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Dark fermentation of complex waste biomass for biohydrogen production by pretreated thermophilic anaerobic digestate

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ABSTRACT

The Biohydrogen Potential (BHP) of six different types of waste biomass typical for the Campania Region (Italy) was investigated. Anaerobic sludge pre-treated with the specific methanogenic inhibitor sodium 2-bromoethanesulfonic acid (BESA) was used as seed inoculum. The BESA pre-treatment yielded the highest BHP in BHP tests carried out with pre-treated anaerobic sludge using potato and pumpkin waste as the substrates, in comparison with aeration or heat shock pre-treatment. The BHP tests carried out with different complex waste biomass showed average BHP values in a decreasing order from potato and pumpkin wastes (171.1 ± 7.3 ml H_2/g VS) to buffalo manure (135.6 ± 4.1 ml H_2/g VS), dried blood (slaughter house waste, 87.6 ± 4.1 ml H_2/g VS), fennel waste (58.1 ± 29.8 ml H_2/g VS), olive pomace (54.9 ± 5.4 ml H_2/g VS) and olive mill wastewater (46.0 ± 15.6 ml H_2/g VS). The digestate was analyzed for major soluble metabolites to elucidate the different biochemical pathways in the BHP tests. These showed the H_2 was produced via mixed type fermentation pathways.

1. Introduction

During the last decades, several technologies for biohydrogen production have been proposed (Hallenbeck et al., 2012). One of the most promising and widely investigated processes is dark fermentation (Das and Veziroglu, 2008). The possibility of using several types of renewable biomass (including organic waste), the wide range of operational temperature and pressure conditions, and the high biohydrogen production rates makes dark fermentation an attractive option for biohydrogen production. The choice of substrate plays an important role in dark fermentation processes, either in terms of maximizing the biohydrogen yield or in the economy of the process. Moreover, the biohydrogen potential (BHP) mainly depends on the substrate composition in terms of lipids, proteins and most essentially on the carbohydrate content present in soluble form. Several studies have found a strong correlation between the soluble sugar content with the BHP, i.e. a strong positive correlation of the BHP with the initial carbohydrate content of the substrate and a negative correlation with the initial protein content (Guo et al., 2013; Monlau et al., 2013, 2012; Shi et al., 2010). Carbohydrate rich substrates, such as pure glucose, sucrose and starch mixtures are extensively used in dark fermentation studies. In more recent works, however, the use of more complex substrates has been considered, i.e. the organic fraction of municipal solid waste (OFMSW); agricultural lignocellulosic biomasses like wheat straw and corn stalks as well as agro-industrial wastes like waste from the food processing industries, effluents from livestock farms and aquatic plants (De Gioannis et al., 2013; Guo et al., 2010; Wong et al., 2014; Yasin et al., 2013). Based on their availability, novel substrate sources need to be identified and assessed for their BHP.

The Campania Region (Italy) is well known for its agricultural and livestock products, such as buffalo milk (for mozzarella production), fruits and vegetables, olive mill industries (about 25% of the olive mill industry worldwide is located in Italy and most of them are in Campania). The intense agro-industrial activities provide a wide range of organic by-products and waste biomass such as buffalo manure, fruits and vegetables waste, slaughter house waste, olive mill waste. These complex wastes rich in carbohydrates, proteins or lignocellulosic biomass could be valuable

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feedstock sources for bio-energy production. Dark fermentation processes can be set up in order to valorize these waste biomasses by recovering biohydrogen. However, literature data on BHP of these complex agro-industrial waste biomasses are scarce and reported only in a few studies (Gonçalves et al., 2014; Guo et al., 2013). This work has therefore focused on the following objectives: i) to evaluate the effect of inoculum pre-treatment methods on the BHP using real agro-industrial waste substrates, ii) determine the BHP of several agro-industrial wastes and iii) to characterize the dark fermentation effluents in terms of VFA composition and concentration.

This study is aimed at evaluating the BHP of the agro-industrial substrates typical from the Campania Region (Italy). The BHP of complex waste biomass in a mixed dark fermentative system is influenced by a number of parameters such as substrate composition, substrate pre-treatment, inoculum source and enrichment methods, bioreactor design and operation (De Gioannis et al., 2013; Ntaikou et al., 2010; Wong et al., 2014). A mixed culture of hydrogen producing biomass is usually preferred over pure cultures due its practicability for the use of non-sterile waste biomasses. Nevertheless, enrichment of hydrogen producing bacteria and inhibition of hydrogen consumers becomes essential when animal manure, wastewater sludge or soil sediments are used as inoculum sources (Giordano et al., 2014; Wong et al., 2014). When applying different sludge pre-treatment methods to enrich hydrogen producing microorganisms, inconsistent results have been obtained (Luo et al., 2010; O-Thong et al., 2009; Venkata Mohan et al., 2008; Wang et al., 2011). Most of these studies have been conducted using simple carbohydrate sources, such as glucose, as the substrate. Thus, the effectiveness of inoculum pre-treatment methods for the source of inoculum and complex real substrates selected for BHP tests needs to be evaluated.

Parameters like H_2 yield and soluble metabolite production can be used as performance indicator of the BHP tests. Specially, when mixed dark fermentative cultures are applied, fermentation byproducts, which mainly include volatile fatty acids (VFAs) and alcohols, can elucidate the performance of the BHP tests (Hawkes et al., 2007). Some authors (Hawkes et al., 2007; Kim et al., 2006) have proposed to use the B/A (butyric to acetic acid) ratio as a quantitative indicator of biohydrogen yield associated with microbial metabolic pathways. Conversely, Guo et al. (2013) recommended carefulness when using such a ratio because of the effect of homoacetogenesis in the batch tests.

2. Materials and methods

2.1. Inoculum

The sludge collected from the anaerobic digestion plant of the dairy farm “La Perla del Mediterraneo” located in Capaccio (Salerno, Italy) was used as inoculum. The plant features includes a 100 m^3 CSTR operating at 52–56 °C, a hydraulic retention time of 24 days and operating in the pH range of 7.4–7.5. The plant continuously fed with buffalo manure, cheese whey of buffalo milk and sludge from an industrial wastewater treatment plant. The inoculum was stored at 4 °C and its total solids (TS) and volatile solids (VS) contents were 17.7 gTS/L and 10.5 gVS/L, respectively.

Table 1
Characteristics of organic waste types used in this study.

Waste types	Total solids (%)	Volatile solids (%)
Buffalo manure	7.03	5.76
Olive oil mill wastewater	6.55	4.78
Olive pomace	28.8	25.20
Fennel waste	5.25	4.46
Dried blood (Slaughterhouse waste)	93.59	82.90
Potato waste	16.87	15.00
Pumpkin waste	11.61	8.40

2.2. Feedstock preparation

The BHP was measured for different biomasses collected from various agro-industries and local markets located in the Campania region, with TS and VS contents presented in Table 1. The effect of the inoculum pre-treatment methods on the BHP tests was investigated with a mixture of potato and pumpkin (13.45 g and 13.48 g, respectively), prepared in the laboratory and stored in frozen conditions (<−4 °C).

2.3. Experimental design and procedures

All dark fermentation experiments were carried out in air tight 1000 ml transparent borosilicate glass bottles GL 45 (Schott Duran, Germany), placed in a water bath maintained at 35 ± 1 °C by a thermostat (mesophilic conditions). The experiments were carried at the natural pH of the inoculum and substrates, which was in the neutral range of 6.8–7.4, despite that an optimum pH of 4–5.5 has been reported in the literature for mixed culture systems (Ntaikou et al., 2010; Wang and Wan, 2009). The batch reactors were provided with air tight caps sealed with silicon and sampling pipes for collecting digesting mixtures for volatile acids samples and measuring the biogas produced from the reactors. The experiments were carried in duplicate and the average values were considered for the evaluations, while the data range based on the duplicate samples is provided and indicated by “±”.

The inoculum of the BHP tests was obtained after the pretreatment the anaerobic sludge. The performance of three common inoculum pre-treatment methods, i.e. sodium 2-bromoethanesulfonic acid (BESA), heat shock and aeration pretreatment, was evaluated in BHP tests. To carry out the aeration pre-treatment, the inoculum was aerated for 10 days with an air flow rate of 100 L air/kg sludge/h. The thermal shock treatment was given by keeping the anaerobic digestate in an oven for 4 h at 105 °C (Giordano et al., 2011), whereas up to 25 mM BESA was added to the inoculum for the chemical pre-treatment (Li and Fang, 2007).

In all experiments, the substrate to inoculum ratio (food to microorganism, F/M) was fixed at 1:2 (0.5 gVS substrate/gVS inoculum). The effectiveness of the three inoculum pre-treatment methods was studied using 3.15 g VS of potato and pumpkin waste mixture (described in section 2.2) as the substrate and 600 ml of inoculum (6.3 g VS) in 1000 ml batch reactors at mesophilic conditions (35 ± 1 °C). The inoculum pre-treatment method giving the highest BHP was selected for the subsequent experiments. Similarly, BHP tests for different waste feedstocks were carried out with 3.15 g VS equivalent weight of waste biomass, i.e. 70.63 g of fennel waste, 54.69 ml of buffalo manure, 65.90 ml of olive mill wastewater, 3.8 g of dried blood (slaughter house waste), 12.5 g of olive pomace, with two blanks without substrates being used as controls. The amount of BESA equal to 25 mM was added to all the reactors to inhibit the methanogenic populations.

The major dark fermentation intermediates acetic, propionic and butyric acids were analyzed from the digestate sample when the cumulative biohydrogen production reached its maximum to define the key biochemical pathways prevailing in the BHP tests. Other soluble metabolites such as lactate and ethanol were not quantified in this study since these two metabolites are expected to be present only in lower concentrations in low loaded systems (Peiris et al., 2006), as represented by the low F/M ratio of 0.5 g VS