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Biomass Enrichment and Scale-up Implications for Dark Fermentation Hydrogen Production with Mixed Cultures

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Biohydrogen (H_2) production via dark fermentation (DF) is a promising renewable energy technology. In the process, the enrichment of microbial communities producing hydrogen is very important as their activities considerably affect the overall performance. To this aim, spore-forming microorganisms as e.g. *Clostridium* species, which are commonly present in anaerobic digestate and wastewater sludge are suitable for enrichment, although pre-treatments of these biomass sources are needed.
This work evaluates the following pre-treatment methods: acid treatment, heat shock (at 95 ^{0}C and 105 ^{0}C) and load shock pre-treatment, keeping into account the scale-up of DF systems. The effectiveness of pre-treatment methods was assessed by conducting bio-hydrogen potential (BHP) tests fed with glucose and comparing the following H_2 production performance parameters: (i) cumulative H_2 production; (ii) H_2 production rate; (iii) length of the lag phase; (iv) process intermediates production. The analysis of results showed that the load shock pre-treatment is the most efficient. Hence, further insights are also given on the safety aspects concerning the production and storage of H_2, and on the importance of operational costs and feasibility of the pre-treatment methods.

1. Introduction

The progressive running down of fossil fuel reserves coupled with the need of reducing the greenhouse gas (GHG) emissions in the atmosphere has made the development of new, renewable and environmental friendly energy sources very crucial. Hydrogen (H_2) biologically produced from organic wastes seems to be really promising, due to its efficient hydrogen to power conversion coefficient (3.0 kWh/Nm^3), high energy density (142 MJ/kg) and harmless combustion by-products (Das & Veziroglu 2008). To this aim, either photo fermentation (PF) or dark fermentation (DF) processes have been successfully used to biologically produce H_2 from organic sources. However, DF is usually preferred to PF due to lower operational costs and process conditions at ambient temperature and pressure (Das & Veziroglu 2008).
The biological conversion of organic sources into H_2 is obtained either by using biomasses consisting of pure cultures or composed of mixed cultures. Mixed systems are generally less performing in terms of H_2 yields, but are easier and less expensive to handle as they do not require any asepsis procedure and can be fed with several different substrates (Valdez-Vazquez 2004; Li & Fang 2007; Wang & Wan 2009; Wong et al. 2014). Here it is worth noting that mixed bacteria communities with the ability of producing H_2 are intrinsically present in soils, sediments, sludge from wastewater treatment plants, compost, cow dungs, municipal organic solid

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wastes (Valdez-Vazquez 2004; Wong et al. 2014). Hence, this communities can be enriched by appropriate pre-treatment methods, although higher H_2 production rate can be only obtained if H_2 consuming organisms such as methanogens and homoacetogens are inhibited (Li & Fang 2007; Wang & Wan 2009).

The most commonly and successfully used biomass pre-treatment methods include heat (Wang & Wan 2008), acid (Wang & Wan 2008), base (Zhu & Beland 2006), and load shock (Luo et al. 2010) as well as aeration (Giordano et al. 2014). These methods are based on the observation that when the biomass experiences inhospitable environmental conditions, H_2 producers survive due to their ability in forming spores (e.g. *Clostridium)* that protect them from the adverse conditions, hence returning to be effective again when the environmental conditions turn to be favourable again as the spores germinate (Li & Fang 2007). Besides, the H_2 consumers may not survive unless with same capacity.

The effectiveness of these pre-treatments on H_2 production depends on nature of biomass, which in turn can cause the occurrence of inconsistency in results from lab scale experiments(Wang & Wan 2009). Therefore a deeper knowledge of the effects that pre-treatment methods have on H_2 production from DF is necessary before operating the scaling up of these methods as well as, being H_2 highly flammable and explosive, safety aspects in large-scale reactors is also a primary concern.

The aim of this paper is to evaluate the effectiveness of the following pre-treatment methods (i) acid shock treatment, (ii) heat shock treatment and (iii) load shock pre-treatment on H_2 production through bio-H_2 potential DF batch (BHP) tests. The evaluation have been done by analysing the following parameters from the BHP tests: (i) cumulative H_2 production; (ii) H_2 production rate; (iii) length of the lag phase; and (iv) production of process intermediates.

Furthermore, this study also deals with the safety aspects concerning the production and storage of H_2 (EPA, 2011) and highlights the relevance of operational costs, feasibility and complexity of the pre-treatment methods in scaled up systems.

2. Materials and methods

Biomass used to perform the BHP tests was collected from the anaerobic digester treating dairy waste produced by the factory "La Perla del Mediterraneo" located in Capaccio (Salerno, Italy). The total solids (TS) and volatile solids (VS) content of biomass were 2.79±0.05% (**w/w** on wet mass) and 67.2±0.4% (**w/w** on dry mass) respectively. The sludge was stored at 4 ^{0}C before being used. The BHP tests were fed with glucose.

All BHP tests were carried out in 1000 mL transparent borosilicate glass bottles GL 45 (Schott Duran, Germany) used as DF batch reactors and placed in a water bath maintained at 34±1 °C by a thermostat (ALEAS AL 2201, 150 W). In the batch reactors, airtight conditions were provided with caps sealed with silicon. Each bottle was equipped to sample the internal mixture and spill out the gas. BHP tests were carried out in duplicates at the initial pH of 7.

Heat shock treatments were carried out by heating the biomass at 105 ºC for 4 hours (HST-105ºC) and at 95 ºC for 45 minutes (HST-95ºC); acid shock treatment (AST) was performed by adjusting the pH of the biomass at pH 3 using 1 M HCl for 24 hours and then turning pH back at 7 using 1 M NaOH; load shock (LST) treatment was carried out by feeding the batch reactors with 85 g COD/L of glucose followed by acidification process for 4 days and finally extracting the supernatant after a settlement process and replacing the extracted liquid volume with distilled water. A substrate to biomass ratio of 0.85 g COD glucose/g VS biomass was maintained in all BHP tests. Once the cumulative H_2 production in the reactors reached a stable value (Load I), the reactors were furthermore fed with 4.5 g of glucose (Load II).

The volume of gas produced from each BHP tests was measured on daily basis by acid solution (1.5 % HCl) displacement method. The biogas volumes were corrected for moisture at 0 ◦C and 1 atm (NmL) and reported as the daily average. H_2, CO_2 and CH_4 content in gas were measured with Varian Star 3400 gas chromatograph equipped with ShinCarbon ST 80/100 column provided with a thermal conductivity detector and argon as carrier gas. Samples of the digesting mixture collected from each reactor to measure the volatile fatty acids (VFAs) content and their composition were preliminarily extracted at 80^{0}C according to the head space-solid phase micro-extraction technique (HS-SPME) (Abalos et al., 2000) and subsequently analysed with gas chromatograph equipped with mass spectrometry provided with helium as carrier gas. The pH was measured with a pH meter (WTW, inolab, pH level 2). The TS and VS content of biomass and organic wastes were determined according to Standard Methods (APHA 2005).

The modified Gompertz relationship (Eq. 1) was used to model the H_2 production from BHP tests (Wang & Wan 2008). The equation contains 3 parameters: i) cumulative H_2 production potential H_o (mL), ii) H_2 production rate R (mL/h), iii) lag time λ (h). H_o, R and λ were estimated from BHP test by using the Curve Fitting Toolbox in MATLAB®.

$$\mathrm{H(t)} = H_o \cdot \mathrm{e}^{-\mathrm{e}^{\left[\frac{R.\mathrm{e}}{H_o}\right](\lambda-\mathrm{t})+1}} \qquad (1)$$

Where t is the time.

3. Results and discussions

The results from BHP tests are shown in Figures 1 and 2 and Tables 1 and 2. In Figure 1, the effects of different biomass pre-treatment methods are represented by plotting the average cumulative H_2 production, whereas in Tables 1 and 2, the same effects are evaluated comparing the specific H_2 production and the parameters calibrated by using equation 1.

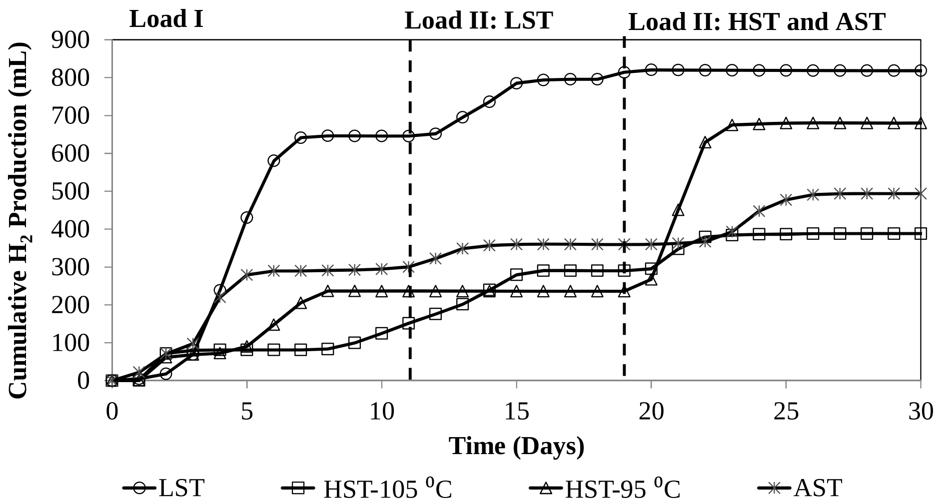

Figure 1: Average Cumulative H_2 Production in BHP tests

From Figure 1 and data in Tables 1 and 2, it can be noted that LST gave better H_2 production performance with highest specific H_2 production (143.5±13.2 NmL/g glucose), H_2 production rate (9.4 NmL/h) and a lag phase slightly longer (0.53 hour) than HST-95ºC, while AST showed the lowest lag time (13.57 h). AST also gave the good performance in terms of cumulative H_2 production (373.1 NmL) whereas unsatisfying results were obtained in BHP tests with HSTs. From the analysis of methane content in biogas, it can be concluded that there were negligible methanogenic activities in the tests with LST, HST-105ºC and AST whereas the BHP tests with HST-95ºC was incompetent to completely inhibit the methanogenic microorganisms, which could explains the lower H_2 production.

Table 1: Effects of biomass pre-treatment methods on biohydrogen production performance during Load I

Pre-treatment method	Modified Gompertz model[a]			
	Ho (Nml)	*R* (Nml/h)	λ (h)	R^2
LST	657.8	9.40	69.94	0.9980
HST-105 ºC	341.6	1.28	138.98	0.9880
HST-95 ºC	238.9	2.44	69.41	0.9910
AST	373.1	1.52	13.57	0.9953

[a]The parameters were determined based on average cumulative daily H_2 production during Load I

After the batch reactors were fed with a second load of glucose (Load II), the H_2 yield decreased in the BHP tests with LST, HST-105ºC and AST whereas it increased in tests with HST-95ºC (Figure 1). In Table 2 the specific H_2 production obtained from the first (Load I) and the second (Load II) feeding operation as well as the respective pH values at the beginning and at the end of the BHP tests are compared. Figure 2 shows the major fermentative products accumulated at the end of the BHP tests. The production of intermediates (VFAs) and pH values were monitored in order to evaluate the performance of DF process. A possible reason for the lower H_2 yield than expected when a LST was performed could actually be explained with the occurrence of the inhibiting effect due to the high butyric acid accumulation in the reactor, as indicated in the study published by Van Ginkel & Logan (2005), whereas a low pH (3.7±0.44) could be the cause of the lower H_2 production in AST during Load II.

Table 2: Comparison between Load I and Load II feeding operations

Pre-treatment Method	NmL H_2/g glucose (Load I)	mL H_2/g glucose (Load II)	Initial pH	Final pH Load I	Final pH Load II
LST	143.5±13.2	38.4±17.4	7±0.01	5.3±0.01	4.9±0.02
HST-105 ºC	64.5±12.7	21.8±5.1	7±0.01	5.2±0.00	4.5±0.02
HST-95 ºC	52.5 ±3.4	98.7±23.9	7±0.01	5.4±0.01	4.6±0.02
AST	79.9±22.3	29.8±5.0	7±0.01	4.5±0.16	3.7±0.44

± indicates data range based on duplicate samples

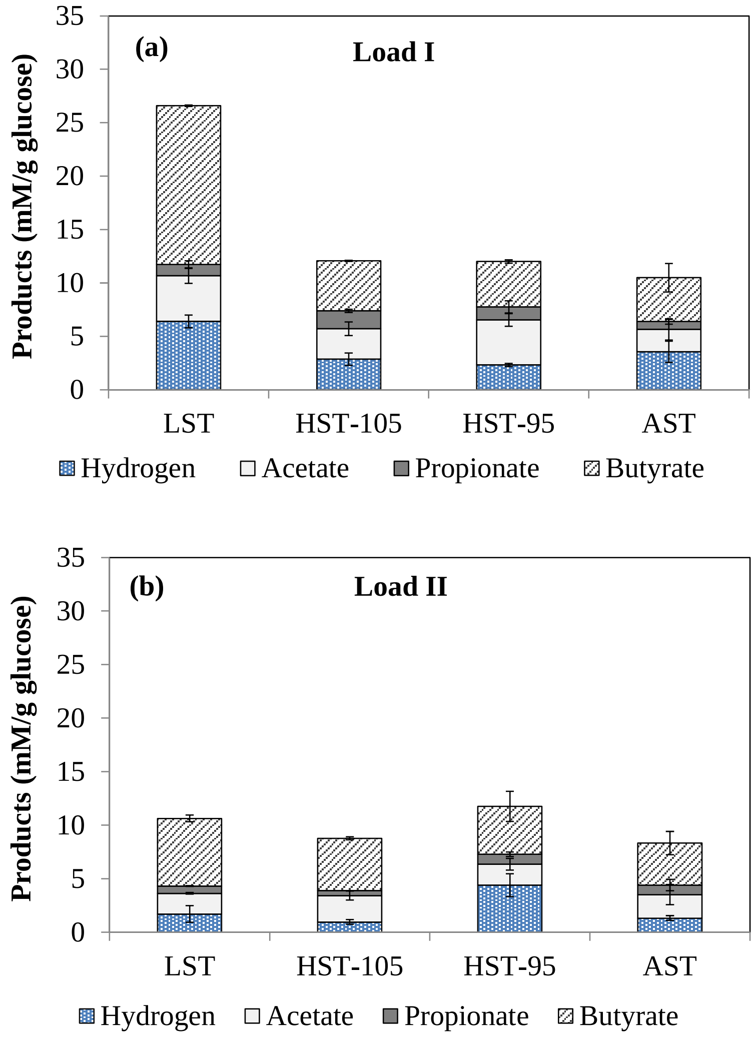

Figure 2: Major fermentative end products in the BHP tests with different biomass pre-treatment methods f (a) Load I and (b) Load

In order to select and set up a method to pre-treat the biomass in full scale reactor, several parameters need to be considered: the operational costs, the feasibility and complexity of the method as well as the time required to enrich the biomass with hydrogen producing bacteria (safety will be considered in the next section) Table 3 shows a simple evaluation of the parameters based on this study and literature data for the four pre-treatment methods investigated in this paper. HSTs show a high energy demand which makes them less attractive in a full scale application. AST requires large amounts of acid and base solutions. LST is more feasible to be used in a full scale reactor due to lower operational costs compared with the other methods.

Table 3: Evaluation of biomass pre-treatment methods for DF process

Pre-treatment method	Energy Requirement	Chemical Requirements	Operational Costs	Scale-up application
LST	+ +	+	+	+ + +
HST-105 ºC	+ + +	+	+ + +	+ +
HST-95 ºC	+ +	+	+ + +	+ +
AST	+	+ + +	+ +	+ + +

+ Less intensive; ++ Moderately intensive; +++ Very Intensive (Adapted and modified from Ghimire et al. 2015)

The H_2 production and process performance are strongly influenced by many factors such as physico-chemical properties of substrate and co-substrates, type of biomass sources, reactor configuration, and operational conditions. Luo et al. (2010) actually evaluated the effects of different pre-treatment methods on mixed culture for H_2 production using cassava stillage as substrate and found differences in H_2 yields only when DF was performed in batch reactors, whereas no difference was noticed in continuous DF processes.

4. Safety considerations on scale-up

Several accidents can be found in the literature due to severe reactivity of biogas. Hence, specific analyses are due for this mixture for the correct design of prevention and mitigation systems (e.g. venting, suppression), and for the structural design of the reactors, including auxiliary and transportation systems (USEPA, 2011).
When batch reactors are adopted, the isochoric-isotherm option should be considered for the hazard of hydrogen mixture. By using the ideal gas equation, the calculated maximum pressure in the lab reactors varied from 2.11 to 2.15 bar, considering a reactor head space 540 mL, reactor temperature of 35 °C and ambient conditions 25 °C and 1 bar for the measurement of the biogas. Quite clearly, due to anaerobic conditions, the reactors are flushed with nitrogen and no hazards are predicable unless oxygen (air) leakage due to rapid depressurisation and oxygen (air entrance). On the other hand, the continuous operations adopted in large scale reactors are normally operated under ambient conditions and air. Hence, a deflagration or even a detonation of the mixture of hydrogen possibly mixed with several other oxidation components which are typical in large-scale biomass operation as CO, CO_2, methane and other low-weight gases, including toxic H_2S, may occur.
The literature on the safety characterisation of complex biogas mixtures is very scarce and mainly based on experimental observations, as no additive methodologies are applicable for the definition of flammability limits, burning velocity, and for the definition of occurrence of dramatic scenarios as deflagration to detonation transition or combustion-induced Rapid Phase Transitions (Cammarota et al., 2009; Salzano et al., 2012).
In large scale reactors, H_2 might ranges between 40% to 50% v/v however with inerts as CO_2 (50-60% v/v) and water vapour (1-5%) and operation are conducted under thermophilic temperature ranges (55-60 °C) in comparison or mesophilic reactors (35-40 °C). For ambient conditions, the analysis reported in Di Benedetto et al. (2009) may be adopted, which clarified the effect of CO_2 on H_2 burning, which is essentially thermal, and the ranges of adiabatic flame temperature (i.e. adiabatic pressure) and laminar burning velocity for the given mixtures obtained by means of both experimental and numerical analysis. Stable flames (for the use in combustion equipment) or, conversely, flame extinguishing (for fire and explosion safety) are obtained, at ambient temperature, for CO_2 larger than 40% v/v in air, hence in the presence of N_2. The effect at higher temperature has to be defined in future works.

5. Conclusions

The evaluation of results from the BHP tests and the analysis of different pre-treatment methods suggest that LST of biomass can favour the development and growth of an efficient H_2 producing bacteria community to start-up and handle up-scaled DF systems. Moreover, a monitoring of metabolites production and pH can give useful information on process performance and its reliability, thus helping to prevent VFAs accumulation and the subsequently occurrence of inhibition phenomena affecting the H_2 producing biomass activity. Also, safety aspects need to be taken into consideration in the up-scaled DF systems during H_2 production, storage and application.

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