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The type of carbohydrates specifically selects microbial community structures and fermentation patterns

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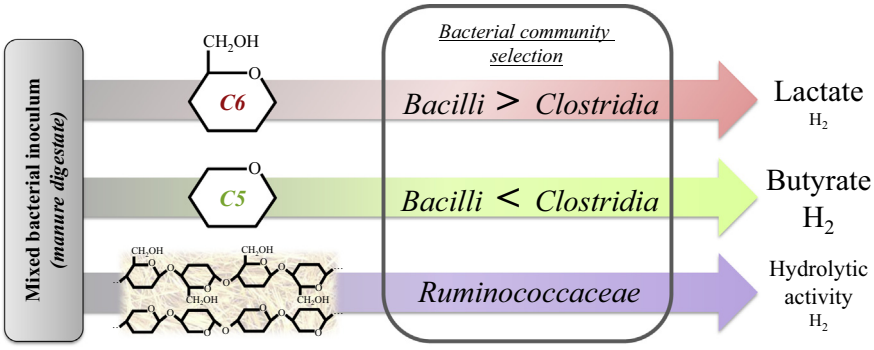
HIGHLIGHTS

- Higher amounts of hydrogen were recovered from pentose than hexose fermentation.
- Lactate pathway was preferentially used from hexoses.
- Carbohydrate types structured bacterial communities and metabolic patterns.
- Degree of polymerisation is detrimental to hydrogen production.
- *Ruminococcaceae* bacteria likely played an important role in hydrolytic activity.

GRAPHICAL ABSTRACT



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ABSTRACT

The impact on dark fermentation of seven carbohydrates as model substrates of lignocellulosic fractions (glucose, cellobiose, microcrystalline cellulose, arabinose, xylose, xylan and wheat straw) was investigated. Metabolic patterns and bacterial communities were characterized at the end of batch tests inoculated with manure digestate. It was found that hydrogen production was linked to the sugar type (pentose or hexose) and the degree of polymerisation. Hexoses produced less hydrogen, with a specific selection of lactate-producing bacterial community structures. Maximal hydrogen production was five times higher on pentose-based substrates, with specific bacterial community structures producing acetate and butyrate as main metabolites. Low hydrogen amounts accumulated from complex sugars (cellulose, xylan and wheat straw). A relatively high proportion of the reads was affiliated to *Ruminococcaceae* suggesting an efficient hydrolytic activity. Knowing that the bacterial community structure is very specific to a particular substrate offers new possibilities to design more efficient H_2-producing biological systems.

1. Introduction

Energetic transition and waste management are ones of the main XXI[st] century challenges (UNFCCC. Conference of the Parties (COP), 2015). Among all the renewable energies nowadays available, hydrogen has a promising future. Its energy power by mass unit (122 kJ.g^{-1}) is higher than most of other energy sources and its combustion produces only water and no carbon dioxide (Saratale et al., 2008). Hydrogen gas can be produced along the bioconversion of organic matter by dark fermentation. This natural biological process is carried out by different types of microorganisms widespread in Nature and found in most of anaerobic environments, such as in lakes, guts, urban sludge or manure digestate (Wang and Wan, 2009). First hydrolytic bacteria convert the organic matter into simpler molecules and the monomeric

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forms can further be assimilated by fermentative hydrogen-producing bacteria. Depending on the type of bacteria and the metabolic pathway used to convert these substrates, accumulation of hydrogenated biochemical intermediates (NADH) in the cell can lead to the production of biohydrogen to recycle the reduced elements in the cell (Cai et al., 2011). More particularly, acetic and butyric acid pathways generate four and two moles of hydrogen per mole of glucose consumed, respectively (Kalia and Purohit, 2008).

In mixed cultures, other metabolic pathways that are detrimental to hydrogen production by consuming carbohydrates, such as lactic acid or alcoholic fermentation, are also observed. Furthermore, the reuse of the hydrogen produced is also possible by other groups of H_2-consuming bacteria to form other volatile fatty acids such as propionate, caproate but also acetate and methane by homoacetogenesis and methanogenesis, respectively (Saady, 2013). Although heat shock treatment has been well described to get rid of the methanogenic activity, other fermentative pathways are often carried out by thermo-resistant microorganisms (Wong et al., 2014).

Nonetheless, using mixed culture is advantageous since complex substrates such as lignocellulose can also be used. The use of complex microbial consortia will increase the chance to find bacteria with efficient enzymatic materials to degrade complex biomass (Kleerebezem and van Loosdrecht, 2007). Lignocellulosic biomass is a substrate of choice for the production of renewable energies. It is the most abundant organic carbon source because it composes the major part of agricultural waste with a low cost (Guo et al., 2010). But the complex structure of lignocellulosic materials can impact bacterial fermentation and mechanisms are not yet well understood.

Lignocellulosic compounds are mainly composed of three polymers linked together to form a complex matrix: lignin, cellulose and hemicelluloses (Menon and Rao, 2012). Lignin is a phenolic polymer known to not be biodegradable for most of bacteria. It is linked to hemicelluloses, bond to cellulose. Whereas hemicelluloses are heteropolymer mainly composed of D-xylose, but also L-arabinose and D-glucose, cellulose is a homopolymer of cellobiose, a dimer of glucose. Because of the polymeric structure, deconstructive pre-treatment are usually applied on lignocellulosic biomass to release fermentable sugars (Nissilä et al., 2014).

Depending on the proportion of each sugar, the polymerisation degree and other biochemical parameters, fermentative pathways and performances can be both impacted. This work aims to evaluate the potential of main lignocellulosic biomass fractions to produce hydrogen by dark fermentation. Pure model substrates representing the main fractions of lignocellulosic materials were fermented in batch reactors with the same initial mixed inoculum (manure anaerobic digestate). Through the analysis of metabolites co-produced during the process and the final bacterial community structures, the impact of the type of carbon source on microbial community function was evaluated.

2. Materials and methods

2.1. Inoculum preparation and experimental setup

The outlet of a solid-state anaerobic reactor fed with bull manure (Montrodat, France), so-called digestate, was used as initial microbial inoculum. Because undigested straw was present in the digestate, microorganisms were first extracted according to a protocole adapted from Zhang et al. (2007). For that, 40 ml of sterile physiological water were added to 20 g of digestate in a 500 ml centrifuge tube. After dynamic hand shaking, the tube was centrifuged for 5 min at 3000*g* (temperature 4 °C). The supernatant was recovered in a sterile bottle and the pellet was washed with 40 ml of sterile phosphate buffer at 10 mM. After shaking, the tube was again centrifuged at 3000*g* during 10 min (4 °C). The supernatant was recovered and added to the first one. The residual juice of the pellet was sieved at 1 mm. Heat shock pre-treatment (90 °C, 30 min followed by ice cooling) was performed before inoculation to get rid of hydrogen consumption by methanogenic archaea.

Biohydrogen production potential (BHP) tests were carried out in batch tests in quadriplicates in 600 ml plasma bottle with 400 ml of working volume. The medium was composed of 12.5 ml of minimal nutrient solution (in $mg.l^{-1}$ – NH_4Cl: 32000; K_2HPO_4: 20000; $FeCl_2,4H_2O$: 1500; H_3BO_3,H_2O: 60; $MnSO_4,H_2O$: 117; $CoCl_2,6H_2O$: 25; $ZnCl_2$: 70; $NiCl_2,6H_2O$: 25; $CuCl_2,2H_2O$: 15; $NaMoO_4,2H_2O$: 25; HCl: 1755) and 100 mM of MES (2-[N-morpholino] ethane sulfonic acid buffer). Glucose (Sigma), cellobiose (Fluka), arabinose (Sigma), xylose and xylan (Sigma) concentrations were fixed at 5 $g_{COD}.l^{-1}$ whereas micro-crystalline cellulose (MCC, Fluka) and wheat straw (Haussmann common wheat straw) were introduced at 10 $v_{olatile\ solid}.l^{-1}$ to increase the production of metabolites and remain above the detection limit of metabolites. Heat treated inoculum was then added to reach a substrate/inoculum (S/X) ratio of 10 $g_{COD\ substrate}.g^{-1}_{COD\ inoculum}$.

Volatile solid analysis of wheat straw and MCC were performed in quadriplicates according to the APHA standard methods (APHA, 1999). Chemical oxygen demand (COD) was analysed using Spectroquant® kit (Merk) according to manufacturer's indications.

After inoculation, bottles were sealed with a rubber stopper and locked with an aluminium screw. Head space was then purged with 0.2 µm-filtered nitrogen gas to remove oxygen traces and keep the conditions anaerobic. The fermentation was stopped when the hydrogen production stabilized to avoid further consumption.

2.2. Metabolites and gas composition analysis

Gas production was monitored every 8 h with an automatic micro-gas chromatograph (SRA µ-GC R3000) equipped with two columns: a Molsieve 10 m/PPU at 80 °C with Argon as vector gas and a VAR 8 m/PPU at 70 °C with Helium, for O_2-CH_4-H_2-N_2 and CO_2 analysis, respectively. The TCD temperature was set at 90 °C.

Gas production was estimated by pressure measurement. From the gas composition and volume analysis, a modified Gompertz model was used to assess hydrogen production kinetic parameters (Eq. (1)):

$$H(t) = Pexp\left\{-exp\left[\frac{Rm.e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where H is the cumulative volume of hydrogen production (ml) along the incubation time (t), P is the maximum cumulative hydrogen production ($ml_{H2}.g^{-1}_{eq\ initial\ COD}$), Rm is the maximum hydrogen production rate ($ml_{H2}.g^{-1}_{eq\ initial\ COD}.d^{-1}$), λ is the lag phase (days) and e is exp(1). The values of P, Rm and λ were estimated using grofit R package (v 1.1.1-1) with nonlinear least square fitting (Kahm et al., 2010).

Soluble metabolites, i.e., volatile fatty acids (VFAs) and organic acids, solvents and residual sugars, were quantified before and after fermentation by Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC), respectively. Samples were filtrated at 0.2 µm prior to analysis. The HPLC was coupled to refractometric detection (Waters R410). Chemicals were separated by an Aminex HPX-87H column (300 × 7.8 mm, Biorad) equipped with a protective precolumn (Microguard cation H refill catbridges, Biorad). The eluting solution corresponded to 2 mM H_2SO_4 under a flow rate of 0.4 $ml.min^{-1}$. The column temperature was set at 35 °C and the refractive index detector (Waters 2414) worked at 45 °C.