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Aerobic–anaerobic biodegradation of beet molasses alcoholic fermentation wastewater

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Abstract

A study of the aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50% (chemical oxygen demand, COD: 82 g/l) was carried out using the following fungi: *Penicillium* sp., *Penicillium decumbens*, *Penicillium lignorum* and *Aspergillus niger*. These four microorganisms produce a decolorization of the wastewater from the first day of incubation, achieving the maximum decolorization level at the fourth day of treatment in all cases. *P. decumbes* showed the maximum decolorization with a percentage of 40%. Simultaneously, a significant reduction in the phenolic content of the wastewater was also observed in all cases, reaching average removals of 70% for the four microorganisms studied. Average COD removals were similar in the four cases, achieving maximum values of 52.1 and 50.7%, respectively, on the fifth day of fermentation with *Penicillium* sp. and *P. decumbens*. Finally, a comparative study of the mesophilic anaerobic digestion of untreated and previously fermented (with *P. decumbens*) beet molasses was carried out in laboratory-scale suspended cell bioreactors. Average COD removals of 93% and methane yield coefficient of 305 ml methane at STP conditions per g of COD removed were found in the anaerobic digestion of pre-treated molasses. The combined aerobic–anaerobic process showed the following advantages in relation to the single anaerobic digestion process: higher average percentages of COD removal (96.5 compared with 90.0%) and a decrease of the hydraulic retention time (HRT) necessary to achieve these COD reductions, increasing the decolorization of the wastewater.

Keywords: Beet molasses; Aerobic pre-treatments; *Penicillium decumbens*; Anaerobic digestion; Combined aerobic–anaerobic treatment

1. Introduction

There are a number of small and medium-sized industries in Spain that produce ethanol by fermentation–distillation. The industrial production of ethanol by fermentation results in the discharge of large quantities of high-strength liquid wastes generally called stillages, distillery slops or vinasses. The production of vinasses in a traditional alcohol factory is in the range of 9–14 l of wastewater per l of ethanol obtained. These wastes are strongly acidic (pH: 4–5), and have a high organic content (chemical oxygen demand (COD) in the range of 50–100 g/l). Their free disposal presents a serious challenge to the natural ecosystem and can cause considerable environmental problems [1].

Some researchers [2] have reviewed several methods for the treatment, utilization and disposal of wastewaters from ethanol fermentation industries. Among these are both chemical and biological treatments (aerobic or anaerobic classical methods, trickling filters, lagoons, etc. evaporation–condensation with or without combustion, direct dispersion on soil as a fertilizer, etc.) [2]. A common feature of all these methods is their relatively high cost and, for some, the simultaneous creation of other hazardous by-products/pollutants [3].

The aerobic biological treatment of high-organic load wastes, such as molasses, is associated with operational difficulties of sludge bulking, inability of the system to treat high BOD or COD loads economically, relatively

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high biomass production and high cost in terms of energy. On the other hand, with the diminishing supply of natural gas and other fossil fuels, bacterial conversion of liquid (or solid) wastes to methane and stabilized by-products through anaerobic digestion would be beneficial [3–5]. These by-products could subsequently serve as food or fertilizer and generally be disposed of with fewer problems (easier dewatering, smaller amounts). Also, the high temperatures and high organic load concentrations of the effluents to be treated, as well as the high-energy requirements of the distillery process, are very suitable conditions for the application of anaerobic digestion.

Anaerobic digestion has a number of advantages. For example, it demands less energy input, anaerobic bacteria are capable of transforming most of the organic substances present into biogas, sludge formation is minimal and nutrient demands are very low. The production of biogas enables the process to generate some energy in addition to the reduced consumption; this can reduce operational costs by a large margin compared with high-energy consumptive aerobic processes [6].

For these reasons, anaerobic digestion of molasses alcoholic fermentation wastewaters have been the subject of a number of studies using laboratory or pilot-scale digesters [7–12], but studies on full-scale mesophilic plants have been reported less often. Several related reviews have been presented and many pilot-scale investigations have been reported, using different anaerobic reactor configurations [13–15].

Although anaerobic digestion of most types of distillery wastewaters is feasible and quite appealing from an energy point of view, the presence of inhibitory substances such as phenolic compounds severely hinders the anaerobic process. This slows down the kinetics, and reduces mean rates of methane production, methanogenic activities and yield coefficients. These problems were previously observed in anaerobic batch cultures of wine distillery wastewaters and cane molasses stillages [16–18].

Many phenolic compounds are known to be toxic and interfere with the activity of methanogenic bacteria. There are numerous reports in the literature showing the toxicity and the inhibitory effects of these compounds on anaerobic digestion processes [19–23]. In addition, the high salinity of this waste (average conductivity of 40 mS/cm) can also cause osmotic pressure problems to the microorganisms responsible for the anaerobic process [24].

Therefore, although the anaerobic digestion of this wastewater is attractive and energetically promising, the presence of a high phenolic content slows down the process, and hinders the removal of part of its organic content, making the utilization of high hydraulic retention times (HRT) necessary. Moreover, the anaerobic digestion process does not remove the intense color from this wastewater or an important fraction of the initial COD, even working at organic loading rates (OLR) as low as 2–4 kg COD/m^3 day.

Table 1
Features and composition of the beet molasses used in the experiments[a]

pH	5.2
Chemical oxygen demand (COD)	80.5 g/l
Soluble COD	74.5 g/l
Total solids (TS)	109 g/l
Mineral solids (MS)	30 g/l
Volatile solids (VS)	79 g/l
Total suspended solids (TSS)	3.6 g/l
Mineral suspended solids (MSS)	1.1 g/l
Volatile suspended solids (VSS)	2.5 g/l
Volatile acidity (acetic acid)	8.5 g/l
Alkalinity ($CaCO_3$)	6.0 g/l
Kjeldahl nitrogen	1.8 g/l
Soluble Phosphorous	0.12 g/l
Sulfates	5 g/l
Conductivity	40 mS/cm
Total phenols (gallic acid)	0.450 g/l
Color (pH 7.5, $\lambda = 580$ nm, dilution 10%)	302

[a] Values are averages of 50 determinations; there was virtually no variation (less than 5%) between analyses.

Thus, the aim of this work was to study a combined aerobic–anaerobic treatment of beet molasses alcoholic fermentation wastewater. In the first step of the treatment most of the phenolic content, color and part of the initial COD will be removed; in the anaerobic step the remaining organic content (not previously removed) will be eliminated. The anaerobic digestion process was carried out in two suspended cell bioreactors operating in continuous mode.

2. Materials and methods

2.1. Wastewater

The beet molasses used were collected from the alcohol factory of el Puerto de Santa María (Cádiz, Spain). The samples of wastewater were collected from the exit of the rectification column, packed in 1 l bottles and, finally, frozen at −20 °C with the aim of carrying out all the experiments with the same type of wastewater.

2.2. Analyses

The following parameters were determined in the wastewater used: pH, solids, COD, volatile acidity, alkalinity, Kjeldhal nitrogen, phosphorous, sulfates, conductivity, and color. All analyses were carried out in accordance with the Standard Methods for the

Examination of Water and Wastewater [25]. The total phenol content was determined according to the Folin–Ciocalteau method [26]. Heavy metals were determined by atomic absorption.

The features and composition of the beet molasses used are summarized in Table 1.

2.3. Microorganisms used

The fungal species used for the study were: *Penicillium lignorum* IFJM-B 22 isolation 0.46 Rio Tinto (Huelva, Spain); *Penicillium decumbens* and *Penicillium* sp. isolated from molasses and *Aspergillus niger* IJFM-A570 from the collection of LNETI (Portugal). All the cultures were supplied by the 'Centro de Investigaciones Biológicas' (C.S.I.C.) of Madrid (Spain) except *P. decumbens* NRRL3388 (synonymous of *Penicillium glauco lanosum*), which was supplied by the culture collection of the Department of Agriculture, Peoria University, Illinois, USA.

The culture medium (PPG) used for growth and formation of the fungi spores was composed of: mashed potatoes, 20; glucose, 20; and agar, 20 g/l. The cultures were maintained in a thermostatic chamber at 20 °C until the phase of spores formation was achieved. Part of these cultures were used to maintain the active population through fresh reseeding that was carried out every 15–30 days. After the phase of spores formation was achieved, conidia were collected using a Triton × 100 solution diluted to 0.1%, this detergent was used given the hydrophobic character of the spores. Finally, these were filtered through a sterile filter and counted in a Bürker Superior W-Germany chamber.

The growth medium used for the count of viable spores (Sutter IV) contained:

- Solution A (per 500 ml of distilled water): Sutter concentrated, 20 ml; L-Asparragine, 2; and H_2KPO_4, 5 g.
- Solution B (per l of distilled water): glucose, 20; and agar, 15 g.

These two solutions were mixed, after each one was previously sterilized separately and, finally, 0.7 ml of a 35% HCl solution was added to acidify the medium and to avoid the extended growth of the population, making it easy to count the colonies.

2.4. Aerobic treatment

Aerobic treatments were carried out in 250 ml glass flasks placed in an orbital shaking incubator operating at 100 rpm and 22 °C. After sterilization of the flasks, 100 ml of beet molasses, diluted to 50% and sterilized, were added to each flask. Each one was inoculated with spores suspended in saline (10^7 viable spores per ml). The air input flow rate was 3 l/h per l of molasses during 5 days. Adjustment of the pH was not necessary as molasses have a pH of 5.2, which is favorable for the growth of the fungus.

2.5. Anaerobic treatment

Two anaerobic reactors, with a working volume of 1 l, equipped with magnetic stirring and placed in a thermostatic chamber at 35 °C were used. The reactors were fed daily by means of external feeders and liquid effluents removed daily through a hydraulic seals, comprising 25 cm liquid columns, designed to prevent air from entering the reactors and biogas from leaving. This reactor has been described in detail elsewhere [27].

The methane volume produced in the process was measured using 5 l Mariotte reservoirs fitted to the reactors. Tightly closed bubblers containing a NaOH solution (3 M) to collect the CO_2 produced in the process were intercalated between the two elements. The methane produced displaced a given volume of water from the reservoirs, allowing ready determination of the gas [27].

The reactors were inoculated with biomass from an industrial anaerobic contact digester operating with vinasses. It was methanogenically active and its content in volatile solids was 59.1 g/l.

The anaerobic reactors were initially charged with 750 ml of a synthetic solution containing peptone (1%), yeast extract (0.5%), sodium chloride (0.5%) and 135 ml of the inoculum. The biomass of each reactor was initially acclimatized by batch feedings of 10–100 ml of diluted wastewater (10 g COD per l) over a period of 2 weeks.

This preliminary step was followed by a series of two continuous experiments in which natural molasses and molasses previously fermented with *P. decumbens*, were added to the digesters with biomass suspended. The influent daily feed volumes were varied to give 18.7, 31.2, 44.0, 56.0, 68.7, 81.0 and 94.0 ml/day of untreated molasses (equivalent to 53.3, 32.0, 22.7, 17.8, 14.7, 12.3 and 10.6 days HRT), respectively, and 65, 108, 152, 195, 239, 283 and 326 ml/day of pre-treated molasses (equivalent to 15.4, 9.3, 6.6, 5.1, 4.2, 3.5 and 3.1 days HRT), respectively. These values of feed flow-rates and HRTs corresponded to organic loading rate (OLR) values of 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 g COD per l day, respectively, for the two substrates studied. The biomass concentration ranged between 10.0 and 10.4 g VSS per l (mean value = 10.2 g VSS per l) and was, therefore, approximately constant for all experiments.

Once steady-state conditions were achieved at each feeding flow-rate, the daily volume of methane produced, pH, volatile acidity, alkalinity and COD of the different effluents studied were determined. The samples were collected and analyzed for at least 5 consecutive

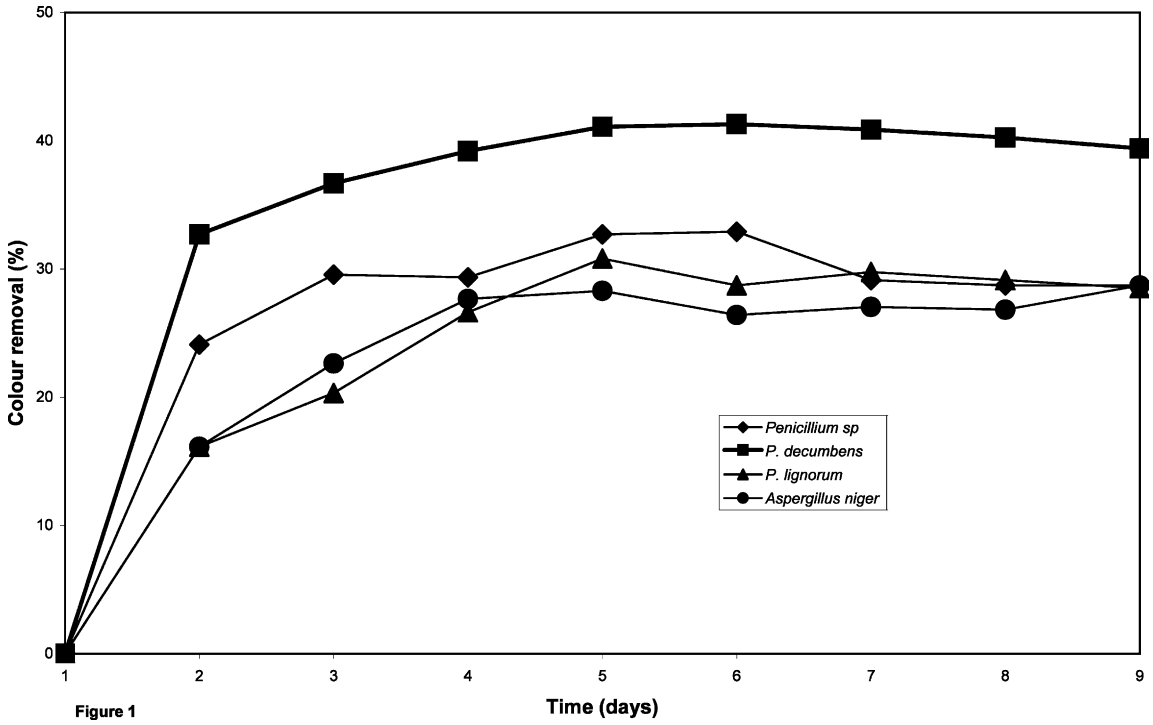

Fig. 1. Variation of the percentage of color removal with time (days) for the four microorganisms assayed.

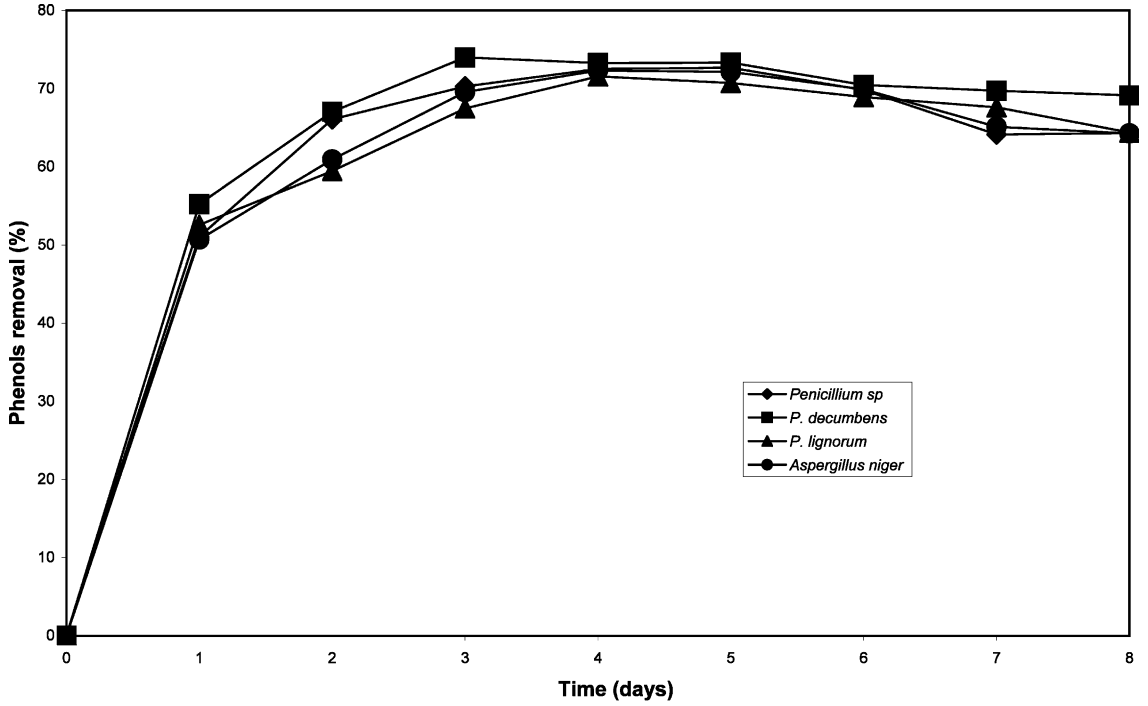

Fig. 2. Variation of the percentage of phenols removal with time (days) for the four microorganisms assayed.

days. The steady-state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations between the observed values were less than 5% in all cases. The organic loadings applied in this investigation were increased in a stepwise fashion in order to minimize the transient impact on the reactors that might be induced by a sudden increase in loadings.

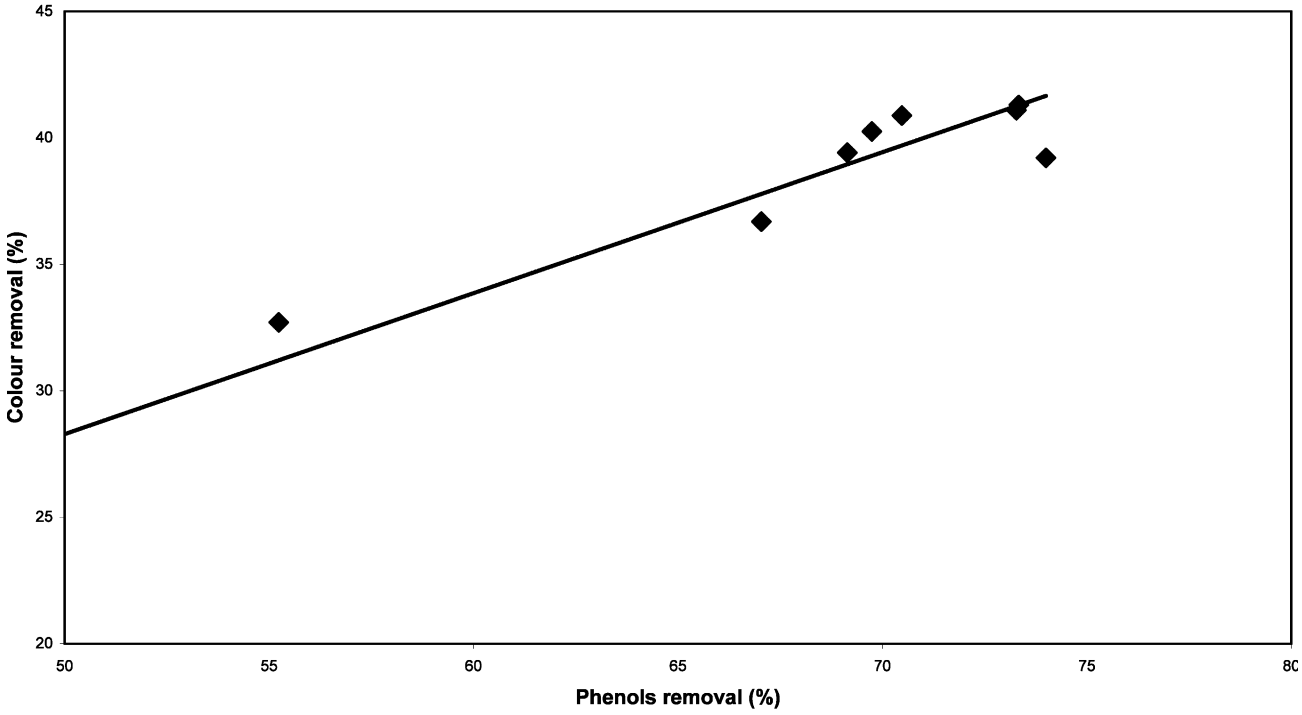

Fig. 3. Variation of the percentage of color removal as a function of the percentage of phenols removal.

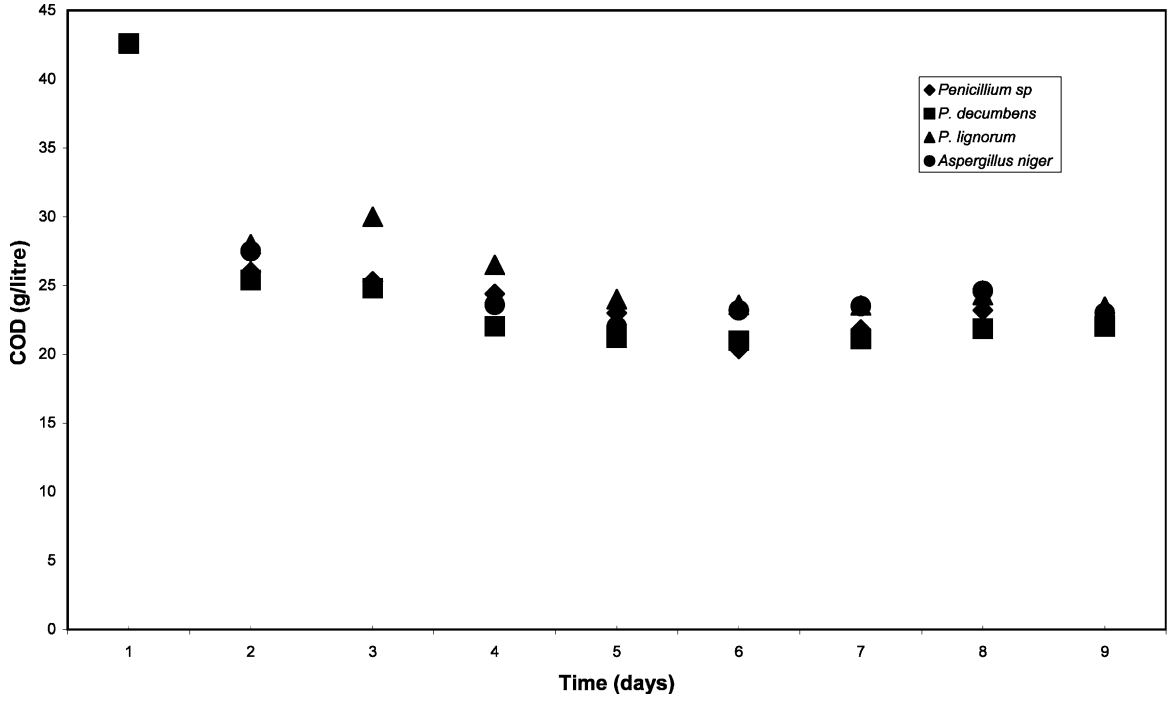

Fig. 4. Variation of the COD of molasses with incubation time for the four microorganisms assayed.

3. Results and discussion

3.1. Aerobic treatment

Fig. 1 shows the variation of the percentage of color removal as a function of time for the four microorganisms assayed. As can be seen, all the fungi studied produced a decolorization of the beet molasses from the first day of incubation, although the first removed percentages were low. The higher reductions in color were achieved between the fourth and fifth day of treatment, with *P. decumbens* achieving the best results

with 41% of the initial color removed after 4 days of treatment. This decrease in the coloration of beet molasses may be attributed to the degradation and/or adsorption of tannins and some phenolic compounds on the mycelium, as was previously observed by Hamdi et al. in the treatment of olive mill wastewater (OMW) with *A. niger* [28].

Similar results were obtained by Sayadi and Ellouz treating OMW with the fungi: *Pycnoporus cinnabarinus*, *Phlebia radiata* and *Polyporus frondosus*, with percentages of color removal ranging between the 32 and 46% after 5 days of treatment [29]. Similar percentages in color removal were also obtained by Hamdi et al. [28] fermenting OMW with *A. niger*.

The variation of the percentage of phenolic compound removal with the operation time is shown in Fig. 2. Although this variation was similar for the four microorganisms used, *P. decumbens* showed a maximum value of phenols removal of 74% after 3 days of treatment. Garcia et al. obtained a 66 and 70% reduction in phenols, treating cane sugar vinasses with *Aspergillus terreus* and *Geotrichum candidum*, respectively, after 5 days of incubation [30]. Values of 90.0 and 94.3%, respectively, in the phenol content removals, were obtained in the treatment of OMW with *Azotobacter chroococcum* and *A. terreus* after 5 and 3 days of treatment [31].

Fig. 3 shows the variation of the percentage of color removal with the percentage of phenol removal for the treatment with *P. decumbens*. As can be observed, there is a strong linear correlation among both variables. By using a linear regression with the least squares method, the following equation was obtained:

$$\%\ \text{of Colour removed} = 0.42 + 0.55(\%\ \text{of Phenols removed}) \quad (1)$$

This equation demonstrates that although the fungi removed 100% of the phenols, only 55.4% of the initial color would be eliminated, which suggests the occurrence of another type of compound, different from the phenols that are not eliminated by the fungi assayed.

The variation of the COD content of molasses with the operation time for the four microorganisms studied is given in Fig. 4. As can be seen, the reduction of the COD with time was similar in all cases, with *Penicillium* sp. and *P. decumbens* achieving the maximum removal values of 52.1 and 50.7%, respectively, at the end of the treatment. The monitoring of this variable is of great interest to measure the growth of the microorganisms, in addition to allowing the study the evolution of the biodegradation process. Sayadi and Ellouz obtained 48 and 51% reductions in COD content of the OMW treated with *P. radiata* 28773 and *P. radiata* FR DAOM 53209 after 5 days of incubation [29]. Garcia et al. found a COD reduction of 29 and 28% in vinasses treated with *A. terreus* and *G. candidum*, respectively [30]. Other researchers [28,32] found COD removals of 62.8 and 55.0%, respectively, in OMWs fermented with *Candida tropicalis* and *Geotrichum* sp., after 7 days of treatment. Finally, values of 63.3, 74.5 and 74.0%, respectively, in COD removal were obtained in the treatment of OMW with *G. candidum*, *A. chroococcum* and *A. terreus* after 5, 5 and 3 days of treatment, respectively [31].

Table 2
Average concentrations (mg/l) of heavy metals before and after treatment of molasses with *P. decumbens*

Cation	Molasses	Pretreated molasses
Na	2500	1875
K	3550	2540
Ca	985	833
Mg	510	500
Fe	83	6.8
Mn	85	0.7
Zn	20	1.0
Cu	7	0.3
Co	2	0.1
Ni	4	0.3
Cr	2	0.2

In parallel to COD removal, an elimination of heavy metals takes place, as can be observed in Table 2; this topic has been widely investigated. In this way, Ross and Townsley [33] observed that the cell walls of the fungi adsorb a great variety of heavy metals. Kiff and Little [34] observed that some exopolymers are optimally located to interact with the metallic ions. Gadd [35] also carried out a study using a number of metals, recommending the use of fungi for the removal of heavy metals. Townsley et al. [36] studied the removal of copper using *Trichoderma viride*. Moreover, other researchers [37,38] were able to remove uranium using the species *A. niger*.

3.2. Anaerobic treatment

Tables 3 and 4 summarize the steady-state operating results including OLR, HRT, pH, methane flow-rates, percentages of COD removal, volatile acidity, alkalinity and volatile acidity/alkalinity ratios, for the anaerobic digestion experiments carried out with untreated molasses and molasses previously treated with *P. decumbens*. The pH in both reactors remained approximately constant for all the HRTs studied, with 7.8 and 8.4 as extreme values. This stability can be attributed to carbonate/bicarbonate buffering. This is produced by the generation of CO_2 in the digestion process, which is not completely removed from the reactor as gas. Buffering in anaerobic digestion is normally due to bicarbonate as carbonate is, generally, negligible if compared with the bicarbonate (carbonate/bicarbonate

Table 3
Results obtained under different experimental conditions in the anaerobic digestion process of untreated molasses

OLR	HRT	pH	q_g	COD removal (%)	Volatile acidity	Alkalinity	Volatile acidity/alkal
1.5	53.5	8.0	0.296	93.7	0.30	7.5	0.04
2.5	32.0	8.4	0.495	91.6	0.65	15.4	0.04
3.5	22.7	7.8	0.700	88.7	0.99	10.2	0.10
4.5	17.8	8.3	0.835	87.7	0.60	14.8	0.04
5.5	14.7	8.2	0.925	85.0	4.70	17.9	0.26
6.5	12.3	8.1	0.761	73.9	5.01	18.7	0.27
7.5	10.6	8.3	0.755	68.6	9.30	18.9	0.49

OLR, organic loading rate (g COD per l day); HRT, hydraulic retention time (day); q_g (l CH_4 per l day), methane production rate; volatile acidity (g acetic acid per l); alkalinity (g $CaCO_3$ per l).

ratio is equal to 0.01 for pH 8.2). The buffering guards against possible acidification of the reactor giving a pH of the same order as optimal for methanogenic bacteria [39]. The high pH values and the buffering capacity is a guarantee as opposed to an acidification of the reactor that could be caused by a sudden overloading of the reactor, an abrupt change of the operation temperature or by the presence of toxic compounds or inhibitors in the substrate.

The VFA/alkalinity ratio can be used as a measure of process stability [39]: when this ratio is less than 0.3–0.4 the process is considered to be operating favorably without acidification risk. As was observed (Tables 3 and 4), the ratio values were lower than the suggested limit value in all cases except in the experiment corresponding to the lowest HRT studied (10.6 days) for untreated molasses. Between HRTs of 53.5 and 12.3 days for untreated molasses and between 15.4 and 3.1 days for pre-treated molasses, the VFA/alkalinity ratio was always lower than the above-mentioned failure limit value, and the VFA values were always lower than 5.01 and 1.02 g/l (as acetic acid), respectively. However, in the case of the anaerobic digestion of untreated molasses, at a HRT of 10.6 days, a considerable increase of the VFA/alkalinity ratio was observed in the reactor (0.49), which was due to an important rise in the VFA concentration (9.30 g/l, as acetic acid) with an alkalinity value of 18.9 g/l as $CaCO_3$. In addition, the VFA/alkalinity ratio of the effluents of both reactors was always lower than those corresponding to the two molasses studied (1.7 and 0.6 for untreated and previously fermented molasses, respectively). This fact confirms the uptake of volatile organic acids present in both feeds and the tendency of the systems to reach a dynamic equilibrium.

The COD removal efficiencies observed at different HRTs and OLRs for both substrates are also summarized in Tables 3 and 4. For untreated molasses, COD removal decreased slightly from 93.7 to 85.0% when OLR increased from 1.5 to 5.5 g COD per l day and HRT decreased from 53.5 to 14.7 days. At an OLR of 7.5 g COD per l day a marked decrease in efficiency was observed (68.6%). In consequence, it appears that the performance of the anaerobic system treating untreated molasses becomes independent of HRT and OLR, provided that the HRT and OLR of the reactor are maintained above 14.7 days and below 5.5 g COD per l day, respectively. In contrast, for molasses previously fermented with *P. decumbens*, the decrease in the percentage of COD removal with increased OLRs was softer than that observed for untreated molasses in the same range of organic loadings (1.5–7.5 g COD per l day).

Table 4
Results obtained under different experimental conditions in the anaerobic digestion process of molasses pre-treated with *P. decumbens*

OLR	HRT	pH	q_g	COD removal (%)	Volatile acidity	Alkalinity	Volatile acidity/alkal
1.5	15.4	7.8	0.337	82.6	0.288	1.50	0.19
2.5	9.3	8.3	0.546	78.3	0.936	7.07	0.13
3.5	6.6	8.2	0.710	76.1	0.740	7.95	0.09
4.5	5.1	8.0	0.867	73.0	0.710	8.05	0.09
5.5	4.2	7.8	1.008	69.6	1.020	10.3	0.10
6.5	3.5	7.8	1.108	65.2	0.552	10.6	0.05
7.5	3.1	8.0	1.331	63.5	0.432	10.2	0.04

OLR, organic loading rate (g COD per l day); HRT, hydraulic retention time (day); q_g (l CH_4 per l day), methane production rate; volatile acidity (g acetic acid per l); alkalinity (g $CaCO_3$ per l).

Table 5
Volume of methane per gram of COD added for the different OLRs used in the anaerobic digestion processes of untreated and previously treated molasses

OLR (g COD per l day)	Untreated molasses l CH_4 STP per g COD	Pre-treated molasses l CH_4 STP per g COD
1.5	0.197	0.224
2.5	0.198	0.218
3.5	0.200	0.203
4.5	0.186	0.193
5.5	0.168	0.183
6.5	0.167	0.170
7.5	0.101	0.177

The values of volumetric methane production rates for each OLR studied in natural and previously fermented molasses are also given in Tables 3 and 4, respectively. In the case of untreated molasses, it can be seen that the volume of methane produced per day increased progressively with increased OLR up to OLR values of 5.5 g COD per l day, after which a marked decrease was observed over the range tested. Apparently, the activity of methanogenic bacteria was not impaired up to OLR values of 5.5 g COD per l day because of the adequate buffering capacities provided in the experimental system. Nevertheless, the methane production rate decreased from 0.925 to 0.755 l/l day when the OLR was increased from 5.5 to 7.5 g COD per l day. This decrease in the methane production at the highest OLR values might be attributed to an inhibition of the methanogenic bacteria at high OLR values, which caused an increase in effluent VFA contents and VFA/alkalinity ratios, as can be seen in Table 4. Specifically, VFA content increased from 4.7 to 9.3 g/l (as acetic acid) when the OLR was increased from 5.5 to 7.5 g COD per l day. In the case of molasses previously treated with *P. decumbens*, the methane production rate increased linearly from 0.337 to 1.331 l/l day with increased OLR in all the range of OLR tested (1.5–7.5 g COD per l day). In addition, the values found for this parameter were always higher than those observed for anaerobic digestion of untreated molasses for the same values of OLR, which clearly shows the advantage of previously fermenting the molasses to be treated by anaerobic digestion.

On the other hand, the methane yield coefficient (volume of methane per COD added to the reactor) can be calculated from Tables 3 and 4. As can be observed in Table 5, a gradual decrease in the fraction of organic matter transformed into methane was found after an OLR value of 5.5 g COD per l day, decreasing the values of methane yield coefficient 49% (untreated molasses) and 20% (pre-treated molasses) for the experiments corresponding to an OLR of 7.5 g COD per l day in relation to the values found at an OLR of 1.5 g COD per l day.

Finally, the values of the substrate removal rate can be calculated from data summarized in Tables 3 and 4. Fig. 5 shows the variation of the HRT with the organic loading removal rate in the cases of the anaerobic digestion of untreated and pre-treated molasses. This

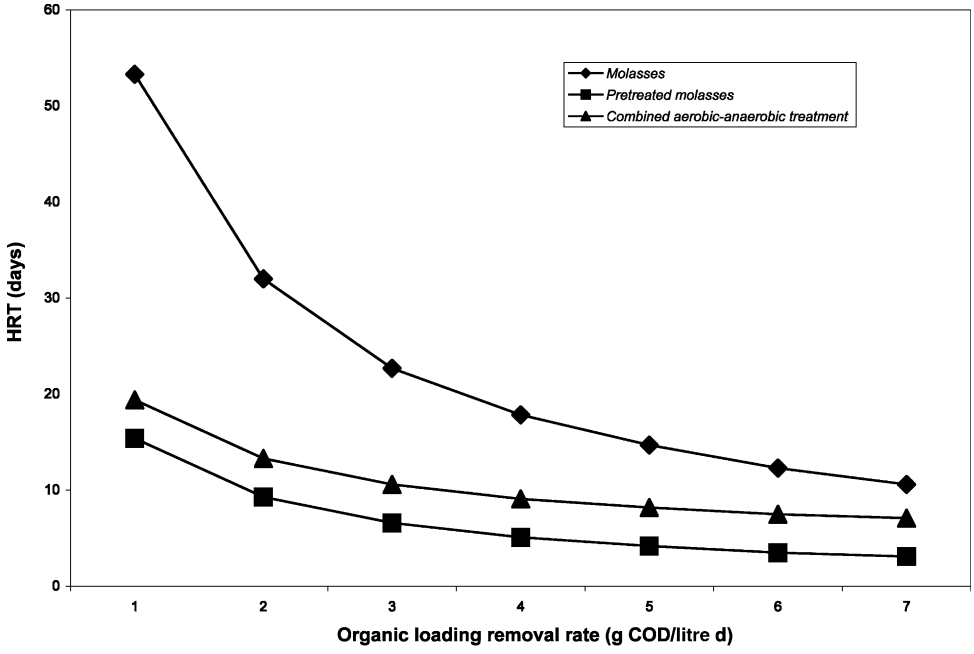

Fig. 5. Variation of the HRT with the organic loading removal rate (g $COD_{removed}$ per l day) in the cases of the anaerobic digestion of untreated molasses, previously fermented molasses and in the combined aerobic–anaerobic digestion process.

plot also clearly shows the advantage of the pre-treatment of molasses with *P. decumbens* previous to its anaerobic digestion. For a given value of the organic loading removal rate, the HRT decreased considerably when the molasses are previously fermented. In order to establish a more rigorous comparison, the time used in the pre-treatment (4 days) has been added to the HRT of the pre-treated molasses, obtaining a curve located in an intermediate position (Fig. 5). It is apparent that, even under these circumstances, the previous statement continues being valid. Therefore, the time necessary for digesting a given organic load, in the case of using a combined aerobic–anaerobic treatment, is always lower than that necessary to anaerobically treat the waste. On the other hand, the performance of the reactor processing untreated molasses starts to become destabilized for OLR values of 5.5 g COD per l day, which does not happen when the wastewater has been pre-treated, since the reactor admits loading rates of up to 7.5 g COD per l day without destabilization symptoms appreciated.

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