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Special Edition

Anaerobic treatment of sugarcane vinasse pretreated with a calcium-based biopolymer

Tratamento Anaeróbio de Vinhaça de Cana-de-Açúcar Pré-Tratada com Biopolímero à Base de Cálcio

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ABSTRACT

This study investigated the influence of a previous sugarcane vinasse coagulation, by using a calcium-based biopolymer, on anaerobic treatment in a lab-scale reactor. Two anaerobic reactors (R1 and R2) were operated for 155 days. R1 was fed only with raw sugarcane vinasse and R2 was fed with pretreated effluent. The operation was divided into three stages: E1 (inoculation and adaptation); E2; and E3. The major difference between E2 and E3 was the fact that R2 failed at the end of E2. Thus, R2 was recovered by applying a low organic loading rate (OLR) of raw vinasse for 29 days. In E2 and E3, for both reactors, pH, alkalinity, COD (influent and effluent samples), and methane production were monitored. During E1, an OLR of 2.0 kgCOD. $m^{-3}.d^{-1}$ 1 was applied, and R1 and R2 sludges showed good adaptation to the wastewater in this condition. Then, the OLR was progressively increased to 7.0 kgCOD.$m^{-3}.d^{-1}$ in both systems. The average COD removal efficiencies were 82.9 ± 4.4% and 72.2 ± 18.1% for R1 and R2, respectively. R2, operated with pretreated vinasse, showed a decrease in the COD removal and methane production, which could be caused by the biopolymer presence. The average COD removal efficiencies in E3 were 77.5 ± 9.4% and 79.2 ± 9.7% for R1 and R2, respectively. After 155 days of operation, a decrease in the COD removal and methane production was identified again in R2, indicating a new failure. The quality of sludge granules from the inoculum and also from the granules developed at the end of R1 and R2 operation was analyzed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). These analyses indicated a rise in the rugosity of R1 and R2 granules, possibly due to the deposition of sedimentable compounds present in both raw and pretreated vinasse. The EDS analyses indicated a high presence of calcium in R2 granules, indicating that this compound acted in the reduction of mass transfer between organic load from vinasse and the granules' microbiota.

Keywords: Anaerobic digestion; Calcium; Methane

RESUMO

Neste trabalho investigou-se a influência da coagulação de vinhaça de cana-de-açúcar, utilizando um biopolímero à base de cálcio, no tratamento anaeróbio em reator de bancada. Operou-se durante 155 dias dois reatores anaeróbios (R1 e R2), sendo o denominado R1 alimentado apenas com vinhaça bruta. Dividiu-se a operação em três etapas, E1 (inoculação e adaptação), E2 e E3, sendo a diferença entre estas duas últimas o fato de o reator alimentado com vinhaça pré-tratada ter apresentado falha no tratamento. Monitorou-se o pH, alcalinidade e DQO na entrada e na saída dos reatores, e a produção de metano durante as etapas 2 e 3. Durante a fase de adaptação (E1), aplicou-se cargas orgânicas volumétricas de aproximadamente (COV) 2,0 kgDQO/m³.d e o lodo dos reatores 1 e 2 apresentaram boa adaptação ao efluente. Após esta etapa, aumentou-se progressivamente a COV até atingir valores próximos a 7,0 kgDQO/m³.d. A eficiência de remoção de DQO do R1 foi de 82,9 ± 4,4%, e do R2 de 72,2 ± 18,1%. O reator 2, operando com vinhaça pré-tratada, apresentou queda na remoção de DQO e na produção de metano, indicando possível influência do biolpolímero no tratamento. Após recuperação do reator, iniciou-se a terceira etapa. A eficiência de remoção de DQO para R1 e R2 nesta etapa foi de 77,5 ± 9,4% e 79,2 ± 9,7%, respectivamente. Após 155 dias de operação, o reator 2 apresentou, novamente, queda na eficiencia de remoção de DQO e na produção de metano, indicando nova falha. Analisou-se por meio de microscopia eletrônica de varredura (MEV) e de espectrometria de energia dispersiva de Raios-X (EDS) a qualidade dos grânulos de lodo do inóculo (início da operação), R1 e R2 (após 155 dias de operação). As imagens indicaram um aumento da rugosidade dos grânulos de R1 e R2, possivelmente devido à deposição de compostos sedimentáveis presentes na vinhaça, tanto bruta quanto pré-tratada. As análises de EDS indicaram elevada presença de cálcio nos grânulos no reator 2, indicando que este composto atuou na diminuição da transferência de massa entre a matéria orgânica da vinhaça e os microrganismos presentes nos grânulos.

Palavras-chave: Digestão anaeróbia; Cálcio; Metano

1 INTRODUCTION

Sugarcane vinasse is the primary residual stream from the sucro-alcohol industry in Brazil. It is characterized by a high content of organic matter (both dissolved and suspended fractions), ranging from 27,000 to 37,000 mg.L^{-1} (GODOI *et al*., 2019). This concentration is substantially higher than that commonly observed in sanitary sewage (600 mg.L^{-1}), indicating the significant potential of sugarcane vinasse to trigger negative environmental issues (FUESS; GARCIA, 2014). To avoid environmental damage, especially into water bodies, the land disposal of *in natura* vinasse through fertirrigation is commonly applied (DIAS *et al*., 2015).

Excessive and long-term soil application of vinasse can result in undesirable effects, such as a reduction in sugarcane quality for sugar production, groundwater contamination, microbial activity losses, and potential soil salinization (FUESS; GARCIA, 2014). Anaerobic digestion stands out as an alternative for vinasse management, by reducing its polluting organic load and generating by-products with potential economic value, such as methane and hydrogen (SANTOS *et al*., 2014; MORAES *et al*., 2014; RUDDER; SEABRA, 2017; NAKASHIMA; OLIVEIRA JUNIOR, 2020). The treated vinasse presents lower impact to the environment when disposed, higher applicability in the soil and a consequent increase in the fertigation potential (FUESS; GARCIA, 2014). Anaerobic reactors applied to sugarcane vinasse treatment present high rates, a relatively low cost and greater operational simplicity, when compared to aerobic reactors (MOTA; ARAÚJO; AMARAL, 2015).

The physical-chemical treatment is also an alternative to vinasse management. An example of this technique is the liquid-solid separation by coagulation/flocculation/sedimentation, which results in clarified final effluent with lower solids and particulate organic matter contents (SAPLA, 2012; SACCHI *et al*., 2020; SICA *et al*., 2020; SYAICHURROZI *et al*., 2020). The coagulation process consists of applying an agglutinating compound (inorganic or organic), which has the capacity to destabilize solid particles. Therefore, solids will join, forming flakes, which will sediment because of their average diameter increasing and then, solids can be removed by gravity. The most used coagulants are metal salts (containing Fe^{+3} and $Al^{+3)}$, tannin-based, natural and polyacrylamide-based polymers. Among the polymers, polyelectrolytes have been satisfactorily used as a coagulant and/or coagulation aid in the wastewater clarification (CASTRO; YAMASHITA; SILVA, 2012; SAPLA, 2012; GUIMARÃES, 2017; SACCHI *et al*., 2020).

There are several studies on vinasse clarification, including in terms of the proposition of specific methodologies for this purpose. In fact, the clarification efficiency is known as a pre-treatment for biological reactors, especially the anaerobic ones (GONÇALVES; SILVA, 2000, HEREDIA; DOMINGUEZ; PARTIDO, 2005; CORAVUBIAS; LUNA, 2007, SOUZA, 2010, SAPLA, 2012). The combination of physical-chemical and anaerobic digestion is a possible arrangement for the treatment of recalcitrant wastewaters as the solid separation would induce a rise in the COD removal and biogas production, reducing its toxicity to bioreactor microorganisms, among other advantages (PEACE-PINO; BARBA-HO; MARRIAGA-CABRALES, 2014).

The present study investigated the influence of a previous sugarcane vinasse coagulation, by using a calcium-based biopolymer, on COD removal and biogas production in an anaerobic lab-scale reactor. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were used to better elucidate the mechanisms associated with calcium-containing compounds on the anaerobic digestion process.

2 MATERIALS AND METHODS

2.1 Characterization of sugarcane vinasse

Vinasse samples were collected from a full-scale sugarcane biorefinery located in Pradópolis, SP, Brazil. Samples were kept at 4°C, prior to use. In total, four samplings were made according to the demand for reactors feeding. Specific chemical analyses were made at each sampling to characterize and determine the variability of vinasse in these different periods. Table 1 summarizes the average physicochemical characterization of vinasse used in this study.

Table 1 – Compositional characterization of sugarcane vinasse applied in this study

Parameter	**Unit**	**Average ± Standard Deviation**
pH	-	4.6 ± 0
COD	g/L	37.8 ± 3.1
Soluble COD	g/L	17.4 ± 8.8
NTK	mg/L	599.9 ± 359.3
$N\text{-}NH_4$	mg/L	38.6 ± 22.6
P	mg/L	200.8 ± 49.0
SO_4^{2-}	mg/L	1403.3 ± 400.8
K	mg/L	4958.3 ± 490.7

Source: Authors, 2022

2.2 Sugarcane vinasse pretreatment: biopolymer and coagulation tests

A biopolymer is a natural-based product, originated from the enzymatic reaction of calcium oxide with polysaccharides extracted from microalgae of *Spirillum Calcium*, and it is classified as a cationic polymer (ROCHA, 2012). Thus, it can coagulate with negatively charged suspended solids, such as from water bodies for water supply, textile, pulp and paper industries and also sugarcane biorefineries (CAVALCANTI, 2009). The calcium-based biopolymer used in this study can also support the flocculation and odor neutralization (ROCHA, 2012).

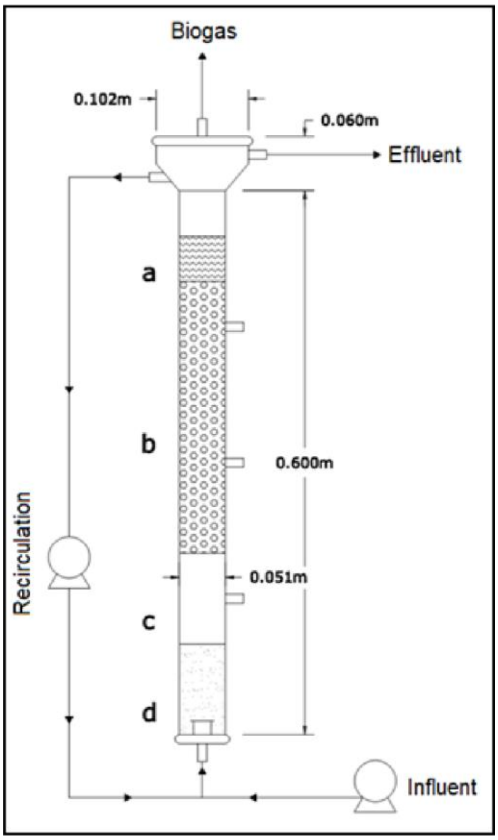
Pretreated vinasse was prepared by jar testing, to simulate a conventional coagulation/flocculation process. The procedure was initiated with a rapid mixing at 200 rpm for 1 min, followed by 1 hour settling. The biopolymer dosage was 14 $g.L^{-1}$, as recommended by Sapla (2012). As a result, COD and turbidity removal efficiencies were 30% and 86%, respectively. As the calcium oxide was present in the biopolymer, the pH of the pretreated vinasse increased to 12.4 after the coagulation/flocculation. Therefore, it was necessary to neutralize the pH up to 8.0 by the addition of concentrated HCl, prior to R2 feeding.

2.3 Anaerobic reactor: experimental apparatus, operating conditions, and analytical techniques

Two identical cylindrical reactors with a working volume of 1.38 L were operated (Figure 1). Both were hybrid in terms of biomass positioning, that is, part of the volume occupied with biomass adhered to the support medium and another part with biomass was fully dispersed at the base of the reactor (flocculent biomass). Both reactors were inoculated with sludge from an up-flow anaerobic sludge blanket reactor (UASB) from a poultry slaughterhouse.

The control reactor (R1) was fed with fresh vinasse and pretreated vinasse was added to R2. The reactor's operation was divided into three stages (Table 2). Stage 1 lasted 22 days and comprised the inoculation, start up and biomass adaptation. Different OLR were applied in the systems during stages 2 and 3 to evaluate the influence of the pre-treatment with biopolymer on the anaerobic digestion in R2 (Table 2). In stages 2 and 3, both reactors were subjected to an effluent recirculation of 3 $L.d^{-1}$ aiming to reuse the total alkalinity detected in the final effluent. Moreover, the recirculation promoted the wastewater dilution, minimizing the impact of the vinasse pollution load to biomass. Nevertheless, alkalinity was supplemented prior to R1 feeding by the $NaHCO_3$ dosing of 1g per 1 g COD. This supplementation also acted in the vinasse neutralization prior to R1, as an average pH of fresh vinasse was 4.6 (Table 1). As the calcium-based biopolymer applied in the pre-treatment was characterized by high alkalinity (calcium oxide), the $NaHCO_3$ dosing in R2 was not necessary.

Figure 1 – Schematic design of the anaerobic reactor a) porous bulkhead; b) granular biomass immobilized in mobile floating support material; c) biomass-free space; and d) flocculent biomass



Source: Rocha, 2022

The COD, sulfate and total alkalinity analyses were conducted via colorimetric methods according to the Standard Methods for Examination of Water and Wastewater (APHA, 2012). The biogas production in terms of methane (CH_4) was determined by the procedure described by Aquino *et al*. (2007).

Table 2 – Characteristics of operational stages applied for both reactors

Stage	Operational period (days)	Reactor	COD ($g.L^{-1}$)	$NaHCO_3$ dosing ($gNaHCO_3.gCOD^{-1}$)	HRT (h)	OLR ($kgCOD.m^{-3}.d^{-1}$)	Recirc.
1	22	1	2.8 ± 1.7	1,0	35.7 ± 0.2	1.9 ± 1.1	No
		2	3.3 ± 1.6	-		2.2 ± 1.1	No
2	69	1	9.8 ± 4.4	1,0	39.5 ± 0.3	5.3 ± 1.3	Yes
		2	9.7 ± 5.6	-		5.3 ± 1.6	Yes
3	37	1	7.6 ± 3.6	1,0	35.7 ± 0.2	5.1 ± 2.4	Yes
		2	7.5 ± 3.4	-		5.0 ± 2.3	Yes

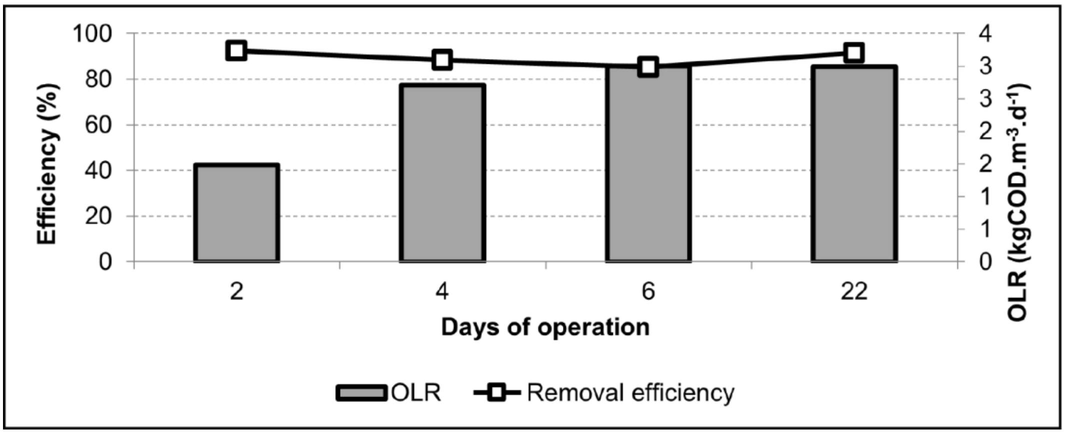
Source: Authors, 2022

3 RESULTS AND DISCUSSION

In terms of HRT, and the consequent influent flow rate, it remained practically constant in all three stages, demonstrating uniformity in the hydraulic application rate. The OLR control in Stage 1 occurred by effluent dilution, to promote the biomass adaptation to both raw and pretreated vinasse. The OLR and HRT, applied in the three stages (Table 2), followed Brown's (2012) recommendations.

3.1 Operational Stage 1 - Biomass adaptation

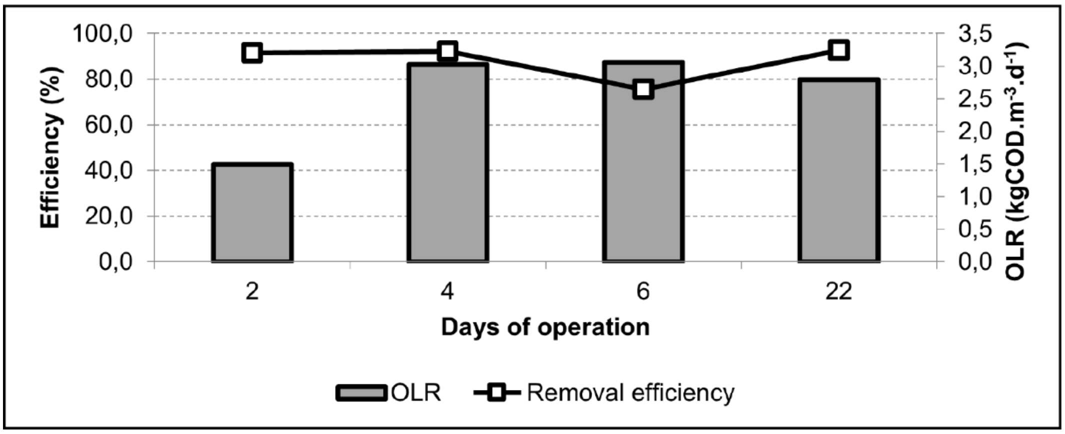
Figure 2 – Temporal profiles of OLR and COD removal efficiency in R1 during the experimental adaptation phase (Stage 1)



Source: Authors, 2022

Figures 2 and 3 display the OLR and COD removal efficiency profiles during Stage 1 for R1 and R2, respectively. It can be observed that both systems showed COD removal efficiencies above 80% (Figures 2 and 3), indicating the successful biomass adaptation.

Figure 3 – Temporal profiles of OLR and COD removal efficiency in R2 during the experimental adaptation phase (Stage 1)



Source: Authors, 2022

3.2 Operational Stage 2

The Stage 2 was started by applying an OLR of 4.0 kgCOD.m^{-3}.d^{-1} for both systems. From this stage onwards, a recirculation rate of 3:1 was used and the OLR was gradually elevated until it reached approximately 7.0 kgCOD.m^{-3}.d^{-1} at the 69th day of operation. This value is recommended by Chernicharo (2007) for up flow anaerobic sludge blanket reactor (UASB) treating high-strength wastewaters.

During almost the whole of Stage 2, R1 and R2 behaved similarly, presenting high COD removal efficiencies, stable effluent pH, alkalinity above 1000 mgCaCO$_3$.L^{-1} (Table 3), and continuous biogas production. These factors indicated the reactor's stability (Table 3, Figures 4 and 5). However, from the 85th day of operation, COD removal efficiency and biogas production in R2 declined sharply (Figure 5), indicating collapse or inhibition of anaerobic metabolism in this reactor.

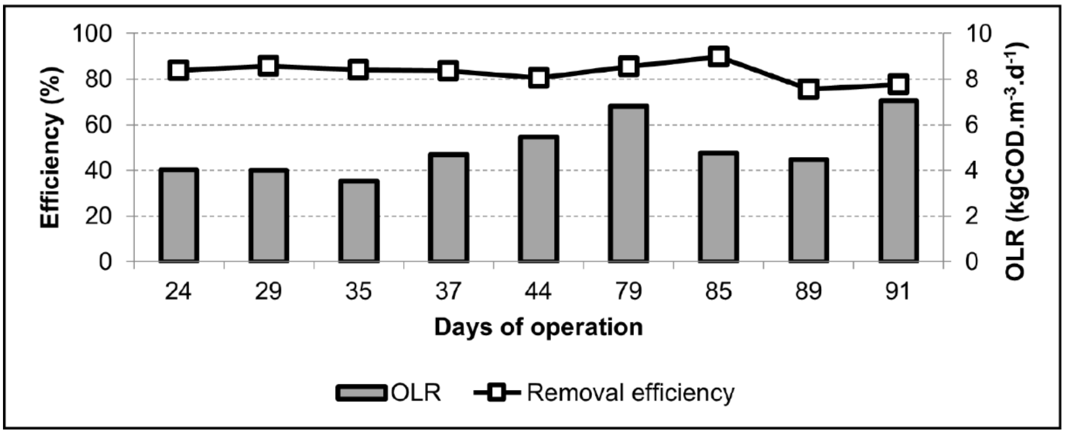
Table 3 – Characteristics of influent and effluent samples from R1 and R2 during Stage 2

Parameters	R1		R2	
	Influent	**Effluent**	**Influent**	**Effluent**
COD (g.L^{-1})	9.8 ± 4.4	1.5 ± 0.5	9.7 ± 5.6	2.8 ± 2.3
pH	6.6 ± 1.2	8.7 ± 0.4	7.5 ± 1.1	7.7 ± 0.2
Total alkalinity (mg CaCO$_3$.L^{-1})	4027.0 ± 3981.4	5846.3 ± 3766.9	1792.8 ±653.7	2260.3 ± 914.7
Sulfate (mgSO$_4^{2-}$.L^{-1})	373	74,2	542,3	ND

ND: not detected
Source: Authors, 2022

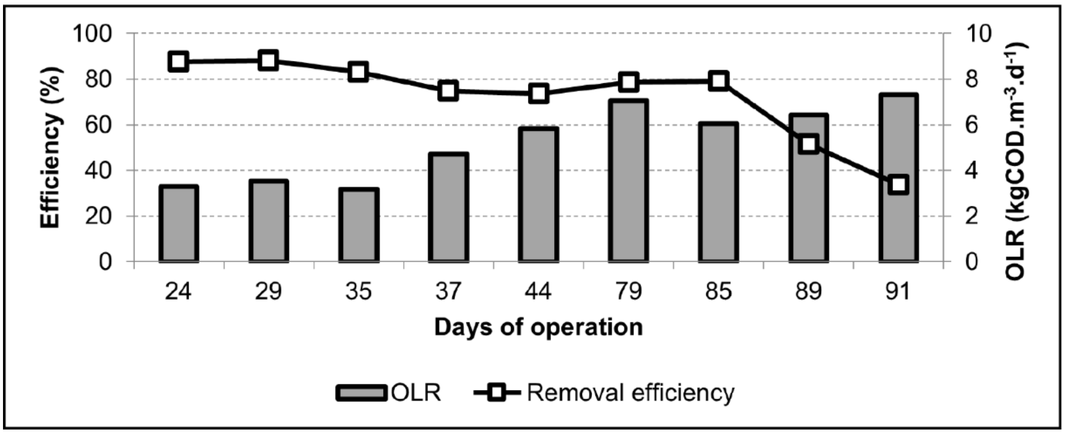
The average COD removal efficiencies from R1 and R2 were 82.9 ± 4.4% and 72.2 ± 18.1%, respectively (Figures 4 and 5). The difference between R2 and R1 performance can be related to a possible inhibition effect caused by the biopolymer to the anaerobic microorganisms in R2. A COD removal efficiency of 80.7 ± 5.8% was observed in R2 before the 85th day of operation, which was similar to that achieved in R1. Therefore, no improvement in COD removal was observed with the vinasse pre-treatment. Therefore, any benefit in the COD removal of observed by the vinasse pretreatment. On the other hand, it was observed that the application of the calcium-based biopolymer neutralized the odor from the raw vinasse, especially considering the high concentration of sulfate in the vinasse, which is reduced to H_2S by sulfate reducing bacteria (SRB) under anaerobic conditions. In fact, sulfate was not detected in the R2 effluent (Table 3), which indicates the biopolymer effectiveness of odor control.

Figure 4 – Temporal profiles of OLR and COD removal efficiency in R1 during Stage 2



Source: Authors, 2022

Figure 5 – Temporal profiles of OLR and COD removal efficiency in R2 during Stage 2



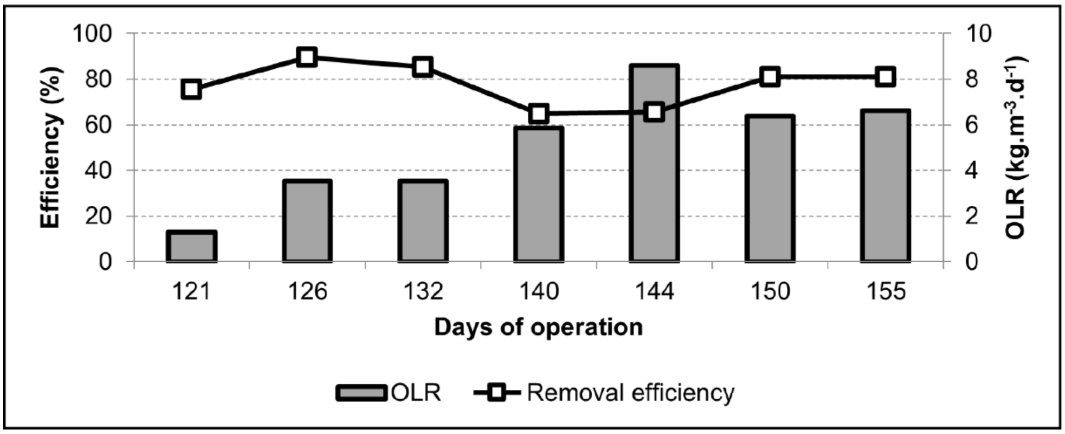
Source: Authors, 2022

Due to the fall in the COD removal efficiency observed in R2 in the 85th day of operation, the feeding with pretreated vinasse was stopped and R2 was subjected to a recovery (29 days) in which an OLR of 3.0 kgCOD.m^{-3}. d^{-1} of raw vinasse was applied.

3.3 Operational Stage 3

In Stage 3, OLR progressively rose until 7.0 kgCOD.m^{-3}.d^{-1}, similar to the value applied in Stage 2. The COD removal efficiencies for both reactors reached an average value of 80% (Figures 6 and 7). On the 150th day of operation, a fall in the COD removal in R2 was detected (Figure 7), which resulted in a new collapse caused by the calcium -based biopolymer pretreatment. To date, the average COD removal efficiencies for R1 and R2 were 77.5 ± 9.4% and 79.2 ± 9.7%, respectively. This fact reveals the potential of anaerobic digestion of raw sugarcane vinasse by using the operating conditions mentioned in this study, without any physical-chemical pretreatment (R1).

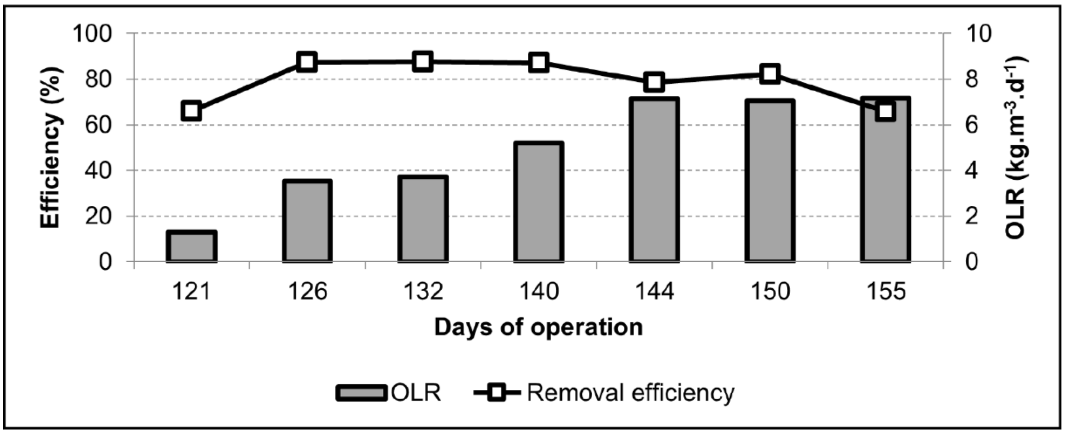
Figure 6 – Temporal profiles of OLR and COD removal efficiency in R1 during Stage 3



Source: Authors, 2022

For both three stages, COD removal was always satisfactory and around 80%, as expected for anaerobic reactors. Another relevant monitoring parameter was biogas production, which corroborated the success of the treatment of raw vinasse, indicating the potential for energy utilization of this by-product.

Figure 7 – Temporal profiles of OLR and COD removal efficiency in R2 during Stage 3



Source: Authors, 2022

Table 4 – Characteristics of influent and effluent samples from R1 and R2 during Stage 3

Parameters	R1		R2	
	Tributary	**Effluent**	**Tributary**	**Effluent**
COD ($g.L^{-1}$)	7.6 ± 3.6	1.8 ± 1.5	7.5 ± 3.4	1.5 ± 1.1
pH	6.2 ± 0.5	8.2 ± 0.4	7.4 ± 0.4	7.3 ± 0.5
Total Alkalinity ($mg.L^{-1}$)	1058.4 ± 342.9	3082.0 ± 772.8	1792.8 ± 563.7	2260.3 ± 914.7
Sulfate ($mg.L^{-1}$)	611.7 ± 1.5	104.6 ± 3.1	814.6 ± 8.5	204, ± 4.2

Source: Authors, 2022

Although different reactor configurations have been applied to the vinasse treatment by an anaerobic process, there are no reports regarding the use of calcium-based coagulants as pre-treatment of this high-strength wastewater. Table 6 summarizes results from studies that aimed at removing COD and producing biogas from sugarcane vinasse under different reactor types and operating conditions. It was observed that, despite the variation of reactor configurations, the results achieved in this study are similar to those described in the literature.

Table 5 – COD removal efficiencies and operational characteristics from different anaerobic reactors applied in the vinasse treatment

Reactor configuration	Material support	HRT (d)	Maximum OLR ($kgCOD.m^{-3}.d^{-1}$)	COD removal efficiency (%)	Reference

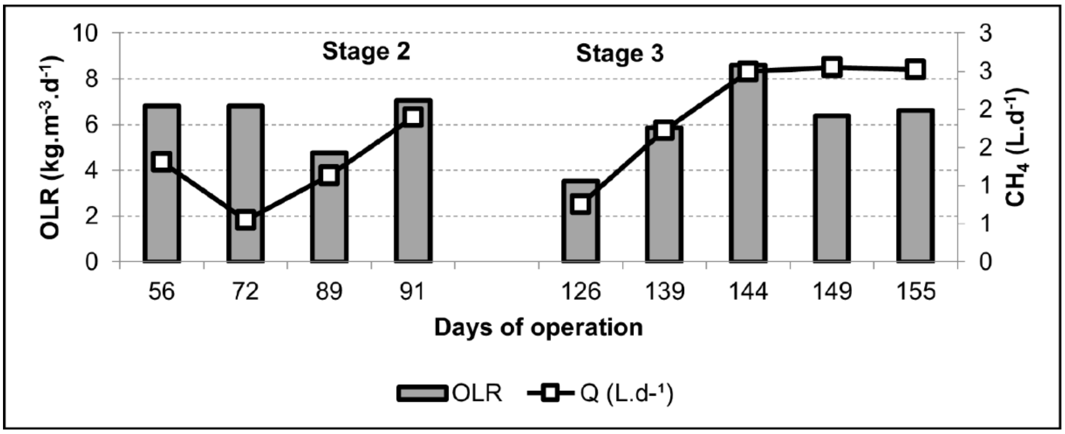
RALF	Activated carbon	0.3	10.0	76	Wang *et al*. (2011)
RALF	Zeolite	0.6	10.0	80	Wang *et al*. (2011)
UASB		3.2	215	58	Lopez, Lopez, Lopez, Borzacconi (2011)
UASB		0.83	183	76	Costa *et al*. (1986)
Aerobic Filter		6.0	34	89	Fernandez *et al*. (2001)
Hybrid anaerobic reactor	PEAD	1.5	129	81	This study

RALF - Fluidized Anaerobic Bed Reactor.
Source: Adapted from Moraes, Zaiat and Bonomi (2015)

3.4 Methane production

Biogas and methane production was monitored during the anaerobic treatment of vinasse in the two reactors in stages 2 and 3 (Figures 8 and 9). The tendency towards increased biogas production followed the values of OLR applied. The same occurs for the decrease in the R2 yield in both stages. The biogas monitoring indicated, quickly and objectively, that this reactor was about to collapse, and that anaerobic processing was under inhibition by biopolymer action.

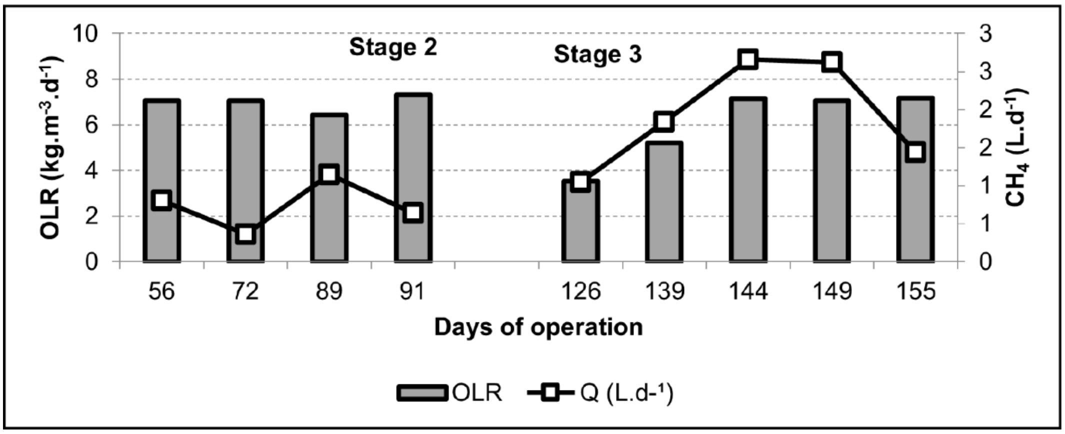
Figure 8 – Temporal profiles of OLR and CH_4 production in R1 during Stages 2 and 3



Source: Authors, 2022

During stage 2 of the reactor operation, it was observed that methane production was slightly higher in R1. Similarly, as occurred with COD removal in R2 around the 91st day, there was a decline in gas flow, indicating a beginning treatment failure. For this stage, the methane production average was 1.22 ± 0.56 L $CH_4.d^{-1}$ for R1 and 0.74 ± 0.33 L $CH_4.d^{-1}$for R2.

Figure 9 – Temporal profiles of OLR and CH_4 production in R2 during Stages 2 and 3



Source: Authors, 2022

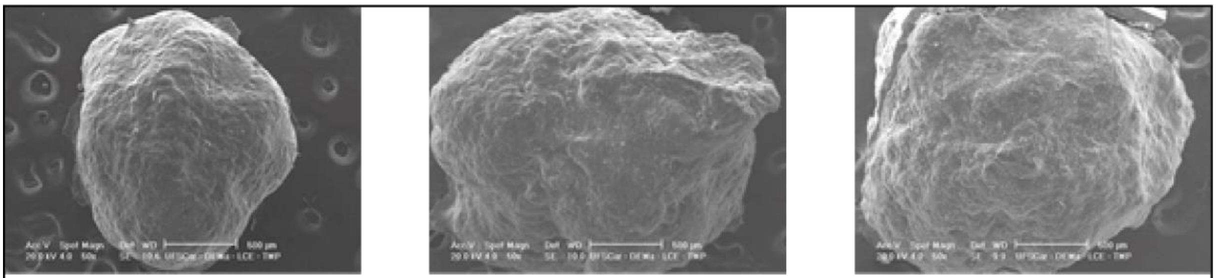
After recovering R2, methane production was monitored and, this time, the by-product flow was similar for both reactors in most of the period (2.01 ± 0.78 L $CH_4.d^{-1}$ for R1 and 1.92 ± 0.71 L $CH_4.d^{-1}$ for R2). However, on the 155th day of operation the methane flow in R2 decreased again, indicating a potential system failure. At that time, it was decided to close the operations and analyze the possible causes of these declines in yields presented in stages 2 and 3.

3.5 Analysis of granular anaerobic sludge

Images of inoculum and monitored reactor granular sludge were analyzed. Besides the images, X-ray dispersive energy spectrometry (EDS) analyses were performed, showing the compounds present in the samples.

During the analysis of the obtained images, an evident change was observed in the surface granule structure. The most evident difference was the high granule surface roughness in Reactors 1 and 2, compared to the inoculum. It is assumed that this roughness is due to the deposit of inorganic substances present in vinasse and experimental biopolymer. Figure 10 illustrates, under scanning electron microscopy (SEM), the surface of the granules of the inoculum (beginning of the operation) and Reactors 1 and 2 after 155 days treating raw and pretreated vinasse.

Figure 10 – Scanning electronic micrographs in the anaerobic granules of the inoculum at the beginning of the operation, and Reactors 1 and 2 after 155 days of operation. (a) inoculum; (b) reactor 1; (c) reactor 2



(a) (b) (c)

Source: Authors, 2022

Differences were observed on the surface between the granules of the two reactors. The granules from reactor 2 were the ones with the highest roughness. The inorganic substance in greater quantity, in this case, is probably calcium, because apparently the roughness is due to deposits of this element on the surface of the granules, as seen by EDS analyses. The results of these analyses are shown in Table 6.

Table 6 – EDS analysis in samples of inoculum granules (a) and Reactors 1 (b) and 2 (c) after 155 days of operation

Element	**Percentage in Sample (%)**		
	Inoculum	**Reactor 1**	**Reactor 2**
Si	35,20	1,81	1,67
S	12,69	41,70	16,35
K	5,51	6,45	4,84
Ca	22,91	39,08	49,90
Fe	23,67	10,96	27,25

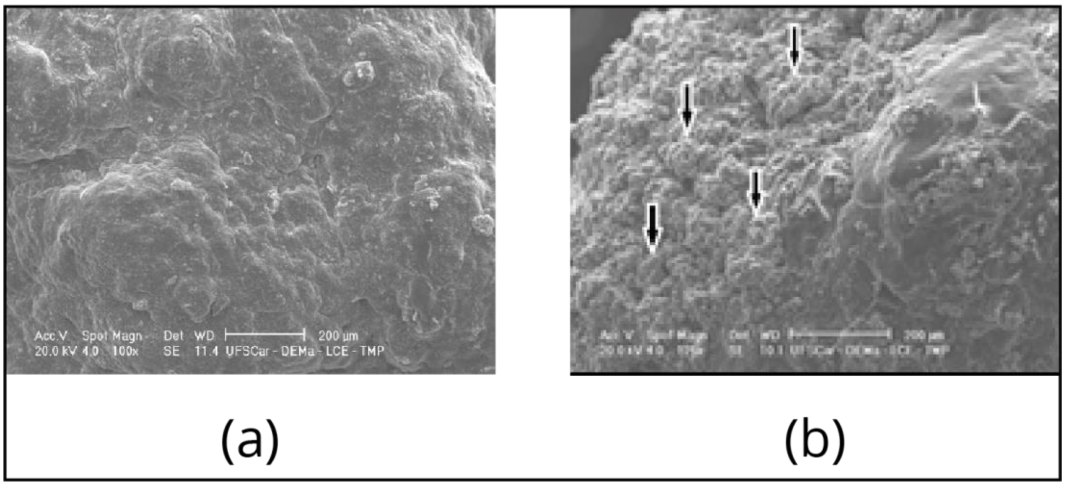
Si - silica; S - sulfur; K - potassium; Ca - calcium; Fe – iron
Source: Authors, 2022

EDS analysis indicated a predominance of silica, sulfur, potassium, calcium and iron on the surface of the granules. Although it did not present a clear pattern of an increase or decrease of the elements for the three samples, the decrease of silica in the reactor granules could be observed in relation to the inoculum. This may be related to the difference in the characteristic of the effluent treated in the reactor where the sludge was collected. This anaerobic reactor was located in a poultry slaughterhouse, which explains the predominantly protein liquid effluent. Vinasse, a by-product of alcohol and sugar production, has high carbohydrate loads.

However, the most evident and relevant result was the increase in the proportion of calcium in granules, especially in Reactor 2. This result was to be expected because the biopolymer has calcium oxide as the main component. Van Langerak *et al*. (1998) considered that calcium carbonate precipitation can influence biomass activity. This precipitation is related to Ca^{+2} ion concentration and the COD removal efficiency. The impact of precipitate on biomass is complex, and its influence on granules is related to the decrease in activity caused by the limitation in mass transfer. However, active biomass can be formed into thin layers of biofilms on the surface of the precipitate. Therefore, the net (total) activity of biomass would be the average of these two effects. (VAN LANGERAK *et al*., 1998). Thus, the failure occurred in Reactor 2, in the two phases of operation, can be based on calcium deposition on the biomass surface, hindering the mass transfer between the granules and the substrate (vinasse).

Figure 11 shows the SEM image of R1 and R2 granules that show evidence of calcium deposition on the surface, thus indicating the negative influence on mass transfer between the liquid and the granule. The arrows indicate the regions where calcium depositions were identified on the surface of the reactor granule 2.

Figure 11 – Scanning electronic micrograph of reactor granules surfaces 1 (a) and 2 (b) after 155 days of operation



Source: Authors, 2022

4 CONCLUSIONS

The coagulation of vinasse through the experimental biopolymer as a pre-treatment to anaerobic processing was not advantageous, because after three months of operation there was a sudden decrease in the efficiency of COD removal, which probably indicates an initial collapse of the system. After the re-establishment of the initial conditions of anaerobic treatment, a tendency to reduce efficiency and a possible collapse of the system were also observed, when it was completed just over a month of operation.

Methane production in both reactors, during stages 2 and 3, was compatible with the observed COD removal efficiencies, indicating the quality of anaerobic digestion. Thus, this parameter was important to indicate system failures, in this case, reactor 2 during the two phases of operation. Thus, this parameter was important to indicate system failures, in this case, Reactor 2 during the two operation phases.

X-ray Dispersive Energy Spectrometry analyses indicate the presence of the calcium element with approximately 23% for the inoculum, 39% for Reactor 1 and 50% for Reactor 2 of the mass of the analyzed elements of the granule samples.

Deposits of some type of compound on the surface of the granules adapted to vinasse were observed in conjunction with the micrographs performed by means of Scanning Electron Microscopy, especially in the samples of Reactor 2. There are indications that these deposits are calcium-containing compounds, and that they interfere with the capacity of substrate assimilation by the biota present in the granular structure, which is the possible cause of Reactor 2 failure. Thus, calcium would act as an inhibitory compound of anaerobic digestion.

In a global analysis of the experiment, the use of a calcium-based biopolymer as a coagulant in a pre-treatment to anaerobic processing of vinasse was not profitable, therefore, not recommended.

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