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Mesophilic Dark Fermentation of Food Waste for Biohydrogen Production in a Mixed Batch Reactor

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Biohydrogen is considered a promising biofuel of strategic interest characterized by high energy content per mass unit, easiness in producing electricity through fuel cells, a process that produces water as the only by-product. Among the bioprocesses aimed at biohydrogen production, dark fermentation is gaining a worldwide interest for being both environmental friendly and relatively efficient. In this perspective, food waste represents a good substrate for biohydrogen production through dark fermentation, being made of biodegradable organic matter and nutrients essential for the growth of microorganisms driving this process.
In this study, biohydrogen production through a mesophilic batch dark fermentation (37 °C) was performed. The effect of the progressive adaption of the inoculum on the food waste feedstock was evaluated. Both the liquid and the gas phases produced during the incubation were analyzed. Microbial biomass, pH, biogas volume and composition were also monitored. Biohydrogen yields increased with progressive inoculum acclimation to the food waste, reaching about 35 mL/gVS_{FW} in 3 days of dark fermentation, with an average biohydrogen in the biogas produced of 55 %, corresponding to a theoretical energy recovery of about 34 kWh for 1 ton of food waste.

1. Introduction

The ever increasing worldwide urbanization and industrialization have considerably augmented the amount of municipal solid waste (MSW) (Al-Zuhairi et al., 2015). An improper management of such waste increases environmental pollution and the greenhouse gases (GHG) emissions contributing to worsen the ecosystem quality (water and land pollution, and biodiversity loss), affecting the global climate (Grimm et al., 2008), and facilitating the diffusion of infectious diseases and degenerative illnesses (Ejaz et al., 2010). The production of biofuels from organic waste helps to alleviate the waste disposal problem producing in the meantime renewable energy.The food waste (FW) content is estimated in the range of 25 % and 70 % of the MSW (Matsakas et al., 2017). Due to its biodegradability and higher carbohydrate content, FW has a greater potential as feedstock for the biofuel production (particularly biohydrogen) than fat- and protein-rich biomasses (Yun et al., 2018; Lay et al., 2003).
Biohydrogen ($BioH_2$) is considered a promising fuel of strategic interest characterized by high energy content per mass unit, easiness in producing electricity through fuel cells (FCs), and generating water as the only by-product. Currently, H_2 is produced by polluting fossil-based processes emitting GHG (as steam reforming of natural gas) while $BioH_2$ production is environmentally friendly and less energy consuming, compared with the thermo-chemical processes currently in use (Yun et al., 2018). Traditional electrolysis process has the disadvantages of being very demanding in energy, that reach about 80 % of the operating cost of H_2 production (Karthic and Joseph, 2012). Biological methods for the H_2 production are a promising alternative to the traditional ones. They include photosynthetic and fermentative processes, however fermentative H_2 production is the most feasible and more used (Karthic and Joseph, 2012).

$BioH_2$ can be produced by the dark fermentation (DF) process through fermentative conversion of organic feedstock, in the absence of light. The complex bioconversion process is carried out by various microbial consortia (facultative and obligate anaerobic bacteria), involving three biochemical steps similar to anaerobic digestion (AD): hydrolysis, acidogenesis and acetogenesis. A key aspect for the DF optimization is the inhibition of methanogenesis that usually follows the acetogenetic phase. This can be achieved simply controlling the pH to a value promoting $BioH_2$ production (Khanal et al., 2004). H_2-producing microorganism's activity found its optimum at pH from 4.5 to 6 (Fan et al., 2004; Zhu et al., 2006), while the optimum pH for methanogenic microorganisms is between 6.0 and 7.5 (Chandrasekhar et al., 2015).
The use of FW as DF feedstock could contribute to reduce the costs of $BioH_2$ production alleviating, in the same time, the problem of waste management and disposal. In addition, the progressive acclimation of the H_2-producing microorganism to the waste feedstock, could allow to increase the $BioH_2$ yield produced without recurring to pre-treatments that are expensive from both an economic and environmental point of view. This is in agreement to Abreu et al. (2009) and Scoma et al. (2013), who carried out inoculum acclimation on different feedstock (L-arabinose and olive mill wastewaters). According to Gomez et al. (2009), acclimatized inoculum to slaughterhouse waste had better performance in terms of stability of the bioprocess.
In this work, $BioH_2$ was obtained through single-stage DF. The effect of the progressive inoculum acclimation to the FW feedstock was evaluated. The incubation was performed in a mixed batch reactor in mesophilic conditions (37 °C).

2. Materials and Methods

2.1 Food waste

Food leftovers was used to prepare FW mixture in laboratory, with the following composition: 30 wt% fruits, 5 wt% cooked meat, 30 wt% vegetable and 35 wt% bread (Florio et al., 2017). The mixture was incubated for 3 days at about 30 °C in an incubator (Infors HT Minitron, Bottmingen/Basel, Switzerland). The waste mixture was at first grossly chopped manually, then finely shredded with a home blender (Termozeta®, Milan, Italy) and finally pressed in a mortar to make a puree before being added to a batch digester. The physical-chemical characteristics of FW were analyzed by thermogravimetric analysis (Table 1), according to Florio et al. (2017).

2.2 Inocula

Semi-liquid digestate obtained from a full scale AD plant operating in the Naples region (Italy) was used as starting inoculum. The inoculum was kept at room temperature in anaerobic conditions for 1 h prior to use and added to the feedstock at the inoculum/substrate ratio of 0.5 (wet weight) (Florio et al., 2017). Total solids (TS) and moisture were measured according to APHA (2005) (Table 1). This inoculum was initially acclimated to FW feedstock in a batch lab-scale AD operating at 37 °C for 21 days.
The liquid digestate coming from this incubation was used in a first DF test (I FW), then the resulting liquid digestate was used in a second DF test (II FW).

Table 1: Characterization of FW and inoculum (for FW: Florio et al., 2017)

	Total Solids (wt%)	Volatile Solids (wt%)	Moisture (wt%)
FW	36.5	31.3	63.5
Anaerobic digestate inoculum	9.0	-	91.0

2.3 Dark fermentation batch experiments

Crimped Pyrex® bottles with perforable butyl rubber septa were used as batch digester. The digesters were filled with 15 % w/v of FW, inoculated with 20 % v/v of I FW and II FW inocula. Then, distilled water was added to obtain a total volume of 100 mL. Before starting DF process, the pH was corrected with H_2SO_4 to reach value around 5 in order to inhibit the methanogenic activity. To prevent shift during incubation, the pH was stabilized with Na_2CO_3. Anaerobic conditions were assured by bubbling N_2 in the inoculated feedstock for 10 min and then the bioreactors were placed in an anaerobic incubator (Binder, Tuttlingen, Germany) at 37 °C continuously mixed by a magnetic stirrer.

2.4 Biogas volume analysis

Batch digesters were connected to an upturned bottle (125 mL) by a capillary tube equipped on both ends with a needle. Upturned bottle was entirely filled by water and the biogas volume was calculated measuring the water displaced through a needle in the upturned glass bottle (Al-Zuhairi et al., 2015).

2.5 Analytical methods

Samples of liquid phase from crimped vials were collected for monitoring bioconversion process. Optical absorbance at 600 nm (OD_{600}) from a 1:10 diluted sample was analyzed to monitor the variation of microbial biomass. pH was measured using a 740 pHmeter (WTW, Weilheim, Germany). Samples of gas phase were collected from upturned bottle and biogas composition was determined by GC analysis using a HP 5890 series II equipped with double packed molecular sieves column Porapack^{TM} and a TCD detector (Ausiello et al., 2017).
Biogas volumes were normalized at standard conditions (25 °C, 1 bar).

3. Results and discussion

3.1 Dark fermentation tests

Figure 1 shows the trend of microbial growth during the fermentation measured as optical density at 600 nm (OD_{600}). In the first 24 h the OD_{600} reached the higher value in treatment II FW (1.93 ± 0.06) compared to treatment I FW. The opposite was observed at 48 h of incubation when I FW outcompeted II FW reaching an OD_{600} value of 2.11 ± 0.06. Between 48 and 72 h there was a slight decrease of the amount of microorganisms in both tests (2.02 ± 0.06 for I FW and 1.95 ± 0.06 for II FW). II FW test had an average OD_{600} value higher by 6.06 % than I FW test during the entire retention time in the bioreactor. In addition, the amount of microbial biomass was higher by 31.6 % at the staring time of incubation for II FW compared with I FW treatment.
In Figure 2 are reported pH values during the incubation of I FW and II FW treatments. A marked acidogenic phase was evident in the first 24 h of incubation in both I FW (pH 3.84 ± 0.04) and II FW (pH 3.61 ± 0.04). The pH remained more or less constant, keeping average values of 3.73 ± 0.09 for both tests between 24 and 48 h. At the end of the incubation the pH tends to stabilize on less acidic values reached the value of 3.95 in both samples, with an increase of about 6 %.
Figure 3 and 4 shows the biogas production and composition during DF. The cumulative biogas production was about 7 % higher in the treatment II FW than in I FW, with a more regular production over time. The quantity of $BioH_2$ contained in the biogas produced by treatment II FW was 39 % higher than I FW, highlighting that the progressive acclimation of the inoculum to FW feedstock improves the $BioH_2$ production and consequently the energy recovery. The amount of $BioH_2$ produced by our lab-scale apparatus is comparable to the yields reported by Pan et al. (2008), Gomez et al. (2009) and Redondas et al. (2012). In addition, a possible scale-up of our process could beneficiate from the short retention time that characterizes our experiment. Final volumes produced and yields of biogas and $BioH_2$ obtained are summarized in Table 2.

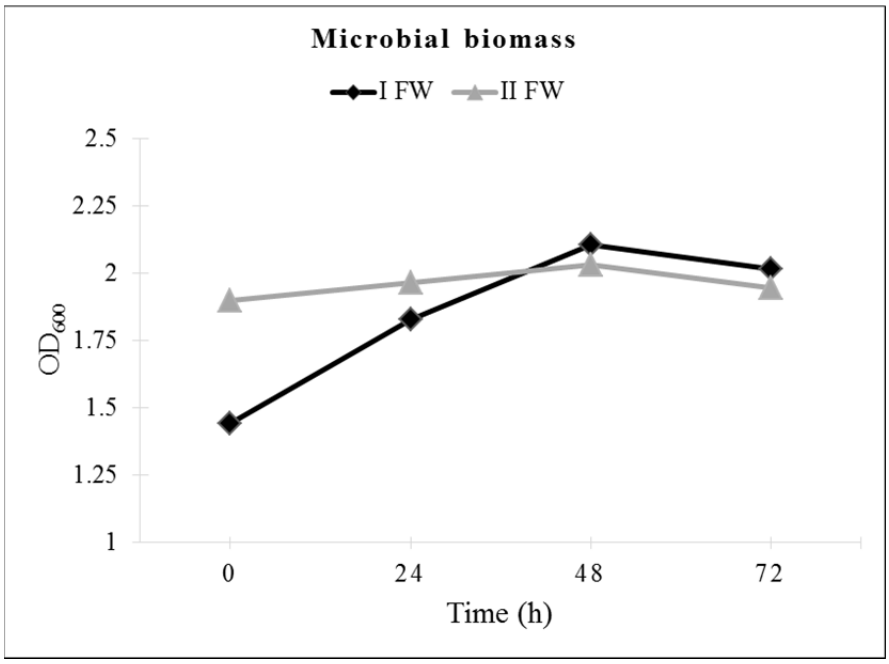

Figure 1: Microbial biomass OD_{600} 1:10 for DF batch experiments (I FW and II FW)

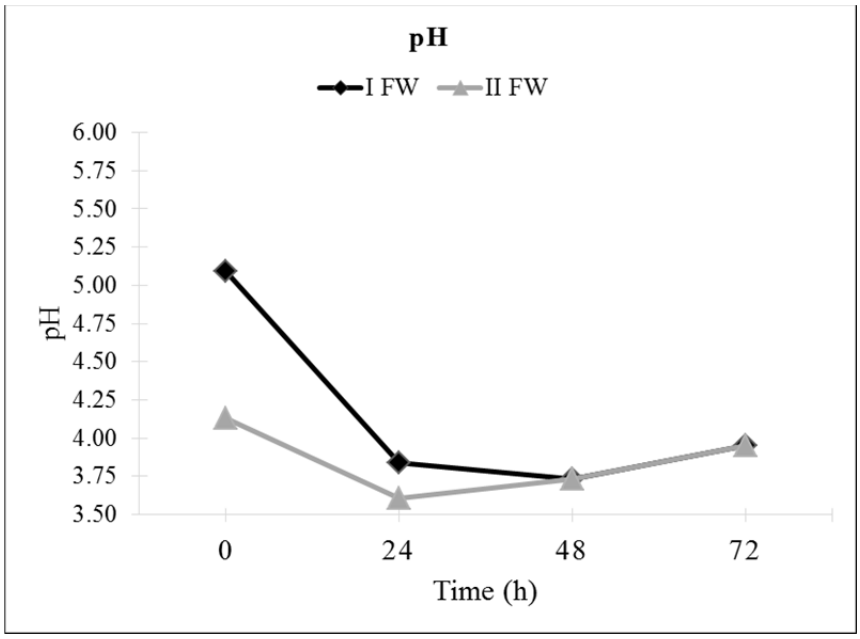

Figure 2: pH for DF batch experiments (I FW and II FW)

Table 2: Cumulative biogas production and $BioH_2$ yields at the end of fermentation

Test	Cumulative biogas (mL)	Cumulative $BioH_2$ (mL)	Average $\%H_2$ in the biogas (%v/v)	$BioH_2$ yields (mL/gVS_{FW})	HRT (days)	Y increase (%)
I FW	277 ± 6.5	117 ± 2.7	42 ± 1	24.9 ± 0.6	3	-
II FW	296 ± 7	163 ± 3.8	55 ± 1.3	34.6 ± 0.8	3	39

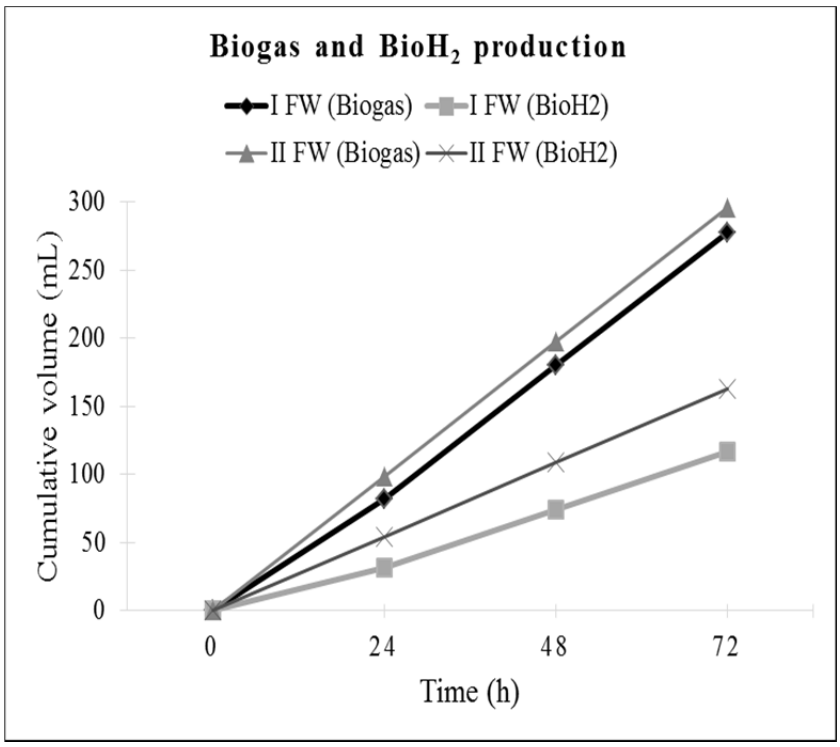

Figure 3: Cumulative biogas production for DF batch experiments (I FW and II FW)

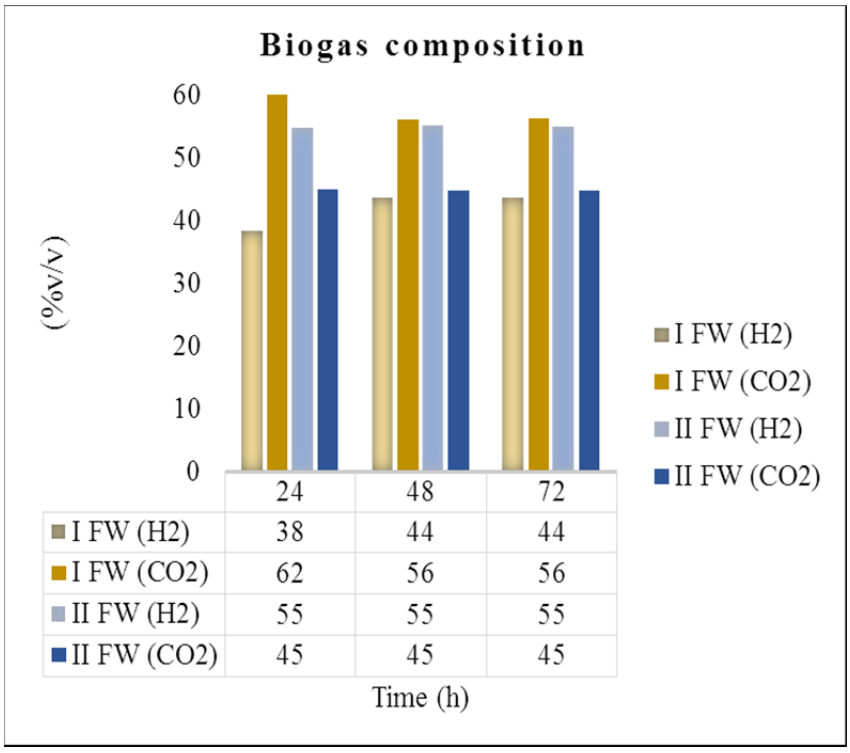

Figure 4: Biogas composition for DF batch experiments (I FW and II FW)

3.2 Energy recovery

Table 3 shows the theoretical kWh produced by our DF experiments in terms of $BioH_2$ yield. For calculation we used a H_2 heating value of 11 MJ/m^3 scaling-up the results of our lab experiment to a hypothetical full-scale DF plant treating 1 ton of FW with a VS content of 31 wt%. Our calculations foresee a theoretical yield of 24.1 kWh/ton_{FW} in case of treatment I FW and 33.6 kWh/ton_{FW} for treatment II FW during a DF of 3 days in mesophilic condition. In line with the increase in the overall yields of the process, progressive adaption of inoculum allows to obtain a raise of theoretical kWh/ton_{FW} yields of about 39 % for treatment II FW compared to the I FW one.

Table 3: Theoretical kWh/ton_{FW} produced during DF tests

Adaption	Yields (mL/gVS_{FW})	$BioH_2$ (m^3 for 1 ton of FW)	kWh/ton_{FW} (theoretical)	HRT (days)	Y increase (%)
I FW	24.9	7.8	24.1	3	-
II FW	34.6	10.9	33.6	3	39

The biogas produced, which contains both H_2 and CO_2, can be used for fueling FCs, in particular solid oxide fuel cells (SOFC). A 100 kWh FCs-SOFC fed with H_2 and CO_2 (η of 50 %) would need a quantity of $BioH_2$ resulting from 6083 kg of FW used as DF feedstock, in case of the II FW inoculum (yield 11 mL/g_{FW}).

4. Conclusions

This work was aimed at optimizing $BioH_2$ production through a DF process using inocula with different degree of acclimation. We obtained the maximum $BioH_2$ yields (34.6 ± 0.8 mL/gVS_{FW}) during 3 days of batch fermentation, using a highly acclimated inoculum (II FW). $BioH_2$ production and yields increased with progressive inoculum adaptions to the feedstock, reaching a $BioH_2$ yield about 39 % higher than those obtained using the intermediate acclimated inoculum (I FW).

Our results demonstrated that one of the most relevant parameters driving the $BioH_2$ production in DF processes, apart the technical characteristics of the DF plant, is the degree of acclimation of the inoculum to the peculiar feature of the feedstock.

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