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Thermophilic and cellulolytic consortium isolated from composting plants improves anaerobic digestion of cellulosic biomass: Toward a microbial resource management approach

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HIGHLIGHTS

- Efficient anaerobic, thermophilic and cellulolytic consortium was isolated from compost.
- Consortium improves anaerobic digestion of cellulosic substrates.
- Main microbial strains are strictly anaerobic and belong to *Clostridia* class.
- Despite weak richness and evenness, consortium adapts to pH evolution.

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ABSTRACT

A cellulolytic consortium was isolated from a composting plant in order to boost the initial hydrolysis step encountered in anaerobic digestion. Improvement of the cellulose degradation, as well as biogas production, was observed for the cultures inoculated with the exogenous consortium. Metagenomics analyses pointed out a weak richness (related to the number of OTUs) of the exogenous consortium induced by the selective pressure (cellulose as sole carbon source) met during the initial isolation steps. Main microbial strains determined were strictly anaerobic and belong to the *Clostridia* class. During cellulose anaerobic degradation, pH drop induced a strong modification of the microbial population. Despite the fact that richness and evenness were very weak, the exogenous consortium was able to adapt and to maintain the cellulolytic degradation potential. This important result point out the fact that simplified microbial communities could be used in order to increase the robustness of mixed cultures involved in environmental biotechnology.

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1. Introduction

Anaerobic digestion of organic matter is recognized as an attractive and commercially viable option as a source of alternative energy. Methane can be obtained by anaerobic digestion of different organic matters including industrial residues, agricultural wastes, industrial wastewaters and municipal solid wastes. The anaerobic degradation process involves synergistic interactions among various bacteria and methanogenic archea existing as a complex consortium. Four biochemical steps are involved, i.e. hydrolysis of substrate polymers to monomers, acidogenesis and conversion of monomers to volatile fatty acids, carbon dioxide, and hydrogen, acetogenesis and production of acetate from the intermediate metabolites, and finally methanogenesis with the conversion of acetate and carbon dioxide to methane. Lignocellulosic biomass is mainly composed of three different polymers i.e. cellulose, hemicellulose and lignin. Vavilin et al. (2008) highlighted the fact that the hydrolysis of lignocellulosic substrate is the rate-limiting step during biogas production from wastes rich in lignocellulosic plant fiber. Thus, effective hydrolysis of recalcitrant substrate is necessary for a profitable biogas production from fiber-rich wastes. Degradability improvement can be achieved by different physical (Pommier et al., 2010), chemical (Monlau et al., 2012) or biological (Zhang et al., 2011) pretreatments. However, pretreatments increase the cost of the global

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process and can generate various compounds interfering with microbial activities (Palmqvist and Hahn-Hägerdal, 2000). Therefore, a direct efficient bioconversion of raw lignocellulosic waste during anaerobic digestion process is more favorable.

As efficient lignocellulosic biomass degradation occurs in diverse ecosystems by the cooperative activities of many microorganisms (cellulolytic and non-cellulolytic), management of consortia involved in these natural process can be a source of sustainable solution. On the basis of this hypothesis, the "Microbial Resource Management" concept was developed by Verstraete et al. (2007). In order to efficiently manage endogenous microbial populations, three parameters must be taken into account, i.e. the diversity of the microbial strains, the functionality or metabolic activity of each strain and the possible interactions between microbial communities (Verstraete et al., 2007). Marzorati et al. (2008) propose three parameters, based on molecular tools, to answer these questions: (i) the range-weighted richness (*Rr*) reflecting the carrying capacity of the system, (ii) the dynamics (*Dy*) reflecting the specific rate of species coming to significance, and (iii) functional organization (*Fo*), defined through a relation between the structure of a microbial community and its functionality (Marzorati et al., 2008).

Moreover, the integration of this concept to the enrichment culture techniques could be a powerful tool for the engineering of microbial consortia with given properties. Zuroff and Curtis (2012), reviewed some works about enriched cellulolytic natural consortia. However, only few works have emphasized on strict anaerobic enrichment. To date, most of the works have been focused on enrichment culture under aerobic or facultative anaerobic conditions. As well in aerobic conditions (Wongwilaiwalin et al., 2010) as in anaerobic conditions (Izquierdo et al., 2010) compost is a frequent microbial resource for enrichment culture. Nevertheless, other types of inocula have been tested for the development of efficient microbial cellulolytic communities, such as forest soil (Feng et al., 2011), or anaerobic digester sludge (Yan et al., 2012). Accordingly, based on enrichment method from compost samples, the present work has led to the design of an anaerobic thermophilic cellulolytic microbial consortium able to degrade cellulose under conditions related to anaerobic digestion process.

2. Methods

2.1. Biological materials

2.1.1. Compost

Compost samples were collected from a composting center in Naninne, Belgium, exclusively supplied with green lignocellulosic waste. Sampling was realized during thermophilic phase of composting process at a temperature of 73.2 °C.

2.1.2. Anaerobic sludge

Anaerobic sludge used as inoculum for BMP tests were sampled from a full-scale anaerobic digester fed with agro-food organic waste and agricultural waste.

2.2. Cellulolytic microbial consortium preparation

BMP assay medium (Wang et al., 1994) supplemented with 10 g l^{-1} of cellulose (Whatman filter paper) was used as enrichment medium. Five grams of compost samples were inoculated in 125 ml bottles containing 45 ml of sterilized medium with a filter paper strip as an indicator for cellulase activity. Cultures were incubated at 37 °C and 55 °C under anaerobic conditions and without stirring. To generate the anaerobic conditions, headspace of bottles, tightly capped with rubber septa and sealed with aluminum caps, was flushed with carbon dioxide and with oxygen-free nitrogen gas in a second step. Once the strip of filter paper began to be degraded, 5 ml of culture were transferred into fresh enrichment medium. This process was repeated several times.

In parallel of this anaerobic enrichment, aerobic tests were led according to the procedure employed by Wongwilaiwalin et al. (2010). Five grams of compost samples were inoculated in flask containing 45 ml of PCS medium (0.1% yeast extract, 0.5% peptone, 0.5% $CaCO_3$, 0.5% NaCl, 1% filter paper, pH 8.0) with a filter paper strip as an indicator for cellulase activity. Cultures were incubated at 37 °C and 55 °C under aerobic conditions and without stirring.

2.3. Cellulose degradation capacity test

The cellulose degradation tests were led in BMP medium supplemented with 10 g l^{-1} of cellulosic material (filter paper and Avicell) for 7 days at 55 °C under anaerobic conditions without stirring and with uninoculated medium as a control. To generate the anaerobic conditions, headspace of bottles, tightly capped with rubber septa and sealed with aluminum caps, was flushed with carbon dioxide and with oxygen-free nitrogen gas in a second step. At the end of the culture, centrifugation (8000×*g*) allowed separation of supernatant and pellet which included residual substrate and microbial biomass. Pellet was then suspended in 100 ml acetic acid/nitric acid reagent (Feng et al., 2011) and heated at 100 °C for 30 min to remove the biological cells. Then, acetic acid/nitric acid treated suspension was filtered. The remaining cellulosic material was washed three times using 100 ml of distilled water each time. After washing and filtration, filtered solids were dried at 105 °C and determined gravimetrically (Feng et al., 2011). Degradation ratio was calculated according to the equation:

$$\text{Degradation ratio } (\%) = \frac{M_t - M_r}{M_t} \times 100 \quad (1)$$

where M_t is the total weight of the cellulosic materials before degradation and M_r is the weight of residual substrates after degradation. All experiments were performed in triplicate and the average values were reported.

2.4. Anaerobic digestion test (biochemical methane potential assay)

BMP assay medium (Wang et al., 1994) was used. Experiments were performed according to the procedure published by Wang et al. (1994) and were carried out in triplicate in 125 ml sterile glass serum bottles. 0.5 g of filter paper, as cellulosic substrate, or 0.5 g of mechanically treated paper paste (composition: 53% of holocellulose, 32% of lignin and 15% of others compounds), as lignocellulosic substrate, were introduced into bottles containing 45 ml of BMP medium, and 5 ml of inocula. Two inocula were compared. One consisted of only anaerobic sludge and the other was a mix 50:50 of anaerobic sludge and isolated cellulolytic consortium. pH was adjusted with a 0.5 M KOH solution to achieve an initial pH of 7.3 in each sample, and a maximum variation during the culture period of pH ± 1 was maintained. The sample bottles were capped tightly with rubber septa and sealed with aluminum seals. To generate the anaerobic conditions, headspace of bottles, tightly capped with rubber septa and sealed with aluminum caps, was flushed with carbon dioxide and with oxygen-free nitrogen gas in a second step. Bottles were incubated at 55 °C.

Biogas production and composition were monitored for 90 days. Biogas was collected using a syringe fitted with a needle and composition was monitored according to procedures described in Section 2.7 (gas phase analysis). First, collected biogas (or a fraction of collected biogas) was injected in 100 ml gas replacement equipment containing a 9 M KOH solution for a fast determination of carbon dioxide concentration in gas phase. In a second time,

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