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Pre-treatments for enhanced biochemical methane potential of bamboo waste

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HIGHLIGHTS

- Bamboo waste is a potential feedstock for methane production.
- Methane yields from bamboo waste increased with decreasing particle sizes.
- Pre-treatments led improvements in both sugars and methane yields.
- Mild alkaline pre-treatment was most efficient in enhancing methane yields.
- Competition for biomass for food/feed is avoided by converting bamboo to methane.

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ABSTRACT

Various pre-treatments (acid, alkaline, enzyme and alkaline aided enzyme also termed combined) were evaluated on different fractions of bamboo waste from a chopstick production factory. Chemical oxygen demand (COD) solubilisation, monomeric/dimeric sugar yield, methane yield enhancement and methane production rate were assessed. The biochemical methane potential was determined in batch assays under mesophilic conditions (37 ± 1 °C) using the Automatic Methane Potential Test System (AMPTS-II).

Pre-treatments led to enhanced COD solubilisation as compared to raw sample. Alkaline aided enzymatic pre-treatment led to the highest sugar yield, comparable to the theoretical yield. However, high sugar yield did not translate to high methane yield. The best pre-treatment in terms of methane yield was alkaline pre-treatment which resulted in a surplus of up to 88% methane yield. There was a positive correlation between dissolved COD and methane yield. Methane yield and methane production rate also increased with decreasing particle sizes. In all investigated scenarios, pre-treatment led to an improved methane production rate as compared to the raw samples.

These results demonstrated that alkaline pre-treatment at ambient temperature was an efficient treatment option to improve methane yield of bamboo waste.

1. Introduction

Plant biomass is a natural renewable resource that can readily be converted to high value compounds and fuel [1]. Bamboo (*Phyllostachys pubescens*) is fast growing perennial woody plant biomass with an annual production of between 6 and 7 million tons [2]. It has diverse uses such as building material, food source, ornaments, medicine and as versatile raw biomass. Bamboo plays a pivotal role in the local economy in some areas of southern China. The bamboo industry provides more than 5 billion USD per year in China as a whole [3]. A large quantity of bamboo waste is produced every year, which contains a high fraction of lignocelluloses. This lignocellulosic waste could be a potential source for renewable energy production.

Anaerobic digestion (AD) is one way of harnessing renewable energy from plant biomass and bio-waste. AD has seen a rapid increase in recent years primarily due to the increasing depletion of fossil fuels and fears over global warming. Renewable energy production from lignocelluloses biomass (referred to as second generation feedstock) is preferred because the competition for biomass for human and animal food/feed is avoided [4]. Bamboo waste is lignocellulosic in nature where holocellulose (cellulose and hemicelluloses) are strongly covered by a hydrophobic lignin network, which might lead to poor anaerobic biodegradability.

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Hydrolysis is therefore the rate limiting step in the AD of lignocellulosic biomass such as bamboo waste [5].

For the effective degradation of cellulose and hemicelluloses into methane, destruction of the lignin network by certain pre-treatments is a must. As a matter of fact, the pre-treatment of lignocellulosic biomass to partially or totally disrupt the lignin structure is a plausible option in order to maximize biogas or methane production from lignocellulosic material [6]. The purpose of any pre-treatment is therefore to partially or totally destroy lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials [7]. An effective pre-treatment should amongst others meet the following requirements: (1) improve the formation of monomeric/oligomeric sugars or the ability to subsequently form monomers by enzymatic hydrolysis; (2) minimise the degradation or loss of carbohydrates; and (3) minimise the formation of toxic by-products to anaerobic bacteria [7]. Physical, physico-chemical, chemical, and biological processes have been used for the pre-treatment of lignocellulosic materials. The following pre-treatment modes have been reported to enhance the biodegradability of lignocellulosic biomass: steam explosion, dilute acid catalysed enzyme treatment, milling, liquid hot-water treatment, etc. [8–10]. The selection of best pre-treatment module depends greatly on the type of lignocelluloses biomass. It has been reported for example, that dilute acid pre-treatment of bark from poplar trees or corn leaf was promising while at the same time ineffective for treating the bark from sweet gum or corn stalks [11,12]. In all, alkaline pre-treatment at ambient temperatures is a plausible cost efficient option since the AD process usually requires a boost in alkalinity [13]. Therefore, to convert lignocellulosic biomass to biogas or bio-methane, an appropriate pre-treated peculiar to the given biomass must be performed to facilitate the release of sugars. This pre-treatment may also aid subsequent enzymatic hydrolysis of cellulose and hemicellulose, to monomeric hexose and pentose sugars which can used by the anaerobic consortium for methane production.

In the present study, various sizes of bamboo waste (from 0.05 mm to 5 mm) generated during chopstick production, as well as the effect of different pre-treatment modules (acid, alkaline, enzyme, and alkaline aided enzyme) were studied as per their impact on the methane yields and methane production rate. The aim was to pinpoint the pre-treatment module best suited for the enhancement of the methane yield of bamboo waste. The methane potential tests were carried out utilising an Automatic Methane Potential Test System, AMPTS II (Bioprocess Control, Sweden). A correlation between dissolved chemical oxygen demand (COD)/released sugars during pre-treatment and methane yields was also evaluated.

2. Methods

2.1. Feedstock and inoculums

Bamboo waste was collected from a chopstick production factory (Qingyuan, China). The bamboo waste was fractionated into different sizes during the chopstick production process wherein sample I represented more than 75% of the total weight whereas sample B accounted for only 0.75% (Fig. 1). Fig. 1 also shows the different particle sizes (A to H) and pre-treatments effectuated in the present study as described below. The total solids (TS), volatile solids (VS), total chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD) of bamboo waste are listed in Table 1. Table 1 also shows the main component of bamboo waste. Anaerobic sludge used as inoculums during the study was collected from a mesophilic biogas digester feed with dewatered sewage sludge from a local wastewater treatment plant (Källby, Lund, Sweden). The inoculums had an average pH of 7.6, partial alkalinity of 6.5 g/l. Other characteristics of the inoculum are listed in Table 1.

2.2. Alkaline and acid pre-treatment

For alkaline pre-treatment, 20 g of bamboo waste (samples A, B, C and I) was mixed with 75 mL of 7% NaOH solution and incubated for 2 h at room temperature (25 °C) as recommended elsewhere [13] and at 45 °C, 65 °C and 85 °C. Since there was no significant difference in methane yield at the different temperatures, subsequent treatments were done at 25 °C only. After the pre-treatment, the alkaline treated paste was neutralized to pH 7.0 with 12% HCl. For diluted acid pre-treatment, 20 g of samples A, C and I were mixed with 140 g of 0.5% H_3PO_4 as has been reported in [8] and heated to 121 °C for 1 h (Fig. 1). (It should be noted that only alkaline pre-treatment was performed for sample B due to the small sample amount and by virtue of the fact that alkaline pre-treatment from a previous experiment (results not shown) had shown substantial improvement in methane yield). Phosphoric acid was chosen in lieu of sulfuric acid because of the potential inhibitory effect hydrogen sulfide and the proliferation of sulfur reducing bacteria which might compete for feedstock with methanogens. As a control, the samples were also autoclaved (121 °C, 1 h) in order to evaluate the effect of thermal treatment. All pre-treated samples were stored at 4 °C for subsequent sugar and COD analysis. The different pre-treatment modules are outlined in Fig. 1. Biochemical methane potential (BMP) assays are presented in Fig. 1 as AD.

2.3. Enzyme hydrolysis

Cellulase (celluclast) with an activity of 84.1 FPU/ml was obtained from Novozyme, Denmark. The cellulase loading was 15 FPU/g cellulose based on compositional analysis (Table 1). For enzyme pre-treatment, the pH of the bamboo waste pre-treated with alkaline and raw bamboo waste was adjusted to 4.8 with diluted HCl for optimal enzyme activity. The bamboo waste/cellulase mixture was then incubated at 50 °C in a shaking water bath for 72 h at 120 rpm. For the alkaline aided enzyme treatment also called combine treatment, 20 g of samples A, C and I were mixed with 150 ml water and adjusted to pH 10 with NaOH and autoclaved (125 °C for 1 h). After cooling to room temperature, the pH was adjusted to 4.8 with HCl. After enzyme hydrolysis, the supernatant was filtered through a 0.2 μm nylon membrane filter for sugar analysis. The sugar concentrations were measured by HPLC as described below.

2.4. Biochemical methane potential analysis

The BMPs of the raw (fractionated) bamboo waste named A, B, C, D, E, F, G, and H were performed without further treatment. Since the methane yields for samples D, E, F, G and H did not differ significantly ($P \leqslant 0.05$), the samples were pooled into one sample type denoted I. Samples A, B, C and I were further pre-treated prior to the BMP (AD) as outlined in Fig. 1. The BMP tests were performed in an Automatic Methane Potential Test System (AMPTS II) (Bioprocess Control AB, Sweden) for 30–33 days. The test was carried out at 37 °C, in triplicates (in some cases duplicates) with an inoculum to substrate ratio (ISR) of 2. Three sets of controls were included in the test. First, where only the inoculum was used to measure the indigenous methane production from the inoculums, which was subtracted from the total methane produced from all samples. A second control with cellulose (Avicel PH-101, Sigma–Aldrich, St. Louis, MO, USA) was used to test the activity of the inoculum. The methane production from celluclast was also evaluated (third control) and subtracted from the enzyme pre-treated samples.