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Uncoupled hydrogen and volatile fatty acids generation in a two-step biotechnological anaerobic process fed with actual site wastewater

Research Paper

Matilde Monti, Alberto Scoma, Gonzalo Martinez, Lorenzo Bertin and Fabio Fava

Department of Civil, Chemical, Environmental and Materials Engineering (DICAM), University of Bologna, via Terracini 28, 40131 Bologna, Italy

Among agro-wastes, olive mill wastewater (OMW) truly qualifies as a high impact organic residue due to its biochemical-rich composition and high annual production. In the present investigation, dephenolized OMW (OMW_{deph}) was employed as the feedstock for a biotechnological two-stage anaerobic process dedicated to the production of biohydrogen and volatile fatty acids (VFAs), respectively. To this end, two identically configured packed-bed biofilm reactors were operated sequentially. In the first, the hydraulic retention time was set to 1 day, whereas in the second it was equal to 5 days. The rationale was to decouple the hydrolysis of the organic macronutrients held by the OMW_{deph}, so as to quantitatively generate a biogas enriched in H_2 (first stage aim), for the acidogenesis of the residual components left after hydrolysis, to then produce a highly concentrated mixture of VFAs (second stage aim). Results showed that the generation of H_2 and VFAs was effectively split, with carbohydrates and lipids, respectively, being the main substrates of the two processes. About 250 ml H_2 L^{-1} day^{-1} was produced, corresponding to a yield of 0.36 mol mol^{-1} of consumed carbohydrates (expressed as glucose equivalents). The overall concentration of VFAs in the acidogenic process was 13.80 g COD L^{-1}, so that 2.76 g COD L^{-1} day^{-1} was obtained. Second generation biorefineries use a selected fraction of an organic waste to conduct a microbiologically-driven pathway towards the generation of one target molecule. With the proposed approach, a greater value of the waste was attained, since the multi-purpose two-stage process did not entail competition for substrates between the first and the second steps.

Introduction

Agricultural, industrial, forestry, fishery and municipal organic leftovers can be used as renewable resources in the development of second generation biorefinery processes. The design of multi-purpose biorefinery schemes has been found to be fundamental to meeting the overall economic sustainability of the process at the large scale [1–6]. Due to the considerable versatility of anaerobic mixed microbial consortia, dark fermentative anaerobic processes dedicated to the production of H_2, volatile fatty acids (VFAs) or CH_4, can be easily adapted and integrated in biorefinery chains fed with renewable complex organic matrices. H_2 and VFAs have been obtained by processing several biowastes under hydrolytic [7,8] and acidogenic [9] conditions, respectively. Both approaches may represent the first step of two steps processes, the second of which being dedicated to the production of biomethane. Despite the higher complexity, hydrolytic processes were often reported as more efficient, because of the possibility of separating microbial populations responsible for the different digestion activities, thus optimizing related process parameters [10].

Among the most investigated agro-wastes for second generation biorefineries, Olive Mill Wastewater (OMW) is one of the most interesting, due to its high availability (more than 10 Mt in 2011) as well as its rich and biochemically-diverse composition (for a review see [11]). OMW was tested as the raw material for the

Corresponding author: Bertin, L. (lorenzo.bertin@unibo.it)

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production of H_2 [12–14] and VFAs [15,16]. Recently, the possibility of processing dephenolized OMWs (OMW_{deph}), under dark fermentative anaerobic conditions in packed bed biofilm reactors (PBBRs), for the production of biofuel and bio-based chemicals, depending on the applied hydraulic retention time (HRT), was demonstrated [17].

In the present investigation, the possibility of simultaneously obtaining both products has been tested employing the same kind of feedstock, by sequentially operating, in a two-step process, two independent PBBRs dedicated to these target products. Since the biological anaerobic consumption of the organic macronutrients, carbohydrates, proteins and lipids follow different kinetics, a first reactor with an HRT of 1 day was operated to produce an H_2-rich biogas, while the effluent of this reactor was fed to another identically configured PBBR operated for 5 days HRT to generate VFAs. Thus, the main rationale was to decouple the degradation of organic macronutrients carried by OMW_{deph}, by physically controlling, in different reactors, the processes leading to these target compounds.

Materials and methods

Dephenolized olive mill wastewater (OMW_{deph})

The OMW employed in this study was provided by the Sant'Agata d'Oneglia (Imperia, Italy) three-phase olive mill. Polyphenols were removed according to a solid phase extraction (SPE) procedure [29] developed and applied according to previous investigations [18]. The main OMW features after removal of polyphenols were: pH, 4.5; density, 0.9 g cm^3; chemical oxygen demand (COD), 51.9 ± 9.9 g L^{-1}; phenols, 0.93 ± 0.19 g L^{-1}; VFAs, 4.70 ± 1.3 g_{COD} L^{-1}; carbohydrates, 7.14 ± 0.6 g L^{-1}; proteins, 0.34 ± 0.04 g L^{-1}; lipids, 5.35 ± 0.6 g L^{-1}.

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 process fed with OMW_{deph} [15]. The inoculum was stored at 4°C before being used in this study.

Packed bed biofilm reactors (PBBRs)

The two PBBRs used in the present investigation had the same configuration. They consisted of a hermetically closed glass column (40 cm in height, 5 cm outer diameter) with an empty volume of about 0.8 L, operated under continuous anaerobic conditions. Columns were packed with ceramic cubes of Vukopor S10® (Lanik, Boskovice, CZ) whose dimensions, porosity and density were 25 mm × 25 mm × 18 mm, 10 ppi and 2.38 g mL^{-1}, 1, respectively. This support was selected according to previous investigations since it favoured acidogenic activity [16]. Reactors were equipped with a recycle line: the recycling ratio, expressed as the ratio between the recycled broth flow and the whole flow entering the column, was about 0.95. PBBRs were fed according to an up-flow scheme with HRTs equal to 1 and 5 days (HRT1 and HRT5, respectively), corresponding to Organic Loading Rates (OLRs) of 38.79 and 7.76 g L^{-1} day^{-1}, respectively. Both systems were initially provided with a mixture of OMW_{deph} and microbial inoculum (10%, v:v). Such a mixture was flushed with nitrogen and pumped up in the columns. Nitrogen gas was also sparged into the reactor's head space during inoculation. Reactors were continuously operated for 53 and 41 days (HRT1 and HRT5, respectively). However, both reactors needed 26 and 14 days, respectively, before biogas or VFA production was stable, that is, oscillating around a value ±20%. In particular, the process was considered stable when such productivity was maintained for 5 full liquid retention times (equal to 5 and 25 days in HRT1 and HRT5, respectively). Finally, the average performance and the confidence interval were calculated as the mean and standard deviation observed during the last 27 days. The process temperature was maintained at 35°C with serpentine silicon tubing continuously recycling temperature controlled water; the pH was corrected to 7 on a daily basis by manual dropwise addition of a 10 M NaOH solution. The two processes were monitored daily for biogas production and composition, VFA and COD concentrations.

Analytical procedures

VFA concentration was monitored with a GC-7890A (Agilent Technologies, Milano, Italy) with a Flame Ionization Detector (FID) under the following conditions: column temperature 170°C, inlet temperature 250°C, detector temperature 280°C, pressure 5 psi, gas carrier nitrogen. Before analyses, samples were diluted with a 60 mM oxalic acid solution. VFA concentration was reported as COD equivalents (g_{COD} L^{-1}) by using stoichiometric conversions. Phenols were evaluated as reported in [18] according to the conventional Folin-Ciocalteu procedure. Soluble COD concentration was determined spectrophotometrically on centrifuged samples (14,000 rpm, 10 min) according to the potassium dichromate colorimetric oxidation method using COD Vario Tube Test (Aqualytic, Dortmund, Germany) following the manufacturer's instructions, while total carbohydrates were determined according to [19] using glucose as a standard (Sigma–Aldrich, Milano, Italy). Total lipids were evaluated as reported in [20] using as a standard the olive oil produced at the industrial plant to which the wastewater belongs. Total soluble proteins were determined with the Bradford method [21], by using the commercial protein assay dye reagent concentrate from BioRad (Milano, Italy). In particular, in order to avoid interference with biomass, samples for soluble proteins were first placed at −20°C for at least 24 h, then brought to room temperature and centrifuged at 14,000 rpm for 5 min.

The amount of biogas was measured as reported elsewhere [22] by using a Mariotte flask, which was hydraulically connected to the reactor head spaces. The biogas composition was determined by gas chromatography using a μGC, model 3000A (Agilent Technologies, Milano, Italy), under the conditions described in [15]. Bioconversion of OMW_{deph} organic matter into VFAs (COD = >VFAs) was calculated as the ratio between produced VFAs and influent net COD excluding its VFA fraction. Analytical measurements always showed a standard deviation below 5%.

Results

Dephenolized three-phase olive mill wastewater (OMW_{deph}) was fed to a PBBR operated with a HRT equal to 1 day. The main activity observed within this reactor (HRT1) was a sustained production of a biogas enriched in H_2, producing H_2 at a rate of 252 ± 38 mL L^{-1} day^{-1}. The biogas had a relative content of $12.7 \pm 2.5\%$ H_2 (Fig. 1a). However, a concomitant small accumulation of VFAs was also observed (total VFA concentration was