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RESEARCH PAPER

A kinetic study on carboxylic acids production using bovine slaughterhouse wastewater: a promising substrate for resource recovery in biotechnological processes

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Abstract

Carboxylic acids (CA) are considered high added-value compounds, and their production from wastes has gained economic and environmental notoriety. However, the CA production and kinetic modeling using some agro-industrial wastewaters, such as bovine slaughterhouse wastewater (SHW), are not well reported in the literature. Therefore, the objective of this work was to evaluate the CA production potential using SHW as a substrate under acidogenic conditions and to apply mathematical models to estimate the kinetic parameters of particulate organic matter hydrolysis, soluble organic matter consumption, and CA production. Tests were carried out in quadruplicate batch reactors with a 250-mL reaction volume, with brewery sludge as inoculum and using chloroform (0.05%, v/v) for methanogenesis inhibition. The obtained yield was 0.55 g acids $gCOD_A^{-1}$, corresponding to 0.76 gCOD $gCOD_A^{-1}$. The production of caproic acid without the addition of electron donors was achieved. Mathematical models that describe exponential growth, such as the first-order exponential model, cone model, and Fitzhugh model, were the most suitable to describe the production kinetics of CA. Finally, SHW seems to be a promising substrate to be investigated in the carboxylic platform.

Keywords Carboxylic acids · Slaughterhouse wastewater · Kinetic modeling · Biotechnological processes

Introduction

Carboxylic acids (CA) are building block chemicals widely applied to the industry to varnishes, paints, perfumes, disinfectants, surfactants, textile auxiliaries, drugs, and food products production [1]. Traditionally, these acids are synthesized from the oxidation or carboxylation of chemical precursors derived from petroleum processing, generating the emission of greenhouse gases (GHG), which cause serious environmental and human health problems [2]. GHG emissions over the entire acetic acid (HAc) life cycle (cradle-to-grave) are estimated to be 3.3 ton CO_{2eq} ton^{-1} HAc in cases where the production process is incinerated without energy recovery [3]. Therefore, the biological prospecting of CA via anaerobic fermentation from agro-industrial wastes is an attractive alternative to the carboxylic platform development to mitigate the environmental impacts of improper waste disposal and reduce the petrochemical dependence sector on CA production [4–6].

CA biological production occurs during carbohydrates, lipids, and proteins fermentation under anaerobic conditions, especially after the inhibition of the methanogenesis and sulfetogenesis steps [7]. Short-chain carboxylic acids (SCCA) are usually the main products in acidogenic processes, but medium-chain carboxylic acids (MCCA) can also be produced. MCCA are more economically attractive compared to SCCA because of their nobler purposes in the industry, such as in the biofuels synthesis, as a feed

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additive, as an antimicrobial agent, and in biopolymers production (e.g., polyhydroxyalkanoates) [8, 9]. SCCA added-value is estimated to be USD 400–2500 ton^{-1}, a higher value than methane (CH_4), which is USD 200–600 ton^{-1}, but lower than the market price of the MCCA, which is 2000–2500 USD ton^{-1} [2, 10–12]. However, MCCA synthesis requires the presence of reduced organic material, usually ethanol or lactate, which provides electrons for SCCA bioconversion into MCCA [13, 14]. This is known as carboxylic chain elongation process (CCEP) and occurs by the metabolic pathway of reverse β-oxidation, in which two carbons are added to an even or odd SCCA chain [15].

Several agro-industrial substrates can be suitable for CA biotechnological production, such as cassava wastewater [16], residual glycerol from biodiesel production [17], swine wastewater [18], and dairy wastewater [19]. In this sense, slaughterhouse wastewater (SHW) may have the potential for CA production due to its high amount of nutrients and organic matter that can be biodegraded under anaerobic conditions [20, 21]. SHW also has a complex composition, including heavy metals, cleaning agents residues and veterinary medical products, hormones, pathogenic and nonpathogenic microorganisms [22]. According to Bustillo-Lecompte and Mehrvar [23], a meat processing plant can generate between 2.5 and 40 m^3 of wastewater per ton of meat produced, which is equivalent to about 3 m^3 of wastewater per slaughtered cattle [21]. These factors encourage the development of wastewater treatment processes to recover bioproducts, such as CH_4 and hydrogen (H_2) [24, 25]. Therefore, the anaerobic processes capacity to treat this wastewater must be investigated.

Although biological CA production from organic waste has been deeply investigated in recent years aiming at industrial-scale production, the technical literature still lacks studies on the acidogenic process kinetic modeling. In this context, data related to the CA production parameters using SHW as a substrate are scarce, making it challenging to obtain optimal performance [26]. The application of mathematical models aiming to describe the acidogenic process stages (hydrolysis, soluble organic matter consumption, and CA production) becomes attractive to obtain kinetic parameters, such as the production rate constant (k), phase lag time (λ), and maximum production rate of the bioproduct of interest (μ_m), that can be used to simulate CA production and to predict and optimize reactor performance [27].

Therefore, the objective of this work was to evaluate the CA production potential using SHW as a substrate under acidogenic conditions and to apply mathematical models to estimate the kinetic parameters of particulate organic matter hydrolysis, soluble organic matter consumption, and CA production.

Materials and methods

Substrate and inoculum

SHW was obtained from the municipal slaughterhouse in the Itarema city, Ceará, Brazil. The batch reactors used in the carboxylic acid production potential assays were inoculated with methanogenic sludge from an upflow anaerobic sludge blanket (UASB) reactor that was operated in the treatment of brewery effluents located in the metropolitan region of Fortaleza, Ceará, Brazil. Total solids (TS), total volatile solids (TVS), and total fixed solids (TFS) concentrations were 81.0, 33.8, and 47.2 g L^{-1}, respectively.

The values of the SHW physical–chemical characterization are shown in Table S1 (Supplementary Material), which were performed according to the Standard Methods for the Examination of Water and Wastewater [29]. In general, all the values obtained are in agreement with previous investigations with SHW [22, 28].

Carboxylic acids production potential assays

Carboxylic acid production potential assays were carried out in quadruplicate batch reactors in borosilicate flasks with 300 mL total volume, 250 mL reaction volume, and 50 mL headspace, with brewery sludge as inoculum, with SHW as a substrate and using chloroform (0.05%, v/v) for methanogenesis inhibition [30]. The basal medium and buffering at pH 7.0, previously adjusted with 1 M HCl or NaOH, were performed according to Dams et al. [17]. The food/microorganism (F/M) ratio was 0.64 ± 0.01 gCOD gVS^{-1}. Reactors were sealed with rubber stoppers and purged with nitrogen (N_2) for 1 min to create an anaerobic atmosphere [31]. They were then kept in an incubator (MA-420, Marconi LTDA, Brazil) under orbital shaking at 150 rpm and 35 °C for 28 days [32, 33].

A 5-mL sample of the liquid phase was collected from the reactors on days 0, 2, 4, 7, 14, 21, and 28 using

syringes (SampleLock syringe, Hamilton Company, EUA). The sample was used to perform triplicate analyses of chemical oxygen demand (COD) and to determine the CA that were produced, aiming to study the substrate bioconversion kinetics. At the end of the experiment (28th day), a 1-mL biogas sample was extracted from the headspace of the reactors to verify the inhibition of methanogenic activity [34, 35].

Inhibition of methanogenic activity was verified by analyzing the gas fraction in a gas chromatography-barrier ionization discharge (GC BID-2010 Plus, Shimadzu Corporation, Japan), and CA concentrations (acetic—HAc, propionic—HPr, butyric—HBu, isovaleric—HIVa, valeric—HVa and caproic—HCa acids) were determined by high-performance liquid chromatography (HPLC) (LC-20A, Prominence, Shimadzu Corporation, Japan), according to Morais et al. [18].

Organic matter fractions and yields

Organic matter fractions and yields were calculated using Eqs. (1–6) shown in Table 1.

The concentration of organic matter (COD in the form of acids) of each acid was obtained by the following conversion factors: 1 mg HAc = 1.07 mgCOD, 1 mg HPr = 1.51 mgCOD, 1 mg HBu = 1.82 mgCOD, 1 mg HIVa = 2.04 mgCOD, 1 mg HVa = 2.04 mgCOD, 1 mg HCa = 2.21 mgCOD [11]. The selectivity and productivity of CA were calculated according to Eqs. (7 and 8).

Table 1 Equations applied in calculating organic matter fractions

Equation	Number
$COD_P(mgCOD) = COD_T - COD_S$	1
$COD_A(mgCOD) = COD_T - COD_{CA(t0)}$	2
$COD_{Bio}(mgCOD) = COD_S - COD_{CA}$	3
$COD_{CA}(mgCOD) = COD_{CA(tf)} - COD_{CA(t0)}$	4
$Y1_{CA}\left(\frac{mgCOD_{CA}}{mgCOD_A}\right) = \frac{COD_{CA}}{COD_A}$	5
$Y2_{CA}\left(\frac{mg_{acids}}{mgCOD_A}\right) = \frac{M_{TCA}}{COD_A}$	6

COD_P particulate COD added in the batch reactor, COD_T total COD added in the batch reactor, COD_S soluble COD added in the batch reactor, COD_A available COD, corresponding to the total COD fraction applied at the beginning of the experiment that is not in the form of CA, $COD_{CA\ (T0)}$ the fraction of soluble COD in the CA form at the beginning of the batch, COD_{BIO} fraction of COD soluble not referring to CA, COD_{CA} COD in terms of CA formed during the incubation period, $COD_{CA\ (TF)}$ the fraction of soluble COD in the CA form at the end of the batch. Units in mgCOD, $Y1_{CA}$ COD_{CA} production yield about the available COD ($mgCOD_{CA}$ $mgCOD_A^{-1}$), $Y2_{CA}$ acids production yield about the available COD (mg acids $mgCOD_A^{-1}$), M_{TCA} the total mass of CA formed during the incubation period (mg acids)

$$\text{Selectivity} = \left(\frac{M_{CA}}{M_{TCA}}\right) \times 100\% \quad (7)$$

where selectivity is the percentage of each acid formed about the total acids produced (%); M_{CA} is the acid mass formed during the batch test (mg acid); and M_{TCA} is the total CA mass formed during the incubation period (mg acids).

$$\text{Productivity}\left(\frac{mg_{CA}}{L\ d}\right) = \frac{C_{CA1D} - C_{CAB}}{\text{Time}} \quad (8)$$

where productivity is the quantity of acid present per working volume and per time (mg acid $L^{-1}{\cdot}d^{-1}$); C_{CA1D} is the acid concentration at one day of collection (mg acid L^{-1}); C_{CAB} is the acid concentration at the beginning of the batch (day zero) (mg acid L^{-1}); and Time is the unit of time (d).

Model fitting

The equations of the kinetic models selected to describe the particulate organic matter hydrolysis process, the soluble organic matter consumption, and the CA production are presented in Table 2. A nonlinear least-squares regression analysis was performed using the Microsoft Office Excel 2019 Solver tool to estimate the parameters of the selected kinetic models [36, 37]. Origin version 8.1 software (OriginLab Corporation, Northampton, MA, USA) was used to plot curves from data obtained by kinetic modeling.

The selection of the model that best describes each organic matter bioconversion process was performed using the coefficient of determination (R^2) and the Akaike information criterion (AIC). The lower the AIC's value, the greater the adequacy of the data estimated by the kinetic model to the experimental data [38]. AIC was calculated according to Eq. (9).

$$AIC = N \ln\left(\frac{SS}{N}\right) + 2k \quad (9)$$

where AIC is the Akaike information criterion (dimensionless); N is the number of observations of experimental data; SS is the square sum of the residuals; and k is the number of model parameters.

Results and discussion

Kinetic modeling of organic matter conversion bioprocesses

A continuous process of particulate organic matter hydrolysis occurred, indicated by the decrease in this fraction in the system over time (Fig. 1a). Due to hydrolysis, the soluble organic matter (soluble COD and available for

Table 2 Models selected to describe the organic matter bioconversion

Kinetic model	Kinetic model equation
	Hydrolysis and biodegradable organic matter consumption
First-order	$C_t = C_0 \exp(-kt)$
First-order with residual	$C_t = C_r + (C_0 - C_r)\exp(-kt)$
Carboxylic acids production	
First-order	$CA_t = CA_f\left[1 - \exp\left(-K_{CA}t\right)\right]$
Second-order	$CA_t = \frac{K''_{CA}(CA_f)^2 t}{1+K''_{CA}(CA_f)t}$
Fitzhugh	$CA_t = CA_f\left[1 - \exp\left(-K_{CA}t\right)^n\right]$
Cone	$CA_t = \frac{CA_f}{1+\left(K_{CA}t\right)^{-n}}$
Modified Gompertz	$CA_t = CA\exp\left\{-\exp\left[\frac{\mu_m e}{CA_f}(\lambda - t) + 1\right]\right\}$
Logistic	$CA_t = \frac{CA_f}{1+\exp\left[\frac{4\mu_m(\lambda - t)}{CA_f}+2\right]}$
Transference	$CA_t = CA_f\left\{1 - \exp\left[-\frac{\mu_m(t-\lambda)}{CA_f}\right]\right\}$
Richards	$CA_t = CA_f\left\{1 + v \times \exp(1+v) \times \exp\left[\frac{\mu_m}{CA_f} \times (1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right]\right\}^{(-1/v)}$

C_t organic matter concentration at a time, C_0 initial organic matter concentration, k rate constant, C_r residual organic matter concentration, CA_t CA concentration at a time, CA_f final concentration of CA, k_{CA} first-order CA production rate constant, k_{CA}'' second-order CA production rate constant, t digestion time, n shape constant, λ lag phase time, μ_m maximum productivity of CA, v constant of the Richards model

bioconversion) was continuously available. Biodegradable COD concentrations (soluble COD that is not in the CA form, therefore available to be bioconverted to CA) also decreased continuously, indicating that the consumption of this fraction of COD occurred faster than that generated from the mechanism of hydrolysis (Fig. 1b, c). This can be confirmed by the kinetic parameters obtained in the kinetic modeling of organic matter conversion bioprocesses (Table 3).

According to Table 3, the value of the degradation rate constant of the soluble substrate ($k_B = 0.26 \pm 0.07$ d^{-1}) was higher than that of the hydrolysis rate constant ($k_H = 0.15 \pm 0.04$ d^{-1}), as estimated by the first-order with residual model, which obtained better fit in the kinetic modeling of these biological processes. As $k_B > k_H$, the consumption rate of the biodegradable COD was greater than the rate of degradation of the particulate COD fraction. This is because the biodegradable COD concentration (1.88 gCOD L^{-1}) was higher than the concentration of particulate COD (0.74 gCOD L^{-1}) at the beginning of the experiment (Fig. 1a).

The first-order with residual kinetic model was the one that best fitted the hydrolysis curve of the particulate organic matter (Fig. 1b) generated by the experimental data with a high R^2 and lower values for AIC. This model was also the one that best fits the curve formed by the consumption of biodegradable organic matter (Fig. 1c) with a high R^2 and lower value for AIC. The first-order model and the first-order with residual model described satisfactorily the biological processes of hydrolysis and consumption of biodegradable soluble organic matter (Table 3). However, the first-order with residual model fitted better to the data, given that it considers the residual concentration of biodegradable organic matter as one of its variables.

Among the models selected to describe the COD_{CA} production, the cone model was the one that best fits the experimental data (Table 3), in which a higher R^2 and lower AIC were found. It can be seen that the CA production from SHW in mixed culture follows a first-order kinetic, as evidenced by the R^2 of 0.985 and AIC of − 30.69 about the second-order model ($R^2 = 0.950$ and AIC = − 22.41). Kinetic models describing exponential functions, such as cone, first-order, and Fitzhugh, can be efficiently applied to describe CA production from this substrate.

According to Labatut et al. [39], the shape of a production curve reflects the biodegradability characteristics of the substrate, also inferring inhibitory processes. As a result, the information can be used to anticipate problems during the anaerobic process. During the lag phase, the initial breakdown of the substrates occurs through hydrolytic bacteria. Substrate physically degraded to soluble compounds becomes available to acidogenic and acetogenic bacteria, starting the exponential phase of production. The stabilization of CA production occurs when the fraction of easily degraded organic matter (soluble in the form of carbohydrates, for example) is depleted, and there is a residual of organic matter difficult to be biodegraded. These bioprocesses can be preliminarily evaluated through

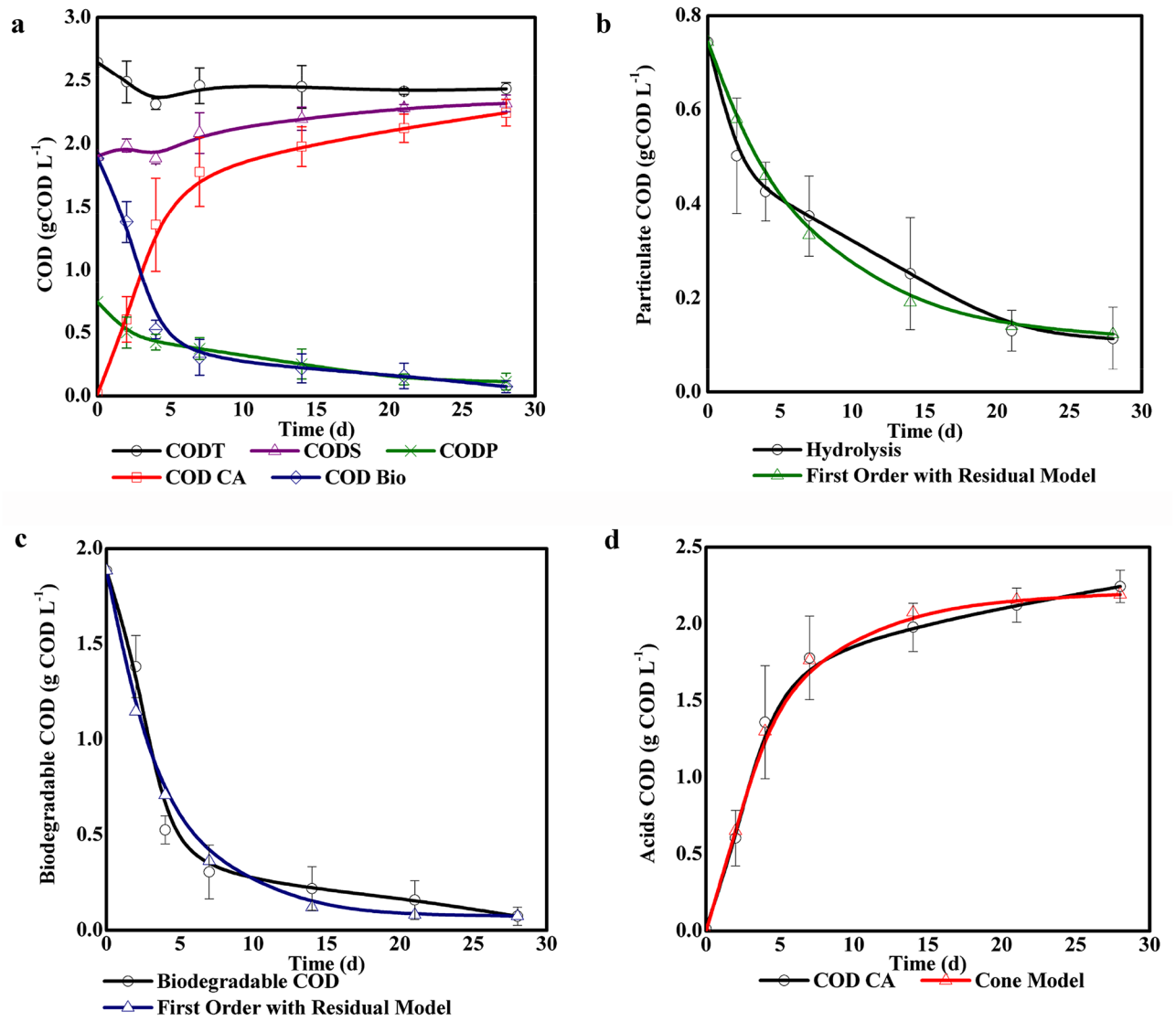

Fig. 1 Organic matter concentration profiles during the batch assays. **a** Organic matter concentration profile curves: total, soluble, particulate, biodegradable, and CA. **b** Kinetic modeling of particulate organic matter hydrolysis (first-order with residual model). **c** Kinetic modeling of biodegradable organic matter consumption (first-order with residual model). **d** Kinetic modeling of COD production in the form of CA (cone model)

the production curve format. The CA production curve presented an exponential format (Fig. 1d), indicating high CA productivity in the first days of incubation, short lag time, and, therefore, high performance of the acidogenic microorganisms. There were no temporary inhibitory processes that could be visually recognized in the production curve by periods of stabilization, followed by new exponential phases of production [40].

The shape factor of the cone ($n = 1.75$) and Fitzhugh ($n = 1.20$) models was greater than unity, indicating the presence of a delay period [38]. However, the estimated time for the lag (λ) phase was short, ranging from 0.04 to 0.37 d, indicating that the microorganisms present in the inoculum were able to adapt quickly to the environmental conditions and that the SHW applied is a highly biodegradable substrate in an acidogenic fermentation process.

The hydrolysis of the particulate fraction of the organic matter, which is, usually, the limiting step of the acidogenic process [41, 42], had little influence on the CA production delay due to the high presence of soluble substrate, which was indicated by the value of k_B being greater than that of k_H. Thus, the short lag phase and the high organic matter concentration in the soluble fraction ($COD_{BIO}/COD_T = 0.71$) at the beginning of the experiment allowed 80% of the CA to be formed in the first seven days of the experiment. Thus, according to data shown in Table 3, the highest value for

Table 3 Kinetic parameters estimated by the modeling of organic matter conversion bioprocesses

Particulate organic matter hydrolysis			Biodegradable organic matter consumption		
First-order	k_H (d^{-1})	0.10 ± 0.04	First-order	k_B (d^{-1})	0.240 ± 0.06
	R^2	0.907		R^2	0.954
	AIC	− 36.79		AIC	− 25.54
First-order with residual	Cr (gCOD L^{-1})	0.11 ± 0.07	First-order with residual	Cr (gCOD L^{-1})	0.07 ± 0.05
	k_H (d^{-1})	0.15 ± 0.04		k_B (d^{-1})	0.26 ± 0.07
	R^2	0.957		R^2	0.963
	AIC	− 42.19		AIC	− 27.21
Carboxylic acids production					
First-order	k_{CA} (d^{-1})	0.20 ± 0.04	Second-order	k_{CA}''(L $gCOD^{-1}$ d^{-1})	0.16 ± 0.04
	R^2	0.985		R^2	0.950
	AIC	− 30.69		AIC	− 22.41
Fitzhugh	k_{CA} (d^{-1})	0.24 ± 0.13	Cone	k_{CA} (d^{-1})	0.30 ± 0.07
	n	1.20 ± 0.95		n	1.75 ± 0.48
	R^2	0.985		R^2	0.995
	AIC	− 29.08		AIC	− 37.08
Logistic	μ_m(gCOD $L^{-1}d^{-1}$)	0.33 ± 0.05	Transference	μ_m(gCOD $L^{-1}d^{-1}$)	0.46 ± 0.09
	λ (d)	0.37 ± 0.74		λ (d)	0.04 ± 0.12
	R^2	0.957		R^2	0.985
	AIC	− 21.58		AIC	− 28.73
Richards	μ_m(gCOD $L^{-1}d^{-1}$)	0.36 ± 0.18	Modified Gompertz	μ_m(gCOD $L^{-1}d^{-1}$)	0.33 ± 0.05
	λ (d)	0.34 ± 0.77		λ (d)	0.16 ± 0.50
	v	1.16 ± 0.57		R^2	0.975
	R^2	0.954		AIC	− 25.24
	AIC	− 19.08			

k_H hydrolysis rate constant (d^{-1}), k_B soluble substrate degradation rate constant (d^{-1}), C_r residual COD concentration (gCOD L^{-1}), k_{CA} first-order COD_{CA} production rate constant (d^{-1}), k_{CA}'' second-order COD_{CA} production rate constant (L $gCOD^{-1}$ d^{-1}), n form constant (dimensionless), λ lag phase time (d), μ_m maximum COD_{CA} productivity (gCOD $L^{-1}d^{-1}$), v constant of the Richards model (dimensionless)

maximum CA productivity was estimated by the transference model ($\mu_m = 0.46 \pm 0.09$ gCOD $L^{-1}d^{-1}$), and the lowest value was predicted by the modified Gompertz model ($\mu_m = 0.33 \pm 0.05$ gCOD $L^{-1}d^{-1}$).

Kinetic modeling of carboxylic acid production

The kinetic parameters obtained in the mathematical modeling of each CA production can be seen in Table 4. The most suitable models to simulate CA production were: cone model for HAc and HCa and Fitzhugh model for HBu and HIVa (Table 4; Fig. 2). These models indicated that the highest first-order production rate constant was estimated for HAc ($k_{CA}' = 0.298$ d^{-1}) and the lowest for HBu ($k_{CA}' = 0.209$ d^{-1}), suggesting that HAc and HBu are the acids that are formed more quickly and slowly, respectively. The second-order model was the one with the best fit for HPr and HVa synthesis (Table 4 and Fig. 2), resulting in a CA production rate constant k_{CA}'' of 5.72 and 17.44 L $g^{-1}d^{-1}$ for HPr and HVa, respectively. Thus, mathematical models that describe exponential growth proved to be adequate to simulate CA production from acidogenic fermentation of SHW.

According to the kinetic parameters presented in Table 4, the maximum productivity rate of acids (μ_m), in g $L^{-1}d^{-1}$, obtained by the models that best fit the experimental data was 0.202 (transference model); 0.089 (transference model); 0.023 (modified Gompertz model); 0.019 (modified Gompertz model); 0.014 (transference model); and 0.015 (modified Gompertz model) for HAc, HPr, HBu, HIVa, HVa, and HCa, respectively. These values of μ_m are very close to those calculated directly with the concentration data obtained on collection days (Table 5), indicating that the applied kinetic models were able to satisfactorily estimate this parameter. Regarding the lag phase, the models indicated time lower than one day for the beginning of the production of all acids, except HCa ($\lambda = 1.79$ d).

Yields, selectivity, and productivity

Detailed monitoring of the organic matter bioconversion (in COD mass) can be seen in Table S2 (Supplementary

Table 4 Mean values of kinetic parameters estimated by modeling carboxylic acids production in the SHW acidogenic fermentation

Model	Parameters	HAc	HPr	HBu	HIVa	HVa	HCa
First-order	k_{CA} (d^{-1})	0.206	0.492	0.132	0.175	0.362	0.149
	R^2	0.983	0.945	0.976	0.986	0.836	0.899
	AIC	−41.76	−58.78	−57.73	−68.84	−72.24	−64.26
Fitzhugh	k_{CA} (d^{-1})	0.291	0.591	0.209	0.273	0.040	0.479
	n	1.526	1.328	1.838	1.787	0.173	4.935
	R^2	0.987	0.945	0.995	0.998	0.898	0.937
	AIC	−41.78	−56.83	−66.82	−81.20	−73.58	−65.63
Cone	k_{CA} (d^{-1})	0.298	0.509	0.189	0.251	1.100	0.233
	n	1.872	25.654	2.148	2.110	0.641	2.530
	R^2	0.993	0.929	0.990	0.997	0.873	0.949
	AIC	−46.45	−55.04	−61.44	−77.90	−72.02	−67.05
Second-order	k_{CA}''($L\ g^{-1}\ d^{-1}$)	0.390	5.725	0.968	2.190	17.443	3.404
	R^2	0.940	0.956	0.884	0.910	0.897	0.852
	AIC	−32.89	−60.29	−46.61	−55.74	−75.50	−61.59
Modified Gompertz	μ_m($g\ L^{-1}d^{-1}$)	0.148	0.466	0.023	0.019	0.009	0.015
	λ (d)	0.340	1.757	0.694	0.505	0.000	1.792
	R^2	0.982	0.929	0.992	0.994	0.795	0.933
	AIC	−39.52	−55.04	−62.93	−72.64	−68.69	−65.16
Logistic	μ_m($g\ L^{-1}d^{-1}$)	0.152	0.062	0.024	0.019	0.008	0.014
	λ (d)	0.612	0.274	1.150	0.749	0.000	1.833
	R^2	0.972	0.942	0.980	0.982	0.763	0.912
	AIC	−36.17	−56.44	−57.02	−64.93	−67.68	−63.22
Transference	μ_m($g\ L^{-1}d^{-1}$)	0.202	0.089	0.033	0.026	0.014	0.012
	λ (d)	0.099	0.000	0.421	0.229	0.000	0.472
	R^2	0.984	0.945	0.981	0.988	0.836	0.907
	AIC	−40.01	−56.78	−57.43	−68.13	−70.24	−62.84
Richards	μ_m($g\ L^{-1}d^{-1}$)	0.001	0.002	0.000	0.000	0.000	0.000
	λ (d)	0.230	1.772	0.283	0.468	0.468	1.769
	v	0.004	0.001	0.006	0.000	0.000	0.000
	R^2	0.979	0.929	0.967	0.994	0.762	0.933
	AIC	−36.39	−53.04	−51.39	−70.62	−65.63	−63.16

k_{CA} first-order CA production rate constant (d^{-1}), k_{CA}'' second-order CA production rate constant ($L\ g^{-1}d^{-1}$), n form constant (dimensionless), λ lag phase time (d), μ_m maximum CA productivity ($g\ L^{-1}d^{-1}$), v constant of the Richards model (dimensionless)

Material). On average, 76 ± 4% of the applied organic matter was converted to CA (501.1 ± 23.7 $mgCOD_{CA}$), representing a yield of 0.55 g acids $gCOD_A^{-1}$ ($Y2_{CA}$), corresponding to 0.76 gCOD $gCOD_A^{-1}$ ($Y1_{CA}$). This result was higher to those reported by Yin et al. [41] that investigated biological CA production from acidogenic fermentation of glucose, peptone, and glycerol in batch reactors inoculated with mixed biomass from a brewery wastewater treatment. They reported average yields of 0.66, 0.60, and 0.51 gCOD $gCOD_A^{-1}$ to these substrates, respectively. Thus, SHW becomes a promising substrate due to its higher yields of CA than that obtained by the fermentation of simple substrates that do not require hydrolysis for the availability of soluble organic matter.

Besides, CA production potential from SHW was also higher than reported by our previous work using swine wastewater (SW) as a substrate [18]. Using SW under the same operating conditions (temperature, pH, rotation, and inoculum) adopted in this study, a yield of 0.33 g acid $gCOD_A^{-1}$ was achieved, corresponding to 0.40 gCOD $gCOD_A^{-1}$. Unlike SHW, SW is a liquid organic waste with a high content of particulate organic matter because it is formed by the wash water of pig farms, excrement, and undigested food residues [43]. Thus, hydrolysis was the main limiting step of CA production from SW, once that it took more than twenty days for the particulate COD consumption to stabilize and fourteen days to produce 60% of the acids formed [18], while SHW proved to be a highly available substrate for acidogenic microorganisms because it presented high production rates of CA in the first days of the experiment (Fig. 1d).

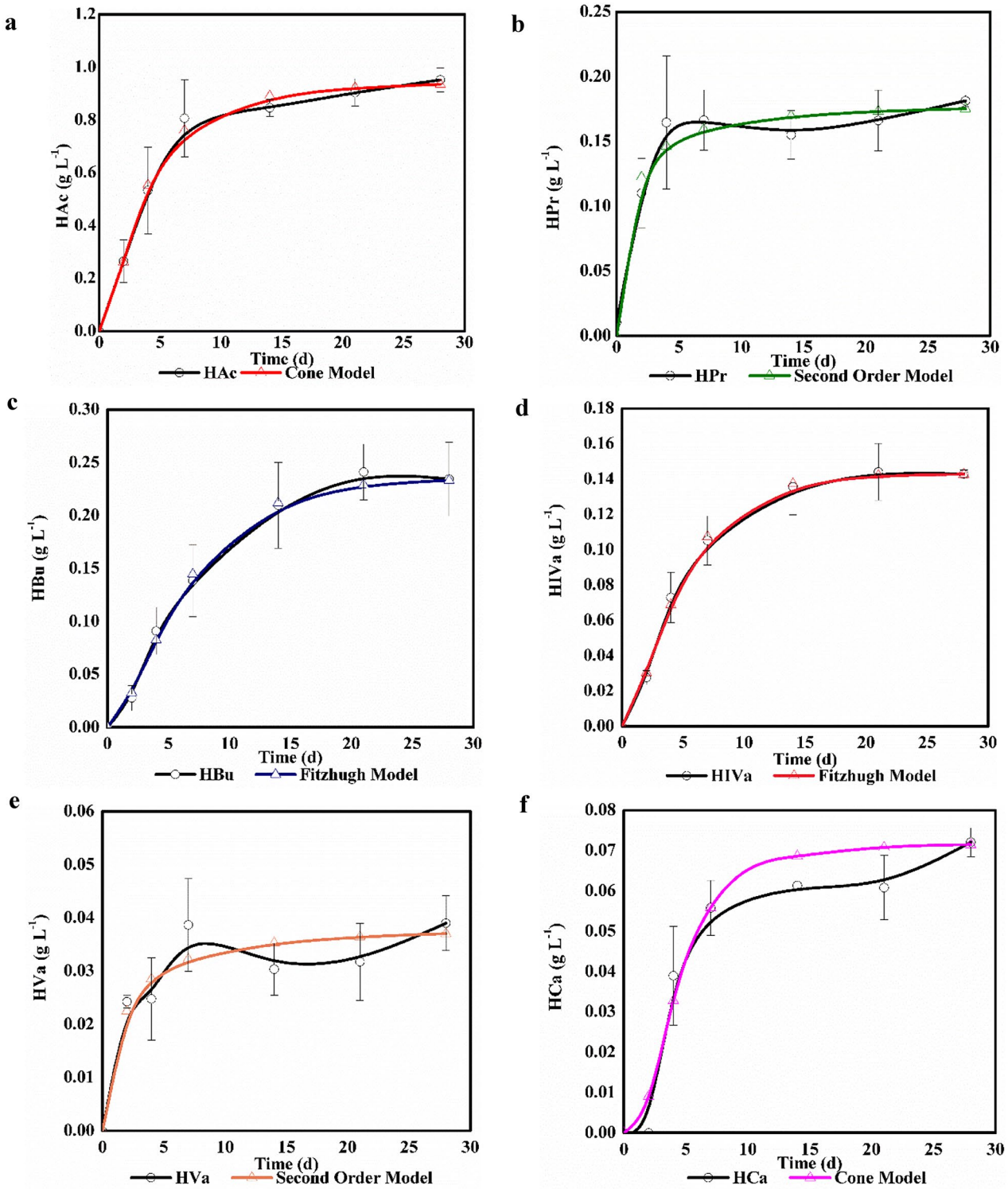

Fig. 2 Experimental production curves and curves generated by the kinetic modeling of CA production. **a** HAc (cone model). **b** HPr (second-order model). **c** HBu (Fitzhugh model). **d** HIVa (Fitzhugh model). **e** HVa (second-order model). **f** HCa (cone model)

The high CA yield obtained in this study can be attributed to SHW having high soluble compound-related biodegradability, high concentration of macro- and micronutrients, natural buffering, and readily available biomolecules that can be easily degraded by anaerobic microorganisms under favorable conditions [21]. Besides, ammonia produced

Table 5 Selectivity, yields, final concentration, and maximum productivity of carboxylic acids

Parameter	HAc	HPr	HBu	HIVa	HVa	HCa
[a]Selectivity(%)	59 ± 2	11 ± 2	15 ± 2	9 ± 0	2 ± 1	4 ± 0
[b]$Y1_{CA}$($mgCOD_{CA}$ $gCOD_A^{-1}$)	347 ± 17	88 ± 17	146 ± 22	100 ± 2	27 ± 7	54 ± 3
[c]$Y2_{CA}$(mg acid $gCOD_A^{-1}$)	326 ± 16	58 ± 11	80 ± 12	49 ± 1	13 ± 3	25 ± 1
[d]Final concentration (mg acid L^{-1})	952 ± 45	181 ± 33	234 ± 35	143 ± 2	39 ± 10	72 ± 3
[e]Maximum productivity (mg acid L^{-1} d^{-1})	133 ± 41	54 ± 36	23 ± 6	18 ± 4	12 ± 10	10 ± 3

[a]Percentage of each acid formed about the total acids produced (%)

[b]COD_{CA} production yield about the available COD ($mgCOD_{CA}$ $gCOD_A^{-1}$)

[c]Acids production yields about the available COD (mg acid $gCOD_A^{-1}$)

[d]Final acid concentration (at the end of the experiment) (mg acid L^{-1})

[e]Maximum productivity (mg acid L^{-1} d^{-1})

during the hydrolytic–acidogenic stage of proteins may have contributed to inhibiting methanogenesis, aided the buffering capacity of the fermentation process, and provided nutrients to microbial biomass [44, 45].

According to Wang et al. [46], SHW is characterized by high organic matter in the form of proteins because it is composed mainly of bovine blood, as can be indicated by the concentration of total nitrogen (503 mg L^{-1}) present in this substrate (Table S1). Thus, although proteins were not quantified in the physicochemical characterization, they are very likely to be the main source of carbon for the acidogenic process. According to Lee et al. [47], proteins are degraded faster than lipids during the hydrolytic–acidogenic stage, which may have contributed to the high CA productivity in the first days of the experiment and to the short lag phase.

The major CA formed were HAc and HBu with a selectivity of 59% and 15%, respectively (Table 5). Several factors may have influenced the higher HAc and HBu selectivities in SHW fermentation, such as temperature, substrate composition, type of biomass, and incubation time applied [48]. According to Chen et al. [49], in a mixed culture system, pyruvic acid proportions converted to CA can be influenced by the type of organic residue used as a substrate, by the main enzymes involved in the CA production, and by the preferential metabolic pathways of the microorganisms present in the inoculum. The high HAc selectivity obtained in this work agrees with the studies reported by Atasoy et al. [3], who observed that HAc represents 30% to 80% of CA produced from the organic waste fermentation.

Temperature is an important operational factor in CA production because it affects microorganisms' growth, enzymatic activity, and hydrolysis rate [50]. Mesophilic temperature (35 °C) was used for the assays with SHW, promoting a selectivity of 59% HAc and close values for HPr (11%) and HBu (15%). This result is in accordance with Jiang et al. [48], who investigated the CA synthesis from food waste and found that HBu was the main product at 55 °C, while HAc and HPr were the main products at 35 °C. This demonstrates that mesophilic temperature is one of the variables that favor HAc metabolic pathways synthesis [3].

Liu et al. [50] reported that HAc was the main fermentation product of protein-rich residues, which is in agreement with the findings in the present research. According to Yin et al. [41], HBu is the dominant product of carbohydrate fermentation, and HAc and HPr are the preferred products of protein and lipid fermentation. Besides, Yu and Fang [51] concluded that even HAc is the preferred product protein-rich fermentation substrates, the HVa production is also favored, even at low concentrations, as can be seen by HIVa (9%) and HVa (2%) selectivities obtained in the current work (Table 5).

On average, 65% of COD converted in CA was directed toward HAc and HBu formation, which were the main CA to be converted in HCa and other MCCA in the β-oxidation reverse process [52]. It is noted that even without the addition of electron donors for CCEP, such as ethanol and lactic acid, the inoculum was able to produce HCa from SHW as the sole source of carbon and energy, even at low concentration (72 ± 3 mg L^{-1}) and selectivity (4%) (Table 5).

Because no external electron donor was added, possibly ethanol and hydrogen, formed in the metabolic pathways of macromolecules degradation, were the main contributors to HVa and HCa production from the CCEP of HAc, HPr, and HBu [53, 54]. Thus, the incubation time adopted (28 days) contributed to the hydrolytic–acidogenic microorganisms having enough time to degrade organic matter and producing CCEP precursors (acetate, butyrate, ethanol, hydrogen), contributing to the MCCA formation [55]. Similar studies that evaluated the biological CA production in batch reactors using mixed biomass from brewery wastewater treatment did not report HCa formation, for instance, using residual glycerol [17], or glucose, peptone, and glycerol [41] as substrates. For this reason, SHW seems to be a potential substrate for MCCA production.

Electron donor's addition to stimulating CCEP is sometimes used to increase MCCA productivity in the acidogenic

anaerobic process [56]. In this experiment, the maximum HAc and HBu yields occurred on the fourth day, and the addition of electron donors at this time might favor the carboxylic chain elongation process and increase HCa synthesis, as well as HIVa and HVa, since CCEP also occurs through HPr [57].

Finally, biogas chromatographic analysis showed that chloroform was able to inhibit methanogenesis, since CH_4 concentrations were not detected in biogas, confirming the methanogenic inhibitor efficiency [30]. Chloroform is capable of inhibiting the methyl-coenzyme M reductase in methanogenic archaea and may also interfere with the metabolism of homoacetogenic and sulfate-reducing bacteria [58]. As methanogenesis was inhibited, the biogas formed in the bioreactors had a predominance of CO_2, H_2S, and H_2.

Conclusions

SHW showed to be a highly available substrate for acidogenic microorganisms because it had high CA production rates in the first days of the experiment. Due to the high presence of soluble substrate at the beginning of the experiment, the hydrolysis step had little influence on the delay of CA production. The first-order with residual kinetic model best simulated the hydrolysis process and the biodegradable organic matter consumption, and the kinetic models that can describe exponential functions, such as cone, first-order, and Fitzhugh models, were the most suitable for simulating CA production. A yield of 0.55 g acids $gCOD_A^{-1}$ was achieved, corresponding to 0.76 gCOD $gCOD_A^{-1}$.

Most of the organic matter applied (76%) was bioconverted to CA. Therefore, SHW seems to be a promising substrate to produce CA from biotechnological processes. For this reason, further studies should be conducted to define strategies focusing on chain elongation to produce MCCA from SHW. Besides, economic feasibility studies must be carried out in future work to investigate the real economic return of this type of bioprocess in a large-scale production context.

Furthermore, SHW's acidogenic fermentation presents itself as a possibility to dispose of this waste in an environmentally appropriate way and to promote the prospecting of high value-added products from low-cost organic matter. In the context of wastewater resource recovery, understanding the process kinetics can assist in the development of new bioreactors for acidogenic fermentation and process scaling. Therefore, the data generated in this work may be useful for future bioprocess scale-up.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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