Environ Sci Pollut Res
DOI 10.1007/s11356-016-7307-8

RESEARCH ARTICLE

# Integrated expanded granular sludge bed and sequential batch reactor treating beet sugar industrial wastewater and recovering bioenergy

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Received: 31 May 2016 / Accepted: 21 July 2016


**Abstract** The exponential rise in energy demand vis-à-vis depletion of mineral oil resources has accelerated recovery of bioenergy from organic waste. In this study, a laboratory-scale anaerobic (An)/aerobic (Ar) system comprising of expanded granular sludge bed (EGSB) reactor coupled to an aerobic sequential batch reactor (SBR) was constructed to treat beet sugar industrial wastewater (BSIW) of chemical oxygen demand (COD) 1665 mg $L^{-1}$ while harnessing methane gas. The EGSB reactor generated methane at the rate of 235 mL/g COD added, with considerably higher than previously reported methane content of 86 %. Meanwhile, contaminants were successfully reduced in the combined An/Ar system, realizing a removal rate of more than 71.4, 97.3, 97.7, and 99.3 % of organic matter as total phosphorus, total nitrogen, biological oxygen demand (BOD), and soluble COD, respectively. Microbial community analysis showed that the bacterial genus *Clostridium* sp. and archaeal genus *Methanosaeta* sp. dominated the EGSB reactor, while *Rhodobacter* sp. dominance was observed in the SBR. The obtained experimental results indicate that the integration of expanded granular sludge bed and sequential batch reactor in treating BSIW obtained competitively outstanding performance.

**Keywords** Aerobic system · Anaerobic system · Biogas production · Mesophilic condition · Microbial community analysis · Wastewater treatment

Responsible editor: Bingcai Pan

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## Introduction

The current environmental degradation and exponential resource depletion trend has compounded pressure on the need of global clean production. This in turn has pilled immense pressure on the polluting industries. Hence, sugar beet processing industries is no exception owing to its high energy consumption and production of large amounts of wastewater (Krajnc et al. 2007; Alkaya and Demirer 2011b). Besides, much attention has shifted to biomass utilization for bioethanol or biogas production, where the latter consists of methane (48–65 %) and carbon dioxide (36–41 %) as observed in biogas plants that generate heat and electricity (Rasi et al. 2007). Biogas production from biomass conversion is a result of biodegradation. Particulate biopolymers undergo microbial digestion in four main processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Bacterial activity in the first three processes produce acetate, hydrogen, and carbon dioxide or formate, of which they are utilized by archaea to produce methane gas in the final stage (Angelidaki and Sanders 2004; Zhu et al. 2013; Moeller et al. 2015).

Sugar industries are known to consume much water during operation, while at the same time generating a lot of wastewater. For instance, to process 1 t of sugar requires about 20 $m^3$ of fresh water, assuming that each stage requires fresh water. Besides, the generated wastewater discharge stream is even larger than, sometimes even more than twice consumption amount, the initial water used since water contained in the beet is also released (Zver and Glavič 2005). The beet sugar industrial wastewater (BSIW), on the one hand, has a strong potential to create serious environmental pollution problems if discharged before treatment, while on the other hand, it is very degradable due to high concentrations of hydrocarbons and sucrose. This has increased its popularity in the biogas generation, leading to increase in beet crop productivity

Published online: 04 August 2016

(3.54 t bioethanol $ha^{-1}$) and consequently, high-quantity wastewater generation (Wang et al. 1986; Alkaya and Demirer 2011b; Alonso et al. 2014; Moeller et al. 2015).

When it comes to wastewater treatment processes, anaerobic techniques have provided outstanding performance. The use of the technology makes it to be economically viable (Kim et al. 2010; Bae et al. 2014) and operationally beneficial by not only removing organic pollutants and reduce its volume, but also recovers bioenergy (Farhadian et al. 2007; Yilmaz et al. 2014). These advantages have further stretched the innovation to focus to not only high-strength anaerobic wastewater treatment, but also to low-strength anaerobic wastewater treatment processes (Kim et al. 2010).

Various technologies in the treatment of BSIW has previously been utilized. For instance, the application of an upflow anaerobic sludge bed (UASB) in treating wastewater of chemical oxygen demand (COD) 4000 mg $L^{-1}$ achieved nearly 97 % soluble COD (sCOD) removing efficiency with remanent sCOD of about 100 mg $L^{-1}$. A parallel pilot-scale fluidized bed (FB) reactor achieved sCOD removal efficiency rate of nearly 90 % and the remanent concentration of about 150 mg $L^{-1}$. A lagoon was used for effluent polishing (Iza et al. 1990). Three upflow anaerobic fixed bed reactor treating influent of 8000–2000 mg $L^{-1}$, filled with standard industrial parking and inoculated with different anaerobic culture achieved sCOD removal efficiency of 75–93 %. However, gas production was not measured with accuracy (Farhadian et al. 2007). Comparison of batch-fed continuously mixed anaerobic reactor (FCMR) and anaerobic sequencing batch reactor (ASBR) treating influent with sCOD of 5318 ± 288 mg $L^{-1}$, showed the former achieving sCOD removal efficiency of 68.7 ± 2.2 %, effluent concentration of 484 ± 40 mg $L^{-1}$, and methane yield of 255 ± 11 mL g COD added, while the latter reported sCOD removal efficiency of 79.7 ± 1.1 %, effluent concentration of 503 ± 10 mg L and methane yield of 337 ± 15 mL g COD added. Methane gas was 81.9 ± 4.7 % (Alkaya and Demirer 2011a). Continuous stirred tank reactor treating wastewater of 6000 mg L with the aim of generating hydrogen gas, realized hydrogen gas generation of 16.2 L day (Zhu et al. 2013). The application of aerobic granular sequencing batch reactor (SBR) treating wastewater of sCOD 3055 ± 183 $mgL^{-1}$ achieved sCOD removal efficiency of nearly 87 ± 1 % (Kocaturk and Erguder 2015). Despite of the fact that BSIW is rich in hydrocarbons and highly degradable, most of the applied technologies have rarely taken advantage of harnessing methane gas, while at the same time, the systems effluent concentration remained higher than the required standards, yet was unpolished. Only at one instance has effluent polishing been applied, albeit the use of lagoons takes much space making it unfavorable.

This study purposes to utilize an expanded granular sludge bed (EGSB) reactor as an alternative in treating BSIW. Its use in the treatment of various types of wastewater has offered numerous advantages and higher treatment efficiency levels (McHugh et al. 2004). Hence, the use of EGSB reactor for anaerobic treatment is a preferred good alternative for this study. It is also worth noting that different types of aerobic treatment technologies such as sequential batch reactor (SBR), activated sludge, and wetlands have been applied to ensuring reduction of organic matter levels in anaerobic effluents. Its application can either be entirety treatment or as a polishing stage (Omil et al. 2003; Mittal 2006). In this study, SBR technology has been utilized for polishing effluent from EGSB with the aim of ensuring complete degradation. Hence, this study evaluated the performance of anaerobic/aerobic (An/Ar) biological treatment system comprising of an EGSB coupled to an SBR. To the best of our knowledge, this dual investigation of BSIW treatment is the first of its kind and the results thereof shall immensely contribute to industrial wastewater treatment as well as the development of bioenergy recovery.

## Materials and methods

### Experiment set-up and procedure

An/Ar system consisted of sequentially coupled EGSB and SBR systems, respectively, each constructed of plexiglass. The EGSB reactor, 6.5 L volume, 90-cm high, and 10-cm diameter was configured with temperature controller, online pH, and oxidation reduction potential (ORP) detectors and peristaltic pumps (BT100-2J, Longer Pump, China) which were used for feeding the reactors and for recycling purposes at controlled flow rate. The influent was introduced into the reactor from the bottom of the reactor in an upflow mode and a recirculation pump (BT100-2J, Longer Pump, China) was set with the aim of diluting the influent (as shown in Fig. 1).

The temperature of the system was maintained at mesophilic conditions of 36.0 ± 1 °C using temperature controller. The EGSB reactor was operated at three different hydraulic retention times (HRTs), decreasing from 48 to 12 h, with corresponding increment of organic loading rate (OLR) as from 0.8 to 3.2 kg COD $m^{-3}day^{-1}$, respectively. The pH range was maintained at 6.9 ± 0.2 by using buffer (sodium hydrogen carbonate). The evolved biogas was collected, measured, recorded, and normalized to negate reactor head space effects. Normalization was done as provided (Richards et al. 1991). Oxidation reduction potential (ORP), pH, biogas generation, and temperature parameters were monitored and recorded daily, while COD, volatile fatty acid (VFA), and biogas composition were observed periodically. The reactor was seeded to 66.7 % (*v*/*v*) of its working volume with granular sludge (8.09 g volatile suspended solids (VSS/L)) which was obtained from the beer industry (latitude 45° 49′ N and longitude 126° 42′ E) Harbin city, Heilongjiang Province. The EGSB effluent was then redirected into the SBR reactor for polishing and nutrient removal.

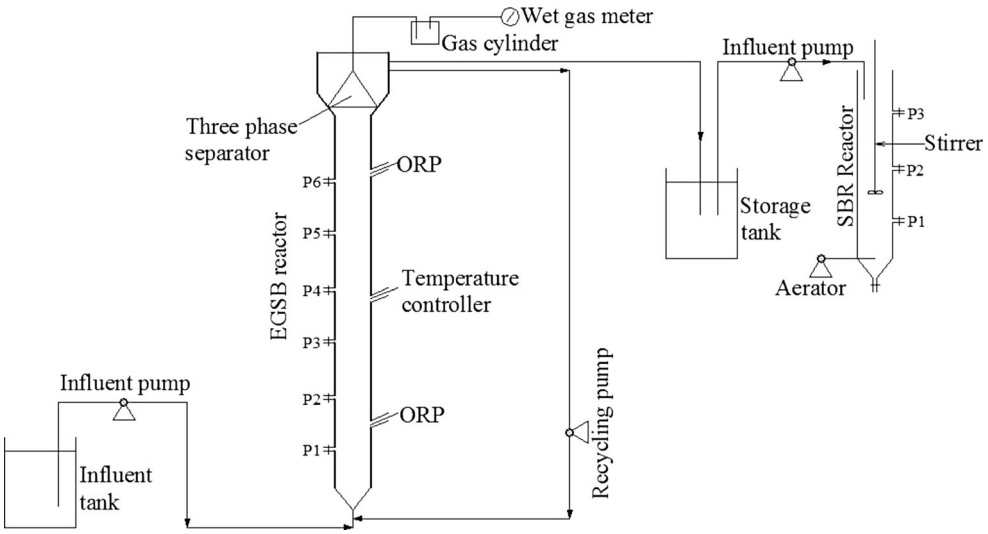


**Fig. 1** Schematic diagram of an EGSB reactor with six sampling ports (*P1*, *P2*, *P3*, *P4*, *P5*, and *P6*), connected to influent tank and gas cylinder and configured with ORP detector, temperature detector and controller, influent tank, influent and recycling pumps, three-phase separator, effluent outlet, and further connected to SBR through a storage tank and configured with pumps, aerator, and stirrer

The SBR reactor, 4.5-L capacity, 40-cm high, and 12-cm diameter was used to treat the effluent from EGSB reactor. Its inoculum (5.67 g VSS/L) was obtained from the municipal sewage treatment plant in Harbin city, Heilongjiang province, in China. The reactor was operating in three cycles per day, 8 h each. The times for each stage of the SBR cycle were as follows: fill, 10 min; anoxic, 1 h and 5 min; aeration, 4 h and 30 min; settle, 1 h and 7 min; and draw 3 min, with HRT of 3 days. It was connected with peristaltic pumps (BT100-2J, Longer Pump, China) which were used for feeding the reactors, aerating pumps, magnetic stirrer, and timers so as to maintain constant flow. The operation of the SBR was under constant monitoring. Samples of treated wastewater were collected after every 3 days.

## Feed and medium composition

The EGSB reactor was fed with a synthetically prepared influent at the start of the experiment for 56 days, with a chemical oxygen demand (COD) of 1600 mg $L^{-1}$ with sucrose (1425 mg $L^{-1}$) as the main carbon source till stabilization of operational condition was realized before introducing the real BSIW. The SBR reactor was fed with the same influent but with COD of 800 mg $L^{-1}$. The basal medium components utilized were as prescribed (Angelidaki and Sanders 2004). The nutrient ratio used when preparing synthetic wastewater was (for COD/nitrogen (N)/phosphorus (P)) 200:5:1. While real BSIW utilized for this experiment was collected from sugar beet factory in Nehe city, Heilongjiang Province, China. Its characterization is as given in Table 1.

## Analytical methods

Chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS), nitrogen (N) removal rate, phosphorus (P) removal rate, and alkalinity determinations were carried out as described in standard methods (Lenore et al. 1998). The pH values were measured using an online pH probe and soluble COD (sCOD) determinations were performed using spectrophotometer after filtration using 0.45-μm pore-sized filters.

Biogas generation rate was measured using a wet gas meter (LML-1, China), while its biogas composition was determined using a gas chromatograph (Agilent GC 7890A, USA).

Volatile fatty acids (VFAs) were analyzed by a gas chromatograph (Agilent GC 7890A, USA) with flame ionization detector (FID) equipped with a HP-INNOWAX column (HP-Innowax 19095N-123, Agilent, USA). The samples for VFA analysis were filtered using centrifuge trim at 1300 rpm in 2 min and then acidified with concentrated formic acid (98 % purity) to adjust pH below 2 in order to convert fatty

**Table 1** Beet sugar industrial wastewater characterized index (all measurements are in mg $L^{-1}$ except pH)

| Component | Value |
|---|---|
| pH | 6.89 |
| SS | 338.5 ± 12 |
| TOC | 550.2 ± 50 |
| TN | 40.02 ± 5 |
| $NH_4$–N | 11.125 ± 1.5 |
| TP | 4.2 ± 1.2 |
| K | 43.34 ± 8 |
| Na | 58.86 ± 3.2 |
| Ca | 209.28 ± 40 |
| Mg | 40.78 ± 5.4 |
| Al | 7.58 ± 2.5 |
| Fe | 5.68 ± 0.5 |
| $Cl^-$ | 37.15 ± 3.2 |
| $SO_4^{2-}$ | 5.9 ± 1.1 |
| $NO_3^-$ | 6.05 ± 2.7 |
| COD | 1665 ± 100 |
| $BOD_5$ | 600 ± 35 |

acids to their undissociated forms. The sample injection volume was 1 μL.

### Bacterial community analysis

Collected samples of the sludge after 126 days of the reactor operation were analyzed for microbial community. The primer for PCR used was fused universal primer of Miseq sequencing platform. The process was done using Sangon agarose recover kit at Sangon Biotech (Shanghai, China) Co. Ltd. In order to check the integrity and concentration of extracted genomic DNA, agarose gel was run. Qubit 2.0 DNA kit for PCR reaction was used to quantify genomic DNA. Agarose gel electrophoresis was also run to test PCR products and recover DNA after amplification process. Recovered products were quantified and all samples mixed in the ratio 1:1 based on DNA and were fully shaken to ensure uniform mixing for subsequent sample library construction and sequencing. In order to merge dual-terminal sequences forming one sequence flash software (FLASH v1.2.7) was utilized. To remove extraterritorial sequences of target area and chimeras, Uchime software was used.

Thereafter, RDP classifier was used for sequence classification whereby sequences with similarity at or above 97 % were clustered and defined as OUT using clustering software (Unclust v1.1.579). Taxonomic unit classification based on Bergey's taxonomy was then carried out. Homology searches of nucleotide sequences were completed with the BLAST server of the National Center for Biotechnology Information (NCBI) using a BLAST algorithm (http://www.ncbi.nlm.gov.library.vu.edu.au/BLAST/) for the comparison of a nucleotide query sequence against a nucleotide sequence database (blastn).

## Results and discussion

In this study, ORP values of the reactor were measured and recorded daily. Throughout the experiment period, ORP in the EGSB reactor ranged −450 ± 6 mV, an indicator of excellent methanogenic activity in EGSB reactor. This is vital since daily biogas productions are primarily affected by conditions influencing methanogenic activities (Alkaya and Demirer 2011b).

### Treatment levels and bioenergy recovery

Figure 2 below shows sCOD removal efficiencies from wastewater in the EGSB reactor at varying HRTs of 48, 24, and 12 h. During the systems operation (EGSB reactor operated for a period of 126 days) sCOD removal efficiency of 50 % and above was achieved after 48 days. Thereafter, up to 94 % of COD removal was realized at 12-h HRT. At the same time,

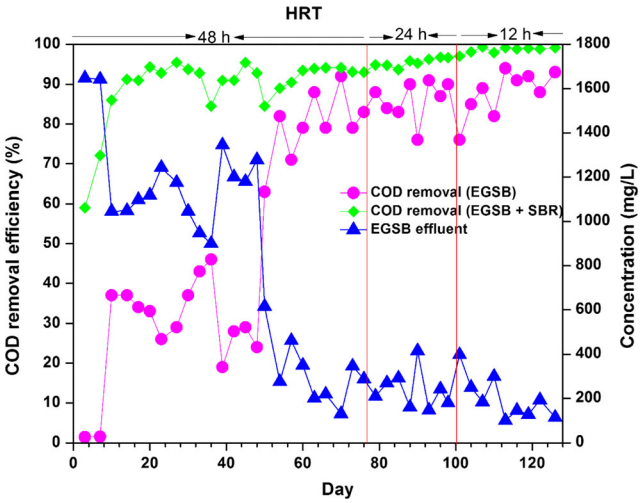


**Fig. 2** Graph showing COD removal rate (%) in the EGSB reactor and in a combined EGSB and SBR together with EGSB effluent COD concentration (mg $L^{-1}$) during the experiment period of 126 days

BOD removal rate realized up to 93 % removal rate. However, even though the highest COD removal rate of 94 % was realized; the effluent was subjected to further treatment using sequential batch reactor in order to improve its quality. These polishing of the EGSB effluent boosted the system's efficiency to COD removal rate of greater than 99 % (using An/Ar system, as shown in Fig. 2), while BOD removal rate recorded was above 97 %. This performance was realized at OLR = 3.2 kg COD $m^{-3}$ $day^{-1}$ and HRT = 12 h in the EGSB reactor (effluent quality is shown in Table 2).

At 48-h HRT, observable scintilla quantities of biogas could only be realized at the end of the third week of operation. This period, as observed earlier, characterized low COD removal efficiency which could hardly surpass 50 % rate. Biogas composition was monitored at an interval of about 5 days. Results (as shown in Fig. 3) realized low methane content but steady performance was realized after 20 days of biogas release. The results show up to about 86 % methane-rich biogas content was obtained during EGSB period of operation, a figure higher than those realized in previous studies. Hydrogen gas production (in percentage) saw a slight initial increase before subsequent drop as methane gas content

**Table 2** Summary of wastewater characteristics before and after treatment (all measurements are in mg $L^{-1}$ except for pH)

| Parameter | Raw wastewater | EGSB effluent | Treated wastewater |
|---|---|---|---|
| pH | 6.89 | 6.90 | 7.0 |
| $BOD_5$ | 600.0 | 40.0 | 14.0 |
| COD | 1665.0 | 184.0 | 12.0 |
| SS | 338.5 | 253.0 | 53.0 |
| TP | 4.2 | 3.8 | 1.2 |
| $NH_4^+$-N | 11.125 | 11.0 | 0.2 |
| TN | 40.02 | 37.2 | 1.1 |

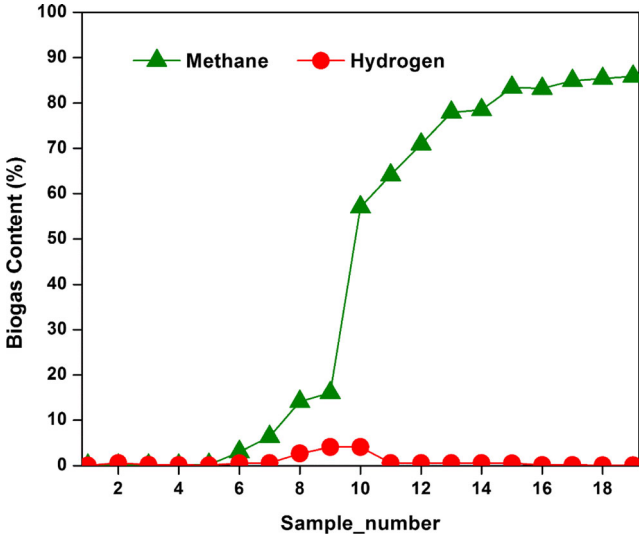


**Fig. 3** Methane gas content taken at intervals of at least 5 days from the time of biogas production in the experiment period

increased (as shown in Fig. 3). The drop of hydrogen in comparison to increase in methane gas could be an indicator that much of the gas produced was utilized for $CH_4$ production, hence a precursor for methanation. When the values of methane production obtained during higher COD removal rate were used in the calculation of methane yield, this resulted to 253 ± 13 mL/g COD added. This was almost equivalent to the yield (255 ± 11 mL/g COD added) obtained while treating beet sugar wastewater using a batch-fed continuously mixed anaerobic reactor (FCMR) with an anaerobic sequencing batch reactor (ASBR) (Alkaya and Demirer 2011a). This recovered amount of methane yield could be useful for heating and providing electricity for the running of industrial activities; hence, promoting self-power sustenance and reducing overdependence on petroleum fuels, a resource that is highly on demand yet scarce due to rapidly depletion.

The observed low COD removal rate and meager biogas generation phenomena at the initial stages of the experiment could be attributed to the onset of biological degradation process due to the growth of biofilm. On the other hand, this remarkable observation of methane content above 85 % in comparison to the typical values of 65 to 70 % (Gerardi 2003) is considerably high. However, it is a little higher than the observed value of 81.9 % realized when treating beet sugar processing wastes (Alkaya and Demirer 2011a). This phenomena can be associated with the abundance of calcium ions ($Ca^{2+}$), as can be seen in Table 1 (209.28 mg $L^{-1}$), commonly found in BSIW due to the use of lime during processing (Iza et al. 1990). In the factories, lime is usually added to flume and wash water in order to adjust its pH and enhance settling characteristics. As a result, lime reacts with soluble carbon dioxide forming bicarbonate alkalinity [Ca $(HCO_3)_2$] and precipitates ($CaCO_3$) (as shown in Eqs. 1 and 2). This reactions, according to Gerardi (2003), can result to lose of carbon dioxide in the sludge, which is readily replaced by carbon dioxide from the biogas. This position then explains scintilla carbon dioxide content of 14 % and considerably higher methane content of 86 % in biogas.

$$Ca(OH)_2 + 2CO_2 \rightarrow Ca(HCO_3)_2 \quad (1)$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \quad (2)$$

On the same note, the methane yield figure attained which was relatively higher than (210 mL g COD added) the one calculated by Weiland (1993) while anaerobically biodegrading beet pulp alone, could be as a result of readily biodegradable BSIW compared to beet pulp. Considering it qualifies the system's efficiency in simultaneous BSIW treatment and bioenergy harnessing realms.

## VFA variation

The set experiment started with 48-h hydraulic retention time (HRT), and this was reduced step wisely in relation to performance up to 12 h. The onset of the experiment showed no emission of biogas. This phenomenon lasted up to 3 weeks before biogas could be noticeably visualized. During this stage of operation, predominance of acetic, propionic, and butyric acid was realized (as shown in Fig. 4). As the experiment progressed, there was drastic reduction in acetic and propionic, especially when the reactor was operating within the 12-h HRT. At this point, the predominance of N-butyric acid was starkly evident as compared to other acids. Considering drawing parallelism in the reduction rate of VFAs, it can be observed that in spite of the abundance of acetic and propionic acid at the start of the experiment, they decreased significantly.

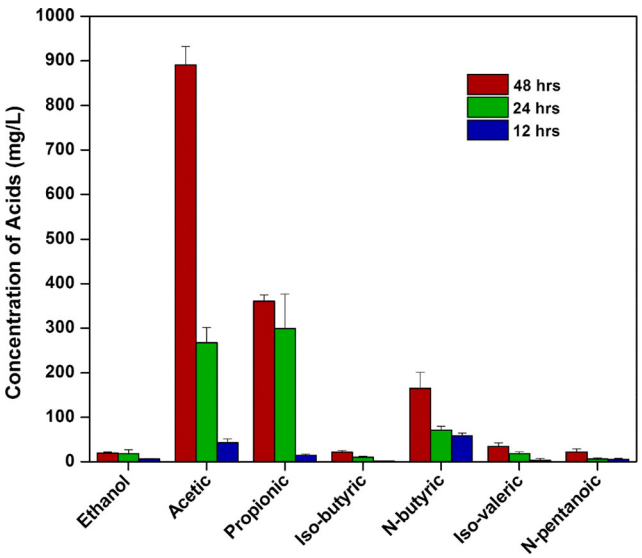


**Fig. 4** Average concentration values of different volatile fatty acids (VFAs) from EGSB reactor within 48-, 24-, and 12-h HRT

The observed predominance of acetic, propionic, and butyric acid at the onset of the experiment is an expected situation in anaerobic systems (Rittmann and McCarty 2001). This is a likely indicator of higher growth rate of acidogenic bacteria in comparison with methanogens, which resulted to carbohydrate degradation (Wang et al. 1986): hence, accumulation of VFAs. However, it took at least 3 weeks for methanogens to mature in the EGSB reactor for VFA conversion to methane to be realized. This was evidenced by the release of biogas after a period of about 3 weeks. This subsequently resulted to reduction of acetic, propionic, and isobutyric acids to below 50 mg $L^{-1}$ concentration at the stable state. In this case, the stable state condition was a period characterized with no change or if any then minimal in pH, ORP, COD removal efficiency, and biogas generation in two or three consecutive measurements.

The observed drastic decrement of propionic acid as opposed to N-butyric acid that remained outstanding in quantity

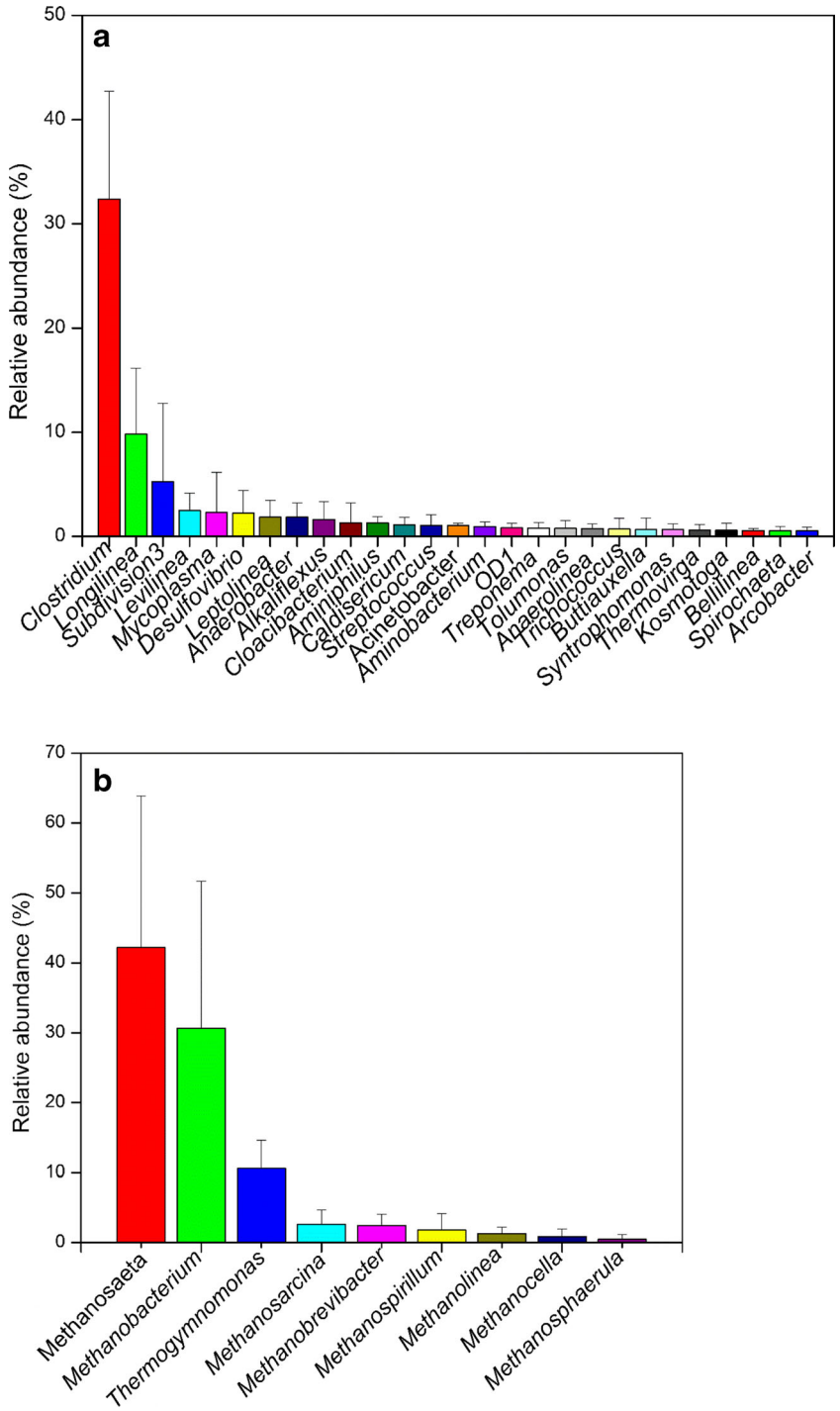


**Fig. 5** Microbial community composition in the EGSB reactor. Bacterial genera **a** and archeal genera **b**. Only genera with assigned read numbers ≥0.5 % of the sequencing effort were included

demonstrated that the bacterial oxidizing propionate did not constitute one of the bottlenecks in the biogas production process as opposed to bacterial butyrate. This finding, however, is contrary with the earlier investigation where Refai et al. (2014) reported that bacterial propionate and butyrate oxidation constituted one of the bottlenecks in the whole process of biogas generation. Hence, the role of propionate bacteria needs further examination. However, it is in agreement with previous findings that have demonstrated the usefulness of acetate utilization capacity as an important phenomenon in monitoring, measuring, and predicting digester stability in anaerobic processes (Yilmaz et al. 2014). This is because when acetic acid was considerably high in the first 3 weeks of the reactor operation (up to 890.84 ± 41.36 mg $L^{-1}$), the reactor was unstable with no biogas observed. As stability was realized in terms of nutrient removal, pH, and biogas generation, acetic acid significantly reduced to the levels below 40 mg $L^{-1}$ at optimal operation. Therefore, observing and monitoring acetic acid production trends can give predictive information on the performance of the reactor. Further, this shows that acetate and propionate constitute dominant intermediates produced during hydrolysis and fermentation processes of organic matter during methanogenic degradation.

## Microbial community of EGSB and SBR

This study showed microbial bacterial and archaeal composition in the EGSB and SBR. After the experiment, the biomass concentration measured was 10.02 g VSS/L. Bacterial community showed predominance of *Clostridium* sp. (32.4 %), *Longilinea* sp. (9.8 %), and *Subdivision3* sp. (5.3 %), *Levilinea* sp. (2.5 %), *Mycoplasma* sp. (2.3 %), and *Desulfovibrio* sp. (2.2 %) of the total bacterial community in the order of dominance as shown in Fig. 5a. On the other hand, the archaeal community comprised of 89.4 % methanogens. The predominance showed genus *Methanosaeta* sp. (42.2 %), *Methanobacterium* sp. (30.7 %), *Thermogymnomonas* sp. (10.6 %), *Methanosarcina* sp. (2.7 %), and *Methanobrevibacter* sp. (2.5 %) as shown in Fig. 5b. The results in Fig. 5 shows that the archaeal community was less diverse in comparison to the bacterial community. They are also parallel to those obtained in a two-stage biogas production by co-digesting molasses wastewater and sewage sludge (Lee et al. 2014).

Microbial community analysis in the SBR indicated prevalence of diverse bacterial genus as shown in Figs. 5 and 6. The predominant genus were *Rhodobacter* sp. (10.7 %), *Pasteuria* sp. (7.1 %), *TM7_genera_incertae_sedis* sp. (5.7 %), *Rhodopirellula* sp. (5.6 %), *Verrucomicrobium* sp. (5.3 %), and *Rhizomicrobium* sp. (3.7 %).

The hydrolytic fermentative bacteria predominance of *Clostridium* sp. and *Longilinea* sp. genera showed *Clostridium intestinale* (99 % identical-Genbank KX261408) and *Longilinea arvoryzae* (99 % identical-Genbank accession KX261407) as the dominant species, respectively. Their presence suggests their responsibility in the degradation of cellulose by providing cellulosomes (Lynd et al. 2002) and their abundance may have provided synergistic influence in degradation of the BSIW known to comprise of hemicellulose and cellulose. Their degradation ability might have as well immensely contributed to production of metabolites in larger quantity, as shown in Fig. 2. *Clostridium* sp. genus type of dominance was also observed in a two-stage biogas production of co-digestion molasses wastewater and sewage sludge (Lee et al. 2014). Since they are known to grow faster than methanogens, the abundance of VFAs at the start of the experiment attests to this.

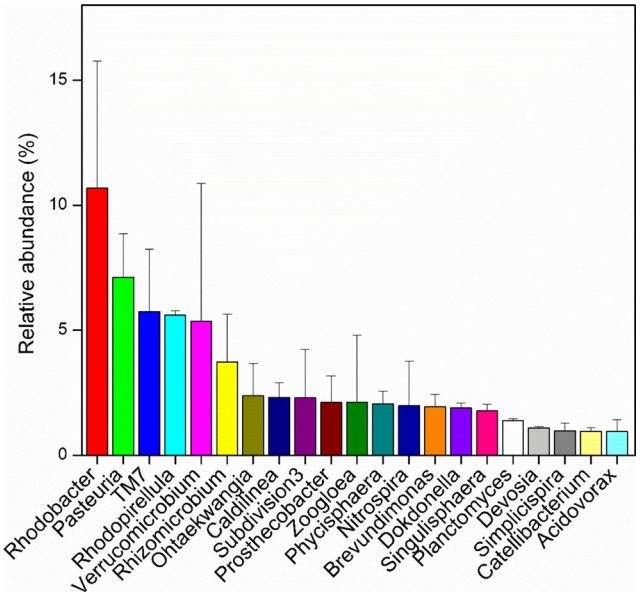


**Fig. 6** Bacterial community composition in the SBR reactor. Genera with assigned read numbers >1.0 % of the sequencing proportion were included

*Methanosaeta* sp. genera predominance in the archaeal microbial community, which is acetoclastic (Shen et al. 2013), may have been made possible due to low content of acetate concentration, and significantly boosted at 12-h HRT due to very low amounts, as shown in Fig. 2. *Methanosaeta concilii* (100 % identical-Genbank accession KX261417) was the highly predominant species. Although the prevalence of *Methanobacterium* sp. C5/51 (99 % identical-Genbank accession KX261415), *Methanosarcina barkeri* (99 % identical-Genbank accession KX261416), *Methanobacterium beijingense* (99 % identical-Genbank accession KX261414), and *Methanosphaerula palustris* (100 % identical-Genbank accession KX261413) might have performed a pivotal role in methanation. They are known to increase fast in numbers as acetate concentration decreases (Zheng and Raskin 2000). Furthermore, the prevalence of *Methanosaeta* sp. and *Methanosarcina* sp. (44.8 %), is a likely indication that methane production took the pathway of acetoclastic methanogenesis. But also, the presence of *Methanobacterium* sp., *Methanobrevibacter* sp., *Methanospirillum* sp.,

*Methanolinea* sp., and *Methanosphaerula* sp., which accounted 36.8 % of archaeal population suggests that besides acetoclastic methanogenesis, hydrogenotrophic methanogenesis pathway could also be a possibility. Their prevalence is mostly attributed to their fast growth while competitively utilizing $H_2$ and $CO_2$. This could be associated with high biogas production as recorded in this study. However, it is intriguing to find *Thermogymnomonas* (10.6 %) in larger proportion in this anaerobic mesophilic condition. This newly found genus is known to thrive in acidophilic, strictly aerobic, and moderately thermophilic condition; with this phenomena having been earlier reported (Kim et al. 2011). It shall be highly fascinating to find its role in anaerobic degradation of beet sugar industrial wastewater at mesophilic conditions and its adaptation mechanism.

*Rhodobacter* sp., the purple nonsulfur bacteria (PNB) which are known to grow aerobically are known to be either chemoheterotrophs thriving in the light or dark. They photoassimilate organic compounds which include fatty acids, other organic acids, alcohols, carbohydrates, and aromatic compounds (Huang et al. 2001). Their abundance in the SBR may indicate a vital role played in further degradation of effluent from the EGSB reactor. *Rhodobacter* sp. EMB 174 (99 % identical-Genbank accession KX261419) was the most predominant species in the reactor. However, there was also *Rhodobacter* sp. TCRI 14 (99 % identical-Genbank accession KX261420) and *Verrucomicrobium spinosum* DSM 4136 = JCM 18804 (94 % identical-Genbank accession KX261422).

Further, the dominance of candidate division TM7 in this study suggests the involvement of the bacterial in the removal of phosphorus by accumulating polyphosphate and polyhydroxybutyrate (PHB). This might have contributed immensely to the removal of phosphorus of up to 71 % (as shown in Table 2). The role of candidate division TM7, though, needs further investigation. Nitrification is a key process in biogeochemical nitrogen cycle. This process involves oxidation of ammonia into nitrite accomplished by autotrophic bacteria of genus *Nitrosomonas* sp. and thereafter oxidation of nitrite to nitrate by bacteria of genus *Nitrospira* sp. The dominance of genus *Nitrospira* sp. is a testament of the crucial role accomplished in nitrification. On the other hand, denitrifiers involvement was evidently observed with the presence of heterotrophic bacteria of genus *Zoogloea* sp. This is where nitrate formed is reduced to nitrogen. The dominance of genus *Pasteuria* sp., *Rhodopirellula* sp., *Verrucomicrobium* sp., and *Rhizomicrobium* sp. vis-à-vis good removal efficiencies of organic compounds, phosphorus, and nitrogen in the system reveals mutual relationship among microorganisms discharging different functionalities.

Although the integration of EGSB and SBR technologies has been practically utilized in coupling methanogenesis and shortcut nitrogen removal (Bai et al. 2013), treatment of cellulosic ethanol production wastewater (Shan et al. 2015) and treatment of pig waste slurry (Lee and Han 2015), it was applied to BSIW treatment for the first time. This study reported better performance in terms of COD removal efficiency (COD effluent concentration of 12 mg L) of BSIW than any other before. In fact, the values for effluent COD, TN and TP characteristics are according to the international set standards. Besides, the system's methane yield recovery rate was better with the biogas realizing the highest methane content of nearly 86 %. The results realized in this experiment indicate that the integrated systems performance is competitive in comparison to already utilized technologies in the treatment of BSIW (as summarized in Table 2).

## Conclusion

The use of this integrated technology will ensure perfect treatment of emitted wastewater from industries while at the same time benefiting immensely from the clean energy recovery. As investigation results reveal, an integrated An/Ar system comprising of EGSB followed by SBR reactor constructed for the treatment of BSIW was able to remove more than 71.4, 97.3, 97.7, and 99.3 % of organic matter as total phosphorus, total nitrogen, BOD, and soluble COD, respectively. Bioenergy recovery of methane gas was achieved at the production rate of 235 mL $CH_4$ per g COD added. This was realized even as 86 % of biogas content released was measured and detected as methane. Further, microbial community analysis revealed that the bacterial genus *Clostridium* sp. and archaeal genus *Methanosaeta* sp. dominated the EGSB reactor, while *Rhodobacter* sp. dominated in the SBR.

**Acknowledgments** This work was supported by the National Science Fund for Distinguished Young Scholars of China (51125033) and the support of the International Cooperating Project between China and European Countries(2014DFE90110). The authors also thank the support of State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (2015DX08).

**Compliance with ethical standards**

**Conflict of interest** All the authors declare that they have no conflict of interest.

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