A kinetic study on caproic acid production using different inocula

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Highlights:

- Key insights for optimizing caproic acid production through mathematical models.
- Ruminal fluid presented the lowest AIC and highest R^2 values in all models.
- Modified Gompertz was the one that best fit the caproic acid production curve.

Keywords: Medium-chain carboxylic acids; caproic acid; mixed cultures.

INTRODUCTION

The chain elongation process presents an opportunity for the biological production of fuels and high-value chemicals, such as medium-chain carboxylic acids (MCCAs). Among MCCAs, caproic acid has various market applications, including antimicrobials, additives, flavoring agents, biofuels, and bioplastics (Cavalcante et al., 2017).

Chain elongation is achieved using short-chain carboxylic acids (e.g. acetic and butyric acids) and electron donors (e.g., ethanol, hydrogen, and lactate) via two possible pathways: reverse β-oxidation pathway or fatty acid biosynthesis (Angenent et al., 2016; Villegas-Rodríguez & Buitrón, 2021).

In this context, data related to the production parameters of caproic acid using different inocula are not well-reported in the literature (Zuo et al., 2020). The application of mathematical models becomes attractive for obtaining kinetic parameters, such as the production rate constant (k), phase lag time (λ), and maximum production rate of the bioproduct of interest (R_m), which can be used to simulate caproic acid production and predict and optimize reactor performance (Wang et al., 2020).

Therefore, the objective of this study was to apply mathematical models to estimate the kinetic parameters for caproic acid production potential using ethanol and acetic acid as substrates with different inocula (ruminal fluid, granular anaerobic sludge, and cassava wastewater).

METHODOLOGY

Inoculum: Three different inocula were selected to evaluate the potential to perform the chain elongation process: 1) Ruminal fluid (RF), including solids, collected from a cattle slaughterhouse; 2) Granular anaerobic sludge (GAS), from an IC reactor used to treat a brewery wastewater; and 3) Cassava wastewater (CW), from a cassava flour mill. The batch reactors were inoculated with a final concentration of 1 g TS/L in the mixed liquor for each microbiome.

Experimental setup: Caproic acid production potential assays were carried out in batch reactors in borosilicate flasks with 600 mL total volume and 426 mL reaction volume, sealed with butyl rubber stoppers and plastic caps. The basal medium used as substrate was composed of ethanol (10 g/L) and acetic acid (6.5 g/L) complemented with the solution of macro and microminerals, sodium bicarbonate buffer (2.4 g/L) and with the pH adjusted to 6.80 by the addition of NaOH (1 M) or HCl (1 M) (Cavalcante et al., 2017). The reactors were maintained at 30±1 °C in an orbital shaker at 100 rpm. 2-bromoethanesulfonic acid (2-BES) (1 g/L) was used as a methanogenesis inhibitor (Grootscholten et al., 2013). The reactors were assembled in triplicate and received 3 feeds in sequential batches, lasting 12 days each, named B-I, B-II, and B-III.

Analytical Methods: Concentrations of caproic acid were determined by a high-performance liquid chromatography (HPLC; Shimadzu LC-20AT) equipped with a RID detector and a Aminex HPX 87H column (300 mm × 7.8 mm).

Model fitting: The equations of the kinetic models selected to describe the caproic acid production are presented in Morais et al. (2021) . A nonlinear least-squares regression analysis was performed using the Statistica 14 software to estimate the parameters of the selected kinetic models. The selection of the model that best describes caproic acid production was performed using the coefficient of determination (R^2) and the Akaike information criterion (AIC). Data from the B-III of the three tested inocula were used to calculate the kinetic parameters of the models.

RESULTS AND CONCLUSIONS

Table 1 presents estimated kinetic parameters for caproic acid production with different models (Modified Gompertz, Richards, Modified Logistic, Transference and Boltzmann), where C_{max} is the maximum potential production of caproic acid (g/L), μ_m is the maximum production rate of caproic acid (g/L.d), λ is the time lag (lag phase) (d), R^2 is the coefficient of determination and AIC is the Akaike information criterion. The Modified Gompertz was the one that best fit the caproic acid production curve generated by experimental data from the three inocula with high R^2 and lower values for AIC. Ruminal fluid (RF) inoculum presented the lowest AIC values and highest R^2 values in all models.

Therefore, the data generated in this work may be useful for future bioprocess scale-up.

Table 1. Kinetic parameters estimated by the modeling of caproic acid production.

Kinetic model	Parameters	CW	GAS	RF
Modified Gompertz	Cmax	8.628	9.413	8.750
	μ_m	23.708	25.938	4.634
	λ	4.557	4.699	2.078
	R^2	0.967	0.977	0.996
	AIC	-1.272	-2.677	-13.382
Richards	Cmax	8.628	9.413	8.750
	μm	4.298	6.116	1.429
	λ	4.512	4.972	2.757
	R^2	0.958	0.971	0.994
	AIC	-0.214	-1.698	-11.384
Modified Logistic	Cmax	8.598	9.175	8.751

Kinetic model	Parameters	CW	GAS	RF
	µm	3.753	18.753	4.526
	λ	2.506	4.624	2.469
	R^2	0.957	0.969	0.994
	AIC	-0.026	-1.179	-11.287
Transference	Cmax	9.784	11.171	10.439
	µm	2.355	2.217	2.278
	λ	0.400	0.459	0.417
	R^2	0.737	0.782	0.776
	AIC	11.243	10.909	10.580
Boltzmann	Cmax	8.618	9.413	8.742
	µm	3.651	4.917	3.436
	λ	0.573	0.077	0.483
	R^2	0.957	0.969	0.994
	AIC	-0.025	-1.179	-11.287

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