

Quantitative Assessment of Seagrass as Bioethanol Feedstock

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ABSTRACT.

The depletion of fossil fuels and the increase of fuel demand lead to the search of more sustainable alternatives. Nowadays, bioethanol is gaining popularity as renewable fuel to replace existing fossil gasoline. Currently, bioethanol is produced from land based crops but in the future, marine biomass such as seagrass and seaweeds are promising alternatives since these do not take up land area for cultivation. In this paper, seagrass, *Thalassia hemprichii* was tested for its potential as bioethanol feedstock via fermentation by yeast, *Saccharomyces cerevisiae*. *Thalassia hemprichii* is highly abundant as it can be easily cultivated in warm seawater in Malaysia for example in Sabah. *Thalassia hemprichii* contains high carbohydrate content, hemicellulose and cellulose which will be hydrolyzed to glucose and other reducing sugars, which in turn is converted to ethanol by yeast. It has been shown that the extracted leaves from *Thalassia sp.* through hot water treatment gives higher concentration of sugar (1.68g/ml) as compared to acid hydrolysis using dilute sulfuric acid (1.38g/ml). Besides that, among the five different inoculum concentrations, it was found that 10% (v/v) concentration of inoculum gives the highest bioethanol production for both types of treatments. Ethanol produced with hot water treatment (2.29g/ml) was higher as compared to sulfuric acid hydrolysis(1.74g/ml). The results from this study showed that *Thalassia hemprichii* has potential to be used as substrate for bioethanol production.

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Introduction

Choice of a suitable species as a substrate is one of key elements for effective ethanol production. Korean research groups were focused on the utilization of red algae, agar weeds and green algae as a substrate. Other than that, the most extensive studies that have been done by researchers throughout the world are seaweed and microalgae (Uchida *et al.*, 2014). Food versus fuel was the problem faced by first generation bioethanol. In second generation, the problem was insufficient supply of biomass to fulfill the demand of bioethanol (Brigs, 2004). Therefore, the third generation is proposed to solve those problems stated where the feedstocks being used are renewable, easily available, cheaper and has sufficient area for its cultivation (Brown, 2006). This is because the third generation mainly focuses on unexploited sea resources including seagrass which is recently gaining attention from biofuel researchers.

Seagrass belong to the subclass Monocotyledoneae within the class Angiospermae. It is considered as true vascular plants. Seagrasses are structurally and functionally similar to terrestrial grasses. They are differentiated into discrete morphological entities such their leaves, stems, roots and reproductive structures. Since they are submerged for either part or all of their life cycle, they have mostly adopted underwater pollination. . So far there are nine seagrass species have been recorded along the coast of Sabah and the study area showed that the most abundant were *Thalassia hemprichii* and *Enhalus acoroides* (Ho *et al.*, 2011).

Based on literature, cell wall of seagrasses contains lignin, cellulose and hemicellulose. Most of the hemicellulose present in the cell wall of the seagrass (1 to 16%) while 37 to 40% hemicellulose are present in the cell wall of the terrestrial grasses. Lastly, lignin is about 0.2 to 5% in seagrass compared to terrestrial monocots (6 to 10%) (Makey *et al.*, 1996). Since the use of seagrass feedstock for ethanol production is still in its pioneer stage, it is important to determine the optimized conditions for seagrass processing in order to enhance the bioethanol utilization in Malaysia (Ismail, 1993).

Materials and methods

Seagrass preparation

The most abundant species of seagrass namely *Thalassia hemprichii* and *Enhalus acoroides* were collected during low tide from Outdoor Development Centre at University Malaysia Sabah. The collected seagrass were washed thoroughly with tap water. The fresh leaves were weighed accurately and washed with 50% (v/v) of ethanol to eliminate low molecular weight sugars. This was followed by addition of 99.5% (v/v) of ethanol, 10% (w/v) of sodium hydroxide and distilled water. The leaves were heated for around 3 minutes in boiling water followed by addition of hydrochloric acid for neutralization. The fresh seagrass then was blended and stored in a blue cap bottle inside refrigerator.

Screening of the two species of seagrass for higher sugar content

The sample was brought to room temperature and neutralized with 25% hydrochloric acid. Sugar composition was measured as follows: 10.0 g of grind leaves were weighed and incubated with 2 ml of 72% (w/w) sulfuric acid for an hour at room temperature, then diluted with 56 ml of distilled water and autoclaved for 1 h at 121°C. The mixture was neutralized with 30% (w/w) sodium hydroxide. Then, the volume was adjusted to 100 ml with distilled water. This was followed by the sonication of the sample. The quantitative analysis of sugar compounds was performed using a HPLC Perkin Elmer Series 200. The species which give the highest glucose content was selected as the best feedstock for bioethanol. According to Ravikumar *et al.* (2011), drying process of the seagrass are not required since it was proven that semi decayed leaves give minimum concentration of sugar compared to fresh leaves that gave higher concentration of glucose.

Acid hydrolysis

5g of the sample was incubated for 2h with different concentrations (0.1%, 0.6%, 1.1%, 1.6% and 2.1%) of 100ml sulfuric acid at 50°C. Later, the samples were sonicated with ultrasonic bath and was

filtered with 0.45 μm Durapore (PVDF) syringe. The samples were analyzed for reducing sugars using HPLC.

Hot water treatment

5g of slurry was heated in a water bath for a period of 2 h at different temperatures of 60°C, 70°C, 80°C, 90°C and 100°C. Similarly, the samples were sonicated with ultrasonic bath and was filtered with 0.45 μm Durapore (PVDF) syringe. Again, the samples were analyzed for reducing sugars using HPLC.

Fermentation process

50 ml of yeast culture (5- 25 % (v/v)) were added into the slurry obtained from acid hydrolysis treatment. The flasks were tightly sealed for anaerobic fermentation. The media were placed in orbital shaker at 30°C and 200rpm for 72 h.

Results

Results for the screening of seagrass species is shown in Figure 1. It shows that *Thalassia sp.* has the highest concentration of glucose compared to *Enhalus sp.* by 65 % using 20 g/L as a response factor. The concentration of glucose that obtained from the *Thalassia sp.* was 3.266 g/ml while for the *Enhalus sp.* was 1.734 g/ml. The lowest concentration of glucose extracted for both species at 5 g/ml where for the *Thalassia sp.* was 1.46 g/ml while for *Enhalus sp.* 0.78 g/ml. Based on the result shown, *Thalassia sp.* was chosen as the seagrass feedstock for bioethanol production since this species showed highest glucose extracted compared to *Enhalus sp.*

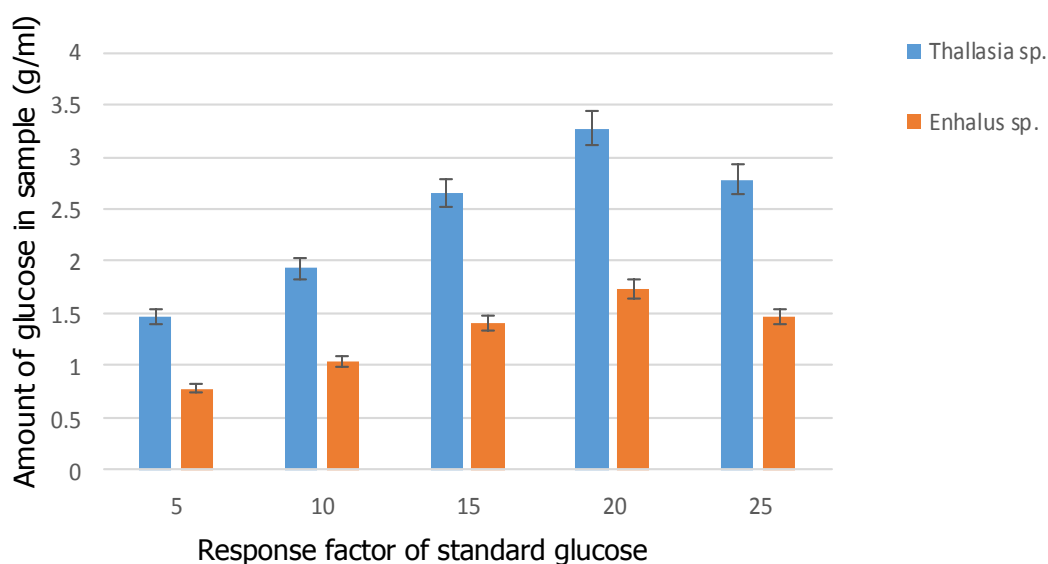


Figure 1. Glucose content from *Thalassia sp.* and *Enhalus sp.*

Figure 2, shows that the amount of ethanol produced from acid hydrolysis treatment with different concentrations of inoculum in fermentation process. The highest amount of ethanol produced from the sample was in 10% (v/v) inoculum percentage which gave 1.74g/ml while the lowest amount of ethanol produced from the sample was at 25% (v/v) which gave 1.13g/ml. Therefore, 10% (v/v) was chosen as the best inoculum concentration that gives high bioethanol production for acid hydrolysis sample.

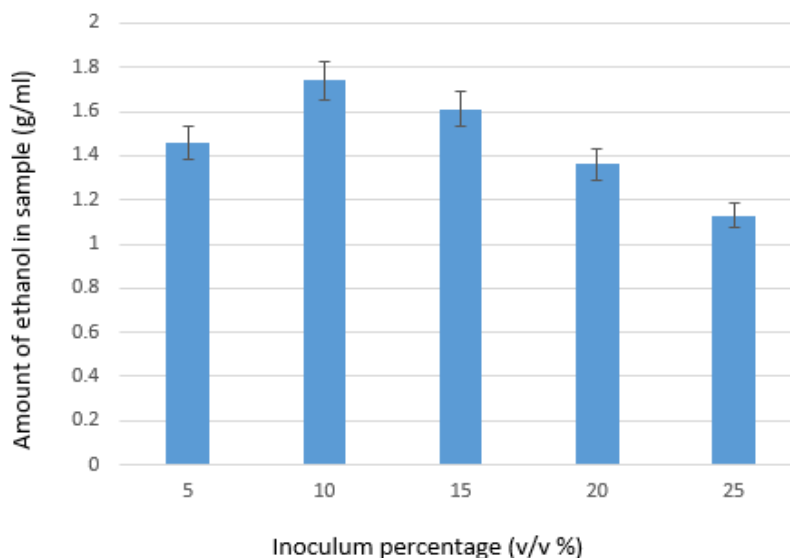


Figure 2. Effect of acid hydrolysis on bioethanol yield

Figure 3 shows the amount of ethanol produced from acid hydrolysis treatment with different concentration of inoculum in fermentation process. Its clear that the highest amount of ethanol produced from the sample was in 10% (v/v) inoculum percentage which gave 2.29g/ml while the lowest amount of ethanol produced from the sample was at 25% (v/v) which gave 1.23g/ml. Therefore, 10% (v/v) was chosen as the best inoculum concentration that gives high bioethanol production for hot water treatment sample.

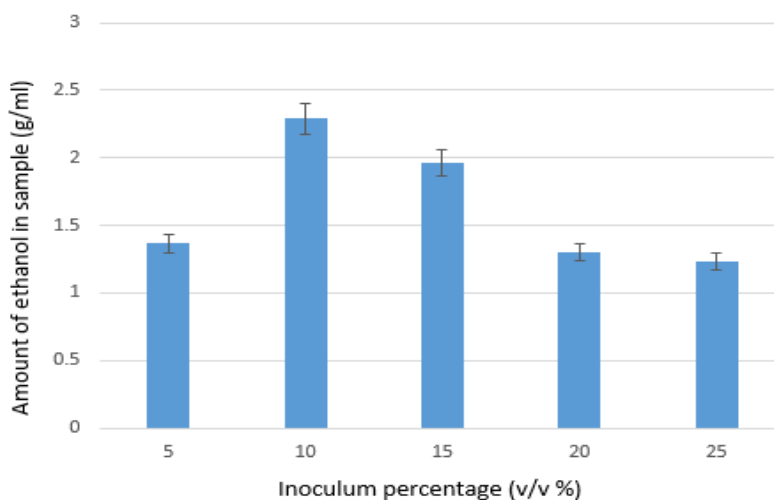


Figure 3. Effect of hot water treatment on bioethanol yield

Comparison of hot water treatment and acid hydrolysis are shown in Figure 4. A 10% (v/v) of inoculum from hot water treatment gave 2.29g/ml while another 10% (v/v) of inoculum percentage from acid hydrolysis treatment was only 1.74g/ml of ethanol. Therefore, hot water treatment was chosen as the best pretreatment that gives high yield of bioethanol.

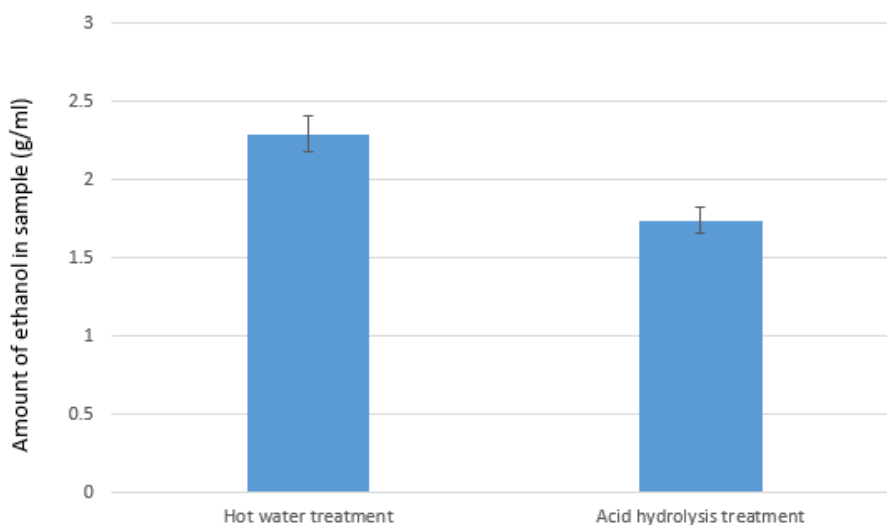


Figure 4. Comparison of effect of hot water and acid hydrolysis treatment on bioethanol yield

Discussion and Conclusion

In this study, seagrass, *Thalassia hemprichii* showed its feasibility and suitability to be used as substrate for bioethanol production via fermentation by yeast *Saccharomyces cerevisiae*. *Thalassia hemprichii* contains higher reducing sugar (3.26g/ml) as compared to *Enhalus acoroides* (1.74g/ml). This is due to lower lignin content in *Thalassia hemprichii* than *Enhalus acoroides*. Moreover the technique of hot water treatment produced higher ethanol content as compared to its acid hydrolysis treatment. So far there is no reported work on bioethanol production from this species of seagrass. Ravikumar *et al.* (2011) showed that the fresh leaves of seagrass gave higher yield of bioethanol as compared to the dried ones. A bioethanol yield of 16.5%v/v was reported by Uchida et al (2014) from the seeds of the seagrass, *Zostera marina*. Thus seagrass holds immense potential as a promising feedstock for bioethanol.

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