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Predictive and explicative models of fermentative hydrogen production from solid organic waste: Role of butyrate and lactate pathways

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ABSTRACT

Solid organic waste represents an abundant, cheap, and available source of biodegradable substrates not yet exploited to produce biohydrogen by dark fermentation. The impact of the composition of solid organic waste on microbial metabolic pathways and subsequently on biohydrogen production, has not been clearly elucidated. The aim of this study is to determine the compositional features of different substrates that influence bioH2 production. For this, we measured Biological hydrogen potentials (BHP) on 26 different substrates and performed a multivariate statistical analysis of the experimental data using a partial least square regression. The results showed that the BHP values correlated well with the initial carbohydrate content measured after mild hydrolysis. A predictive model explaining more than 89% of the experimental variability was then built to predict the maximal biohydrogen yield with a high accuracy and for a large spectrum of organic waste. An explicative model showed that only carbohydrates, butyrate and lactate concentrations were significant variables explaining more than 98% of biohydrogen yield variability. Interestingly, an interaction term between carbohydrates and lactate concentrations was required to explain microbial pathways producing hydrogen.

1. Introduction

Anaerobic digestion is a microbial and ecological process widespread in nature that degrades complex organic matter to methane and carbon dioxide in strict anaerobic environments. The microbial anaerobic digestion process is composed of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. This natural process has been used for many years to treat liquid effluents as well as solid waste, and produce concomitantly methane used as renewable energy source in its biogas form. This latter can be further converted into electricity and heat by combustion [1,2]. Moreover, as an intermediate of the whole anaerobic process, biohydrogen can accumulate when methanogenesis is inhibited and thus can be produced from various sources of biomass [2]. Interestingly, adding 5–20% of biohydrogen to methane biogas generates a new biogas, so called biohythane, that, after combustion, could reduce nitrogen oxide emission and increase engine combustion performances as compared to CH_4 combustion alone [3]. Additionally, hydrogen is considered as one of the most interesting alternative energy carrier since it has the highest energy content (122 kJ g^{-1}) and produces only water after combustion [4].

Besides its production under anaerobic conditions, biohydrogen is a key central metabolic intermediate and can therefore be consumed very efficiently by many

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microorganisms, such as methanogens, homoacetogens and sulfate-reducing bacteria [2]. It is thus necessary to limit the microbial anaerobic process to its first three steps, in a process called "dark fermentation". Besides biohydrogen, some metabolic end-products are produced concomitantly such as acetate, butyrate, propionate, ethanol and lactic acid. Similarly to biohydrogen, these end-products could be highly valuable for industrial purposes, *e.g.* lactate can be reused for PLA (PolyLactic Acid) bioplastic production, a renewable and biodegradable plastic [5]. Metabolic end-products, so-called metabolites, could also serve to improve further methane production in a second step [6]. Liu et al. (2006) showed that methane yield was 21% higher in a two-step than in one step anaerobic biogas process, likely due to an advanced hydrolysis of the substrate during the hydrogen production step [7]. Better description of the distribution of these end-products according to the kind of organic matter is therefore essential to better evaluate the biomass conversion potentiality in a biorefinery concept, where biohydrogen, methane as well as metabolic intermediates are recovered as valuable industrial products.

Among potential and easily available substrates, agricultural solid waste represents an abundant, cheap and highly biodegradable source of substrates that can produce hydrogen by dark fermentation. About 1.5 million tons of agricultural, forestry and fishing waste are annually produced in France [8]. Worldwide, lignocellulosic biomass residues are evaluated to exceed 220 billion of tons produced per year [9]. Over the past decades, the possibility to convert many different types of waste into biohydrogen has been investigated. In particular, food waste showed relative high performances of conversion to biohydrogen. So far, a range of 2.68–8.75 $mmol_{H2}\ g_{VS}^{-1}$ has been reported for food waste collected in food restaurants [10–13]. Nevertheless, the "food waste" category, when investigated as feedstock for hydrogen production, represents a wide variety of substrates including kitchen refuse, municipal waste, food industry co-products such as oil mill, cheese whey and starch-manufacturing waste [2]. Similarly, a wide group of agri-industrial waste has been investigated for hydrogen production and constitutes a very promising category of feedstock. Indeed, high biohydrogen production yields were observed from 3.77 $mmol_{H2}\ g_{VS}^{-1}$ to 11.67 $mmol_{H2}\ g_{hexose}^{-1}$ and 12.95 $mmol_{H2}\ g_{VS}^{-1}$ for palm oil mill effluent, molasses and cheese whey, respectively [14–17]. The use of a third type of waste, called agricultural residues that are generated from the primary agricultural sector, such as maize stalks or rice straws is usually reported in association with acidic, enzymatic or microwave pretreatments. This results in few available data dealing with hydrogen production from raw agricultural waste. Overall, reported hydrogen production yields vary a lot for a given category of feedstock, mainly because of the high variability in nature and composition of the substrates, as well as the differences in experimental procedures, *e.g.* batch or continuous reactors [10–12]. Moreover, structural features as well as chemical composition of organic substrates could have both an important role on hydrogen production in dark fermentation bioprocesses [18]. When considering the whole anaerobic digestion process of complex organic solid waste, only few studies dealt with the link existing between the complex chemical composition of such organic substrates and methanogenesis. Buffiere et al. (2006) reported a strong correlation between the composition in cellulose and lignin of seven solid organic substrates and their biological methane potential [19]. They showed that the sum of cellulose and lignin of organic substrates presented a linear and negative impact on methane production yields. The authors suggested that such linear correlation was mainly due to bio-accessibility constraints for hydrolysis of complex solid substrates.

Although many different types of substrates have already been assessed for their potential of hydrogen production, the impact of experimental set up, type of substrate as well as microbial ecosystem variability make difficult to predict accurately biohydrogen yields when using different categories of waste. Furthermore, indicators of well-established microbial metabolic routes are still missing. Therefore, the purpose of the present study was to investigate biological hydrogen yields of a large range of solid organic waste to further evaluate the impact of the biochemical composition of these substrates on fermentative microbial processes. For this, we performed a multivariate analysis by partial least square regression method to build a predictive model of biohydrogen production yield according to the biochemical composition of the solid waste. Concomitantly, we built an explicative model emphasizing the role of the main microbial metabolic pathways occurring in dark fermentation.

2. Material and methods

2.1. *Experimental substrates and chemical composition analysis*

Samples of 26 different solid organic substrates were used in this study. We distinguished four groups: (i) the first group corresponded to the substrates rich in carbohydrates, including apples (*royal gala*), carrots, Jerusalem artichoke roots, maize flour, oats, potatoes, and wheat flour; (ii) the second group corresponded to the substrates rich in proteins, i.e. soybean milk cake, chicken meat, cow manure with straw, fish residues, and meat waste from restaurants, (iii) the third group corresponded to agri-industrial waste, including food waste from restaurants, rapeseed oil cakes, sunflower oil cakes, grape marc, vegetable waste from restaurants, fruit peels (orange peels and banana peels) and maize cob, (iv) and the last group corresponded to agricultural end-products, such as Jerusalem artichoke leaves and stalks, giant reed stalks and leaves, maize stalks, rice straw and sorghum stalks.

To homogenize the samples, all substrates were freeze-dried for 48 h and milled (particle size <3 mm) in a blender. Total solids (TS) were quantified according to standard methods [20]. Carbohydrates were extracted using a gentle acid-extraction method with starch as reference substrate. For this, around 500 mg of samples were added to 40 mL of 2 N HCl in a sealed glass vial and put in an ultrasonic bath for one hour at room temperature. The liquid fraction was then centrifuged and carbohydrate concentration was measured using the anthrone method [21]. The carbohydrate concentration was expressed in glucose(Glc)-equivalent. Protein composition was assessed by extraction in sodium hydroxide