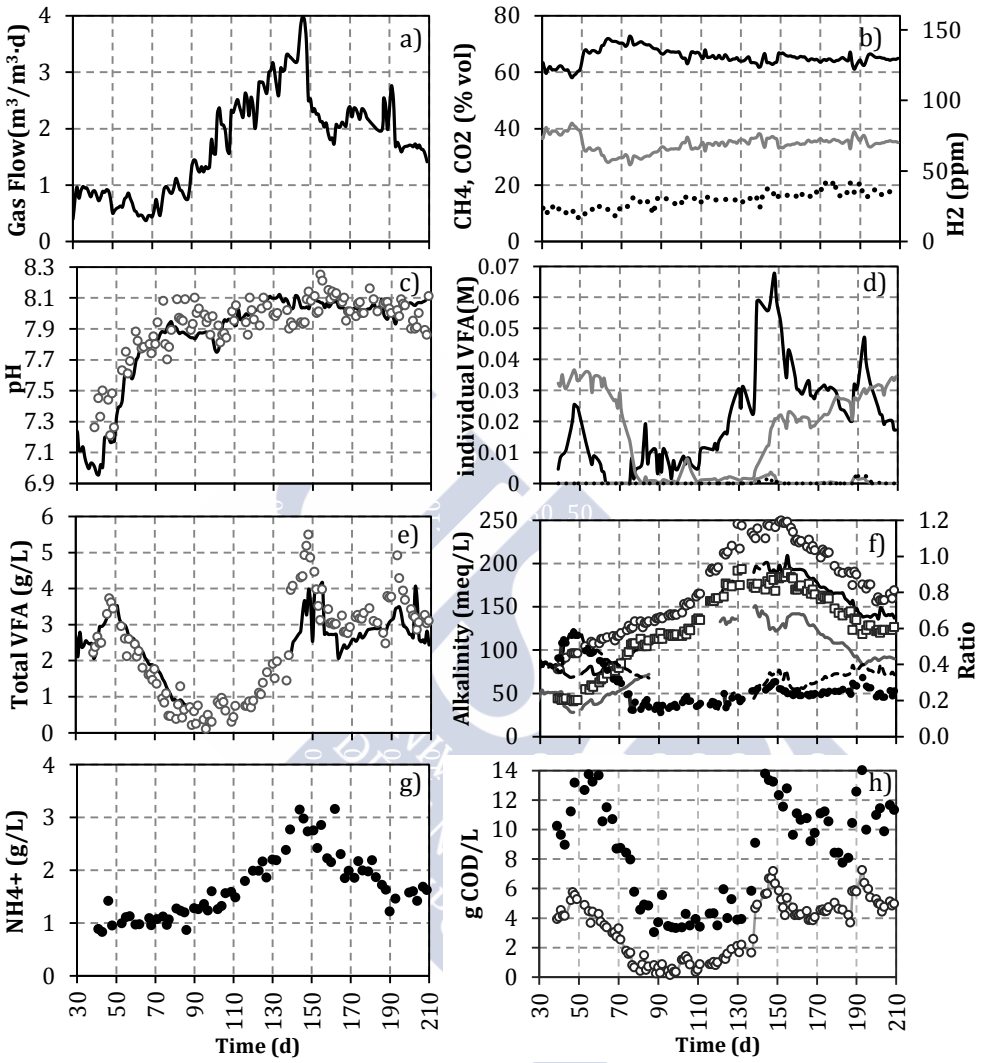

Figure 6.6. Experimental results of the controlled pilot-scale anaerobic co-digestion experiment. a) biogas production, b) biogas composition (CH_4, CO_2, H_2), c) pH, d) Individual VFA (off-line), e) Total VFA (Anasense and off-line), f) PA, TA and alkalinity ratio (Anasense and off-line) g) NH_4^+, h) Effluent CODs and CODs related to VFA; (▬) Gas flow, CH_4 composition (%vol.), pH, acetate (off-line), VFA (Anasense), TA (Anasense); (▬) CO_2 (%vol.), propionate (off-line), PA (Anasense); (...) H_2 gas (ppm), Alkalinity ratio (Anasense); valerate (off-line); (○) pH (off-line), total VFA (off-line), TA (off-line); (●) alkalinity ratio (off-line), NH_4^+, Total effluent CODs; (▬) butyrate (off-line); (□) PA (off-line).

The individual VFA are shown in Figure 6.6d. Acetate and propionate showed different behaviours along the operation. As acetate could recover rapidly from high concentrations, propionate was more persistent and remained longer at high concentrations. However, the propionate/acetate ratio, used as indicator of process imbalance in other studies (Hill et al., 1987), was not useful in assessing system overload as it varied in a wide range within unstable periods. On the other hand, butyrate and valerate accumulated up to 3 mM in periods with high acetate accumulation and disappeared when acetate concentration decreased. These concentrations were already found as thresholds of methanogenic toxicity (Nielsen et al., 2007), and could be used as indicators of severe acidification.

Both VFA and alkalinity (PA and TA) were measured off-line and on-line. The use of Anasense® analyser reinforced the monitoring, diagnosis and control of the AcoD experiment. VFA estimations with Anasense® (expressed as meq/L of acetate) matched to a large extent off-line VFA measures (Figure 6.6e). However, although Anasense® was successfully validated in previous studies (Molina et al., 2009), the analyser underestimated PA and extended the error to TA estimations (Figure 6.6f). Therefore, in this study, only the conventional off-line alkalinity ratio data were considered for the diagnostics during the control action. Alkalinity ratio was sensitive to acidification along the operation: very sensitive at the first stages of the operation when TA was low and moderately sensitive as TA increased. Based on these results it is concluded that this parameter is an effective indicator of process imbalances during AcoD treating different blend of substrates at different operating conditions. Its use allowed for the stable operation of the process and recovery from failure. Beyond that, the possibility of monitoring this parameter on-line makes the alkalinity ratio an attractive diagnosis indicator for assessing the performance of AcoD processes.

6.4. Conclusions

The proposed optimum substrate blend control strategy has been validated in a continuous pilot-scale co-digestion experiment treating soluble substrates. It was proven capable of changing the operating conditions to increase methane productivities and to recover the system from transient acidifications. The constrained linear programming method appears as a suitable approach to calculate optimal feedings of co-digestion systems based on conventional substrates characterisation and biomethane potential tests. Both alkalinity ratio and methane flow rate appear as

key diagnosis indicators of stability and performance of co-digestion processes due to their sensitivity to disturbances occurring during the operation.

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6.6. Supplementary material

Biochemical methane potential (BMP) tests

Biodegradability assays of gelatine, glycerine-containing biodiesel residue and pig manure supernatant (Fig. 6.A – 6.C) were carried out in 500-mL glass flasks with coiled butyl rubber stoppers. All tests were performed in triplicate assays under the following operating conditions: 5 g COD/L substrate, 5 gVSS/L of inoculum at 35 °C and mixing of 120 rpm. Control assays with only inoculum were also performed. Anaerobic conditions were maintained by using an anaerobic basal medium composed of macro- and micro-nutrients solution, cysteine (0.5 g/L) and $NaHCO_3$ (5 g/L), at pH of 7.0–7.2. Before flushing the liquid and headspace with N_2, 1.2 mL of Na_2S (20 g/L) was added to each assay as a reducing agent (Alvarez et al., 2010). An initial liquid volume of 385 mL was used in all assays. A pressure transducer was used to measure the pressure increase. The biogas was sampled regularly, and its composition was determined by gas chromatography.

Calculations

Cumulative methane production was plotted as accumulated CH_4 expressed in COD units (gCOD-CH_4) divided by the initial COD added in the assay (gCOD initial) versus time (d). Firstly, moles of methane were calculated by the ideal gas law:

$$CH_4\ moles = \frac{P \cdot X_{CH4} \cdot V_{GAS}}{R \cdot T}$$

where P is the total pressure measured by the transducer (mmHg); X_{CH4} is the methane molar fraction; V_{GAS} is the headspace volume (mL); R is the ideal gas constant (62,364 mmHg mL/mol K), and T is the temperature of the assay (K).

Finally, cumulative methane expressed in COD units is calculated by multiplying the moles of CH_4 by 64 (gCOD/CH_4 mole).

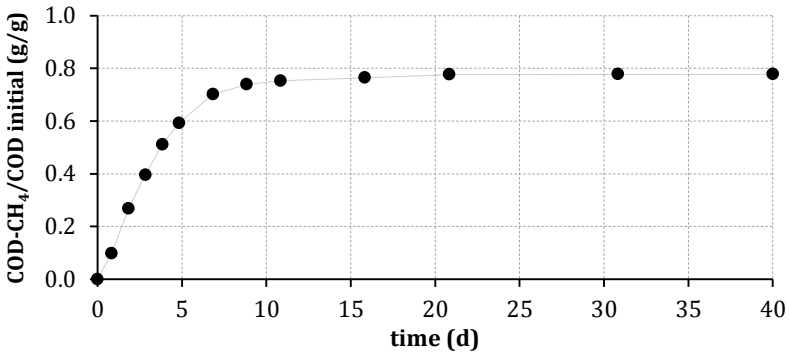

Figure 6.A. Biodegradability curve of gelatine BMP test.

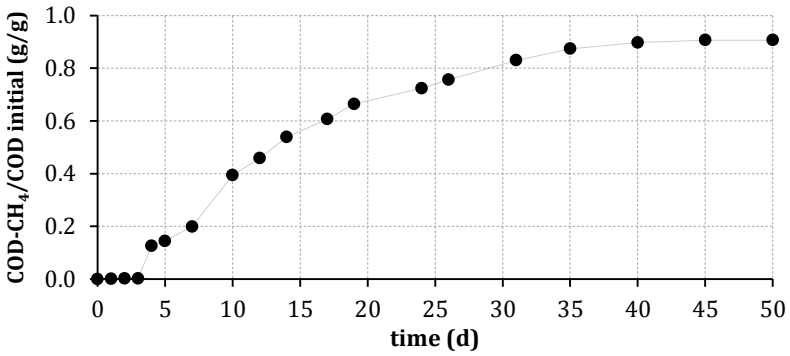

Figure 6.B. Biodegradability curve of glycerine BMP test.

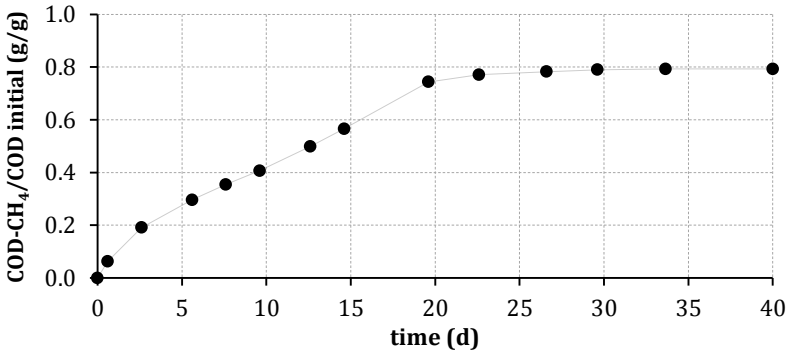

Figure 6.C. Biodegradability curve of pig manure supernatant BMP test.

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General Conclusions

This Thesis contributes to the modelling, optimisation and control of anaerobic co-digestion (AcoD) processes. In the modelling section (Chapter 3 and Chapter 4) a generalised modelling approach for soluble fermentable substrates is proposed and validated in a continuous experiment (Chapter 3). In addition, a new modelling approach for the disintegration and hydrolysis processes of complex particulate substrates is proposed and validated with available data from both batch and continuous operation modes (Chapter 4). In the optimisation section (Chapter 5) a method based on linear programming to obtain optimal feedings blends for co-digestion systems is presented and validated in a continuous experiment, aiming at maximising the methane production of an AcoD process. Finally, in the control section (Chapter 6) a novel control strategy for anaerobic co-digestion is developed and validated in a continuous experiment, aiming at maximising the energy recovery and reaching the required quality of digestate while maintaining the stability of the operation. The following general conclusions are drawn for these three areas of contribution of the thesis:

I. Modelling of soluble and solid substrates in AcoD within the ADM1framework

1. The generalised modelling of soluble fermentable substrates as glucose-equivalent fermentation by one single microbial group is a feasible applicable approach to methanogenic AcoD since acidogenesis is faster than methanogenesis.

2. This approach for fermentable substrates extends the application of the Anaerobic Digestion Model No. 1 (ADM1) to soluble substrates not originally implemented in the model.

3. Real continuous AcoD systems can be accurately described in both steady and dynamic states by using the ADM1-based generalised approach including fermentable substrates (such as ethanol, glycerol or methanol) in long-term operation.

4. Default ADM1 parameters values can be used to predict the system performance to a large extent. Only the inhibition of the acetoclastic methanogenesis kinetics by free ammonia (parameter $K_{I,NH3}$,) required specific calibration to fit acceptably the experimental behaviour.

5. A modelling approach considering a decoupled disintegration of the readily and slowly biodegradable fractions of particulate matter is proposed. The approach allows

for the estimation of disintegration and hydrolysis kinetic parameters for to a good number of different fruit and vegetable wastes, FVW.

6. The modelling approach presented for the disintegration and hydrolysis of complex particulate substrates is able to predict batch and continuous experimental dynamics of both anaerobic mono- and co-digestion of solid wastes.

7. The readily biodegradable fractions (f_R) of solid substrates are determined from experimental biodegradability assays of individual substrates (BMP tests) and not estimated from model calibration.

8. The kinetic parameters estimated by batch assays calibration with FVW can be applied to the accurate prediction of a continuous AcoD system of FVW in a long-term operation.

II. Optimisation of feedings for anaerobic-codigestion systems

1. Linear programming optimisation can be used to calculate optimum blends of substrates with individual different methane potentials and physicochemical characteristics, aiming at maximising methane productivity.

2. Optimum blends can be calculated by linear optimisation using typical information available in biogas plants, characterisation of substrates and BMP tests.

3. Linear restrictions to the optimisation method are defined based on heuristic knowledge and expertise on the process as linear function of the physical and chemical characteristics of the substrates. These restriction boundaries are modified along the operation for enhanced performance.

4. Different optimal operating conditions (hydraulic retention time and volumetric flows of substrates) are obtained with linear programming depending on the OLR to be applied.

5. The optimisation method was validated in a long continuous pilot scale AcoD experiment at different OLR values under dynamic conditions.

III. Control in anaerobic co-digestion

1. A control strategy based on an iterative calculation of optimal feedings, with restrictions modified based on the outcome of a diagnosis system, allows for the long-term operation optimisation of AcoD plants treating blends of different substrates.

2. The alkalinity ratio and methane productivity are used as the key diagnosis parameters of process stability and energy recovery, respectively. They appear to provide sufficient sensitivity to any changes on the operating conditions and feeding composition in AcoD processes.

3. The control action is implemented as a change in the boundaries of the linear optimisation restrictions applied to calculate the feeding to the AcoD systems. This strategy brings about new optimum operation points that enable the improvement of the AcoD process performance.

4. The proposed control strategy was demonstrated to serve two objectives: (i) the maximisation of methane production when the system is stable, and (ii) the recovery of the system from acidification when the process is destabilised, both always maintaining the required quality of digestate at all times.

Annex. Curriculum Vitae

EDUCATION

2011 – Present **Doctorate in Chemical & Environmental Engineering**
University of Santiago de Compostela (Spain)

2010 – 2011 **MPhil in Engineering of Chemical & Environmental Processes**
University of Santiago de Compostela (Spain)

2000 – 2006 **MEng in Chemical Engineering**
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Other

2013 – 2014 **Master in Instrumentation and Process Control**
International Society of Automation & Repsol (Madrid, Spain)

EXPERIENCE

08/14 - Present **Research coordinator**. Núcleo Biotecnología Curauma
Pontifical and Catholic University of Valparaíso (Chile)

11/09 – 07/14 **Researcher**. Group of Environmental & Bioprocesses Engineering
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09/08 – 08/09 **Process Engineer**
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03/07 – 08/08 **Analyst** (IT Consultancy)
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PUBLICATIONS

García-Gen, S., Rodríguez, J., Lema, J.M., 2015. Control strategy for maximum anaerobic co-digestion performance. Water Res. 80, 209-216.

García-Gen, S., Sousbie, P., Rangaraj, G., Lema, J.M., Rodríguez, J., Steyer, J.-P., Torrijos, M., 2015. Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes. Waste Manage. 35, 96–104.

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PATENTS

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Rodríguez, J. **García, S.**, 2010. Modelling anaerobic digestion of complex particulate/lipid-protein rich substrates: Balancing complexity and model utility using ADM1. Proceedings of International Workshop on Anaerobic Digestion of Slaughterhouse Waste, 11th June 2010, Barcelona (Spain). (*Oral presentation*)

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