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A Study on the Production of Bioethanol from *Portieria hornemannii* Seaweed

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ABSTRACT

Bioethanol production was investigated in red algae Portieria hornemannii which belongs to the family Rhizophyllidaceae. Saccharomyces cerevisiae was cultured on Potato Dextrose Agar (PDA) media that had been sterilized at a temperature of 121°C and at a pressure of 1 atm for 15 min. It was incubated at 30°C for 3 days. Microbes on PDA media as culture stock was regenerated on the Yeast Extract Peptone Dextrose (YPD) media at 30°C for 24 h before mixing with hydrolysate. Reducing sugar was determined by 3,5-dinitrosalicylic acid (DNS) method. The seaweed hydrolysate from 60 g of seaweed gel (15 g of seaweed) yielded alcohol maximum in fermentate using S. cerevisiae as fermentation agent. Fermentation to produce alcohol was carried around 36-168 h. Bread yeast S. cerevisiae was found suitable for the bioethanol fermentation of seaweed hydrolysate, and gave an alcohol content of fermentate after 20-40 h of fermentation at room temperature.

Keywords: *Portieria hornemannii*, Bioethanol, Hydrolysate

INTRODUCTION

Bioethanol is a renewable energy which can be produced from seaweeds which makes the process inexpensive. Seaweeds are the new resource for alternate energy production which is termed as the third generation biofuel resource. For economical production, seaweeds can be aqua cultured in a mass scale and they have the uniqueness of growing at a fast rate yielding high amount of biomass. The current demand for bioethanol outstrips supply and with rising oil prices, improved technologies and tax breaks, the production of bioethanol is becoming increasingly viable. However, demand for land use is increasing and the conversion of land use from food to fuel is a contentious issue. By considering marine biomass as a source of carbon for bioethanol production, these issues are negated, with phaeophyta producing 3.3-11.1 kg m^{-2} $year^{-1}$ dry material [1]. Saccharification of the seaweed *S. japonica* was performed using thermal acid hydrolysis with and without the addition of the isolated marine bacteria *Bacillus sp. SSF* for ethanol production gave a theoretical yield of 33.3% with an ethanol concentration of 7.7 g/L with *Bacillus* sp. Thermal acid hydrolysis gave 14.5 ± 2.1 g/L of reducing sugars with a yield of 21% of the total carbohydrates of *S. japonica* JS-1 for the saccharification and the yeast *Portieria angophorae* for ethanol production [2]. Investigation studies on 20 species of seaweed evaluated, *P. palmata* showed the highest potential for the large- scale production of bioethanol. Acid hydrolysis in 0.4 M sulphuric acid (H_2SO_4) at 125°C for 25 min produced the highest carbohydrate yield. Fermentation of this hydrolysate for 72 h with a 1.5 mg/ml $^{-1}$ inoculation concentration of commercial brewer's yeast (freeze-dried *S. cerevisiae*) at pH 5.25 produced the highest yield of ethanol [3]. The carrageenophyte *K. alvarezii* is now produced in mass scale by aquaculture simply using asexual tissue propagation in tropical areas of Indonesia and Philippines. Indonesia produced at least 1.5 million tons of dry cottonii from 1.1 million hectare of farming area in 2009. The ethanol yield is increased after removing fermentation inhibitors [4] studied that industrial production of ethanol from seaweed would require an optimization of the extraction process, to yield a higher ethanol concentration. The process would be most economical in combination with a total utilization of the seaweed material [5]. Studies on optimal condition for ethanol conversion from Floating Residue (FR) were carried at a relatively lower (0.1% w/v) acid concentration added to FR which improved the enzymatic hydrolysis of cellulose [6]. Seaweeds are also explored to produce biodiesel apart from being considered for bioethanol production. *Sargassum myriocystum* and *Caulerpa peltata* were successfully used as a resource to produce biodiesel and engine and emission tests proved it to be a competent alternate for commercial diesel [7].

EXPERIMENTAL METHODOLOGY

Seaweed was soaked for 2 days with the replacement of water during the soaking process. Salt was removed by immersion process. It was further cleaned using distilled water and then crushed to a powder. The crushed seaweed was dried under the sun for 5-7 days for removal of moisture content. Growth media used was Potato Dextrose Agar (PDA) purchased commercially. *S. cerevisiae* was cultured on PDA media that had been sterilized at a temperature of 121°C and a pressure of 1 atm for 15 min, and then incubated at 30°C for 3 days. Microbes on PDA media as culture stock was regenerated on the Yeast Extract Peptone Dextrose (YPD) media at 30°C for 24 h before mixing with hydrolysate. YPD media was prepared in laboratory with composition of yeast extract 1 g/ml, peptone 2 g/ml and dextrose 2 g/ml to make the solution to 100 ml. Media was sterilized at a temperature of 121°C and a pressure of 1 atm for 15 min.

The detoxification process was conducted in two ways. Overliming was used to eliminate the toxic content on the hydrolysate. Overliming was done by adding $Ca(OH)_2$ and NH_4OH in the hydrolysate to maintain a pH 10. Neutralization of the hydrolysate with $Ca(OH)_2$ and NH_4OH was done to maintain a pH 6.4-6.8. When overliming with $Ca(OH)_2$, insoluble calcium was separated by filtration. The hydrolysate was lowered to a pH of 5.5-6, with the addition of 10% H_2SO_4. Acid hydrolysis was carried out to elaborate a polysaccharide in the seaweed so that it becomes a simple structure. The acid used in hydrolysis was H_2SO_4 with a concentration of 3% through two stages of hydrolysis. The first stage was hydrolysis for 30 min and the second stage was by mixing 15 g crushed seaweed for 30 min. Hydrolysis was carried out using an autoclave at 121°C. Hydrolysate was neutralized to a pH of 5.5-6 and then reducing sugar was determined by 3,5-dinitrosalicylic acid (DNS) method. Fermentation was carried out in 250 ml Erlenmeyer flask with a working volume of 100 ml. Prior to fermentation; pasteurized hydrolysate at 121°C for 15 min was sterilized so that the microbes will not disrupt the fermentation process. Hydrolysates are added with 0.5% urea to enrich the substrate. Microbial fermentation used was *S. cerevisiae*. Temperature of 28-30°C was maintained. Time required for the fermentation process was about 36-168 h. After the fermentation process, analysis was performed to measure pH, reducing sugar, ethanol content. The fermentation broth consists of 1 g of seaweed, 1 g of glucose, 0.05 g of Na_2PH_4, 0.225 g of $(NH_4)_2SO_4$ and 100 ml of distilled water. Figure 1a shows the powdered seaweed after shade drying and grinding to a fine powder using mortar and pestle. Figure 1b shows the cultured yeast by using PDA which was obtained after four days. Figure 2 shows the produced bioethanol after 48 h of time duration.

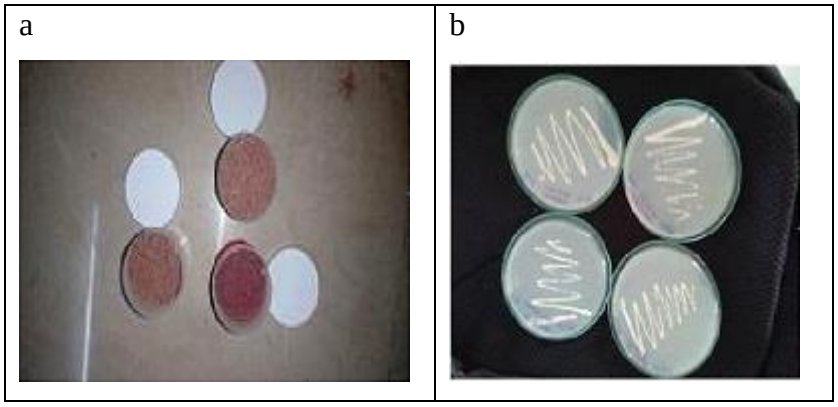

Figure 1: powdered seaweed and cultured yeast

Figure 2: Production of bioethanol

RESULTS AND DISCUSSION

Overliming treatment with $Ca(OH)_2$ reduces the HMF content. Overliming process using $Ca(OH)_2$ and NH_4OH was lower than the neutralization process. HMF has inhibitory effects on the growth of microbes. HMF will interfere with metabolic processes of yeast, so the yield of ethanol produced in fermentation process will not be optimal. The use of $Ca(OH)_2$ in detoxification process not only reduces toxic compound such as HMF but also reducing sugar. During the detoxification process, the seaweed was kept as such for 10 min and it was kept for drying. After drying in sun the seaweed becomes slightly milky, because the $Ca(OH)_2$ is insoluble in water. The process of acid hydrolysis with 3% H_2SO_4 by overlimimg used two types of base as $Ca(OH)_2$ and NH_4OH. Hydrolysis process produced hydrolysate and residual solid after separation process. Type of base will affect the amount of residual solid. The use of $Ca(OH)_2$ resulted in the high amount of residual solid. The addition of 1 M NaCl in the media will reduce the growth rate of *S. cerevisiae* by 70%. The higher the salt content in the hydrolysate there will be disturbance of the growth of microorganisms in the fermentation process. It will result in low yield of ethanol. During the acid hydrolysis process, the acid should be diluted and the pH should be checked in order to avoid damage to the seaweed, which may not lead to the optimal production of bioethanol.

Seaweed hydrolysate (sugar concentration) produced by acid hydrolysis using 3% H_2SO_4 at 100°C for 2 h was used as raw material of fermentation process. The seaweed hydrolysate from 60 g of seaweed gel (15 g of seaweed) yielded alcohol maximal in fermentate using *S. cerevisiae* as fermentation agent. Time for fermentation to produce alcohol was around 36-168 h or about 3-6 days. Fermentation gave significant effect on alcohol content of fermentate. The made up solution is kept in shaker incubator at 130 rpm and at a temperature of 30°C for different period of time. The fermentate residue can be again used for the production of bioethanol.

But it would not be economical because less amount of bioethanol will be produced and the chemical requirement will be the same. Hydrolysed and unhydrolysed solution is processed for fermentation. In hydrolysed, acid hydrolysis of seaweed was used and in unhydrolysed, powdered seaweed was used. Ethanol formation was achieved from 20th h. More amount of ethanol can be produced from hydrolysed process due to maintenance of pH and acid content. In unhydrolysed neither pH nor acid hydrolysis is maintained, so the ethanol formation was less in comparison to hydrolysed. Small amount of solution was taken for centrifugation to measure the DNS of the fermentate. Centrifugation was done to get the clear solution, so that good absorbance values can be achieved. The hydrolysed and unhydrolysed solution was kept in water bath for 15 min and absorbance values were obtained. Glucose content was further inferred through colorimeter. The temperature in the water bath was maintained at 100°C for 10 min.

Bioethanol produced was separated by using filter medium (filter cloth, filter paper etc.), till clear solution is obtained. It is performed in laminar flow to prevent any contamination. Small amount of solution is taken for centrifugation to measure the DNS of the fermentate. The hydrolysed and unhydrolysed solution was kept in water bath for 15 min and absorbance values were measured. From hydrolysed and unhydrolysed graph, we can infer that the hydrolysed process is more appropriate than that of unhydrolysed one. Because in hydrolysis process for detoxification of hydrolysate, pH is maintained and acid wash is done to remove the toxic content of the sample and contamination can be prevented in comparison to unhydrolysed process. Figure 3 shows the high liquid performance chromatography of produced bioethanol.

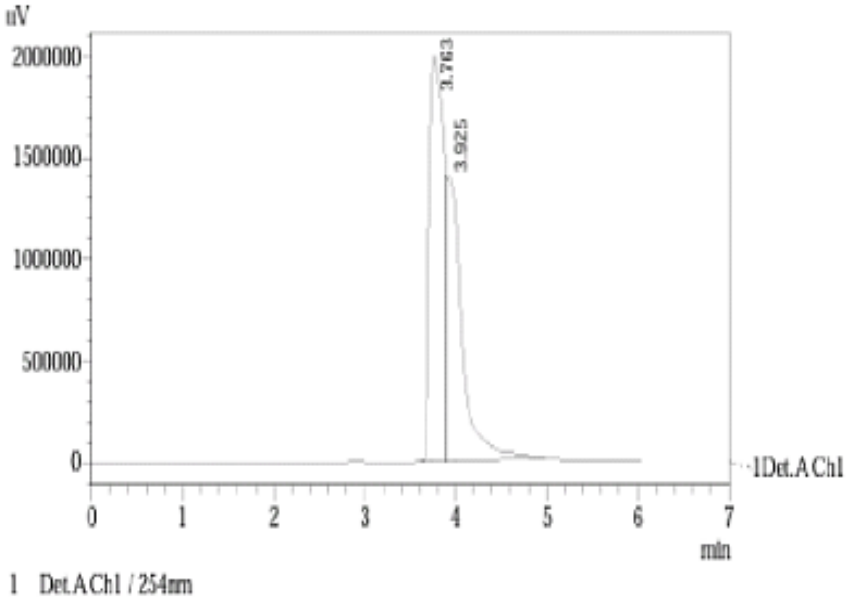

Figure 3: HPLC of bioethanol

CONCLUSION

Acid hydrolysis for 2 h using H_2SO_4 of 3% at 121°C of seaweed gel derived from 60 g of seaweed *Portieria hornemonnii* yielded sugar content in hydrolysate. Bread yeast *S. cerevisiae* was found suitable for the bioethanol fermentation of seaweed hydrolysate, and gave an alcohol content of fermentate after 20-40 h of fermentation at room temperature.

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