

# Kinetics of anaerobic biodegradation of synthetic dairy wastewater pretreated with lactase

*Cinética de biodegradação anaeróbia de água residuária sintética de laticínios pré-tratada com lactase*

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## ABSTRACT

The aim of this study was to evaluate the anaerobic degradation of dairy wastewater with and without the application of lactase. Two UASB reactors (16 L) were built to treat synthetic dairy wastewater (DWW). Four organic loads (0.5, 1.0, 2.0, and 3.0 kg m<sup>-3</sup> d<sup>-1</sup> COD) were applied for 45, 95, and 45 days, keeping the HRT at 1 day. Ammonium sulfate and potassium phosphate were added to the DWW (COD:N:P of 500:5:1) and sodium bicarbonate (pH equal to 7.0). UASB1, which received DWW with lactase, showed no difference in COD removal efficiency compared to UASB2, which received DWW without lactase. The average COD removal efficiencies were 69, 77, 70, and 56% for UASB1 and 68%, 72%, 69%, and 57% for UASB2 in each phase. From the Monod kinetics,  $Y$  is the growth coefficient (mg mg<sup>-1</sup> d<sup>-1</sup>);  $K_d$  is the endogenous decay coefficient (d<sup>-1</sup>);  $\mu_{max}$  is the maximum microbial growth rate (d<sup>-1</sup>); and  $K_s$  is the limiting substrate concentration (mg L<sup>-1</sup>) equal to 1.16 and 1.20; 0.05 and 0.04; 0.18 and 0.13; and 248 and 109, respectively, for UASB1 and UASB2. It can be concluded that the analysis of the anaerobic degradation of DWW in the UASB reactor proved to be adequate, with COD removal efficiencies considered satisfactory (70-76%). The application of lactase as a pre-treatment was not effective in increasing the biodegradability of synthetic dairy wastewater.

**Keywords:** agro-industrial wastewater; anaerobiosis; UASB; *Kluyveromyces lactis*.

## RESUMO

O objetivo do presente trabalho foi avaliar a degradação anaeróbia de efluentes de laticínios com e sem a aplicação de lactase. Foram construídos dois reatores tipo UASB (16 L), para o tratamento de efluente sintético de laticínios. Quatro cargas orgânicas (0,5, 1,0, 2,0 e 3,0 kg m<sup>-3</sup> d<sup>-1</sup> de DQO) foram aplicadas durante 45, 95, 95 e 45 dias, mantendo o HRT de 1 dia. À ARL foi adicionado sulfato de amônio e fosfato de potássio (DQO:N:P de 500:5:1), e bicarbonato de sódio (pH igual a 7,0). O UASB1 que recebeu a ARL com lactase não apresentou diferença de eficiência de remoção de DQO em relação ao UASB2, que recebeu a ARL sem a lactase. As eficiências médias de remoção de DQO foram de 69, 77, 70 e 56% para o UASB1 e de 68, 72, 69 e 57% para o UASB2, em cada fase. A partir da cinética de Monod, o coeficiente de crescimento -  $Y$  (mg mg<sup>-1</sup> d<sup>-1</sup>); o coeficiente de decaimento endógeno -  $K_d$  (d<sup>-1</sup>); a taxa máxima de crescimento microbiano -  $\mu_{max}$  (d<sup>-1</sup>); e a concentração do substrato limitante -  $K_s$  (mg L<sup>-1</sup>) foram iguais a 1,16 e 1,20; 0,05 e 0,04; 0,18 e 0,13; e 248 e 109, respectivamente para os UASB1 e UASB2. Pode-se concluir que a análise da degradação anaeróbia da ARL no reator UASB se mostrou adequada com obtenção de eficiências de remoção de DQO consideradas satisfatórias (70-76%). A aplicação da lactase como pré-tratamento não se mostrou efetiva, no aumento da biodegradabilidade da água residuária sintética de laticínios.

**Palavras-chave:** água residuária agroindustrial; anaerobiose; UASB; *Kluyveromyces lactis*.

## INTRODUCTION

The dairy industry is known for its social, environmental, and economic contributions. In the State of Minas Gerais, Brazil, the production of milk and dairy products has a significant impact. Regulatory agencies ensure wastewater is treated before discharge (Tabelini *et al.*, 2023).

In 2020 and 2021, several sectors suffered from the negative impacts of the COVID-19 pandemic, especially the derivatives sector, with reduced availability of inputs for the production chain and an increase in the prices of dairy products. In Brazil, production in 2021 was approximately 3.6% lower than in 2020 (EMBRAPA, 2022). As of 2023, the adverse effects of the health

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crisis abated, resulting in a restoration of equilibrium between the supply and demand of the products.

The wastewater generated by this sector contains organic compounds that require treatment. The techniques employed include sequential treatment under anaerobic and/or aerobic conditions, which may involve fat-removing units (Stasinakis; Charalambous; Vyrides, 2022).

Aerobic methods are biological treatments that are generally used to remove pollutants from dairy wastewater. However, these methods have high-energy requirements to operate, making them non-sustainable. However, anaerobic methods are found to be active in stabilizing the biodegradable part of organic matter to an extent of 80–90%, without energy consumption (Bella; Rao, 2023).

Anaerobic methods offer advantages while removing pollutants and recovering energy from wastewater. Moreover, less sludge production, no artificial aeration requirement, and less area demand are some other benefits of anaerobic treatment. Anaerobic treatment methods are especially suitable for treating highly organic wastewaters (Chernicharo, 2007), such as dairy wastewater.

Despite the advantages of anaerobic treatment, it is important to understand the biochemistry and microbiology of the process in order to achieve greater treatment efficiencies. In addition, the kinetics of the treatment process are influenced by environmental issues, such as temperature. The kinetic models showed the effect of temperature on microbial growth and substrate consumption. The increase in temperature resulted in an increase in the removal of organic matter (Boshagh; Rostami; van Niel, 2022). The kinetics of the treatment process are greatly influenced by the characteristics of the wastewater. In the case of lactose intolerance, it is possible to add the enzyme lactase to the production process of milk products. This addition has caused an alteration in the final characteristics of the wastewater generated, without lactose (Natrella; Gambacorta; Faccia, 2023).

Slavov (2017) verified that the by-products in cheese manufacturing are rich in soluble compounds, over 80% of which is lactose. And about 90% of BOD and COD loading is caused by lactose. Göbös *et al.* (2008) concluded that the yeast *Kluyveromyces lactis* converts the lactose in the first fermentation stage into ethanol, avoids the relatively fast acidogenic step, and reduces the production of volatile fatty acids. Thus, the application of *K. lactis* changes the characteristics of dairy wastewater, due to the removal of lactose, and changes the steps of anaerobic degradation.

Meanwhile, from the standpoint of design, prediction, and evaluation of bioreactors, it is vital to use mathematical modeling and kinetic study, which are the most promising tools for indicating microbial activity and can be simply used by design engineers (Ahmadi *et al.*, 2020). However, there is limited or even no information about the kinetic and mathematical modeling of anaerobic reactors used in the treatment of dairy wastewater generated in the production of lactose-free dairy products.

Then, the objective of this study was to investigate the anaerobic degradation of synthetic dairy wastewater using Upflow Anaerobic Sludge Blanket (UASB) reactors, with and without lactase  $\beta$ -galactosidase obtained from the *K. lactis* fungus.

## METHOD

### Description of the experimental apparatus

The experiment was executed at the Wastewater Treatment Laboratory within the Department of Environmental Engineering at the Federal University of Lavras.

The experimental setup consisted of two identical treatment lines, each featuring an acidification and equalization tank (TAE), a solenoid metering pump for wastewater pumping, a coil system heater, and a UASB reactor (Figure 1).

The TAEs consisted of fiberglass boxes with a capacity of 250 L each. The pumps were solenoid dosing machines, with a maximum flow rate of 23 L h<sup>-1</sup>, of the Prominent® brand. The heaters consisted of a glass container measuring 0.15 m × 0.15 m × 0.30 m (width, length, and height), into which a coil (spiral) of copper tube of 10 mm diameter was willing, which filled the entire height of the heater. The electric boiler, which heated the water containing a submerged coil, featured a connection to a thermostat responsible for regulating the water temperature within the range of 35°C ± 5°C. The wastewater was pumped from the TAE, directing it through the coil for heating before being conveyed to the UASB reactor.

The UASB reactors were constructed using acrylic material and had a height of 0.80 m, an internal diameter of 0.25 m, and a usable volume of 0.016 m<sup>3</sup>. The base of the reactors is an inverted cone that is 0.15 m deep, where sludge accumulates the most. In monitoring sludge profiles, we installed four samplers equidistantly throughout the reactors at intervals of 0.15 m. We positioned the first sampler at 0.05 m above the base of the inverted cone, and we situated the last sampler at 0.15 m below the reactor's DWW exit point.

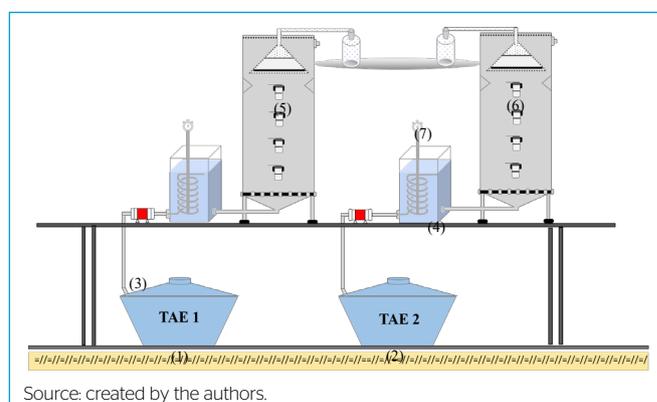
### Synthetic dairy wastewater

In this study, we prepared weekly synthetic dairy wastewater (DWW), diluting long-life skimmed milk (173,000–200,000 mg L<sup>-1</sup> of COD) with water from the UFLA supply network, adding it to TAE in a COD range of 600–3,000 mg L<sup>-1</sup> in the four phases. COD concentrations were defined according to monitoring of wastewater from a dairy plant in the study region.

We corrected the nutrient content by adding ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) and potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) to achieve a COD:N:P ratio of 500:5:1, according to Chernicharo (2007). Additionally, we supplemented the alkalinity of the DWW with a sodium bicarbonate solution (NaHCO<sub>3</sub>) to maintain the DWW pH at 7.0.

### Experimental procedure

Distinguishing two treatment lines based on the application of the enzyme lactase  $\beta$ -galactosidase, we applied Prozyn Lactase® 5,000 NLU g<sup>-1</sup> in line 1



**Figure 1** - Schematic drawing of the experimental apparatus composed of acidification and equalization tanks (1 and 2), metering pumps (3), coil heaters (4), UASB1 (5), UASB2 (6), and methane gas collectors (7).

(UASB1) using the enzyme derived from the fungus *K. lactis*, produced by Prozyn Biosolutions®, São Paulo, Brazil. In contrast, line 2 (UASB2) did not receive the enzyme. Afterward, we added the enzyme directly to the milk at a concentration of 0.5 mL L<sup>-1</sup> and diluted it in TAE with water from the supply network.

We inoculated the two reactors with sludge from the UASB reactor of the sanitary wastewater treatment plant at the Federal University of Lavras (WWTP/UFLA). We used an amount of 1.55 L of sludge per reactor, resulting in an initial biological organic load (BOL) of 0.10 kg kg<sup>-1</sup> d<sup>-1</sup> expressed in terms of [COD] [STV]<sup>-1</sup> [d]<sup>-1</sup> (Chernicharo, 2007).

The study consisted of four phases, each with a distinct organic loading rate (OLR) (0.35, 0.70, 1.4, and 2.8 kg m<sup>-3</sup> d<sup>-1</sup>) and durations (45, 95, 95, and 45 days). HRT (24 h) and flow rate (0.016 m<sup>3</sup> d<sup>-1</sup>) remained constant throughout all phases. Also, we monitored the soluble COD data to determine changes in the experimental stages.

## Monod kinetics

To conduct kinetic studies on UASB reactors with and without lactase, we assessed the average influent and effluent concentrations of organic matter (COD) and total volatile solids (TVS), theoretical hydraulic detention time (HRT), and results of sludge profiles performed with TVS analyses along the UASB reactors. We obtained laboratory-scale kinetic parameters for treating synthetic dairy wastewater using the equations described by von Sperling (2014) and Liu *et al.* (2017), both in the presence and absence of lactase.

The COD and TVS were analyzed in the influent and effluent using the closed backflow method for COD and colorimetry and gravimetry for TVS twice a week. During each phase, the sludge samplers collected the TVS in six samplings (APHA; AWWA; WEF, 2005).

## First-order kinetics of organic matter degradation

In the anaerobic biodegradability test, sludge from the UASB reactor of the WWTP/UFLA, collected in concentrated form at the base of the reactor, was used as inoculum. This sludge was characterized in terms of TVS by the gravimetric method (APHA; AWWA; WEF, 2005).

To obtain the results, two assays were performed with the same synthetic dairy wastewater (DWW), one with the inoculation lactase enzyme and the other without the presence of the enzyme.

The BOL of the reactor (capacity of 2.5 L) was determined based on the concentration of TVS present in the sludge (60.97 g L<sup>-1</sup> TVS) and the COD concentration of the DWW (3,000 mg L<sup>-1</sup>). We establish BOL 0.10 kg kg<sup>-1</sup> in the form of [COD][TVS]<sup>-1</sup> following the recommendations of Chernicharo (2007) for domestic wastewater.

The biodegradability tests were conducted in a reactor composed of a glass flask equipped with a magnetic stirrer and placed on a magnetic plate. In both assays, we introduced 1.5 L of the substrate with a COD concentration of 3,000 mg L<sup>-1</sup> COD (DWW, or DWW+lactase) into the reactor, prepared according to the procedure outlined in Section Synthetic dairy wastewater. Furthermore, we inoculated the reactor with 0.74 L of inoculum (sludge from the UASB reactor—WWTP/UFLA).

The contents were mixed for approximately 10 h at room temperature using a magnetic stirrer, positioning the cap to allow the release of gases generated during degradation into the external environment. The agitation was sufficient to maintain biomass suspension without requiring aeration.

The COD analysis was performed using the closed reflux method and colorimetry (APHA; AWWA; WEF, 2005). All experiments were conducted in triplicate to ensure robustness and reproducibility. The resulting COD concentrations, observed over the designated time frame, were subjected to the first-order organic matter degradation model, according to von Sperling and Paoli (2013).

## RESULTS AND DISCUSSION

### Monod kinetics

In this study, we determined the kinetic values by evaluating four distinct phases with a constant HRT (16 h) while introducing a variable and incrementally increasing organic load. Table 1 presents the mean values for COD and TVS.

Upon scrutinizing the interior TVS profile of the reactor (Xr), as outlined in Table 1, it is evident that UASB1 experienced difficulty in sustaining solids concentration while incrementing organic load, leading to a reduction in the mean concentration from 17,050 to 12,794 mg L<sup>-1</sup>. In the UASB2 system, a decline in concentration was observed during the initial phase (9,310 mg L<sup>-1</sup>), followed by a successive escalation in subsequent phases, reaching a TVS concentration of 16,594 mg L<sup>-1</sup> in phase IV.

**Table 1** - Mean values of Qe (influent flow), Vr (reactor volume), Xe (average concentration of total volatile solids effluent to the reactor), Xr (average concentration of volatile solids inside the reactor), Co (influent COD concentration), and C (effluent COD concentration).

Reactor	Phases	HRT(h)	Qe (L d <sup>-1</sup> )	Vr (L)	Xe (mg L <sup>-1</sup> )	Xr (mg L <sup>-1</sup> )	Co (mg L <sup>-1</sup> )	C (mg L <sup>-1</sup> )
UASB1	I	24	16	16	312	17,050	640	157
	II	24	16	16	383	12,941	1,343	263
	III	24	16	16	709	11,757	1,926	538
	IV	24	16	16	1,514	12,794	2,990	1,311
UASB2	I	24	16	16	287	9,310	539	158
	II	24	16	16	404	13,994	1,065	254
	III	24	16	16	698	14,917	1,895	586
	IV	24	16	16	1,546	16,549	3,046	1,317

HRT: average hydraulic detention time; UASB1: fed with DWW+ lactase; UASB2: powered with DWW.

Note that the COD affluent to the system ( $C_0$ ) from the DWW and, therefore, prepared and submitted to the same operating conditions showed a slightly higher value for UASB1 (except in phase IV), which may be related to the COD of the enzyme itself. Piao *et al.* (2019) obtained 1.03 g of COD per gram of yeast extract. Due to its composition, residual brewer's yeast has high levels of chemical oxygen demand (210,000 mg L<sup>-1</sup>) (Devolli *et al.*, 2018). The authors evaluated different yeasts, but we still believe that the application of *K. lactis* may have caused a small increase in the COD values of the effluent, especially for the lower concentrations of wastewater. However, we believe that the small increase in COD did not affect the results obtained.

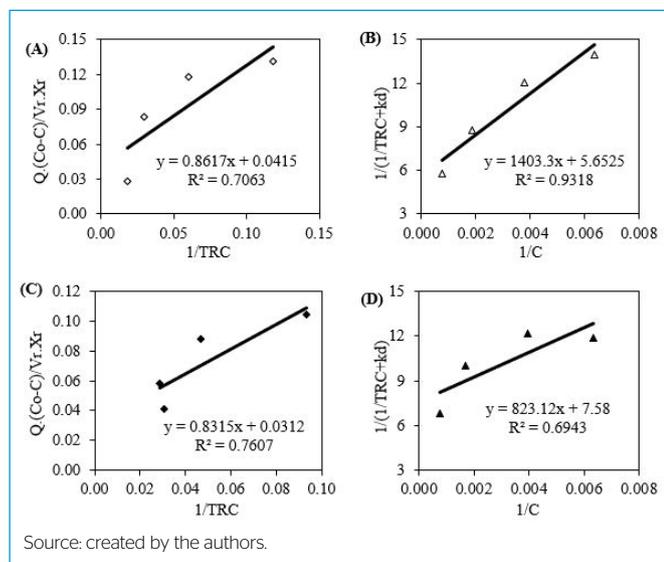
The kinetic parameters were determined through linear regression analysis using the data provided in Table 1. The results are presented in Table 2 and illustrated in Figure 2.

Upon scrutinizing the values acquired through kinetic modeling as delineated in Table 2, the proximity of coefficients  $Y$ ,  $K_d$ , and  $\mu_{max}$  between UASB1 and UASB2 is evident. Nevertheless, UASB1, subjected to lactase, exhibited a reduction in solids production rate ( $Y$ ) and an augmented growth rate ( $\mu_{max}$ ). This observation is likely attributable to the initial partial degradation of lactose, which constitutes approximately 70–90% of the COD in dairy wastewater (Göblös *et al.*, 2008; Slavov, 2017). Consequently, with a reduced substrate readily available, there was a concurrent increase in microbial decay ( $K_d$ ) due to a diminished quantity of biomass within the reactor.

**Table 2** - Kinetic parameters for an upflow anaerobic reactor treating synthetic dairy wastewater—DWW with added lactase (UASB1) and DWW (UASB2) subjected to different organic loads.

Reactors	$\theta_c$	$Y$	$K_d$	$\mu_{max}$	$K_s$
UASB1	8.4-54.7	1.16	0.05	0.18	248
UASB2	10.7-32.5	1.20	0.04	0.13	109

$\theta_c$ : cell detention time (d);  $Y$ : growth coefficient (mg mg<sup>-1</sup> d<sup>-1</sup>);  $K_d$ : endogenous decay coefficient (d<sup>-1</sup>);  $\mu_{max}$ : maximum microbial growth rate (d<sup>-1</sup>);  $K_s$ : concentration of the limiting substrate (mg L<sup>-1</sup>).



**Figure 2** - Determination of the kinetic coefficients of the model proposed by Monod for the degradation of organic matter in UASB1 (A and B) and UASB1 (C and D) in each experimental phase.

The higher  $K_s$  value for UASB1 may be due to the high concentration of soluble substrate provided by the breakdown of lactose (Sankar; Chandran; Pandiyan, 2018). Thus, microorganisms that demand a high value of  $K_s$  will exhibit a higher specific growth rate, as observed for UASB1, will degrade the substrate, and grow rapidly (Al-Malack; Aldana, 2016).

Even in this scenario, when evaluating UASB2, which received DWW, the values were quite discrepant when compared to domestic sewage and agro-industrial wastewater (Table 3), probably due to the specific characteristics of dairy wastewater, with specific organic characteristics and high nutrient rates (Krishna B. *et al.*, 2022).

Due to the scarcity of data on dairy wastewater in the literature and the discrepancy in the observed values, the initial analysis of this study uses reference values for raw domestic wastewater. While von Sperling (2014) cautions about the presence of an optimal range for each analyzed parameter, contingent upon the effluent's source, it is essential to consider these distinctions. Thus, comparing diverse wastewaters, or even those originating from similar sources but exhibiting distinct characteristics and compositions, can result in discrepant kinetic values between them.

The growth coefficient range ( $Y$ ) for raw domestic sewage is typically between 0.03 and 0.15 g g<sup>-1</sup>, expressed as [SSV] [COD removed]<sup>-1</sup> (Chernicharo, 2007). The values observed in this study, 1.16 and 1.20 g g<sup>-1</sup>, were higher than the average values reported in the literature, indicating the presence of microbial activity in the reactor and a high capacity for organic matter reproduction and degradation. However, the anticipated rise in reactor sludge volume did not materialize. The  $\theta_c$  levels are related to  $Y$  due to the food/microorganism (A/M) ratio and microbiological activity. According to Belli *et al.* (2019), these characteristics are crucial for the dynamics of biological reactor operation.

The endogenous decay coefficient ( $K_d$ ) for untreated domestic sewage typically falls within the range of 0.05–0.12 (mg mg<sup>-1</sup> d<sup>-1</sup>). Such values indicate a daily loss of 0.05–0.12 mg of volatile suspended solids (SSV) for each milligram of SSV initially present in the reactor. The  $K_d$  values obtained in this study align with those reported in the literature for domestic wastewater.

The microbial production coefficient ( $Y$ ) exhibited elevated levels, and the biomass loss within the reactor conformed to the average standards observed in domestic sewage. Nevertheless, the microbial growth rate was comparatively modest (0.13–0.18 d<sup>-1</sup>) when juxtaposed with the values advocated by van Haandel and Lettinga (1994), who propose a maximum microbial growth rate ( $\mu_{max}$ ) ranging from 2 d<sup>-1</sup> for acidogenic bacteria to 0.4 d<sup>-1</sup> for methanogenic archaeal bacteria.

The observed values in the current study are inferior to those previously reported, corroborating the value-limiting substrate ( $K_s$ ) concentration during phases I and II. For phases I and II, the substrate quantity partially restricted microbial degradation, consequently influencing biomass production. As a result, there was a reduced sludge volume in UASB1 and constrained sludge production in UASB2. After an escalation in organic load during phases III and IV, an enhanced structuring of the microbial community was evident, accompanied by a discernible trend toward solids recovery in UASB1 and UASB2 (Table 2).

Mohan and Vivekanandhan (2017) operated a bench-scale UASB-type anaerobic reactor to treat dairy wastewater. They found a  $Y$  value of 0.135 mg mg<sup>-1</sup> d<sup>-1</sup>, lower than the value in the current study. Additionally, they obtained a  $K_d$  value of 0.003 d<sup>-1</sup>, similar to the values observed in the present research.

**Table 3** - Summary of anaerobic kinetic constants obtained from the literature for agro-industrial wastewater in the present study.

Wastewater	COD (g L <sup>-1</sup> )	$\mu_{\max}$ (d <sup>-1</sup> )	$K_s$ (mg L <sup>-1</sup> )	Y (mg mg <sup>-1</sup> d <sup>-1</sup> )	$K_d$ (d <sup>-1</sup> )	References
Dairy	70-200	1831	8,594	-	-	Najafpour <i>et al.</i> (2009)
Dairy	26	1.69	174	0.23	0.14	Kaewsuk <i>et al.</i> (2010)
Dairy	15.0	15.36	15,890	-	-	Gadhe, Sonawane and Varma (2014)
Synthetic (diluted milk)	31	7.22	450	-	-	Mazzucotelli <i>et al.</i> (2014)
Dairy	176	0.70	5,860	-	-	Coelho <i>et al.</i> (2020)
Swine	15	0.05	282	0.09	0.01	Matangue and Campos (2011)
UASB1	0.64-2.99	0.18	248	1.16	0.05	Present study
UASB2	0.54-3.05	0.13	109	1.20	0.04	Present study

COD: chemical oxygen demand;  $\mu_{\max}$ : maximum microbial growth rate;  $K_s$ : concentration of the limiting substrate; Y: growth coefficient;  $K_d$ : endogenous decay coefficient.

The authors justify the values due to reduced organic loading rates applied to the reactor, which provided a reduction in the volatile solids.

Saléh, Campos, and Figueiredo (2009) modeled the removal of organic matter in a UASB reactor on a pilot scale, subjected to different HRT (20–44 h) and volumetric organic loads (0.5–1.1 kg m<sup>-3</sup> d<sup>-1</sup> COD). The study found Y values ranging from 0.59 to 0.82 mg mg<sup>-1</sup> d<sup>-1</sup>,  $K_d$  between 0.006 and 0.008 d<sup>-1</sup>,  $\mu_{\max}$  between 0.18 and 0.25 d<sup>-1</sup>, and  $K_s$  between 612 and 850 mg L<sup>-1</sup>, at a mean temperature of 24.8°C±1.8°C.

When comparing the values obtained in the present study to those of previous studies, it is evident that the solids production capacity was lower, as indicated by the lower values of Y.

Nevertheless, Saléh, Campos, and Figueiredo (2009) found that the lower microbial decay rate may lead to increased sludge accumulation in the reactor. The authors found that even with the increase in the organic load, there was a reduction in the concentration of TVS in the reactor (13,612 to 8,973 mg L<sup>-1</sup>). This fact may be related to the internal processes of microbial conversion because even with the highest HRT, it would not be sufficient to promote the washing of the sludge from the system.

The Y values in this study may be related to the wastewater's characteristics, which can affect the diversity and abundance of microorganisms in the biological system (Yang *et al.*, 2019).

Eldyasti, Nakhla, and Zhu (2012) noted that the microbial yield coefficient is significantly affected by the characteristics of wastewaters. In comparison to the elevated Y values in the present study, Sankar, Chandran, and Pandiyan (2018) achieved a solids production rate of 0.62 mg mg<sup>-1</sup> d<sup>-1</sup> in an aerobic reactor (membrane and biofilm bioreactor) employed for the treatment of dairy effluent.

Concerning  $\mu_{\max}$  and  $K_s$ , for both, the study found lower values. Thus, the reduced  $K_s$  value would limit constraints on microbial growth. However, notwithstanding the lower  $K_s$  value, microbial growth rates were still lower for UASB2 and equivalent for UASB1.

Najafpour *et al.* (2009), Gadhe, Sonawane, and Varma (2014), and Coelho *et al.* (2020) evaluated anaerobic systems with higher COD influent concentrations (Table 3), and they observed higher values of  $\mu_{\max}$  and  $K_s$  for dairy wastewater, which may indicate that influent concentration plays an important role in the process. Mazzucotelli *et al.* (2014) treated dairy wastewater with COD values similar to those in the present study; however, the  $\mu_{\max}$  and

$K_s$  values were higher than those in the present study, which may be related to the microorganisms employed, as the authors worked with isolated cultures of microorganisms.

Kaewsuk *et al.* (2010) treated dairy wastewater with a similar concentration of COD; however, the authors operated an anaerobic membrane sequencing batch reactor, which may have resulted in a higher growth rate ( $\mu_{\max}$ ) and greater solids production (Y), but also in higher endogenous decay ( $K_d$ ). Matangue and Campos (2011) evaluated the UASB reactor in swine wastewater treatment, but the lower organic matter concentration applied to the reactor in addition to the characteristics of the wastewater may have resulted in a lower rate of microbial growth, lower production of solids, and greater endogenous decay, compared to the present study.

In Table 3, it can be observed that even when comparing the kinetic coefficients of anaerobic degradation for dairy wastewater, the values diverge compared to the present study. This is because the kinetic parameters are specific to each studied condition.

### Anaerobic biodegradability

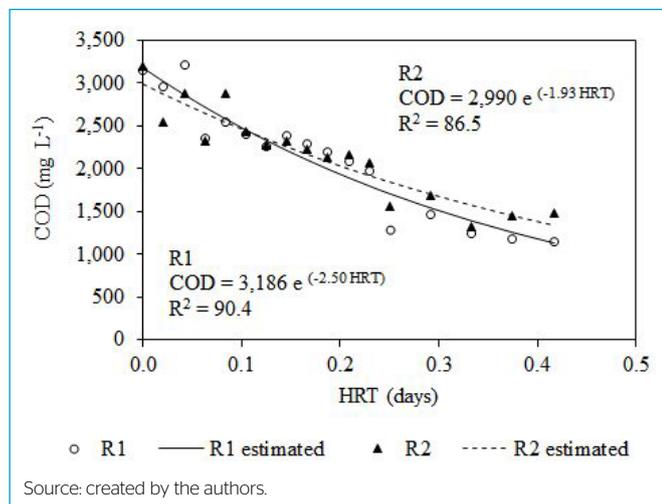
Throughout the anaerobic biodegradability tests, the pH of the liquid remained within the range of 7.3–7.7. These values confirm the optimal range for the growth and microbial activity in the degradation of organic matter, ranging from 5.0 to 9.0 for neutrophil microorganisms (Jin; Kirk, 2018).

Hence, it is apparent that the biodegradability assays do not show significant pH variations that could compromise their performance. The values were consistently maintained within the optimal range for the development of methanogenic microorganisms, as proposed by Mauerhofer *et al.* (2018).

The anaerobic biodegradability assays showed an exponential decay of COD concentration over time in both reactors, evidencing efficient decomposition of organic material in a short period of time (Figure 3).

The obtained equations exhibited a robust fit to the experimental data, with coefficients of determination exceeding 86%. The organic matter degradation coefficient, k, was higher for test 1 (k = -2.50 d<sup>-1</sup>) than for test 2 (k = -1.93 d<sup>-1</sup>). In R1, exposure to synthetic dairy wastewater containing lactase may have contributed to the breakdown of lactose into smaller, more easily biodegradable sugars.

Glucose and galactose, resulting from the enzymatic breakdown of lactose, exhibit higher solubility than lactose alone (Namvar-Mahboub; Pakizeh, 2012;



**Figure 3** - Observed and estimated COD values obtained while monitoring organic matter degradation in reactors that received synthetic dairy wastewater with lactase (R1) and solely synthetic dairy wastewater (R2).

Slavov, 2017). Moreover, fewer microorganisms can metabolize lactose than glucose and galactose (Rosolen *et al.*, 2015).

The presence of substantial quantities of fats and proteins in dairy wastewater can impact the biodegradability of the wastewater (Longaretti *et al.*, 2019). Morais *et al.* (2020) determined  $k$  values in anaerobic biodegradability assays in dairy wastewater, reaching  $0.395 \text{ d}^{-1}$ .

The rate of organic matter removal observed in the biodegradability trials did not correlate with the removal of organic matter from the UASB reactors. Otherwise, it could surpass the 56% COD removal attained in the fourth phase, potentially reaching estimated values ranging from 85% to 92%. This discrepancy is likely due to the heightened operational control implemented in the biodegradability tests.

## CONCLUSIONS

The present study demonstrated that the application of lactase in synthetic dairy wastewater did not result in significant variations in the kinetic parameters of Monod when evaluating anaerobic treatment in UASB reactors. For the two UASB reactors, with no lactase added, high values of the microbial growth coefficient ( $1.16 \leq Y \leq 1.20 \text{ mg mg}^{-1} \text{ d}^{-1}$ ), but also of the microbial decay coefficient ( $0.04 \leq K_d \leq 0.05 \text{ d}^{-1}$ ) were verified, due to the reduced organic loads applied to the reactors, which led to a gradual increase in the concentration of sludge in the reactor fed with dairy wastewater without lactase (UASB2) or even the loss of sludge from the system with the application of lactase (UASB1).

Concerning the anaerobic biodegradability test, values for the organic matter degradation coefficient obtained from the first-order model were higher ( $1.93 \leq k \leq 2.50 \text{ d}^{-1}$ ) than those reported in the literature for domestic and agro-industrial wastewaters. However, such elevated values were not observed in the UASB, indicating that the efficiency of organic matter removal in this system might be lower than initially anticipated.

Based on the results, we believe that new experiments should be conducted involving the monitoring of wastewater from dairy facilities that produce lactose-free dairy products. Additionally, segregating this wastewater for conducting biodegradability tests in both anaerobic and aerobic environments could enhance the results obtained in the present study.

## AUTHORS' CONTRIBUTIONS

Oliveira, F.A.D.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. Antônio, T.S.: Investigation, Writing – original draft. Cruz, T.C.: Investigation, Writing – original draft. Matos, M.P.: Formal analysis, Writing – original draft, Writing – review & editing. Fia, R.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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