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# Anaerobic co-digestion of excess brewery yeast in a granular biomass reactor to enhance the production of biomethane

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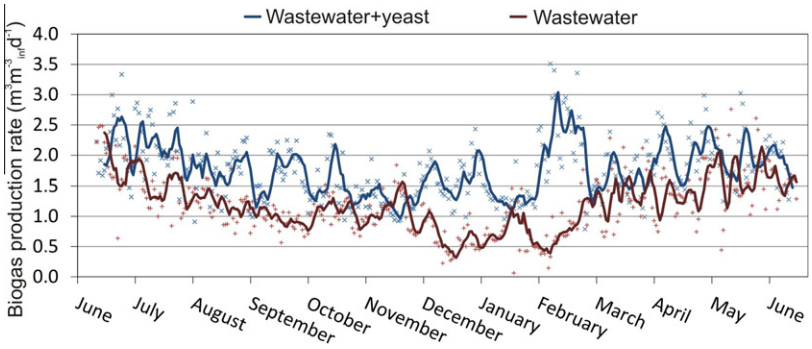
## HIGHLIGHTS

- Brewery yeast was used to produce biogas in an upflow granular anaerobic reactor.
- Mixtures up to 1.1 v/v% yeast are anaerobically degradable without adverse effects.
- In full-scale operation biogas production increased by 38.5%.
- Up to 16% natural gas were replaced in brewery operation.
- Up to 7% in archeal and a 32% dissimilarity in bacterial community were shown.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The anaerobic co-digestion of brewery yeast using granular biomass was studied on the lab, pilot and full-scale. The study shows no adverse effects in the co-digestion of yeast and wastewater in concentrations up to 1.1 (v/v)%. In concentrations up to 2.3% the process is manageable; however, not advisable. In concentrations over 2.8% the process exhibits failure due to the overload with suspended solids. An average specific biogas production of 0.560 $m^3 kg^{-1}$ of volatile solids was achieved. Full-scale operation with 0.7% yeast concentration showed a 38.5% increase in the biogas production and a 26.2% increase in the organic loading rate, which resulted in an increase of the biomethane/natural-gas substitute ratio from 10% to 16%. The influence of the yeast addition on the structure of the microbial biomass showed up to 7% dissimilarity in the archaeal and a 32% dissimilarity in the bacterial biomass community, which did not present any difficulties.



## 1. Introduction

The brewing industry produces large quantities of wastewater with a high concentration of degradable organic pollutants, which are ideal for the production of biogas. There are many conventional ways reported in the recent literature about how to successfully treat brewery wastewater, ranging from fluidised-bed bioreactors (Alvarado-Lassman et al., 2008; Ochieng et al., 2002) anaerobic sequencing batch reactors (Shao et al., 2008) to the mostly commonly used granular sludge reactors (Baloch et al., 2007; Parawira et al., 2005; Leal et al., 1998; Cronin and Lo, 1998) such

*Abbreviations:* BMP, biochemical methane potential; BPR, biogas production rate (per reactor volume); BPRi, biogas production rate (per influent volume); COD, chemical oxygen demand; EGSB, expanded granular sludge bed; FID, flame ionisation detector; OLR, organic loading rate; PCR, polymerase chain reaction; Q, wastewater flow; SBP, specific biogas production; TCD, thermal conductivity detector; TKN, total kjeldahl nitrogen; TRFs, terminal restriction fragments; T-RFLP, terminal restriction fragment length polymorphism; TS, total solids; UASB, upflow anaerobic sludge blanket; UPGMA, unweight pair-group method with arithmetic means; VFA, volatile fatty acids; VS, volatile solids.

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as the upflow anaerobic sludge blanket (UASB) reactors or the upgraded versions, the EGSB (expanded granular sludge bed) reactors (Zoutberg and de Been, 1997). Such processes are well established and known; they appear in many varieties and are used in a range of temperature regimes as well (Connaughton et al., 2006). Although the previously cited scientific papers (Alvarado-Lassman et al., 2008; Baloch et al., 2007; Connaughton et al., 2006; Parawira et al., 2005; Ochieng et al., 2002; Leal et al., 1998; Cronin and Lo, 1998) mainly focus on the treatment procedure itself and its efficiency, it has been the trend in recent years to devote more attention to energy production. As a result, the operators of such anaerobic treatment plants devote their attention to biogas production and its increase, mainly due to carbon-footprint reduction, as well as due to the economic benefits of biogas production. Therefore, in the process operation of such treatment plants, possible additional substrates are identified in order to increase the biogas production and possibly lower the operational costs. Bocher et al. (2008) showed an 8.1% increase in methane production when treating secondary residuals in brewery-wastewater treatment, while Agler et al. (2010) showed a 7.6% increase in methane production when treating primary sludge. In a study done by Neira and Jeison (2010) brewery yeast was already tested as a co-substrate in brewery wastewater treatment with no adverse effects; however the authors expressed concerns about long term operation and its effect on granular sludge. In our case study, Brewery Laško, one of the largest Slovenian breweries, produces approximately 400,000 $m^3$ of wastewater annually and has a Biothane's Biobed® EGSB reactor installed for the wastewater treatment, where the produced biogas is used as a supplement for natural gas. The brewery also produces 3000 tons of waste yeast annually, which has always been considered a secondary resource, although it has never been identified as an energy substrate. In the traditional way, waste yeast is dried and sold as an alimentary substrate to the food-processing industry. However, our previous research (Zupančič et al., 2009) has shown that such processing is very energy demanding due to the large amount of natural gas required for the drying, and with ever-increasing energy prices such a conventional procedure is becoming more economically unsustainable. Waste yeast is high in organic solids' content and can be used as an additional substrate in a UASB reactor to produce more biogas and, consequently, save the natural gas that is used in the brewing process. This research paper looks at the possibilities of using waste yeast as an energy substrate to increase biogas production in brewery-wastewater treatment. In addition to previous studies done in this field (Neira and Jeison, 2010), in the presented study higher concentrations of brewery yeast in the wastewater/yeast mixture were used and the impact of yeast to microbial biomass was addressed in pilot as well as in full scale. Our previous research (Zupančič et al., 2009) has shown that waste yeast can deliver a biogas yield of 0.45–0.72 $m^3\,kg^{-1}$ of volatile solids, which at first glance makes it an ideal substrate for biogas production; however, it cannot be anaerobically digested as a mono substrate due to its high nitrogen content (total nitrogen concentration 11–13 $g\,L^{-1}$), which is above the concentration that might induce ammonia inhibition (Sung and Liu, 2003; Chen et al., 2008). Yeast treated as a co-substrate to brewery wastewater is massively diluted; therefore, the ammonia concentration is drastically lowered and the organic material is available for biogas production.

## 2. Methods

### 2.1. Lab-scale experiments

A biochemical methane potential assay (BMP) was conducted according to the SIST EN ISO 11734:1999 standard and independently replicated twice. Oxitop® bottles (1170 mL) equipped with pressure sensors (WTW, Germany) were used as the anaerobic batch reactors. The side-neck sampling ports were sealed with butyl rubber stoppers to prevent any gas leakage. The granulated inoculum biomass was sampled from the full-scale Biothane's EGSB reactor of Brewery Laško and its concentration was adjusted to 2 $g\,L^{-1}$ of volatile solids. Inoculum with no carbon-substrate addition served as a negative control for the residual metabolic activity; positive control was supplied with 0.2 g COD of glucose. All of the experimental variants were supplied with a substrate mixture (brewery wastewater and wastewater supplemented with 0.74% (v/v), 1.85% and 3.7% waste brewery yeast) of 0.2 $g_{COD}g_{VS}^{-1}$ inoculum. The average COD, TS, VS and TKN of the waste brewery-yeast suspension throughout all the experiments were 277, 188, 177 and 11.4 $g\,L^{-1}$, respectively. The Oxitop® bottles were flushed with nitrogen gas for 15 min to ensure anaerobic conditions and were incubated at 37 °C and 120 rpm in a temperature-controlled incubator (Infors, Switzerland). Hourly measurements of the total biogas produced during 21 days of incubation were recovered from pressure-sensor data loggers. The headspace gas composition and the concentration of the volatile fatty acids (VFAs) were determined on days 0, 2, 9, 15 and 21.

Throughout the experiments the total solids (TS), the volatile solids (VS) and the COD values were determined according to the Standard Methods Online (2010). The biogas and VFAs were determined by gas chromatography on Shimadzu, GC14A-TCD and Shimadzu, GC14A-FID, respectively. The gasses were separated using a 4-m-long steel column with an inner diameter of ¼ of an inch, filled with Porapak Q. Helium was used as a carrier gas. The injector and column temperatures were 30 °C and the detector temperature was 80 °C. The chromatographic signals were evaluated by the integrator Chromatopack CR-4A (Shimadzu) based on an absolute calibration. The VFAs were determined as described in Sežun et al. (2011). The pH was measured using a pH meter (Orion 520A). All the methods were kept under continuous statistical control. Control charts were created from the results obtained in the analysis of the RMs (laboratory working reference standards). In addition, our laboratory also participated in proficiency tests (AQUACHECK, WRc plc, UK) and a good performance was recorded in all the determinations. The measurement uncertainty of the measured COD, TS and VS was 8% and the uncertainty of the biogas measurements was 3% (95% confidence limit). The measurement uncertainty was evaluated according to Drolc et al. (2003) and the principles of the Guide to the Expression of Uncertainty in Measurement (BIPM-IEC-IFCC-ISO-IUPAC-IUPAP-OIML, 1995).

### 2.2. Pilot-plant experiments

To evaluate the impact of the waste-yeast addition to the brewery wastewater a 12-l lab-scale pilot UASB reactor was used (Fig. 1). The reactor was equipped with influent and effluent tanks; the pre-conditioning took place in the influent tank. A biogas treatment unit ($H_2S$ and moisture trap) and a biogas flowmeter (Agilent ADM 2000) were used as well. The influent was supplied to the reactor by a membrane pump. The testing procedure was simple: we mixed the waste yeast with the wastewater in the influent tank, adjusted the pH to 6.5, and treated the mixture in the reactor that was operated under various organic loading rates, similar to the situation in the full-scale EGSB reactor.

To determine the sustainability of the wastewater and the yeast treatment we performed several experiments in consecutive order, starting with the plain brewery wastewater as a control, and continuing with the mixtures of 0.7%, 1.1%, 1.6%, 2.3% and 2.8% yeast in wastewater. We performed the experiment at different organic loading rates, ranging from 3.8 to 16.2 $kg_{COD}\,m^{-3}\,d^{-1}$. The duration of the control was 14 days (0% brewery yeast), and the latter experiments 228 days (0.7% brewery yeast), 37 days (1.1%),