

Production of biofuel ethanol from pretreated seagrass by using *Saccharomyces cerevisiae*

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Abstract

The present study was aimed to produce the bioethanol from seagrass biowastes using *Saccharomyces cerevisiae*. It reveals that, the maximum production of ethanol (0.047 ml.g⁻¹) was recorded from the fresh seagrass leaves in acid pre treatment. Hence, the fresh seagrass leaves could be used one of the suitable substrates for the production of bioethanol.

Keywords: Bioethanol; Fermentation; *Saccharomyces cerevisiae*; Seagrass.

Introduction

Biofuel refers to liquid or gaseous fuels mainly for the transport sector that are predominantly produced from the lignocellulosic materials. Generally, biofuels offer many benefits that include sustainability, reduction of greenhouse gas emissions, engine compression ratio and also reduce the environmental pollution etc., (Reijnders, 2006). A variety of liquid fuels such as bioethanol, methanol, biodiesel and gaseous fuels such as hydrogen and methane can be produced from various biomass resources such as wheat, sugar beet, corn, paddy straw and wood etc. Recent decade, bioethanol is a best alternative fuel to replace the current fossil fuel. The combustion of fossil fuels is responsible for 73% of the CO₂ production which leads to global warming (Wildenborg & Lokhorst, 2005).

The seagrasses are marine flowering plants which are used as raw materials in paper industry and biofertilizer for coastal plants. In general, microbes especially yeast (*Saccharomyces cerevisiae*) and bacteria (*Zymomonas mobilis*) plays vital role in the production of bioethanol. However, studies related with seagrass biowastes on ethanol production are too limited. Hence, the present study was made an attempt to find out the possibility of sustainable utilization of seagrass wastes by using *Saccharomyces cerevisiae*.

Materials and Methods

Collection and preparation of substrates

The fresh and semidecayed leaves of *Cymodocea serrulata* seagrass were collected along the Thondi coast, (Lat. 9°44'N and Lon. 79° 10'E) Ramanathapuram district, Tamilnadu, India. The collected seagrasses were washed with tap water thrice and once by distilled water to remove the adhering soil crystals and other gravel particles. Then the substrates were chopped into small pieces. After that, the materials were sun dried for four days to remove the moisture content.

Fermentation medium

About 100 ml of fermentation medium(g.l⁻¹) [glucose-10; yeast extract-0.1, potassium hydrogen phosphate-0.5, magnesium sulphate-0.1] was prepared and it was autoclaved at 121°C for 15 minutes. The pH of the medium is 4.5 with 1N NaOH. The flasks were allowed for cool at room temperature. Then *Saccharomyces cerevisiae* obtained from local confectionery were inoculated (10⁸ cells/ml) aseptically into conical flask and incubated at 28°C for 24 hrs.

Acid hydrolysis

Two hundred grams of dried seagrass leaves were weighed and 250 ml of oxalic acid was sprayed on to the samples and mix thoroughly. This slurry was steaming for 20 minutes. The exploded materials both fresh and semidecayed leaves were extracted with 300 ml of hot water (65°C) by using thermostat waterbath and filtered by using Whatman filter paper No. 1 (pore size 11 µm). The pH of the extract was adjusted to 5.3±0.2 with 10N NaOH. Then the extracted samples were kept for sterilization and cooled. Then the flasks were mixed with fermentation medium and incubated for 72 hrs in room temperature.

Steam explosion

Two hundred grams of dried seagrass was weighed and mixed with 200 ml distilled water and minced thoroughly. The pH of the minced samples were adjusted to 5.5 with the addition of 10N NaOH and 3N HCl. The slurry was sterilized and cooled. After that, the samples were mixed with fermentation medium and incubated for 72 hrs in room temperature.

Ethanol estimation

The ethanol produced from the fermentation medium was measured by standard procedure as described by Hormitz (1980). About 1 ml of the fermented broth was

mixed with 25 ml of water and distilled by using rotary flash evaporator (Superfit, India). From this 1.5 ml of distillate was taken and 2.5 ml of potassium dichromate solution was added and made up to 5 ml with distilled water. This mixture was incubated at 60°C for 30 minutes. The optical density was measured at 600 nm after the reaction is completed. The amount of ethanol was calculated by using the following formula. Volume of ethanol (ml.g^{-1}) = Std. OD value \times OD value of sample/Weight of the sample. Standard OD value was calculated with 100% of purified ethanol (AR Grade).

Total sugar Estimation

The content of total sugar was also measured in the fermentation medium before and after fermentation process as described by Dubios *et al.* (1956). Briefly, 1ml of prepared substrate sample was taken in test tube. To this 1 ml of 5% phenol and 5 ml of concentrated sulphuric acid was added. This mixture was incubated at 29°C for 15 minutes to develop the color. The optical density was measured at 490 nm. The content of sugar was determined by using D-Glucose as a standard.

$$\text{Total sugar (mg.g}^{-1}\text{)} = \frac{\text{Std. OD value} \times \text{OD value of sample}}{\text{Total volume} \times \text{Weight of the sample} \times \text{Volume of sample taken for estimation}}$$

Results

The results of the present study reveal that, the production of ethanol was found maximum in fresh seagrass leaves than the semidecayed leaves (Fig. 1 & 2). In the pretreatment analysis reveals that, the acid hydrolysis showed maximum production of ethanol than the steam explosion. In acid treatment reveals that, the content of ethanol was found maximum (0.047 ml.g^{-1}) in the fresh leaves and found minimum (0.033 ml.g^{-1}) in the semidecayed leaves. In the case of steam explosion, the content of ethanol was found maximum (0.0008 ml.g^{-1}) in fresh leaves and found minimum (0.0004 ml.g^{-1}) in the semidecayed leaves.

After the treatments the content of total sugar was also estimated. The acid treatment reveals that, the content of total sugar was found maximum ($0.071 \mu\text{g.g}^{-1}$) in the fresh seagrass leaves and found minimum ($0.045 \mu\text{g.g}^{-1}$) in the semidecayed leaves. In the case of steam explosion the content of total sugar was found maximum ($0.014 \mu\text{g.g}^{-1}$) in fresh leaves and found minimum ($0.0089 \mu\text{g.g}^{-1}$) in the semidecayed leaves.

Discussion

Biofuels have been derived from the renewable resources which can readily displace petroleum fuels and low emission of CO_2 . Moreover, during the last decade the cost of oils and other transporting energy materials are too high. So, there is need to develop the alternative

energy from the biological sources like plants and its wastes. Several authors already reported that, the ethanol is produced from different fermentable substrate such as cellulosic waste materials (Karaki, 1974; McCann & Prince, 1978), waste potatoes (Tewari *et al.*, 1982), fruit waste (Cooper, 1976; Bartholomew, 1979; Hang *et al.*, 1981; Pontiveros *et al.*, 1978; Sendlewski, 1980).

In the present study, the ethanol production was observed maximum in the fresh seagrass leaves than the semidecayed leaves. This might be due to the presence of unutilized sugars present in the fresh leaves compared to semidecayed leaves. Earlier findings reveal that, the ethanol production was recorded in eel grass (Viola *et al.*, 2008) and switchgrass (Keshwani & Cheng, 2009). Among the seaweeds species, *Laminaria hyperborean* has reported to be the suitable substrate for the ethanol production (Horn *et al.*, 2000).

The present study also noticed that, the acid treatment showed maximum production of ethanol than the steam explosion. In the acid treatment, it removes 100% of

Fig. 1. Content of ethanol (ml.g^{-1}) and total sugars ($\mu\text{g.g}^{-1}$) in fresh seagrass leaves after pretreatment

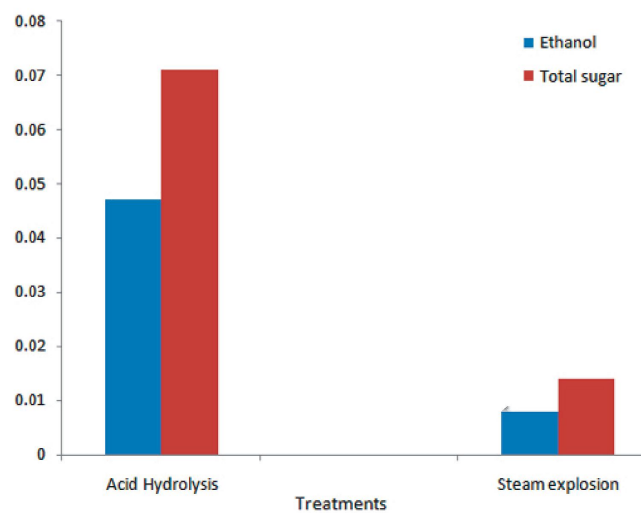
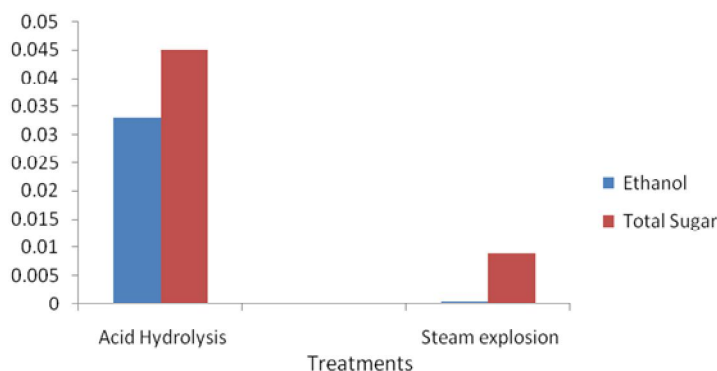


Fig. 2. Content of ethanol (ml.g^{-1}) and total sugars ($\mu\text{g.g}^{-1}$) in semidecayed seagrass leaves after pretreatment



hemicellulosic compounds from the substrates and this could be increase the production of ethanol (Wu & Lee, 1997). The steam explosion is one of the treatments which induce the auto hydrolysis and defibration (Kaar *et al.*, 1988). Previous reports revealed that, the ethanol production was found maximum in steam exploded olive wastes (Cara *et al.*, 2008). The efficiency of steam explosion depends mainly on the temperature, residence time, particle size and moisture.

Conclusion

It is concluded from the present study that, the bioethanol production is higher in the fresh seagrass leaves as substrate than the semidecayed leaves.

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