



## 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  $\text{KH}_2\text{PO}_4$  and 1 g/L of  $\text{MgSO}_4$ . 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.

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### 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 (*Saccharomyces 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. Diastitatus*, *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 hydrolysate 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 peptone, 2 g/L of  $\text{KH}_2\text{PO}_4$  and 1 g/L of  $\text{MgSO}_4$  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

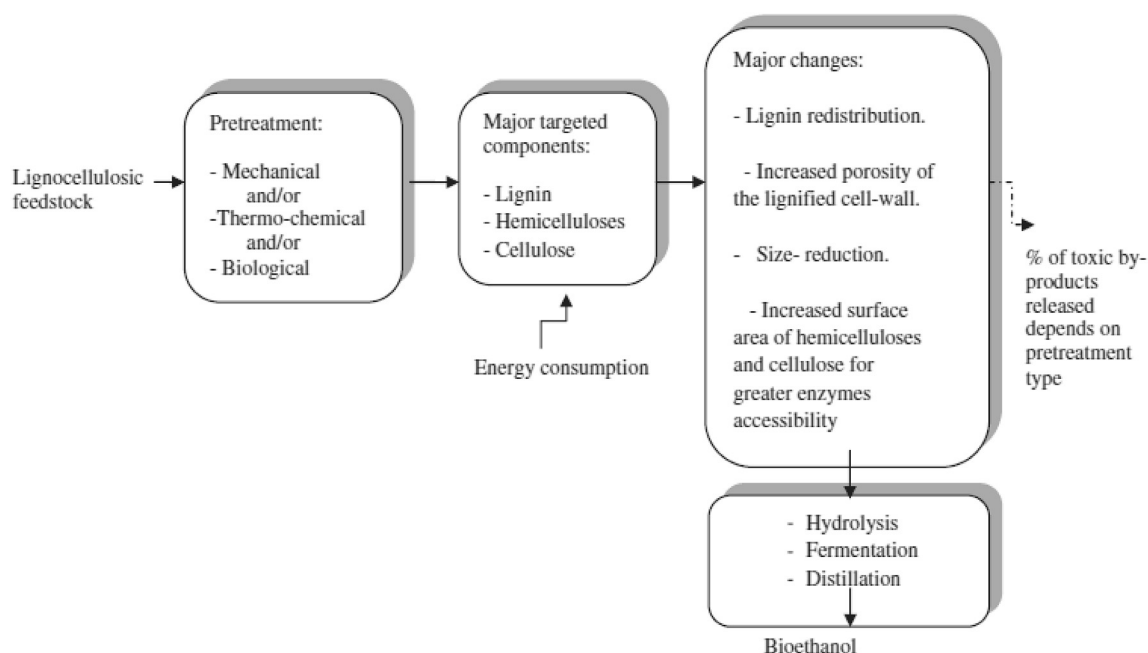


Fig. 1. Bio ethanol production from lignocelluloses material (Limayem and Ricke, 2012).

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