

Biogas Production from Brewer's Yeast Using an Anaerobic Sequencing Batch Reactor

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Received: November 24, 2016

Accepted: February 24, 2017

Summary

Renewable energy sources are becoming increasingly important in the beverage and food industries. In the brewing industry, a significant percentage of the used raw materials finishes the process as secondary resource or waste. The research on the anaerobic digestion of brewer's yeast has been scarce until recent years. One of the reasons for this is its use as a secondary resource in the food industry and as cattle feed. Additionally, market value of brewer's yeast is higher than its energy value. Due to the increase of energy prices, brewer's yeast has become of interest as energy substrate despite its difficult degradability in anaerobic conditions. The anaerobic co-digestion of brewer's yeast and anaerobically treated brewery wastewater was studied using a pilot-scale anaerobic sequencing batch reactor (ASBR) seeded with granular biomass. The experiments showed very good and stable operation with an organic loading rate of up to 8.0 kg/(m³·day), and with a maximum achieved organic loading rate of 13.6 kg/(m³·day) in a single cycle. A specific biogas productivity of over 0.430 m³/kg of the total chemical oxygen demand (COD) inserted, and total COD removal efficiencies of over 90 % were achieved. This study suggests that the brewer's yeast can be successfully digested in an ASBR without adverse effects on the biogas production from brewer's yeast/wastewater mixtures of up to 8 % (by volume). By using the brewer's yeast in the ASBR process, the biogas production from brewery wastewater could be increased by 50 %.

Key words: anaerobic digestion, ASBR, biogas, brewer's yeast

Introduction

As a result of rising fuel costs and urgent need for the reduction of industrial carbon footprint (1), renewable energy sources are becoming increasingly important in the beverage and food industries. In the brewing industry, a significant percentage of the used raw materials finishes the process as secondary resource or waste. The anaerobic digestion offers the possibility to treat these substrates

successfully in order to produce biogas, which can substitute natural gas needed in the brewing process.

The brewing wastewater is the dominant waste stream in the brewing industry and as such has received significant attention in recent times. Usually, this brewing wastewater is treated in anaerobic systems using granular biomass (2). Reactors used for these treatments are up-flow anaerobic sludge blanket (UASB) systems (3,4), adapted

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expanded granular sludge bed (EGSB) reactors (5) and more recently anaerobic sequencing batch reactors (ASBR) (6). However, flow-through UASB reactors are conventionally high-load reactors and have significant problems when degrading high-solid substrates (7), which is most certainly the case when adding yeast to the wastewater. As a result, equipment suppliers usually prescribe a limit for total solids of 5 g/L. In such cases, the ASBR technology can offer a solution for the mentioned problem regarding the degradation of high-solid substrates. In these reactors the granulated sludge tolerates higher concentrations of solids, due to the settling stage of the operation.

The biogas production by anaerobic digestion of the brewery wastewater can offer substantial savings with respect to the energy demands of the brewery. Kormelinck (8) reports that up to 20 % of the natural gas can be substituted with methane from the produced biogas. Moreover, recent research suggests that these savings can even be increased, since there are additional substrates suitable for biogas production already available in most of the breweries. Agler *et al.* (9) reported the anaerobic digestion of the primary brewery wastewater sludge in an ASBR system and achieved a 7.6 % increase in methane production. On the other hand, Bocher *et al.* (10) offered the treatment of secondary residuals from an UASB effluent and achieved an 8 % increase in methane generation. Other substrates available in the breweries that have been identified as suitable for biogas generation are yeast and brewer's spent grain.

The research on the anaerobic digestion of brewer's yeast and brewer's spent grain has been scarce until recent years. One of the reasons for this is because these substrates have so far been used as a secondary resource in the food industry and as cattle feed (11). Furthermore, they have had a market value higher than their energy value. It was only recently, due to the increase of energy prices, that these substrates have become of interest as energy substrates. The other reason for these substrates being of little interest is their difficult degradability in anaerobic conditions. Neira and Jeison (12) were the first to report a successful anaerobic digestion of brewer's yeast in combination with the brewery wastewater. In our previous work (13), we followed and thoroughly researched the possibilities of brewer's yeast co-digestion with the brewery wastewater under different conditions. We have found that with brewer's yeast/wastewater mixtures of up to 1.1 % (by volume) there were no negative impacts on the digestion process, provided that the organic loading rate of the brewery wastewater treatment reactor (EGSB) allowed additional loading. Experiments with more concentrated mixtures showed adverse effects, which were reflected in reduced total chemical oxygen demand (tCOD) degradation efficiencies and biogas production, regardless of the organic loading rate. A full-scale anaerobic co-digestion plant treating brewer's yeast and brewery wastewater mixtures has now been in operation for over five years in a local brewery in Slovenia, and it has only shown positive results (14). There are no adverse effects on technical operation of the plant. Moreover, an average product value of 0.2 euro cent per litre of brew through increased methane usage is achieved.

The objective of this study is to demonstrate a feasible use of brewer's yeast as an energy co-substrate by digestion of yeast/wastewater mixtures in an ASBR system at volume ratios higher than 1.1 %. Such cases would occur when the brewery wastewater treatment system is at full capacity and does not allow additional organic loading by adding the brewer's yeast to the raw brewery wastewater. The treatment in an additional ASBR with a mixture of anaerobically digested brewery wastewater and yeast could offer a solution for the brewer's yeast digestion.

Materials and Methods

ASBR reactor

A pilot-scale anaerobic sequencing batch reactor (ASBR) made of Schott glass (Büchi Glass, Uster, Switzerland) with an operational volume of 30 L and an exchange volume of 10 L was used during the experimental work (Fig. 1). A stirrer RZR 2021 (Heidolph Instruments, Schwabach, Germany) at 70 rpm provided mixing of the substrate and biomass inside the reactor. The biogas production was continuously recorded with an ADM 2000 gas flow meter (Agilent Technologies, Wilmington, DE, USA). The temperature in the reactor was controlled at (32 ± 1) °C. The reactor was also equipped with an on-line differential pH probe (pHD-S sc; Hach-Lange, Düsseldorf, Germany). The volume fraction of methane (φ) in biogas was estimated by installing a custom-made semi-dry absorption unit during the fourth cycle of every experiment, operating with NaOH pellets (Merck Millipore, Darmstadt, Germany). The same pellets were used to make a 20 % (by mass) solution for neutralisation.

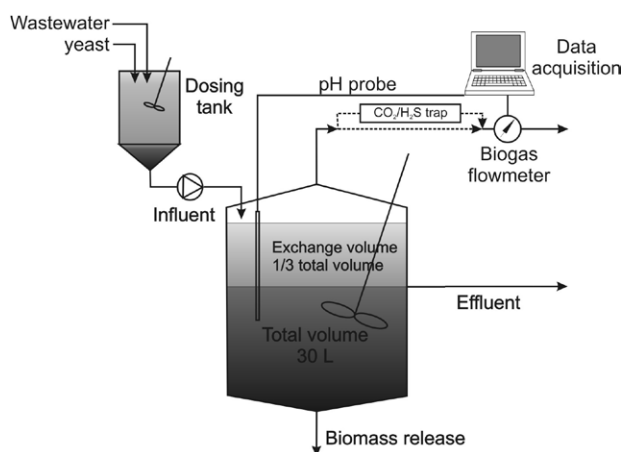


Fig. 1. Experimental anaerobic sequencing batch reactor (ASBR) setup

Seed sludge (inoculum)

The seed sludge was taken from an operational expanded granular sludge bed (EGSB) reactor at the local brewery (Laško, Slovenia) in which the brewery wastewater was treated at (35 ± 2) °C, depending on the seasonal weather. The sludge was typically granular with a total solid concentration $\gamma(\text{TS})=80\text{--}100$ g/L, of which 90 % were

volatile solids (VS). A total of 15 L of the sludge was used as inoculum, which constitutes 50 % of operational reactor volume.

Wastewater and brewer's yeast

The wastewater used in the experiments was taken directly from the effluent of the same EGSB reactor from which the inoculum was collected. Typically, it contained mass concentrations of TS and VS of (1.0 ± 0.2) and (0.3 ± 0.1) g/L, respectively, with the total chemical oxygen demand concentration γ (tCOD) in the range of 300–600 mg/L and no anaerobic biodegradability. The anaerobic degradability of the collected wastewater was investigated in the first cycle of the first experiment and it showed to be negligible and within the measurement uncertainty of the ADM 2000 biogas flow meter (Agilent Technologies). The temperature of the collected and used wastewater was (35 ± 2) °C, depending on the seasonal weather. The excess yeast was collected directly from the yeast collection tank of the lager beer production line of the local brewery at a temperature of approx. (8 ± 3) °C. Typically, the mass concentrations of TS, VS and tCOD in yeast were (185.0 ± 5.0) , (175.0 ± 5.0) and (265.0 ± 15.0) g/L, respectively. The yeast and wastewater were mixed in a dosing tank of the pilot plant prior to the filling of the reactor. Each batch (10 L) was neutralised to pH=6.5 using a 20 % (by mass) NaOH solution. As previously stated, the effluent from the EGSB reactor had a temperature of (35 ± 2) °C, while the waste yeast had a temperature of (8 ± 3) °C. By mixing the substrates on site, a mixture temperature of (32 ± 1) °C was readily achieved without the use of any additional heating.

ASBR operation

A series of eight experiments were performed, each of them having five cycles. The cycles were adapted to the actual beer production process in the local brewery and the consequent wastewater and yeast discharge, which were repeated conventionally on a weekly basis. Therefore, the first four cycles were operated for 24 h each, while the fifth cycle (over the weekend) was operated for 72 h. Each cycle consisted of four phases: fill, react, settle and release. The fill phase lasted approx. 1 h, when the mixture of wastewater and yeast was pumped into the reactor. The react phase was already on-going while pumping, and it continued for an additional 20 h (68 h in the fifth cycle). The settling phase lasted approx. 2 h. This was followed by the release phase, lasting 1 hour.

The experiments were carried out using various yeast and wastewater mixture ratios. In experiment 1, a mixture of 2 % (by volume) of yeast in the wastewater was used in all 5 cycles. Experiments 2–6 were performed with 4, 6, 8, 10 and 12 % (by volume) mixture in all cycles, respectively. During the first cycle in experiment 7 or 10 % (by volume) of yeast in the wastewater was used, in cycles 2–4 11 % (by volume) and in the fifth cycle 17 % (by volume) mixture was used. In experiment 8, during the first two cycles 9 % (by volume) mixture was used, in cycles 3 and 4 10 % (by volume) and in cycle 5 22 % (by volume) mixture was used. The mixtures in experiments 7 and 8 were selected to match the quantity of waste yeast in experiment 6 and to improve the efficiency of the process by

taking into account the prolonged fifth cycle. Samples were taken from each influent and effluent analysing total chemical oxygen demand (tCOD). The dissolved COD (dCOD) was determined only in the samples from the effluent. The volatile fatty acids (VFAs) of the effluents were analysed for every experiment cycle with the highest organic loading rate (OLR). After experiment 6, the biomass accumulation was estimated based on the accumulated TS and VS.

Analytical methods

The mass concentrations of total solids (TS), volatile solids (VS), total Kjeldhal nitrogen (TKN) and ammonia nitrogen ($\text{NH}_4\text{-N}$) were analysed according to APHA standard methods (15). The mass concentrations of tCOD and dCOD were monitored and analysed during all cycles of the conducted experiments. The VFA content was determined only once in all cycles, and mass concentrations of TKN and $\text{NH}_4\text{-N}$ only once in cycles 2, 3, 4, 5, 6 and three times in cycles 7 and 8 in average samples. The COD was determined in accordance with the procedure of ISO 6060:1989 (16). The dissolved portion of the COD was determined by filtration through an ashless black ribbon filter (Whatman, Dassel, Germany). The VFAs were extracted from the samples with diethyl ether according to the procedure of Holdeman *et al.* (17) and analysed with a HP5890 gas chromatograph (Hewlett Packard, Wilmington, DE, USA) with a split/splitless injector and a flame ionization detector, equipped with a fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm stationary phase thickness (model 20988-03A; Supelco, Bellefonte, PA, USA). The VFA content was determined using an internal standard method. The temperatures of the injector, detector and the column were 185, 290 and 75 °C, respectively. All methods were kept under continuous statistical control. The control charts were created from the results obtained in the analysis of the reference material (laboratory working reference standards). In addition, the laboratory participated in proficiency tests (18) and a good performance was obtained in all determinations. The measurement uncertainties of the measured concentrations of COD, TS and VS were always below 8 % and the uncertainty of the biogas measurements was 3 % (95 % confidence limit). The measurement uncertainty was evaluated according to Drolic *et al.* (19) and the principles of the Guide to the Expression of Uncertainty in Measurement (20).

Results and Discussion

Process operation

The experiments of the anaerobic co-digestion of brewer's wastewater and brewer's yeast in an ASBR system were performed during a total of 40 cycles divided into eight experiments as described in previous sections. The major expected drawback during the operation of such process was the accumulation of yeast solids throughout the duration of the process in the settled biomass of the reactor. However, the settleability of the yeast solids was very low compared to the settleability of the granulated biomass, and the expected accumulation of solids did not

present a problem. In addition, in the first cycle of experiment 1, the anaerobically digested wastewater, which was used for dilution was tested for any eventual residual biodegradability. The result is presented in Fig. 2 and it shows that no statistically significant increase of biogas production was observed, compared to the background (respiration of inoculum).

Biogas production and pH were monitored on-line and are shown in Figs. 2 and 3. The average mass concen-

trations of the tCOD in the influent ($tCOD_{in}$) and dCOD in the effluent ($dCOD_{eff}$), the mass concentrations of TKN and NH_4-N and the volume fraction of methane in biogas are shown in Table 1. The COD removal, specific biogas production (SBP) and organic loading rate (OLR) are shown in Figs. 4 and 5. The VFA mass fractions are shown in Table 2. From the obtained results, it could be concluded that the process operated very well with yeast/wastewater mixtures of up to 8 %, by volume (experiments 1 to

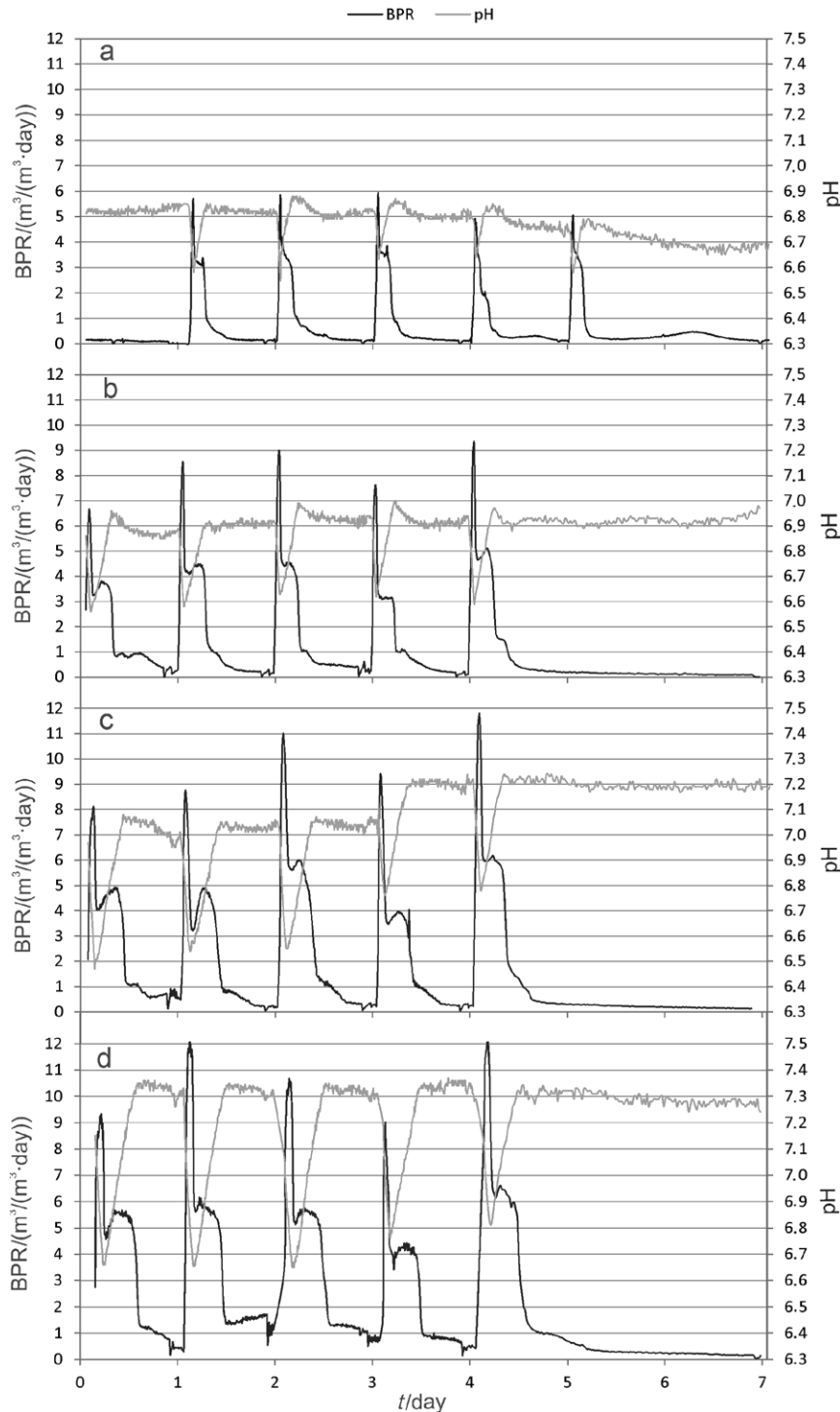


Fig. 2. Biogas production rate (BPR) and pH values during: a) experiment 1, b) experiment 2, c) experiment 3, and d) experiment 4

4, Figs. 2 and 4). This corresponds to an OLR of 7.0 kg/(m³·day), tCOD removal of over 80 % and dCOD_{eff}/tCOD_{inf} removal of over 94 %. The VFA concentrations were very low, which confirms the very good operation. Similar results of the treatment of brewery slurry were reported in our previous research (21) and of the treatment of brewery wastewater by other authors (6). By increasing the OLR of the treated yeast/wastewater mixtures from 10 to 12 % (by volume), the COD degradation was substantial-

ly lower with tCOD removal of below 80 % and dCOD_{eff}/tCOD_{inf} of below 88 %. However, the process was still stable. During the longer lasting fifth cycle, the performances slightly improved, which is evident also when 17 % yeast/wastewater mixture (by volume) with a cycle time of 3 days was used, where the tCOD removal increased to 81.4 % and the dCOD_{eff}/tCOD_{inf} to 88.5 % (Fig. 5). The process efficiency was reflected in the change of volatile fatty acid mass fractions in the effluent as well (Table 2),

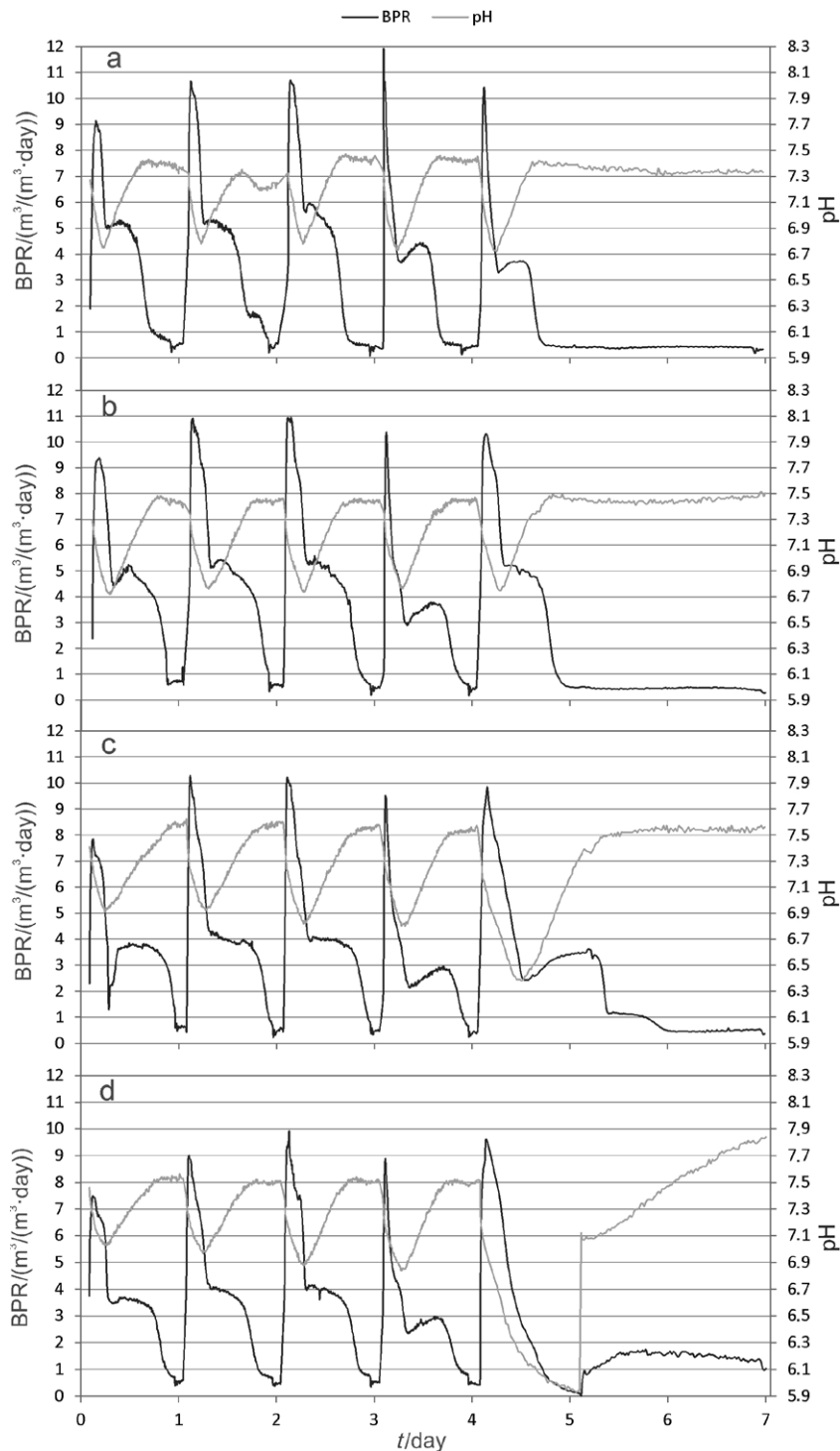


Fig. 3. Biogas production rate (BPR) and pH values during: a) experiment 5, b) experiment 6, c) experiment 7, and d) experiment 8

Table 1. Process parameters in experiments

| Experiment | tCOD | | dCOD | | NH ₄ -N | | TKN | | OLR | SBP | BPR | φ(methane) in cycle 4 | |
|------------|------------|-----------|----------|--------|--------------------|----------|----------|----------|-----------|-----------|-----------|-----------------------|---------------------------------------|
| | influent | effluent | effluent | | influent | effluent | influent | effluent | | | | m ³ /kg | m ³ /(m ³ ·day) |
| 1 | 5701±317 | 755±42 | 139±8 | n.d. | n.d. | n.d. | n.d. | n.d. | 1.90±0.11 | 0.44±0.05 | 0.80±0.09 | 0.80±0.09 | 72.2 |
| 2 | 10450±578 | 856±48 | 207±12 | 119±10 | 176±12 | 327±18 | 250±13 | 250±13 | 3.48±0.19 | 0.46±0.02 | 1.67±0.06 | 1.67±0.06 | 72.4 |
| 3 | 16271±903 | 1409±78 | 617±34 | 152±11 | 458±48 | 697±55 | 557±53 | 557±53 | 5.42±0.30 | 0.44±0.04 | 2.44±0.22 | 2.44±0.22 | 72.8 |
| 4 | 20800±1150 | 4014±224 | 1240±70 | 211±13 | 660±62 | 873±87 | 980±92 | 980±92 | 6.93±0.38 | 0.45±0.02 | 3.35±0.21 | 3.35±0.21 | 72.4 |
| 5 | 25522±1420 | 5778±322 | 3235±180 | 246±13 | 752±73 | 1019±99 | 973±92 | 973±92 | 8.51±0.47 | 0.43±0.02 | 3.89±0.30 | 3.89±0.30 | 71.9 |
| 6 | 31369±1760 | 9371±524 | 4783±268 | 327±19 | 941±90 | 1249±109 | 1238±108 | 1238±108 | 10.5±0.59 | 0.44±0.04 | 4.55±0.15 | 4.55±0.15 | 70.8 |
| 7/cycle 1 | 25416±1420 | 7331±407 | 4061±227 | n.d. | n.d. | n.d. | n.d. | n.d. | 8.47±0.47 | 0.42±0.02 | 3.56±0.24 | 3.56±0.24 | n.d. |
| 7/cycle 4 | 25659±1430 | 7017±390 | 4374±244 | n.d. | n.d. | n.d. | n.d. | n.d. | 8.55±0.48 | 0.46±0.03 | 3.95±0.26 | 3.95±0.26 | 69.9 |
| 7/cycle 5 | 40719±2290 | 7569±425 | 4697±264 | 520 | 1160 | 2104 | 1493 | 1493 | 13.6±0.76 | 0.44±0.03 | 3.13±0.21 | 3.13±0.21 | n.d. |
| 8/cycle 1 | 21253±1190 | 7578±423 | 4392±245 | n.d. | n.d. | n.d. | n.d. | n.d. | 7.08±0.40 | 0.47±0.02 | 3.48±0.12 | 3.48±0.12 | n.d. |
| 8/cycle 4 | 25292±1410 | 5813±325 | 3509±196 | n.d. | n.d. | n.d. | n.d. | n.d. | 7.10±0.47 | 0.45±0.02 | 3.83±0.13 | 3.83±0.13 | 70.0 |
| 8/cycle 5 | 52738±2960 | 14086±790 | 7922±445 | 548±53 | 2250±227 | 2238±212 | 2630±252 | 2630±252 | 17.6±0.99 | 0.34±0.01 | 2.04±0.07 | 2.04±0.07 | n.d. |

n.d.=not determined

tCOD and dCOD=total and dissolved oxygen demand respectively, TKN=total Kjeldahl nitrogen, OLR=organic loading rate, SBP=specific biogas production, BPR=biogas production rate

which increased by increasing the load and slightly decreased during the fifth cycle of the experiment with the 17 % yeast/wastewater mixture (by volume). When a yeast/wastewater mixture of up to 17 % (by volume) was used, the observed mass fractions of the acetic and propionic acids were very low. This indicated a very good performance of the methanogenic microorganisms and a limiting step of the degradation of yeast solids. The maximum achieved operational OLR was 13.6 kg/(m³·day), which is comparable with the results obtained by Baloch *et al.* (4). They managed to successfully treat the brewery wastewater in a phase-separated granular bed reactor at a similar OLR of 13.38 kg/(m³·day). Further increase of the OLR to 17.6 kg/(m³·day) with a higher volume ratio of yeast/wastewater mixture of 22 % resulted in process failure. The process resembled a classical organic overload, with a massive increase in VFA production and a consequent decrease of pH to 6.0 (Fig. 3). Therefore, we can safely state that the OLR was too high. Although an immediate intervention was performed by an automated system, by adding NaOH when the pH reached 6.0 and correcting it to pH=7.0 (Fig. 3), the system never fully recovered to its original performance capability. Even after completing the cycle (minimum biogas production), the mass fraction of the acids was still very high (Table 2). A few later process recovery attempts were made and recovery was achieved after 5 days (effluent γ(tCOD)<10000 mg/L), at an OLR of approx. 6.0 kg/(m³·day). The performance was less than 45 % of its original efficiency, prolonging the original daily cycle to two days. Moreover, a visual disintegration of the granular sludge was detected, considerably reducing the granular size and consequently its settleability. In all, the performance was severely compromised, although not completely suspended. We estimated that an OLR of 2.0 kg/(m³·day) could be achieved in these conditions, however, such a low OLR would not represent a feasible performance when compared to the original capabilities.

Biogas production

The biogas production and pH values during the experiments are presented in Figs. 2 and 3. The values of the specific biogas production (SBP) are presented in Figs. 4 and 5. On average, when the process performance was good, a SBP of over 0.430 m³/kg was achieved. As expected, the biogas production rate (BPR) was higher at a higher OLR, peaking at 4.55 m³/(m³·day) during experiment 6 (yeast/wastewater mixture of 12 % by volume). The methane content was in the range of 70–73 %. Similar results were also presented by Shao *et al.* (6), where a SBP of 0.480 m³/kg and a BPR of 2.4 m³/(m³·day) were achieved at an OLR of 5.0 kg/(m³·day). The BPR showed a typical profile. At first, the biogas from dissolved organic matter was produced (a distinctive peak at the beginning of the reaction phase). Later on, a less distinctive peak of degrading solids, which was dissipated over a longer period of time due to the slower degradability, was observed. Fig. 3 presents the biogas production in experiments 5–8, where it is clear that the biogas production in experiments 6–8 was not completely finished before the cycle was over, and this was reflected in the acid mass fractions in the effluent as well (Table 2).

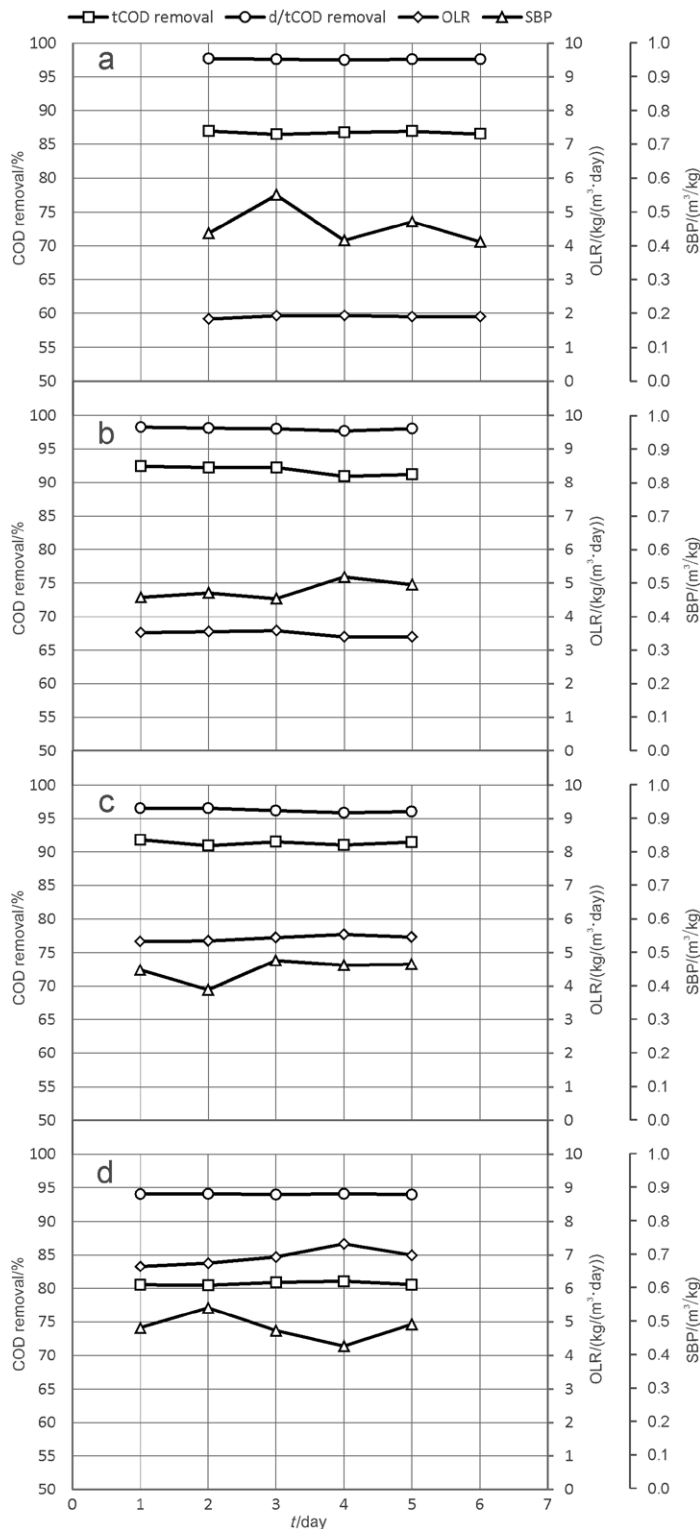


Fig. 4. Chemical oxygen demand (COD) removal, organic loading rate (OLR) and specific biogas production (SBP) on insoluble COD basis during: a) experiment 1, b) experiment 2, c) experiment 3, and d) experiment 4

Taking into account the obtained results, it can be stated that under the presented conditions of a 1-day cycle, an OLR of over $8.0 \text{ kg}/(\text{m}^3 \cdot \text{day})$ would not be advisable over longer periods of time. Moreover, a maximum OLR of $13.6 \text{ kg}/(\text{m}^3 \cdot \text{day})$ is achievable in a single cycle but with a cycle duration of at least two days. It can also be concluded that a

mixture of brewer's yeast and wastewater does act, in a certain way, very similarly in anaerobic digestion as does the brewery wastewater itself, which is confirmed by comparing the results of this study with the results obtained by other authors (4,6). The concentration of yeast in the yeast/wastewater mixture does not affect the anaerobic digestion

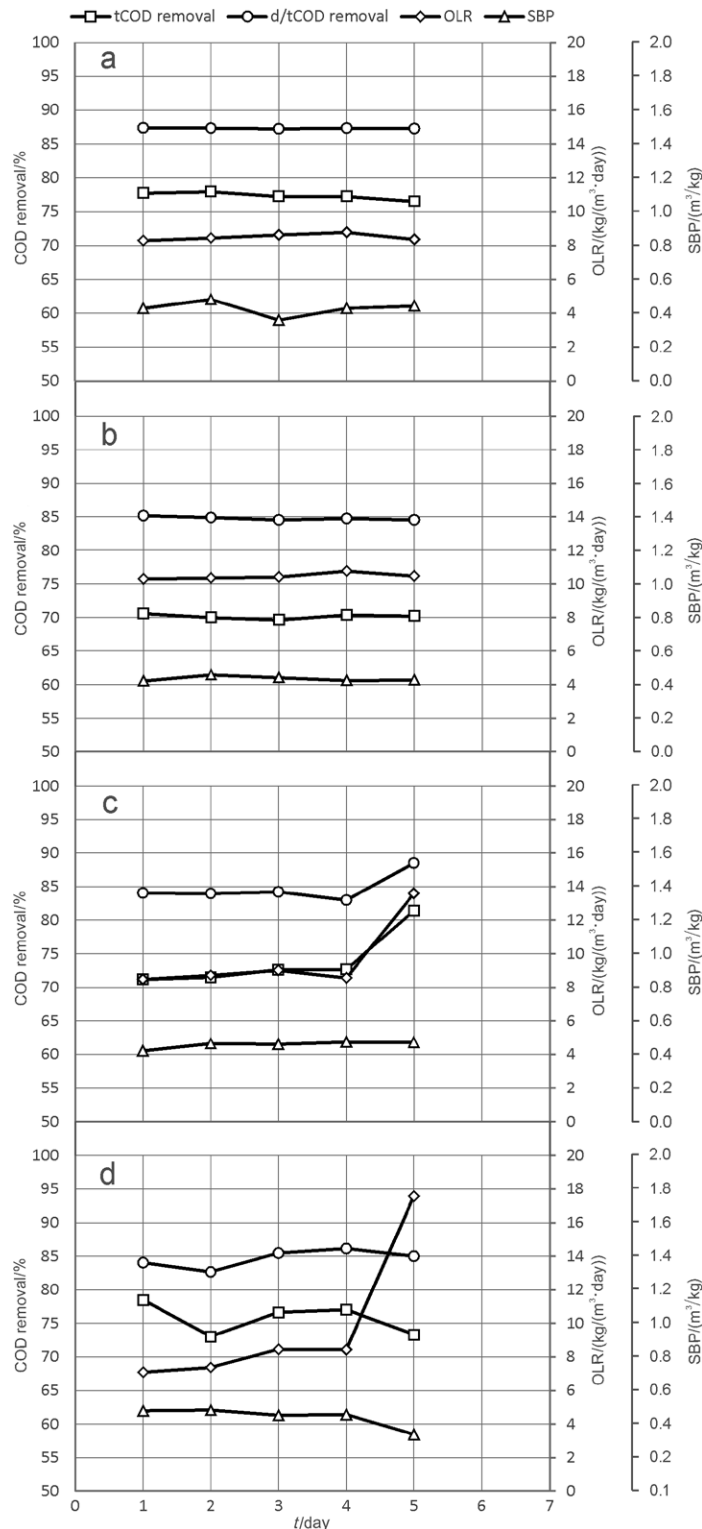


Fig. 5. Chemical oxygen demand (COD) removal, organic loading rate (OLR) and specific biogas production (SBP) on insoluble COD basis during: a) experiment 5, b) experiment 6, c) experiment 7, and d) experiment 8

in ASBR significantly; the increase of OLR due to yeast addition to the mixture has the predominating impact. Contrary to our previous research (13), high ratios of yeast in yeast/wastewater mixture (over 1.1 % by volume) do not hinder the anaerobic digestion process in the ASBR as much as the resulting OLR does. Therefore, we can state that as

long as the OLR is within the limits, in our case less than 13.6 kg/(m³·day), anaerobic digestion can be operated safely using any yeast volume fraction up to 17 % in a single cycle. This confirms that ASBR is more suitable for anaerobic digestion of yeast at higher ratios than 1.1 % (by volume) than the EGSB reactor.

Table 2. Mass fractions of volatile fatty acids in experiments

| Experiment/ cycle | $w(\text{acid})/(\text{mg}/\text{kg})$ | | | | | | | | |
|----------------------|--|-----------|------------|-------------------|---------|----------------------|----------|-----------|----------|
| | Acetic | Propionic | Isobutyric | <i>n</i> -Butyric | Valeric | 4-Methyl- valeric | Capronic | Heptanoic | Total |
| 1/4 | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. |
| 2/4 | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. |
| 3/4 | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. | b.d.l. |
| 4/4 | 2±1 | 1±1 | 8±2 | 1±1 | b.d.l. | b.d.l. | b.d.l. | 11±5 | 24±5 |
| 5/4 | 4±1 | 5±1 | 211±59 | 320±93 | 126±44 | 1±1 | 18±6 | 9±4 | 955±153 |
| 6/4 | 5±1 | 7±1 | 303±106 | 607±212 | 184±64 | 4±1 | 27±7 | 6±2 | 1611±242 |
| 7/5 | 7±1 | 4±1 | 345±121 | 193±68 | 194±69 | 276±97 | 28±7 | 1±1 | 1429±229 |
| 8/5 | 260±13 | 701±95 | 341±120 | 882±296 | 185±59 | 397±115 | 17±4 | 2±1 | 3160±474 |

b.d.l.=below detection limit

Additional concerns and impacts

Although the anaerobic digestion of brewer's yeast has been proven to be feasible, some additional concerns should be addressed. The biomass growth was estimated after experiment 6 and compared to the biomass growth in the EGSB reactor treating brewery wastewater from which the inoculum was collected. The biomass growth was 0.110 and 0.123 kg of TS per kg of tCOD, added and degraded, respectively, whereas in the EGSB reactor 0.087 kg of TS per kg of tCOD were added (13). This increase can be attributed partially to the higher OLR (the EGSB reactor was operating at an average OLR of 4.0 kg/(m³·day)), but mainly to the accumulation of degraded yeast solids. Although no adverse effects were observed as a consequence of the accumulation of solids, a longer-term study should be performed in order to confirm whether there are any negative effects after longer-term operation. The excess biomass was removed after experiment 6 and there were no negative effects on the process observed in experiment 7.

The yeast has a $\gamma(\text{TKN})=(12.0\pm 1.0)$ g/L, which may cause some problems in terms of ammonia inhibition (22) as well as in any subsequent aerobic treatment, which is a conventional step in brewery wastewater treatment. In our research, we observed maximum mass concentrations of NH₄-N and TKN of 2250 and 2630 mg/L, respectively, at the maximum ratio of yeast/wastewater mixture of 22 % (by volume). The mass concentrations of NH₄-N and TKN were much lower at cycles with lower yeast/wastewater mixture ratio (data shown in Table 1). These concentrations were well below the ammonia inhibition limit, which according to Chen *et al.* (22) is between 1400 and 4000 mg/L. The ratio of yeast in the yeast/wastewater mixtures of our interest was up to 17 % (by volume); therefore, it does not constitute any concern regarding ammonia inhibition. However, such anaerobic digestion of yeast represents a several-fold increase in the nitrogen load in aerobic treatment, which usually follows such anaerobic digestion. The results of the presented study were extrapolated to the brewery wastewater treatment plant where the substrates were collected, and the nitrogen content in the aerobic treatment stage increased on average by 60 %. This is a considerable increase and it should be considered. Analyses of samples of the substrates taken from the

full-scale plant operation, anaerobically digesting all the excess brewer's yeast available (14), showed that in the case of local brewery such an increase of nitrogen load was well managed in the aerobic treatment stage with no significant increases in the used resources. Moreover, yeast digested to biogas can offer up to a 50 % increase of natural gas/biogas substitution ratio, which can be increased from 20 (8) to 30 %.

Conclusions

Employing an anaerobic sequencing batch reactor (ASBR) is an effective way of using brewer's yeast as an energy co-substrate for the biogas production, which can then substitute natural gas used in the breweries. In comparison with digesting the yeast in an expanded granular sludge bed (EGSB) reactor, where maximum allowable volume ratio of yeast/wastewater mixture was 1.1 %, much higher volume ratios of yeast/wastewater mixture can be applied in the presented ASBR system. Up to 8 % (by volume) yeast in yeast/wastewater mixture presented no adverse effects in anaerobic digestion. Applying mixtures with volume ratios of 9–12 %, process performance slightly diminished; however, it was still satisfactory. Higher volume ratios of yeast/wastewater mixture require longer than 1-day cycle to be successful. Maximum viable anaerobic digestion process was achieved with yeast/wastewater mixture volume ratio of 17 %. This corresponds to organic loading rate (OLR) of 13.6 kg/(m³·day), where we can conclude that this is the maximum organic load in one cycle under which the anaerobic process of this type can operate safely. Moreover, the anaerobic digestion process is more affected by the OLR than by the yeast ratio in the mixture; therefore, in such operation the main attention should be given to the OLR in the process. The mass concentrations of NH₄-N and TKN in the influent and effluent do not represent a significant obstacle at achieved OLR. They are below inhibition limit and the increase to aerobic stage can be managed with optimisation of the aerobic process.

By extrapolating the experimental results to the conditions of the local brewery, an increase in biogas production and natural gas substitution of up to 50 % could be achieved. The application of the presented technology

may offer substantial savings in terms of financial and energy resources in the brewing process.

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