



# Effect of the substrate/microorganism ratio on the anaerobic production of carboxylic acids from residual glycerol

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## Abstract

The production of carboxylic acids and 1,3-propanediol was assessed from the anaerobic digestion of residual glycerol from biodiesel production to investigate the influence of substrate/microorganism ratio on the bioproduct profile and the kinetic of the process through the application of different mathematical models to the data. So, the experiments were performed in batch reactors for 28 days, and four substrate/microorganism ratios were tested (0.7, 1.1, 1.5, and 2.1 gCOD gVSS<sup>-1</sup>). About 64–77% of the initial organic matter estimated by the chemical oxygen demand of residual glycerol was converted into carboxylic acids and 1,3-propanediol (substrate/microorganism ratio 1.5 showed the highest yield). In addition, acetic acid was the bioproduct formed in the highest concentration for all experiments. Propionic acid had a higher selectivity in the substrate/microorganism ratio 1.5 and butyric acid and 1,3-propanediol in the ratio 2.1. About kinetics results, exponential models were better suited to describe process kinetics. However, sigmoidal models have also shown good fit, making it possible to correlate the substrate/microorganism ratio with velocity constant, maximum productivity rate, and lag phase time.

**Keywords** Anaerobic digestion · Carboxylic acids · Kinetic modeling · Residual glycerol · Substrate/microorganism ratio

## Introduction

Biodiesel is one of the most important biofuels to reduce the demand for petroleum-based fuels, with an estimated world production of around 3.7.10<sup>10</sup> L year<sup>-1</sup> in 2020 (OECD/FAO 2016). In its production process, the transesterification of oilseeds and animal fats generates residual glycerol (RG) as a by-product in the proportion of 10% (mass of glycerol/mass of biodiesel), whose purity ranges from 55 to 90%, making it difficult to industrial use (Leng et al. 2017; Veras et al. 2019). So, RG is the major component of wastewater from biodiesel production, which contains high chemical oxygen demand (COD) (1023–1900 gCOD L<sup>-1</sup>) (Silva et al. 2017; Dams et al. 2018).

The glycerol has several industrial applications, such as the production of cosmetics, automotive paints, food, medicines, cellulose and paper, leather, and textiles. However, it is economically unfeasible to purify all the RG generated, making it a waste, which results in an additional cost in biodiesel production due to its disposal (Saini et al. 2017). Therefore, it is important to analyze biotechnological platforms that convert RG into value-added products. In this context, RG presents itself as a promising raw material for biological processes, such as anaerobic digestion (AD), a technology considered as a low-cost alternative technique to convert solid and liquid wastes into products with high added value, such as methane (CH<sub>4</sub>), hydrogen (H<sub>2</sub>), alcohols, and carboxylic acids (CA) (Saini et al. 2017). Among these compounds, CA stands out due to its high gross added value and several industrial applications, such as the production of varnishes, paints, perfumes, disinfectants, surfactants, textile auxiliaries, medicines, and food products (Du et al. 2015).

Traditional AD converts the complex substrate into methane. However, when the focus is on the production of intermediate compounds, such as CA and alcohols, the methanogenesis step must be inhibited. The longer the CA chain,

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the higher its added value. Among the alcohols produced from RG bioconversion, 1,3-propanediol (1,3-PD) stands out due to its high yield and many applications in polymer manufacturing (Pan et al. 2019). Many types of research have been conducted with the acidogenic fermentation of agro-industrial wastes to better understand the biochemistry of this bioproduct formation, the process limitations, the forms of extraction/purification, and the kinetic processes involved (Silva et al. 2020).

The RG has a low COD/BOD ratio (approximately 1.1), indicating ease biodegradation. However, the direction of the metabolic pathway in acidogenic fermentation is influenced by several factors such as type of substrate and inoculum, environmental conditions (pH, temperature, nutrients, presence of toxic or inhibitory compounds etc.), and operational parameters (organic loading rate—OLR, hydraulic retention time—HRT, and substrate/microorganism ratio—S/X) (De Groof et al. 2019). Moreover, some of these parameters, like the optimum S/X ratio, are varied among the substrates (Reungsang et al. 2016). There are some studies about S/X ratio influence on maximum biogas formation by using different substrates such as food waste, a mixture of food waste and green waste, and RG (Elbeshbishy et al. 2012; Liu et al. 2009; Pereira et al. 2019). However, studies about CA production in different S/X ratios are rather scarce in the literature, and no report concerning the use of RG was found.

In addition, mathematical modeling is an alternative that can provide a better understanding of AD process, being a widely used tool to simulate bioreactors' performance (Mousavian et al. 2019). Mathematical models estimate important kinetic parameters to design and operate biological treatment plants more efficiently, promoting improvements in systems and thus generating significant economic payback (Abou-Elela et al. 2016). These tools are already studied for traditional AD, mainly to optimize the production of biogas from organic waste (Çetinkaya and Yetilmezsoy 2019). However, few studies on kinetic modeling evaluate the acidogenic fermentation of agro-industrial residues, such as dairy (Morais et al. 2019) and swine (Coelho et al. 2020) wastewaters as substrate, with no report for RG.

In this context, this study aims to investigate the effect of different S/X ratios on the production of CA and 1,3-PD from the AD of RG from biodiesel production. Moreover, it evaluates the degree of anaerobic degradation in terms of the experimental CA yield and theoretical CA yield. Different mathematical models are utilized to estimate the maximum CA and 1,3-PD production potential to compare with the experimental results. The investigation was carried out in the Environmental Sanitation Laboratory of the Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Fortaleza, Ceará, Brazil, from August to September 2019.

## Materials and methods

### Substrate and inoculum

The RG used was obtained from the company Petrobrás S.A. (Quixadá, Ceará, Brazil). A methanogenic sludge was used as inoculum which was obtained from an Upflow Anaerobic Sludge Blanket (UASB) reactor that treated sewage (Fortaleza, Ceará, Brazil). The concentrations of total solids (TS), total volatile solids (TVS), and total fixed solids (TFS) were 72.7, 44.8, and 27.9 g L<sup>-1</sup>, respectively.

### Carboxylic acid production potential (CAPP) assays

The CAPP assays were performed in borosilicate reactors, with 300 mL of total volume, 270 mL of reaction volume, and 30 mL headspace. The pH correction to 7.0, buffering (sodium bicarbonate), and addition of nutrients were carried out according to Morais et al. (2019). Four S/X ratios were evaluated, where the substrate concentrations were varied, and the inoculum concentration was maintained (2.5 g VSS L<sup>-1</sup>), reaching 0.7, 1.1, 1.5, and 2.1 gCOD gVSS<sup>-1</sup>. Each statistical treatment was performed with three repetitions, totaling twelve assays. Inhibition of methanogenic activity was performed by adding chloroform 0.05% (v/v) (Viana et al. 2019). Nitrogen gas was used to purging each flask for 1 min. The batch assays were subjected to a temperature of 35 °C and 150 rpm for 28 days (Coelho et al. 2020). Liquid samples were collected on days 0, 2, 4, 7, 14, 21, and 28 for the analysis of COD and quantification of the bioproducts formed (CA and 1,3-PD). At the end of the experiment (28<sup>th</sup> day), a gas sample was collected from the reactor headspace to determine the average concentrations of CH<sub>4</sub>, H<sub>2</sub>, CO<sub>2</sub>, and H<sub>2</sub>S present in the biogas, and the pH was checked again to verify the buffering capacity of the system.

### Analytical methods

The pH measurement and the COD and solid series analyses were performed according to APHA (2017). For chromatographic and soluble COD analyses, the samples were filtered through a 0.45- $\mu$ m pore glass fiber membrane (EMD Millipore, USA). For liquid chromatography analysis, in order to avoid interference in the method and contamination of the equipment, the samples were also centrifuged at 13,000 rpm for six minutes (Eppendorf AG, Germany). The CA (lactic—HLA, acetic—HAc, propionic—HPr, butyric—HBu, isovaleric—i-HVa, and valeric—HVa) were quantified using high performance liquid chromatography (HPLC) (model 20-AT, Shimadzu Corporation, Japan), with UV/VIS detector and Supelcogel 8H cross-linked column (30 cm  $\times$  7.8 mm, ID 9  $\mu$ m), following the methodology of Dams et al. (Dams et al.



2016) with modifications. The oven temperature was 60 °C, 5 mM H<sub>2</sub>SO<sub>4</sub> was used as a mobile phase in a flow ranging from 0.6 to 1.2 mL min<sup>-1</sup>, and running time was 40 min.

The 1,3-PD was analyzed in a gas chromatography–flame ionization detection (GC–FID) (Trace GC Ultra, Thermo Scientific, USA), equipped with capillary column FFAP-CP (25 m × 0.32 mm, ID 0.3 μm) from Agilent Technologies (The Netherlands). The samples were diluted with ultrapure water (Milli-Q system, EMD Millipore, USA) in a 1:1 ratio to a final volume of 2 mL directly in borosilicate glass vials (20 mL) for extraction of the headspace (10 min at 120 °C) (Supelco, USA), which were sealed with PTFE/silicone septa and aluminum seals (Supelco, USA), according to Monteiro et al. (2016) with modifications. The detector, injector, and oven temperatures were 250, 200, and 40 °C, respectively. Hydrogen was the carrier gas, and the flow conditions were 1.5 mL min<sup>-1</sup> for 9 min. The qualitative analysis of the biogas was performed by gas chromatography using the method described by Coelho et al. (2020).

### Mass balance, yields, and kinetic study

The fractions of organic matter necessary for calculating the mass balance, yields, selectivity, and productivity of the bioproducts were determined according to the equations present in the Supplementary material (Online Resource 1). The mathematical models used to describe the kinetics of consumption of soluble organic matter that can be converted into bioproducts were the first-order model, first-order with residual, monod with growth and logistic; for the generation of bioproducts were the first-order model, second-order, Fitzhugh, cone, BPK, monomolecular, modified Gompertz, logistic, transference (Supplementary material, Online Resource 2).

The calculation of kinetic parameters and the adherence of mathematical models to the kinetic process was performed according to Yang et al. (2016). The results of this work were analyzed statistically using the Microcal Origin 8.1 software (Microcal Software Inc., Northampton, MA, USA), through analysis of variance (ANOVA) with a 95% confidence level and a probability of 5% ( $p < 0.05$ ). Tukey's tests were used to compare the different treatments (S/X ratios of 0.7, 1.1, 1.5, and 2.1). Thus, the data were presented using the mean value followed by the statistical treatment letter, where equal letters mean that there was no significant difference for  $p < 0.05$ .

## Results and discussion

### Effect of the S/X ratio on the distribution of bioproduct profile and productivity

As previously explained, the change in S/X ratio was achieved by manipulating the concentration of RG added.

After the experimental time, the pH in all batch assays remained close to 7. The particulate organic matter was converted into soluble organic matter, mainly in the form of bioproducts (CA and 1,3-PD), as can be seen in the Supplementary Material (Online Resource 3). Because RG has a low amount of particulate organic matter, the residual organic matter present in the inoculum probably contributed to the initial particulate COD.

The conversion of the applied organic matter into bioproducts usually varied with the S/X ratio (Table 1). However, it is noticed that the increase in the S/X ratio from 0.7 to 1.1 did not promote a significant difference. However, for the ratio of 1.5, there was an increase in the conversion efficiency of the applied organic matter into bioproducts from 71 to 77%. For the S/X ratio of 2.1, there was a reduction to 64%, likely due to the increment of impurities, such as NaCl, which can reach a concentration that is toxic to microorganisms, consequently reducing the organic matter conversion (Sittijunda and Reungsang 2012). Regarding the fraction of organic matter destined for cell growth, there was no significant difference when varying the S/X ratio. Furthermore, no methane in the biogas was found, which proves the effective inhibition of methanogens by chloroform, promoting the accumulation of CA, as also found elsewhere (Dams et al. 2018).

Moreover, it is important to highlight that the profile of bioproducts varies over time. There is probably a bioconversion from one product to another (Lonkar et al. 2016), as shown in Fig. 1. At the beginning of the experiments, no bioproducts were identified, but on day 2, carboxylic acids and 1,3-PD were already present, indicating a bioconversion of the organic matter added. At the end of the experiments, the bioproduct formed in the highest quantity was acetic acid, a result also obtained by Forrest et al. (2010) while

**Table 1** Final mass balance of the anaerobic digestion process of residual glycerol in different S/X ratios

S/X	Final mass balance		
	$\text{COD}_{\text{SNB}} / \text{COD}_{\text{T apl}}$	$\text{COD}_{\text{BP}} / \text{COD}_{\text{T apl}}$	$\text{COD}_{\text{VSS}} / \text{COD}_{\text{T apl}}$
0.7	0.22 <sup>a</sup>	0.71 <sup>a</sup>	0.07 <sup>a</sup>
1.1	0.22 <sup>a</sup>	0.72 <sup>a</sup>	0.06 <sup>a</sup>
1.5	0.16 <sup>b</sup>	0.77 <sup>b</sup>	0.07 <sup>a</sup>
2.1	0.30 <sup>c</sup>	0.64 <sup>c</sup>	0.06 <sup>a</sup>

$\text{COD}_{\text{T apl}}$  Total COD applied to the reactor at the beginning of the experiment (residual glycerol + organic matter from seed sludge);  $\text{COD}_{\text{BP}}$  COD converted into bioproducts identified in the chromatographic methods used (HLA, HAc, HPr, HBU, HIVa, HVa, and 1,3-PD);  $\text{COD}_{\text{SNB}}$  Soluble COD except identified bioproducts (recalcitrant, liable to bioconversion, and unidentified bioproducts);  $\text{COD}_{\text{VSS}}$  COD for cell growth

Equal letters mean that there was no significant difference ( $p < 0.05$ )

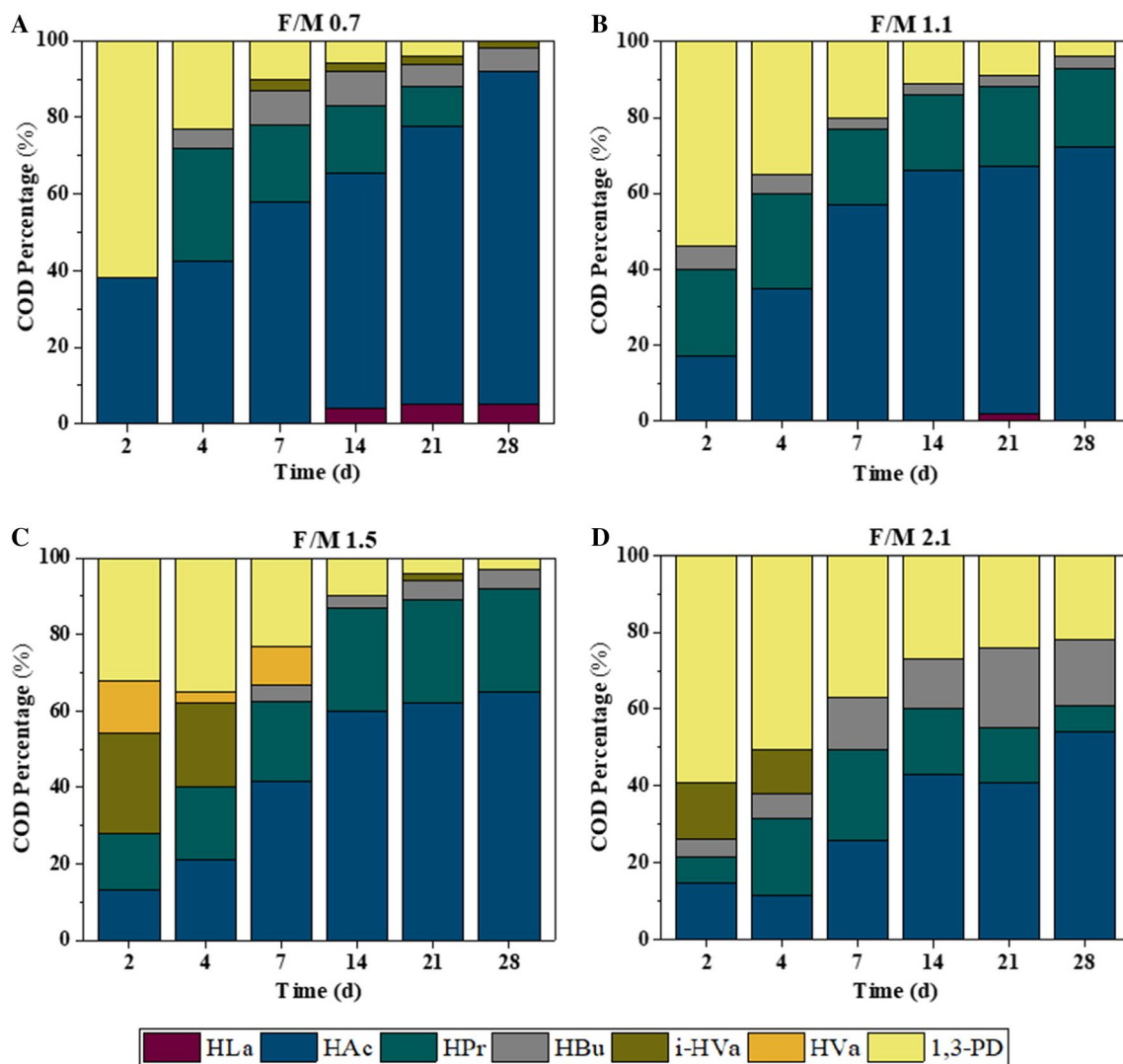


using RG in batch fermentation performed at 55 °C and pH 7.0 during 32 days of incubation time.

However, the increase in the S/X ratio favored the formation of other biocomposites. The production of HPr was more pronounced in the S/X ratio of 1.5, while the production of HBU and 1.3-PD increased in the S/X ratio of 2.1. In addition, i-HVa was found only in the S/X ratio of 1.5, probably due to the chain elongation of HPr to i-HVa in the presence of HAc (Veras et al. 2019). HLa was rarely formed and only found in a low concentration at the S/X ratio of 0.7, showing that RG is not a favorable substrate to produce this acid under the current conditions adopted. Thus, the choice of the S/X ratio depends on the desired final destination, in other words, which bio-product has the greatest interest. This conclusion can be better understood when analyzing the concentration and

yield of these bioproducts (Table 2). It is also important to highlight that there is an increase in the concentration of acetic acid (980–1700 mg L<sup>-1</sup>) with an increase in the S/X ratio from 0.7 to 1.5; however, the yield ( $Y_{2BP}$ ) of HAc decreased, probably because there was a microbial kinetic saturation (Yu and Wensel 2013), which can be better understood with the discussion presented in the topic 3.2. When increasing the S/X ratio to 2.1, there was no significant difference.

The present study showed a total CA yield ( $CA_T = 413\text{--}639 \text{ mgCOD}_{BP} \text{ gCOD}_{T \text{ apl}}^{-1}$ ) (Table 2) significantly higher than that obtained by Reungsang et al. (2013)—99 mgCOD<sub>BP</sub> gCOD<sub>T apl</sub><sup>-1</sup>—where the RG was submitted to AD in an upflow anaerobic sludge blanket reactor (UASB) with immobilized *Enterobacter aerogenes* ATCC13048, using thermal treatment of the inoculum and slightly acidic



**Fig. 1** Profile of bioproducts over time in the process of anaerobic digestion of residual glycerol for the S/X ratios of 0.7 (a), 1.1 (b), 1.5 (c), and 2.1 (d)

**Table 2** Concentration, selectivity, and yield of the bioproducts identified at the end of the experiments of anaerobic digestion from residual glycerol in different S/X ratios

Bioproduct	Concentration (mg L <sup>-1</sup> )				Yield—Y <sub>2BP</sub> (mg gCOD <sub>T apl</sub> <sup>-1</sup> )			
	S/X Ratio							
	0.7	1.1	1.5	2.1	0.7	1.1	1.5	2.1
HLa	50	–	–	–	30	–	–	–
HAc	980 <sup>a</sup>	1352 <sup>b</sup>	1700 <sup>c</sup>	1686 <sup>c</sup>	579 <sup>a</sup>	487 <sup>b</sup>	468 <sup>b</sup>	324 <sup>c</sup>
HPr	–	274 <sup>a</sup>	495 <sup>b</sup>	162 <sup>c</sup>	–	99 <sup>a</sup>	136 <sup>b</sup>	31 <sup>c</sup>
HBu	39 <sup>a</sup>	30 <sup>b</sup>	–	301 <sup>c</sup>	23 <sup>a</sup>	11 <sup>b</sup>	–	58 <sup>c</sup>
i-HVa	13	–	–	–	7	–	–	–
HVa	–	–	–	–	–	–	–	–
1,3-PD	–	44 <sup>a</sup>	58 <sup>b</sup>	423 <sup>c</sup>	–	16 <sup>a</sup>	16 <sup>a</sup>	81 <sup>b</sup>
CA <sub>T</sub>	1082 <sup>a</sup>	1629 <sup>b</sup>	2195 <sup>c</sup>	2091 <sup>c</sup>	639 <sup>a</sup>	597 <sup>b</sup>	604 <sup>b</sup>	413 <sup>c</sup>

*HLa* Lactic acid; *HAc* acetic acid; *HPr* propionic acid; *HBu* butyric acid; *i-HVa* Isovaleric acid; *HVa* valeric acid; *1,3-PD* 1,3-propanediol; *CA<sub>T</sub>* total carboxylic acid

Equal letters mean that there was no significant difference ( $p < 0.05$ )

pH (pH 5–6.5) as a methanogenic inhibition method. The results obtained in the current investigation were also superior to those presented by Chookaew et al. (2015)—54 mgCOD<sub>BP</sub> gCOD<sub>T apl</sub><sup>-1</sup>, where the RG was bioconverted by dark fermentation in a batch reactor using a pure culture of *Klebsiella sp.* TR17. These researchers probably obtained much lower yields because they focused on hydrogen production from the RG, likely indicating that the operational configurations were not the most suitable for CA formation. However, Dams et al. (2018) obtained a yield of approximately 1250 mg gCOD<sub>T apl</sub><sup>-1</sup>, more than double of this work, likely because they used both an external electron donor (ethanol) and bio-augmentation, two techniques that promote a higher formation of CA (De Groof et al. 2019).

Figure 2 shows the productivity of bioproducts throughout the experiments, in which the S/X ratio of 0.7 usually showed the lowest productivity. The highest productivity of HAc (131 ± 8 mg L<sup>-1</sup> d<sup>-1</sup>) was reached in the S/X ratio of 1.1, while i-HVa (51 ± 5 mg L<sup>-1</sup> d<sup>-1</sup>) was at S/X ratio of 1.5, and 1,3-PD (134 ± 5 mg L<sup>-1</sup> d<sup>-1</sup>) at S/X ratio of 2.1. In general, bioproducts had maximum productivity between days 2 and 4, but acetic acid reached a maximum productivity around day 7. It is also noticed that the increase in the productivity of HAc is accompanied by the decrease in productivity of the other by-products. Therefore, this increase is possibly due to the degradation of these other compounds due to a low partial pressure of hydrogen (PPH) and potential redox or equivalently, on the ratio Nicotinamide Adenine Dinucleotide in the reduced form (NADH) to its oxidized form (NAD<sup>+</sup>) [NADH]/[NAD<sup>+</sup>] still achieved in the microbiome, even for the highest S/X ratio used in the present investigation (Kleerebezem et al. 2015; Lonkar et al. 2016).

Another important point is that the productivity of 1,3-PD increased fast, reaching its maximum value very quickly. However, a fast decrease was also verified after day 2. This probably occurs because, at the beginning of the process,

the reductive pathway that leads to the production of 1,3-PD prevails. Afterward, the oxidative pathway is predominant, so the 1,3-PD starts to be degraded, providing more CA formation. Leng et al. (2017) also found a similar pattern while studying the bioconversion of RG with an anaerobic sludge in batch assays incubated at pH 7 and 37 °C. The productivity of 1,3-PD on the 28th was zero at S/X ratios of 0.7, 1.1, and 1.5. Only on the S/X ratio of 2.1 the productivity stabilized from the 14th day, presenting at the end of the experiment (28 days) a value of about 15 mg L<sup>-1</sup> d<sup>-1</sup>. So, high S/X ratios possibly might favor the oxidative pathway required to form CA instead of 1,3-PD.

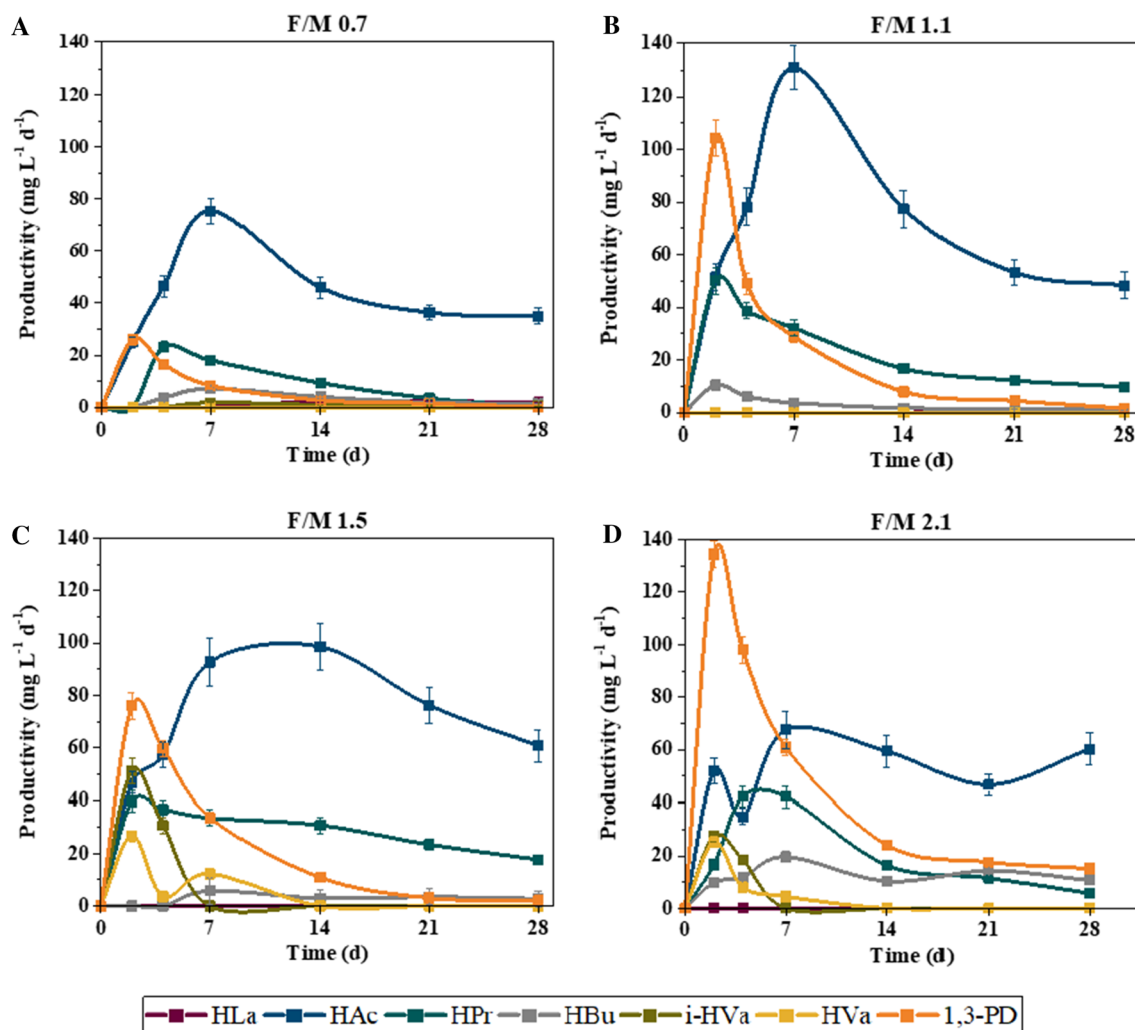
### Kinetic modeling

Mathematical models are also extremely important for the field of bioprocess engineering. So, the results of kinetic studies obtained from laboratory-scale experiments can be used for full-scale design reactors under similar operational conditions and estimating its treatment efficiency (Borghesi et al. 2008). Such models are very mature for the AD focusing on methanization (Çetinkaya and Yetilmezsoy 2019). However, the kinetic modeling of CA formation from RG is not reported in the literature.

Among the different mathematical models used to describe kinetics, exponential models, such as first order with residual (Fig. 3a–d), were more suitable to describe the soluble substrate degradation curve, with a high coefficient of determination ( $R^2$ ) and lower value for Akaike information criterion (AIC) (Supplementary material, Online Resource 4). The soluble substrate degradation rate constant ( $K_B$ ), which represents the substrate degradation velocity, showed to be influenced by the S/X ratio (Fig. 4a), being the kinetics more favored in the S/X ratio of 1.1. The correlation of the S/X ratio can be described by the second-degree polynomial equation  $y = -0.43x^2 + 0.95x - 0.35$  in S/X ratios







**Fig. 2** Productivity of bioproducts over time in the anaerobic digestion process of residual glycerol for the S/X ratios of 0.7 (a), 1.1 (b), 1.5 (c), and 2.1 (d)

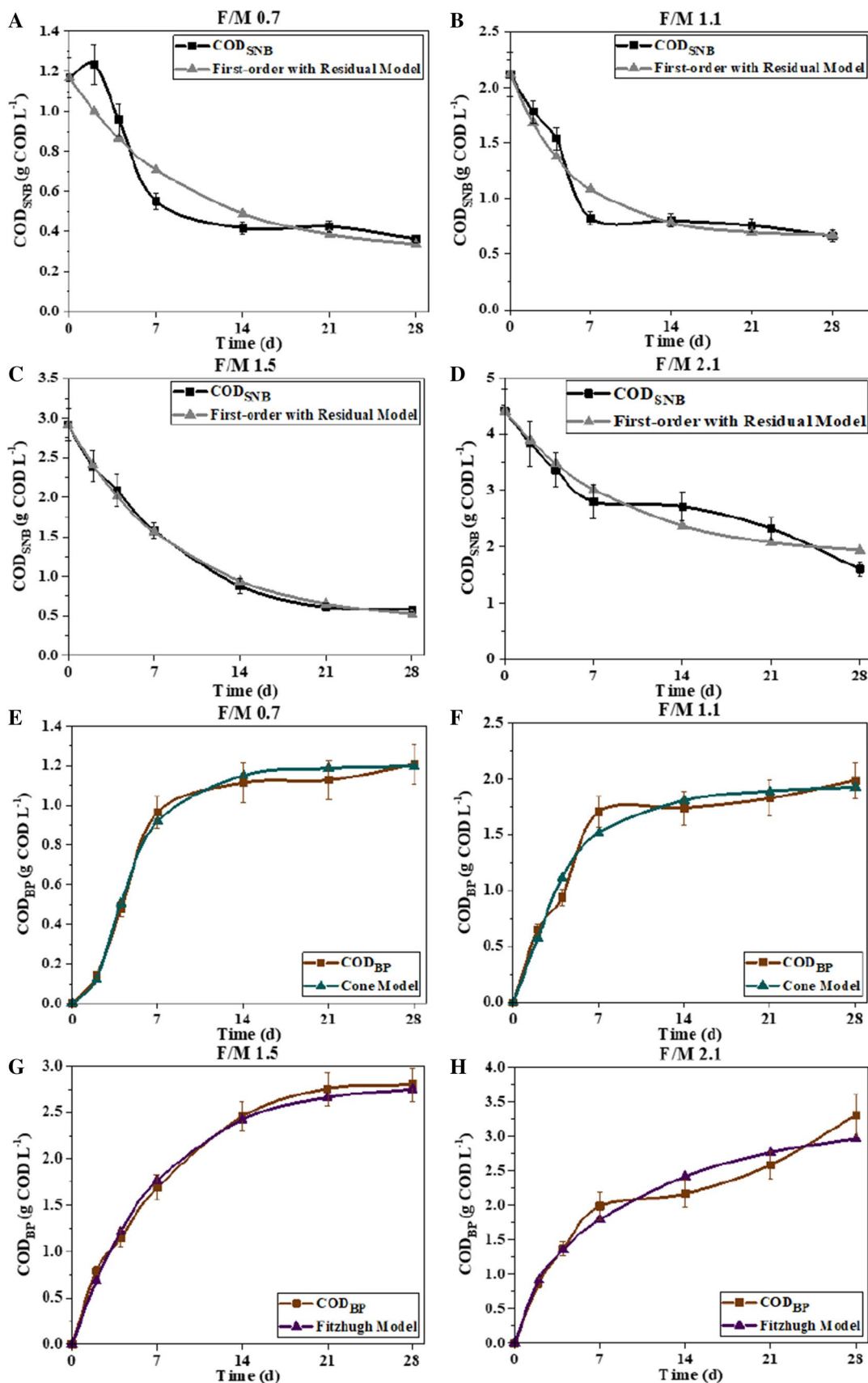
from 0.7 to 1.5 ( $R^2=0.998$ ) then becoming constant until S/X ratio of 2.1. The  $K_B$  decreases at S/X of 1.5, probably due to the microorganisms' inhibition by a high concentration of substrate (Sittijunda and Reungsang 2012) or impurities present in the RG. Another important kinetic parameter is the maximum microbial growth rate ( $\mu_{max}$ ) which was estimated using the Monod with Growth model. The values found were low for all experiments, decreasing with the S/X ratio increase (Fig. 4b).

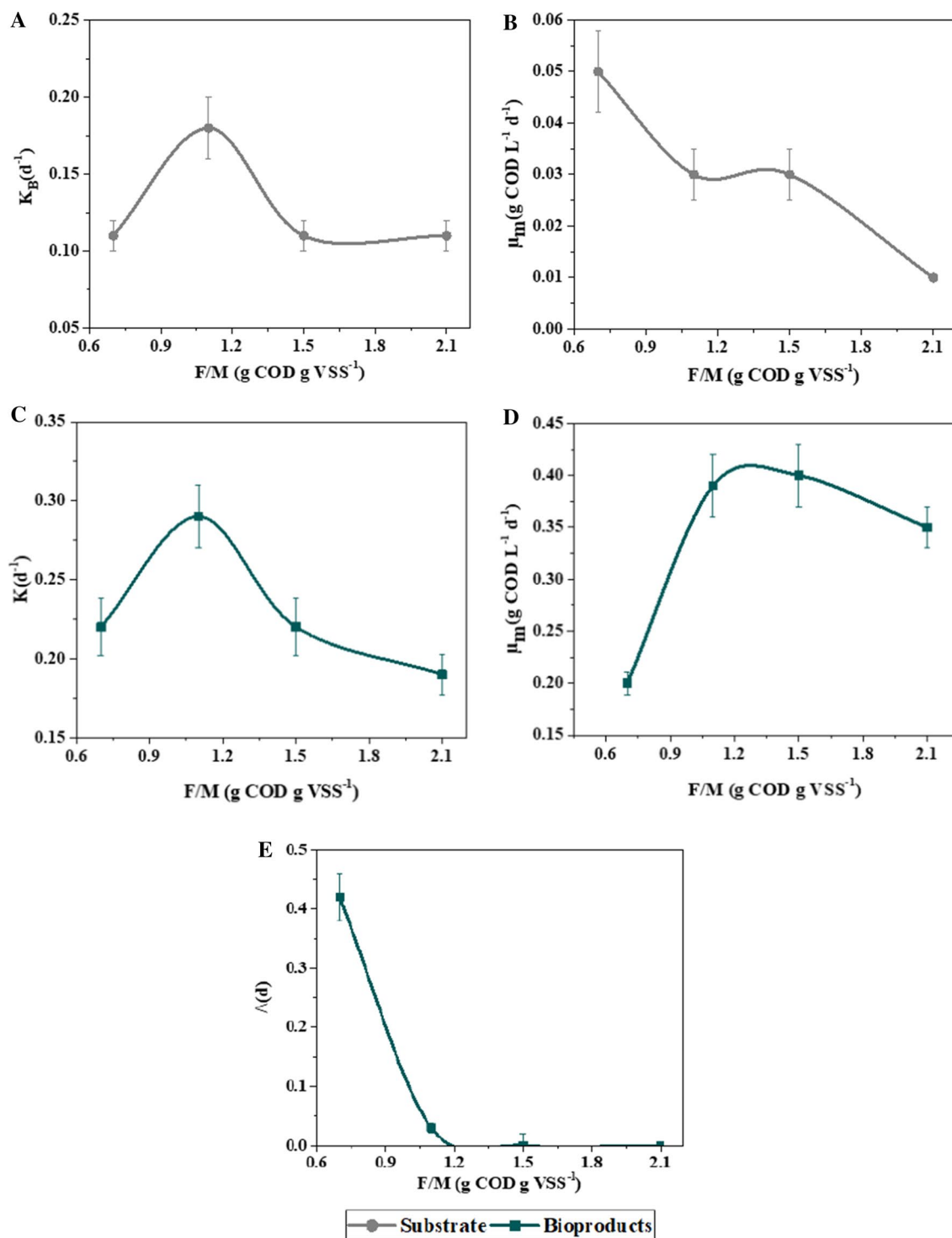
Analyzing the kinetics of bioproduct production, exponential models are also better suited to describe this production, such as cone and Fitzhugh models (Fig. 3e–h) that presented the highest coefficient of determination ( $R^2$ ) and lowest values for AIC (Supplementary material, Online Resource 4). Figure 4c shows the correlation between S/X ratio and the first-order bioproduct production rate constant ( $K_{BP}$ ) estimated by cone model, which can be described by the second-degree polynomial equation

$y = -0.43x^2 + 0.95x - 0.24$  for S/X ratios from 0.7 to 1.5 ( $R^2=0.994$ ). It then presents a lower reduction rate for S/X 2.1. So, among the studied S/X ratios, the relation 1.1 is the most favorable kinetic condition for the formation of bioproducts and the degradation of the soluble organic matter.

In addition, the maximum production rate ( $\mu_m$ ) was calculated using the modified Gompertz, logistics, BPK, and transference (Supplementary material, Online Resource 4). The correlation between S/X ratio and  $\mu_m$  estimated by the transference model (Fig. 4d) showed that the maximum production rate of bioproducts increased with the S/X ratio

**Fig. 3** Kinetic curves of substrate consumption (first order with residual model) in the S/X ratios of 0.7 (a), 1.1 (b), 1.5 (c), and 2.1 (d) and kinetic modeling of bioproduct production in the form of COD in the S/X ratios of 0.7 (cone model) (e), 1.1 (cone model) (f), 1.5 (Fitzhugh model) (g), and 2.1 (Fitzhugh model) (h) from the batch assays using residual glycerol as substrate





**Fig. 4** Correlation of the S/X ratio with the degradation of the soluble substrate constant ( $k_B$ ) (a), maximum microbial growth rate ( $\mu_{max}$ ) (b), bioproduct production rate constant (K) (c), maximum bioproduct productivity ( $\mu_m$ ) (d), and lag phase time ( $\lambda$ ) (e)

increase from 0.7 to 1.1. However, any significant difference was found when increasing to the S/X ratio of 1.5, and a decrease in the maximum production rate of bioproducts was obtained for the S/X ratio of 2.1. Furthermore, these

four sigmoidal models also estimated the lag phase, where increasing the S/X ratio from 0.7 to 1.1 reduces this delay period from 0.42 to 0.03 d<sup>-1</sup> (Fig. 4e). The form factor n



of the Fitzhugh model also indicates that there was a delay period only to S/X ratios of 0.7 ( $n=3.55$ ) and 1.1 ( $n=1.11$ ).

Regarding the kinetics of individual bioproducts (Supplementary material, Online Resource 5), the formations of HAc and HPr were better described by exponential models, such as cone and Fitzhugh, for the S/X ratios of 0.7 and 1.1. However, for the S/X ratios of 1.5 and 2.1, sigmoidal models were more appropriate, such as modified Gompertz and Richards. The HBU production curve, in general, was better described by exponential models, except for S/X of 2.1, which showed a better fit to the modified Gompertz sigmoidal model. The remaining acids were not modeled individually due to their low production or no detection. The sigmoidal curves consist of three phases: the slow phase of production or acclimatization of microorganisms, the phase of rapid production (exponential phase) with higher consumption of substrate and growth of biomass, and phase of production stabilization, in which the production rate decreases and it reaches zero. Therefore, an elongated S-shaped curve indicates the continuous availability of soluble organic matter and a more continuous CA production over time (Morais et al. 2019). So, the best fit of sigmoidal models for higher S/X ratios can be likely justified by the increased inhibition of the process, resulting in a greater lag phase, typical of sigmoidal models.

Analyzing the mathematical models that best described the CA production curves, it can be seen that the velocity constant ( $K$ ) and the maximum productivity rate ( $\mu_m$ ) of HAc were higher at S/X ratios of 1.1 and 1.5 ( $K=0.16\pm 0.01$  and  $0.19\pm 0.02$  d<sup>-1</sup>;  $\mu_m=0.14\pm 0.01$  and  $0.14\pm 0.02$  mg L<sup>-1</sup> d<sup>-1</sup>). However, the lag phase time ( $\lambda$ ) was higher at the S/X ratio of 1.5 (2.45 d). For HPr, the highest values of  $K$  and  $\mu_m$  were found in the S/X ratio of 2.1 ( $0.72\pm 0.05$  d<sup>-1</sup> and  $0.08\pm 0.01$  mg L<sup>-1</sup> d<sup>-1</sup>), which presented a lag phase of 1.81 days. Finally, for HBU, the S/X ratio of 1.1 provided higher values of  $K$  ( $1.23\pm 0.11$  d<sup>-1</sup>) and  $\mu_m$  ( $0.12\pm 0.01$  mg L<sup>-1</sup> d<sup>-1</sup>) when a  $\lambda$  value of 1.82 was used.

In addition, the lag phases for the formation of these acids in the most favorable kinetics S/X ratio were around two days, indicating that the microorganisms do not immediately transform the RG into CA. As hydrolysis is not the limiting step of the process since the RG is practically all in the soluble and non-complex form, this occurred probably because at the beginning of the process, the reductive pathway that leads to the production of 1,3-PD prevails, reaching its maximum productivity within the first two days, and after it starts to be degraded (predominance of the oxidative pathway), providing more CA formation. Leng et al. (2017) found similar results while evaluating glycerol fermentation at pH 7 and 37 °C, where 1,3-PD formation occurred within 3 days, decreasing afterward.

Therefore, according to the estimated kinetic parameters, the kinetic of bioproduct formation is favored at S/X ratio 1.1, where the highest productivity of HAc was also reached. However, the maximum productivity of i-HVa and 1,3-PD was found at S/X ratios of 1.5 and 2.1, respectively. In other words, such effects can be reflected as increased productivity, accelerated biodegradation processes, or a combination of both (Xie et al. 2017).

## Conclusion

The increase in the S/X ratio from 0.7 to 1.5 had a positive effect on the percentage of RG conversion into bioproducts (71 to 78% of organic matter was converted into bioproducts), but the higher ratio (2.1) caused a conversion reduction (64%). Moreover, HAc was the bioproduct formed in the highest concentration for all assays. The S/X ratio of 1.5 provided higher selectivity for HPr, and S/X 2.1 for HBU and 1,3-PD. The S/X ratio 1.5 showed the best results in terms of concentration and total CA production yield. Furthermore, exponential models were better suited to describe this kinetics process. However, sigmoidal models have also shown a good fit, mainly in higher S/X ratios, making it possible to correlate the S/X ratio with coefficients  $K$ ,  $\mu_m$ , and  $\lambda$ .

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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