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Influence of Temperature on Performance of an Anaerobic Sequencing Biofilm Batch Reactor With Circulation Applied to Treatment of Low-Strength Wastewater

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Abstract

The effect of temperature on the performance of an anaerobic sequencing biofilm batch reactor (ASBBR) with liquid-phase recirculation was assessed. Assays were performed using a recirculation velocity of 0.20 cm/s, 8-h cycles, and an average treated synthetic wastewater volume of 2 L/cycle with a concentration of 500 mg of Chemical Oxygen Demand (COD)/L. Operation temperatures were 15, 20, 25, 30, and 35ºC. At 25, 30, and 35ºC, organic matter removal efficiencies for filtered samples ranged from 81 to 83%. At lower temperatures, namely 15 and 20ºC, removal efficiency decreased significantly to 61 and 65%, respectively. A first-order model could be fitted to the experimental concentration profile values. The first-order kinetic parameter value of this model varied from 0.46 to 0.81 h^{-1} considering the lowest and highest temperature studied. Moreover, analysis of the removal profile values allowed fitting of an Arrhenius-type equation with an activation energy of 5715 cal/mol.

Index Entries: Temperature; anaerobic sequencing biofilm batch reactor; circulation; low-strength wastewater; anaerobic treatment.

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Introduction

During the last 40 yr, different anaerobic reactor configurations have been proposed. Most have been shown to be efficient in treating wastewaters and present satisfactory efficiency when treating high-strength wastewaters (>1000 mg of COD/L) near mesophilic temperature (35°C). Dague et al. *(1)* reported that until 1998 virtually all full-scale anaerobic treatment plants were restricted to wastewaters with temperatures higher than 18°C. Many wastewaters have temperatures lower than mesophilic conditions (<25°C) and typically require heating for efficient treatment.

Rebac et al. *(2)* reported that in moderate climatic conditions, the temperature of many wastewaters is considerably lower than the optimum for biologic treatment processes, affecting nitrification, denitrification, and mesophilic methanogenesis. One of the major problems in anaerobic reactors operating at low temperatures is the very low biogas production rate, which may result in low mixing intensity and poor contact between biomass and substrate. According to Kato et al. *(3)*, many industrial and domestic wastewaters present concentrations less than 1000 mg of COD/L, and many of these diluted wastewaters also have low seasonal temperatures. Successful anaerobic treatment of these wastewaters without heating will significantly reduce energy requirements and treatment costs.

Sewage treatment in highly efficient anaerobic reactors is economically feasible only when reactor heating can be dispensed with. This restriction may limit successful application of anaerobic reactors in treating domestic wastewater to places where liquid temperature remains higher than 20°C *(4)*. Although investigations have been reported regarding treatment in the range of 10–15°C, efficiencies attained were hardly superior to those obtained in primary treatment units *(5)*.

The kinetic parameter directly affected by temperature is the specific substrate consumption rate. In the temperature range between 20 and 25°C, this parameter drops to less than half that at 35°C. One should consider, however, that the overall substrate removal rate is related to the product of the specific rate by the concentration of active microorganisms in the reactor. Therefore, the same overall removal rate can be accomplished at different temperatures provided that the reactor operates at high microorganism concentration. This way, reactor performance will depend on the capability to retain biomass. However, low specific organic matter (or substrate) consumption rate might not be the limiting parameter of the anaerobic treatment process. At temperatures lower than 20°C, solubilization of fats, particulate material, and organic material is quite slow and may constitute the limiting step of the process. If these constituents are not solubilized, they may be washed out of the reactor or accumulate at the surface or in the solid/gas/liquid separation systems. On the other hand, because part of the organic material in the wastewater may consist of particulates, as well as lipids, instability of the reactor may result when this substrate is not available for supporting microbial growth *(5)*.

Anaerobic processes are more sensitive to temperature variations than aerobic ones. Conversion of acetate into methane is more dependent on temperature than production of volatile acids (VA). Hence, an increase in VA, associated with lower temperatures, may exceed the buffer capacity of the system, reducing pH. Sensitivity to temperature increases with increasing organic load *(5)*.

In this context, the main objective of the present study was to assess the operation of an anaerobic sequencing biofilm batch reactor (ASBBR) with recirculation of the liquid phase containing immobilized biomass on polyurethane foam on treating synthetic wastewater submitted to different temperatures at constant influent concentration and cycle time.

Materials and Methods

The ASBBR containing immobilized biomass and operating at 30.0 ± 0.5°C was made of acrylic with a 500-mm height, a 100-mm external diameter, and a 3.5-mm thickness. The reactor also contained a liquid-phase recirculation system composed of a reservoir with a 400-mm height, a 60-mm external diameter, and a 3.5-mm wall thickness with a 2-L working volume. The reactor was divided into four levels by stainless steel screens, in order to prevent bed packing. At the lower part of the reactor, a 20-mm-high compartment provided for improved wastewater distribution, and at the upper part a 40-mm-high region functioned as a biogas collecting chamber (CH_4 and CO_2) (Fig. 1).

The inert support consisted of 5-mm polyurethane foam cubes and was inoculated with sludge from a upflow anaerobic sludge blanket reactor treating poultry slaughterhouse wastewater. The immobilization procedure, after Zaiat et al. *(6)*, consisted of crushing the sludge through a 0.5-mm mesh nylon sieve, completely immersing the foam with the obtained suspension, followed by conducting intense homogenization and a 2-h rest. Poorly adhered solids were washed off and the medium was drained. This inoculum presented total volatile solids (TVS) and total solids (TS) of 51 and 62 g/L, respectively. After immobilization bioparticles presented 1.0 g of TVS/g of foam and 1.1 g of TS/g of foam, with TVS in the reactor of approx 52 g, which corresponded to 26 g of TVS/L considering 2 L of volume treated/cycle.

The synthetic wastewater used, based on 500 mg of COD/L, consisted of carbohydrates (35 mg/L of sucrose, 114 mg/L of amide, 34 mg/L of cellulose), proteins (208 mg/L of meat extract), lipids (51 mg/L of soybean oil), and trace metals (250 mg/L of NaCl, 7 mg/L of $MgCl_2 \cdot 6H_2O$, 4.5 mg/L of $CaCl_2 \cdot 2H_2O$), as well as NaHCO3 (200 mg/L) as buffer. The synthetic wastewater was sterilized (121°C for 15 min) to maintain its characteristics throughout the assays.

A cycle length of 8 h (or 480 min) was used (i.e., three cycles per day). At the beginning of a cycle, the reactor was fed for 10 min with an approximate volume of 2 L of wastewater. Reaction phase was 459 min with a

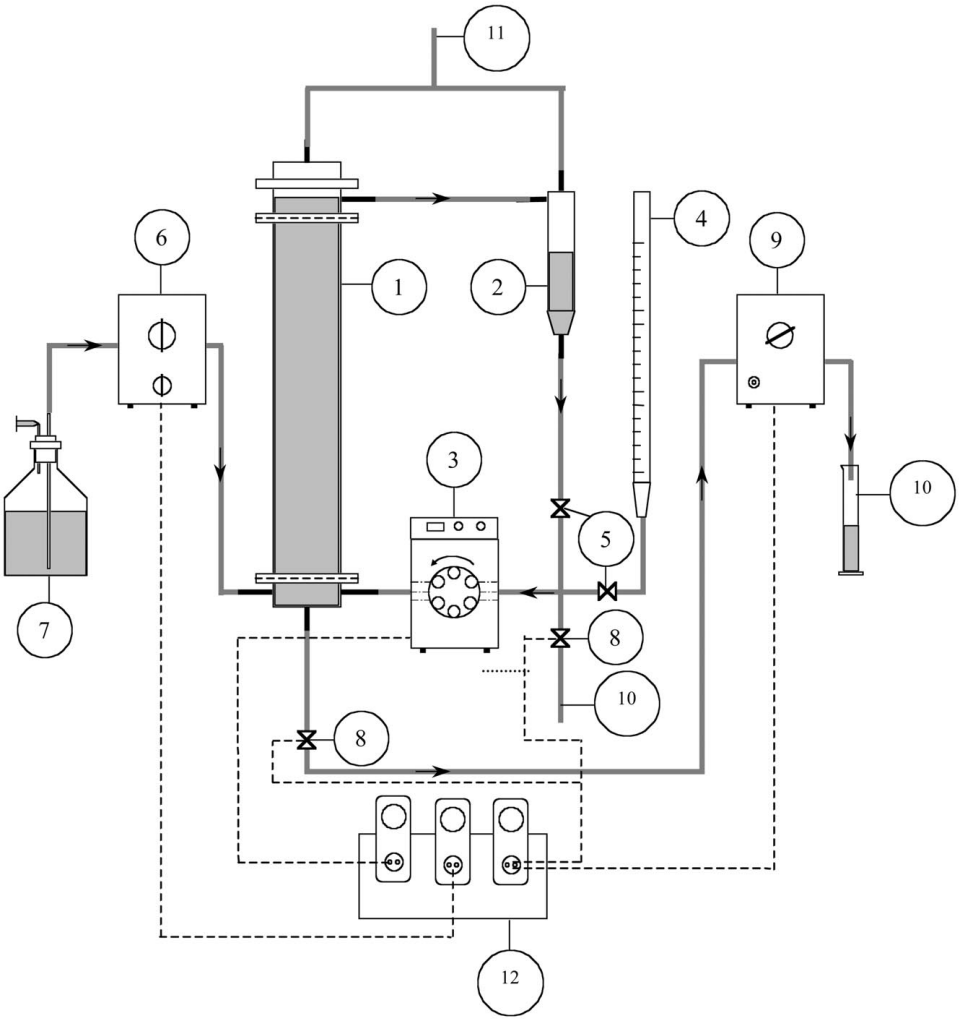

Fig. 1. Scheme of ASBBR with liquid-phase circulation and immobilized biomass: 1, reactor containing immobilized biomass; 2, external reservoir; 3, circulation pump; 4, flow rate meter; 5, valve; 6, feed pump; 7, wastewater reservoir; 8, discharge valve; 9, discharge pump; 10, effluent outlet; 11, gas outlet; 12, control unit. Solid lines represent hydraulic lines and dashed lines represent electric lines.

recirculation flow rate of 6 L/h (upflow rate of 0.20 cm/s). At the end of the cycle, the effluent was discharged within 10 min. The next cycle was started 1 min after the reactor had been emptied. This delay time was established to ensure synchronism of the feed and discharge pumps during operation.

During reactor operation, influent and effluent samples were taken for the following analyses: filtered and nonfiltered effluent organic matter concentration as COD (CES and CET, respectively); nonfiltered influent organic matter concentration as COD (CI) (spectrophotometric); total vola-

tile acids (TVA) measured by potentiometric method (TVA) and TVA measured by chromatographic method (VA); bicarbonate alkalinity (BA) (potentiometric); TS and TVS, total suspended solids (TSS) and volatile suspended solids (VSS) (gravimetric); pH and volume fed per cycle (measured in a measuring cylinder). Methods employed were according to Standard Methods for the Examination of Water and Wastewater (7).

Experiments were carried out in five stages that differed according to reactor temperature: 30, 35, 25, 20, and 15°C. The first condition was at 30°C, to allow evaluation of system behavior by comparison with results obtained from previous investigations in other ASBBRs treating the same type of wastewater.

In each assay, after attaining operation stability, i.e., approximately constant values of the monitored values, profiles were taken during the operation cycle and the following were quantified: filtered substrate concentration, BA, intermediate VA and TVA, methane concentration, and pH. Samples were collected at time intervals of 20 min for the first 2 h, 30 min for the next 2 h, and 60 min for the remaining hours. After the profile acquisition, the operation condition was changed; that is, a new operation phase was started.

For each temperature condition, a first-order kinetic model was fitted to the experimental data from filtered organic matter profiles (CSF), taking into account residual filtered organic matter concentration (CSR), which was determined as the value of organic matter concentration in the reactor at which reaction rate equals zero. The model of the process is given by Eq.1, in which CSF is the filtered organic matter concentration in the reactor, CSO is the filtered organic matter concentration in the reactor at the beginning of the cycle, k is the first-order apparent kinetic constant, t is the cycle time, and CSR is the residual filtered organic matter concentration *(8)*. This modified first-order model was fitted to the experimental organic matter concentration profile values by the Levenberg-Marquardt method (Microcal Origin 6.1"). It should be mentioned that the kinetic model proposed was formulated assuming homogeneous reaction kinetics; that is, the kinetic parameter k is actually an apparent constant, because it includes in its synthesis the internal and external mass transfer resistance to the biomass as well as the biochemical reaction phenomenon.

$$C_{SF} = C_{SR} + \left(C_{SO} - C_{SR}\right) \cdot \exp\left(-k \cdot t\right) \tag{1}$$

The reaction rate constant or, in this case, the apparent kinetic parameter k is the Arrhenius-type time-dependent term of the kinetic model (Eq. 2), in which k0 is the frequency or preexponential factor, E is the activation energy, R is the gas constant, and T is the absolute temperature:

$$k = k_0 \cdot \exp\left(\frac{-E}{R \cdot T}\right) \tag{2}$$

Table 1
Average Values of Monitored Variables in Influent and in Effluent[a]

T (°C)	CI (mg COD/L)	CET (mg COD/L)	εT (%)	CES (mg COD/L)	εS (%)
15	496 ± 35 (16)	250 ± 43 (15)	50 ± 9 (15)	195 ± 27 (15)	61 ± 6 (15)
20	499 ± 60 (19)	233 ± 24 (25)	53 ± 5 (25)	182 ± 23 (25)	64 ± 5 (25)
25	507 ± 92 (9)	104 ± 18 (11)	80 ± 4 (11)	94 ± 20 (11)	81 ± 4 (11)
30	531 ± 44 (21)	110 ± 35 (23)	79 ± 7 (23)	91 ± 14 (23)	83 ± 3 (23)
35	506 ± 66 (10)	104 ± 7 (10)	79 ± 1 (10)	85 ± 9 (10)	83 ± 2 (10)

[a]Values in parentheses refer to the number of samples taken. The average value of the volume treated per cycle was 1.9 ± 0.2 L for all investigated temperatures with at least 10 measurements in each condition.

Table 2
Average Values of Monitored Variables in Influent and in Effluent[a]

T	BA (mg $CaCO_3$/L)		TVA (mg HAc/L)		pH	
(°C)	Influent	Effluent	Influent	Effluent	Influent	Effluent
15	121 ± 5 (12)	138 ± 13 (17)	25 ± 5 (12)	59 ± 13 (17)	8.9 ± 0.3 (12)	7.3 ± 0.6 (17)
20	116 ± 12 (13)	137 ± 32 (20)	28 ± 4 (13)	53 ± 33 (20)	9.0 ± 0.2 (13)	7.2 ± 0.3 (20)
25	119 ± 7 (8)	190 ± 14 (11)	30 ± 5 (8)	20 ± 7 (11)	10.3 ± 0.2 (8)	7.4 ± 0.2 (11)
30	116 ± 27 (15)	202 ± 18 (17)	47 ± 23 (15)	32 ± 14 (17)	8.0 ± 2.2 (15)	7.4 ± 0.3 (17)
35	108 ± 7 (8)	191 ± 17 (13)	32 ± 6 (8)	19 ± 5 (13)	9.9 ± 0.3 (8)	7.2 ± 0.2 (13)

[a]Values in parentheses refer to the number of samples taken.

Table 3
Average Values of Monitored Variables in Influent[a]

T °C)	TS (mg/L)	TVS (mg/L)	TSS (mg/L)	VSS (mg/L)
15	1796 ± 43 (6)	489 ± 65 (6)	46 ± 8 (6)	27 ± 9 (6)
20	913 ± 70 (8)	509 ± 67 (8)	40 ± 25 (8)	41 ± 14 (8)
25	1013 ± 75 (5)	592 ± 55 (5)	39 ± 16 (5)	37 ± 21 (5)
30	920 ± 51 (6)	546 ± 51 (6)	56 ± 25 (6)	51 ± 23 (6)
35	926 ± 43 (6)	492 ± 42 (6)	51 ± 15 (6)	46 ± 27 (6)

[a]Values in parentheses refer to the number of samples taken.

Results and Discussion

Tables 1-4 provide the average values of the main monitored values in the ASBBR. Figure 2 shows the removal efficiencies (ε) for filtered and nonfiltered samples at varying operation temperature. The first operation temperature used was 30°C, considered standard, since other works car-

Table 4
Average Values of Monitored Variables in Effluent[a]

T (°C)	TS (mg/L)	TVS (mg/L)	TSS (mg/L)	VSS (mg/L)
15	1629 ± 22 (6)	310 ± 44 (6)	67 ± 23 (6)	48 ± 27 (6)
20	705 ± 60 (8)	312 ± 52 (8)	72 ± 13 (8)	73 ± 14 (8)
25	665 ± 39 (5)	224 ± 41 (5)	20 ± 9 (5)	15 ± 9 (5)
30	628 ± 66 (7)	243 ± 105 (7)	25 ± 17 (7)	21 ± 11 (7)
35	665 ± 8 (5)	236 ± 27 (5)	36 ± 12 (5)	27 ± 19 (5)

[a]Values in parentheses refer to the number of samples taken.

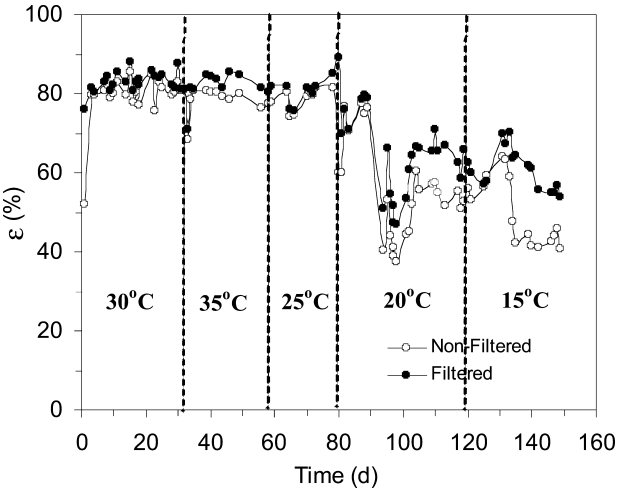

Fig. 2. Removal efficiencies for filtered and nonfiltered samples.

ried out in the same laboratory and utilizing a similar system have been operated at this temperature. The system operated for 32 d (96 cycles) and attained a removal efficiency of 83 ± 3% in terms of filtered samples. Next, system temperature was increased by 5°C (to 35°C) and operation took place for 24 d (72 cycles). In terms of filtered samples, removal efficiency was unaltered; that is, a value of 83 ± 2% was attained. The system was then operated at 25°C for 24 d (72 cycles). In terms of filtered samples, removal efficiency practically did not change; that is, a value of 81 ± 4% was attained, similar to the previous ones considering the deviation.

Operation temperature was next further reduced to 20°C for 40 d (120 cycles). In terms of filtered samples, the removal efficiency dropped to 64 ± 5%. Despite this drop, BA was generated in relation to the effluent. Influent alkalinity was 116 ± 12 mg of $CaCO_3$/L, and effluent alkalinity was 137 ± 32 mg of $CaCO_3$/L. The generation of alkalinity was less compared with the standard condition of 30°C, at which values amounted to 116 ± 27 mg

of $CaCO_3$/L in the influent and 202 ± 18 mg of $CaCO_3$/L in the effluent. Regarding TVA concentration at 20ºC, values were 28 ± 4 mg of HAc/L in the influent and 53 ± 33 mg of HAc/L in the effluent, compared with the standard condition (30ºC) values of 47 ± 23 mg of HAc/L and 32 ± 14 mg of HAc/L, in the influent and effluent, respectively. As to effluent pH, no variation was observed in relation to the standard condition value.

Finally, when the system temperature was further reduced to 15ºC and operation lasted 29 d (87 cycles), a removal efficiency of 61 ± 6% was obtained, i.e., a drop in relation to the standard condition at 30ºC. Analogously to the previous condition, BA was generated, yielding values of 121 ± 5 mg of CaCO3/L in the influent and 138 ± 13 mg of $CaCO_3$/L in the effluent. Hence, more alkalinity was generated at the standard condition. VA concentrations at this condition of 15ºC were 25 ± 5 mg of HAc/L in the influent and 59 ± 13 mg of HAc/L in the effluent, showing accumulation in relation to the standard condition. As to pH, no variation was seen in relation to the standard condition.

The reduction in removal efficiency in the 15–20ºC range might be attributed to the dependence between temperature and the factors affecting microbial growth. Temperature is also one of the most important factors in species selection, because microorganisms do not possess ways of controlling their inner temperature, and, hence, the temperature inside the cell will be determined by the external environment. Moreover, temperature affects biologic processes and influences enzyme reaction rates as well as substrate diffusion rates. Furthermore, according to Ndon and Dague *(9)*, at temperatures less than 20ºC solubilization of fats, particulate material, and organic polymers is slower and might be the limiting step in the process

Figure 3A shows the substrate concentration profiles for all operation conditions. Analysis of the organic matter concentration profiles shows that the ASBBR submitted to the same influent concentration and the same cycle length but at different temperatures presented similar behavior along the cycle for all implemented conditions.

Table 5 provides values of the initial organic matter concentration (C_{S0}), residual organic matter concentration (C_{SR}), organic matter removal efficiency (ε), and apparent kinetic parameter (k) obtained from the fit of a first-order kinetic model to the experimental values (with respective squared correlation coefficient, R^2) for ASBBR operation at the different implemented temperatures.

The organic matter concentration profiles are seen to present similar behavior along a cycle at the different operation temperatures. There was practically no difference among the organic matter profiles for operation conditions at 35, 30, and 25°C. At these conditions, the first-order kinetic parameter and residual concentration values were also similar.

At lower temperatures (20 and 15ºC), the values of the first-order kinetic parameter, as well as of the residual substrate concentration, were seen to be very different from those of the other implemented conditions.

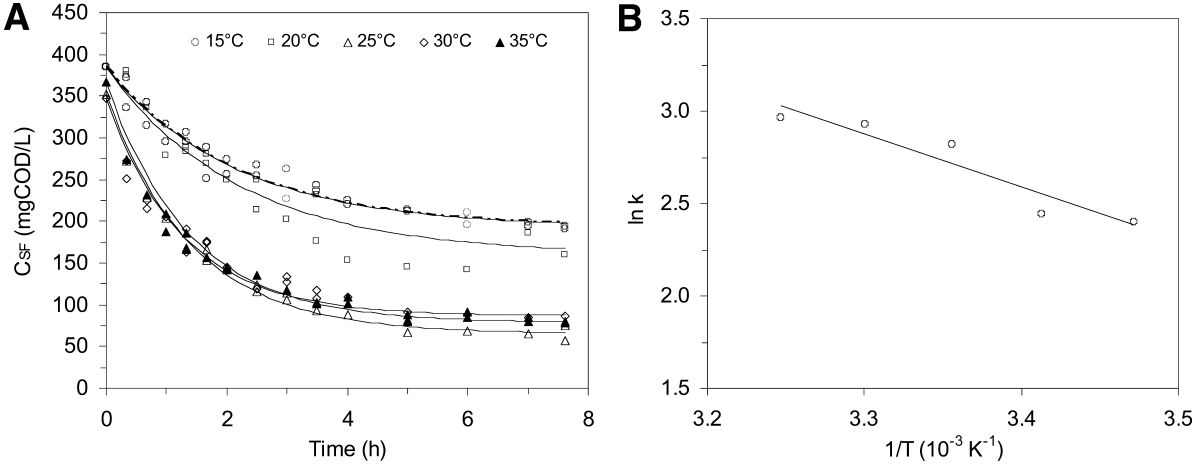

Fig. 3. Profiles of **(A)** organic matter concentration and **(B)** apparent kinetic parameter.

Table 5
Summary of Kinetic Fitting

T (°C)	C_{S0} (mg/L)	C_{SR} (mg/L)	ε (%)	k (h^{-1})	R^2
15	384	192	61 ± 6	0.46	0.960
20	384	160	64 ± 5	0.48	0.932
25	352	65	81 ± 4	0.70	0.986
30	347	87	83 ± 3	0.78	0.965
35	366	79	83 ± 2	0.81	0.984

A temperature reduction of 10 and 15ºC resulted in a decrease in reaction rate in relation to the standard operation condition. This may be attributed to the influence of reducing temperature on equilibrium between production and consumption of intermediate products (such as volatile acids); the methanogenic populations; and the hydrolysis rate of proteins, lipids, and particulates, with consequent reduction in overall efficiency.

To assess the effect of temperature on organic removal rate in the ASBBR, an Arrhenius-type model was fit to the experimental data, which resulted in the profile shown in Fig. 3B. From this fit the following parameters were obtained: $k_0 = 235{,}626\ h^{-1}$, $E = 5715$ cal/mol, and $R^2 = 0.898$. Eq.3 shows the relation between the first-order kinetic parameter (k) and temperature for the ASBBR with liquid-phase circulation treating synthetic wastewater submitted to different operation temperatures:

$$k = 235626 \cdot \exp\left(\frac{-5715}{R \cdot T}\right) \tag{3}$$

Equation 3 is useful in reactor design because it correlates removal rate from the system with operation temperature. A reduction in temperature implies a decrease in the value of the first-order kinetic parameter (k), and, consequently, an increase in cycle length or in reactor volume would be necessary to maintain efficiency.

According to Fogler (10) high activation energy reactions are extremely sensitive to temperature, and low activation energy reactions are relatively insensitive to temperature. It should be mentioned that activation energy values for anaerobic sequencing batch processes with immobilized microorganisms on polyurethane foam could not be obtained. Dinopoulou et al. *(11)* operated a two-phase mechanically stirred anaerobic reactor treating complex meat extract-based substrate in the first stage of anaerobic digestion (production of acetic and propionic acid). They found that the effect of temperature on acidification rate followed an Arrhenius-type equation, with an activation energy of 4736 cal/mol. Guan et al. *(12)* reported activation energy values for substrate removal in biologic aerobic processes. For domestic waste, they reported values between 9895 and 11,998 cal/mol; for amide-based wastewater, a value of 16,492 cal/mol; and for wastewater containing TPD (dye industry-related waste), a value of 14,460 cal/mol.

The BA profiles shown in Fig. 4A reveal that alkalinity was generated at all conditions and that at 15 and 20°C an increase in BA was much slower than at 25, 30, and 35°C. Regarding the pH profiles in Fig. 4B, all implemented conditions showed similar behavior; pH decreased owing to initial accumulation of VA and subsequently increased owing to consumption of the VA (characteristic of intermediate metabolism).

The TVA concentration profiles shown in Fig. 5A reveal that all implemented conditions demonstrated similar behavior with initial accumulation followed by consumption of the VA (characteristic of intermediate metabolism). Considering reduction in temperature, the residual value at the end of the cycle was seen to be higher at 15 and 20°C, with great variation between these conditions, indicating that an increase in cycle length might be necessary. At 25, 30, and 35°C, TVA concentrations were very similar and low. At 15 and 20ºC, higher VA concentrations were seen, although they did not exceed the buffering capacity of the system.

TVA concentration profiles obtained by chromatographic analysis, shown in Fig. 5B, revealed that all implemented conditions showed similar behavior with initial accumulation of VA followed by consumption of the VA (characteristic of intermediate metabolism), as already described for the profiles obtained by titrimetric analysis. Similar to that obtained by titrimetric analysis, a residual value was seen at the end of the cycle at 15 and 20°C, with great variation between these conditions, indicating that an increase in cycle length might be necessary, as in the previous case. At 25, 30, and 35°C, TVA concentrations were very similar, not being detected after 240 min from the start of the cycle. The difference encountered for the two types of analyses might have been caused by not measuring formic and lactic acid in the chromatographic analysis, in addition to errors inherent to titrimetric analysis.

Figures 6 and 7 show the methane gas concentration and molar fraction profiles. At all operation conditions, methane formation was more pronounced in the beginning and decreased along the cycle (typical behavior of a final metabolite), because methane is one of the compounds generated at the end of the process. The results at 30 and 35ºC were almost the same. At the remaining conditions, biogas concentration decreased owing to a reduction in removal efficiency, which was affected by reduction in temperature. When methanogenic microorganisms are submitted to unfavorable environmental conditions, such as low temperatures, they do not utilize the intermediate acids as quickly as when these are formed by acidogenic microorganisms, resulting in their accumulation and, thus, lower biogas concentration.

Conclusion

The ASBBR with liquid-phase recirculation submitted to different operation temperatures was found to be stable and efficient at 25, 30, and 35°C, presenting removal efficiency of 81-83% in terms of filtered samples.

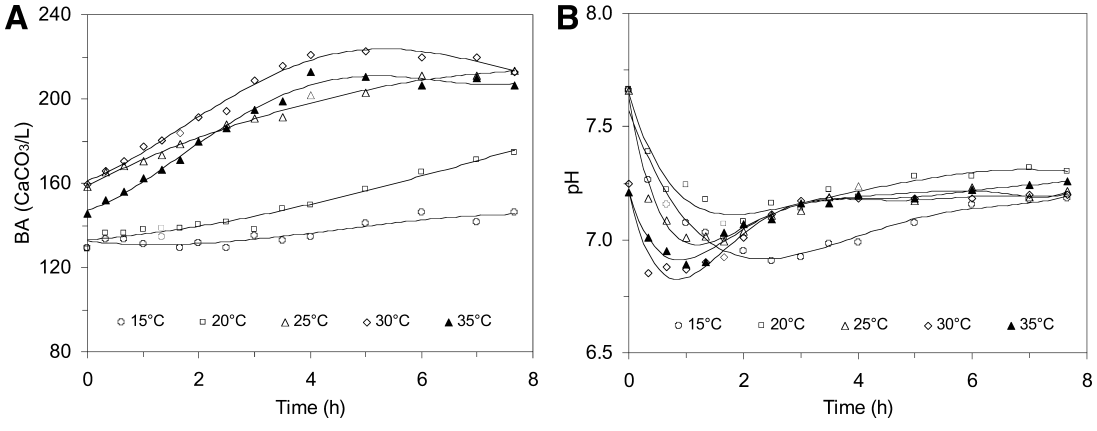

Fig. 4. Profiles of **(A)** BA and **(B)** pH.

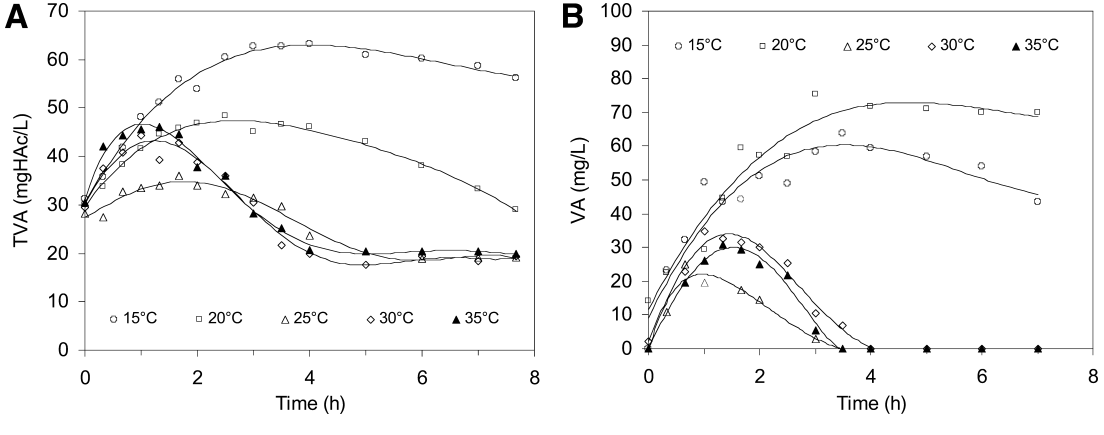

Fig. 5. Concentration profiles of TVA by **(A)** potentiometric method and **(B)** chromatographic method.

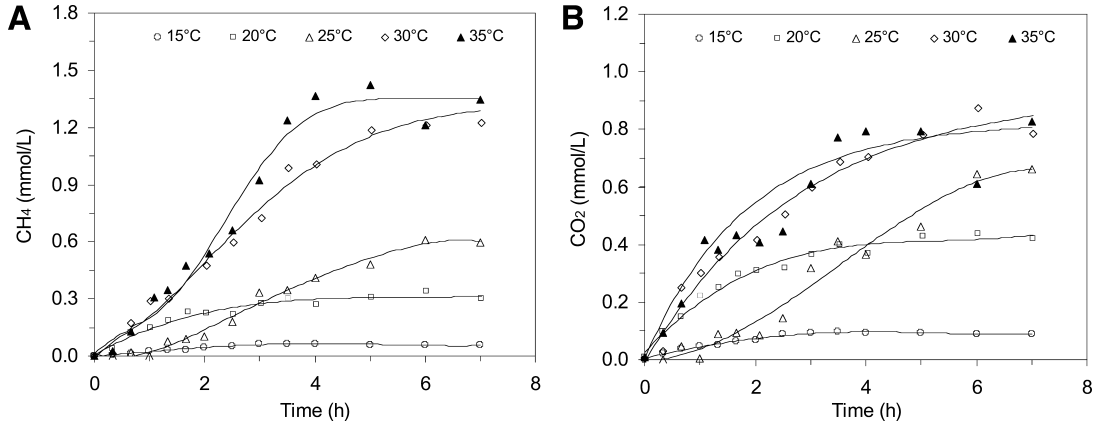

Fig. 6. Concentration profiles of **(A)** CH_4 and **(B)** CO_2.

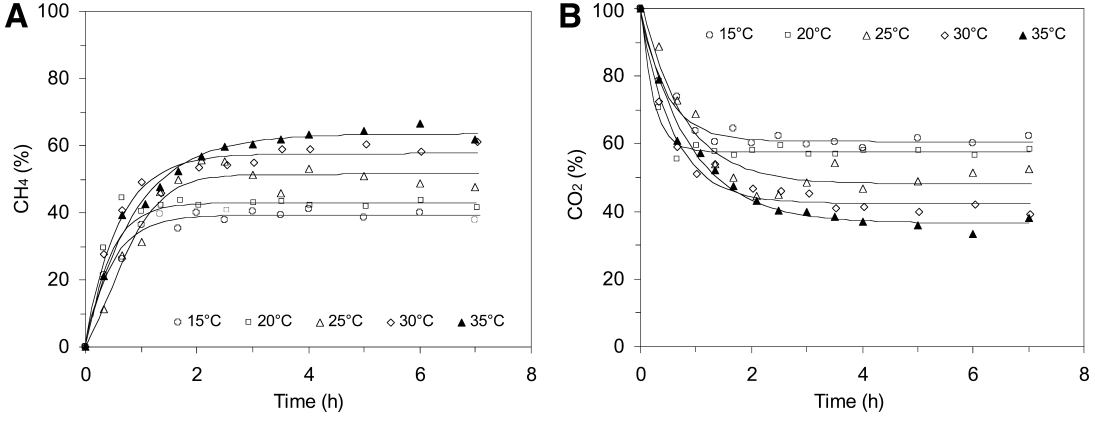

Fig. 7. Molar fraction profiles of **(A)** CH_4 and **(B)** CO_2.

Removal efficiency decreased at reduced temperatures of 15 and 20°C. Despite VA accumulation, the system buffering capacity was not exceeded and the system presented a new stable condition with a removal efficiency of 61-64%.

Analysis of the system in terms of removal efficiency showed that a 5°C increment or reduction in relation to the reference temperature of 30°C practically did not alter efficiency. Regarding the kinetic parameter, a variation was seen from 0.70 to 0.81 h^{-1} considering reduction and increment, respectively. However, this parameter varied significantly at the reduced temperatures; in this case, values were 0.46 and 0.48 at 15 and 20ºC, respectively.

Analysis of the removal profile values allowed fitting of an Arrhenius-type equation with an activation energy of 5715 cal/mol. This correlation might be very useful in reactor design because removal rate is correlated with operation temperature.

Acknowledgments

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