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# Effect of hydraulic retention time on biohydrogen and volatile fatty acids production during acidogenic digestion of dephenolized olive mill wastewaters

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## ABSTRACT

The influence of Hydraulic Retention Time (HRT) on the performances of a recently developed biotechnological anaerobic acidogenic process fed with dephenolized Olive Mill Wastewater (OMW) was investigated. The study was carried out under mesophilic conditions in Packed Bed Biofilm Reactors (PBBRs), filled with ceramic cubes and inoculated with a characterized and acclimated acidogenic microbial consortium. The PBBRs were fed with a HRT of 7, 5, 3 or 1 day, which corresponded to Organic Loading Rates (OLRs) of about 5.5, 7.8, 12.9 and 38.8 g L<sup>-1</sup> d<sup>-1</sup>, respectively. A significant production of a H<sub>2</sub>-rich biogas was observed when shorter HRTs were applied: in particular, H<sub>2</sub> relative amount and productivity increased from 3% to 32% and from 0.20 to 6.10 dm<sup>3</sup> m<sup>-3</sup> h<sup>-1</sup>, respectively, by decreasing the HRT from 7 to 1 day. On the contrary, shorter HRTs turned into a lower accumulation of Volatile Fatty Acids (VFAs), whose highest amounts were found with HRTs of 7 and 5 days (about 18.4 and 19.7 g L<sup>-1</sup> COD equivalents, respectively). The highest conversion yield of COD into VFAs (36%) was obtained with a HRT of 5 days, when VFAs represented about 78% of the effluent COD. HRT also influenced the composition of the VFA mixture: acetic, propionic and butyric acid were the most prominent VFAs, being their relative amounts higher when PBBRs were operated with shorter HRTs (up to 19, 12 and 42% of the whole mixture, respectively, when HRT was 1 day).

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## 1. Introduction

The management of agroindustrial residues represents to date a serious environmental and economic concern [1]. However, the possibility of employing such matrices as renewable feedstock [2–4] has fostered the development of the biorefinery concept as a sustainable approach to extensively exploit some of these residues for production of biomolecules [5–7], biofuels [8–10] and bio-based materials [11].

For instance, recent studies have shown that a number of integrated processes can be applied to the case study of Olive Mill Wastewaters (OMWs), allowing a wide exploitation of this effluent for the sustainable recovery of natural antioxidants such as polyphenols [12] and other natural compounds [13], and for the production of biofuels (CH<sub>4</sub> [14,15] or photoheterotrophically generated H<sub>2</sub> [16]) and biopolymers such as polyhydroxyalkanoates (PHAs) [17]. In this latter case, OMWs need to be previously digested under acidogenic conditions, since Volatile Fatty Acids (VFAs) are the major substrates for

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PHA-accumulating bacteria [18]. In this respect, a dedicated continuous Packed Bed Biofilm Reactor (PBBR) performing OMW organic matter bioconversion into VFAs was recently developed [19]. According to the biorefinery approach mentioned above, the feasibility of a preliminary recovery of the polyphenolic fraction of OMWs before their submission to acidogenic digestion was recently demonstrated [20]. Interestingly enough, in that case, production of some  $H_2$  gas was observed together with accumulation of VFAs.

All this considered, the aim of the present work was to study the influence of Hydraulic Retention Time (HRT) on the developed acidogenic process fed with a dephenolized OMW, in the perspective of inducing the production of a  $H_2$ -rich biogas in concomitance with the acidification of the effluent. Furthermore, the robustness of the most performing process was assessed by operating the reactor for a considerably longer experimental period with respect to the applied HRT. The effectiveness of the acidogenic inoculum, which was fully microbiologically characterized in a previous study [20], was also evaluated by comparing the performances of acidogenic digestion processes carried out in the presence and absence of both the inoculum and the active OMW indigenous microflora.

## 2. Materials and methods

### 2.1. Dephenolized olive mill wastewater

The OMW employed in the present study was provided by the Sant'Agata d'Oneglia (Imperia, Italy) three phase olive mill during the olive oil production campaign of 2010/11. Polyphenols occurring in the OMW were removed according to a solid phase extraction procedure previously developed [20]. The employed actual site OMW had the following characteristics: COD,  $51.66 \pm 1.97 \text{ g L}^{-1}$ ; total phenols,  $1.21 \pm 0.05 \text{ g L}^{-1}$ . After phenols removal, the main features of the dephenolized OMW (OMW<sub>deph</sub>) were those reported in Table 1; OMW<sub>deph</sub> total phenols concentration was  $0.37 \pm 0.04 \text{ g L}^{-1}$ .

### 2.2. Microbial consortium

The acidogenic microbial consortium employed as the inoculum was obtained and microbiologically characterized within a previous investigation dedicated to the development of an acidogenic biotechnological process fed with OMW<sub>deph</sub> [20]. The inoculum was stored at 4 °C before being used in this study.

### 2.3. Effectiveness of the inoculum in the acidogenic digestion process

The effectiveness of the inoculum in the bioconversion of OMW<sub>deph</sub> organic matter into Volatile Fatty Acids (VFAs) under anaerobic acidogenic conditions was preliminary investigated in batch tests. In particular, its capability of producing  $H_2$  and VFAs was evaluated at 35 °C and pH 7.0, provided that such conditions were found to be ideal for the acidogenic digestion of OMW<sub>deph</sub> by the same inoculum [20]. Acidogenic digestion was studied in statically incubated

**Table 1 – Chemical features of the dephenolized olive mill wastewater (OMW<sub>deph</sub>) fed to the Packed Bed Biofilm Reactors (PBBRs), and of the produced effluents according to the applied Hydraulic Retention Time (HRT, days), along with the related process yields. Standard deviations (s.d.) are also reported.**

		Process effluents						Process yields					
		VFAs			COD			pH			Density		
		mean		s.d.	mean		s.d.	mean		s.d.	mean		s.d.
		$\text{g L}^{-1}$ COD eq.	$\text{g L}^{-1}$ COD eq.	$\text{g L}^{-1}$ COD eq.	$\text{g L}^{-1}$ COD eq.	$\text{g L}^{-1}$ COD eq.	$\text{g L}^{-1}$ COD eq.	pH value	pH value	pH value	$\text{g cm}^{-3}$	$\text{g cm}^{-3}$	$\text{g cm}^{-3}$
Feed	OMW <sub>deph</sub>	8.92	1.42	38.79	1.60	4.43	0.09	0.985	0.022	—	—	—	—
PBBRs	HRT 7	18.38	2.40	30.02	1.50	5.99	0.29	0.983	0.023	61.2	31.7	22.6	22.6
	HRT 5	19.67	3.68	25.28	3.14	5.94	0.11	0.986	0.044	77.8	36.0	34.8	34.8
	HRT 3	16.27	0.53	26.94	1.13	5.79	0.10	0.978	0.017	60.4	24.6	30.5	30.5
	HRT 1	11.93	2.78	30.08	0.76	5.04	0.10	0.994	0.004	39.7	10.1	22.5	22.5

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