VALORIZATION OF OLIVE MILL WASTEWATERS AS A RENEWABLE RESOURCE FOR THE BIOLOGICAL PRODUCTION OF POLYHYDROXYALKANOATES

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SUMMARY: An innovative biotechnological anaerobic-aerobic integrated three-stage process for the production of polyhydroxyalkanoates (PHAs) is being studied. A low-cost renewable row material such as the liquid waste resulting from olive oil producing processes (olive mill wastewater, OMW) is employed to feed the first anaerobic step, by which obtaining an effluent rich in volatile fatty acids (VFAs). Four anaerobic packed-bed biofilm reactors filled with granular active carbon (GAC) or ceramic cubes (VS) were developed and employed in batch and continuous conditions in the OMW acidogenic fermentation. The effect on the process of temperature and organic loading rates were studied. Higher COD conversion in VFAs were obtained in the reactor filled with VS. In the second aerobic process stage, a sequencing batch reactor (SBR) is fed with the anaerobic effluent, with the aim of selecting microbial populations able to store PHAs through the metabolic conversion of VFAs. A SBR inoculated with an activated sludge was fed with a synthetic VFA mixture by different pH conditions. The highest substrate removal and polymer production rates were obtained at pH 8.5. The selected microflora is finally employed in a third aerobic batch stage fed with the VFA-rich anaerobic effluent. A batch experiment was carried out at pH 8.5. PHAs were produced at a rate of approximately 350 mgCOD/gCOD/h, with a storage yield of 45% (as COD). The produced polymer was a P(HB-HV) co-polymer, with 13% (mol/mol) HV content.

1. INTRODUCTION

Polyhydroxyalkanoates (PHAs) are bioplastic produced by bacteria or plants whose properties and applicabilities are quite similar to these of polypropylene (Dionisi *et al*., 2005). Despite that, high PHA production costs, mainly due the employment of pure cultures and synthetic specific substrates, actually limit their diffusion (Reddy *et al*., 2003). In this research, an innovative anaerobic-aerobic integrated process for PHA production, employing a mixed microbial consortium

and fed with a non-cost renewable resource, is being studied. This process consists of three stages. In the first anaerobic stage olive mill wastewaters (OMWs), i.e. the liquid effluent resulting from olive oil producing processes, are fermented to obtain an effluent rich in volatile fatty acids (VFAs), which represent the substrate for PHA production. OMWs are generally considered effluents of high environmental concern due to their high COD load (Bertin *et al.*, 2004). Thus, their employment as the process feedstock could also represent an alternative solution for their disposal. In the aerobic second stage, the VFA-rich anaerobic effluent resulting from the first stage is periodically fed to a sequencing batch reactor (SBR) inoculated with an activated sludge. The alternance of excess and lack of substrate ("feast and famine conditions") enriches the mixed culture of the PHA-producing microorganisms, able to store the polymer during the feast phase and to reuse it for growth during the famine phase. This stage operates at high organic loads so to maintain strong selective pressure on the sludge. Finally, in the third aerobic batch stage the excess sludge selected within the second stage is fed with the effluent of the OMW acidogenic fermentation at a considerably higher organic load in order to saturate its PHA storage capacity.

Packed-bed biofilm reactor (PBBR) technology was chosen to develop the anaerobic stage, as immolized cells systems allow to reduce the risks of shock loading and/or washout problems, which can compromise the productivity of dispersed growth reactors operating with low-growth rate biomass and high and fluctuating organic loads as it typically happens in the anaerobic digestion of OMWs (Bertin *et al.*, 2004). The effect on the process of the packing material and of the temperature were studied by developing four identically-configured PBBRs using two different packing materials, particularly granular activated carbon (GAC) and high-porous ceramic cubes (Vukopor S10, VS), and allowing to operate one PBBR for each of them under mesophilic conditions (35°C) and the other two under thermophilic conditions (55°C). Two preliminary one-month batch experiments along with a two-months continuous mode experiment were carried out by feeding the reactors with a diluted and amended OMW.

The second stage of the process was studied in an thermostated (25°C) aerobic sequencing batch reactor, inoculated with an activated sludge and fed with a synthetic mixture of acetic and propionic acid. Particularly, the selection at a high organic load of a mixed culture with high PHA storage rates was verified under different pH values (in the range 7.5 ÷ 9.5). The third stage of the process was finally studied by feeding the selected culture under the pH value (8.5) which gave rise to the best PHA pruduction rate and storage yield.

2. EXPERIMENTAL STUDY

2.1 Apparatus

2.1.1 Anaerobic first stage: packed bed biofilm reactors

Four PBBRs were developed as reported in Bertin *et al*. (2004). In brief, they were 2.400 l, hermetically closed and thermostated glass column reactor equipped with a recycle line in which the cultural broth was continuously recycled from the top to the bottom of the column (Fig. 1). GAC or VS were employed as packing materials. One reactor for each packed-bed operated at 35°C, while the other two PBBRs operated at 55°C. As a result of support addition, the actual raction volumes of the PBBRs filled with GAC and operated at 35°C or 55°C (GAC35 and GAC55, respectively) became 1.50 and 1.80 l, respectively, while the ones of the columns filled with VS and operated at 35°C or 55°C (VS35 and VS55, respectively) became 2.25 and 2.28 l, respectively. The reactors were inoculated with the anaerobic, OMW-digesting microbial consortium employed in a previous research (Bertin *et al.*, 2004) and then employed under strictly anaerobic conditions.

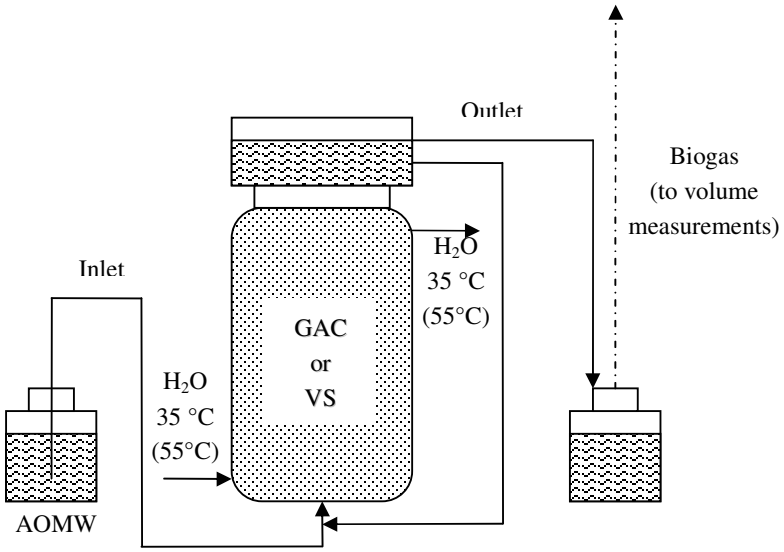

Figure 1. Scheme of the anaerobic PBBRs employed in the acidogenic OMW fermentation

2.1.2 Aerobic second and third stage: sequencing batch reactor

A 2-l SBR stirred by a mechanical impeller at 900 rpm was used in the PHA-producing mixed culture selection and enrichment under periodic conditions. Before its employment, it was inoculated with an activated sludge collected from the ''Roma Nord'' full-scale wastwater treatment plant. The pH was maintained constant through an automatic pH-stat system by the addition of concentrated NaOH and H_2SO_4 solutions. The temperature was maintained at 25°C by a thermostatic jacket.

2.2 Experimental procedure

2.2.1 Anaerobic first stage: batch and continuous mode experimental approach

The OMW employed in the anaerobic stage was kindly purchased by the Sant'Agata d'Oneglia olive mill (Imperia, Italy). Its main chemical-physical parameters are reported in Table 1.

Table 1: Chemical-physical OMW main parameters

COD g/l	Dry weight g/l	VSS g/l	pH	Phenols g/l	VFAs g_{COD}/l
25	20	12	4.3	2.9	0.683

An amended OMW (AOMW) was prepared as follows and fed to the reactors: the OMW was diluted with water (1:4), urea was added at the concentration of 0.45 g/l and the pH was corrected to 7 with NaOH. The AOMW initial COD was so about 6.33 g/l. The AOMW employed in the first batch experiment was amended with glucose at the concentration of 0.5 g/l in order to sustain the microbial growth and the biofilm development. The two batch experiments lasted about one month.

Under continuous mode of operations, the AOMW was fed to the PBBRs at a dilution rate (D) of about 1.27 (d^{-1}), so that the organic loading rate (OLR) was about 8 g/(l*d), for a 2-month period. Six ml samples of wastewater were taken daily through sampling ports placed along the PBBR inlet

and outlet lines. The collected samples were filtered on 0.22 μm cellulose-nitrate filters (Millipore, MO, USA) and then analysed for COD and concentration of total phenolic compounds as detailed elsewhere (Bertin *et al*., 2004). Amount and composition of the total biogas were as well determined (Bertin *et al*., 2004).

2.2.2 Aerobic second and third stage: experimental approach

The SBR cycle representing the second stage of the process consisted of 10 min feed, 1 h 48 min reaction, and the final (2 min) withdrawal of the mixed liquor from the mixed vessel. All excess biomass was withdrawn with the mixed liquor, so that the biomass retention time was equal to hydraulic retention time (1 d). The SBR was fed with a synthetic mixture of acetic (85% on a COD basis) and propionic (15 %) acids, with an overall concentrations of 8.5 g_{COD}/l, so that the OLR was 8.5 g_{COD}/(l*d). SBR cycles were characterized by measurements of biomass concentration (at the end of the cycle) and of substrates, PHA and dissolved oxygen concentration (during the whole cycle). The effect of pH was investigated by carring out three experiments at pH 7.5, 8.5 and 9.5, respectively.

Identical loading conditions were chosen to carried out a batch experiment (representing the final third stage of the process) with the enriched culture resulted from the SBR operations. pH value (8.5) which gave rise to the best PHA pruduction rate and storage yield was applied.

3. RESULTS AND DISCUSSION

Under batch conditions, a significant biological activity occurred within all the PBBRs employed in the anaerobic fermentation of the AOMW. Considering any single PBBR, similar results related to the two sequencial batch experiments were observed. In particular, the acidogenic fermentation was the main metabolic process measured in the PBBR packed with VS under mesophilic conditions (VS35), where VFAs accumulated to concentration values of about 4.49 and 4.59 g_{COD}/l (batch experiment n. 1 and 2, respectively), which are about 25 times higher then the initial one (~0.17 g_{COD}/l). In general, VFAs accumulated in all the developed systems during both batch experiments; however, a higher methanogenic activity appeared in the reactors packed with GAC, where lower VFA concentrations where measured (data not shown). Conversely, only slight methane production occurred in the VS-PBBRs, and expecially in the VS35. Very high COD removal yields were observed in the GAC-PBBRs, where significant part of the removed COD was converted into CH_4 (data not shown). This could have also been due to different phenol removals exherted by reactors packed with different material, as higher concentrations of such substances measured in the VS-PBBRs (where only 33÷35% of the phenols were removed, whereas they were almost completely depleted by GAC-systems) could have inhibited the methanogenic activity (Beccari *et al*., 2002), so allowing higher VFA accumulations in the VS-reactors. Acetic acid was main component of the final VFA mixtures. Propionic and butirric acid were the other VFAs significantly detected. The final VFA mixture compositions seemed to depend by the employed packed material and not by the temperature data not shown).

Similar evidences where observed under continuous mode of operations. In particular, VFAs accumulated only in the VS-PBBRs, whereas part of their initial load was depleted in the reactors packed with GAC (Table 2). VS55 reactor, where more than 1 g/l of VFAs were produced and about the 26% of the COD was converted into VFAs, gave rise to the strong acidogenic OMW fermentation; correspondently, the lower COD depletion occurred in such reactor (Table 2). In general, only slight methane productions yields were calculated for VS-PBBRs, where again phenols tent to persist so probably contributing to inhibit the methanogenic microbial activity (Table 2). Less of the 50% of the initial COD was collected as VFAs in the VS55 anaerobic effluent

(Table 2): this is encouraging in the perspective of determining stronger acidogenic conditions by which also avoiding COD consumption trough the methanogenic activity, so trying to obtain higher final VFAs concentrations.

Table 2: main experimental parameters determined within the continuous mode study: VFA and CH_4 productions, COD and phenols removal percentages, COD conversions into VFAs along with final VFA concentrations with respect to the initial COD value

	VFA production	COD removal	CH_4 production yield	Phenol removal	COD conversions into VFAs*	VFA_{out} / initial COD
	g_{COD}/l	%	$l/g_{CODremoved}$	%	%	%
VS35	0.83	41.5	0.05	15.5	18.5	42.2
VS55	1.16	25.6	0.06	31.7	25.9	47.5
GAC35	-0.17	60.7	0.16	74.0	-3.78	26.5
GAC55	-0.67	75.3	0.09	68.2	-14.9	18.6

* COD conversions into VFAs are calculated as the produced VFAs (VFA_{out} - VFA_{in}) divided per the initial COD excluding its initial VFA fraction (CODin - VFA_{in})

As for the VFA mixture composition, acetic acid was again its main component. Significant concentrations of propionic and butyric acids were also measured. Confirming what observed during the batch experiments, packed-bed materials appeard to be variables able to influence the different acids production, whereas temperature didn't exert significant effects on such a parameter. In particular, following percentages were calculated for the above mentioned acids in the VS55: 56, 15 and 21%, respectively.

Concerning the second stage of the process, three runs were carried out with the SBR fed with a synthetic VFA mixture at three different pH values: 7.5, 8.5 and 9.5. The highest substrate removal rates and polymer production rates were obtained at pH 8.5. A batch experiment was carried out employing the biomass selected within the SBR under these latter conditions (pH 8.5): PHAs were produced at a rate of approximately 350 mgCOD/gCOD/h, with a storage yield of 45% (as COD). The produced polymer was a P(HB-HV) co-polymer, with 13% (mol/mol) HV content. This represent a very promising result, as such a co-polymer exhibited chemical-physical properties much more interesting with respect the pure PHB ones in the perspective of its industrial production and employment (Dionisi *et al*, 2005).

4. CONCLUSIONS

Four anaerobic packed-bed biofilm reactors were developed and employed in batch and continuous conditions in the mesophilic or thermophilic OMW fermentation, with the aim of obtaining an effluent rich in VFAs representing the feedstock of the following two aerobic stages of an innovative integrated process for PHAs production. Strong acidogenic fermentation was obtained in the reactors packed with VS, where low COD depletions were observed and a low phenolic removal could have contributed to inhibite the methanogenic activity. In particular, VS55 reactor, operating at 55°C, gave rise to the highest VFA production.

pH 8.5 seemed to represent an optimal condition in order to maximize the polymer production rate performed within the aerobic stages.

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