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Effect of the addition of glycerol on hydrogen production from industrial municipal solid waste

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ABSTRACT

The purpose of this study was to investigate the effect of glycerol supplementation (1% v/v) on the hydrogen production (HP) steps in thermophilic-dry dark fermentation of industrial municipal solid waste (IMSW) in batch mode. For this purpose, physicochemical and microbiological parameters were considered. The rates of HP and specific hydrogen production (SHP) almost doubled when adding 1% v/v crude glycerol to the IMSW. This result was in keeping with the high values of hydrolysis yield (75 ± 4%) and microbial activity ($7.3 * 10^{-12} \pm 0.9 * 10^{-12}$ l H_2/cell). Microbiological studies showed that the percentages of *Eubacteria* decreased slightly after the dark fermentation process. *Archaea* contents were not significant and the biogas produced was methane-free.

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1. Introduction

The overexploitation of fossil fuels has now brought humankind to a dead end from both an environmental and energy resource standpoint. It is undeniably true that fossil fuel reserves are not endless [16]. A pressing need has thus emerged to identify and exploit alternative energy sources; i.e., energy sources that will be both renewable and environmental friendly.

Biohydrogen, i.e., hydrogen produced from renewable sources, has the highest specific energy and generates zero CO_2 emissions when burned for energy recovery [14,13,16]. Dark fermentation is an attractive treatment strategy for hydrogen production (HP) from waste [12,24,26,27,9] as well as for reducing the problem of disposal. HP from IMSW, which is ultimately converted into H_s and CO_2, includes three steps [24–28] and is performed by microbial consortia comprising *Eubacteria* (hydrolytic-acidogenic bacteria and acetogenic bacteria). In the first step (hydrolysis), complex organic polymers are hydrolysed into simpler soluble organic compounds by hydrolytic-acidogenic bacteria. Large quantities of H_2 are produced in this step, particularly in dry anaerobic digestion (AD). In the second step (acidogenesis), hydrolytic-acidogenic bacteria produce volatile fatty acids (VFA), alcohols, H_2 and CO_2. In the third step (acetogenesis), acid acetic, H_2 and CO_2 are produced by acetogens, organisms that consume fermentation products such as propionate, butyrate, lactate and ethanol. These bacteria (acetogens) require very low H_2 partial pressure to promote the thermodynamics of the reactions. Therefore, the acetogens in a H_2-producing reactor are inactive [2,7,23,19,11,27]. Previous studies on dark fermentation of IMSW have shown that destabilization of HP in continuously stirred tank reactors without recycling of biomass occurs due to wash-out of the bacteria and not because of over-loading [26,27]. Hence, the interesting option of co-digestion of readily biodegradable organic substances, such as glycerol, could increase the load of biodegradable organic matter and lead to a higher biogas yield [3,8], without having to wash out the microorganisms.

Although [8], published a study on the effect of adding 1% v/v glycerol to the AD of the synthetic organic fraction of municipal solid waste (OFMSW) (40% fruit, 25% potatoes, 25% vegetables, 8% bread and 2% paper), no prior studies have been published on the effect of glycerol addition to dark fermentation of real IMSW. The effect of this addition on the microbial groups involved in the digestion process, which have varying sensitivities to environmental changes [24–28], has not been investigated either.

The aim of the present study was to investigate the effect of 1% supplementations of glycerol on the dark fermentation of IMSW, a waste that is a well-known substrate for HP [24,26,28]. Anaerobic batch reactors were used for this purpose.

The following parameters were considered in this study: HP, SHP, soluble chemical oxygen demand (COD_D), VFA, total volatile solid (TVS), hydrolysis yield, acidification yield and microbial activity. Fluorescence *in situ* hybridization (FISH), employing different

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Nomenclature

AD	anaerobic digestion
COD_D	soluble chemical oxygen demand
FISH	fluorescence *in situ* hybridization
HP	hydrogen production
IMSW	industrial municipal solid waste
OFMSW	organic fraction municipal solid waste
OLR	organic loading rate
SHP	specific hydrogen production
TVS	total volatile solid
VFA	volatile fatty acid

oligonucleotide probes, was used to determine the main groups involved in the anaerobic process (*Eubacteria* and *Archaea*).

2. Material and methods

2.1. Inoculum and substrate

Anaerobic digester effluent from a thermophilic-dry dark reactor was used as inoculum. In previous studies, the thermophilic inoculum was found to be suitable for anaerobic digestion of solid waste [24,27]. Its main characteristics were: TVS = 37 ± 2 g TVS/l, COD_D = 42 ± 5 g O_2/l, VFA = 7.4 ± 1.2 g acetic/l and pH = 5.6 ± 0.3.

The IMSW and glycerol used to feed the reactors came from an industrial trommel (30 mm) at Las Calandrias MSW treatment plant (composting plant), located in Jerez de la Frontera, Cádiz-Spain, and Abengoa Bioenergy biofuel company, based in San Roque, Cádiz-Spain, respectively.

The IMSW was stored in 25 kg drums at 4 °C to avoid anaerobic degradation by the microorganisms found in the solid waste itself. Characterization of the substrates used in the assay is shown in Tables 1 and 2.

2.2. Experimental reactors

The assays were carried out in eight batch discontinuous reactors, three for each condition (0% and 1%) +2 blanks (inoculum without waste), with a total volume of 2.0 l and a useful volume of 1.7 l. Each reactor had an independent agitation system capable of maintaining a uniform moisture content and of redistributing soluble substrate and microorganisms at a rate of 23 rpm, in addition to electrical temperature control. The biogas was collected in 5 l capacity Tedlar (a polyvinyl fluoride plastic polymer) bags. The digesters (except for the blanks) were initially loaded with a mixture of inoculum (700 g) and substrate (351 g IMSW in 1 l tap water), resulting in a final concentration of 40% v/v inoculum, which is considered optimum for biogas production and substrate acclimatization [15]. Glycerol was then added to the reactors to provide glycerol percentages in the 0% v/v (Test 1) and 1% v/v (Test 2) range. Blanks were loaded with inoculum (700 g) to determine background gas production. All the results presented in this paper were corrected by subtracting the HP obtained from the blanks.

The reactors were monitored until reaching a plateau of HP; this represented a monitoring period of at least 12 days.

Table 1
Physicochemical and microbiological characterization of the IMSW used in the tests.

Parameter	Average	Range
VFA (g acetic/l)	5 (1)	4–6
TVS (g/kg)	250 (20)	230–270
Total population (10^7 cells/ml)	36 (7)	29–43
EU (%)	88 (2)	86–90
Archaea (%)	12 (2)	10–14

Average values are shown with standard deviations in parentheses.

Table 2
Characterization of the glycerol used in the tests.

Parameter	Average	Range
COD_D (g O_2/l)	1400 (100)	1300–1500
VFA (g acetic/l)	<0.1	<0.1
TVS (g/kg)	820 (10)	810–830
H_2O	12 (1)	11–13
K	<0.1	–
Na	5.5 (0.5)	5.0–6.0

Average values are shown with standard deviations in parentheses.

2.3. Analytical methods

The analytical determinations performed in this study can be grouped into two categories: physicochemical analysis and microbiological analysis.

2.3.1. Physicochemical analysis

For process monitoring and control of the reactors, the following analytical determinations were performed: biogas, COD_D, pH, TVS and VFA. All analyses were carried out in duplicate according to APHA [1] and Zahedi et al. [24–28]. The TVS, VFA and COD_D of the digester liquor were measured on days 0, 3, 7 and 12. VFA were determined by gas chromatography, using a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector (FID) and a capillary column filled with Nukol. The digesters were monitored daily for biogas (production and composition) and pH. The gas volume produced in the reactor was directly measured using a high-precision flow gas meter: TG-01-Series (Wet-Type) Ritter drum-type gas meter. The composition of the biogas was determined by gas chromatography separation (SHIMADZU GC-2010). H_2, CH_4, CO_2, O_2 and N_2 were analyzed by means of a thermal conductivity detector (TCD) using a Supelco Carboxen 1010 Plot column. Samples were taken using a 1 ml Dynatech Gastight gas syringe under the following operating conditions: split = 100; constant pressure in the injection port (70 kPa); 2 min at 40 °C; ramped at 40 °C/min until 200 °C; 1.5 min at 200 °C; detector temperature: 250 °C; and injector temperature: 200 °C. Helium was used as carrier gas (266.2 ml/min). Commercial mixtures of H_2, CH_4, CO_2, O_2, N_2 and H_2S (Abelló Linde S.A.) were used to calibrate the system.

2.3.2. Microbiological analysis

Microbiological analyses were performed by FISH. Two specific probes (EUB338 and ARC915) were used to determine the cellular concentration and relative percentages of *Eubacteria* and *Archaea*. The overall population was calculated as the sum of the relative amounts of *Eubacteria* and *Archaea*, estimated as 100% [29]. Prior to incubation and once the digestion period had ended, samples were collected in each condition from each reactor in sterile universal bottles. Absolute ethanol was added to the bottles at a volume ratio of 1 sample:1 ethanol. Samples were stored at −20 °C

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