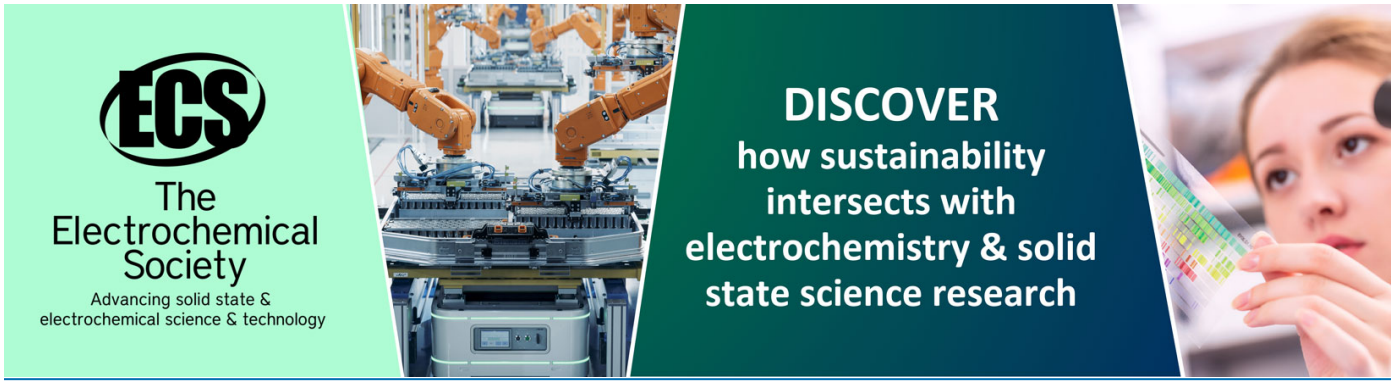
Preliminary Study on Biethanol Production from Starchy Foodwastes by Immobilized *Saccharomyces cerevisiae*



Preliminary Study on Biethanol Production from Starchy Foodwastes by Immobilized *Saccharomyces cerevisiae*

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Abstract. Dumping of food wastes into the landfill resulted in major environmental pollution. However, attempted had been made to develop these wastes into a new renewable and sustainable energy. Liquid biofuels, bioethanol can be produced from a variety of feedstock including biomass and food crops or wastes. Therefore, in this study, starchy food wastes of bread, rice and potatoes were utilized as a potential feedstock for the bioethanol production. Yeast *Saccharomyces cerevisiae* was immobilized in 2% calcium alginate beads using entrapment technique. Then, the effect of temperature on bioethanol efficiency was investigated using the immobilized yeasts. From the result, highest fermentation efficiency of 1.24% was obtained at temperature 30^{o}C, 48 h with agitation speed of 150 rpm. However, further research and studies are required in order to optimize the bioethanol production from fermentation process of starchy foodwastes.

1. Introduction

Depletion of energy resources, fossil fuels and crude oil happened due to their limited in supply. Liquid biofuels, bioethanol is a potential green bioenergy sources that are able to replace gasoline in transportation sector [1]. Bioethanol is more competitive to fossil fuels because its production utilizes cheaper and non-food feedstocks such as lignocellulosic biomass and municipal solid waste [2,3]. In recent years, utilization of food waste as an alternative feedstock for bioethanol become a hot topic for researcher [4]. This is because bioethanol produced from food wastes can reduce both the cost of waste disposal and bioethanol production [5]. However, composition of the food waste is important as it will influence the bioethanol production efficiency [6].

Food waste can be defined as kitchen leftover waste or precooked agricultural waste which discharged from various industries such as households, restaurants or food processing industries [7]. Food waste accumulation is often associated with environmental pollution issues [8] not only in Malaysia but also in developing and developed countries. Kiran and Liu [9] reported that almost 1.3 billion tons of food is lost or wasted throughout the food supply chain. Hence, efficient and effective ways on handling food wastes are necessary to counter this problem. Food waste is a non-edible and no-value resource that contain lipid, carbon and carbohydrate which can be converted into bioethanol [10]. Konti *et al*. [1] reported that production of bioethanol from food waste is an eco-friendly process

which aids in reducing green house gas emissions. Food waste is a promising bioethanol feedstock that can be produced through microbial fermentation [11].

Yeasts such as *Saccharomyces cerevisiae* play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. They are used in industrial plants due to valuable properties in ethanol yield (>90.0% theoretical yield), ethanol tolerance (> 40.0 g/L), ethanol productivity (> 1.0 g/L/h), growth in simple, inexpensive media and undiluted fermentation broth with resistance to inhibitors [12]. *S. cerevisiae* cells can be used either as immobilized or free cells. However, cells immobilization have the advantages over free cells as they can be reused in new production cyles, improved the resistance to inhibitors in the hydrolysates and the utilization of sugars. They also facilitate the separation of cells from the medium and products thus reducing the production costs [13]. Numerous immobilization techniques had been reported including entrapment, covalent bonding, cross-linking, adsorption and encapsulation [14]. Even so, the most extensively used technique is entrapment of cells in calcium alginate beads as it is less expensive, non-toxic and can be prepared easily [15]. In immbilization process, microbes are being used instead of enzyme, calcium alginate as the matrix and entrapment as a mode of attachment between the *S. cerevisiae* and calcium alginate. Therefore, these immobilized microbes will be able to catalyze the starchy food wastes to produced bioethanol [16].

In this study, bioethanol was produced from a thermo-acidic pretreated food waste hydrolysate using yeast *S. cerevisiae*, immobilized in calcium alginate beads. Effect of time, temperature and agitation rate on bioethanol production from starchy food wastes using immobilized *S. cerevisiae* were optimized during fermentation process. It is hoped that this study could helps in established an effective strategy for bioethanol production from different starchy food wastes.

2. Materials and Method

2.1. Collection of samples

The samples used in this study were starchy food wastes consisting of cooked wastes of white bread, white rice and potatoes. Cooked rice waste were obtained from Nasi Kukus restaurant in 1Borneo, white bread waste from UMS hostel canteen while cooked potatoes waste were taken from Filipino market, Kota Kinabalu, Sabah. Each samples were either blended or grounded using mortar for 2-3 min. Then, the samples were stored at -80^{o}C overnight then transferred into freeze dryer until no weight change. Next, the samples were grounded again into powder form and stored separately in a sealed plastic bags at -20^{o}C until further use.

2.2. Thermo-acidic pretreatment

Pretreatment process was carried out according to Vucurovic *et al.* [17] with slight modification. 10g of each powdered food wastes was mixed together. Then, 300 mL of distilled water was added into the mixture at ratio 1:10. Next, 1M of hydrochloric acid was added until the mixture pH turn to 1 and it was autoclaved at 121^{o}C for 20 min. The pretreated samples were cool down at room temperature and neutralized up to pH 6.8 using 1M of sodium hydroxide.

2.3. Enzymatic hydrolysis

A mixture of 50 mL treated samples and 25 mL of phosphate buffer solution was added into a beaker. Then, the pH was calibrated at 6.8 as it was the optimum pH for amylase. Enzyme amylase (2mL) was added into the mixture and heated at 35^{o}C for 30 min. The mixture was cooled down and centrifuged at 10,000 x g for 15 min. The reducing sugar content of hydrolysates were analyzed using dinitrosalicylic acid (DNS) method.

2.4. Cultivation of S. cerevisiae

The cultivation was done by weighing 1g of *S. cerevisiae* which was dissolved in a 10 mL of sterilized YPD broth. The *S. cerevisiae* was then cultured on agar plate and incubated at 37^{o}C for 48-72 h to observe the single colony.

2.5. Immobilization of S. cerevisiae

Preparation of 2% (w/v) of sodium alginate solution by weighing 2g of sodium alginate and was diluted in 100 mL of distilled water. The mixture was then stirred until sodium alginate completely dissolved in distilled water forming sodium alginate solution. To prepare 2% (w/v) of calcium alginate solution, 2g of calcium chloride was added into 100 mL of distilled water. The mixture was stirred well and the calcium chloride solution was stored at 4ºC. Entrapment method and the immobilized beads were prepared according to Gao *et al*. [16] with modifications. About 10 mL of enriched *S. cerevisiae* was added into 100 mL of 2% (w/v) sodium alginate solution. The mixture of immobilized cells with sodium alginate solution was added drop by drop into $CaCl_2$ solution using a syringe needle (21G x 3 cm) to form beads. The distance between the syringe and $CaCl_2$ solution was 10 cm. The beads were left inside $CaCl_2$ solution for 25 min and then it was filtered using a sieve. The beads were rinsed with distilled water and left to dry on Whatmann filter paper.

2.6. Optimization parameters for bioethanol production

Parameters involved in the optimization process were temperature (20, 30, 40) ^{o}C, agitation rate (150, 180, 250) rpm and fermentation time (24, 48, 72) h. The baseline was set at temperature 30^{o}C, 150 rpm agitation rate and 48 h of fermentation time. Fermentation was carried out using 50 mL of food wastes hydrolysate and 5g of immobilized calcium-alginate beads incubated at different temperature, agitation rate and time. Steriled YPD broth of 5 mL was added to provide nutrients for the immobilized *S. cerevisiae* beads.

2.7. Ethanol determination from fermentation

Ethanol content from the fermentation broth was determined using potassium dichromate oxidation in aqueous solution by applying a redox titration method. First, the fermentation broth was diluted with distilled water at ratio of 1:20 (1 mL sample: 19 mL distilled water). Then, 1 mL from the mixture was added into 4 mL of distilled water to make a ratio of 1:5. Next, 10 mL of potassium dichromate (0.01 mol/L) was added into the 1:5 sample followed by the addition of another 100 mL of distilled water. Colour formation of orange-red should be observed after addition of 4 mL of potassium iodide (1.2 mol/L). Then, 2-3 drops of 1.0% starch solution was added until blue-black colour was formed. Lastly, 0.03 mol/L sodium thiosuplhate was dropped slowly until the solution turn to colourless. Amount of sodium thiosulphate used was recorded and calculated accordingly. Ethanol concentration in % (v/v) were calculated and determined at the end of the experiment.

3. Results and Discussion

3.1. Sample Preparation

Food waste were stored at -80^{o}C overnight before being transferred into freeze dryer. Figure 1 shows the powdered 72 h freeze dried food wastes from three main sources such as potato, rice and white bread. The food wastes were freeze dried to induce dehydration, since presence of water inside the samples may lead to contamination [18]. The samples were ground into powder form to increase the surface area of the samples making it useful for the ezymatic hydrolysis process. Moreover, Vaitheki and Deepa [19] stated that bioethanol yield was higher when using powdered mixed fruit waste.

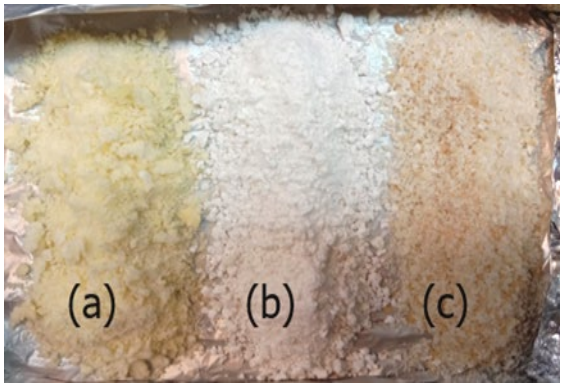

Figure 1. Powdered freeze dried food wastes, a) Potato, b) Rice and c) white bread.

3.2. Cultivation of Saccharomyces cerevisiae

After 48 h of incubation, a single colony was formed on YPD agar medium as shown in Figure 2. The single colony formed was in round shape, smooth, flat, moist and had creamy white colour. Similar result was reported by Elbashiti *et al*. [2] which observed that the colonies of *S. cerevisiae* isolated were slightly convex, white to cream in color, and had smooth creamy consistency. Dash *et al*. [20] also observed that the *S. cerevisiae* cells had a smooth, white and shiny surface.

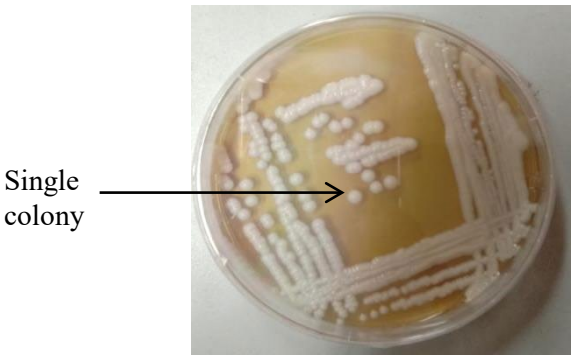

Figure 2. Formation of single colony of *Saccharomyces cerevisiae* on YPD agar

3.3. Immobilization of S. cerevisiae

Exponential phase was determined from growth monitoring of *S. cerevisiae*. The yeast cells reached its exponential phase at 24 h. Hence, when preparing inoculum, the inoculum was left for 24 h at 30^{o}C while shaking at 150 rpm before immobilization with calcium alginate via entrapment method. Figure 3 shows the immobilized beads of *S. cerevisiae* with different sizes. Since the immobilized beads were differ in size; the weight of beads used were kept constant at 5 g to avoid inconsistent result.

Bhadana *et al.* [21] reported that the immobilization of yeast cells helps in improving bioethanol productivity. From the study, immobilized *S. cerevisiae* using calcium alginate able to produce higher amount of bioethanol at 89.68%. According to Waluyo *et al*. [22], immobilized *S. cerevisiae* produced the highest bioethanol concentration compared to free cells at 3.9% and 3.8%. Although the difference is not much significant but immobilized cells displayed faster fermentation process. Previous study of Duarte *et al*. [23] reported that bioethanol concentration using immobilized cells in calcium alginate beads were 32.9 ± 1.7 g/L and 30.7 ± 1.4 g/L for using immobilized cells in chitosan-covered calcium alginate beads. Thus, immobilization of *S. cerevisiae* in calcium alginate produced higher bioethanol yield compared to in chitosan-covered calcium alginate. Study by Mathew *et al.* [24] showed that bioethanol yield was 25% higher by using immobilized cells compared to free cell.

Figure 3. Immobilized beads of *S. cerevisiae*

3.4. Optimization of bioethanol production in fermentation

Bioethanol production was analyzed using titration with potassium dichromate ($K_2Cr_2O_7$) solution. According to Vucurovic *et al*. [17], there are several factors that contribute to incomplete hydrolysis such as temperature, pH and autoclaving time. Therefore, in this study, three parameters; time, temperature and agitation rate were optimized to maximize the bioethanol production in the fermentation process.

3.4.1. Effect of fermentation time

From Figure 4, the highest fermentation efficiency was at 48 h. At 24 h, the fermentation efficiency was 1.19%, 1.24% at 48 h, 1.06% at 72 h and 1.03% at 96 h. Hence, the optimum fermentation time was at 48 h since it produced higher bioethanol yield. Similarly, Ojewumi *et al*. [25] also reported that higher amount of bioethanol was produced at 48 h and the fermentation efficiency were declined after 48 h. Study conducted by Sakharkar [26] observed a higher glucose concentrations at 24 h followed by 48, 72 and 90 h. However, the bioethanol production was the lowest at 24 h. This might happened due to substrate inhibition. Active growth of *S. cerevisiae* contributed to higher bioethanol production as a lot of yeast cells able to produce bioethanol from glucose. At 48 h, the growth of *S. cerevisiae* became slower and soon stagnant. After 48 h, the nutrients content was depleted. According to Han *et al.* [27], bioethanol fermentation process should be done within 50 h because the soluble nutrient pretreated by enzyme was easy to be utilized by the yeast at that time. Availability of nutrients in media will affect the logarithmic growth phase of *S. cerevisiae*. Therefore, lower fermentation efficiency were observed at 72 and 96 h due to lack of nutrients available to support the growth of *S. cerevisiae.* Moreover, increase in accumulation of metabolites and low pH in the fermentation medium is not suitable for the growth of *S. cerevisiae* cells. Thus, the cells are unable to produce bioethanol anymore [28].

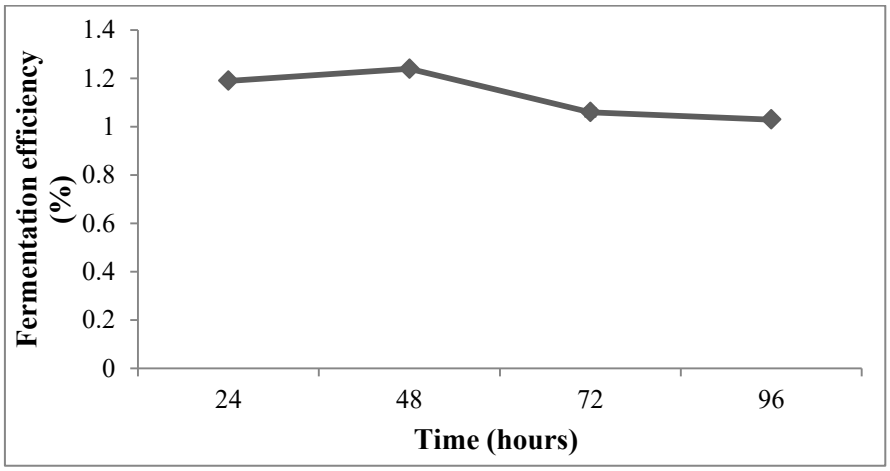

Figure 4. Fermentation efficiency at different time

3.4.2. Effect of temperature

Based on Figure 5, the fermentation efficiency at temperature of 30°C was higher as compared to others. The fermentation efficiency at 20°C, 30°C were 1.07% and 1.24%, respectively. The fermentation efficiency remained the same at 40°C and 50°C with 1.0%. Temperature play a crucial role in producing bioethanol from starchy food waste. Previous study by Ma *et al*. [29] determined that the optimal temperature for bioethanol production from food waste was at 30°C with 64.9 g/L of bioethanol yield. Similarly, Abdulla *et al*. [18] also obtained higher bioethanol yield of 0.514 g/L from papaya peels waste using immobilized *S. cerevisae* at the optimized temperature of 30°C and 48 h of fermentation.

Changes in temperature affected the microorganisms activities by influencing the growth of the yeast cells in the fermentation medium [30]. Temperature also can affect the enzymatic activity and turgidity of yeast cells membrane greatly. It was reported that the performance of *S. cerevisiae* in bioethanol production is reduced at temperatures higher than 37°C [31]. This result is in accordance with the study of Vohra *et al.* [32] in which the fermentation temperature for food waste for bioethanol production is in the range of 30-37°C. This is because the microorganism will becomes more active when warm and dies at high temperature. Liu *et al.* [33] also mentioned that yeast cells cannot survive higher temperatures more than 36°C. Thus, lower bioethanol production was observed at temperature of 40°C and 50°C as the yeast cells experience denaturation at high temperature.

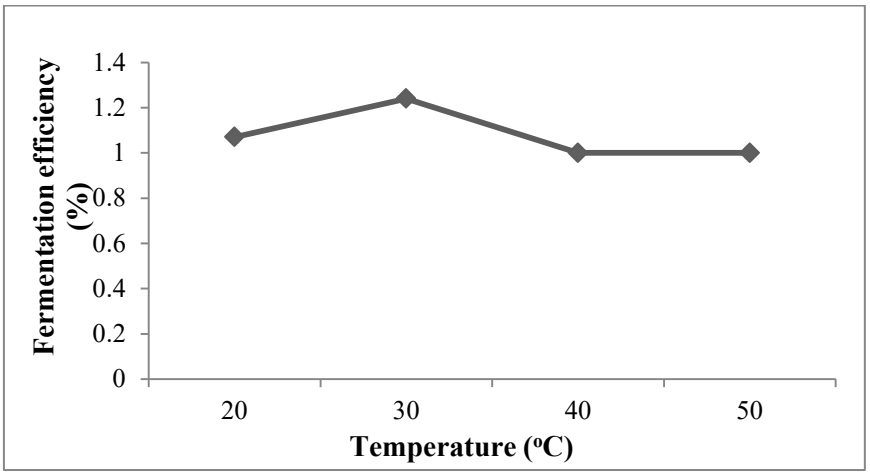

Figure 5. Fermentation efficiency at different temperatures

3.4.3. Effect of agitation rate

Based on Figure 6, the fermentation efficiency was higher at 150 rpm agitation rate with 1.24%. The fermentation efficiency at 100 rpm, 200 rpm, 250 rpm were 1.04%, 0.96% and 0.99% respectively. Agitation rate was able to enhance growth and performance of yeast cells by improving the mass transfer characteristics with respect to substrate, products and oxygen. Moreover, agitation rate helps in better mixing of fermentation broth and maintaining the concentration gradient between inside and outside of yeast cells. For instance, it helps in supplying enough sugars and nutrients to the cells and then removes gases, wastes and other byproducts from the cells.

The importance of agitation is to supply oxygen to the *S. cerevisiae* cells. Study conducted by Rodmui *et al*. [34] reported a maximum bioethanol production at 100 rpm after 16 h while at 150 and 200 rpm, a maximum amount of bioethanol were produced after 14 h. Faster agitation rate contributes to a faster bioethanol production as the yeast cells had enough supply of oxygen. At the end of the fermentation process, cultures that agitated at 150 rpm produced more bioethanol followed by 200 rpm and 100 rpm with 2.93 g/L, 2.69 g/L and 2.45 g/L, respectively.

According to Mad Saad *et al*. [35], higher agitation rate between 100 rpm to 175 rpm contributed to higher growth of yeast cells. Therefore, increase in agitation speed provided a lot amount of oxygen for aerobic fermentation and produced larger cells. Lower fermentation efficiency were observed using agitation rate at 200 rpm and 250 rpm as the immobilized *S. cerevisiae* cells might be damaged due to the aggressive agitation. Increase in agitation rate also causes increase in assimilation of glucose. Further increase in agitation speed caused decline in bioethanol concentration as it may shorten the log phase. Rodmui *et al.* [34] stated that higher dissolved oxygen concentration was beneficial only during cell growth but not in bioethanol production as it decrease bioethanol concentration. This is due to the fact that agitation speed promotes turbulence and shear force in the cultivation process.

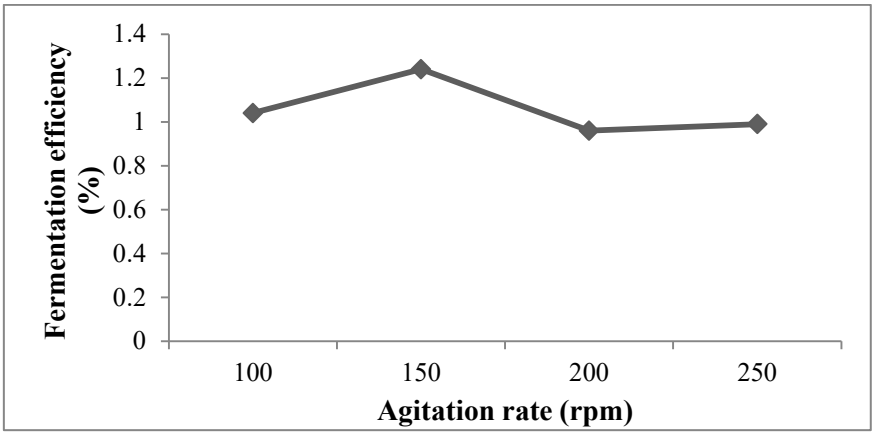

Figure 6. Fermentation efficiency at different agitation rate

4. Conclusion

Starchy food wastes consisting of cooked wastes of potatoes, white bread and rice were used as a feedstock for bioethanol production in this study. To improve the fermentation efficiency on bioethanol production, immobilization was done on yeast *S. cerevisiae* using calcium alginate via entrapment method. Furthermore, parameters of time, temperature and agitation rate were optimized during the fermentation process. From the investigation, the optimal conditions for immobilized *S. cerevisiae* reported were at 30 ^{o}C with an agitation speed of 150 rpm for 48 h. The fermentation efficiency for these optimized parameters were 1.24%. Bioethanol produced from crude oil and fossil fuels resources are non-renewable and limited. Therefore, it is hoped that starchy food wastes can serve as an excellent feedstock for bioethanol production and reduced the formation of unwanted chemicals which cause environmental pollution.

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