



## Biogas production within the bioethanol production chain: Use of co-substrates for anaerobic digestion of sugar beet vinasse



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### HIGHLIGHTS

- Co-substrates addition enhanced the anaerobic digestion of sugar beet vinasse in CSTR.
- Cow manure and lime fertiliser provided trace elements and straw improved C/N ratio.
- Biogas production in the CSTR was close to the BMP of vinasse at steady state.
- Stable CH<sub>4</sub> yields and low VFA accumulation were detected at the maximum OLR.
- Modified Gompertz model fitted to the experimental data to describe BMP.

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### ABSTRACT

Bioethanol production generates large amounts of vinasse, which is suitable for biogas production. In this study, the anaerobic digestion of sugar beet vinasse was optimised using continuous stirred-tank reactors (CSTR) supplemented either with lime fertiliser or with 3% cow manure. In both reactors, the C/N ratio was adjusted by adding straw. The biochemical methane potential (BMP) of vinasse was  $267.4 \pm 4.5$  L CH<sub>4</sub> kg VS<sup>-1</sup>. Due to the low content of macro- and micronutrients and low C/N ratio of vinasse, biogas production failed when vinasse alone was fed to the reactor. When co-substrate was added, biogas production achieved very close to the BMP of vinasse, being  $235.7 \pm 32.2$  L CH<sub>4</sub> kg VS<sup>-1</sup> from the fertiliser supplied reactor and  $265.2 \pm 26.8$  L CH<sub>4</sub> kg VS<sup>-1</sup> in manure supplied reactor at steady state. Anaerobic digestion was the most stable when cow manure was supplied to digestion of vinasse.

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### 1. Introduction

The aim worldwide is to enhance renewable energy production using organic waste for the purposes of reducing fossil fuel consumption and pollution (Eriksson et al., 2014). Biogas production from organic waste is one of the most socio-economic renewable energy biotechnologies (Triolo et al., 2012). It is the policy of the European Union (EU) that production of biogas from sewage sludge, animal manure, organic waste and energy crops shall contribute to the supply of energy needed for heat and power production (Eurobserv'ER, 2012).

Large amounts of residues are produced by ethanol biorefineries, and of these residues, vinasse or stillage is produced in large

amounts when distilling ethanol produced by fermenting sugar from sugarcane, sugar beet, hydrolysed straw etc. (Moraes et al., 2014). Vinasse contains easily digestible organic matter and is a potential substrate for anaerobic digestion producing biogas (Moraes et al., 2015). However, vinasse's suitability for the anaerobic digestion process depends on the raw material and the fermentation feedstock for ethanol production (Parnaudeau et al., 2008), e.g. the macro- and micronutrient content could be low and/or the C/N ratio could be outside the optimal range for the biological process depending on the biomass source. Little research has been successfully reported with regard to the use of sugarcane vinasse for anaerobic digestion (Costa et al., 1986; Souza et al., 1992) in which the vinasse composition did not need adjusting to optimise the C/N ratio. The optimum range is reported as being between 25 and 35 for biogas production (Metcalf and Eddy, 2003). However, in the case of sugar beet vinasse, a high nitrogen content

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is reported, which makes the C/N ratio below the optimum range (Vlissidis and Zouboulis, 1993). Thus, carbon rich materials may be added to optimise the C/N ratio, when using sugar beet vinasse for biogas production, (Holm-Nielsen et al., 2009; Fang et al., 2012).

For stable anaerobic digestion performance, sugar beet vinasse may be co-digested with manure to make up for the low content of macro- and micronutrients (Demirel and Scherer, 2011). Alternatively, biorefinery by-products in the form of plant biomass, filter cake and lime fertilisers are potential supplement sources to provide macro- and micronutrients.

Furthermore, use of these by-products for co-digestion in biogas plants might be economically advantageous for biorefineries because they are obtained within the biorefinery itself and their use will reduce the need for external feedstocks for co-digestion.

The need for the use of co-substrates in biogas production from vinasse is unclear, in contrast to the many successful studies on the use of animal manure in co-digestion with industrial organic waste (El-Mashad and Zhang, 2010; Comino et al., 2012; Marañón et al., 2012). The aim of the study is to examine the potential of biogas production and to study stable performance of anaerobic digestion from sugar beet vinasse against system instability. We examined the effect of using by-products from biorefineries and manure from the livestock industry as co-substrates. We focused on stable anaerobic digestion performance and further on feasible biogas yield that can be close to the methane potentials of sugar beet vinasse. The experiments were carried out using continuous stirred tank reactors (CSTR) in the laboratory and the optimal addition of co-substrates, with a focus on adjustment of the C/N ratio plus the effect of adding nutrients in the form of chemicals and the option of replacing them by adding co-substrates. Furthermore, the organic loading rate (OLR) was varied with the purpose of defining the optimal OLR. All the biomass used was characterised by measuring biochemical methane potential (BMP), organic matter and mineral composition.

## 2. Methods

### 2.1. Substrate and inoculum

Vinasse from sugar beet ethanol production was obtained from Nordic Sugar (Copenhagen, Denmark). Two samples (1st and 2nd batches) of concentrated vinasse were provided and the chemical composition of each analysed prior to their use in the reactors. To feed to the reactors, vinasse was previously diluted in order to achieve a similar composition to sugarcane vinasse as regards organic matter content (in terms of volatile solids, VS). The dilution rate was initially 1:7 to reach the final organic content of about 60 g VS L<sup>-1</sup>, corresponding to the vinasse from sugarcane ethanol production from molasses (Costa et al., 1986). When the organic co-substrates were added, the dilution rate was changed to 1:10 to keep the same final VS content in the reactors. In the last phase of the reactors' operation, a more concentrated vinasse was provided (2nd batch), causing a higher final organic content in the diluted vinasse used in the feed and, consequently, altering the final VS content in the reactors. The inoculum was obtained from the Fangel biogas plant (Funen, Denmark), which operates under mesophilic conditions (37 °C) and is fed with a mixture of 80% animal slurry and 20% industrial organic waste from the food processing industry.

### 2.2. Continuous stirred tank reactor (CSTR)

Anaerobic IMT bioreactors (Andtec, Ski, Norway), made from 316 AISI steel, and casting acrylic tubes were used. Each reactor

had a total volume of 20 L and was made of acrylic cylinder (diameter 360 mm and height 360 mm) fitted with stainless steel plates at the top and bottom. The top plate supported a mechanical stirrer (two radial flow-impeller turbines 20.0 cm in diameter), a mixer motor, a feed tube, a temperature-measuring port and a gas-measuring/sampling port. The bottom plate was connected to four 50-watt temperature elements, producing a total of 200 watts, and a thermostat with a sensor in the reactor tank. The bottom plate also had two valves for tapping substrate from two different levels. The top plate had one valve to feed the reactor. Gas production was measured continuously with a flow metre.

The effluent tube was located at the bottom plate. The agitation was kept at 90 rpm and the operating temperature was 37 °C. Total ammoniacal nitrogen (TAN = NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup>) and VFA concentrations in the digestate and methane and carbon dioxide content in the biogas were regularly monitored.

### 2.3. Manure free anaerobic digestion of vinasse performance

Two reactors were used for the experiments (Table 1). In one of the reactors, anaerobic digestion of vinasse was operated without manure supplement. In this reactor, chemical nutrition, i.e., FeCl<sub>3</sub>·6H<sub>2</sub>O, nickel and zinc were added and afterwards, lime fertiliser replaced the chemical nutrition. The other reactor was fed with vinasse and 3% cow manure as a co-substrate. For both reactors, the C/N ratio was adjusted by the addition of microcrystalline (Avicel PH 101) cellulose that was replaced posteriorly by straw. During the start-up, the reactors were filled with inoculum (13.2 L) and after 24 h the reactors were operated in a fed-batch mode of 24 h, during which effluent was discharged and aliquots of feed were added to the reactors. After an acclimation period of 10 days, the reactors were subjected to different OLRs and hydraulic retention times (HRT). The reactors were operated for 100 days, during which different co-substrates were added with the vinasse and OLR was adjusted to between 20 and 36 days. Detailed anaerobic digestion performance at different phases is presented in Table 2.

### 2.4. Biochemical methane potential (BMP)

The BMP of vinasse and co-substrates (cow manure, straw and cellulose) were measured according to VDI 4630 (2006) using triplicate 1-L batch infusion digesters. The inoculum was degassed for 2 weeks at 37 °C prior to the BMP tests. Each substrate was mixed to the inoculum at a ratio of 1:3 on a dry matter (DM) basis, according to the VDI 4630 procedure (2006). Reactors containing only inoculum were used as the control. After filling the batch digesters with biomass, they were flushed with nitrogen gas to ensure an anaerobic atmosphere and incubated under mesophilic conditions (37 °C). Digestion was finished when the daily biogas production per batch was less than 1% of cumulative gas production (approximately 60 days of batch fermentation). The wet biogas read-off at room temperature was corrected into dry biogas at standard conditions for temperature and pressure according to VDI 4630 (2006).

### 2.5. Physicochemical analyses

The influent and effluent composition of the reactors was analysed three times per week, being the feed materials and digestates stored at 4 °C immediately after sampling. The composition of the biogas was determined daily. The methane composition in the biogas was determined using a gas chromatograph (HP 6890 series), equipped with a thermal conductivity detector and a 30 × 0.320 mm column (J&W 113-4332). The injector temperature was 110 °C, and the detector and oven temperature was 250 °C.

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