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OLIVE MILL WASTEWATER VALORISATION BY USING PASSIVELY IMMOBILIZED ANAEROBIC BIOMASS

Immobilized cell processes were often observed to be more effective over the freely suspended cell ones in biotechnological applications. In general, the formers are less affected by shock–load and wash–out problem and can be fed with significantly higher organic loads; more, the intrinsic activity and the metabolic versatility of microorganisms often significantly increase upon passive cell immobilization on porous carriers [1,2], which can contribute to further enhance the process by exerting a role in the substrate and/or microbial inhibitor bioavailability [2,3,4]. Olive mill wastewaters (OMWs) are the aqueous effluent from olive oil producing processes; they have a high COD content, part of which constituted by phenolic compounds, mostly responsible for the high phytotoxicity and antibacterial potential of such a waste [5]. Anaerobic digestion is a promising biological technology for OMW disposal, as it combines both the wastewater decontamination and valorization through methane production; however, methane volumetric productivity (produced gas as a function of the time and of the reaction volume) in dispersed growth bioreactors is limited by the low organic load with which those plants are generally fed; more, OMW toxic phenols tend to persist in conventional digestor effluents. Considering the above evidences, the possibility of passively immobilizing in packed–bed reactors an OMW–degrading anaerobic consortium, in order to intensify its metabolic activity versus the OMW phenolic fraction and to enhance the methane productivity of the process, was here investigated. Granular active carbon (GAC) and silica beads (SB), which are immobilization supports commonly employed in the bioremediation of industrial wastewaters [2,4], have never been tested in this field: thus, two identically configured anaerobic reactors packed with GAC or SB, along with a conventional dispersed growth digestor operating with the same inoculum and OMW, were developed and compared, under batch mode, for their bioremediation and biomethanization potential. Then, the performances of the two biofilm reactors were investigated in continuous mode at increasing OMW organic loads. Finally, GAC–biofilm reactor, that exhibited the best performances, was studied to determine its stability under higher OMW organic loads as well as the composition and spatial distribution of its bacterial biomass.

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MATERIALS AND METHODS

Anaerobic microbial consortium and experimental OMWs

The OMW–degrading microbial consortium was the same already employed in a dispersed growth anaerobic reactor fed with diluted OMWs [6]. Two experimental OMWs, EOMW1 and EOMW2, were prepared by diluting (1:1) two OMWs (OMW1 and OMW2, respectively) with water. OMW COD and phenolic compound contents were about 20 and 50 g/l, respectively, and 1.5 and 2.5 g/l, respectively; diluted OMWs were then amended with $Ca(OH)_2$ (to adjust their pH to 6.5), urea (0.45 g/l) and NaOH (to adjust their pH to 7.8 $\pm$ 0.2). Both EOMWs were purged with 0.22 µm filter–sterilized O_2–free N_2 at room temperature for 3 h before being employed in the experiments.

Bioreactor systems

A stirred tank anaerobic bioreactor (STB) was only employed under batch conditions and it had the same configuration of the reactor developed and described by Beccari et al. [6]. It had 1.50 l of reaction medium volume, an external jacket recycling water at 35°C, and a recycle line (that moved medium from the top to the bottom at 25 ml/min) equipped with a closed glass bottle allocating a redox potential and a pH probe. The produced biogas volume was measured by a 4 l "Mariotte" system connected to the reactor head–space. Two identically configured anaerobic packed–bed biofilm reactors (PBBRs), employed both in batch and in continuous mode, were developed by using glass columns, wrapped with a silicon tubing serpentine recycling water at 35°C, with an inner diameter of 8 cm and a height of 45 cm. The medium was recycled from

the top to the bottom of both columns by high recycling ratios in order to guarantee a good homogeneity. When continuous operational mode was performed, the Mariotte system was connected to a first reservoir in which the exhausted medium was collected. The reactors were filled with 1.175 or 2.296 kg (dry weight) of autoclave–sterilized GAC or SB, respectively, thus reducing their reaction volume to 1.04 and 1.16 l for the GAC– and SB–PBBR, respectively. STB and the PBBRs were started up by deoxygenating them with filter–sterilized O_2–free N2 for 1 h, and filling them with deoxygenated EOMW2 inoculated with Beccari et al.' inoculum (201.7 mg dried biomass/l). PBBR content was then recycled at high rates for two weeks to induce a preliminary biofilm formation.

EOMW digestion in the STR and in the PBBRs

STB and the two PBBRs were preliminary compared for their ability to digest EOMW2 through three successive batch experiments of two weeks. Under continuous mode of operation, PBBRs were fed with EOMW1 or EOMW2 and operations were carried out until steady state conditions were attained.

Biofilm analyses

Triplicate 3 g–samples of GAC carrier were collected at 5, 18 and 36 cm of height (from the bottom) at the end of the study and subjected to analysis of their protein content as described by Bertin et al. [7]. A second set of GAC samples (of about 20 g) collected from the same places of the reactor were washed and subjected to DNA extraction as described in Zhou et al. [8].

RESULTS AND DISCUSSION

EOMW digestion in the STB and PBBRs under batch conditions

A significant depletion of COD and total phenols initially occurring in EOMW2 was averagely attained in all bioreactors. The process was more rapid and extensive in the PBBRs than in the parallel freely suspended–cell STB; in particular, GAC–bioreactor was found to be more effective than the parallel SB–one, as reported in Bertin et al. [9]. This suggested that the bioremediation potential of the employed inoculum significantly increased upon cell immobilization; it is also possible that other factors, such as a higher biomass availability and/or OMW pollutant bioavailability, also have significantly contributed to favor PBBRs over STR.

EOMW digestion in the PBBRs operating under continuous mode

The PBBRs were then forced to operate under continuous mode. Both reactors were fed with EOMW1 or EOMW2 at dilution rates (D) corresponding to 0.415 day^{-1} and 0.692 day^{-1} (Table 1). Marked depletion yields of COD and total phenols introduced in both reactors along with high yields of COD conversion in CH_4 were attained in all 5 successive experiments of 3 weeks. COD depletion yields slightly decreased by increasing the COD load by using both waters, whereas phenol removal was not significantly affected by this parameter, in particular in the case of the GAC–PBBR (Tab. 1). However, both PBBRs exhibited volumetric productivities in COD removal and phenolic compound biodegradation (expressed as removed compounds per day per reaction volume) that increased markedly with the OMW organic loads (Fig. 1); in particular, GAC–reactor provided productivities significantly higher than those attained with the Baccari's contact reactor [6] developed with the same inoculum and operating with a similar diluted OWM (Fig. 1). CH_4 yields were in general slightly higher in the GAC–reactor than in the SB–one and not affected by the organic load. At the end of the experiments, both PBBRs were opened and analysed for their content of immobilized biomass that was estimated to be 48.52 and 9.17 g (on dry weight basis) in the GAC and SB–PBBR, respectively. GAC exhibited a significantly larger EOMW2 COD and phenols adsorbing capability with respect to SB. On the basis of these

Table 1. COD and phenol depletion yields and methane production achieved in the first set of experiments in the GAC– and SB–PBBR as a function of the different organic loads at which the two PBBRs were subjected.

EOMW	EOMW organic concentrations			EOMW organic loads		COD depletion yields		Phenol depletion yields		Methane production yields (l CH_4 produced/g of COD depleted)	
	D (d^{-1})	COD (mg/l)	Phenols (mg/l)	COD loads $(g(ld)^{-1})$	phenol loads $(g(ld)^{-1})$	GAC–PBBR	SB–PBBR	GAC–PBBR	SB–PBBR	GAC–PBBR	SB–PBBR
EOMW1	0.415	10466±539	857.3±35.9	4.34	0.36	0.38	0.10	0.73	0.23	0.20	0.15
EOMW1	0.692	10256±446	719.6±50.8	7.80	0.50	0.32	0.04	0.70	0.23	0.15	0.13
EOMW2	0.415	26211±1857	1419.5±206.7	10.88	0.59	0.65	0.18	0.74	0.10	0.19	0.16
EOMW2	0.692	24068±407	1315.4±34.2	16.66	0.91	0.57	0.09	0.75	0.05	0.18	0.15
EOMW2	0.692	25574±138	1305.1±29.9	17.70	0.90	0.51	0.11	0.66	0.16	0.20	0.14

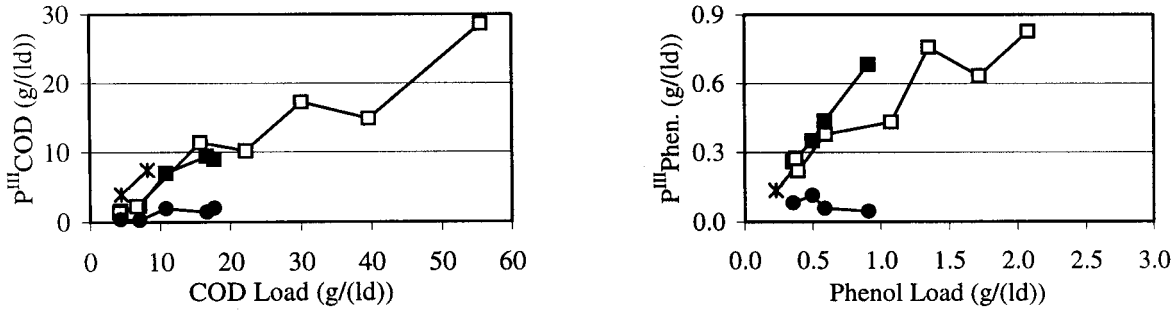

Figure 1. COD and phenol removal volumetric productivity (PIII) in the GAC- and SB-PBBR during the first set of continuous experiments (■ and ●, respectively), in the newly developed GAC-PBBR during the second set of experiments performed at higher organic loads (□), and in the anaerobic digestor developed by Beccari et al. [4] (x).

findings, it has been hypothesized that GAC-reactor was much more effective than the SB-one because of a) its 5 fold-higher content of immobilized biomass, and/or b) GAC potential to optimize, through a combination of adsorption and desorption phenomena, the pollutant bioavailability.

GAC-bioreactor: analysis of its stability and of microbial composition

Given the promising performances exhibited by the GAC-PBBR, new investigations on this type of reactor systems were planned. A new GAC-PBBR was developed as described above to investigate its stability and productivity when fed under continuous mode with high OMW organic loads and the composition and the distribution of its microbial biomass. Thus, the bioremediation efficiency and the biogas productivity of the new GAC-reactor were studied by feeding it with EOMW1 or EOMW2 organic loads that ranged, in terms of COD and phenolic compounds, respectively, from 4.21 and 0.38 g/(l·day) to 55.58 and 2.26 g/(l·day), through a number of sequential 3 weeks experiments, as described in Bertin et al. [10]. In general, the reactor productivity in COD and phenol bioremediation increased proportionally with the organic load (Fig. 1). CH_4 yield seemed not to depend on this parameter and was in general closed to 0,19 l of CH_4 produced per g of COD removed [10]. To further demonstrate the stability of the system, a two-month experiment was carried out in the same conditions in which the GAC-reactor offered the best performances in term of COD and phenol removal and corresponding to an organic load significantly higher than those achieved during the first part of this study; so, it was fed with EOMW2 at D=2.077 day^{-1}, to which corresponded COD and phenol organic loads of 33.00 and 1.76 g/(l·day), respectively. COD and phenol depletion yields were similar to those achieved in the previous experiments carried out with comparable organic loads of EOMW2, while CH_4 yield reached the value of 0.26 l of CH_4 produced per g of COD removed; considering the organic load at which the plant was fed and the relative COD removal yield, obtained methane volumetric productivity was 3.82 l of produced methane per day per reaction volume, that appear to be a very high value. These additional experiments supported previous findings indicating that the GAC-biofilm technology developed in this study was capable of stably mediating an effective EOMW2 bioremediation and biomethanization under a large variety of high organic loads. The reactor was finally opened and analysed for the distribution of archeal and eubacterial communities in its different districts; terminal restriction fragment (T-RF) analyses of amplified 16S-rDNA were performed. Marked differences in fingerprints were present among the inoculum and the various samples taken throughout the GAC-reactor, indicating that many members of the starter community are lost during the reactor operations. Therefore, most of the microbial inhabitants were introduced with EOMW2 or, alternatively, were derived from minor forms originally occurring in the inoculum. RsaI-digestions of amplicons obtained with primers for Archea generated profiles with a major peak in samples from both middle region of the GAC-reactor and recycled reaction medium. This result indicated the presence of a dominant specie among Archea and was confirmed by analysis of T-RFs generated with different enzymes.

CONCLUSIONS

In this work, the possibility of significantly enhancing the performances of an OMW anaerobic digestion process upon biomass immobilization in a SB- and GAC-packed-bed loop reactor was demonstrated. In particular, GAC-biofilm reactor tolerated high and variable OMW organic loads, thus exhibiting high volumetric productivities in terms of OMW bioremediation and biomethanization. The GAC biofilm system was prevalently composed by eubacteria species that, on the basis of the T-RF fingerprint, probably derived from a progressive replacement of many members of the starter community with species naturally occurring in the EOMW2 introduced in the reactor during the treatment operations.

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