

Effect of the Influent COD Concentration on the Anaerobic Digestion of Winery Wastewaters from Grape-Red and Tropical Fruit (Guava) Wine Production in Fluidized Bed Reactors with Chilean Natural Zeolite for Biomass Immobilization

S. Montalvo,^a L. Guerrero,^b R. Borja,^{c,*} I. Cortés,^d E. Sánchez,^e and M. F. Colmenarejo^e

^aDept. of Chemical Engineering, University of Santiago de Chile, Ave. Bernardo O'Higgins 3363, Santiago de Chile

^bDept. of Chemical, Biotechnological and Environmental Processes, Federico Santa María Technical University Casilla 110 – V, Valparaíso, Chile

^cInstituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012 Sevilla, Spain

^dEnvironment Nacional Center, Chile University, Ave. Larraín 9975, La Reina, Santiago de Chile

^eCentro de Ciencias Medioambientales (CSIC), Serrano 115 duplicado, 28006 Madrid, Spain

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The effect of the influent COD concentration on the performance of anaerobic fluidized bed reactors treating winery wastewaters from grape-red wine (GRWW) and guava wine production (GWW) was studied at laboratory scale. Two reactors were used: one treating GRWW (AFB1) and the other processing GWW (AFB2). The behaviour of these reactors packed with Chilean zeolite as biomass immobilization support was compared at mesophilic temperature (35 °C). Influent COD varied from $\gamma = 1\text{--}24 \text{ g L}^{-1}$ and the *HRT* was maintained constant at 1 day throughout the experiment. During the experiment, influent and effluent pH, TVFA, COD and methane gas production were determined. COD removal efficiency increased with the influent COD up to a maximum of around $\gamma = 19 \text{ g L}^{-1}$ for GRWW and up to around 22 g L^{-1} for GWW due to the increase of the concentration of phenols. Process performance was slightly better with guava winery wastewater than with grape-red winery wastewater due its lower phenolic content. During the period of non-inhibition the methane yield was virtually constant.

Key words:

Influent COD, anaerobic fluidized bed reactors, chilean zeolite, process performance, inhibition

Introduction

Wine production generates large volumes of liquid wastewaters with a high concentration of soluble organic matter.^{1–5} Winery wastewaters from red wine production usually have ethanol, as well as tartaric acid, carbohydrates, organic acids and polyphenols, while vinasse from tropical fruit (guava) wine production also contains sulfur compounds and a high content of sugars.^{6–9} Wine production has become an important agricultural industry in Chile and has a relevant position with more than 400 wineries for red and white wine production. However, wine is also produced from tropical fruits. For the reduction of the pollutant organic matter present in winery wastewaters, different anaerobic processes have been applied.^{10–14} Among

the different models of digesters commonly used, anaerobic fluidized bed reactors (AFBRs) have been successfully applied.^{10,15–18} The fluidized bed reactor is a digester configuration which has been demonstrated in various studies to be feasible for the treatment of both low and high strength industrial wastewaters.^{18–20} The use of small, porous, fluidized media enables the reactor to retain high biomass concentrations and thereby to operate at significantly reduced hydraulic retention times (HRT). Fluidization also overcomes operating problems, such as bed-clogging and a high pressure drop which would be encountered if such high surface area media were used in a packed bed reactor.¹⁸ A further advantage of using media to retain the biomass within the reactor is the possible elimination of the secondary clarifier.

One of the most important parameters to determine the feasibility of selecting the adequate reac-

*Corresponding author: R. Borja

(E-mail: rborja@cica.es; phone: + 34 95 4689654; fax: + 34 95 4691262).

tor for a given waste is the influent concentration. As is well-known, the anaerobic fluidized reactor can operate with good efficiencies at high organic loading rates (OLR), achieving COD removals of up to 80–85 % at OLR of around $20 \text{ g L}^{-1} \text{ d}^{-1} \text{ COD}$.²⁰ Natural zeolite has been widely used in environmental technology for filtration, ionic exchange and the immobilization of microorganisms.^{18,21–28} Zeolite is composed mainly of two minerals – clinoptilolite and mordenite.^{29,30} The feasibility of the use of natural zeolite as a support media in AFBR for the treatment of wastewaters generated in alcohol distilleries from the fermentation of sugarcane molasses has recently been demonstrated.¹⁸ In addition, natural zeolite, with its favorable characteristics for microorganism adhesion, has been widely used as an ion exchanger for ammonia removal due to the presence of Na^+ , Ca^{2+} and Mg^{2+} cations in its crystalline structure. This property can also be useful for improving the anaerobic process performance in the treatment of wastewaters with high concentrations of nitrogen compounds, such as cattle and pig wastes, with the aim of preventing process inhibition.^{18,28}

Although anaerobic digestion of most types of winery wastewaters is feasible and quite appealing from an energetic point of view, the presence of inhibitory substances such as phenolic compounds severely hinders the anaerobic process.^{6,9} This slows down the kinetics, and reduces mean rates of methane production and yield coefficients, making the utilization of high HRTs necessary.¹⁸

The aim of this work was to evaluate the effect of the influent COD concentration on the performance of anaerobic fluidized bed reactors operating with GRWW and GWW at mesophilic temperature ($35 \text{ }^\circ\text{C}$). These reactors were packed with Chilean zeolite for the immobilization of the microorganisms responsible for the process.

Materials and methods

Experimental set-up

Two anaerobic fluidized bed reactors consisting of acrylic plastic cylindrical columns with 6 L of effective volume were used in the experiments. The immobilization media consisted of natural zeolite from a Chilean depot obtained from Minera Formas, Chile (ZeoClean®). The chemical composition of the zeolite used as support is summarized in Table 1. Other characteristics of the zeolite used were: framework density (FD), 20.6 of tetra-hedral (T)-atoms per 100 nm^3 , 32.03 % porosity and grain density, 2.12 g cm^{-3} . The average diameter of the zeolite was in the range of 0.5 mm to 0.8 mm which is in line with previous results obtained.¹⁸ Each lab-

Table 1 – Chemical composition of Chilean natural zeolite

Chemical composition/w/%		Phase composition/w/%	
SiO_2	66.62	clinoptilolite	35
Al_2O_3	12.17	mordenite	15
Fe_2O_3	2.08	montmorillonite	30
CaO	3.19	others ^b	20
MgO	0.77		
Na_2O	1.53		
K_2O	1.20		
IW ^a	11.02		

^aIgnition wastes, ^bcalcite, feldespate and quartz

oratory-scale reactor was composed of a fluidization section of 6.6 cm in diameter and 74 cm high and a decantation section consisting of a truncated cone 14.4 cm in diameter and 20 cm high located at the top of the cylindrical section. A gas-liquid separator was placed at the top of the decantation section in order to guarantee the separation of solid, liquid and gas fractions. The gas produced in the process was bubbled in a NaOH solution at $w = 15 \%$ to remove the CO_2 . Later on, the volume of the gas was measured with a wet type gas meter. The operating temperature of the reactors ($35 \pm 1 \text{ }^\circ\text{C}$) was maintained virtually constant by placing them in a room at controlled temperature. A schematic diagram of the experimental set-up used is given in Fig. 1.

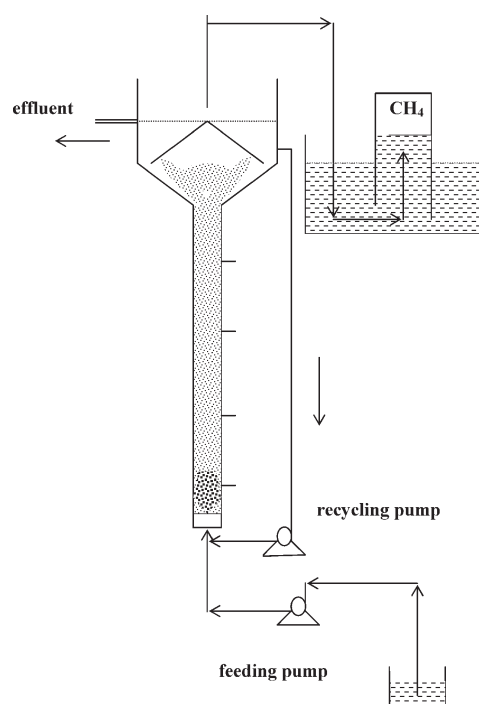


Fig. 1 – Schematic diagram of the experimental set-up used

Inoculum used

Each reactor was inoculated with 1.2 L of methanogenically active biomass from an anaerobic conventional digester processing winery wastewater. The inoculum had a concentration of volatile suspended solids (VSS) of $\gamma = 81 \text{ g L}^{-1}$ and a specific methanogenic activity (SMA) of $0.71 \text{ g g}^{-1} \text{ d}^{-1} \text{ COD}_{\text{CH}_4}$. The SMA was defined as the substrate-dependent methane production rate per unit mass of volatile solids biomass, i.e. the rate with a saturating concentration of substrate present when the background methane production rate had been diluted to an insignificant level.^{17,18}

Winery wastewater used

The winery wastewaters used in the experiments were the liquid effluents generated in both grape-red wine (GRWW) production and the guava fruit wine production (GWW). The average characteristics of these wastewaters are shown in Table 2. Reactor AFB1 operated with GRWW while reactor AFB2 operated with GWW.

Start-up of the reactors and acclimatization stage

The start-up of the reactors was carried out in batch mode with 25 % bed expansion. The biomass of each reactor was initially adapted by batch feedings of diluted wastewater (1 : 30) over a period of 35 d. The changes in volumes fed to the systems depended on the stabilization of biogas production and methane concentration. Once the acclimatization stage was achieved, the reactors operated in continuous mode starting at an influent concentration of $\gamma = 1 \text{ g L}^{-1} \text{ COD}$. The HRT was fixed at 1 day throughout the experiments, in order to evaluate only the effect of the influent COD concentration on the process performance.

Experimental procedure

When the acclimatization period concluded, the reactors were operated in continuous mode increasing only the influent concentration at $\gamma = 1, 3, 5, 8, 10, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23$ and $24 \text{ g L}^{-1} \text{ COD}$ by reducing the dilution ratio. The dilution was carried out by adding distilled water to obtain the desired influent COD. Steady-state conditions were considered to be reached after at least three times the nominal value of the HRT selected. The total duration of the experimental set was 138 d. During the experiment the influent pH was maintained in the range of 6.8–7.2 by adding a sodium hydroxide solution at $w = 10 \%$ in distilled water.

Sampling and analytical determinations

Once steady-state conditions were achieved at each experiment, samples from the effluent of each reactor were collected and analyzed for at least three consecutive days. The steady-state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the standard deviation between the observed values was less than 5 % in all cases. For the processing of the experimental data the Stat graphics plus 5.0 program was used. A previously developed method³¹ for determining methanogenic activities (SMA) was applied. Total volatile fatty acids (TVFA) were determined using a gas chromatograph equipped with a $15 \text{ m} \times 4 \text{ mm}$ Nukol-silica capillary column and a flame ionization detector. The oven temperature was gradually increased from $100 \text{ }^\circ\text{C}$ to $150 \text{ }^\circ\text{C}$ at a rate of $4 \text{ }^\circ\text{C min}^{-1}$. Helium (28.6 kPa), nitrogen (28.6 kPa), hydrogen (14.3 kPa) and air (28.6 kPa) were used as the carrier gas at a flow-rate of 50 mL min^{-1} . Chemical oxygen demand, volatile suspended solids and pH analyses were carried out according to Standard Methods for the Examination of Water and Wastewater.³² Phenolic compounds were determined by using the standard Folin-Ciocalteu reagent technique.³³ The phenolic compounds were expressed as pyrogallol equivalents.³³

Results and discussion

pH and TVFA

Fig. 2 shows the effect of the influent COD concentration on the pH and TVFA concentrations of the effluents of both reactors. As can be observed, for influent COD in the range of $\gamma = 1\text{--}18 \text{ g L}^{-1}$, the pH in the effluents of AFB1 and AFB2 varied between 6.8–7.2, a range that is considered as optimum for the anaerobic process. However, at influent COD values higher than $\gamma = 18 \text{ g L}^{-1}$, the pH in the effluent of AFB1 decreased suddenly to values that may be considered as inhibitory for the process. In the case of AFB2 effluent, the pH started to decrease to values lower than the optimum range at influent COD concentrations higher than $\gamma = 22 \text{ g L}^{-1}$. These results corresponded to the variability observed in the TVFA concentrations. The concentrations of TVFA at the effluents of reactors AFB1 and AFB2 were very similar at influent COD in the range of $1\text{--}10 \text{ g L}^{-1}$. However, at influent COD higher than $\gamma = 10 \text{ g L}^{-1} \text{ COD}$, the TVFA concentration for the effluent of AFB1 was significantly higher than the TVFA concentration in the effluent of AFB2. This showed that inhibition started first in the case of grape-red winery wastewater. It was found that the increase in the TVFA concentration at the effluents was an exponential function of the influent COD concentration as follows:

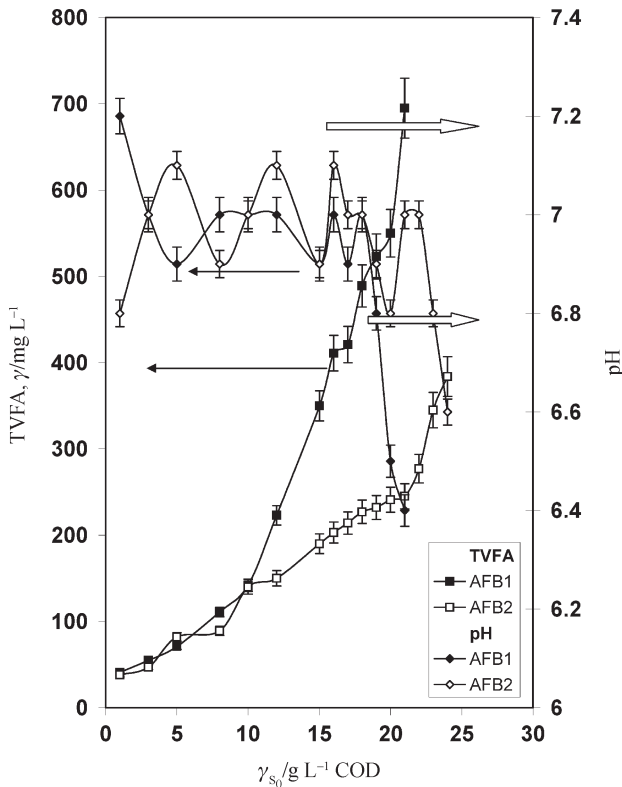


Fig. 2 – Effect of the influent COD concentration (γ_{s_0}) on the effluent pH and TVFA (as acetic acid) for AFB1 and AFB2

$$\text{Reactor AFB1: TVFA} = 36.2 e^{(0.14) \gamma_{s_0}} \quad (1)$$

$$\text{Reactor AFB2: TVFA} = 43.9 e^{(0.09) \gamma_{s_0}} \quad (2)$$

where γ_{s_0} is the influent COD and TVFA is the concentration of total volatile fatty acids at the effluent expressed as acetic acid (AcH). The determination coefficients of the exponential functions obtained were 0.99 and 0.95 for AFB1 and AFB2 respectively. These equations demonstrate that TVFA concentration at the effluent increased faster for grape-red winery wastewater than for guava winery wastewater due to a higher concentration of inhibitory compounds in the former. For influent COD concentrations lower than $\gamma = 16 \text{ g L}^{-1}$, the concentration of TVFA at the effluents of both reactors can not be considered as inhibitory for the anaerobic process. However, for an influent COD value of 16 g L^{-1} COD, the phenol concentrations were 192 mg L^{-1} and 112 mg L^{-1} for the influents of AFB1 and AFB2, respectively, which may be considered as inhibitory for AFB1 and non-inhibitory for AFB2, in line with previous results reported in the literature.^{6–9,34,35} In the case of reactor AFB2 inhibitory phenolic compounds concentration appeared at an influent COD of 22 g L^{-1} because the concentration of phenolic compounds was 154 mg L^{-1} for this substrate concentration, a phe-

nolic concentration that may be considered toxic for methanogenic microorganisms.^{34,36} Therefore, this different behaviour may be determined by the higher concentration of phenols presented in GRWW when compared with GWW as can be seen in Table 2. The characteristics of both wastewaters were very similar except for their phenol concentrations, which was 1.85 times higher for GRWW than for GWW. In this way, the anaerobic digestion process of untreated vinasses, with a phenolic compound content of 450 mg L^{-1} (as gallic acid) was initially inhibited at an OLR of $7.5 \text{ g L}^{-1} \text{ d}^{-1}$ COD showing a considerably decrease in methane production when HRT decreased from 12.3 to 10.6 d.⁹ On the contrary, this same study revealed that the anaerobic digestion of these same vinasses previously fermented with *Penicillium decumbens* (and a phenolic content of $\gamma = 145 \text{ mg L}^{-1}$, as gallic acid) did not show inhibition phenomena when the reactor operated at the same OLR ($7.5 \text{ g L}^{-1} \text{ d}^{-1}$ COD) and a lower HRT (3.1 d).⁹ These results are also in line with data reported in the literature, which demonstrates that other complex substrates such as olive mill wastewaters and olive mill solid wastes with higher phenolic compound concentrations (14.9 g L^{-1} , as caffeic acid) are much more difficult to degrade anaerobically than similar diluted or fermented substrates with lower phenolic content ($\gamma = 60 \text{ mg L}^{-1}$, as caffeic acid).^{34,36}

Table 2 – Characteristics of the winery wastewaters used in the experiments (mean values \pm standard deviations)

Parameters	Grape-red winery effluent	Guava winery effluent
COD/mg L ⁻¹	36100 \pm 1200	33300 \pm 1890
total nitrogen/mg L ⁻¹	450 \pm 25	515 \pm 31
total phosphorus/mg L ⁻¹	250 \pm 11	287 \pm 14
sulfides/mg L ⁻¹	148 \pm 10	184 \pm 12
total volatile fatty acids (TVFA)/g L ⁻¹	7800 \pm 189	6900 \pm 160
total polyphenols/mg L ⁻¹	433 \pm 45	233 \pm 26
pH	4.1 \pm 0.2	4.2 \pm 0.3

The effect of influent COD on the rate of TVFA removal is illustrated in Fig. 3. As can be seen, the rate of removal increased proportionally with the influent COD for AFB1 to an influent COD of $\gamma = 16 \text{ g L}^{-1}$. A further increase in the influent COD caused a progressive diminution of the removal rate coinciding with a progressive increase in the TVFA concentration and the decrease in the pH. In the case of AFB2, the removal rate increased

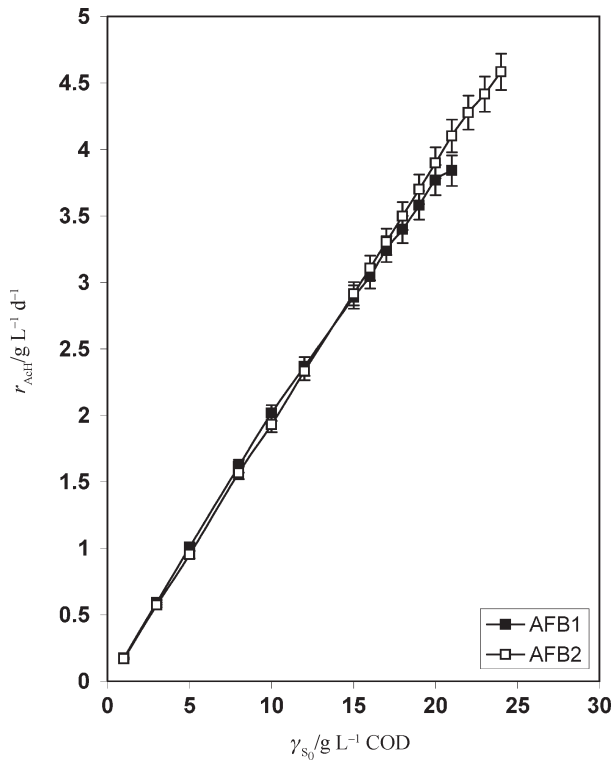


Fig. 3 – Effect of the influent COD concentration (γ_{s_0}) on the rate of TVFA removal in AFB1 and AFB2

proportionally with the increase of the influent COD to a value of $\gamma = 20$ g L⁻¹, while at influent COD higher than 20 g L⁻¹, the increase in the removal rate slowed down. At the same time, the TVFA removal rate values decreased significantly causing an increase in the TVFA concentration and a decrease of the pH to values for which process failure may occur.

Effluent COD

Fig. 4 shows the effect of influent COD concentration on the effluent COD for AFB1 and AFB2. Effluent COD of AFB1 and AFB2 were very similar when influent COD ranged from 1 to 12 g L⁻¹. At influent COD higher than 15 g L⁻¹, the COD of the AFB1 effluent started to be significantly higher when compared with the COD values of the effluents of AFB2. A drastic increase in the effluent COD of AFB1 was found when the influent COD increased to 19 g L⁻¹, showing that the process performance was affected. In the case of AFB2, a sudden increase in the effluent COD occurred at an influent COD of 22 g L⁻¹, but to values always lower than those obtained in AFB1 effluents. These results corroborate that inhibition of anaerobic processes occurs during the experiment due to the decrease in pH values and the increase in TVFA concentration as a consequence of the presence of inhibitory substances such as polyphenols in the substrate.

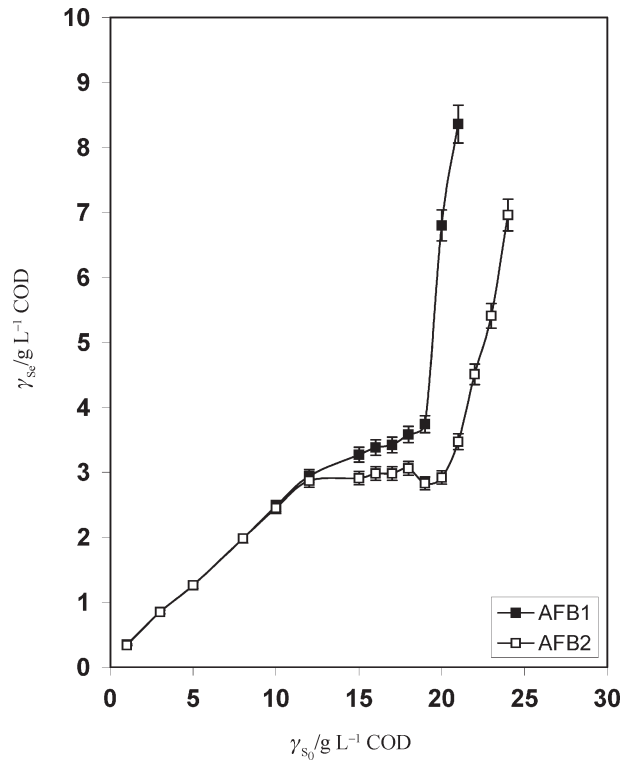


Fig. 4 – Effect of the influent COD concentration (γ_{s_0}) on the effluent COD (γ_{sef}) for AFB1 and AFB2

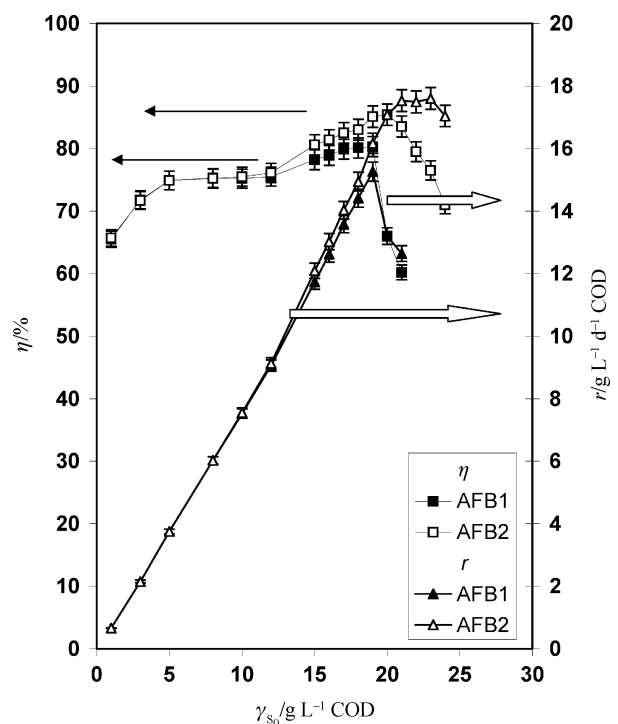


Fig. 5 – Effect of the influent COD concentration (γ_{s_0}) on the COD removal efficiency (η %) and COD removal rate (r) for AFB1 and AFB2

Fig. 5 shows the effect of influent COD concentration on the COD removal efficiency (η %) and the rate of COD removal (r) for AFB1 and

AFB2. As can be seen, the COD removal efficiency was higher with the increase in the influent COD to as much as $\gamma = 19 \text{ g L}^{-1}$ for AFB1, for which values of around 80 % were achieved. These values began to decrease at higher COD values. In the case of AFB2, the removal efficiency increased up to an influent COD of 20 g L^{-1} , achieving values higher than 85 %. In addition, the COD removal efficiencies were very similar in both reactors up to an influent COD of 12 g L^{-1} . At influent COD higher than $\gamma = 12 \text{ g L}^{-1}$, the differences were significantly higher, AFB2 showing the better performance. These results may also be associated with the fact that the mass transfer of organic matter to the biofilm is governed by the gradient of concentration between the bulk liquid and the biofilm surface^{37,38} according to the following equation:

$$J = k_d (\gamma_s - \gamma_{sB}) \quad (3)$$

where J is the flux of substrate ($\text{g dm}^{-2} \text{ d}^{-1}$), k_d is the mass transfer coefficient (dm d^{-1}), γ_s is the concentration of substrate in the bulk liquid ($\text{g L}^{-1} \text{ COD}$) and γ_{sB} ($\text{g L}^{-1} \text{ COD}$) is the substrate concentration at the biofilm surface.³⁸

A previous laboratory-scale investigation conducted to assess the effectiveness of a single-fed flow anaerobic filter reactor for methane production from rice winery effluents at ambient temperatures ($19\text{--}27 \text{ }^\circ\text{C}$), revealed that at a fixed HRT of 24 h, the COD removal efficiency also declined from $\eta = 85 \text{ \%}$ to 73 \% when the influent COD concentration increased from $\gamma = 8.3$ to 25.7 g L^{-1} .⁴

It was also found that the COD removal rate (r) was proportional to the influent COD up to a value of 19 g L^{-1} for AFB1 and to an influent COD of 22 g L^{-1} for AFB2 (Fig. 5). The determination coefficients (r^2) for the above-mentioned influent COD ranges were 0.98 and 0.99 for AFB1 and AFB2 respectively. Under these conditions, the numerical values of the COD removal rate were very similar for both reactors. However, at higher values of influent COD, the removal rate decreased faster in reactor AFB1 than in reactor AFB2, increasing the differences in the values of the COD removal rate with increasing influent COD concentrations. Another study on anaerobic digestion of winery effluents derived from two different wine making processes carried out in a laboratory-scale upflow filter was also previously reported.³⁹ This study revealed that white winery effluents were more easily degradable (average COD removal $\eta = 92 \text{ \%}$) than red winery effluents (average COD removal $\eta = 85 \text{ \%}$). With both wastewaters, the reactor promptly reacted to organic loading rate stress (when tripling from $4\text{--}12 \text{ g L}^{-1} \text{ d}^{-1} \text{ COD}$).³⁹

According to the results obtained, the influence of the influent COD on the COD removal rate may be expressed by the following equation:

$$r = K \gamma_{s0} \quad (4)$$

where r is the rate of COD removal ($\text{g L}^{-1} \text{ d}^{-1} \text{ COD}$) and K is a coefficient (d^{-1}). The values of k were calculated for each reactor at a range of influent COD when non-inhibition was appreciated ($1\text{--}19 \text{ g L}^{-1}$ for AFB1 and $1\text{--}22 \text{ g L}^{-1}$ for AFB2). These values were 0.78 d^{-1} and 0.83 d^{-1} for AFB1 and AFB2 respectively, with variance coefficients of 1 % in both cases (probability level of 95 %, $P \leq 0.05$). As can be seen, although the differences in K values were not considerable, the value obtained for AFB2 was slightly higher than that achieved in AFB1. These results demonstrate that the biodegradability of guava winery effluent was slightly higher than red-grape winery effluent probably due to the lower concentration of inhibitory compounds in the former effluent. Therefore, the results obtained demonstrated that guava winery wastewater was less inhibitory than grape-red winery wastewater. This same behaviour was observed when the anaerobic biodegradability of white winery effluents and red winery wastewaters were compared, the former with a phenolic content lower (350 mg L^{-1}) than the latter (540 mg L^{-1}).³⁹

Other empirical models have been established in the anaerobic digestion of winery wastewater using laboratory-scale filters.⁴ For this type of digester, the proposed models correlated the effluent (γ_{se}) and influent substrate concentrations (γ_{s0}) through equations of the type:

$$\gamma_{se} = k \gamma_{s0}^a \tau^b \quad (5)$$

These models were found to be adequate for reproducing the experimental results. Given that the HRT used in the present work was constant (1 d), the above equation could be transformed into an expression relatively similar to eq. (4).

Methane production

Fig. 6 shows the effect of the influent COD concentration on the methane yield (Y_M) which expresses the volume of methane produced per g COD added to the reactor. As can be seen, the methane yield ranged from 0.20 to 0.24 L CH_4 per g COD added and from $0.25\text{--}0.28 \text{ L CH}_4$ per g COD added for AFB1 and AFB2 respectively, at influent COD in the range of $\gamma = 1\text{--}19 \text{ g L}^{-1}$, with slightly higher values for AFB2. However, when the influent COD increased at $\gamma = 20 \text{ g L}^{-1}$, the methane yield decreased to 0.16 L CH_4 per g COD added for AFB1, while it increased to 0.27 L CH_4 per g COD added for AFB2. At influent COD higher than

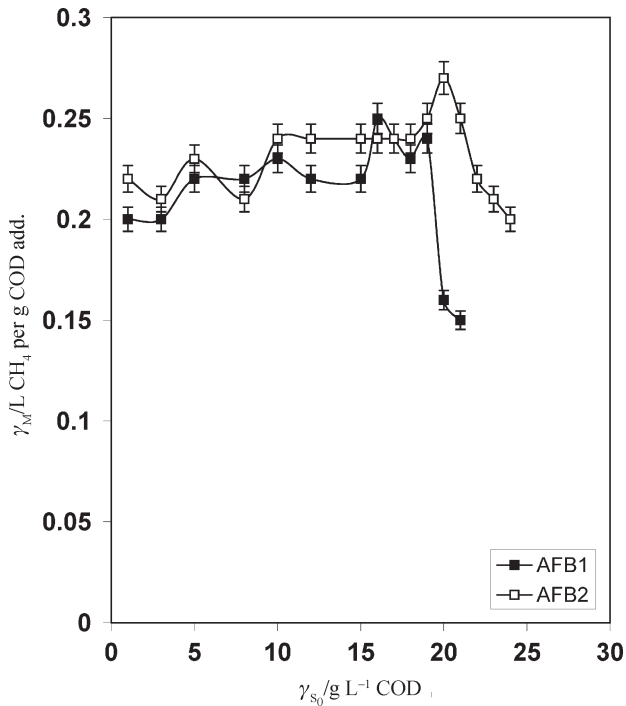


Fig. 6 – Effect of the influent COD concentration (γ_{s0}) on the methane yield (Y_M) for AFB1 and AFB2

$\gamma = 20 \text{ g L}^{-1}$, the methane yield continued decreasing in AFB1 and decreased sharply in AFB2, but reactor AFB2 always showed higher values when compared to AFB1. These results were in line with those obtained for the other parameters evaluated and showed the fact that the anaerobic digestion process of grape-red winery wastewater was more inhibited than the anaerobic treatment of guava winery wastewater due to the higher concentration of phenolic substances in the former. By the same token, the methane yield coefficient obtained in the anaerobic digestion process of vinasses previously treated with *Penicillium decumbens* with a phenolic content of $\gamma = 145 \text{ mg L}^{-1}$ (as gallic acid) was 81 % higher than that obtained in the anaerobic treatment of untreated vinasses whose phenolic content was $\gamma = 450 \text{ mg L}^{-1}$ when both reactors operated at an organic loading rate of $7.5 \text{ g L}^{-1} \text{ d}^{-1} \text{ COD}$.⁹

In addition, the methane yield values obtained in the present work were similar to those obtained for other authors^{4,40} where values of around 0.30 L CH_4 per g COD added operating at higher values of HRT and very similar values of influent COD were achieved. Moreover, methane yield coefficient values lower (0.147 L CH_4 per g COD added) than those obtained in the present work were achieved in the anaerobic digestion process of winery effluents carried out in laboratory-scale CSTR reactors operating at mesophilic temperature ($35 \text{ }^\circ\text{C}$) and a HRT of $\tau = 20 \text{ d}$.⁴¹

Conclusions

The feasibility of using Chilean natural zeolite as microbial immobilization support in fluidized bed anaerobic reactors treating winery wastewaters from grape-red and tropical fruit (guava) wine production was again demonstrated. It was found that at influent COD concentrations higher than $\gamma = 15 \text{ g L}^{-1} \text{ COD}$, process failure occurred. In the anaerobic digestion process of grape-red wine production wastewater (GRWW) inhibition phenomena appeared to lower initial influent COD concentrations compared to the anaerobic digestion process of guava wine wastewater (GWW), probably due to the higher concentration of phenolic compounds in the former. It was found that the efficiency of COD removal increased with the influent COD up to a maximum of around $19 \text{ g L}^{-1} \text{ COD}$ for grape-red wine wastewater and up to around $22 \text{ g L}^{-1} \text{ COD}$ for guava wine wastewater probably due to the increase of the phenolic concentration when processing the first substrate (GRWW). During the period of non-inhibition the COD removal rate was proportional to the influent COD.

Methane yield was slightly higher with guava wine wastewater than with grape-red wine wastewater. During the period of non-inhibition the methane yield was practically constant and independent of the influent COD concentration.

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List of symbols

- FD – framework density, number of tetra-hedral (T) atoms per 100 nm^3
- J – flux of substrate, $\text{g dm}^{-2} \text{ d}^{-1}$
- k_d – mass transfer coefficient, dm d^{-1}
- K – kinetic constant in eq. (4), d^{-1}
- k – constant in eq. (5), d^{-1}
- P – probability level
- r – COD removal rate ($\text{g L}^{-1} \text{ d}^{-1} \text{ COD}$) or TVFA removal rate ($\text{g L}^{-1} \text{ d}^{-1} \text{ acetic acid}$)
- w – mass fraction, %
- Y_M – methane yield, L CH_4 per g COD added
- γ – mass concentration, mg L^{-1} , g L^{-1} .
- γ_S – mass concentration of substrate in the bulk liquid, $\text{g L}^{-1} \text{ COD}$

- γ_{SB} – mass concentration of substrate in the biofilm, g L⁻¹ COD
 γ_{S_0} – influent substrate mass concentration, g L⁻¹ COD
 γ_{S_e} – effluent substrate mass concentration, g L⁻¹ COD
 η – COD removal efficiency, %
 τ – hydraulic retention time, h

Abbreviations

- AFB – anaerobic fluidised bed
 AFBR – anaerobic fluidised bed reactor
 COD – chemical oxygen demand
 GRWW – grape red wine production wastewater
 GWW – guava wine production wastewater
 HRT – hydraulic retention time
 OLR – organic loading rate
 SMA – specific methanogenic activity
 TVFA – total volatile fatty acids
 VSS – volatile suspended solids

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