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Bio ethanol from sewage sludge: A bio fuel alternative

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ABSTRACT

In this study, the potential to fully exploit sewage sludge as a raw material for bio ethanol a source of bio fuel is investigated. Sewage sludge hydrolysate was first made by introducing *Bacillus flexus* in order for saccharification to take place before fermenting to bio ethanol using yeast. The hydrolysate was then prepared for fermentation by introducing 10 g/L of peptone, 2 g/L of KH_2PO_4 and 1 g/L of $MgSO_4$. Afterwards, fermentation was allowed to take place at varying pH (4.0–7.0), temperature (15–45 °C), incubation time (10–70 h) and yeast concentrations (2–10% (v/v). Bio ethanol concentrations were characterized through spectrophotometry and its physicochemical properties analyzed by standard methods. Pearson Correlations Coefficients in MATLAB 13.0 were used to determine the coefficients of interaction between the various parameters in bio ethanol production at 95% confidence interval. Highest bio ethanol yields of greater than 40 mL/L were achieved at an incubation period of 10 days, with an operating temperature of 30 °C and pH of 6.5 with yeast concentration of 6% wt. The interactions between incubation temperature and pH had the best interaction coefficient of 0.9759 being achieved for optimal bio ethanol yield. The bio ethanol produced had a flash point of 19.2 °C, pour point of 4.9 °C, cloud point of 20 °C and viscosity of 1.30 cP.

1. Introduction

Energy usage is increasingly on a daily basis and most developing countries are faced with energy deficit creating the need to look for alternatives that are renewable and affordable. The generation of renewable energy from waste biomass have been explored in numerous studies in the need for promoting a circular bio economy and also mitigation of climate change effects due to greenhouses gases emissions from the biomass (Jang et al., 2011). Bio ethanol is a source of second generation renewable energy that can be obtained from waste lignocelluloses material and can be used in place of the conventional fuels in the transport industry (Mtui, 2009; Jang et al., 2011). Lignocelulose materials are mainly divided into 3 segments which are cellulose 30–50%, hemicelluloses 15–35% and lignin 10–20% and it is because of these characteristics that they are attractive raw materials for bio fuels (Knauf and Moniruzzaman, 2004). Biocatalysts are also employed in order to enhance the bio ethanol production process from biomass. Lignocelluloses materials such as sugarcane, sorghum, corn stover, wheat straw and rice straw have been previously used in the production of bio ethanol employing yeast (*Sacchromyces Cerevisiae*) as the fermentation bio catalyst (Azad et al., 2014; Irfan et al., 2014). In a study by Irfan et al. (2014) process temperatures of 30 °C with incubation times of up to 4 days (96 h) were employed with bio ethanol concentrations of more than 44 g/L being reported (Irfan et al., 2014). Employing bio catalysis during bio ethanol production from lignocelluloses such as rice straw enhanced the product yield by more than 40% in comparison to systems that were not catalysed (Jalil et al., 2010). Several microorganisms as bio catalysts have been used for bio ethanol production from lignocelluloses material including *Saccharomyces Cerevisiae, S. Diastatitus, Kluyveromyces Marxianus, E. coli, Zymomonas mobilis, Klebsiella oxytoca* and *Pichia kudriavzevii* (Yu et al., 2009). However yeast (*Saccharomyces Cerevisiae*) has been found to be the most

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efficient in increasing the bio ethanol yield (Taouda et al., 2017). The process for producing bio ethanol from sewage sludge and other related lignocelluloses material is shown in Fig. 1 whereby the key production processes are shown. The various processes involved in bio ethanol production include pre-treatment (chemical, physical and biological methods), hydrolysis, fermentation and distillation (Limayem and Ricke, 2012).

Currently in Southern Africa, bio ethanol is being produced from sugar cane and baggase and there is need to explore other raw materials for generation of bio fuels like sewage sludge as a waste utilization strategy. In this study, the potential to produce bio ethanol from municipal sewage sludge is investigated. Further to that, the impact of several parameters and how they affect bio ethanol production from sewage sludge was also investigated.

2. Materials and methods

2.1. Materials

Bacillus flexus from Sigma Aldrich was used for saccharification of sugars from the sewage sludge to make a hydrosate. Yeast was then used as the fermentation biocatalyst for the conversion of the sewage sludge hydrolysate to bio ethanol. Sewage sludge was obtained from a local municipal sewage treatment plant.

2.2. Characterization of the sewage sludge

The physicochemical characteristics of the sewage sludge were measured in accordance to standard methods. The moisture content was measured according to CEN/TS 14 774 methodology, ash content was measured according to CEN/TS 14 775 methodology and volatile solids content were determined according to the CEN/TS 14 774 methodology and the volatile solids were determined using the CEN/TS 14 780 methodology. The ultimate analysis for ash content, fixed carbon and the carbon to nitrogen ratio was conducted in accordance to CEN/TS 15 104. The pH was determined using an HI 9124 pH probe. The pH was maintained constant using liquid lime.

2.3. Preparation of the hydrolysate

The collected sewage sludge was first dried and cut to small pieces of less than 3 mm using a grinder and then passed through a sieve to ensure uniformity in the particle sizes. A sample of 10 g of the sewage sludge was put in a 250 mL conical flask and was moistened to 40% using distilled water. These were then autoclaved for 30 min at 121 °C. The sterilized flask with the moistened sewage sludge was inoculated with 3 mL of *Bacillus flexus*. Afterwards the samples were incubated for 48 h at 30 °C. Afterwards, the flasks were distilled with 50 mL distilled water and shaken at 200 rpm in an Innova 43 shaker.

After shaking the contents were filtered off using a muslin cloth to a new dry flask. The filtrate was then centrifuged for 10 min at 800 rpm. This filtrate was the sewage sludge hydrolosate that was formed due to the enzymatic activities of *Bacillus flexus* through saccharification.

2.4. Bio ethanol production through fermentation

Bio ethanol was produced from the sewage hydrolysate through fermentation. For the preparation of the fermentation medium, 10 g/L of pepotone, 2 g/L of KH_2PO_4 and 1 g/L of $MgSO_4$ were added to the sewage hydrolysate and then sterilized at 121 °C for 20 min. Afterwards yeast was added at various concentrations from 2 to 10% (v/v).

The medium was then incubated at varying temperatures of 15–45 °C and retention times of 10–70 h. The pH was ranged between 4.0 and 7.0. After fermentation, the medium was centrifuged for 10 min at 10 000 rpm and the concentration of the bio ethanol and the reducing sugars determined. The effect of each parameter was studied whilst the others were kept constant.

2.5. Determination of reducing sugars content

The total reducing sugars were measured using the 3.5 dinitro salicylic acid DNS methodology in accordance to Miller (1959). Glucose was used as the standard for measurement with samples

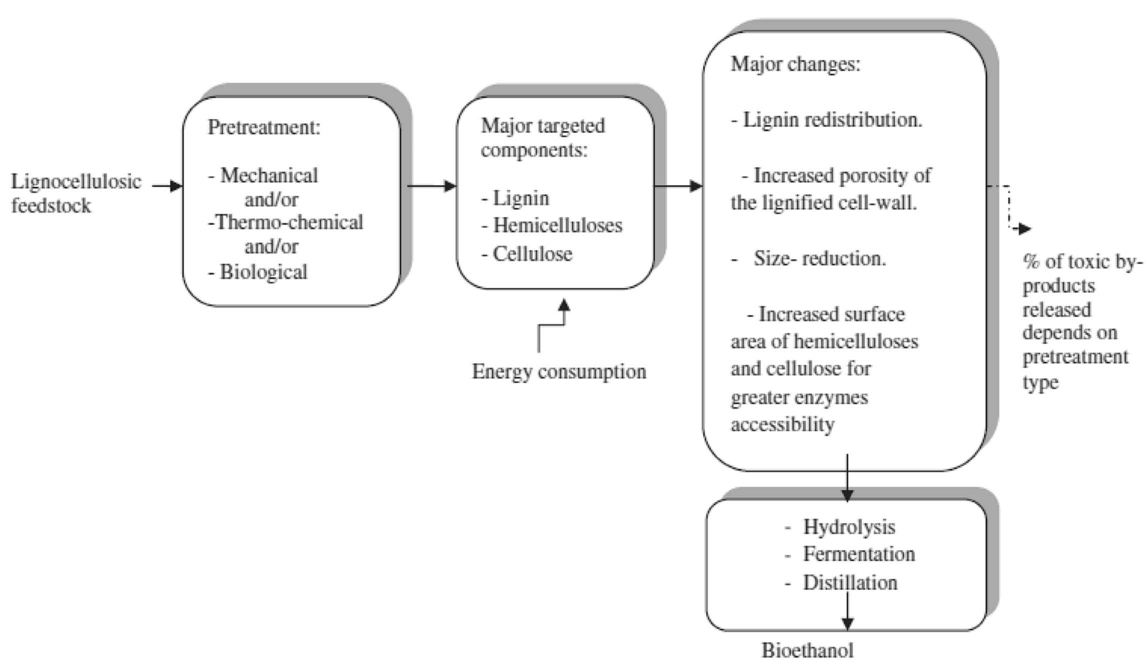

Fig. 1. Bio ethanol production from lignocelluloses material (Limayem and Ricke, 2012).

being stored at 5 °C to avoid bio ethanol loss as well as prevent potential spoilage by micro organisms.

2.6. Characterization of the bio ethanol

The bio ethanol produced was analyzed using an Agilent gas chromatography. The concentration was measured in millilitres of bio ethanol produced per litre of the reducing sugars introduced. The physicochemical characteristics of the bio ethanol were measured in accordance to standard methods indicated in Table 1.

2.7. Statistical analyses of correlations between parameters

A statistical analysis was conducted to determine the correlations between the various parameters on bio ethanol production from sewage sludge. Pearson Correlations Coefficients were determined using MATLAB 13.0 based on the 3 observations made each parameter for the effect of incubation time, pH, glucose concentration and yeast concentration at 95% confidence interval.

3. Results and discussion

3.1. Sewage sludge characteristics

The sewage sludge used in this study had characteristics as indicated in Table 2.

3.2. Characteristics of the hydrolysate

The hydrolysate was obtained by hydrolysis of sewage sludge using *Bacillus flexus* as the hydrolysis media. The end hydrolysate contained 10.5 g/L of reducing sugars.

3.3. Factors affecting bio ethanol production

The factors that affect bio ethanol production from sewage sludge were assessed individually and these included: incubation time, temperature, the yeast concentration, pH as well the available glucose concentration.

3.3.1. Effect of incubation period

The incubation period refers the time at which the bio ethanol can be harnessed from the sewage sludge broth. Reduced incubation times results in inadequate growth of the yeast cells which will eventually reduce the amount of bio ethanol produced. At the same time if the incubation periods are too long, the high concentrations of bio ethanol produced can become toxic to the sewage sludge broth. From this study, increase in the incubation period from 10 h to 70 h resulted in a decreased bio ethanol concentration production from 35 mL/L to 20 mL/L (Fig. 2). This trend showed that the fermentation was fastest at incubation times of around 10 h and the bio ethanol production equilibrium is reached early. Prolonged incubation time have been reported to result in decreased bio ethanol concentration and this can be attributed to bio ethanol evaporation or its consumption by yeast cells during their kinetics (Dash et al., 2017). As the incubation time increased, the amount of sugars available for fermentation becomes less hence the low bio ethanol yields at incubation periods of more than 40 h in this study. Dash et al. (2017) reported an almost similar trend when they produced bio ethanol from sweet potatoes with the maximum bio ethanol yield being achieved at 127.2 g/kg at a pH of 5 and incubation period of 72 h. Therefore, the trend observed in the bio ethanol generation from sewage sludge hydrolysate can be concluded to be a typical trend in bio ethanol production.

Table 1
Bio ethanol physicochemical characteristics measurement methods.

Parameter	Measurement method
Moisture content (%)	SS ISO 760
Density (g/cm^3)	SS-ISO 750
Refractive index	Abbe refractometer
Specific gravity	Pycnometer model SG-16A 2000
Flash point (°C)	ISO/CD 3679
Viscosity (cP)	EN ISO 310
Sulphur (%)	D1266
Ash content (%)	DIN EN 1 806 245
Cloud point (°C)	Petrotest (ASTM D97)
Pour point (°C)	Petrotest (ASTM D97)

Table 2
Sewage sludge characteristics.

Parameter	Value
Total solids (%)	6.3–7.5
Volatile solids (VS)	3.9–4.2
Ash content (%)	1.6–1.8
Fixed carbon (%)	2.7–2.9
Carbon to nitrogen ratio	8.9–10.1

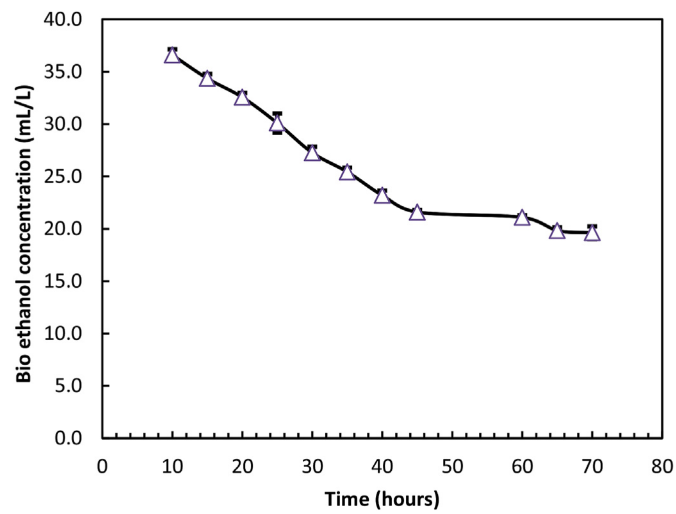

Fig. 2. Effect of incubation time on bio ethanol production from sewage sludge.

3.3.2. Effect of temperature

Bio ethanol production from sewage sludge is a temperature sensitive process since the process is biological in nature. High temperatures are considered a stress factor to microorganisms and can therefore hinder the bio process. From this study, as the temperature increased from 20 °C to 30 °C, the bio ethanol concentration increased and reached a peak of 30 mL/L at 30 °C, afterwards the bio ethanol concentration decreased to 22 mL/L (Fig. 3). The behaviour was attributed to the enzymatic behaviour of yeast, as the temperature is either too low or too high, the yeast is denatured and reduces the rate at which fermentation occurs resulting in low bio ethanol yields. Yeast being a micro-organism is sensitive to high temperatures hence it can become denatured decreasing the yield of the bio ethanol (Zabed et al., 2014). Temperature therefore becomes a critical factor in bio ethanol production from sewage sludge which must be maintained at 30 °C.

3.3.3. Effect of pH changes

Bio ethanol production from sewage sludge is a very pH

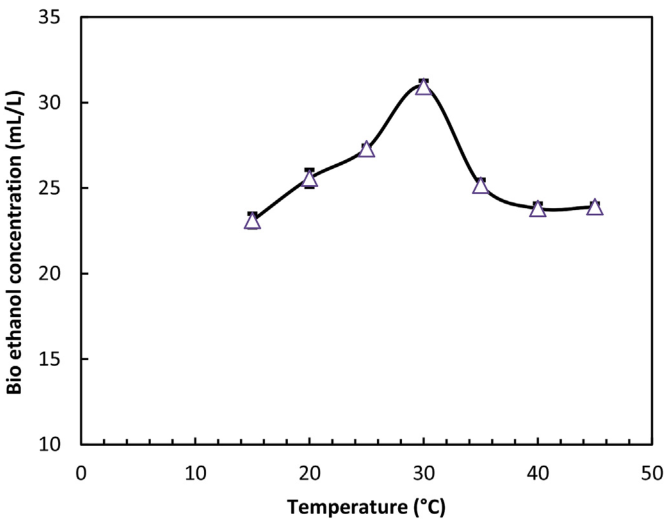

Fig. 3. Effect of temperature on bio ethanol yield from sewage sludge.

sensitive process since the process is biological in nature. The fermentation process, which is very critical during the bio ethanol production, is very sensitive to pH due to the fact that the H+ ions in the fermentation media can change the charge of the plasma membrane resulting low permeability of nutrients into the yeast cells. In this study, as the pH increased from 4.0 to 6.5, the bio ethanol concentration increased (Fig. 4). The highest bio ethanol concentration was 32.9 mL/L which was observed at a pH of 6.5 (Fig. 4). Afterwards, the bio ethanol concentration significantly reduced for pH between 6.5 and 7.0 and this can be attributed to the denaturing of the yeast cells, thereby reduced bio catalytic activity in the process. Since yeast was the bio catalyst used for fermentation, acidic pH was ideal. Tahir et al. (2010) reported the same trend in pH variations when bio ethanol was produced from sugar cane molasses and the highest yield of 65 g/L was achieved at a pH of 4.5. From the observations, it must therefore be noted that bio ethanol production is pH dependent and this must be closely monitored in order to achieve the required yield.

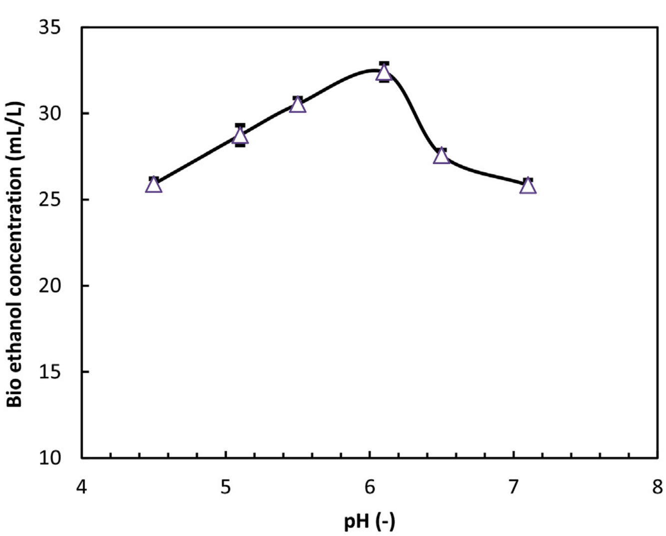

Fig. 4. Effect of pH on bio ethanol production.

3.3.4. *Effect of glucose concentration*

The availability of glucose is critical and has direct linkage on the fermentation rate as well as the growth of the yeast cells. Generally, the rate of fermentation will increase with the increase in the glucose concentration to a steady rate. In this study, as the bio ethanol concentration increased to 60 mL/L, the glucose concentration increased from 20% to 80% afterwards the bio ethanol concentration drastically reduced by almost 50% (Fig. 5). The pronounced increase in the bio ethanol concentration after 60 mL/L was attributed to the decreased nutrient availability for fermentation at high glucose concentrations (Zabed et al., 2014). However, as the glucose concentration increased above 80%, it tended to have inhibitory effects thereby deactivating the yeast catalyst therefore lowering the amount of bio ethanol produced by almost 100%.

3.3.5. *Effect of yeast (Innoculum) concentration*

Yeast was used as the Inoculum bio catalyst during the production of bio ethanol from sewage sludge. The yeast concentration used affects the amount of glucose produced as well as the bio ethanol productivity. In this study, as the yeast concentration increased from 2 wt % to 6 wt %, the amount of bio ethanol

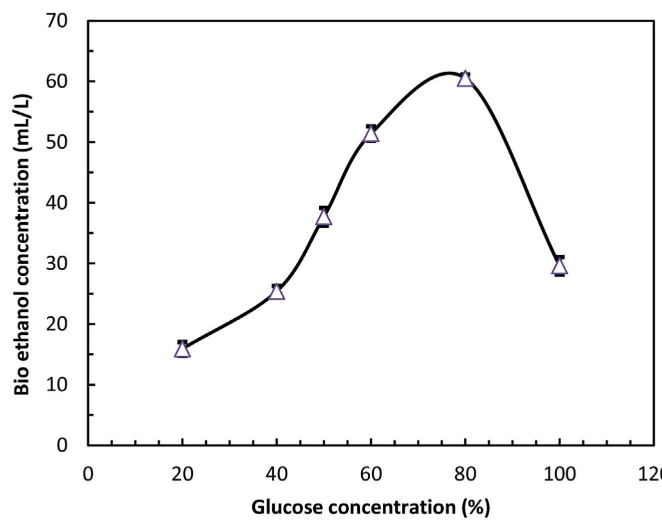

Fig. 5. Effect of glucose concentration on bio ethanol production.

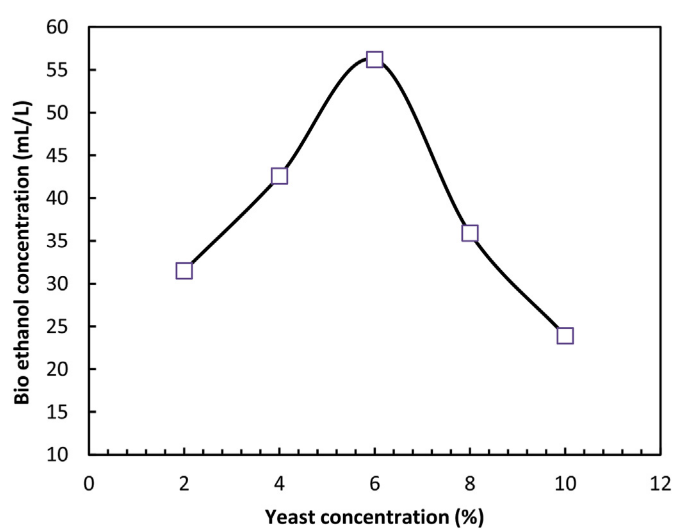

Fig. 6. Effect of yeast concentration on bio ethanol production from sewage sludge.

Table 3
Pearson Correlations coefficients between the parameters.

	Incubation time	Temperature	pH	Glucose concentration	Yeast concentration
Incubation time	1	−0.4549	−0.3724	−0.9930	0.3486
Temperature	−0.4549	1.000	0.9759	0.5419	0.2916
pH	−0.3724	0.9759	1.000	0.4581	0.4837
Glucose concentration	−0.9930	0.5419	0.4581	1.0000	−0.2971
Yeast concentration	0.3486	0.2916	0.4837	−0.2971	1.0000

Table 4
Physicochemical characteristics of bio ethanol from sewage sludge.

Parameter	Value	ASTM Standards
Moisture content (%)	0.45–0.47	20
Density (g/cm^3)	0.97–0.98	0.99
Refractive index	1.35–1.36	1.36
Specific gravity	0.91–0.92	0.87
Flash point (∘C)	19.1–19.3	18.6
Viscosity (cP)	1.28–1.32	1.20
Sulphur (%)	0.1–0.2	20
Ash content (%)	0.6–0.7	30
Cloud point (∘C)	20.8–21.1	23
Pour point (∘C)	4.8–5.0	5.2

produced also increased (Fig. 6). The highest bio ethanol concentration was 58 mL/L and it was observed at the yeast concentration of 6%. However, after a concentration of 6 wt %, the bio catalysts would have saturated the system thereby decreasing the amount of bio ethanol produced. The same trend was observed by Zabed et al. (2014) when they increased the cell concentration from 1×10^4 to 1×10^7 cells/mL of yeast cells over a 72 h incubation period for yeast concentration of 3% (v/v) to 6% (v/v). From the study, it can be concluded that high yeast concentrations of more than 6 wt % are not ideal for optimal bio ethanol productions since they tend to saturate the system and will not contribute towards the bio catalysis to enhance bio ethanol productivity.

3.3.6. *Pearson Correlations coefficients between the parameters*

From the MATLAB Pearson Correlations analysis on the interactions of the parameters on bio ethanol yield from sewage sludge, the interaction between increase in incubation time and yeast concentration, temperature and pH, temperature and glucose concentration, pH and glucose concentration as well as pH and yeast concentration had a positive effect on the amount of bio ethanol produced (refer to Table 3). The interaction between increase in temperature and pH resulted in a correlation coefficient of 0.9759. However, the interactions between increase in incubation time and temperature, incubation time and pH incubation time and glucose concentration as well as increase in yeast and concentration resulted in low bio ethanol yield. The lowest yield can be obtained at high incubation temperature and glucose concentration with coefficient 0.0–0.9930 and these conditions must be avoided.

3.3.7. *Bio ethanol from sewage sludge characterization*

The bio ethanol was characterized and its physicochemical properties compared to the ASTM standards. In accordance to Table 4, the bio ethanol product quality compared well with the ASTM standards for the standard ethanol.

4. Conclusion

Sewage sludge is a good raw material for bio ethanol production which is a good alternative to fossil fuels. High bio ethanol yields of up to 60 mL/L were obtained at an incubation period of 10 days, with an operating temperature of 30 °C and pH of 6.5 with yeast of 6% wt as the ideal fermentation catalyst. The interaction between increase in incubation temperature and the pH had the most positive effect on bio ethanol yield with a Pearson coefficient of 0.9759 being realized. Bio ethanol which is comparable to the standard bio fuel was obtained. The conversion of sewage sludge to bio ethanol promotes both energy potential for developing countries as well as waste management strategy for managing municipal waste.

Conflicts of interest

The authors declare there is no conflict of interest.

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