



# Anaerobic treatment of opaque beer wastewater with enhanced biogas recovery through Acti-zyme bio augmentation

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## ABSTRACT

This study investigates the potentiality of biologically treating opaque beer wastewater using the bio augmentation technology at the same time harnessing biogas and bio solids as value added products. Wastewater sample were collected in 5L containers and the sludge was separated from the liquid. The liquid and sludge were bio augmented with Acti-zyme with loadings of 5 g/L, 10 g/L and 15 g/L and were left to settle over a period of 30 days under anaerobic conditions. The wastewater and treated effluent BOD, COD, TKN, TP, TSS, TDS and pH were measured using standard methods. Whereas the amount of biogas generated was measured using the water displacement and its composition quantified. The BOD, COD, TKN, TP, TSS, TDS were significantly reduced by 93%, 77%, 87%, 89%, 90% and 90% respectively. The BOD/COD ratio was 0.72 initially and reduced to 0.21 for all the Acti-zyme loadings. Biogas with a methane composition of 65–70% was recovered.

## 1. Introduction

The opaque beer (millet beer) manufacturing industry is one of the booming brewery industries in the sub-Sahara Africa due to the abundance of raw materials used in the opaque beer processing. Despite the use for leisure purposes, opaque beer is also associated with application in traditional ceremonies and rituals. Huge amounts of this opaque beer are manufactured on a daily basis and given that the process is a wet process, wastewater is generated in the process. The opaque beer manufacturing processes include the soaking of the millet in warm water until they sprout so as to achieve high content of maltose from the millet grain. This is then followed by drying so as to stop the germination process (Zvidzai et al., 2014). The malted grain is then crushed and mixed with water to form wort. The wort is then boiled as a sterilization measure and then yeast is added after cooling and fermentation which results in the production of the opaque beer is allowed to take place for 5 days.

The wastewater generated is rich in biological components from the grain used in the opaque beer manufacturing process. This wastewater if not properly managed has potential to pollute the environment and can cause greenhouse gases emissions from the decomposition of the

biological matter in the wastewater. This wastewater can therefore be considered for treatment using upcoming technologies like bio augmentation at the same time recovering value added products from the biological content.

Bio augmentation refers to the utilization of microorganisms to conduct accelerated chemical transformations (Nzila et al., 2016; Maulin and Hiral, 2017; Shah, 2017). Bio augmentation presents an opportunity for optimal brewery wastewater treatment at the same time recovering biogas and the anaerobic digestate as bio fertilizers (Karim and Moss, 2017). Bio augmentation has been applied in various wastewaters such as sewage, textile wastewater, petrochemical wastewater (Bhattacharya et al., 2008; Fang et al., 2009; Manyuchi et al., 2018). Fig. 1 shows the potential routes that can be taken in wastewater treatment with bio augmentation as a treatment option.

### 1.1. Biogas from wastewater

Biogas can be generated from the biodegradable sludge generated from the wastewater treatment process (Jingura and Kamusoko, 2017). Bio augmentation then allows for the enhancement of the quantity and quality of biogas generated (Manyuchi et al., 2018). Resource recovery

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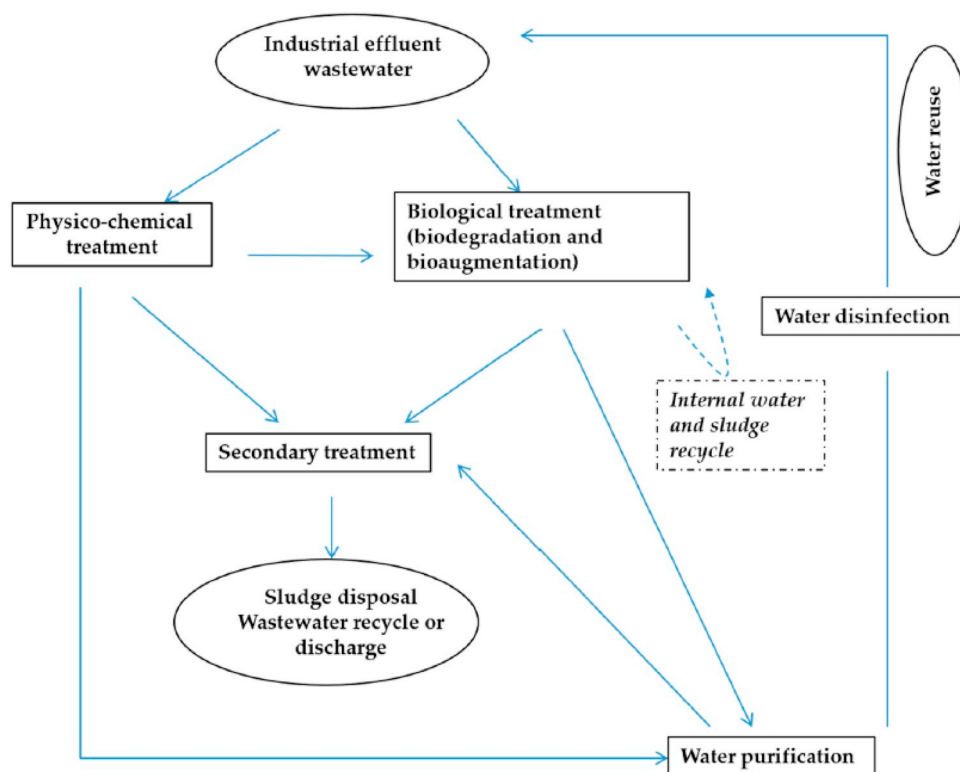


Fig. 1. Wastewater treatment incorporating the bio augmentation process (Fung et al., 2012).

through harnessing of biogas is of utmost importance in terms of created circular economies in wastewater treatment (Neira and Jeison, 2010; Gulizar et al., 2014). Biogas is generated from wastewater of high biological content through the multistage processes which include: Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis in that order (Ma et al., 2008). The biogas generated is mainly composed of methane (CH<sub>4</sub>), carbon di oxide (CO<sub>2</sub>), nitrogen (N<sub>2</sub>) and hydrogen sulphide (H<sub>2</sub>S). However, some bio augmentation media like Acti-zyme also known as Hycura promotes the generation of a biogas that is H<sub>2</sub>S free (Manyuchi et al., 2018).

A summary of bio augmentation applications and the resources that were recovered is shown in Table 1.

The present study focused on the biological treatment of opaque beer wastewater through Acti-zyme bio augmentation with biogas and bio solids being recovered as value added products. An integrated process for adoption of this technology was also proposed.

## 2. Materials and methods

### 2.1. Materials

A sample of 5L of opaque beer wastewater was collected from a local brewery for use in the study. Acti-zyme a biocatalyst from Acti-zyme Australia was used as the bio augmentation media. Acti-zyme has been reported to enhance biological wastewater treatment as well as biogas production due to its composition which include catalase and protease (Manyuchi et al., 2015).

### 2.2. Methods

#### 2.2.1. Wastewater treatment

Acti-zyme loading of 5 g/L, 10 g/L and 15 g/L were used as the bio augmentation media in both the wastewater and in biogas production. Anaerobic treatment of the wastewater and the sludge was allowed to take place over a period of 30 days at atmospheric pressure. The wastewater and treated effluent was characterized for chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), total suspended solids (TSS), total Kjeldhal nitrogen (TKN) and

Table 1  
Application of bio augmentation in wastewater treatment.

Type of wastewater	Resource recovered	Bio augmentation media	Reference
Brewery spent grain	Biogas	<i>P. Xylanivorans Mz5T</i>	Cater et al., 2015
Lipid rich waste	Biogas	<i>Clostridium lundense (DSM 17049T)</i>	Cirne et al., 2006
Textile mill wastewater	Treated effluent	<i>Augment P5</i>	Bhattacharya et al., 2008
Sewage	Treated effluent, biogas, bio solids	<i>Acti-zyme (Hycura)</i>	Manyuchi et al., 2018
Municipal wastewater	Treated effluent, biogas	<i>Micro flora D83</i>	Wang et al., 2018
Pharmaceutical wastewater	Treated effluent	<i>Paracoccus denitrificans W12</i>	Wen et al., 2013
Dairy wastewater	Treated effluent	<i>Commercial inocula</i>	Schneider & (2011)
Municipal wastewater	Treated effluent	<i>SludgeHammer, B. subtilis, B. laterosponus and P. aeruginosa</i>	Hesnawi et al., 2014
Pulp and paper wastewater	Treated effluent	<i>Gordonia strain JW8</i>	Chen et al., 2012
Industrial wastewater	Treated effluent	<i>Micrococcus, Pseudomonas, and Nocardia</i>	Pandya, 2007

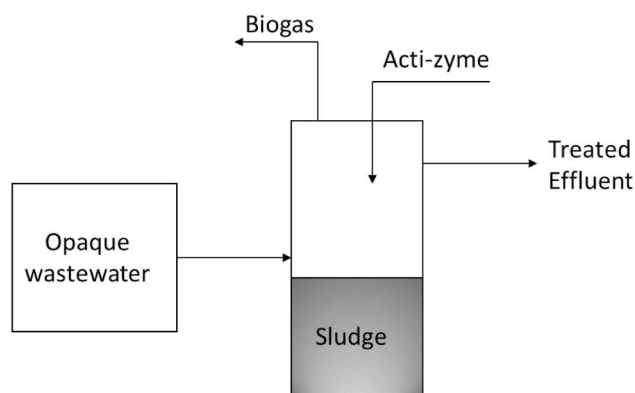


Fig. 2. Experimental set up for anaerobic treatment of opaque beer wastewater harnessing biogas.

total phosphates (TP) using the APHA (2005) standard methods. The Ph was measured using a Hanna HI11B ph probe.

### 2.2.2. Biogas generation

Biogas generation was done under mesophilic conditions of  $35 \pm 1^\circ\text{C}$  over a period of 30 days. The amount of biogas produced was determined using the water displacement method. The biogas composition was quantified using a Biogas 5000 analyser from GeoTech Instruments. The experimental set up for this study is as shown in Fig. 2.

### 2.2.3. Statistical analyses

The average values at the different Acti-zyme loadings were used for plotting the graphs and the standard deviations were indicated.

## 3. Results and discussion

### 3.1. Opaque beer wastewater characteristics

The opaque beer wastewater used in this study had high COD and BOD values. These values indicated the potential the opaque beer wastewater had in terms of bio augmentation and also biogas generation. The summary of the characteristics of the opaque beer wastewater are shown in Table 2.

The effect of bio augmentation with Acti-zyme on the opaque beer wastewater's physicochemical parameters is described in detail below.

#### 3.1.1. COD

The COD levels decreased significantly with increase in the treatment time as shown in Fig. 3. A 77% reduction in the COD was observed and this was attributed to the removal of contaminants during the bio augmentation process through Acti-zyme catalysis. As the time increased, the Acti-zyme cells also multiplied resulting in the usage on the chemical pollutants in the wastewater resulting in reduced COD levels. Schneider and Topalova (2011) also reported the same reduction trend in COD with a 70% decrease being observed during municipal wastewater treatment with *Commercial Innocula*.

Table 2

Opaque beer wastewater characteristics.

Physicochemical characteristic	Value
Ph	$8.4 \pm 0.1$
COD	$2271.7 \pm 7.64$
BOD	$1629.3 \pm 15.50$
TKN	$59.7 \pm 6.11$
TP	$60.0 \pm 5.00$
TSS	$678.7 \pm 7.09$
TDS	$445.0 \pm$

Effect of bio augmentation on physicochemical properties.

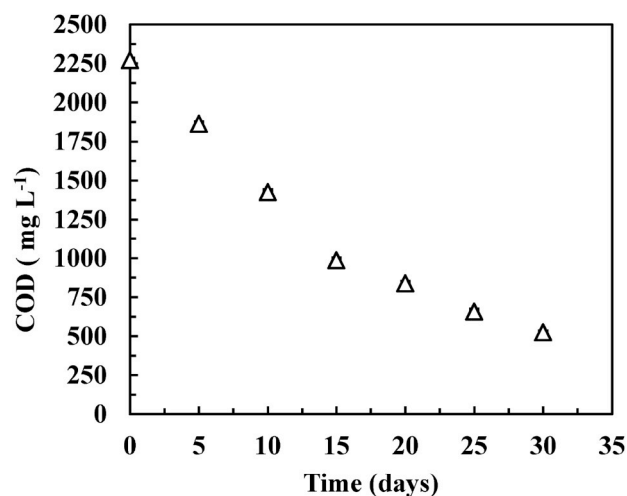


Fig. 3. Effect of bio augmentation on opaque beer wastewater COD.

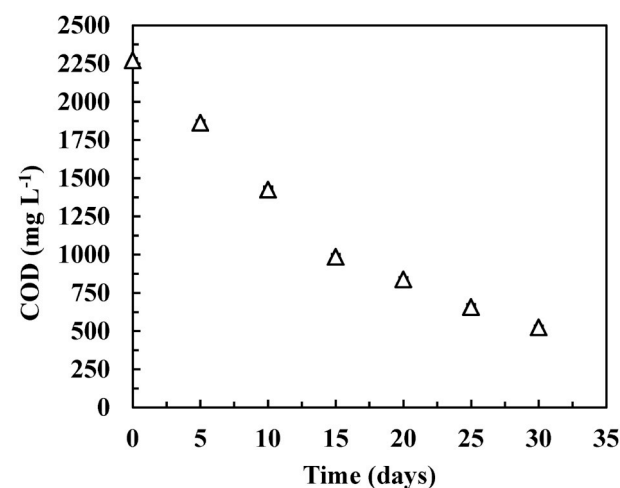
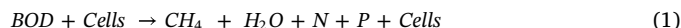


Fig. 4. Effect of bio augmentation on opaque beer wastewater BOD.

#### 3.1.2. BOD

The BOD in the opaque beer wastewater decreased with increase in treatment time with Acti-zyme at the various loadings as indicated in Fig. 4. The BOD concentration significantly decreased by 93% and this was attributed to the Acti-zyme action as the bio augmentation media. Acti-zyme as a micro-organism has potential to feed on the bio contaminants in the wastewater resulting in the decreased BOD concentration. The BOD in the wastewater is reduced to  $\text{CH}_4$  water and other nutrients that are utilized by Acti-zyme as per Equation (1) below during anaerobic treatment. Chen et al. (2012) also reported a 96% decrease in BOD when paper and pulp wastewater was treated using *Gordonia* strain JW8 as a bio augmentation organism.



#### 3.1.3. TSS

The TSS concentration in the opaque beer wastewater decreased with increases in the treatment time with Acti-zyme (Fig. 5). The TSS was reduced by 90% through bio augmentation with Acti-zyme. The reduction in the TSS is attributed to the removal of the suspended bio contaminants in the wastewater through the Acti-zyme activity. The same trend was observed by Bhattacharya et al. (2008) when they inoculated Augment P5 and noted a TSS decrease of 47% in textile mill wastewater.

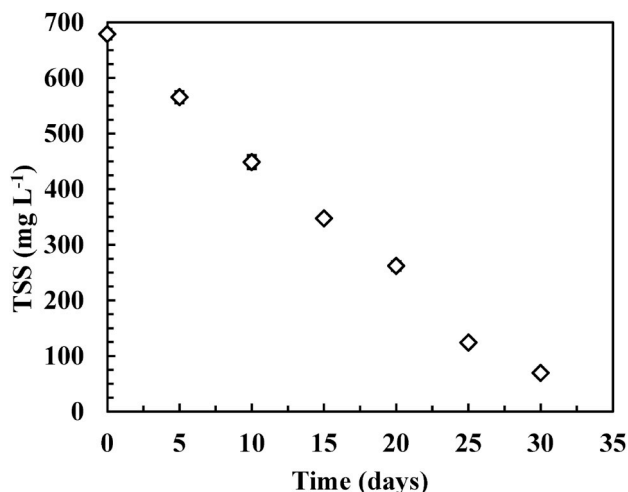


Fig. 5. Effect of bio augmentation on opaque beer wastewater TSS.

### 3.1.4. TDS

The TDS concentration decreased as the treatment period increased (Fig. 6). A 90% decrease was observed during the 30 day treatment period. The TDS acted as a source of nutrients for growth and reproduction of the Acti-zyme cells resulting in their significant decrease. Mostafa et al. (2015) reported a 41% decrease in industrial oil wastewater that was treated using *Candida utilis* bio augmented system which was almost 50% less the change observed for the current study.

### 3.1.5. TKN

The TKN decreased significantly with increase in the treatment time in a system with Acti-zyme as the bio augmentation media (Fig. 7). An 87% decrease was noted for all the systems loaded with Acti-zyme. The Acti-zyme potentially used the nitrogen compounds from the wastewater for its growth and reproduction. Increased removal efficiencies of TKN due to bio augmentation were also reported by Pei et al. (2014) with a similar trend as observed in this study.

### 3.1.6. TP

The TP concentration reduced significantly decreased with increase in treatment time in a system that was loaded with Acti-zyme (Fig. 8). An 89% decrease was observed. The TP reduction can be attributed to the utilization of phosphates by Acti-zyme during its growth and reproduction. Kim et al. (2013) also reported a similar 90% decrease in TP for tannery wastewater that was treated using Proteobacteria,

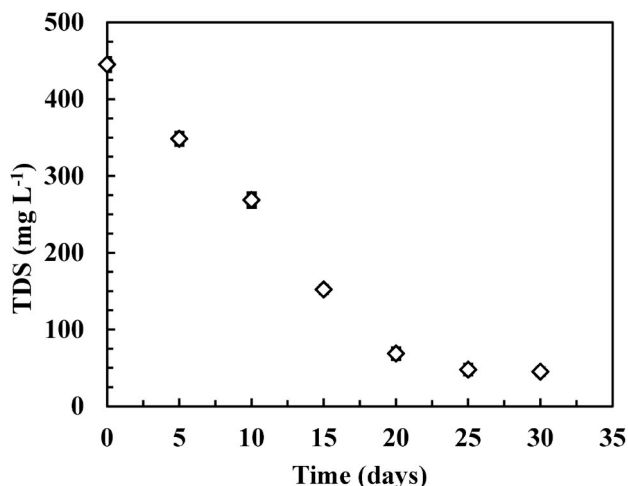


Fig. 6. Effect of bio augmentation on TDS.

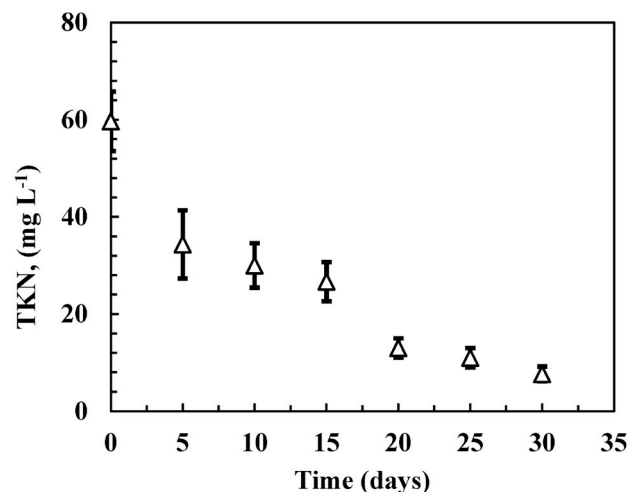


Fig. 7. Effect of bio augmentation on opaque beer wastewater TKN.

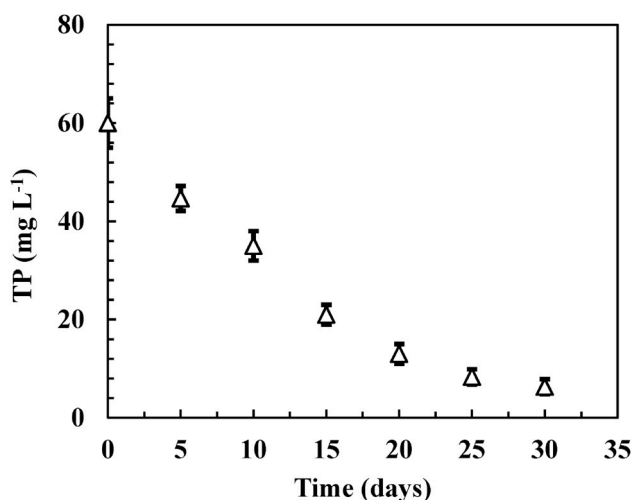


Fig. 8. Effect of bio augmentation on TP.

Firmicutes, Bacteroidetes, Planctomycetes and Deinococcus-Thermus as bio augmentation media.

### 3.1.7. pH

The pH in the opaque beer changed from being alkaline to neutral during the bio augmentation process with Acti-zyme (Fig. 9). The neutralization on the pH was attributed to the removal of the bio contaminants in the wastewater due to Acti-zyme activity. The same trend for pH changes was reported by Kaur and Chaman (2012) during the treatment of dairy wastewater using *Alternaria* sp., *Fusarium* sp and *Aspergillus* sp.

## 3.2. Biodegradability of the opaque wastewater

The opaque beer wastewater was highly biodegradable as indicated by the BOD/COD ratio over the 30 days period (Fig. 10). BOD/COD ratios of between 0.3 and 0.8 indicate that the wastewater has a high biodegradability rate (Aboulhassan et al., 2008; Lee and Nikraz, 2014). The initial BOD/COD ratio was 0.72 and it decreased linearly to 0.21 after 30 days for the final treated effluent. The same ratios for biodegradability were reported by Murunga et al. (2016) for brewery wastewater.

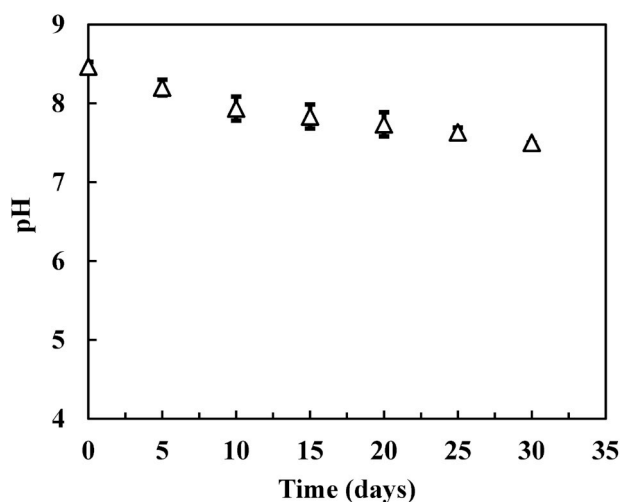


Fig. 9. Effect of bio augmentation on Ph.

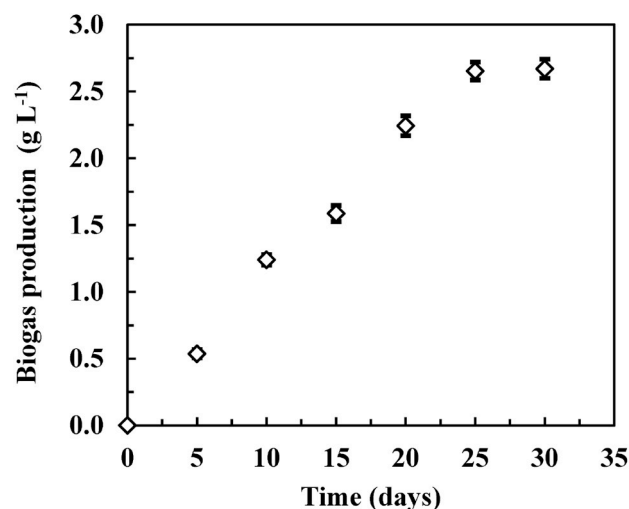


Fig. 11. Cumulative biogas production from opaque sludge.

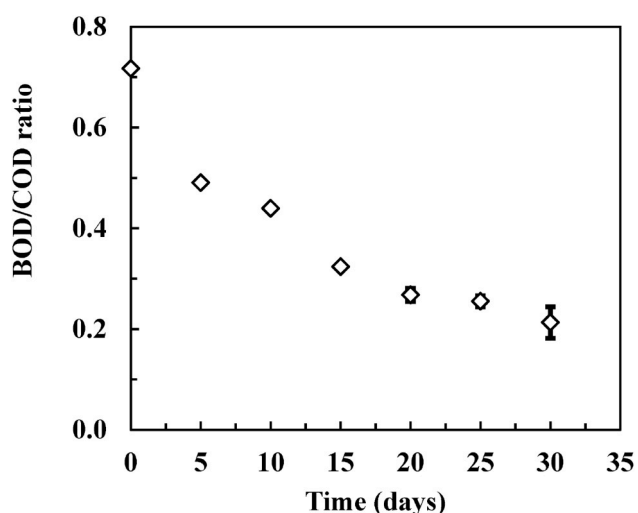


Fig. 10. Effect of bio augmentation on opaque beer wastewater biodegradability.

### 3.3. Biogas generation from the opaque beer sludge

The amount of biogas produced increased with increase in retention time of the opaque beer sludge bio augmented with Acti-zyme (Fig. 11). The increase in the biogas quantity with time was attributed to the increase in Acti-zyme activity with time for up to 25 days. Thereafter the quantity of biogas produced almost became constant and this was attributed to the stationary phase whereby both there were no more growth and reproduction of the Acti-zyme cells. Strang et al. (2017) reported a 22–24% increase in biogas quantity produced upon bio augmentation of cellulose and corn stover with *Thermoanaerobacterium thermosaccharolyticum*, *Caldanaerobacter subterraneus*, *Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum*.

The biogas generated from the opaque beer sludge was rich in methane composition. The high methane composition of 65–70% was attributed to the bio augmentation of Acti-zyme which enhanced the performance of methane generated bacteria and hindered the production of H<sub>2</sub>S. The composition of the biogas from the opaque beer sludge is shown in Table 3.

**Table 3**  
Opaque beer sludge biogas composition.

Biogas component	Composition
CH <sub>4</sub> (%)	65–70
CO <sub>2</sub> (%)	30–35
N <sub>2</sub> (%)	1–5
H <sub>2</sub> S (ppm)	600–800

## 4. Process design

An integrated process for the resource recovery from opaque beer wastewater was developed. Biogas, digestate and a clean effluent that can be re-used back in the system are recovered and the process is presented in Fig. 12. The wastewater treatment will take place at room temperature whilst the biogas generation will take place at 35 °C at atmospheric pressure. Acti-zyme can be loaded at 5–15 g/L for both wastewater treatment and biogas generation.

## 5. Conclusion

Opaque beer wastewater which is highly biodegradable can be efficiently treated using Acti-zyme as the bio augmentation catalyst. A reduction in COD (77%), BOD (93%), TKN (87%), TP (89%), TSS (90%) and TDS (90%) was achieved for Acti-zyme loading of 5–15 g/L. Anaerobic digestion of the opaque beer sludge from the process resulted in the generation of biogas with 70% methane content as well as nutrient rich bio solids.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgements

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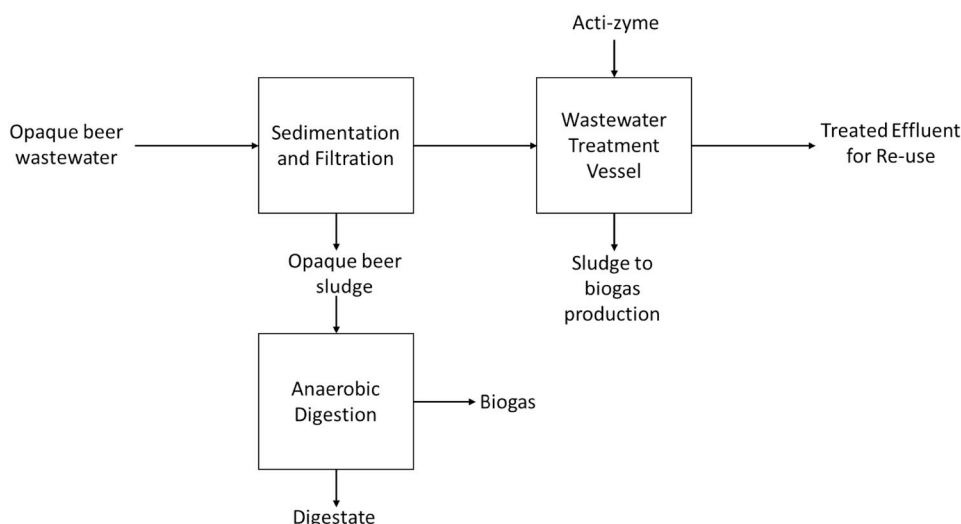


Fig. 12. Integrated process for opaque beer wastewater treatment recovering biogas.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sajce.2018.10.002>.

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