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# Effect of enzymatic pretreatment on anaerobic co-digestion of sugar beet pulp silage and vinasse

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## HIGHLIGHTS

- Anaerobic co-fermentation of sugar beet pulp silage (SBPS) supplemented with vinasse was conducted.
- The effect of enzymatic pretreatment of SBPS and vinasse blends on methane co-fermentation was checked.
- The highest biogas yield was achieved from enzyme-digested SBPS supplemented with 25% vinasse.
- The modified Gompertz's model fits well to batch co-fermentation of SBPS and vinasse blends.

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## ABSTRACT

Results of sugar beet pulp silage (SBPS) and vinasse (mixed in weight ratios of 3:1, 1:1 and 1:3, respectively) co-fermentation, obtained in this study, provide evidence that addition of too high amount of vinasse into the SBPS decreases biogas yields. The highest biogas productivity (598.1 mL/g VS) was achieved at the SBPS–vinasse ratio of 3:1 (w/w). Biogas yields from separately fermented SBPS and vinasse were by 13% and 28.6% lower, respectively. It was found that enzymatic pretreatment of SBPS before methane fermentation that caused partial degradation of component polysaccharides, considerably increased biogas production. The highest biogas yield (765.5 mL/g VS) was obtained from enzymatic digests of SBPS–vinasse (3:1) blend (27.9% more than from fermentation of the counterpart blend, which was not treated with enzymes). The simulation of potential biogas production from all the aforementioned mixtures using the Gompertz equation showed fair fit to the experimental results.


## 1. Introduction

Production of a fossil fuel alternative has become an attractive method of utilization of lignocellulosic residues from agriculture and food processing because it not only reduces amounts of harmful wastes released to the environment but also enables production of second generation biofuels from renewable resources. Also sugar beet pulp (SBP) and molasses that are valuable process residues from sucrose manufacturing may be used for biofuel production. One third of world sucrose production is derived from sugar beets. In European Union countries the annual sucrose production reaches 15.62 million tons that is equivalent to around 14 million tons of the sugar beet pulp dry weight (Altundogan et al., 2007). Poland is the third sucrose producer in Europe, with the annual production of around 1.87 million tons. It means the necessity of utilization of around 0.6 million tons of sugar beet pulp dry weight and 600 thousand tons of molasses (www.stat.gov.pl). Seasonal production of sugar causes that fresh sugar beet pulp is accumulated in much higher amounts than those, which can be immediately utilized. Because of the high water content (70–80%) and the presence of monosaccharides, this material undergoes quick spoilage (Zheng et al., 2011). Therefore, SBP is often conserved by lactic acid fermentation and as ensiled forage is used all round the year. However, this method does not solve the problem of complete SBP utilization.

An increase in electricity prices caused that sugar factories utilize all process residues and byproducts in order to reduce production costs. As much as 170–330 kWH of electric energy is necessary to produce 1 ton of sugar (Brooks et al., 2008). In 2012, a biogas plant supplied with 50 thousand tons of fresh and ensiled sugar beet pulp per year and producing 2 MW of power begun to operate in a sugar factory in Strzelin (Poland). Some other biogas plants based on this feedstock are expected to be launched soon. The crucial factor deciding of further development of these biogas plants is an income from the biogas and therefore the yield of the

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latter has to be as high as possible. The yield of anaerobic fermentation depends not only on the configuration of reactors and technological process parameters but also on feedstock characteristics (Ward et al., 2008). The presence of lignin and crystalline cellulose and the limited access of microorganisms to the surface of polysaccharides suppress the anaerobic biodegradation of sugar beet pulp and reduce the rate and yield of fermentation (Frigon and Guiot, 2010). The development of efficient pretreatment methods, bringing about deconstruction of polymers to simpler compounds that are substrates in the anaerobic fermentation, is a prerequisite of complete utilization of lignocellulosic wastes. Therefore, partial hydrolysis of polymers contained in sugar beet pulp is a key step in its further processing (Zheng et al., 2014). Enzymatic preparations have been increasingly applied for degradation of lignocellulosic wastes and their large scale use seems to be attractive. Enzymatic hydrolysis outperforms other methods because of the low water and energy consumption, reduced costs of wastes utilization and the lack of problems caused by the corrosion of equipment (Kumar et al., 2009).

To maximize yields of biogas synthesis from lignocellulosic feedstocks, also from sugar beet pulp, the biomass is mixed with sufficient amounts of water (Hutnan et al., 2000; Demirel, 2009), the C:N ratio is modified and mineral salts contents are balanced (Suhartini et al., 2014). It was also demonstrated that the anaerobic fermentation of mixtures of two or more substrates increased methane yields (Ward et al., 2008). Therefore, we decided to study the co-fermentation of sugar beet pulp silage (SBPS) and vinasse because this may be an effective way to improve buffer capacity and achieve stable performance of a biogas plant.

Vinasse is a process residue from ethanol production from molasses that has been quickly developing in recent years. Complete utilization of large amounts of vinasse has not been hitherto achieved. Its biodegradation is a challenge both in terms of economy and environment protection. In Poland, 25% of ethanol has been produced from molasses. In contrast to residues from starch-based alcohol production, vinasse cannot be used for fabrication of feed for animals (www.mir.gov.pl). Production of 1 L of ethanol generates as much as 8–15 L of vinasse (Syaichurrozi et al., 2013), which is characterized by the low solid substance content, COD varying from 15 to more than 100 g $O_2$/L, acidic pH, strong, characteristic smell and dark brown color (Satyawali and Balakrishnan, 2008). Reports on co-fermentation of sugar beet pulp and vinasse have not been published yet.

In this study we attempted to determine the dependence of biogas yields on the composition of SBPS–vinasse mixtures and their enzymatic pretreatment before methane fermentation.

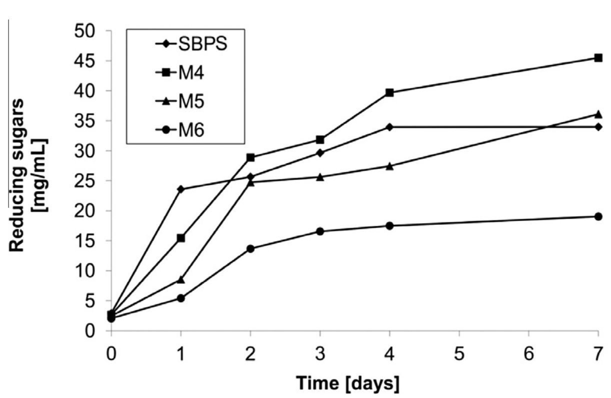


**Fig. 1.** Changes in reducing sugars concentration during enzymatic hydrolysis of sugar beet pulp silage and its mixtures with vinasse (mixed at ratios of 3:1(M4), 1:1 (M5), and 1:3 (M6)).

## 2. Methods

### 2.1. Sugar beet pulp silage, vinasse, inoculum

Sugar beet pulp was used in this study as silage. SBP is a perishable commodity, produced in the period from September to February. As silage it becomes storable throughout the year. Parameters of the SBPS, vinasse and anaerobic sludge that were used in this study are presented in Table 1. These parameters were determined as described in Section 2.5. The seed anaerobic sludge was harvested from an agricultural biogas plant, which was supplied with sugar beet pulp. It was concentrated by sedimentation (for 24 h) in an Imhoff funnel.

### 2.2. Enzymatic pretreatment

Hydrolysis of SBPS and its mixtures with vinasse (in 3 weight ratios of 3:1, 1:1 and 1:3) was conducted using a mixture of commercial enzymatic preparations Celustar XL and Agropect pomace (3:1, v/v), presented in Table 2. The vinasse was added to reduce the solid substance contents in SBPS suspensions. The optimal ratio of the two enzymatic preparations and the dose of 0.15 IU/g TS were determined as described elsewhere (Ziemiński et al., 2012). Processes of enzymatic hydrolysis were conducted for 7 days at the temperature of 50 °C. Their control was based on measurements of reducing sugars concentration each day.

### 2.3. Batch methane fermentation of sugar beet pulp silage and vinasse

Batch methane fermentation processes were conducted at mesophilic conditions (37 °C) with stirring at the rate of 4 rpm in identical glass chambers, with working volume of 1 L each. Volumes of methane synthesized daily were measured using a system consisting of an electronic Aalborg® GFM17 flow-meter governed by a computer system. The same system was used for the continuous temperature measurements during fermentation processes. The constant fermentation temperature was maintained using a thermostat connected to a water jacket of the fermentor.

Parameters of SBPS, vinasse and their mixtures (weight ratios of 3:1, 1:1, and 1:3), either enzymatically digested (for 4 days) or not (the controls), are shown in Table 3. Before methane fermentation, the pH of each batch of the substrates was adjusted to around 7.2 using $Na_2CO_3$. To initiate the fermentation process, the inoculum derived from the agricultural biogas plant was added to the substrates in a dose of 20 g TS/L.

### 2.4. Kinetic model of biogas production

Kinetics of biogas production was modeled using a modified Gompertz equation (Lo et al., 2010). Kinetics of biogas production in batch conditions was determined based on the assumption that it is affected by the specific growth rate of methanogenic bacteria in a digester (Budiyono et al., 2010; Zhu et al., 2009). The modified Gompertz equation is as follows:

$$y(t) = A \cdot \exp\left\{-\exp\left[\frac{\mu e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, $y$ is the biogas accumulation (mL/g VS) at time $t$ (day), $t$ is the time (day) over the digestion period. A is the biogas production potential (mL/g VS), $\mu$ is the maximal biogas production rate (mL/g VS × d) while $\lambda$ is the lag phase (day) or minimum time to produce biogas and e is a mathematical constant (2.718282). Kinetic constants: $A$, $\mu$ and $\lambda$ were determined using the non-linear regression and Matlab software.