Effective Microorganism as New Cellulose Degrading Bacteria to Produce Biofuels

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Ab tract

Nowadays, biofuel such as bioethanol and biomethanol has been widely used by many countries. Example application of biofuels is used as fuel for transportation. Study found that this biofuel can be produced by using cellulose in living things. Then it was related from a study which is production of bioethanol from newspaper using *Cythophagahutchnosonni* bacteria as biodegradable bacteria for hydolysis proces and fermentation process using common yeast, *Sacacharomyces cerevisa*e. Newspaper which is one of municipal waste which contain cellulose has been used as a substrate in producing bioethanol. Fermentation process of newspaper has been proved by a study to produce bioethanol. The purpose of this experiment is to produce biomethanol using *Effective Microorganisms* (EM) as biodegradable bacteria. It undergo several processes which are pre-treatment of substrate using hydrochloric acid, hydrolysis of substrate using EM and addition of yeast *S. cerevisae* for fermentation process. Biofuels produced was analysed using Refractive Index method. As a result, 91% - 93% concentration of biomethanol were produced. Based on analysis, it contains average of 33% biomethanol, 4% of water in each sample taken.

Keywords: Fermentation, Biofuels, Newspaper, *Saccharomyces Cerevisae* , biomethanol

1.0 Introduction

Biomass also known as bio-energy is one of the major stakes in meeting global future energy (Oropeza-De la Rosa et al., 2017). Bio-energy refers to the use of plant and animal-based matter to generate renewable energy (Gasparatos et al., 2017). It is believed that bio-energy can supply more than quarter of global energy supply by 2050. According to a study in Taiwan, bio-energy is used to reduced and limit the emission of CO_2 (Tsai et al., 2008). One of the most major bio-energy is methanol which can be obtained from non-fossil source. This increasing in global demand has affected the production of methanol as well improvement of technology used during the process and in order to improve the process efficiency, it is necessary to study the behavior of raw material and its transformation during the process (Oropeza-De la Rosa et al., 2017). Methanol is mainly used in chemical production as feed-stock as well as solvent. Methanol was then undergo experiments of large potential uses such as for transportation. (Yasin et al., 2015). The advantage of methanol production is the raw materials used in its production are easily to be found and also cheap. The new invention of raw materials in methanol production by using waste newspaper (Zabed et al., 2017).

According to the previous estimation made by the United Nations, it was about seven billion of world population in October 2011 (Roberts, 2011). Due to this increment, the municipal solid waste will increase and potentially to be a major problem worldwide since it could lead to a rising of landfill cost (Chintagunta et al., 2017). Apart from that, the excessive of municipal solid waste may cause variety of problems such as air pollution and water pollution. It was recorded that about 35% to 40% of municipal solid waste came from paper and newspaper. In comparison, food waste, wood, leaf, rubber, textile, leather etc have low percentage. Based on the above concern, the municipal solid waste such as newspaper is wisely used to produce something which is far more beneficial. This new invention may result in a reduction of the major waste materials in the world.

Since biomethanol was found to be used as fuel for transportation, it may give a lot of advantages for both economic strategic and environmental perspectives. Petroleum based is commonly used as fuel for transportation. Consumption of petroleum produces energy from hydrocarbons, which are made-up of carbon and hydrogen (Chen et al., 2017). Carbon dioxide, a greenhouse gas that gets trapped in the atmosphere were produced during the burning of hydrocarbons. It is indefinitely and plays a major role in global warming. It was recorded that the Americans consume 19.4 million barrels of crude oil every day, which is refined to make gasoline, jet fuel and propane. The emission of hazardous waste can be controlled by using bio-fuels such as methanol (Santos et al., 2013).

Increasing in population over the last century is in line with the national development. Therefore, the municipal solid waste will increase and potentially to be a major problem worldwide since it could lead to a rising of landfill cost. Based on the above concern, the municipal solid waste such as newspaper is used to make methanol (Byadgi & Kalburgi, 2016a). It was recorded that about 35% to 40% of municipal solid waste came from paper and newspaper. In comparison, food waste, wood, leaf, rubber, textile leather etc have low percentage. Therefore, by recycling all the used newspaper around the world, we can reduce one of the major waste materials in the world.

The main process in methanol production is fermentation of sugar. Yeast, S*accharomyces cerevisiae* is a commercial yeast that has been used in many process and it also known as *basidiomycetous* fungi that has ability to reproduce itself (Jambo et al., 2016) by fission and form spores. This yeast was used to convert the maximum sugars into methanol (Byadgi & Kalburgi, 2016b). At first this yeast, *S. Cerevisae* has been used for making alcohol, studied found that this yeast was able to produce methanol. Hence, it was used as main material for fermentation process for the last two decades. Further and various researched have been made to give some innovation in production of methanol efforts (Sikarwar et al., 2017).

The way of hydrolysis pre-treated substrate was by using *Effective Microorganisms(EM)*. EM were added to the pre-treated substrate to release maximum sugar which then taken for the fermentation process. EM is defined as mixed culture of natural occurring organism that can increase the quality of soil ecosystem. They consist mainly of the photosynthesizing bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi. EM was originally used to improve composting. It was found that EM are able to reduce odor, improve the decomposition rate (Fan et al., 2017) and produce higher nutrition content. At larger scale of studied, application of Effective microbe can be further improved such as on industrial scale. For example, EM can be used on fermentation using different feedstock. It was proven by a study which EM can act as cellulose degradation (Mayer et al., 2010).

In addition, the main objective of this research was to study the effective of newspaper as feedstock to produce methanol. Besides, to compared the effectiveness of EM as cellulose biodegrade bacteria. Other than that, to analysed biofuels produces using Refractive Index method.

1 Material:

Newspaper was collected from the households and cellulose biodegradable bacteria which is *Effective Microorganisms* (EM) was bought from EMRO Malaysia Sdn Bhd, Skudai, Johor. Yeast for fermentation proce s, *S. Cerevisae*, biodegradable bacteria was bought at mini market in Masai, Johor.

.2 ample preparation

The substrate (newspaper) was collected and washed for several times using distilled water to make it dust free and fungus free state. It was then put in oven to be dried at 104°C for overnight. bstrate was weighed for 30 grams before shred into small pieces and filled into two 1000mL beakers. EM were prepared for 45 mL and 60mL in each different jar by using pipette. While yeast was weighed at 50g and 60g as manipulate variables. Distilled water with amount of 2L was measured using a 1000mL beaker and concentrated Sulphuric Acid was diluted until 1.5% concentration for pre-treatment process.

2.3 Pr -treatment process of the substrate

Sulphuric acid was diluted with distilled water in fume chamber until it reached 1.5% concentration and the substatre was added into the diluted sulphuric acid with ratio 1g substrate to 15 mL diluted sulphuric acid (1:15). Then, the diluted sulphuric acid with substrate was through heating process at 121°C for 45 minutes for rearrangement structure of cellulose (Byadgi & Kalburgi, 2016a).

2.4 Hydrolysis of the pretreated substrate

The pre-treated substrate was washed with distilled water several times to neutralize the acid concentration until the pH was adjusted to 7.0. The substrate then was oven dried at 104°C for overnight. EM was used to substitute *Cythophagahutchisonni* which act as isolated cellulose degrading bacteria. It was mixed with substrate and distilled water. Each jar were mixed with dried substrate, distilled water and EM with different ratio; jar 1, (2L distilled water : 45mL EM) and jar 2, (2L distilled water: 60mL EM). Each mixture was stirred and jars were tidely closed. The mixtures were left for 7 days.

2.5 Fermentation of hydrolysed substrate

S. cerevisiae, commercially available yeast was used to carried out fermentation process at room temperature. Both jar then was equally separated into 2 jars for further fermentation process. Yeast with mass of 50g and 60 g were added into one of each jar of same volume of EM. Each mixture was stirred and jars were tidely closed. This steps were duplicated with jar 2 with same properties. The mixtures were left for 2 days.

2.6 Extraction of biomethanol

Sample was taken within 48 hours interval for 10 days. 50 mL of the solution in each jar was taken into 50mL beaker. Solution contains biomethanol then was extracted using Heildoph Hei-VAP Platinum 1 Rotary Evaporator and amount of solution extracted was measured using measuring cylinder. The solution was kept in test tube for further analysis and all the results were recorded.

2.7 Analysis of biomethanol

Biomethanol was analysed using Refractometer. The reading of refractometer was recorded for further discussion.

.0 Results and discussion

1 Determination of biomethanol by using Refractive Index Method

E traction of solution from each fermentation sample was using rotary evaporator. Each solution extract from the sample then was taken for RI measurement. It was taken at reference of 22°C which have been nsidered as room temperature. The data gathered were taken to be measured using equation from tandard methanol reference graph. A graph of methanol with concentration range between 90% to 96% was plotted to get the equation for the curve which is to be $y = 1.1875X^2 - 2.2923X + 2.4348$. A study from (Chu & Thompson, 1962) stated the range of methanol RI is to be exactly between 1.3330 to 1.3287 which supported the result from this experiment. The data gathered then was calculated using curve equation from standard ethanol reference graph to get the exact concentration of each sample. It was studied that the range of the sample concentration is to be between 91% to 93%.

3.2 Parameter's time affected the concentration of biomethanol

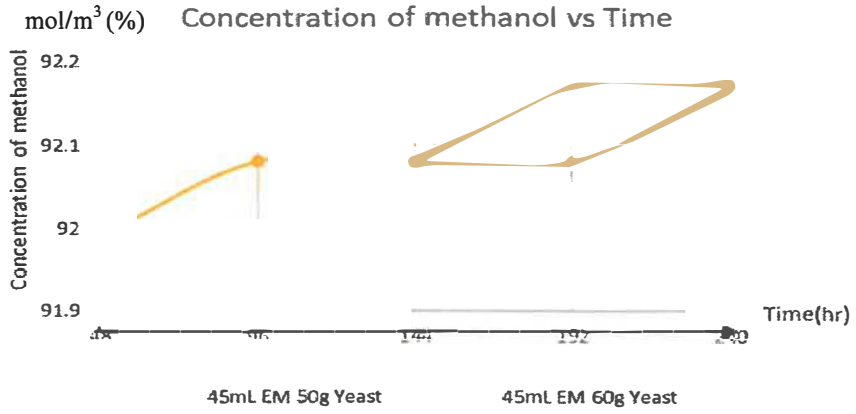

Figure 3.2 Concentration of methanol produced increased as fermentation time increased

From the figure 3.2 above, there was only different on the yeast amount. The concentration of both sample increase with time. Fermentation was carried out for 10 days with ferment each of the sample being collected every 48 hours.

Fermentation time affect the growth of microorganisms. The shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms. However, the longer fermentation time gives toxic effect on microbial growth. After 10 days, it was found that the fermentation is still ongoing due to increasing of concentration. This means the growth of microorganisms still working in 10 days. The factor affecting the production is temperature. The growth rate of the microorganisms is directly affected by the temperature. High temperature which is unfavourable for cells growth becomes a stress factor for microorganisms (Mohd Azhar et al., 2017). In this experiment, fermentation undergo the process at room temperature which is 22°C. The ideal temperature for fermentation process is range 20°C to 35°C. This means the temperature of 22°C is a good range temperature for the fermentation process based on previous paper.

The other factor was increasing in sugar concentration up to a certain level will increase the fermentation rate. On the other hand, the use of excessive sugar concentration will cause steady fermentation rate. This is because the concentration of sugar use is beyond the uptake capacity of the microbial cells (Mohd Azhar et al., 2017). EM is added to help in converting cellulose to sugars in hydrolysis process which is indirectly will produce higher initial sugar concentration. As shown in graph, both sample rise dramatically from 48 hours to 96 hours. This means that the initial sugar concentration considered as the important factor production.

3.3 Volume of solution extracted using rotary evaporator

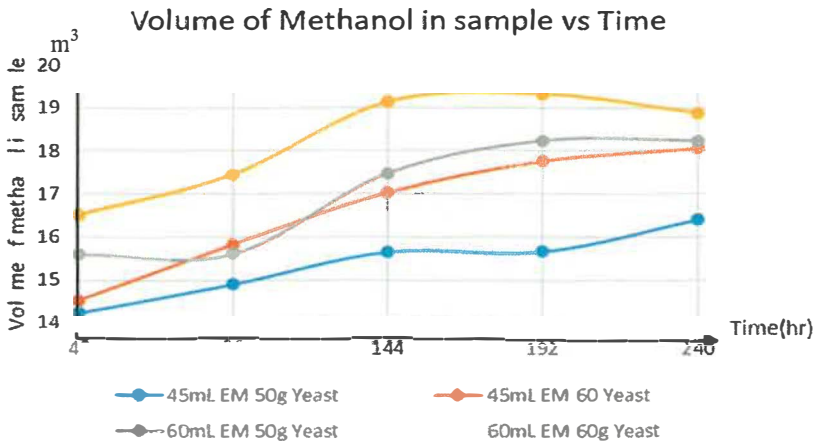

Figure 3.3 Volume of methanol in each sample taken

Figure above show the result of the conversion or yield of the biomethanol after completed testing for 5 times. The result shows an increment in terms of volume of the methanol as the time increase. From the graph above, it shows that the quantity of the bacteria used will effect to the volume the methanol produced. Microorganism that are metabolically engineered to redirect renewable carbon sources into desired fuel products are contemplated as best choices to obtain high volumetric productivity and yield. There is some difference of the production of the methanol between the 45mL and 60 mL quantity of bacteria with the same amount of yeast. The graph showed that the higher number of bacteria resulting higher amount of methanol produced. The production of methanol also is due to the use of EM which have its own characteristic that can produce methanol from the fermentation process where its provide nutrient in the fermentation process.

The time to extract the sample also become one of the factor that influenced the methanol production. Based on the journal about fermentation process, it shows that variation fermentation period had the greatest impact on the percentage composition of the biofuel and the volume of biofuel produced showing the best fermentation period for obtaining optimal biofuel production to be at 96 hours (Nambu-Nishida et al., 2017). For our result, the best fermentation period to obtain the methanol is at the 96 hours. This is due to the type of bacteria that is used which is EM and the raw material used that is treated newspaper.

3.4 Manipulation amount of yeast affected rate of fermentation

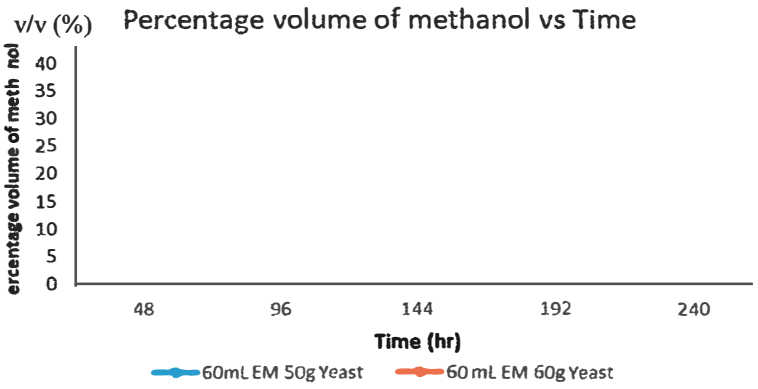

Figure 3.4 Differences of percentage of methanol produce affected by changing amount of yeast