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Effect of Inoculum/Substrate Ratio on Dark Fermentation for Biohydrogen Production from Organic Fraction of Municipal Solid Waste

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Hydrogen (H_2) is meeting with increasing interest as possible alternative to fossil fuels for energy production. It can be produced both thermochemically and biologically from a number of organic substrates. H_2 is usually employed in fuel cells, a technology aimed at producing electricity, characterized by low environmental impact and high efficiency. Similarly, dark fermentation (DF) of organic waste is gaining popularity because of its environmental compatibility and relatively high efficiency in the production of biogas (a mixture of CH_4, CO_2 and H_2 in variable proportions). In this perspective, the organic fraction of Municipal Solid Waste (OFMSW) is an interesting substrate for biohydrogen ($BioH_2$) production because of its large supply and its chemical composition able to sustain a vigorous growth of H_2 producing microorganisms.
In this work, the effect of inoculum to substrate ratio (ISR) (0.01, 0.07 and 0.14, dry weight basis) on mesophilic DF of OFMSW for $BioH_2$ production was evaluated. Primary sewage sludge heated at 95 °C for 30 min was used as inoculum. Both the liquid and the gas phase produced during the DF were analysed, together with the microbial biomass growth, the amount of reducing sugars, the pH, and the biogas volume and composition. Our results show the possibility of using OFMSW as substrate in mesophilic DF process for $BioH_2$ production. In particular, the best performance in terms of $BioH_2$ production was obtained with ISR of 0.07.

1. Introduction

The decline in the availability of fossil fuels and the growing interest in environmental protection have focused the attention of researchers on renewable energies and in particular on the so called bioenergies (Scarlat et al., 2015). Renewable energy production is aimed at contributing to the reduction of greenhouse gases emissions into the atmosphere, lessening in the same time our dependency on fossil fuels (Singh et al., 2015). In this perspective, H_2 is considered as the fuel of the future for its high heating value and for the production of water as the only by-product during its conversion into energy. H_2 can be easily converted with high efficiency into energy through the Fuel Cells system, an electrochemical device converting a fuel chemical energy into electricity with high efficiency and without an intermediate heat cycle. Fuel Cells are currently considered the ultimate solution for automotive transportation and electricity production (Ausiello et al., 2015). At the present time, however, H_2 is mostly produced through technologies exerting a detrimental impact on the environment, as the thermochemical steam reforming from hydrocarbons (the most widely used) requires high working temperature and produces polluting by-products. The main challenge to face in the use of this technology is, then, the sustainable production and storage of H_2 (Ghimire et al., 2015).
For this reason, recently H_2 biological production ($BioH_2$) DF of organic waste is gaining interest for sustainable H_2 production. DF consists of the fermentative conversion of organic substrate to $BioH_2$ involving biochemical pathways similar to those of the methane producing anaerobic digestion. Numerous feedstock

can be used, such as: sugars (optimal but too costly), carbohydrate-rich food crops, food wastes, agricultural residues, lignocellulosic biomasses and energy crops, wastewaters from paper and pulp industries, urban wastewaters (Singh et al., 2015). The use of organic waste in $BioH_2$ production is particularly interesting due to the reduction of both economic and environmental costs of its treatment and the reuse of the spent substrate as biomass for compost production and as feedstock for anaerobic digestion (due to its high content of volatile fatty acids) for the biogas (a mixture of CH_4 and CO_2) production (Ghimire et al., 2015). In addition, this process is considered as C-neutral (Hawkes et al., 2002).
From this point of view, the organic fraction of Municipal Solid Waste (OFMSW) is an excellent feedstock for DF process, thanks to its easily biodegradable organic matter and its richness in essential nutrients for the microorganisms driving the process (Florio et al., 2016). In addition, its use as DF feedstock could alleviate the problem of OFMSW management and the connected environmental negative impact, producing in the same time energy and heat.
DF process involves different microorganisms: facultative anaerobes (*Enterobacter, Citrobacter, Escherichia coli*), strict anaerobes (genus *Clostridia*) or even aerobes (genus *Bacillus*). Fang et al. (2002) have identified *Clostridia* as the dominant H_2-producing microorganism in mesophilic DF inoculated with a mixed culture of microorganism at pH of 5.5, a value that maximizes the $BioH_2$ yields through the inhibition of the methanogenic activity.
The inoculum to substrate ratio (ISR) and the inoculum composition are two important process parameters for the optimisation of anaerobic digestion of organic waste (Sri Bala Kameswari et al., 2012) and the whole anaerobic digestion process (Kalloum et al., 2014). In this work, DF of OFMSW was carried out for $BioH_2$ production in mesophilic conditions (37 °C), using a wet single-stage process in a batch reactor employing different amounts of thermally pre-treated sewage sludge as inoculum (0.01, 0.07 and 0.14 g/g OFMSW DW). Both the liquid and the gas phase produced were analysed during the DF process for the microbial biomass, the amount of reducing sugars, the pH, the biogas volume and composition.

2. Materials and Methods

2.1 Organic fraction of Municipal Solid Waste

OFMSW was prepared in laboratory using food leftovers: 30 wt% fruits, 5 wt% cooked meat, 30 wt% vegetable, 35 wt% bread. Before being added to a bioreactor, the mixture was grossly chopped, finely shredded with a home blender and finally pressed manually in a mortar to make a puree. OFMSW was characterized by thermogravimetric analysis to evaluate its composition (see Table 1).

2.2 Inoculum

Primary sewage sludge (pSS) obtained from the wastewater treatment plant of Nola (Naples, Italy) was used as inoculum. pSS was kept at room temperature for 1 h prior to use, and then filtered through two sieves (212 µm and subsequently 125 µm) to remove coarse particles. pSS total solids and moisture were measured according to APHA (2005) (Table 1).
Before inoculation pSS was pre-treated at 95 °C for 30 min to inhibit the methanogenic activity and select sporogenic hydrogen-producing microorganism.

Table 1: Characterization of OFMSW and pSS

	Total Solids (wt%)	Volatile Solids (wt%)	Moisture (wt%)	Ash (wt%)	Fixed Carbon (wt%)	pH
OFMSW	36.50	31.34	63.50	1.27	7.04	5.62
pSS	5.10	-	94.90	-	-	7.69

2.3 Dark fermentation batch experiments

Crimped pyrex bottles with perforable butyl rubber septa were used as batch bioreactor. The bioreactors were filled with 15 % w/v OFSMW, inoculated with different amount of pre-treated pSS (1.5 % v/v, 7.5 % v/v and 15 % v/v): ISR0.01 (0.07 g pSS/5.47 g OFMSW, DW), ISR0.05 (0.38 g pSS/5.47 g OFMSW, DW) and ISR0.14 (0.76g pSS/5.47 g OFMSW, DW). Then distilled water was added to obtain a total working volume of 100 mL. pH was corrected with few drops of H_2SO_4 (48 % v/v) to reach a value between 5 and 5.2. During fermentation the pH was eventually corrected with Na_2CO_3 (5 %w/v) to restore the starting value. Anaerobic conditions were ensured by sparging the fermentation medium with nitrogen for 10 min, then bioreactors were placed for

72h in a basculating incubator (Infors HT Minitron, Bottmingen/Basel, Switzerland) at 150 rpm and 37 °C for mesophilic DF.
Each bioreactor was connected to an upturned bottle (125 mL), entirely filled by water, by a capillary tube equipped on both ends with a needle; the biogas volume was calculated measuring the water displaced through a needle in the upturned glass bottle (Al-Zuhairi et al., 2015).

2.4 Analytical methods

Samples of gaseous and liquid phase from crimped vials were collected every 24h. Microbial biomass was determined by optical absorbance at 600 nm (OD_{600}) from a 1:10 diluted sample. The concentration of reducing sugars was calculated by modified Nelson-Somogy method (Nelson, 1944; Pirozzi et al., 2013). pH was measured using 740 pHmeter (WTW, Weilheim, Germany) and biogas composition was determined by GC analysis using a HP 5890 series II equipped with a TCD detector and a double packed molecular sieves Porapack^{TM} column (Ausiello et al., 2015).

3. Results and Discussion

3.1 Dark fermentation

The evolution of microbial biomass during incubation is shown in Figure 1a. In the first 24h OD_{600} for ISR0.14 increases by 36.9 %, while decreased by 47.5 % for ISR0.01 and to a lesser extent for ISR0.07 (13.5 %). These results are in agreement to the reduced biogas volume produced in the first step of the process (39.5 mL is the average of all tests). Between 24h and 48h a bacterial exponential growth phase occurred, after 48h the OD_{600} of ISR0.14 decreased while it went on to increase by 11.7 % for ISR0.07, in agreement with the volume of $BioH_2$ produced (3.48 mL), which was higher than produced by ISR0.14 (1.87 mL).
The modification of pH, as function of the ISR, is shown in Figure 1b. No differences among the means of different ISR at 24h (3.75 SD 0.04), 48h (4.38 SD 0.02) and 72h (4.47 SD 0.19) were reported. All the values show a significant acidification during the DF process due to acidogenic microbial activity, which started around 12h of incubation.

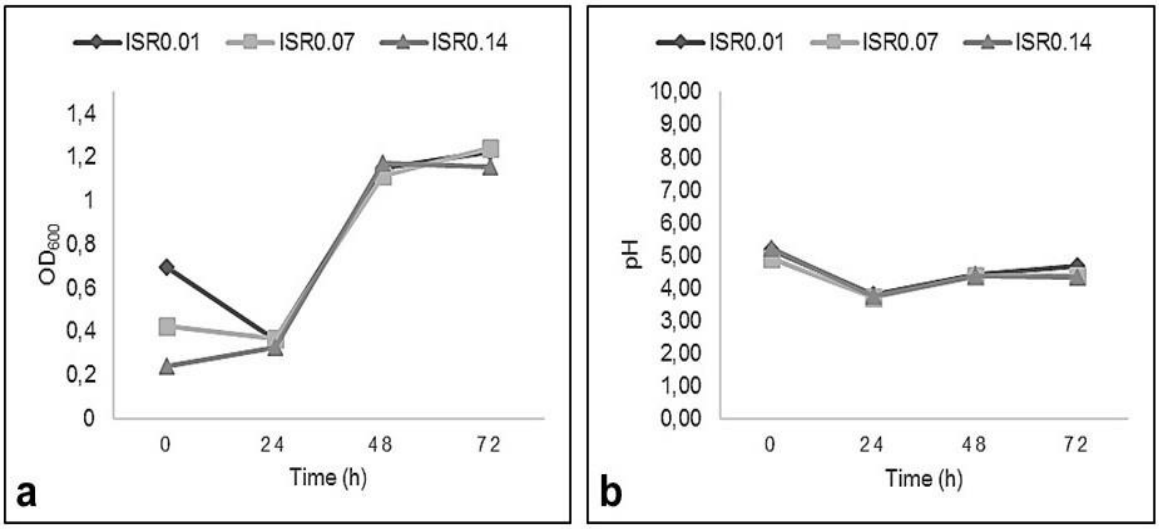

Figure 1: Microbial biomass OD600 1:10 (a) and pH (b) for DF batch experiments

Figure 2a illustrates the sugars biodegradation, that appears to increase over time, reaching a reducing sugars concentration <1 g/L at 72h. The reducing sugars concentration in the feedstock biomass reached 4 % of the total amount at the beginning of the incubation ISR0.07, 5 % for ISR0.14 and 18% for ISR0.01. The increase of the feedstock-inoculum ratio had a marked effect on the amount of bioavailable sugars in the bioreactor at starting time. Although the total sugars reduction in ISR0.01 is less than that recorded for ISR0.14, the cumulative $BioH_2$ production is slightly higher (33.83 mL compared to 32.44 mL for ISR0.14), even though not statistically different. The highest production of $BioH_2$ was recorded for ISR0.07 (39.42 mL). As reported in Figure 3a, the biogas production was quite in the same range of values for all the treatments. It is worth to note that at 72h, the cumulative biogas production of ISR0.14 reached the highest values recorded in this experiment (171 mL), even though this treatment was the worst performing in terms of $BioH_2$

production. In particular, at 24h and 48h, ISR0.07 was the best treatment in terms of $BioH_2$ production (Figure 2b).

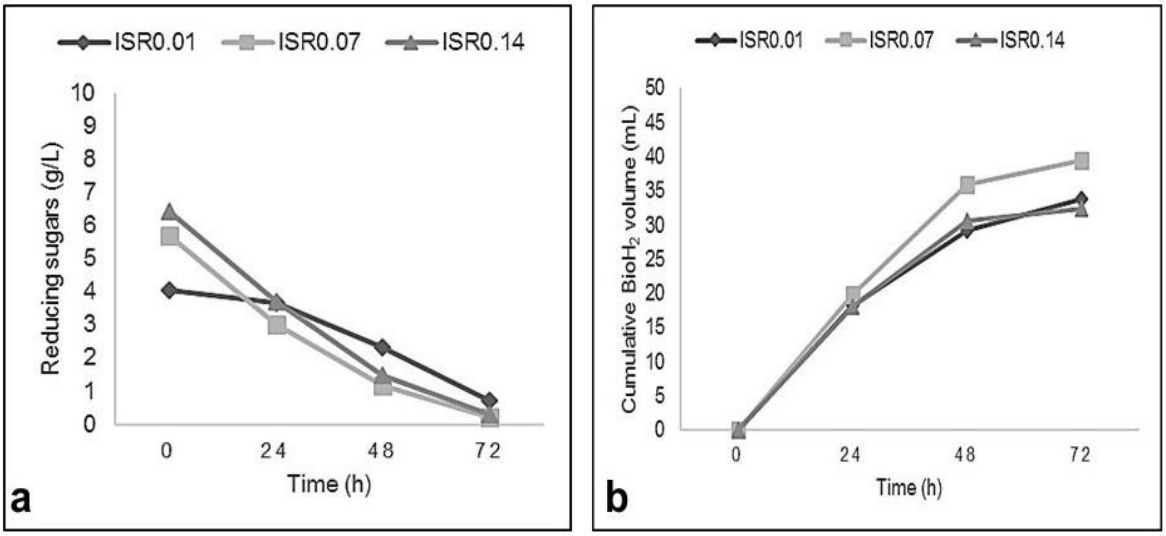

Figure 2: Reducing sugars g/L (a) and $BioH_2$ production mL (b) for DF batch experiments

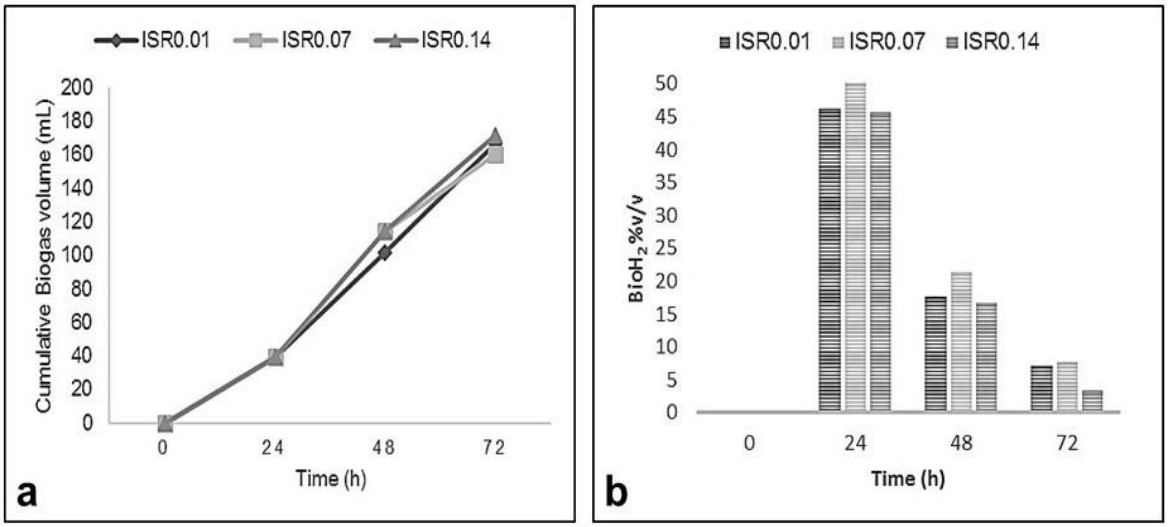

Figure 3: Cumulative Biogas production mL (a) and Biogas composition % v/v (b) for DF batch experiments

The best performance in terms of $BioH_2$ yields (mL/gVS) was obtained in the treatment ISR0.07, which increased the $BioH_2$ concentration in the biogas of about 20.8 % compared to ISR0.14, and an increases the $BioH_2$ yield of 16.5 % compared to ISR0.01 (Table 2).

Table 2: Cumulative Biogas production and $BioH_2$ yields at the end of incubation

ISR (DW)	Cumulative Biogas (mL)	Cumulative $BioH_2$ (mL)	Average $\%H_2$ in the biogas (%v/v)	$BioH_2$ yields (mL/gVS)
0.01	166.50	33.83	23.66	7.20
0.07	160.00	39.42	26.45	8.39
0.14	171.00	32.88	21.89	6.90

Even though many studies are available on the $BioH_2$ production from OFMSW and food waste (FW), in which different yields are reported according to the different operating conditions, it is not easy to compare these results to those obtained here, as the authors not always completely describe the process parameters.

Pan et al. (2008) analysed the effect of feedstock to microorganism ratio (calculated on VS basis) on $BioH_2$ production during a 44h fermentation of FW. They obtained a H_2 yield of 39 mL/gVS at F/M 6 (corresponding to our ISR0.16) under mesophilic conditions (35 ± 2 °C). Gomez et al. (2009) obtained a H_2 production ranging from a minimum of 19 to a maximum of 67 mL/gVS from FW fermentations (of 20 and 40 days) in mixing and static conditions using as inocula an anaerobically digested slaughterhouse waste and a sludge from a wastewater treatment plant. Redondas et al. (2012) obtained from FW fermentation a $BioH_2$ production from to 13.1 to 20.5 mL/gVS at 34 °C. Their reactor was operated using four feeding cycles per day for hydraulic retention time of 1-2 days, five feeding cycles for a hydraulic retention time of 0.75 day, and six cycles for a hydraulic retention time of 0.5 day. Karlsson et al. (2008) have studied the fermentation of food industry residues and manure. They carried out 17 tests varying temperature, hydraulic retention time and the N_2 flow rate obtaining yields equal to 16 mLH_2/gVS at 55 °C, with a sparging rate of 125 mL N_2/min. These authors, under operating conditions similar to ours (37 °C and 2 day of incubation time), have obtained 2.2 mL H_2/gVS. In general, the yields ranging from 0 to 16.5 mL H_2/gVS (hydraulic retention time from 2 days to 8 days), more comparable to our values. Shin et al. (2004) have studied H_2 production from FW in anaerobic mesophilic and thermophilic acidogenesis, obtaining also a yields ranging between 1.3 and 5 mL H_2/gVS. Also in this case the obtained yields of H_2 values are comparable with those reported here. Our yields, even though obtained from a lab-scale apparatus, are in the range of the above mentioned results. The composition of the starting feedstock is another key aspect in $BioH_2$ production. Alibardi and Cossu (2015) found that the highest H_2 production was obtained by the bread–pasta fraction of the OFMSW, while the lowest productions were measured for the meat–fish–cheese fraction. Authors showed that the different content of these two fractions, both present in our lab-made OFMSW, had a direct influence on the H_2 production.

4. Conclusions

In this work, aimed at optimizing the amount of inoculum for $BioH_2$ production through DF, we obtained a maximum $BioH_2$ yields around 8.40 mL/gVS (ISR0.07) during 3 days of fermentation.

On the basis of our results, considering a H_2 lower heating value of 121 MJ/kg and a potential amount of OFMSW undergoing a DF process in the amount of 1 t, we obtain a theoretical yields of 6.95 kWh for ISR0.07 (the best performer) and 5.71 kWh for ISR0.1 (the worst performer) at 37 °C.

In our case, the best performance in terms of $BioH_2$ yields (mL/gVS) was obtained in the treatment ISR0.07, which increased the $BioH_2$ concentration in the biogas of about 20.8 % compared to ISR0.14 and an increases the $BioH_2$ yield of 16.5 % compared to ISR0.01.

Future investigations, aimed at maximizing the $BioH_2$ production from DF of OFMSW by optimizing both FW composition and ISR, should be carried out.

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