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# Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments

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#### ABSTRACT

A multi-channel analyzer was used to evaluate biogas potential of sugarcane bagasse (SCB). The Automatic Methane Potential Test System contained fifteen parallel reactors and the same number of gas flow meters attached to the acquisition system. The set of reactors – gas flow meters gave reproducible results during anaerobic digestion of chemically defined carbon source and the units were used to evaluate the biomethane potential of SCB after different pretreatments, such as treatment with water, acid, acid followed by enzymatic treatment and acid followed by treatment with inactive enzymes. Combined pretreatment with 2% sulphuric acid and enzymatic hydrolysis (3.5% enzymes) resulted in conversion of 79% to monomeric sugars present in SCB. SCB treated with acid followed by enzymatic hydrolysis achieved the methane yield of 200 NL per kg VS<sub>added</sub>. Enzymatic saccharification of acid pretreated SCB resulted in increase of methane yield by 16 ± 5% compared to that from acid treated SCB.

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## 1. Introduction

Interest in the production of biofuels is currently increasing due to the expected shortage of conventional energy resources, emissions of green house gases by fossil fuels, increased demand of energy for transportation, heating and industrial processes, increase in oil prices, environmental concerns, and an existing infrastructure used for the distribution and use of biofuels (Börjesson and Mattiasson, 2008; Hahn-Hagerdal et al., 2006; Linde et al., 2008; Wingren et al., 2008; Zhao et al., 2007).

Lignocellulosic biomass is an attractive renewable feedstock for biofuel production (second generation technology) because of its availability in high quantities and at reasonable cost (Kaparaju et al., 2009; Sanchez and Cardona, 2008). Sources of lignocellulosic biomass that potentially can be used for the biofuel production are agricultural residues (sugarcane bagasse, corn stover, straw, etc.), forest residues such as softwood (spruce) and hardwood (*Salix*), waste fibre sludges from paper and pulp industry, newsprint, office paper, municipal solid waste, dedicated energy crops (fast growing switchgrass, hybrid poplar, etc.) and recently also algae (Sanchez and Cardona, 2008; Sassner et al., 2008; Sjöde et al., 2007; Vergara-Fernández et al., 2008; Wingren et al., 2008). Pakistan is an agricultural country and sugarcane is the largest agricultural commodity produced. Pakistan is the fifth largest producer of sugarcane in the world with a production of 53 million tons annually (2003–2008) (http://faostat.fao.org) with 2.5% of annual growth rate (Harijan et al., 2009). In general, approximately 27% of the sugarcane crop is recovered as sugarcane bagasse (SCB) (50% moisture) in the sugar production process (Xu et al., 2006). Therefore, it can be estimated that the yield of SCB (50% moisture) is 14 million tons per year, approximately while annual global production of dry sugarcane is 540 million tons (Minavari, 2010). About 50% of the SCB is burnt for steam and electricity generation.

Hydrolysis of solids is commonly the slowest reaction and rate limiting step in anaerobic digestion (Vavilin et al., 2008). Pretreatment of SCB removes the hemicelluloses, reduces the crystallinity of cellulose and increases the porosity of material (Cardona et al., 2010) and hence enhances the hydrolysis of SCB during anaerobic digestion while the pretreated SCB can be enzymatically hydrolyzed prior to anaerobic digestion which can further enhance the hydrolysis.

The primary aim of the study was to use a multi-channel analyzer the Automatic Methane Potential Test System (AMPTS) for studying the biomethane potential of the SCB under different treatments.

## 2. Methods

#### 2.1. Experimental setup

The volume of the biomethane produced were quantified during anaerobic digestion of sugarcane bagasse (SCB) treated in



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different ways (Fig. 1) using the AMPTS (Bioprocess Control AB, Lund, Sweden, Fig. 2). Before evaluating the biomethane potential (BMP), the best pretreatment conditions and optimum enzyme loading were studied for the hydrolysis of polymeric carbohydrates in SCB.

## 2.2. Substrate

The Pakistani SCB used as substrate in this experiment was collected in July 2009 from Pattoki Sugar Mill located in south of Pakistan. The substrate was stored at +8  $^\circ$ C until use.

#### 2.3. Acid pretreatment

The effect of varying concentrations of sulphuric acid required for pretreatment of SCB was studied. SCB samples of 9.3 g (dry weight) were mixed in 500 mL flasks with several concentrations of dilute sulphuric acid i.e. 0.5%, 1%, 1.2%, 1.4%, 1.6%, 1.8% and 2% (w/v). The total working volume of the mixtures was 200 mL resulting in 4.6% of total solids (TS). Each mixture was autoclaved at 121 °C for 15 min. Controls were run as described above, except that deionized water was used instead of dilute acid. After the treatment the samples were cooled down to room temperature and neutralized with 5 M NaOH solution. The liquid fraction (hydrolysate) and the solid fraction (pulp) of the pretreated bagasse were separated from the slurry by vacuum filtration using 180 µm sieve. The samples were stored at -20 °C until analysis.

#### 2.4. Enzymatic saccharification

Accellerase<sup>®</sup> 1500 enzyme preparation kindly provided by Dr. Shukun Yu (Genencore, Danisco/DuPont Buisness, Denmark) was studied with regard to its ability to hydrolyze cellulose and hemicellulose in bagasse. Accellerase<sup>®</sup> 1500 is a mixture of enzymes mainly with exoglucanase, endoglucanase, hemi-cellulase and  $\beta$ -glucosidase activities.

For evaluating the enzymatic convertibility of the polymeric carbohydrates, 0.5 g (dry weight) of raw SCB, water treated SCB and solid fraction (pulp pretreated with dilute acid 2% w/v and neutralized before use by 5 M of NaOH) were resuspended in 20 mL glass vials containing 0.1 M phosphate buffer (pH 4.8) and Accellerase<sup>®</sup> 1500, the final dry matter content was 5% in all vials. The vials were incubated for 72 h in a shaking water bath at 50 °C and 70 rpm. Three different concentrations of enzyme were used in the study: 2.5%, 3% and 3.5% of total working volume. Controls having enzymes only were also evaluated in test reactors. Traces of sugar from the enzymes preparations were subtracted from respective incubations. Each experiment was run in triplicates and samples were collected at 12, 24 and 72 h of incubation.

#### 2.5. Inoculum preparation and characteristics

An active inoculum was collected from a mesophilic biogas plant that digests pig manure mixed with wastes from food industry and some slaughter house waste (Wrams Gunnasrstorp located at Bjuv, Sweden). The particulate matter (>1 mm) was removed

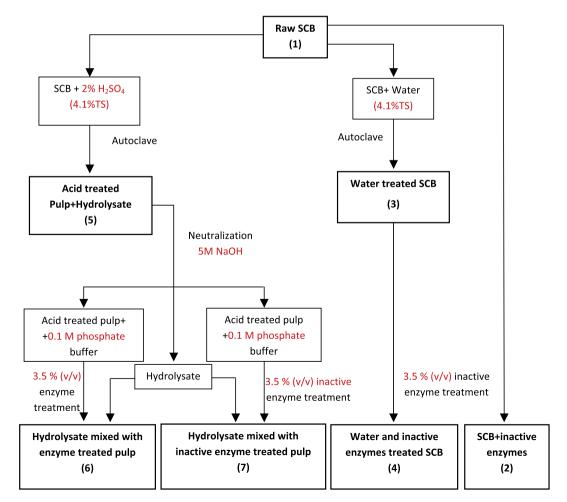


Fig. 1. Schematic presentation of treatments of SCB used in anaerobic digestion. The bold boxes are used for anaerobic digestion.

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