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Use of biosurfactant surfactin produced from cassava wastewater for anaerobic treatment of effluent from a poultry slaughterhouse

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The use of a biosurfactant surfactin produced by *Bacillus subtilis* LB5a in cassava growth medium (cassava wastewater) was evaluated to treat anaerobically, the effluent from a poultry slaughterhouse. During the effluent pretreatment, effects of surfactin concentration factors were evaluated, considering the ones which were superior and below its critical micelle concentration (CMC = 28 mg L^{-1}): 6, 13, 27, 31, 48, 73 and 56 mg L^{-1} and temperature (25, 30, 42.5, 55 and 60°C) up to 6 h, using the rotational central composite design. During anaerobic treatment, flasks were filled with anaerobic sludge as inoculum and a pretreated effluent for 4.5 h in its different concentrations of surfactin. Reactors were connected to eudiometers under static system at 34°C. During the pretreatment phase, there was a direct ratio between temperature and surfactin concentrations according to the increase of organic matter solubilization, measured by soluble chemical oxygen demand (SCOD). These results have shown surfactin applicability produced by *B. subtilis* LB5a during the anaerobic treatment of effluent from a poultry slaughterhouse; as for all treatments, there was no inhibition of microbial consortium of the anaerobic sludge. SCOD removal was above 80%, while oil and greases removal was above 70%, plus a propitious specific methane yield.

Key words: *Bacillus subtilis*, agro-industrial effluents, anaerobic sludge.

INTRODUCTION

The slaughter and poultry processes have produced high volumes of effluent with high organic load, mainly due to lipids (Dallago et al., 2012). Lipid fraction is characterized by oils, greases, fats and fatty acids, and it is one of the most important components in wastewater from food industries (Mendes et al., 2005; Chipasa and Medrzycka,

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Table 1. Average values for the characterization parameters of raw effluent from poultry slaughterhouse.

Parameters	Average values
Total alkalinity (TA) (mg L^{-1})	185.25
Volatile acidity (mg L^{-1})	243.63
Chemical oxygen demand (COD) (mg L^{-1})	2360.49
Soluble chemical oxygen demand (SCOD) (mg L^{-1})	590.30
Oils and greases (OG) (mg L^{-1})	535.33
pH	6.17
Total dissolved solids (TDS) (mg L^{-1})	515.00
Fixed dissolved solids (FDS) (mg L^{-1})	217.50
Volatile dissolved solids (SDV) (mg L^{-1})	297.50
Total solids (TS) (mg L^{-1})	1594.66
Fixed total solids (FTS) (mg L^{-1})	208.67
Volatile total solids (VTS) (mg L^{-1})	1386.00

2006), since it may cause operational problems to the effluent treatment system. Limiting the transfer of gas is an example, since it is necessary for biological degradation, fouling in reactors, biomass flotation and the absence of methanogenesis and acetogenesis phases during anaerobic treatment processes (Cammarota and Freire, 2006; Cirne et al., 2007; Gomes et al., 2011).

The physico-chemical pretreatment phase is generally used to remove lipid fraction and ensure efficiency of the subsequent anaerobic biological treatment (Cammarota and Freire, 2006; Cirne et al., 2007). However, the chemical reagents applied are expensive and the produced sludge is difficult to dispose (Semerjian and Ayoub, 2003). Microbial enzymes have been applied and evaluated to hydrolyze and dissolve fats from wastewater during the pretreatment of fish industry effluent (Valente et al., 2010), dairy effluent (Rosa et al., 2009; Mendes et al., 2006; Leal et al., 2002; Cammarota et al., 2001), effluent from a poultry slaughterhouse (Valladão et al., 2007), and effluent from a swine slaughterhouse (Masse et al., 2003).

Gallert and Winter (2005) described that maximum hydrolytic activity, provided by enzymes present in medium, can be achieved when lipid fraction of effluent is emulsified, that is, when there is dispersion of lipids in water like microscopic droplets (Desai and Banat, 1997). Emulsion can be obtained by the action of chemical surfactants or microbial origin (biosurfactants).

A biosurfactant is known as an additive when it stimulates biodegradation (Cammarota and Freire, 2006) as well as remove environmental oily substances due to their high molecular weight and micelle formation that are able to reduce the surface and interfacial tension. So, there is an increase in solubility and bioavailability of hydrophobic organic compounds (Pacwa-Plociniczak et al., 2011).

The use of surfactants produced by microorganisms is also evaluated by environmental sanitation area as well as for the treatment of contaminated soil and water by fats, oils and derivatives (Nitschke and Pastore, 2002; Nakhla et al., 2003; Kumar et al., 2008).

In order to treat specifically effluents, Damasceno et al. (2012) evaluated combined application of an enzymatic preparation obtained from *Penicillium simplicissimum* and a kind of rhamnolipid biosurfactant, produced by *Pseudomonas aeruginosa* to treat wastewater from a poultry slaughterhouse. Daverey and Pakshirajan (2011) also evaluated the use of a kind of sophorolipid biosurfactant produced by *Candida bombicola* in the treatment of effluent from dairy industry.

Therefore, based on brief discussions, this study aimed at evaluating the application of surfactin as a biosurfactant produced by *B. subtilis* LB5a in a growth medium of cassava effluent (cassava wastewater), during the anaerobic treatment of effluent from a poultry slaughterhouse.

MATERIALS AND METHODS

Collection and characterization of effluent from a poultry slaughterhouse and anaerobic sludge

The raw effluent used in this study was collected during slaughtering period in a poultry agro-industry in South of Brazil. This effluent is produced with a 180 $m^3.h^{-1}$ average flow. After collection, it was correctly preserved and aliquots were taken for their initial characterization (Table 1), while the remaining part was stored and frozen.

The sludge, used as inoculum during anaerobic treatment phase, was collected from an anaerobic digester of an agro-industry that produces cassava starch. It showed the average values for the following parameters: series of suspended solids - total (40.301 mg L^{-1}), fixed (20.448 mg L^{-1}), volatile (19.852 mg L^{-1}), and specific methanogenic activity (SMA) (0.12 $gCOD_{CH4}$ $gVSS^{-1}$ d^{-1}).

Biosurfactant production

The microorganism that produces the biosurfactant, surfactin in

Table 2. Coded values and real factor: Biosurfactant concentration (BC) and temperature (T).

Variable	Levels				
	-1.41	**-1**	**0**	**1**	**1.41**
BC (mg L^{-1})	6	13.27	31	48.73	56
T (°C)	25	30	42.5	55	60

BC = biosurfactant concentration, T = temperature.

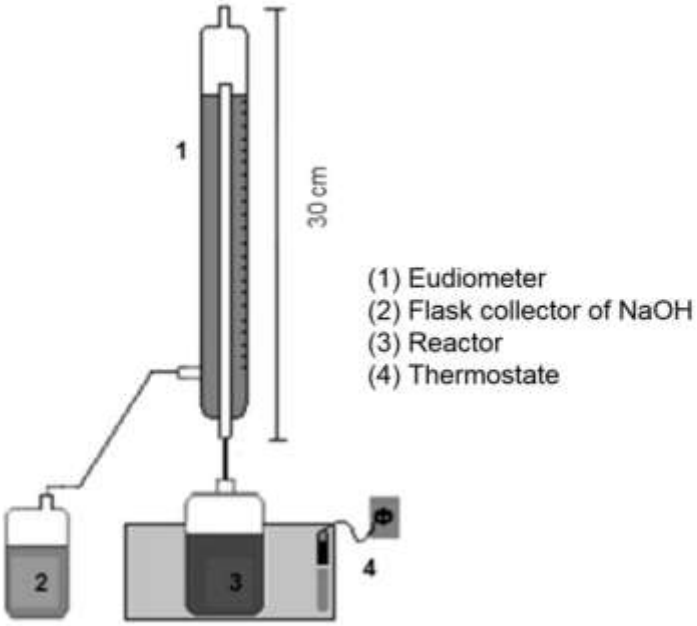

Figure 1. Schematic model of an experimental unit used in anaerobic treatment of wastewater.

cassava wastewater is the bacterium, *B. subtilis* LB5a, which was stored in agar nutrient at 4°C, at Bioaromas Laboratory of FEA/UNICAMP (Nitschke et al., 2004). Cropping of this microorganism for biosurfactant production in cassava was carried out in a pilot bioreactor, New Brunswick Scientific®, model Mobile Pilot Plant fermentor 8000 MP 80 with 56 L of cassava wastewater as growth medium and 4 L of inoculum. The experimental conditions of such bioreactor, procedures for obtaining semi-purification of biosurfactant, are described by Nitschke and Pastore (2003), Nitschke et al. (2004) and Barros et al. (2008).

After three growing days, biosurfactant was obtained and characterized as: emulsification index (in gas: 61%; diesel: 74%); concentration in crude extract (0.3 mg mL^{-1}); surface tension (25.97 MN m^{-1}); critical Micelle concentration (CMC = 28.33 mg L^{-1}) and chemical oxygen demand (COD = 1.26 gCOD/g biosurfactant).

Pretreatment of the effluent with biosurfactant

During the pretreatment, surfactin emulsified lipid fraction was evaluated, and thus solubilization increase of organic matter in the effluent was promoted. The effects of temperature and concentration factors concerning biosurfactant on soluble COD increase (SCOD) were evaluated by using rotational central composite design (RCCD) (Rodrigues and Iemma, 2009). Value ranges used for the factors were determined according to the study of Damasceno et al. (2012). The conditions in which the pretreatment tests were carried out are shown in Table 2.

The eleven pretreatment assays were carried out in 250-ml glass beakers containing 140 ml of medium consisting of raw effluent and 10 ml of a medium consisting of surfactin in its different concentrations, totaling 150-ml net volume. In a shaker incubator, a 150-rpm stirring was determined for up to 6 h, and aliquots were withdrawn for analysis at fixed intervals of 1.5 h (Damasceno, 2013). The results were analyzed by the Statistica 8 software and for statistical analysis of variance (ANOVA), the maximum value of soluble COD (SCOD) was used as a variable response.

Anaerobic treatment of a pretreated effluent

Based on the results obtained in the pretreatment tests, anaerobic biodegradability of the effluent previously exposed to surfactin was evaluated. So, an experimental apparatus (Figure 1), which consists of: 1) a 62-L plastic box, filled with water and used as a water bath container; (2) Two 100-W thermostats were used to heat and keep temperature at 34 ºC water bath; (3) Eighteen glass bottles (reagent grade) of 610 ml volume, with a 450-ml net volume were used as reactors; (4) Eighteen glass eudiometers were filled up with NaOH (20%) and a 50-mm diameter, 300-mm length and

Table 3. Coded matrix and soluble COD values during the evaluated periods in response to temperature and biosurfactant concentration.

Assay	Coded		Real		Soluble COD (mg L^{-1}) Incubation period					Maximum Value
	BS	T	BS	T	0 h	1.5 h	3 h	4.5 h	6 h	
1	1	1	48.73	55	650.20	1048.55	1282.07	1428.59	1275.20	1428.59
2	1	-1	48.73	30	634.17	670.80	771.54	1135.55	1176.76	1176.76
3	-1	1	13.27	55	583.81	936.37	1144.71	1163.02	1197.36	1197.36
4	-1	-1	13.27	30	570.07	677.67	1087.47	723.46	1014.21	1087.47
5	0	1.41	31	60	553.38	944.48	1108.06	1190.79	1292.33	1292.33
6	0	-1.41	31	25	523.29	820.38	856.10	946.36	950.12	950.12
7	1.41	0	56	42.5	500.73	797.81	873.03	997.12	952.00	997.12
8	-1.41	0	6	42.5	410.48	636.11	747.05	771.49	728.24	771.49
9	0	0	31	42.5	886.01	986.74	1121.81	1005.05	780.69	1121.81
10	0	0	31	42.5	636.46	989.03	1087.47	1050.84	1039.40	1087.47
11	0	0	31	42.5	654.78	998.19	1087.47	1053.13	936.37	1087.47

BS = Biosurfactant surfactin; T = temperature.

output with 8-mm tubes, whose total volumetric capacity was 450 ml were used. They were used to measure, through NaOH solution displacement, the volume of methane produced in effluent biodegradability.

The reactors flasks were sealed with a rubber cork and connected to eudiometers by silicone hoses. This experimental apparatus was operated in accordance with Suarez et al. (2012). The inoculum used in this phase was the anaerobic digester sludge from the starch manufacturer. The volumes of effluent and sludge were calculated in order to obtain an initial ratio of COD : VSS- 1: 1 in the reactor flasks.

Three replications were developed for each of the six effluent compositions containing different concentrations of surfactin (0, 6, 13.27, 31, 48.74 and 56 mg L^{-1}), which consisted of a 415-mL volume of each unit regarding the pretreated effluent during 4.5 h. Also, a 35-mL anaerobic sludge was used as inoculum, and the total was 450 ml of net volume in each reactor. The pH of the pretreated effluent (pH = 6.17) was not adjusted prior to mixing with sludge, and each mixture was submitted to purging of oxygen by bubbling N_2 for five minutes.

Daily, there was a reading to record the produced gas volume by 20-cm rules, fixed in glassware previously calibrated as Aquino et al. (2007) methodology states. At the same time, 500-ml plastic bottles with 450-ml volume were used under the same conditions of the treatments observed in eudiometers. They were incubated at 34°C in BOD chamber aiming at evaluating the time profile of the anaerobic treatment for each composition. Weekly, two bottles of each treatment were analyzed to record the effluent biodegradability by removing SCOD, oils and greases (OG), solid series, pH, alkalinity and acidity/alkalinity (VA/TA).

Aliquots were taken to obtain the initial characterization from each composition just before contact with sludge, and the sludge itself. The results obtained during anaerobic treatment phase were evaluated by descriptive statistics and Tukey test with 95% significance with the Statistica 8 software.

Analytical methods

Parameters such as total alkalinity (TA), volatile acidity (AV), volatile acidity/total alkalinity (VA/TA) ratio, chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), oils and greases (OG), pH, total solid series (TS, FTS and VTS) and dissolved solid series (TDS, FDS and VDS) were obtained from the methods described in APHA (2005). The specific methanogenic activity (SMA) of anaerobic sludge and the specific production of methane (SPM) were determined according to the adapted methodologies of Chernicharo (1997), Rocha (2001) and Aquino et al. (2007). The emulsification index (EI%) of biosurfactant was determined according to Cooper and Goldenberg (1987). Surfactin concentration in crude extract was determined by HPLC according to Slivinski (2012). The surface tension of surfactin was obtained by Krüss (1994) cited by Slivinski (2012) and the critical Micelle concentration (CMC) followed the technique described by Sheppard and Mulligan (1987).

RESULTS AND DISCUSSION

Pretreatment of effluent with surfactin

Table 3 shows the values of increased SCOD based on the evaluated periods for each condition applied to the assays of effluent pretreated with surfactin as well as coded and real matrix of planning. It was observed that the initial COD (time 0 h) differed among treatments. This was due to the composition of each treatment, which there was application of different concentrations regarding surfactin.

Considering the eleven treatments applied in RCCD, three assays showed the maximum value of COD solubilization at 4.5-h incubation. Eight assays showed the maximum value at other incubation times, but the concentrations were very close to the ones obtained at 4.5 h. From the foregoing, it is possible some solubilizing increase up to 4.5 h incubation, except for the fourth assay, which showed reduction of solubilization at this time (RCCD). Laboratory analysis was repeated for this sample, but the result remained at the same trend (decrease of soluble COD in 4.5 h).

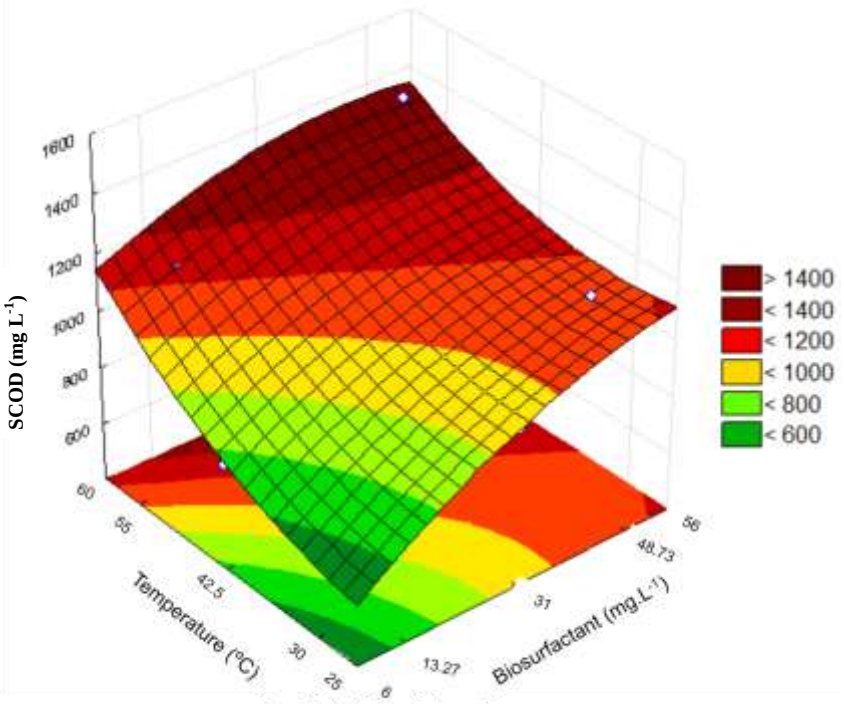

Figure 2. Response surface to increase soluble COD according to temperature and biosurfactant concentration.

Authors such as Valladão et al. (2007) and Leal et al. (2002) pointed out as the best hydrolysis time (action of enzymes on effluent), a total of 4 h to solubilize COD. For biosurfactants, Damasceno (2013) was a pioneer in evaluating the associated action of enzymatic pool and biosurfactant of rhamnolipid on COD solubilization that can be seen in wastewater from a poultry slaughterhouse. The author observed that 4.5 h was enough to promote such response.

Thereby, this trial applied 4.5 h as maximum time for the wastewater pretreatment, since the treatments in which there was an increased solubility of COD varied from 4.5 to 6 h, and such increase was little significant. It was also considered that, in some treatments, SCOD decreased during this time interval. After statistical analysis regarding the responses obtained in 4.5 h period, it was possible to determine variables that showed some effects on the increase of soluble COD.

The parameters considered significant were the ones with p-values lower than 10% ($p < 0.1$), due to the large variability inherent to the processes. It was observed that variables such as biosurfactant and temperature, both in linear or quadratic ways, present a significant effect on the response variable. The effect of interaction between biosurfactant and temperature was excluded from the model because it was not significant. This answer differed from Damasceno (2013), who observed that when temperature was analyzed as a variable, there was a significant positive effect on the response variable, both in linear and quadratic forms, but there was also a significant interaction between biosurfactant and temperature.

Silva et al. (2013) evaluated the use of an enzyme produced by fermentation in solid state using babassu oil residue as a culture medium by *P. simplicissimum* and by a rhamnolipid biosurfactant, produced by *P. aeruginosa* in pretreatment and anaerobic treatment of poultry effluent from a poultry slaughterhouse. The authors selected 8 h as the time of pretreatment with the enzyme and biosurfactant. They also observed that enzyme and biosurfactant concentrations as well as the interaction between biosurfactant and temperature have shown the most significant effect on hydrolysis of fat effluent.

The analysis of variance (ANOVA) was calculated based on SCOD average of time as pretreatment (4.5 h) that showed significant values for regression, that is, the biosurfactant concentration or temperature have affected organic matter solubilization, expressed as soluble COD. Thus, the following model equation was obtained:

$$SCOD = 1035.801 + 124.847*BS - 41.854*BS^2 + 135.059*T + 50.831*T^2$$

Where: BS = biosurfactant and T = temperature.

Based on the generated model to an increase of soluble COD, a response surface was built (Figure 2), according to temperature and biosurfactant concentration.

Table 4. Results from the anaerobic biodegradability assays of the pretreated effluent with surfactin.

Treatment	Initial VA/TA ratio	Final VA/TA ratio	Initial SCOD (mg L^{-1})	Final SCOD (mg L^{-1})	SCOD removal (%)	Initial OG (mg L^{-1})	Final OG (mg L^{-1})	OG removal (%)	Methane Volume (mL)	SPM (g$CODCH_4$ / gVSS d^{-1})
1 = Control	1.76	0.10	505.63	85.89^{a}	83.01	519.93	62.54	87.97	3500	0.78
2 = 6 mg L^{-1} BS	1.06	0.07	529.69	104.07^{bc}	80.35	519.93	75.27	85.52	4822	0.98
3 = 13.27 mg L^{-1} BS	0.86	0.07	492.92	85.04^{a}	82.74	519.93	97.26	81.29	4665	0.83
4 = 31 mg L^{-1} BS	1.26	0.09	508.24	92.65^{ab}	81.77	519.93	0	100	5130	0.91
5 = 48.73 mg L^{-1} BS	1.21	0.08	629.3	97.30^{ab}	84.53	519.93	141.62	72.76	5020	0.87
6 = 56 mg L^{-1} BS	1.28	0.10	763	113.37^{c}	85.14	519.93	144.07	72.29	4115	0.87

*The same letters represent equal averages. VA/TA = Volatile acidity ratio/total alkalinity; SCOD = soluble chemical oxygen demand; OG = oils and greases; BS = biosurfactant surfactin; SPM = specific production of methane.

Temperature and biosurfactant concentration conditions can be observed by analyzing the response surface generated by the model, which resulted in higher COD solubilization. The highest values of soluble COD concentration are recorded at temperatures above 60°C and at higher biosurfactant concentrations (56 mg L^{-1}). This implies that despite the applied factor, there will be an increase in COD solubilization. Therefore, biosurfactant applicability can be extremely advantageous for effluent treatment, since a heating system for treating this kind of effluent demands financial resources for energy as well as a structure designed to implement and keep it during the treatment.

Anaerobic biodegradation of a pretreated effluent

During the anaerobic biodegradation assays, it was observed that pH samples have not changed much and their initial range stayed from 6.0 to 7.0 (initial and final) for all the treatments. The maintenance of a relatively neutral pH range shows that the anaerobic process has been developed (Keefer and Urtes, 1962). This was appropriate for the survival of methane producing bacteria (Sosa et al., 2004; Chernicharo, 2007). The results obtained during the anaerobic biodegradability assays of the pretreated effluent with surfactin are shown in Table 4. The VA/TA ratio indicates stability of a reactor and it was observed that after the first week of digestion, VA/TA ratio was already in the reactor stability range, which according to Barana and Cereda (2000) varies from 0.1 to 0.3. Values of about 0.4 have shown some instability and when they were superior to 0.8, there could be a collapse of digestion.

The differences in the initial concentrations of SCOD among treatments may be due to the addition of surfactin or the presence of pieces of floating organic material in the effluent, which were broken during the pretreatment. The analysis of variance (ANOVA) was obtained based on the final results (Week 5) of SCOD and the test of multiple comparison of Tukey averages. Thus, it was observed that, statistically, treatments 1, 3, 4 and 5 presented the same average of soluble COD removal, consequently, they differed from averages of 2 and 6 treatments. Regarding the tested level of significance, averages of 2, 4 and 5 treatments do not differ, as well as averages from 2 and 6 treatments.

Nakhla et al. (2003) studied the effect of biosurfactant addition from cactus for anaerobic wastewater treatment with a high content of oils and greases (OG = 38,800 mg L^{-1}) from an animal diet industry. The authors concluded that after 16 days of anaerobic digestion, the addition of biosurfactant in raw effluent removed SCOD from 11,200 to 7,050 mg L^{-1}.

Daverey and Pakshirajan (2011) evaluated the pretreatment of an effluent from a dairy industry using a sophorolipid biosurfactant produced by *Candida bombicola*. The results showed that COD removal efficiency was 93% after 96 h of operation. The synthetic surfactant Tween 80 has been used by Kumar et al. (2008) as a substrate during anaerobic treatment process of tannery residues. The addition of surfactant in this process significantly increased hydrolytic and fermentative activities of enzymes (proteases and deaminases). These results indicated that when

this surfactant is added to the treatment process, there is an increase in using residues by microorganisms, and in turn, there is an improvement on metabolic conversions.

Damasceno et al. (2012) evaluated the combined use of rhamnolipid biosurfactant and an enzymatic preparation to treat wastewater from a poultry slaughterhouse with high fat concentration (2,403 mg L^{-1}) and 8,692 COD mg L^{-1}. The authors carried out the pretreatment of such effluent with this enzyme and biosurfactant, and, subsequently, the anaerobic treatment at the best condition was evaluated. It was found that enzyme concentrations above 0.5% (w/v) or biosurfactant concentrations below CMC (<205 mg L^{-1}) promoted inhibitory or toxic effects to anaerobic bacteria. At optimal concentration, both microbial metabolites showed simultaneous action on the availability/hydrolysis of fats, and the authors concluded that there is potential to treat wastewater from a poultry slaughterhouse and there will be no need for a flotation phase.

Jacobucci et al. (2009) observed, by COD reduction, the application of two bacterial species (*Pantoea agglomerans* and *Planococcus citreus*) that are biosurfactant producers and of a biosurfactant produced by them, in a greasy effluent that comes from a soap and margarine industry. The effluent presented 4,400 mg L^{-1} COD and 70 to 76% of removals that were obtained from COD with an application of bacterial strains and biosurfactants after 24-contact hours with the effluent.

The samples' results developed as replicas showed that there was higher conversion of soluble organic matter at the first three weeks of anaerobic biodegradation and the percentage of SCOD removal ranged from 80.35 to 85.14%. Based on these results, it can be inferred that anaerobic treatment of wastewater from a poultry slaughterhouse is a feasible option, since Chernicharo (2007) considers as efficient, the anaerobic system on wastewater treatment when the removal of organic matter exceeds 65%. It is noted that, in these samples, there is almost no difference on OG removal among the treatments, that is, either with or without biosurfactant application, there was an excellent OG removal from wastewater.

In this trial, an anaerobic sludge sample from each treatment was evaluated at the end of digestion to check some possible adsorption of fat to sludge; therefore, OG concentration in this material was not detected. This indicates that fat present in wastewater from a poultry slaughterhouse was degraded in such process. Accordingly, Gomes et al. (2011) reviewed the application of porcine pancreatin enzyme in the pretreatment of synthetic dairy wastewater with a subsequent anaerobic treatment in UASB reactor, and they recorded that lipids were completely removed from the anaerobic sludge digestion used as inoculum.

On the other hand, regarding methane production (mL), it was observed that in the early days of samples incubation, there was slight displacement of NaOH solution from eudiometers. It lasted effectively almost until the 25th incubation day, consequently, after this term, the treatments tended to stabilize methane production. The assays ended at 36 days of incubation, when some stabilization was observed for three consecutive days of methane production. Treatment T1, which had no surfactin, showed the least and final volume of methane production. This may be an evidence that biosurfactant may have promoted some effect on microbial consortium.

Valladão et al. (2007) reported that the pretreatment of wastewater from a poultry slaughterhouse with a hydrolase pool obtained from fungi increased methane production, reduced time as well as reaction volume of reactors. When comparing the cumulative methane production with data of SCOD and OG removal for the studied period, there already had a removal superior to 80% SCOD as well as 90% OG of destructive samples, which are replicas of treatments applied to eudiometers. Therefore, there was no high concentration of organic material to be degraded or converted into biogas.

Huang et al. (2015) studied the improved volatile fatty acid production during waste activated sludge anaerobic fermentation by different bio-surfactants. They reported that volatile fatty acid production was increased to approximately 4-fold versus the blank using surfactin, rhamnolipid and saponin. Surfactin mainly increased the dissolution of organic matters to reach a high volatile fatty acid accumulation.

ANOVA analysis was carried out with maximum SPM values for each of the treatments' replicas. The p-value and calculated F showed that there is no statistical difference among the maximum SPM values from the applied treatments.

Conclusions

With RCCD, it was observed that at higher temperatures and concentrations of surfactin (superior to their CMC), there are highest values of increased COD solubilization, but, effectively heating an effluent to carry out its pretreatment is not feasible.

The removal of soluble organic matter was statistically equal among the applied treatments as well as for SPM. The control treatment was the lowest one observed among the others concerning the cumulative volume of methane at the end of this trial, when surfactin was not added to the pretreatment.

These results demonstrated that the application of biosurfactant surfactin produced by *B. subtilis* LB5a above its CMC (> 28 mg L^{-1}) did not inhibit the activity of microbial consortium responsible for anaerobic biodegradation, and this process took place appropriately in all applied treatments.

Conflict of interests

The authors have not declared any conflict of interests.

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