



Country report

Fostering the valorization of organic wastes into carboxylates by a computer-aided design tool

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ABSTRACT

The carboxylate platform has the potential to constitute an outstanding opportunity for converting organic wastes into chemicals and other value-added products within a circular economy framework. However, its development is still hampered by technological and financial constraints due to difficulties at forecasting the carboxylates yields by different wastes. This work provides a framework that can be the key to foster circular economy and bridge the development risks, allowing early-stage evaluation of process performance. This framework, which is implemented as a computer-aided design tool, is comprised by: (i) a library of substrates including their characterization and appropriate kinetic parameter selection, (ii) an integral kinetic and stoichiometric model which solves both identified gaps regarding the disintegration mechanisms and the acidogenic stoichiometry variability in the anaerobic mono and cofermentation of complex organic wastes, and (iii) a set of indicators to interpret simulation results and assist the decision making; and presents a showcase of applications supported by two case studies. These case studies show that the optimal conditions to steer VFA spectrum towards odd-chain VFA in MCF of regrind pasta are neutral pH (6.5–7) and a relatively low HRT (3–4 days), while cofermentation of tuna canning wastewater and regrind pasta follows interactive mechanisms that cannot be captured by a “naïve approach”, i.e. by adding up the individual contributions. Finally, it is discussed how value chain actors with different interests can benefit from the proposed tool: identifying technical, economic, and environmental bottlenecks, and proposing innovative solutions prior to costly lab research and piloting.

1. Introduction

Growing attention to resource recovery is reflected in the new circular economy action plan, adopted by the European Commission in March 2020 (European Commission, 2020). A biorefinery approach would allow the transition towards circular upcycled value chains, thereby contributing to the sustainability growth goals (Teigiserova et al., 2019). Given its growing market demand and wide range of possible substrates, as well as a cost-effective and environmentally friendly approach, the carboxylate platform is gaining attention in the resource recovery framework (Ramos-Suarez et al., 2021). In this biorefinery approach, waste, side streams and wastewaters are initially converted into volatile fatty acids (VFA), organic solvents (e.g., ethanol

and hydrogen through mixed culture fermentation (MCF) (Naresh Kumar et al., 2022). These intermediates can be further purified or employed as a platform for the obtention of a wide range of high-value products with multiple applications, such as biofuels, chemicals, pharmaceuticals or bioplastics, in particular polyhydroxyalkanoates (Atasoy et al., 2018).

H2020 project USABLE Packaging (H2020 USABLE Packaging, 2019) aims to overcome the polyhydroxyalkanoates (PHA) production drawbacks, building an entirely new value chain from food industry byproducts and wastes, and using innovative PHA production routes. The first step in the conversion of organic wastes to PHA is an acidogenic fermentation yielding a mixture of VFA. The performance of the PHA depends highly on the obtained VFA composition. The advantage of this

Abbreviations: CH, Rapidly biodegradable carbohydrates; COD, Chemical oxygen demand; CSTR, Continuous stirred tank reactor; I, Inert; LCFA, Long chain fatty acids; LI, Lipids; MCF, Mixed culture fermentation; PHA, Polyhydroxyalkanoates; PR, Proteins; sCH, Slowly biodegradable carbohydrates; VFA, Volatile fatty acids.

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first acidification step is that MCF can handle the complexity and variability of complex organic substrates such as food wastes and has both economic and environmental advantages (Naresh Kumar et al., 2022), e.g., MCF allows continuous operation at non-sterile conditions and thus, reducing substantially the process operating costs. In contrast, a major technical challenge of the carboxylate platform is that acidogenic fermentation has a very variable stoichiometry (Bhatia and Yang, 2017; Domingos et al., 2017; Zhou et al., 2018), depending on its design parameters, i.e., (i) the pH, which can change radically the obtained VFA spectrum (Bevilacqua et al., 2021a; Jin et al., 2019; Lu et al., 2020); (ii) the substrate composition (carbohydrates, proteins, lipids, inert) can modify metabolic pathways and determines the VFA composition (Alibardi and Cossu, 2016; Bevilacqua et al., 2020; Yin et al., 2016) and, (iii) the hydraulic retention time (HRT), which affects the hydrolysis degree of compounds with different hydrolysis rates and thus, the composition of the acidified substrate (Atasoy et al., 2018; Jankowska et al., 2018; Strazzera et al., 2018).

The use of computer-aided design tools, such as mathematical models for predictive, environmental, and techno-economic assessment, can be the key to facilitate process development decisions. Concretely, it is possible to assess a large set of alternatives, identify technical bottlenecks and propose innovative solutions prior to expensive lab research and piloting (Varghese et al., 2022). Mathematical models can be a valuable tool to screen complex organic wastes as potential VFA producers. However, current models cannot predict the selectivity and productivity of VFA from a given substrate due to the variability of acidogenic stoichiometry which depends, itself, on previous disintegration and hydrolysis steps. Most published mathematical models for open mixed cultures in anaerobic conditions are characterized by fixed stoichiometry (Alexandropoulou et al., 2018; Bai et al., 2017) and in many cases focus on methane production. Only recently, models at metabolic level have provided predictive capabilities on the VFA stoichiometry in function of substrate and operational conditions, hence predicting the effect of pH on the VFA selectivity in glucose MCF (González-Cabaleiro et al., 2015), protein MCF (Regueira et al., 2020b) and cofermentation with sugars (Regueira et al., 2020a). These models focus on the MCF stoichiometry for model substrates and can predict the actual VFA production in mono- or cofermentation of substrates ready to be acidified, e.g., glucose or casein. However, real substrates are composed of different fractions that undergo, at different rates, several transformation steps before being available for acidification. Other challenges in modelling the MCF real substrates are (i) the characterization of organic substrates and their representation as model variables; and (ii) different (and unknown) disintegration and hydrolysis rates that change the substrate composition capable of being acidified. Therefore, new models that address all these challenges are required to enable the valorization of organic wastes into VFA.

The current work covers the identified gaps regarding the production and selectivity of VFA by acidogenic fermentation of organic wastes. Additionally, our contribution goes beyond and proposes a framework to connect multiple levels of actors in the value chain with different interests, expertise levels, available data and knowledge. The development of the framework is described in Section 2: (i) substrate library, (ii) integral kinetic and stoichiometric model, and (iii) set of indicators of MCF performance. Section 3 presents its implementation as a computer-aided design tool as well as a showcase of its functionality. Then, the limitations and applicability of the framework within the carboxylate value chain are discussed in Section 4, and final remarks and conclusions are provided in Section 5.

2. Framework development

The framework developed in this section aims to cover a gap in the early-stage development of the carboxylate platform and includes: (i) a catalogue of complex organic wastes that includes their characterization and representation as model variables; (ii) a mathematical model which

allow the MCF of different complex organic substrates and captures the variability on acidification stoichiometry; and (iii) a set of indicators that can be employed in assisting decision making in the early-stage design of MCF processes.

2.1. Substrate library

The substrate library characterizes several real substrates as variables that are represented in a mathematical model, i.e., (i) the substrate composition, i.e., primary data referred to moisture, proteins (and aminoacids), carbohydrates, lipids and ash content; (ii) the substrate chemical oxygen demand (COD) fractionation, i.e., the characterization of substrate chemically degradable fractions in terms of COD, and (iii) the description of the disintegration kinetic behavior.

Basic composition data, i.e., moisture, carbohydrates, proteins, lipids, and ash content, provided by USABLE Packaging partners or extracted from food databases is employed as input to obtain the substrate fractionation factors on COD (f_i [=] g i-COD · g total-COD⁻¹) for particulate (f_{XC}) and soluble compounds (f_S), carbohydrates (f_{CH} and f_{SCH}), proteins (f_{PR}), lipids (f_{LI}) and inert (f_I). Given their different hydrolysis rates into sugars (García-Gen et al., 2015), carbohydrates are decoupled into rapidly biodegradable (CH, e.g., starch) and slowly biodegradable (sCH, e.g., cellulose). Their fractions on COD basis (f_{CH} and f_{SCH}) are calculated assuming a carbohydrates COD factor of 1.18 g COD/g, while protein fraction (f_{PR}) is calculated according to their AA composition. As some substrates could already contain most of its organic matter partially or totally solubilized, COD is also fractionated into particulate (f_{XC}) and soluble (f_S) components. Both fractions are assumed to have the same carbohydrates, proteins, and lipids composition, i.e., a particulate substrate which is partially solubilized would have the same proportion of carbohydrates/sugars or proteins/aminoacids in both particulate and soluble fraction. However, when complex organic substrates such as household food waste are considered, particulate and soluble fractions can have different proportions of carbohydrates/sugars or proteins/aminoacids and thus, a characterization of these fractions may be necessary.

Substrate specific kinetic parameters (e.g., kinetic parameter for the disintegration of particulate matter into macromolecule compounds) were gathered through a literature review of anaerobic digestion and MCF studies. For that purpose, the following query string was introduced in Scopus web search engine: 'TITLE-ABS-KEY (((anaerobic AND digestion) OR (anaerobic AND fermentation) OR (VFA AND production)) AND ((food AND waste) OR (solid AND waste) OR (complex AND organic AND substrates) OR (fruits AND vegetables) OR (wastewater)) AND kinetic)'. Among the document results sort by relevance, those containing information regarding the disintegration of particulate substrates and kinetic parameters were selected. The procedure to choose disintegration kinetic parameters follows a hierarchical order: (1) parameters that are specific to a particular substrate in our library, (2) parameters of substrates with similar composition and physicochemical characteristics, (3) general parameters for complex organic wastes.

Besides, substrate library can be easily expanded to any complex organic substrate (if there is no data available) following the method proposed by (Fisgativa et al., 2020) for the characterization of complex organic substrates and their representation as model variables for the anaerobic digestion model N°1.

2.2. Mathematical model

The mathematical model follows an integral approach, i.e., the kinetic model which describes the production of the different VFA (acetate, propionate, butyrate and valerate), hydrogen, methane and other subproducts (e.g., ammonia) during the MCF of complex organic substrates is coupled to a bioenergetic model which predicts the acidogenic stoichiometry in function of substrate composition and operational conditions (Regueira et al., 2021). In subsequent subsections the kinetic

model structure and biochemical reaction involved are presented, while the insights on how the bioenergetic model is integrated into the kinetic model are provided later in Section 3.2.

2.2.1. Kinetic model structure and mass balances

The kinetic model is built on the mass balances in a continuous stirred tank reactor (CSTR) of the thirty different compounds which make up the states of the model. Two different compartments are considered: reactor bulk and gas phase. The 30 states represent the mass hold-up of different soluble compounds (13), macromolecule compounds (5), particulate compounds (3), gaseous compounds (3) and biomass (6). The mass balances of the system are defined by the following equations (Eqs. (1)–(3)).

Soluble, macromolecule and particulate compounds

$$\frac{dS_i}{dt} = D_{liq} \cdot (S_{i,inlet} - S_i) + R_i + R_{i,T} \tag{1}$$

where S_i is the concentration (g COD·L⁻¹), D_{liq} is the dilution rate of the liquid fraction (d⁻¹), R_i and $R_{i,T}$ are, respectively, the reaction rate and liquid–gas transport rate of volatile compounds (g COD·L⁻¹·d⁻¹).

Biomass

$$\frac{dX_i}{dt} = -D_{liq} \cdot X_i + R_{i,ana} - R_{i,decay} \tag{2}$$

where X_i is the biomass concentration (g COD·L⁻¹), $R_{i,ana}$ is the anabolism rate and $R_{i,decay}$ the decay rate.

Gas compounds

$$\frac{dG_i}{dt} = -D_{gas} \cdot G_i + R_{i,T} \tag{3}$$

where G_i is the concentration (g COD·L⁻¹) and D_{gas} is the dilution rate of the gas fraction (d⁻¹). The related concentrations and processes are referred with respect to the headspace volume of the reactor (V_{gas}).

The kinetic model of the reactor is solved as a system of 30 non-linear algebraic equations by integration with MATLAB (R2021a) command ode15s to steady state (pseudo time stepping). Steady state was assumed when all the state absolute derivatives values were under 1·10⁻¹² g COD·L⁻¹·d⁻¹.

2.2.2. Biochemical processes

This kinetic model covers all biochemical reactions from the disintegration of particulate compounds to the acidogenesis, including also the acetogenesis and methanogenesis to evaluate the risk of part of the COD being converted to methane, possibly decreasing the VFA yield (Fig. 1). Biomass decay, degradation of composites into macromolecules, and their hydrolysis into monomers, which are extracellular reactions, follow a first-order kinetics (Eq. (4)). The considered intracellular biochemical reactions are divided in several sets: (i) acidogenesis from sugars, (ii) acidogenesis from aminoacids, (iii) acetogenesis from long chain fatty acids (LCFA), (iv) acetogenesis from propionate, (v) acetogenesis from butyrate and valerate, (vi) acetoclastic methanogenesis, and (vii) hydrogenotrophic methanogenesis. These reactions are considered to follow Monod kinetics (Eq. (5)), with the inclusion of an inhibition term. Several mechanisms of inhibition and kinetic control were considered: (i) pH inhibition, which affects all intracellular processes (with different severity for acidogens and acetogens, hydrogenotrophic methanogens, and acetoclastic methanogens); (ii) free ammonia and hydrogen inhibition, which affect acetoclastic methanogens and acetogens respectively; (iii) substrate (butyrate and valerate) uptake competition (for C4 biomass); (iv) inorganic nitrogen limitation, which is modelled as a secondary substrate inhibition and affects all uptake reactions. A detailed and comprehensive description of stoichiometric matrix, kinetic rate equations, parameters and inhibition forms is available in Supplementary materials.

Integration of particulate components to the acidogenesis, including also the acetogenesis and methanogenesis to evaluate the risk of part of the COD being converted to methane, possibly decreasing the VFA yield (Fig. 1). Biomass decay, degradation of composites into macromolecules, and their hydrolysis into monomers, which are extracellular reactions, follow a first-order kinetics (Eq. (4)). The considered intracellular biochemical reactions are divided in several sets: (i) acidogenesis from sugars, (ii) acidogenesis from aminoacids, (iii) acetogenesis from long chain fatty acids (LCFA), (iv) acetogenesis from propionate, (v) acetogenesis from butyrate and valerate, (vi) acetoclastic methanogenesis, and (vii) hydrogenotrophic methanogenesis. These reactions are considered to follow Monod kinetics (Eq. (5)), with the inclusion of an inhibition term. Several mechanisms of inhibition and kinetic control were considered: (i) pH inhibition, which affects all intracellular processes (with different severity for acidogens and acetogens, hydrogenotrophic methanogens, and acetoclastic methanogens); (ii) free ammonia and hydrogen inhibition, which affect acetoclastic methanogens and acetogens respectively; (iii) substrate (butyrate and valerate) uptake competition (for C4 biomass); (iv) inorganic nitrogen limitation, which is modelled as a secondary substrate inhibition and affects all uptake reactions. A detailed and comprehensive description of stoichiometric matrix, kinetic rate equations, parameters and inhibition forms is available in Supplementary materials.

$$\rho_i = k_i \cdot X_i \tag{4}$$

$$\rho_i = \frac{k_{max} \cdot S_i}{K_S + S_i} \cdot X_i \cdot I \tag{5}$$

where ρ_i is the specific consumption rate (g COD·g COD biomass⁻¹·d⁻¹), ρ_{max} is the maximum specific consumption rate (g COD·g COD biomass⁻¹·d⁻¹), S_i is the soluble component concentration (g COD·L⁻¹), K_S is the half-saturation constant (g COD·L⁻¹), and X_i is the biomass concentration (g COD biomass·L⁻¹), and I is the inhibition term.

2.3. Indicators to support decision-making

Mathematical model outputs must be transformed into easy interpretable indicators that provide valuable information to both expert and non-expert user, since these indicators determine the technical, environmental, and economic performance of MCF processes. The following indicators were considered relevant here:

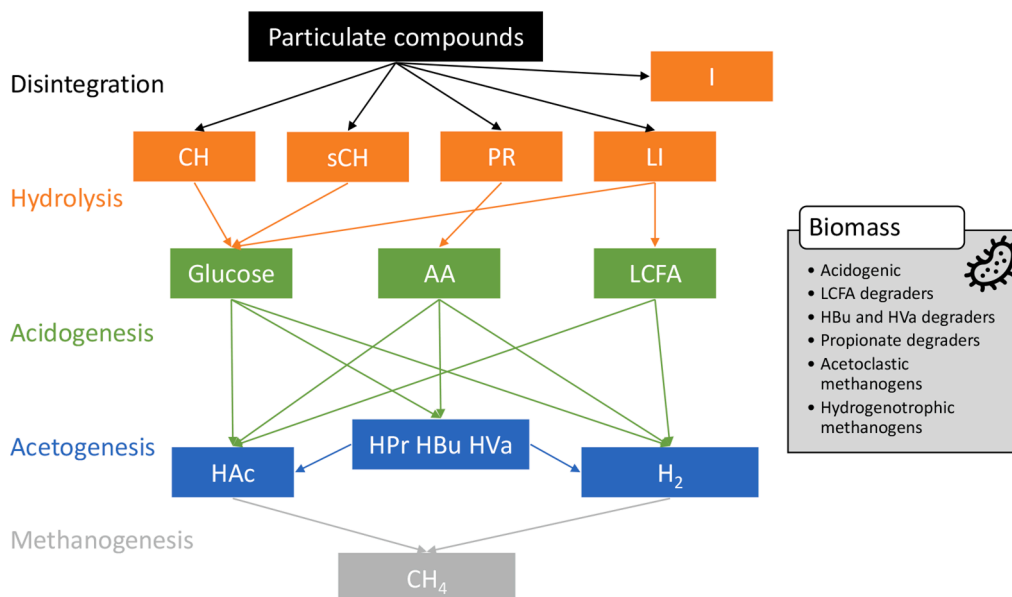


Fig. 1. Biochemical reactions of the MCF of complex organic substrates.

i) Substrate conversion (%), which measures what percentage of inlet COD is converted into biomass and products and indicates substrate biodegradability.

ii) VFA yield (g VFA-COD·g feed-COD⁻¹) and productivity (g VFA-COD·L⁻¹·d⁻¹) indicate the ratio of substrate converted to VFA and the amount of VFA produced per volume and time, respectively, and are relevant indicators to reduce capital costs.

iii) Selectivity measures the fraction of each VFA within the spectrum (e.g., g COD-acetate·g COD-VFA⁻¹) for targeted production of a given VFA.

iv) As VFA can be employed as precursor for PHA production an additional equivalent hydroxyvalerate (HV) ratio indicator is proposed which measures the molar ratio of precursors of HV, i.e., acetate plus propionate and valerate, within the total precursors of both HV and hydroxybutyrate (HB), i.e., acetate plus acetate and butyrate.

3. Computer-aided design tool utility assisting the process early-design

Following the proposed framework, a modular tool for computer-aided design was implemented. The purpose of this section is to provide the results of the built substrate library (Section 3.1), insights on how these modules are interconnected (Section 3.2), and present a showcase of applications of this computer-aided design tool (Sections 3.3 and 3.4), e.g., screening substrates as potential precursors of VFA that are employed to produce PHA.

3.1. Substrate library

The substrate library (Table 1) covers a wide range of substrates with diverse compositions and characteristics. Additionally, it can be easily expanded to any kind of complex organic substrates following the method described in Section 2.1. The substrate library majorly contains

fruit and vegetable wastes (spinach, cucumber, tomato, and lettuce), food industry byproducts (regrind pasta, wheat bran and bread crust) and side streams (vinasses and tuna canning wastewater). Kinetic parameters for disintegration were chosen according to criteria established in Section 2.1: a value of 1.7 d⁻¹ for fruit and vegetable wastes (García-Gen et al., 2015), 0.41 d⁻¹ for wheat byproducts and vinasses (Vavilin et al., 1998), and 1.4 d⁻¹ for fishery wastes (García-Gen et al., 2015).

3.2. Solution strategy: Bioenergetic model integration into the kinetic model

To account for the well-known variability of the acidification stoichiometry with respect to substrate composition and operating conditions the yields of acidification products are estimated by a bioenergetic model which determines the most likely metabolic pathways in MCF and has been already validated for the cofermentation of sugars and aminoacids (Regueira et al., 2021). The bioenergetic model is integrated with the kinetic model by providing the stoichiometry of the acidification products given the acidification substrates, i.e., the fraction of the substrate that can be directly acidified and the products of hydrolysis (Fig. 2).

For a given substrate (or substrate mixture) and operational conditions (i.e., pH and HRT), in first place, the information related to its fractioning in terms of components (i.e., soluble fraction, rapidly and slowly biodegradable carbohydrates, proteins, lipids, inert and aminoacids molar composition) and its disintegration kinetics is taken from the substrate library. After that, acidogenic stoichiometric coefficients, which do not depend on disintegration and hydrolysis, are determined considering only the pH, substrate(s) aminoacids composition and the actual consumed fraction of sugars and aminoacids (HRT is not considered since its influence on acidogenic stoichiometry is negligible). However, as the different organic fractions comprising the wastes can have different fractioning between soluble and particulate matter, with

Table 1

Substrate library comprised by basic characterization of substrates (TS, VS, TCOD) and inputs to the kinetic model (COD fractionation, AA molar fraction composition and disintegration kinetic parameter). Acronyms: S: spinach, C: cucumber, T: tomato, L: lettuce, V: vinasses, RP: regrind pasta, WB: wheat bran, BC: bread crust, TWW: tuna canning wastewater.

Substrate	S	C	T	L	V	RP	WB	BC	TWW
Gross substrate characterization									
TS (% ww)	28.0	3.0	4.2	4.0	49.0	94.0	91.0	93.0	1.0
VS (% ww)	28.0	2.7	3.4	3.5	40.0	74.0	74.0	75.0	1.0
tCOD (g COD·L ⁻¹)	35.8	33.3	48.5	43.0	485.1	896.0	941.9	968.2	15.2
Disintegration kinetic parameters									
k _{dis} (d ⁻¹)	1.7	1.7	1.7	1.7	0.42	0.42	0.42	0.42	1.4
Substrate fractionation									
f _{xc}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.26
f _s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74
f _{ch}	0.40	0.49	0.43	0.56	0.10	0.78	0.27	0.70	0.00
f _{pr}	0.29	0.26	0.19	0.27	0.09	0.15	0.18	0.10	0.92
f _{sCh}	0.22	0.22	0.11	0.14	0.75	0.02	0.41	0.05	0.00
f _l	0.10	0.04	0.28	0.04	0.07	0.05	0.14	0.15	0.08
Aminoacid composition									
Arginine (% molar)	5.1	6.5	2.0	5.0	7.1	2.1	2.1	2.1	4.8
Alanine (% molar)	8.8	6.9	4.9	7.5	7.9	4.0	4.0	4.0	9.5
Aspartate (% molar)	1.0	3.1	7.9	5.0	0.9	0.6	0.6	0.6	9.7
Lysine (% molar)	6.6	5.1	3.0	6.9	2.6	0.9	0.9	0.9	8.8
Glutamate (% molar)	3.8	9.8	16.6	4.6	8.4	2.1	2.1	2.1	12.8
Serine (% molar)	5.5	4.9	4.0	4.5	7.8	6.5	6.5	6.5	5.5
Threonine (% molar)	5.7	4.1	3.7	5.9	3.9	3.8	3.8	3.8	5.2
Cysteine (% molar)	2.2	1.2	1.6	2.3	3.0	1.5	1.5	1.5	1.7
Glycine (% molar)	9.9	8.2	4.1	9.4	6.6	12.2	12.2	12.2	9.0
Proline (% molar)	5.4	3.3	2.1	5.0	4.8	15.2	15.2	15.2	4.3
Valine (% molar)	7.6	4.8	2.5	7.2	4.0	3.8	3.8	3.8	6.2
Isoleucine (% molar)	6.2	4.1	2.2	7.7	1.1	3.1	3.1	3.1	4.9
Leucine (% molar)	9.4	5.7	3.1	7.2	2.7	6.2	6.2	6.2	8.7
Methionine (% molar)	2.5	1.3	0.8	1.7	4.7	0.8	0.8	0.8	3.6
Glutamine (% molar)	9.2	24.6	31.3	10.6	15.7	34.9	34.9	34.9	1.4
Asparagine (% molar)	9.0	4.9	8.6	7.9	14.8	1.1	1.1	1.1	1.1
Histidine (% molar)	2.3	1.7	1.5	1.7	4.0	1.4	1.4	1.4	2.7

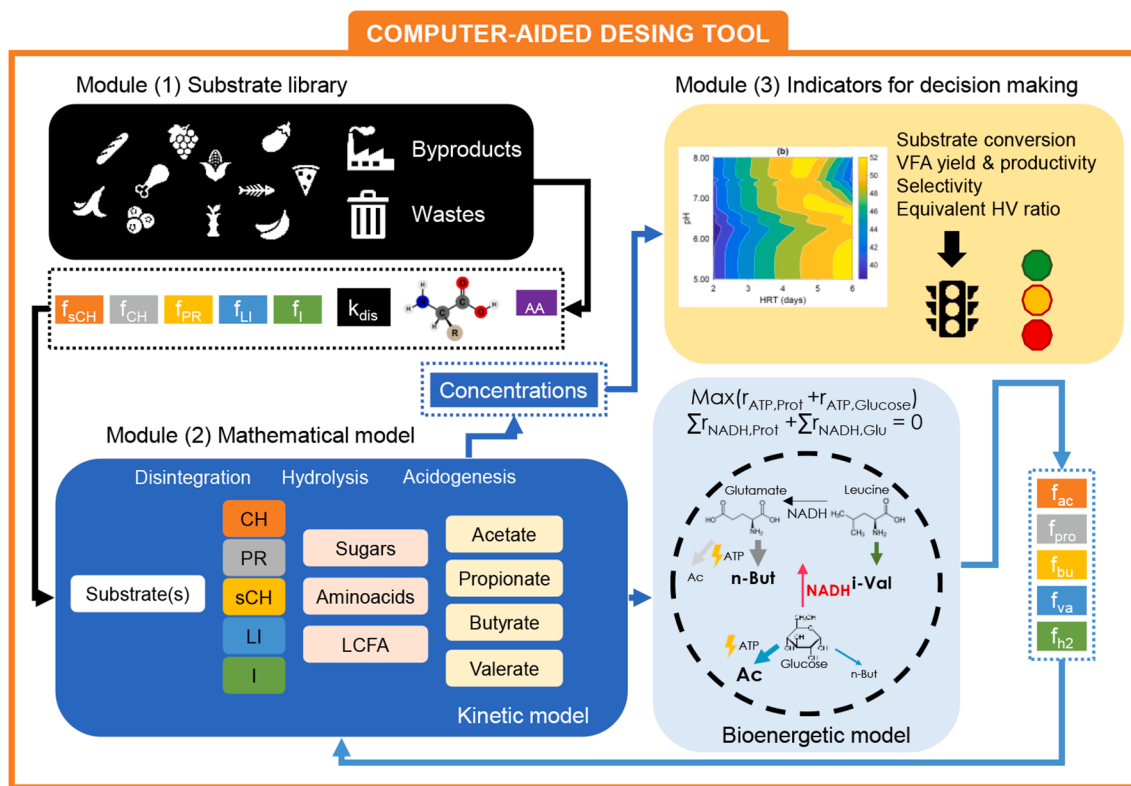


Fig. 2. Structure of computer-aided design tool developed in this research.

intrinsic distinct disintegration kinetics, the resulting macromolecules are also hydrolyzed at different rates. All of this makes determining *a priori* the consumed sugars and AA in the acidification step a non-obvious task.

To solve this challenge, a preliminary run of the kinetic model coupled with a mass balance function which determines the net consumption of monomers (glucose and aminoacids) to the bioenergetic model is carried out. When only one substrate is fermented, this function calculates the net consumption of monomers in the MCF. If two substrates are fermented, then this function estimates the corresponding net fractions of consumed AA, and after that, determines the AA mixture that is acidified in MCF (an example is included in Fig. 3).

Once the function calculates the corresponding net fractions of consumed monomers, the yields of acidification products are estimated by the bioenergetic model. To determine the product spectrum, the bioenergetic model main hypothesis states that the most efficient microorganisms harvesting energy from the substrate are dominant in an anaerobic mixed microbial culture in a CSTR. Namely, microorganisms employ the metabolic branches that yield the maximum net ATP, i.e., the sum of the ATP produced in catabolism and the ATP spent in the active transport of compounds, ensuring a neutral net electron balance (Table 2), since anaerobic process lack external electron acceptors that could act as electron sinks (Regueira et al., 2020b). Once the process stoichiometry is determined, one function transforms the bioenergetic model outcome into the stoichiometric coefficients needed by the kinetic model and loads them into the stoichiometric matrix. Finally, the kinetic model is solved again considering the determined stoichiometric coefficients and the concentrations at steady state are transformed into easy interpretable indicator to assist decision-making: substrate conversion, VFA yield, productivity, and selectivity.

3.3. Steering the VFA spectrum by modifying operational conditions: A case study on MCF of regrind pasta

Regrind pasta is a byproduct of pasta production, which is currently

employed as animal feed. As carbohydrate-rich substrate, it could be a suitable substrate to produce VFA and therefore the application of the developed tool can support the selection of the appropriated values for the operational conditions. A fast evaluation of the range of MCF design parameters, i.e., pH and HRT, was carried out. HRT and pH ranges were from 2 to 6 days and, from 5 to 8 respectively according to MCF literature (Bevilacqua et al., 2020; Domingos et al., 2017; Strazzera et al., 2018). Then, the program was run for these design parameters ranges and model outputs were transformed into a map for each indicator defined in Section 2.3 (Fig. 4). Employing an Intel(R) Core (TM) i5-8250U (@3.4 GHz) the average time employing per simulated pH and HRT was 400 s.

Regrind pasta is mainly composed by rapidly biodegradable carbohydrates (78% of COD) such as starch, which are hydrolyzed at higher rates than other macromolecules. As both disintegration and hydrolysis are the MCF limiting steps and independent to pH, substrate conversion map (Fig. 4a) only shows dependency on the HRT. Substrate conversion map also confirms the good biodegradability degree of regrind pasta. Regarding the VFA yield (Fig. 4b) and productivity (Fig. 4c), both maps show a dependency on the HRT and pH, especially at neutral to basic pH. Operating at a high HRT (>5 days) and a neutral to basic pH (from 6.5 to 8) leads to methanization (see methanization graph enclosed in Supplementary materials), while acidic pH values inhibit the methanogenesis and thus, enhance the VFA yield. Therefore, it is preferable to operate at low HRT, which also implies a higher productivity due to an increased feeding flow rate.

With regards to the equivalent HV precursors ratio (Fig. 4d), high values of HV are obtained at neutral pH values. In this region, the VFA spectrum is dominated by both acetate and propionate, which are precursors of HV, and whose production is favored by this pH range. However, highest values, which are obtained at high HRT (>5 days), must not be considered since VFA concentration is rather low due to methanization (see total VFA concentration graph enclosed in Supplementary materials). HV ratio values at acid pH are low due to these conditions favor the butyrate production, which is a precursor of HB. If

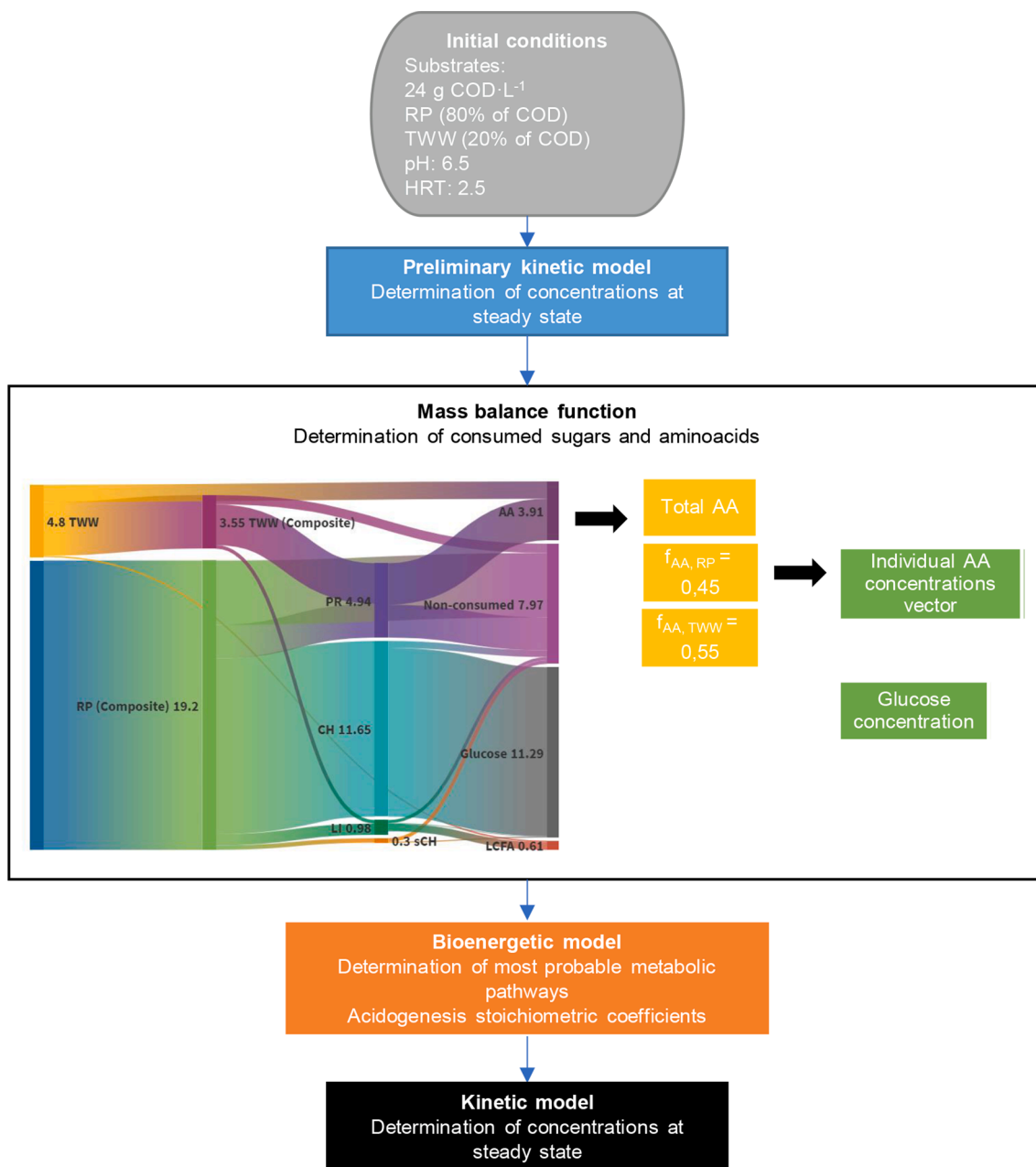


Fig. 3. Diagram flow for mathematical model solution strategy. MCF design parameters: pH 6.75, HRT of 3.5 days, and a substrate concentration of 24 g COD·L⁻¹ (80% RP, 20 % TWW). Numbers appearing in the Sankey diagram have units of g COD·L⁻¹.

Table 2
Bioenergetic model formulation.

Premises	Formula	Equation
ATP net production	$r_{ATP} = r_{transport} + r_{PMF} + r_{catabolism}$	(6)
ATP maximum production (objective function)	$\max(r_{ATP,AA} + r_{ATP,glucose})$	(7)
NADH conservation (restriction)	$\sum r_{NADH,AA} + r_{NADH,glucose} = 0$	(8)
Yield of each metabolic branch (optimization variable)	$0 \leq z_{ij} \leq 1$ $\sum_j z_{ij} = 1, i = 1, \dots, n_{substrates}$	(9)

obtaining a VFA spectrum with high content of equivalent HV precursors was a priority, then best optimal conditions would be a neutral pH (6.5–7) and a relatively low HRT (3–4 days) to prevent methanogenesis and sustain a substantial VFA yield (Fig. 4b).

To understand better the fate of available COD in the substrate, Fig. 4e displays a Sankey diagram with the mass balance of the MCF simulation of regrind pasta to obtain at least a 40% of equivalent HV precursors. Most of VFA come from the acidogenesis of sugars, while hydrolysis is the limiting step for VFA production from proteins. Similarly, sCH fermentation is limited by their hydrolysis rate. However, it does not suppose a drawback to produce VFA since these two components have a low fraction in regrind pasta. Therefore, from the model results it can be inferred that those wastes mainly composed of particulate protein or sCH may not be suitable for VFA production or will need a pretreatment step to increase its biodegradability.

Both the Sankey diagram (Fig. 4e) and the stoichiometric model provide valuable insights regarding the formation of HV precursors: (i) acetate ($f_{ac} = 0.20$) and propionate ($f_{pro} = 0.19$) production are favored by neutral pH and, (ii) valerate production was negligible ($f_{va} = 0.01$) since the percentage of protein in the acidified substrate was also low

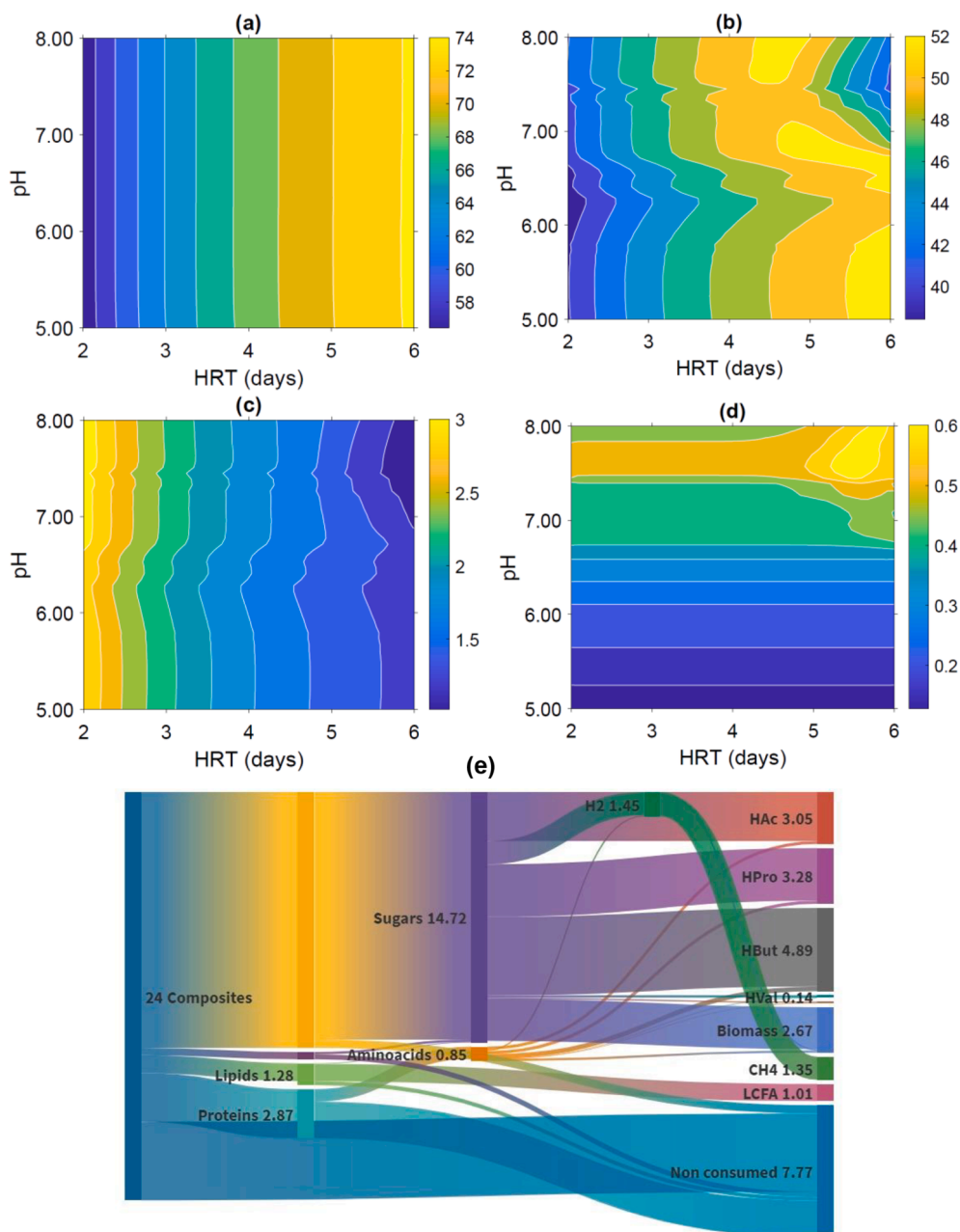


Fig. 4. Indicators for the simulation of MCF of regrind pasta, $24 \text{ g COD}\cdot\text{L}^{-1}$. (a) Substrate conversion (%), (b) VFA yield ($\text{g VFA}\cdot\text{COD}\cdot\text{g feed}\cdot\text{COD}^{-1}$), (c) productivity ($\text{g VFA}\cdot\text{COD}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$), (d) equivalent HV ratio ($\text{mol precursors HV}\cdot\text{mol precursors HB and HV}^{-1}$), (e) Sankey diagram which accounts the mass balance of the MCF of regrind pasta at pH 6.75, HRT of 3.5 days. Numbers appearing in the diagram have units of $\text{g COD}\cdot\text{L}^{-1}$.

(5% total of acidified substrate-COD).

3.4. How can substrates be mixed to produce a targeted profile of VFA? A case study on cofermentation of regrind pasta and tuna canning wastewater

Obtaining the desired HV ratio is not possible with regrind pasta. A strategy to increase the equivalent HV ratio could be to mix regrind pasta with a soluble substrate with a high content in valerate precursors, i.e., a protein-rich substrate with high content in arginine, proline, and leucine/isoleucine. Given substrates from Table 1, canning tuna wastewater could be a potential substrate to mix with, since is majorly soluble ($f_S = 0.74$) and composed by proteins ($f_{PR} = 0.92$) with a considerable content in AA precursors of valerate, i.e., 5, 4 and 14% on molar basis of arginine, proline, and leucine/isoleucine, respectively. Following

Section 3.3 results, HRT was fixed at a value of 3 days, and a map of pH conditions (from 5 to 8) and substrate mixing ratio was performed to evaluate if the cofermentation of regrind pasta and tuna canning wastewater could boost the content of equivalent precursors of HV within the VFA spectrum (Fig. 5). Employing an Intel(R) Core (TM) i5-8250U (@3.4 GHz) the average time employing per simulated pH and substrate mixing ratio was 300 s.

Tuna canning wastewater has a sensible higher biodegradability than regrind pasta (Fig. 5a), as it is majorly composed of solubilized organic matter (74% of COD). Analyzing both Fig. 5b and c, both pH and substrate composition affect VFA yield and productivity. A higher fraction of hydrogen (up to 15% of COD fermented by acidogenic bacteria) is obtained when MCF is performed at acidic pH values and the substrate has a higher content on carbohydrates, i.e., regrind pasta, and thus, both VFA yield, and productivity decrease for this sugar-rich substrate.

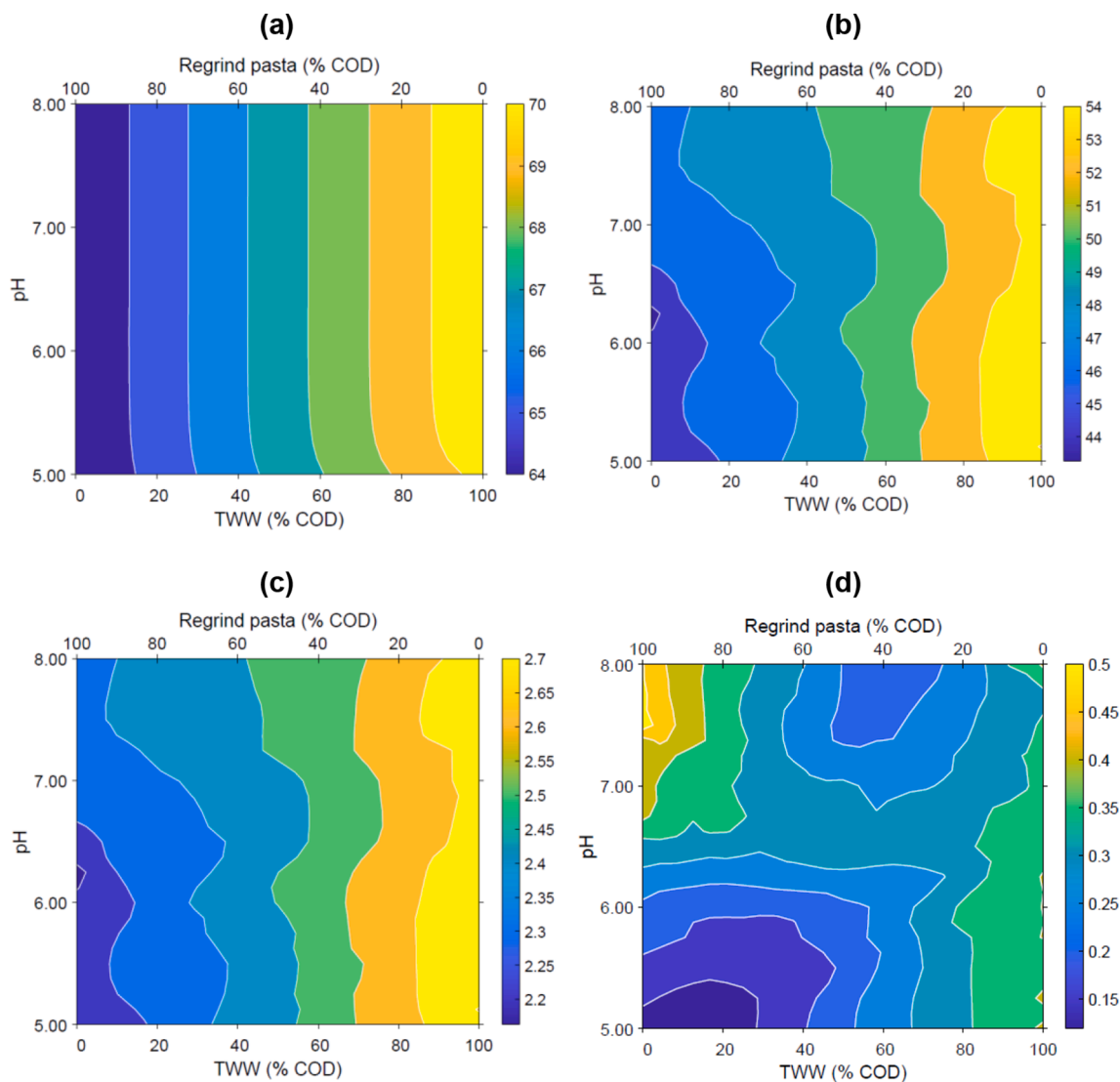


Fig. 5. Indicators for the simulation of MCF of regrind pasta, substrate concentration of $15 \text{ g COD}\cdot\text{L}^{-1}$ and HRT of 3 days. (a) Substrate conversion (%), (b) VFA yield ($\text{g VFA}\cdot\text{COD}\cdot\text{g feed}\cdot\text{COD}^{-1}$), (c) productivity ($\text{g VFA}\cdot\text{COD}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$), (d) equivalent HV precursors ratio ($\text{mol equivalent HV/mol equivalent HB}\cdot\text{HV}^{-1}$).

As showed in Fig. 5d, equivalent HB precursors dominate the VFA spectrum at (i) acidic pH conditions ($\text{pH} < 6$) and high to low regrind pasta ratio on COD basis (from 15 to $5 \text{ g COD}\cdot\text{L}^{-1}$ of $15 \text{ g mixture}\cdot\text{COD}\cdot\text{L}^{-1}$), and (ii) neutral to basic pH conditions (from 7 to 8) and medium to low regrind pasta ratio (from 9 to $4 \text{ g COD}\cdot\text{L}^{-1}$ of $15 \text{ g mixture}\cdot\text{COD}\cdot\text{L}^{-1}$). Given that butyrate production is clearly favored at acid pH, and since valerate depends majorly on the presence of its precursors, i.e., arginine, proline, and leucine/isoleucine (see valerate concentration graph in [Supplementary material](#)), equivalent HV ratio can be only boosted by increasing the TWW content. When mixing regrind pasta and TWW at similar concentrations and neutral to basic pH, acetate production is favored, since glucose stoichiometry is shifted towards acetate production as this pathway yields the maximum ATP and the surplus of NADH produced is consumed in the fermentation of AA supplied by the TWW. This is clearly shown in Fig. 5d, since equivalent HV ratio is substantially lower than for monofermentation of regrind pasta or TWW.

As a conclusion, cofermentation of TWW and regrind pasta yields, as major products, acetate at neutral to basic pH values and butyrate at acidic pH values. The motivation for this cofermentation, i.e., increasing the HV indicator, cannot be reached by blending these two substrates as their interactions shift the fate of HV precursors. It is seen that acidification of real substrates follows interactive mechanisms that cannot be

captured by a “naïve approach” by adding up the individual contributions. The complexity of the process outcome highlights the usefulness of the proposed tool for screening substrates and operational conditions.

4. Discussion

The proposed framework, which is implemented as a computer-aided design tool, goes further than prior studies on modelling of MCF and offers multiple advantages. The mathematical model predicts the acidogenic stoichiometry on the MCF of organic wastes regarding the pH, HRT and substrate composition. Furthermore, its modular configuration enables the modification of each module without altering the entire tool. For instance, it could be feasible to add a layer that estimates the CAPEX and OPEX or a new composite or reaction to the mathematical model. This approach also empowers practitioners to use the tool regardless their expertise level and thus, fosters the connections between different actors throughout the carboxylate platform. However, the results must be taken as a first evaluation of its potential and further confirmed by experimental tests. Moreover, these experimental tests can, in turn, be designed using this tool. Regarding the limitations of the bioenergetic model, it is implemented for a CSTR configuration and does not assess the lipid cofermentation. Thus, its reliability when employing

other reactor configurations or screening lipid-rich wastes must be considered. Another shortcoming is the lack of description of chain elongation reactions, which are a means to obtain more valuable products from VFA. Unfortunately, the drivers of chain elongation are not sufficiently known (Bevilacqua et al., 2021b; Wang et al., 2020) and therefore, chain elongation reactions are not included in this model.

Through the insights provided by the computer-aided design tool, practitioners can gather information on the potential feedstock and establishing relations with raw materials providers, and thus, boosting the implementation of circular economy-based value chains by bridging development risks, allowing early-stage evaluation of process performance. Food industry actors can study the feasibility of their wastes to be integrated into the carboxylate platform perspective in a resource recovery perspective, i.e., assessing wastes potential to be valorized as VFA. Supported by the set of indicators developed in Section 2.3, they can perform a preliminary screening of their wastes' potential. As demonstrated in Section 3.3, regrind pasta could be a suitable substrate to produce VFA, which can be further processed into PHA and finally converted into pasta packaging. Considering that Italy produces 3.6 million tones (67% of EU pasta production) (EUROSTAT, 2018) and 24.4 g of regrind pasta are generated per kg of pasta produced (EPD International AB, 2019), PHA production from regrind pasta through carboxylate platform could fulfill 20% of total required packaging (assuming an overall yield of 55% in the PHA production and downstream). Therefore, it could be possible to establish a circular economy strategy that could reduce the dependency on oil-based plastics.

In process development, it is possible to explore the space of operational conditions, in particular pH and HRT when designing processes for the valorization of organic wastes in VFA, facilitating the experimental validation at pilot or demonstration scale. Furthermore, experimental data can be employed to calibrate and optimize the computer-aided design tool. Likewise, in process optimization, the tool can assist in finding co-substrates for an existing VFA. Namely, a retrofitting function which selects the suitable co-substrates to achieve a determined VFA spectrum could be easily implemented. For instance, pasta producers in collaboration with R&D institutions and companies could study the optimal operational conditions for their substrates.

PHA producers can employ the computer-aided design tool to solve the supply chain problem: screening potential substrates to obtain a suitable VFA mixture that can be further transformed into a PHA material with specific properties. By using the computer-aided design tool, concretely the substrates library, they could find a food industry actor that provides them a suitable substrate. In prospective cost and environmental assessment of novel processes, MCF design parameters and mathematical model inputs/outputs can be employed in the preliminary scale-up of the MCF process and the life cycle inventory construction for the life cycle cost and life cycle assessment.

Therefore, the knowledge integration of different tools, such as the developed under the current framework, can help to move from the evaluation and optimization of individual processes to the global vision. Concretely, through sharing knowledge generated by the computer-aided design tool among stakeholders, it can be possible to help identifying technical, environmental, and economic obstacles and constraints along the whole value chain, and thus, giving recommendations and proposals during the early development stages when there are still opportunities to use environmental guidance for major alterations (Arvidsson et al., 2018).

5. Conclusion

As conclusion, valuable insights are extracted from the discussion carried out: (i) the identified gaps regarding the disintegration and hydrolysis mechanisms and the acidogenic stoichiometry variability in the MCF of these complex organic substrates are completely addressed, (ii) tool utility for the screening of potential substrates to produce VFA and optimizing design parameters under both monofermentation and

cofermentation of complex organic substrates is demonstrated in Sections 3.3 and 3.4; (ii) as discussed in Section 4, it is demonstrated that tool applications foster circular economy through resource recovery and connect multiple levels of stakeholders in the value chain with different interests and expertise levels, from those involved in the optimization and design of acidification processes to those studying the feasibility of valorizing wastes from an economic and environmental perspective.

Under this research, it is demonstrated that data driven models can help to assist the decision making at different stakeholders' levels, identifying technical bottlenecks, and proposing innovative solutions prior to expensive lab research and piloting.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2022.02.008>.

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