6.0. ASSESSMENT OF BIOFUEL ETHANOL PRODUCTION FROM SEAGRASS BY CONTINUOUS FERMENTATION

6.1. Introduction

Due to the increasing amount of greenhouse gases to the atmosphere causes global warming. About 73% of the CO₂ is liberated due to fossil fuel. So, there is an urgent need for the alternative energy to solve the above problems. Recently, bioethanol, biodiesel and biogas have been used worldwide. Among them, bioethanol is the leading alternative energy derived from the various lignocelluloses substrates such as vegetable wastes, fruit peels, wheat straw, sugar beet, corn, paddy straw, wood and agricultural wastes. Moreover, they are the rich source of hexoses and pentoses which used for the chemical and food productivity (Kuhad and Singh, 1993; Kuhad et al., 1997; Herrera, 2004).

The hydrolyzing of cellulose and hemicellulose fractions can be fermented by several microbes particularly bacteria and yeast (Chandel et al., 2007; Olsson and Hahn-Hagedal, 1996). However, details related to biowastes from marine resources are too limited. In view of this, the seagrass ecosystem plays an important role in the nutrient cycling and nitrogen fixation of the inshore coastal areas. This can be used as the raw materials in paper industry and in the production of fertilizer, fodder and
feed. Most of the seagrass are used extensively as biofertilizer for coconut and other plantations. A variety of medicines and chemicals are also prepared from them. Large amount of seagrass has been deposited in the Palk Strait region when compared with the other coastal regions of Tamil Nadu. The deposited seagrass on the shore produce unpleasant odour along the coast which may create an anoxic condition to the marine organisms. Keeping this view in mind, the present study made an attempt to find out the possible bioethanol production from seagrass wastes by using marine microbes and thus it also helps to clean-up the coastal areas.

6.2. Materials and Methods

6.2.1. Collection site description

Fig. 6.1. Map showing the study area
Thondi is a small village situated in the Palk Strait region of Tamil Nadu. The study area lies in the latitude of 9° 44’ 12” N and longitude of 79° 10’ 14” E. The rainfalls in Thondi region are mainly due to North East and South West monsoon. This coast has a very minimal wave action. Turbidity of the seawater is moderately low and also rich in nutrients. Hence, it serves as a treasure house for valuable marine resources like seagrass, seaweeds and invertebrates like coelenterates, echinoderms and shell fishes. The major occupation of the people is fishing. This area is also considered to be a one of the good source of seagrass diversity in Indian coast (Fig. 6.1).

6.2.2. Collection of seagrass

The seagrass was collected from Thondi coast along Palk Strait region, Tamil Nadu, India. The collected seagrass were carefully washed with ambient seawater and thrice with tap water to remove the adhering soil particles and associate animals. Then it was separated based on the morphological characters. Further, it was dried at 60°C for 24 hrs for further use.
6.2.3. Characteristics of seagrass

6.2.3.1. Taxonomical position of seagrass

6.2.3.1.1. Cymodocea serrulata

Kingdom : Plantae
Class : Lilopsida
Order : Alismatales
Family : Potomoceracea
Genus : Cymodoce
Species : serrulata

Identification features of Cymodocea serrulata

Habit  Male and Female plants are separate, perennial; plant with creeping rhizome, rhizome with scales and scars; shoot erect, with 2-6 leaves, shoot covered by old decayed leaves.

Habitat  Purely a marine, not seen in backwaters or estuaries; plants grow in shallow water areas up to 1m depth on fine to coarse sand with mud.

Leaves  2-5, in each branch; leaf sheaths broadly triangular

Inflorescence  Flowers solitary, terminal and become lateral in due course due to the production of successive lateral shoots.

Perianth  No organized sepals.

Ovary  Globose

Fruits  2, ellipsoid, 4-angular, rarely an immature fruit is seen on each developed fruit.
6.2.3.1.2. *Syringodium isoetifolium*

Kingdom : Plantae
Class : Liliopsida
Order : Hydrocharitales
Family : Potomogetonaceae
Genus : *Syringodium*
Species : *isoetifolium*

**Identification features of *Syringodium isoetifolium***

**Habit**
Herbaceous plant; rhizomes creeping, shoots erect, branched, bearing 2-3 leaves; rhizomes and shoots having scars; rhizomes produce branched roots at each node.

**Habitat**
It generally grows well on coral flats, but also grows on sandy to muddy bottoms. It is not seen and pointed at the apex.

**Leaves**
Leaf tubular, narrowed at base and pointed at the apex.

**Inflorescence**
Flowers in terminal cymes, growing upto 29 cm long.

**Perianth**
Aries are protected by reduced leaves; no organized sepals

**Ovary**
2, ovoid, 1style with bifid stigma

**Fruits**
Ellipsoid with hard pericarp.
6.2.3.1.3. *Halophila ovalis*

**Kingdom**: Plantae

**Class**: Liliopsida

**Order**: Hydrocharitales

**Family**: Hydrocharitaceae

**Genus**: *Halophila*

**Species**: *ovalis*

**Identification features of *Halophila ovalis***

**Habit**
Plant shows morphological diversity due to habitat variations; separate male and female plants with branched, creeping, slender rhizomes; root single with root hairs, at each node of the rhizome.

**Habitat**
Plants occur in both purely marine environs and in backwater areas. Marine forms grow on coarse sands in the sea and on the muddy substratum in tidal and subtidal zones. The backwater forms grow along the shallow margins of estuaries and mangrove creeks where the substratum may vary from black mud to clay.

**Leaves**
Paired at each node with long petiole; a leaf with petiole may measure upto 4 – 12 cm

**Inflorescence**
Flowers solitary, auxiliary, covered by two spathes.

**Perianth**
Tepals 3, broad elliptic.

**Ovary**
Ellipsoid in shape, 1-celled with pointed apex

**Fruit**
Ovoid to ellipsoid with 18-27 seeds

**Seeds**
Globose, white in colour when young but brown when mature.
6.2.3.1.4. *Halodule pinifolia*

Kingdom : Plantae
Class : Liliopsida
Order : Najadales
Family : Cymodoceaceae
Genus : *Halodule*
Species : *pinifolia*

**Identification features of *Halodule pinifolia***

**Habit** Male and female plants are separate; rhizomes slender and branched, roots creeping, formed at each node, branched.

**Habitat** It generally grows on sandy to muddy soils along the coasts, mangrove creeks, coral platforms etc.

**Leaves** Measure about 1-6 cm long and 1-5 mm width, linear with entire margin and with 3 prominent midribs, lateral ribs form lateral teeth on leaf apex

**Inflorescence** Two flowers enclosed by leaf sheaths.

**Tepals** Not well organized.

**Ovary** Ovoid with filiform terminal styles and becomes sub terminal or lateral in fruits.

**Fruit** Globose, seen in pairs with persistent lateral styles; seed coat hard with ornamentations.
6.2.3.2. Biochemical composition of seagrass

6.2.3.2.1. Moisture

Moisture content of the substance was calculated by the NREL procedure described by Sluiter (2005a).

6.2.3.2.2. Ash

Ash content of the substance was calculated by the NREL procedure described by Sluiter (2005b).

6.2.3.2.3. Estimation of cellulose, hemicellulose and lignin

The cellulose, hemicellulose and lignin were estimated by a method developed by Goering and Van soest (1975) as mentioned below.

6.2.3.2.4. Neutral detergent fibre (NDF)

(i) Preparation of neutral detergent solution

About 200 ml of distilled water was taken and mixed with 18.61 g of sodium borate decahydrate was dissolved by heating. To this, 30 g of sodium lauryl sulphate and 10 ml of 2-ethoxy ethanol were added. Finally, 4.5 g of disodium hydrogen phosphate was also added. The final volume was made up to one litre and pH was adjusted to 7.0.
(ii) Procedure

About 0.5 g of delignified samples was taken in a refluxing flask. To this, add 100 ml of cold neutral detergent solution, 2 ml of decahydronaphthalene (Decalin) and 0.5 g of sodium sulphite were added. The mixture was boiling and the heat was reduced to avoid foaming and refluxed for 1 h. After cooling, the sample was filtered through a previously weighed gooch crucible of G-1 grade suction using a vacuum pump. The residue remained in the gooch crucible was washed with hot water repeatedly. Finally, the residue was washed two times with acetone and the washed residue was dried at 100°C for 8 h in a hot air oven. Then it was cooled with desiccators and the dry weight was recorded.

\[
\% \text{ NDF} = \frac{Y - X}{W} \times 100
\]

Where,

- Y-Weight of crucible+NDF
- X-Weight of empty crucible
- W-Weight of the sample

6.2.3.2.5. Acid detergent fibre (ADF)

(i) Preparation of acid detergent solution

About 1 litre of 1N sulphuric acid, 20 g of cetyl trimethyl ammonium bromide was dissolved.
(ii) Preparation of 72% sulphuric acid (H\textsubscript{2}SO\textsubscript{4})

About 72 ml of concentrated sulphuric acid (98% pure) was added to a beaker containing distilled water of 26.5 ml.

(iii) Procedure

About 0.5 g of sample was taken in a refluxing flask. To this, 100 ml of acid detergent solution and 2 ml of decahydronaphthalene were added. The mixture was heated to boiling and the heat was reduced to avoid foaming and refluxed for one hour. After that, the mixture was cooled and filtered through a previously weighed gooch crucible of G-1 grade under suction using a vacuum pump. The sample was washed with hot water to remove the acid followed by washings two times with acetone. The crucibles were dried at 100°C for 8 h. Finally, the crucibles were cooled and the dry weight was recorded.

\[
\% \text{ ADF} = \frac{Y - X}{W} \times 100
\]

Where,

Y-Weight of crucible+ADF
X-Weight of empty crucible
W-Weight of the sample
6.2.3.2.6. Estimation of lignin

(i) Procedure

Crucible containing ADF (acid detergent fibre) were placed in 50 ml beaker and the crucibles were covered with cooled 72% H$_2$SO$_4$. The contents were stirred with a glass rod to break lumps of residue if any. As the acid drain away the crucibles were filled half way with acid and frequent stirring was done. After, 3 h of intermittent stirring, the contents were filtered off under suction to retain the residue and to remove acid using hot water. Then the crucibles with residues were dried at 100° C for 8 h. after this, the crucibles were cooled in a desiccator and weighed (L). After weighing, the contents in crucibles were kept inside a muffle furnace for ashing at 500° C for 2 h. After the furnace temperature came down, the crucibles were taken out, cooled partially in air, then in a desiccator and the weight (A) of the ash was recorded.

\[
\%\text{Hemicellulose} = \%\text{NDF} - \%\text{ADF} \\
\%\text{Cellulose} = \frac{Y - L}{W} \times 100 \\
\%\text{Lignin} = \frac{L - A}{W} \times 100
\]

Where,

- \( Y \) - Weight of ADF + crucible
- \( L \) - Weight of crucible + lignin
- \( A \) - Weight of crucibles + ash
- \( W \) - Weight of the sample
6.2.4. Pretreatment

The dried seagrass were grinded individually with electrical mixer (Preethi pvt ltd, India) to attain a particle size between 2 to 10 mm for further use. Each ground samples were hydrolyzed with 1% HNO₃ separately at 121°C for 40 min at a solid liquid ratio of 1:30. After that, the hydrolysate was filtered through double layered muslin cloth (hemicellulosic fraction) and further used for the detoxification process.

6.2.5. Detoxification

The filtered hydrolysate was detoxified with Ca(OH)₂ to enhance the fermentation. The pH of the hydrolysate was increased up to 10.8 with regular stirring for 1 h at room temperature. After filtration with Whatman No.1 filter paper, the pH of the hydrolysate was adjusted to 5.5 with 1N HCl for the fermentation process.

6.2.6. Characterization of pretreated biomass

6.2.6.1. Fourier Transform Infrared spectroscopy analysis (FT-IR)

The properties of the untreated and treated samples were also characterized by FT-IR (SCHIMADZU, Japan). The analyses were run using the KBr pellet technique. The KBr pellets of samples were prepared by mixing 1.5–2.00 mg of samples, finely grounded, with 200 mg KBr (FT-IR grade) in a vibratory ball mixer for 20s. The 13 mm KBr pellets were
prepared under vacuum in a standard device under a pressure of 75 kN cm\(^{-2}\) for 3 min. The spectral resolution was 4 cm\(^{-1}\) and the scanning range was from 400 to 4000 cm\(^{-1}\).

6.2.6.2. X-Ray diffraction analysis (XRD)

The crystallinity of the untreated and pretreated samples was examined by using XPERT-PRO diffractometer system. The monochromatic CuK\(\alpha\) radiation \((\lambda = 1.5406 \text{ nm})\) generated at 40Kv and 30 mA with a divergence slit size of 0.5° were used. The continuous scan was conducted with the scattering angle \(2\theta = 10° - 80°\) and the step size was 0.05°. Each step has 10 seconds which consider for the accumulation time. The crystallinity index was calculated as described by Buschle-Diller and Zeronian (1992).

\[
CI = \frac{I_{max} - I_{min}}{I_{max}}
\]

Where the \(I_{max}\) is the maximum intensity (\(I_{002}\)) and \(I_{min}\) is the minimum intensity (\(I_{101}\)).

6.2.6.3. Thermogravimetric analysis and Differential thermal analysis (TGA-DTA)

Thermogravimetric analysis (TGA) and Differential thermal analysis (DTA) were performed on the untreated and pretreated samples with the thermal analyzer (EXSTAR 6000, TG/DTA) under air atmosphere. Samples were heated from the room temperature to 900°C at a rate of 10°C/minute.
6.2.6.4. Scanning electron microscopy analysis (SEM)

The morphological changes of the untreated and treated samples were examined by using scanning electron microscope (HITACHI, S3000) with Kv and the samples were coated with gold by sputtering.

6.2.6.5. Estimation of pretreated biomass and dissolved solids

The yield percentage (%) of water soluble (WS) and water insoluble (WIS) components of each fraction from pretreatment were calculated by the following formula:

\[
\text{(WIS) Pretreated biomass (\%) = } \frac{\text{Weight of pretreated biomass}}{\text{Weight of the starting biomass}} \times 100
\]

\[
\text{(WS) Dissolved solids (\%)} = 1 - \frac{\text{Weight of pretreated biomass}}{\text{Weight of the starting biomass}} \times 100
\]

6.2.7. Fermentation studies

6.2.7.1. Inoculum

The isolated marine yeasts were used for the fermentation process. It was cultivated with yeast peptone dextrose (YPD) medium and maintained on the same medium slants and stored at 4°C. For the fermentation process, the cultures were multiplied with the following medium
<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (g/l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3.6</td>
</tr>
<tr>
<td>Peptone</td>
<td>4.0</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>3.0</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.0</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>1.0</td>
</tr>
</tbody>
</table>

pH-5.8±0.2

6.2.7.2. Fermentation

The fermentation was carried out using bench top fermentor (Lark innovative Teknowledge, India). The acid hydrolyzed sample was autoclaved at 121°C and 15 lbs for 15 min. After cooling, the sample was inoculated with 10% (v/v) of inoculum at the pH level of 5.5. The pH level was adjusted with 1N HCl and 1N NaOH automatically with the help of pH probe. This setup was maintained at 30°C with 150 rpm of agitation. The foam level was monitored with the foaming sensor and the airflow at 0.5 l/min was maintained. The sample was withdrawn every 24 hrs intervals and it was centrifuged at 10,000 rpm for 15 min. The supernatant was used to determine the total reducing sugar, total phenolic content and ethanol concentration.

6.2.7.3. Cell mass

The dry cell mass was estimated by centrifuging the known volume of sample in a pre-treated and pre-weighed eppendorf tube for 20 min.
After that, the pellets were resuspended with 2 ml of sterile distilled water and further centrifuged at (10,000 rpm for 15 min). Then, the cell mass was dried at 105°C for 24 hrs (Pramanik and Rao, 2005).

6.2.7.4. Analytical methods

6.2.7.4.1. Estimation of total reducing sugar

The amount of total reducing sugar was estimated by DNS method as described by Miller (1959).

(i) Preparation of Dinitrosalicylic acid reagent (DNS reagent)

About 1 g of dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg of sodium sulphite were dissolved in 1% NaOH and stored in 4°C until use.

(ii) Rochelle salt solution

About 40 g of potassium sodium tartrate was dissolved in 100 ml of distilled water.

(iii) Procedure

About 0.5 ml of sample from each treatment was taken in a test tube. Then the volume was made up to 3 ml by using sterile distilled water. About 3 ml of DNS reagent was added to the each test tube. All the tubes were kept in the water bath for 10 minutes. Then, 1 ml of Rochelle salt
solution was added to the contents in the warm condition. After cooling, the absorbance was taken at 510 nm and the glucose was used as a standard.

6.2.7.4.2. Optimization

The temperature and time were also optimized to enhance the maximum release of total reducing sugar from the seagrass species.

6.2.7.4.3. Estimation of total phenols

The total phenolic content of the fermentation broth was carried out by the method of Cho et al. (2010).

(i) Reagents required

1. Folin : Ciocalteau reagent (1:2)

2. 7.5% Sodium carbonate (Na₂CO₃)

3. Stock gallic acid solution: 10 mg of gallic acid was dissolved in methanol and made up to 10 ml standard flask (concentration 1 mg.ml⁻¹)

4. Working standard: 1 ml of stock solution was diluted to 10 ml in methanol in a standard flask.
(ii) Procedure

About 1 ml of the fermentation broth was mixed with 5 ml of Folin:Ciocalteau reagent and 4 ml of sodium carbonate. The mixture was allowed to stand for 60 minutes under room temperature. The blue colour was developed was read at 765 nm using an UV-visible spectrophotometer (Cyber UV-1, Mecasys Co Ltd). The total phenolic content was calculated and the gallic acid was used as a standard.

6.2.7.4.4. Estimation of ethanol (Caputi et al., 1968).

(i) Preparation of reagents

a) Preparation of potassium dichromate (K$_2$Cr$_2$O$_7$)

About 34 g of K$_2$Cr$_2$O$_7$ was dissolved in 500 ml of distilled water. To this 325 ml of concentrated sulphuric acid was added and the volume was made up to 1000 ml with distilled water.

(ii) Preparation of stock solution

It was prepared by making 12.6 ml of analytical grade ethanol (789 mg.ml$^{-1}$) with little amount of distilled water and making up the volume to 100 ml using distilled water, this gives 100 mg ethanol.ml$^{-1}$.

(iii) Procedure

About 1 ml of fermented broth sample was transferred to 250 ml round bottom flask connected to the condenser and was diluted with 30 ml
of distilled water. The sample was distilled at 75°C. The distillate was collected in 25 ml of K$_2$Cr$_2$O$_7$ reagent, which was kept at the receiving end. The distillate containing alcohol was collected till the total volume of 45 ml was obtained. The sample was kept in water bath at 60°C for 20 min and then cooled. The volume was made upto 50 ml with distilled water and further optical density was measured at 600 nm. The standard curve was prepared with the analytical grade ethanol.

6.2.7.4.5. Simple sugar test

The sugars which released during the pretreatment was analyzed by using the following procedure:

<table>
<thead>
<tr>
<th>S. NO</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MOLISCH’S TEST</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>To the test solution added 2 drops of Molisch’s reagent mixed well and add 1 ml of concentrated sulphuric acid along the sides of the test tube without shaking.</td>
<td>A purple colour ring was formed at the junction of the two liquids</td>
<td>Presence of carbohydrates.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>IODINE TEST</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>To 1 ml of test solution added 2 drops of iodine and mix well.</td>
<td>A blue color solution was obtained.</td>
<td>Presence of starch.</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td><strong>BENEDICT’S TEST</strong></td>
<td></td>
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<td>---</td>
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</tr>
</tbody>
</table>
|   | To 2 ml of benedict’s reagent, few drops of test solution was added and heated in a boiling water bath for few minutes. It was then allowed to cool spontaneously. | i) First the solution turns green color then a reddish brown precipitate was obtained.  
ii) No characteristic color change | Presence of reducing sugar.  
Absence of reducing sugar. |
|   |   |   |   |
|   | **FEHLING’S TEST** |   |   |
|   | To the test solution added 0.5 ml of Fehling’s solution A & B, mixed well and heated in a boiling water bath. | i) A reddish brown colour was obtained.  
ii) No characteristic change. | Presence of reducing sugar.  
Absence of reducing sugar. |
|   |   |   |   |
|   | **BARFOED’S TEST** |   |   |
|   | To 2 ml of Barfoed’s reagent, added 2ml of test solution. Mixed well and heated in a boiling water bath for 2 minutes. | i) A brick red precipitate was obtained.  
ii) No red precipitate was obtained. | Presence of monosaccharide.  
Presence of Disaccharide. |
|   |   |   |   |
|   | **BIAL’S TEST** |   |   |
|   | To 2 ml of Bial’s reagent, 2 ml of test solution was added and heated in a direct flame. | i) Green color solution was obtained.  
ii) No green color solution was obtained. | Presence of Xylose.  
Absence of Xylose. |
6.3. Results

The results of the biochemical parameters reveals that, the moisture content of the seagrass samples were estimated maximum by 45.33±0.53% in *Syringodium isoetifolium* followed by 37±0.16% in *Cymodocea serrulata*, 28.30±0.50% in *Halophila ovalis* and 25.66±0.01% in *Halodule pinifolia* respectively. Moreover, the maximum (13.5±0.35%) ash content was recorded in *Halophila ovalis* followed by *Syringodium isoetifolium* (11.5±0.40%) and the minimum level was recorded in *Cymodocea serrulata* (9.3±0.15%). The level of cellulose, acid detergent fiber and neutral detergent fibre were increased after treatment. But in the case of hemicelluloses and lignin, the percentage level was recorded minimum in the treated samples when compared with the control samples (Table 6.1).

The FTIR spectrum was carried out by the present study and represented in Fig. 6.2. It reveals that, the FTIR spectrum of untreated and pretreated *Cymodocea serrulata* reveals that, the absorption peaks 3313 and 3195 cm\(^{-1}\) indicates the –OH stretching vibration and it has many numbers of hydroxyl groups in the cellulose rich fraction. The –CH stretching was observed in CH\(_2\) and CH\(_3\) groups from cellulose, hemicelluloses and lignin at the peak 2974 and 2883 (2885) cm\(^{-1}\). The absorption peak at 1670 cm\(^{-1}\) attributed to C=\(\text{C}\) stretching vibration through the intramolecular
dehydration. The other peak at 1454 cm\(^{-1}\) was recorded due the presence of C-H asymmetric deformation in CH\(_3\) and CH\(_2\) that are present in the lignin and hemicelluloses. The peak at 1334 (1336) cm\(^{-1}\) is attributed to C-C and C-O skeletal vibration. The absorption band at 1195 cm\(^{-1}\) arose from C-O-C stretching at the β-(1→4)-glycosidic linkages between the sugar units in the cellulose and hemicelluloses. The peak at 1112 cm\(^{-1}\) indicates that, the associate –OH groups from cellulose and hemicelluloses. The –CH bending peak was recorded at 752 cm\(^{-1}\) and 808 (810) cm\(^{-1}\) respectively (Fig. 6.2. a & b).

The spectrum of Syringodium isoetifolium reveals that, the absorption peaks 3313 and 3197 (3195) cm\(^{-1}\) indicates the –OH stretching vibration. The –CH stretching was recorded in CH\(_2\) and CH\(_3\) groups from cellulose, hemicelluloses and lignin at the peak 2972 (2974) and 2887 (2883) cm\(^{-1}\). In addition to that, the peak at 2935 cm\(^{-1}\) was recorded in the untreated sample however; the bond has been removed after pretreatment. The absorption peak at 1670 cm\(^{-1}\) attributed to C=C stretching vibration through the intramolecular dehydration. The other peak at 1454 cm\(^{-1}\) was recorded due the presence of C-H asymmetric deformation in CH\(_3\) and CH\(_2\) that are present in the lignin and hemicelluloses. The peak at 1334 cm\(^{-1}\) is attributed to C-C and C-O skeletal vibration. The absorption band at 1195 cm\(^{-1}\) arose from C-O-C stretching at the β-(1→4)-glycosidic linkages between the
sugar units in the cellulose and hemicelluloses. The peak at 1112 cm\(^{-1}\) indicates that, the associate –OH groups from cellulose and hemicelluloses. The –CH bending peak was recorded at 810 cm\(^{-1}\) in the pretreated *Syringodium isoetifolium* (Fig. 6.2. c & d).

The absorption peaks of the *Halophila ovalis* reveals that, the absorption peaks 3313 (3315) and 3195 (3197) cm\(^{-1}\) indicates the –OH stretching vibration and it has many numbers of hydroxyl groups in the cellulose rich fraction. The –CH stretching was recorded in CH\(_2\) and CH\(_3\) groups from cellulose, hemicelluloses and lignin at the peak 2970 (2974) and 2860 (2885) cm\(^{-1}\). The absorption peak at 1670 cm\(^{-1}\) attributed to C=C stretching vibration through the intramolecular dehydration. The other peak at 1454 cm\(^{-1}\) was recorded due the presence of C-H asymmetric deformation in CH\(_3\) and CH\(_2\) that are present in the lignin and hemicelluloses. The peak at 1336 (1334) cm\(^{-1}\) is attributed to C-C and C-O skeletal vibration. The absorption band at 1195 cm\(^{-1}\) arose from C-O-C stretching at the β-(1→4)-glycosidic linkages between the sugar units in the cellulose and hemicelluloses. The peak at 1112 cm\(^{-1}\) indicates that, the associate –OH groups from cellulose and hemicelluloses. The –CH bending peak was recorded at 806, 752 (750), 605 (603) cm\(^{-1}\) in the pretreated *Halophila ovalis* (Fig. 6.2. e & f).
The FTIR results of the *Halodule pinifolia* reveals that, the absorption peaks 3313 and 3195 (3193) cm\(^{-1}\) indicates the \(-\text{OH}\) stretching vibration and it has many numbers of hydroxyl groups in the cellulose rich fraction. The \(-\text{CH}\) stretching was recorded in CH\(_2\) and CH\(_3\) groups from cellulose, hemicelluloses and lignin at the peak 2974 and 2881 (2883) cm\(^{-1}\). The absorption peak at 1670 (1668) cm\(^{-1}\) attributed to C=C stretching vibration through the intramolecular dehydration. The other peak at 1454 (1452) cm\(^{-1}\) was recorded due the presence of C-H asymmetric deformation in CH\(_3\) and CH\(_2\) that are present in the lignin and hemicelluloses. The peak at 1334 cm\(^{-1}\) is attributed to C-C and C-O skeletal vibration. The absorption band at 1195 cm\(^{-1}\) arose from C-O-C stretching at the \(\beta-(1\rightarrow4)\)-glycosidic linkages between the sugar units in the cellulose and hemicelluloses. The peak at 1114 cm\(^{-1}\) indicates that, the associate \(-\text{OH}\) groups from cellulose and hemicelluloses. The \(-\text{CH}\) bending peak was recorded at 808, 750 (752), 653, 603 (605) cm\(^{-1}\) in the pretreated *Halodule pinifolia* (Fig. 6.2. g & h).

To investigate the crystallinity changes during the pretreatment, the XRD analysis was also carried out by the present study and the results are represented in Figs. 6.3(a-d). The results suggested that, the samples exhibit the diffraction angles (2\(\theta\)) around 15\(^{\circ}\) and 23\(^{\circ}\) in all the pretreated and untreated seagrass. The crystallinity was modified during the pretreatment and it could be confirmed by the crystallinity index (CrI)
values. It reveals that, the CrI values of untreated *Cymodocea serrulata* (18.4%), *Syringodium isoetifolium* (11.6%), *Halophila ovalis* (28.3%) and *Halodule pinifolia* (17.8%) respectively. However, the index values in pretreated seagrass viz., *Cymodocea serrulata, Syringodium isoetifolium, Halophila ovalis* and *Halodule pinifolia* were recorded by 24.7%, 43.12%, 35.1% and 47.7% respectively. After pretreatment, the crystallinity index values are increased than the untreated samples.

The TGA and DTA of pretreated and untreated seagrass samples were recorded to compare their thermal prosperities and are represented in Figs. 6.4 (a-d). The result of the TGA curves reveals that, the water and other volatiles materials were eliminated between 100°C-200°C. After the elimination of water and other molecules, the hemicelluloses were degraded between 220°C-315°C. The peaks at 240°C, 242°C, 237°C and 242°C in pretreated *Cymodocea serrulata, Syringodium isoetifolium, Halophila ovalis* and *Halodule pinifolia*. On the other hand, the untreated *Cymodocea serrulata* showed the peak at 235°C followed by the peak at 204°C in *Syringodium isoetifolium, 214°C* in *Halophila ovalis* and 212°C in *Halodule pinifolia* respectively. Moreover, the cellulose rich peaks were observed between 300°C-420°C and the results of the pretreated samples showed the peak at 345°C in *Cymodocea serrulata* and *Syringodium isoetifolium, 355°C* in *Halophila ovalis* and the *Halodule pinifolia* showed the peak at 348°C.
respectively. However, the untreated samples of Cymodocea serrulata and Syringodium isoetifolium showed the peak at 337°C. Further, the Halophila ovalis and Halodule pinifolia showed the peaks at 345°C and 335°C respectively.

The DTA curves reveal that, the curves started with the endothermic region because of the removal of absorbed water and further moved into the exothermic direction. The peak recorded at 335°C correspond to the pretreated Cymodocea serrulata and Syringodium isoetifolium, 330°C for Halophila ovalis and 340°C for Halodule pinifolia. Further, the peaks recorded at 322°C, 310°C, 315°C and 320°C in untreated Cymodocea serrulata, Syringodium isoetifolium, Halophila ovalis and Halodule pinifolia respectively.

Above 450°C, the lignin and other remaining char can be oxidized into CO₂, CO and water vapour. As the temperature increases, the production of volatile compounds can be completely removed. The peaks of the pretreated samples were 504°C in Cymodocea serrulata, 499°C in Syringodium isoetifolium, 453°C in Halophila ovalis and 489°C in Halodule pinifolia. But in the case of untreated samples, the peaks were recorded at 479°C in Cymodocea serrulata, 458°C in Syringodium isoetifolium, 501°C in Halophila ovalis and 468°C in Halodule pinifolia respectively. Finally, the
thermal stability of the pretreated seagrass was increased than that of the untreated seagrass samples (Figs. 6.5. a-d).

In order to understand the structural changes on the surface of the seagrass during the pretreatment, the pretreated and untreated samples were examined by scanning electron microscope (SEM). It reveals that, the untreated sample shows the compact rigid particle structure. However, the surface of the pretreated sample was damaged severely and it has numerous shrinkages (Figs. 6.6. a-h). In addition to that, the result of the EDX images reveals that, the level of carbon and oxygen was randomly increased however; the level of other elements was slightly fluctuated in the pretreated seagrass when compared with the untreated seagrass (Fig. 6.7. a-h)

The percentage of pretreated biomass and dissolved solids result reveals that, the Cymodocea serrulata showed maximum (62.9%) pretreated biomass followed by Syringodium isoetifolium (49.9%), Halodule pinifolia (45.9%) but minimum pretreated biomass was recorded in Halophila ovalis (39.5%). The maximum (60.5%) dissolved solids was recorded with Halophila ovalis and the minimum (37.1%) dissolved solids was recorded with the Cymodocea serrulata (Fig. 6.8).
The estimation of total reducing sugar was also analyzed by the addition of various acids, alkali and other organic solvents. In nitric acid treatment, the maximum (452.79±0.62 mg.g⁻¹) reducing sugar was recorded from *Cymodocea serrulata* and the *Syringodium isoetifolium* showed minimum by 173.88±0.53 mg.g⁻¹. In orthophosphoric acid treatment, the maximum (134.19±0.28 mg.g⁻¹) total reducing sugar was recorded in *Cymodocea serrulata* and found minimum by 12.69±0.12 mg.g⁻¹ in *Halodule pinifolia*. In sodium carbonate treatment, the maximum (168.75±0.31 mg.g⁻¹) total reducing sugar was recorded in *Halodule pinifolia* and found minimum by 132.3 ±0.65 mg.g⁻¹ in *Halophila ovalis*. In general, the amount of reducing sugar was increased in all the treatment when compared with the control (Table 6.2). Moreover, an experiment was conducted to optimize the temperature and time to enhance the maximum release of total reducing sugar from the selected seagrass. It reveals that, the maximum amount of reducing sugar was released at 120°C in *Cymodocea serrulata* (466.8±0.6 mg.g⁻¹) and the incubation time was found at 40 min (Tables 6.3 & 6.4).

The total reducing sugar utilization during the hydrolysate fermentation is depicted in Fig. 6.9 - 6.12. It reveals that, the reducing sugar was gradually utilized by the microbes with increased fermentation period. In addition to that, all the microbes utilized the reducing sugar maximum
at 120 hrs in *Cymodocea serrulata* substrate, 72 hrs in *Syringodium isoetifolium* substrate and 96 hrs in *Halophila ovalis* and *Halodule pinifolia* substrates.

The results of the percentage of reducing sugar utilization reveals that, the *Saccharomyces cerevisiae* utilized maximum by 89.9% followed by YPD-4 (89.7%), YPD-10 and YPD-11 (89.1%) and found minimum (75.2%) by YPD-1 in *Cymodocea serrulata* hydrolysate with the fermentation time of 120 hrs. But, the maximum percentage (85%) of sugar was utilized by the YPD-11 and the minimum (75.9%) was utilized by YPD-3 at 72 hrs in *Syringodium isoetifolium*.

However, the YPD-8 showed maximum (76.2%) sugar utilized in *Halophila ovalis* and minimum (62.8%) sugar was utilized by *Saccharomyces cerevisiae* at 96 hrs of fermentation. Similarly, the YPD-8 showed maximum (78.1%) percentage of sugar utilization and minimum percentage (63.7%) of sugar was recorded in YPD-1 at 96 hrs in *Halodule pinifolia* hydrolysate fermentation. All the microbes showed the maximum sugar utilization in *Cymodocea serrulata* substrate at 120 hrs and *Syringodium isoetifolium* substrate at 72 hrs fermentation. In *Halophila ovalis* and *Halodule pinifolia* hydrolysate fermentation, the maximum sugar utilization percentage was observed at 96 hrs (Figs. 6.13-6.16).
The ethanol production by the chosen microbes while supplied the seagrass as substrate, the YPD-5 showed maximum (2.7 g.l⁻¹) ethanol production in *Cymodocea serrulata* and the *Zymomonas mobilis* (MTCC-92) showed minimum (0.4 g.l⁻¹) ethanol production in *Syringodium isoetifolium*. Moreover, the MTCC microbes showed minimum ethanol production when compared with the marine yeast in all the seagrass hydrolysate fermentation. In *Cymodocea serrulata*, all the tested microbes showed the maximum production of ethanol at 120 hrs and in *Syringodium isoetifolium*, the ethanol production was found maximum at 72 hrs and at 96 hrs the ethanol production was found maximum in *Halophila ovalis* and *Halodule pinifolia* (Figs. 6.17-6.20).

The cell density of all the chosen microbial strains during fermentation was also recorded by the present study. It reveals that, the cell density was gradually increased with the increasing time of fermentation. The maximum cell density was recorded at 120 hrs in *Cymodocea serrulata* substrate, 72 hrs in *Syringodium isoetifolium* substrate and 96 hrs in *Halophila ovalis* and *Halodule pinifolia* substrates (Figs. 6.21-6.24).

The present study also made an attempt to find out the fermentation inhibitors which could suppress the ethanol production. The average detoxification percentage reveals that, the maximum average detoxification
percentage (40.7%) was recorded in *Halophila ovalis* and *Halodule pinifolia* hydrolysate. But, the minimum detoxification percentage was recorded in *Cymodocea serrulata* (39.3%) and *Syringodium isoetifolium* (38.8%) (Fig. 6.25).

The different mono sugars released during the hydrolyzing process were also observed by the present study and the results are summarized in Table 6.5. The results of the Molisch’s test showed the presence of all carbohydrates. The Benedict’s reagent test, Fehling’s solution test, Barfoed’s test and Bial’s test confirmed the presence of the reducing sugars (glucose, fructose, galactose, lactose, maltose and xylose). However, the iodine test showed the absence of starch.
Table 6.1. Biochemical composition of the chosen seagrass species (% dry substance)

<table>
<thead>
<tr>
<th>Name of the seagrass</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
<th>Acid detergent fibre (%)</th>
<th>Neutral detergent fibre (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Cymodocea serrulata</td>
<td>37.0 ± 0.16</td>
<td>22.2 ± 0.20</td>
<td>63.6 ± 0.43</td>
<td>10.2 ± 0.50</td>
<td>14.3 ± 0.55</td>
<td>43.2 ± 0.95</td>
<td>53.4 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>9.3 ± 0.15</td>
<td>1.0 ± 0.15</td>
<td>1.0 ± 0.15</td>
<td>9.9 ± 0.88</td>
<td>77.4 ± 1.25</td>
<td>1.25.</td>
<td>78.4 ± 1.57</td>
</tr>
<tr>
<td>Syringodium isoetifolium</td>
<td>45.3 ± 0.53</td>
<td>21.0 ± 0.30</td>
<td>60.6 ± 0.30</td>
<td>15.6 ± 0.34</td>
<td>9.1 ± 0.40</td>
<td>27.4 ± 0.52</td>
<td>43.0 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>11.5 ± 0.40</td>
<td>1.0 ± 0.30</td>
<td>8.8 ± 0.30</td>
<td>2.0 ± 0.32</td>
<td>66.0 ± 1.90</td>
<td>1.90</td>
<td>74.8 ± 0.20</td>
</tr>
<tr>
<td>Halophila ovalis</td>
<td>28.3 ± 0.50</td>
<td>29.0 ± 1.00</td>
<td>58.6 ± 0.40</td>
<td>16.4 ± 0.72</td>
<td>9.2 ± 0.50</td>
<td>20.2 ± 0.91</td>
<td>36.6 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>13.5 ± 0.35</td>
<td>3.6 ± 0.40</td>
<td>3.6 ± 0.40</td>
<td>2.0 ± 0.34</td>
<td>69.6 ± 0.86</td>
<td>0.86</td>
<td>73.2 ± 1.10</td>
</tr>
<tr>
<td>Halodule pinifolia</td>
<td>25.6 ± 0.01</td>
<td>26.6 ± 0.36</td>
<td>56.8 ± 1.00</td>
<td>17.0 ± 0.15</td>
<td>17.7 ± 1.05</td>
<td>34.4 ± 2.66</td>
<td>51.4 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>9.5 ± 0.25</td>
<td>8.0 ± 0.60</td>
<td>8.0 ± 0.60</td>
<td>5.3 ± 0.41</td>
<td>79.4 ± 1.41</td>
<td>1.41</td>
<td>87.4 ± 0.75</td>
</tr>
</tbody>
</table>

T<sub>1</sub> – Control (Untreated); T<sub>2</sub> – Pretreated; Values are found significant (p<0.05) between parameters and seagrass species
Fig. 6.2. Picture shows the FTIR analysis of untreated and pretreated seagrass samples

(a) *Cymodocea serrulata* untreated
(b) *Cymodocea serrulata* pretreated
(c) *Syringodium isoetifolium* untreated
(d) *Syringodium isoetifolium* pretreated

Cont…
(e) Halophila ovalis untreated

(f) Halophila ovalis pretreated

(g) Halodule pinifolia untreated

(h) Halodule pinifolia pretreated
Fig. 6.3. Picture shows the crystallinity changes of the pretreated and untreated seagrass samples

(a) *Cymodocea serrulata*  
(b) *Syringodium isoetifolium*

(c) *Halophila ovalis*  
(d) *Halodule pinifolia*
Fig. 6.4. Picture shows the TGA curves of the pretreated and untreated seagrass samples

(a) *Cymodocea serrulata*  
(b) *Syringodium isoetifolium*  
(c) *Halophila ovalis*  
(d) *Halodule pinifolia*
Fig. 6.5. Picture shows the DTA curves of the pretreated and untreated seagrass samples

(a) *Cymodocea serrulata*  
(b) *Syringodium isoetifolium*  
(c) *Halophila ovalis*  
(d) *Halodule pinifolia*
Fig. 6.6. SEM pictures show the morphological changes of the pretreated and untreated seagrass samples (25X)

(a) Untreated *Cymodocea serrulata*  
(b) Pretreated *Cymodocea serrulata*

(c) Untreated *Syringodium isoetifolium*  
(d) Pretreated *Syringodium isoetifolium*  
Cont…
(e) Untreated *Halophila ovalis*

(f) Pretreated *Halophila ovalis*

(g) Untreated *Halodule pinifolia*

(h) Pretreated *Halodule pinifolia*
Fig. 6.7. Picture shows the EDX images of the pretreated and untreated seagrass samples

(a) Untreated Cymodocea serrulata
(b) Pretreated Cymodocea serrulata

(c) Untreated Syringodium isoetifolium
(d) Pretreated Syringodium isoetifolium

Cont…
(e) Untreated Halophila ovalis

(f) Pretreated Halophila ovalis

(g) Untreated Halodule pinifolia

(h) Pretreated Halodule pinifolia
Fig. 6.8. Picture shows the percentage of pretreated biomass and dissolved solids.
Table 6.2. Total reducing sugar in different seagrass species with various pretreatment agents at the temperature: 100°C and the Time: 30 minutes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Pretreatment agent</th>
<th>Cymodocea serrulata</th>
<th>Syringodium isoetifolium</th>
<th>Halophila ovalis</th>
<th>Halodule pinifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.g⁻¹</td>
<td>%</td>
<td>mg.g⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>1.</td>
<td>Control (Distilled water)</td>
<td>56.16±0.14</td>
<td>5.61</td>
<td>57.24±0.37</td>
<td>5.72</td>
</tr>
<tr>
<td>2.</td>
<td>HCl (1%)</td>
<td>408.78±0.45</td>
<td>40.78</td>
<td>167.67±0.75</td>
<td>16.76</td>
</tr>
<tr>
<td>3.</td>
<td>H₂SO₄ (1%)</td>
<td>150.39±0.31</td>
<td>15.03</td>
<td>108.54±0.96</td>
<td>10.85</td>
</tr>
<tr>
<td>4.</td>
<td>HNO₃ (1%)</td>
<td>452.79±0.62</td>
<td>45.27</td>
<td>173.88±0.53</td>
<td>17.38</td>
</tr>
<tr>
<td>5.</td>
<td>H₃PO₄ (1%)</td>
<td>134.19±0.28</td>
<td>13.41</td>
<td>60.21±0.36</td>
<td>6.02</td>
</tr>
<tr>
<td>6.</td>
<td>Acetic acid (1%)</td>
<td>164.16±0.52</td>
<td>16.41</td>
<td>135.81±0.27</td>
<td>13.58</td>
</tr>
<tr>
<td>7.</td>
<td>Oxalic acid (1%)</td>
<td>321.57±0.18</td>
<td>32.15</td>
<td>149.31±0.85</td>
<td>14.93</td>
</tr>
<tr>
<td>8.</td>
<td>NaOH (1%)</td>
<td>194.94±0.25</td>
<td>19.49</td>
<td>146.07±0.46</td>
<td>14.60</td>
</tr>
<tr>
<td>9.</td>
<td>Na₂CO₃ (1%)</td>
<td>166.32±0.66</td>
<td>16.63</td>
<td>141.21±0.53</td>
<td>14.12</td>
</tr>
<tr>
<td>10.</td>
<td>Ca(OH)₂ (1%)</td>
<td>158.76±0.91</td>
<td>15.87</td>
<td>137.97±0.48</td>
<td>13.79</td>
</tr>
<tr>
<td>11.</td>
<td>H₂O₂ (7.5%)</td>
<td>146.88±0.82</td>
<td>14.68</td>
<td>137.16±0.11</td>
<td>13.71</td>
</tr>
<tr>
<td>12.</td>
<td>Hotwater</td>
<td>153.36±0.32</td>
<td>15.33</td>
<td>139.86±0.48</td>
<td>13.98</td>
</tr>
<tr>
<td>13.</td>
<td>Ethanol-water (1:1)</td>
<td>152.28±0.19</td>
<td>15.22</td>
<td>139.59±0.17</td>
<td>13.95</td>
</tr>
<tr>
<td>14.</td>
<td>Methanol-water (1:1)</td>
<td>148.23±0.10</td>
<td>14.82</td>
<td>138.24±0.25</td>
<td>13.82</td>
</tr>
<tr>
<td>15.</td>
<td>Chloroform-water (1:1)</td>
<td>148.50±0.59</td>
<td>14.85</td>
<td>148.50±0.14</td>
<td>14.85</td>
</tr>
<tr>
<td>16.</td>
<td>Acetone-water (1:1)</td>
<td>152.01±0.62</td>
<td>15.20</td>
<td>138.24±0.83</td>
<td>13.82</td>
</tr>
</tbody>
</table>

Values are found significant (p<0.05) between treatments and seagrass species
Table 6.3. Time optimization for total reducing sugar (mg.g\(^{-1}\)) among chosen seagrass species with HNO\(_3\) treatment at 100°C

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Substrate</th>
<th>Cymodocea serrulata</th>
<th>Syringodium isoetifolium</th>
<th>Halophila ovalis</th>
<th>Halodule pinifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
<td>452.7±0.11</td>
<td>173.8±0.20</td>
<td>265.1±0.11</td>
<td>331.2±0.34</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>455.2±0.20</td>
<td>180.0±0.20</td>
<td>272.9±0.92</td>
<td>350.7±0.23</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>442.5±0.40</td>
<td>170.1±0.15</td>
<td>252.9±0.60</td>
<td>324.2±0.30</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>426.6±0.37</td>
<td>156.8±0.10</td>
<td>246.2±0.52</td>
<td>317.5±0.76</td>
</tr>
</tbody>
</table>

Values are found significant (p<0.05) between time and seagrass species
Table 6.4. Temperature optimization for total reducing sugar (mg.g\(^{-1}\)) among chosen seagrass species with HNO\(_3\) treatment with 40 minute incubation

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Substrate</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Cymodocea serrulata</em></td>
<td><em>Syringodium isoetifolium</em></td>
<td><em>Halophila ovalis</em></td>
<td><em>Halodule pinifolia</em></td>
</tr>
<tr>
<td>90</td>
<td>442.5±0.15</td>
<td>164.9±0.25</td>
<td>261.9±0.10</td>
<td>339.1±0.52</td>
</tr>
<tr>
<td>100</td>
<td>445.7±0.20</td>
<td>170.6±0.36</td>
<td>267.0±0.55</td>
<td>343.1±0.30</td>
</tr>
<tr>
<td>110</td>
<td>458.1±0.47</td>
<td>177.3±0.25</td>
<td>269.4±0.30</td>
<td>344.5±0.55</td>
</tr>
<tr>
<td>120</td>
<td>466.8±0.60</td>
<td>189.8±0.40</td>
<td>281.0±0.36</td>
<td>350.1±0.92</td>
</tr>
<tr>
<td>130</td>
<td>459.2±0.61</td>
<td>189.2±0.5</td>
<td>272.4±0.30</td>
<td>345.8±0.20</td>
</tr>
</tbody>
</table>

Values are found significant (p<0.05) between temperature and seagrass species
Fig. 6.9. Picture showing the total reducing sugar utilization in *Cymodocea serrulata* hydrolysate fermentation with different microbial strains.

Fig. 6.10. Picture showing the total reducing sugar utilization in *Syringodium isoetifolium* hydrolysate fermentation with different microbial strains.
Fig. 6.11. Picture showing the total reducing sugar utilization in *Halophila ovalis* hydrolysate fermentation with different microbial strains.

![Graph showing total reducing sugar utilization in *Halophila ovalis* hydrolysate fermentation with different microbial strains](image1)

Fig. 6.12. Picture showing the total reducing sugar utilization in *Halodule pinifolia* hydrolysate fermentation with different microbial strains.

![Graph showing total reducing sugar utilization in *Halodule pinifolia* hydrolysate fermentation with different microbial strains](image2)
Fig. 6.13. Picture showing the percentage of total reducing sugar utilization in *Cymodocea serrulata* hydrolysate fermentation at different time of incubation

![Graph showing percentage of total reducing sugar utilization in *Cymodocea serrulata* hydrolysate fermentation at different time of incubation.](image)

Microbial Strains

0 h, 24 h, 48 h, 72 h, 96 h, 120 h

Fig. 6.14. Picture showing the percentage of total reducing sugar utilization in *Syringodium isoetifolium* hydrolysate fermentation at different time of incubation

![Graph showing percentage of total reducing sugar utilization in *Syringodium isoetifolium* hydrolysate fermentation at different time of incubation.](image)

Microbial Strains

0 h, 24 h, 48 h, 72 h
Fig. 6.15. Picture showing the percentage of total reducing sugar utilization in *Halophila ovalis* hydrolysate fermentation at different time of incubation

![Graph showing percentage of total reducing sugar utilization in *Halophila ovalis* hydrolysate fermentation at different time of incubation.](image1)

Fig. 6.16. Picture showing the percentage of total reducing sugar utilization in *Halodule pinifolia* hydrolysate fermentation at different time of incubation

![Graph showing percentage of total reducing sugar utilization in *Halodule pinifolia* hydrolysate fermentation at different time of incubation.](image2)
Fig. 6.17. Picture showing the ethanol production in *Cymodocea serrulata* hydrolysate fermentation at different time of incubation

Fig. 6.18. Picture showing the ethanol production in *Syringodium isoetifolium* hydrolysate fermentation at different time of incubation
Fig. 6.19. Picture showing the ethanol production in *Halophila ovalis* hydrolysate fermentation at different time of incubation

Fig. 6.20. Picture showing the ethanol production in *Halodule pinifolia* hydrolysate fermentation at different time of incubation
Fig. 6.21. Picture showing the cell density in *Cymodocea serrulata* hydrolysate fermentation

Fig. 6.22. Picture showing the cell density in *Syringodium isoetifolium* hydrolysate fermentation
Fig. 6.23. Picture showing the cell density in *Halophila ovalis* hydrolysate fermentation

![Graph showing cell density in *Halophila ovalis* hydrolysate fermentation](image)

Fig. 6.24. Picture showing the cell density in *Halodule pinifolia* hydrolysate fermentation

![Graph showing cell density in *Halodule pinifolia* hydrolysate fermentation](image)
Fig. 6.25. Picture showing the removal of total phenolic content during the detoxification
<table>
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<tr>
<th>S.No</th>
<th>Name of the test</th>
<th>Name of the test (Carbohydrates)</th>
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<th>Syringodium isoetifolium</th>
<th>Halophila ovalis</th>
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<td>Glucose Fructose Galactose</td>
<td>Glucose Fructose Galactose</td>
<td>Glucose Fructose Galactose</td>
<td>Glucose Fructose Galactose</td>
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