9.0. SUMMARY AND CONCLUSION

Bioethanol has a great worldwide importance nowadays which can be produced from several terrestrial renewable resources which reduce the level of green house gases in the atmosphere. Due to the high cost and lack of frequent availability of the terrestrial renewable energy sources, the alternative renewable sources have been highly warranted. However, studies related with the bioethanol production from the marine renewable resources are too limited. Palk Strait regions of Tamil Nadu have rich seagrass deposition along the coasts. In view of this, the present study has mooted out for the bioethanol production from the seagrass bio-wastes for the sustainable utilization. The salient features of the present study are listed as below:

1. The maximum above ground biomass of *Cymodocea serrulata* (166.4±36.72 g.dry wt/m²) was found at Mimisal and *Syringodium isoetifolium* (84.8±22.46 g.dry wt/m²) was found at S.P.Pattinam and *Halodule pinifolia* (68.8±13.93 g.dry wt/m²) was found at Manora and *Halophila ovalis* (62.4±17.45 g.dry wt/m²) was found at Manamelkudi in November-2011. The minimum above ground biomass of *Cymodocea serrulata* (16.0±16.0 g.dry wt/m²) was recorded at Manora and *Syringodium isoetifolium* (16±14.6 g.dry wt/m²) was recorded at
Mimisal and *Halodule pinifolia* (11.2±2.8 g.drywt/m²) was recorded at Kottaipattinam in May-2012 and *Halophila ovalis* (11.2±9.9 g.drywt/m²) was recorded at Manamelkudi during the months of April and May-2012.

2. The maximum below ground biomass of *Cymodocea serrulata* (406.4±114.5 g.drywt/m²) and *Syringodium isoetifolium* (156.8± 37.37 g.drywt/m²) were recorded in Thondi during the month of December-2011. The maximum below ground biomass level of *Halodule pinifolia* (80±22.5 g.drywt/m²) was recorded during the month of November-2011 and December-2011 at Manamelkudi and the *Halophila ovalis* was recorded maximum (80±22.9 g.drywt/m²) at Manamelkudi during the month of December-2011. The minimum below ground biomass of *Cymodocea serrulata* (33.6±20.63 g.drywt/m²) was recorded during the month of April-2012 at Manora and *Syringodium isoetifolium* (24±21.81 g.drywt/m²) was recorded at Mimisal in May-2012 and *Halodule pinifolia* (11.2±16.2 g.drywt/m²) was recorded at Kottaipattinam during the month of April-2012 and *Halophila ovalis* (14.4±22.9 g.drywt/m²) was recorded at Manamelkudi during the month of May-2012.
3. The maximum total biomass of *Cymodocea serrulata* (571.2±78.94 g. drywt/m²) and *Syringodium isoetifolium* (240±24.92 g.drywt/m²) and *Halodule pinifolia* (145.6±57.41 g.drywt/m²) and *Halophila ovalis* (142.4±21.83 g.drywt/m²) were recorded during the month of December-2011 at Thondi and Manamelkudi stations. Further, the minimum total biomass of *Cymodocea serrulata* (52.8±8.92 g.drywt/m²) was recorded at Manora and *Syringodium isoetifolium* (49.6±21.83 g.drywt/m²) was recorded at Mimisal and *Halophila ovalis* (25.6±7.42 g.drywt/m²) was recorded at Manamelkudi during the month of May-2012. However, the minimum total biomass of *Halodule pinifolia* (22.4±6.27 g.drywt/m²) was recorded at Kottaipattinam during the month of April-2012.

4. The maximum shoot density of *Cymodocea serrulata* (1068.8±46.09 shoots/m²), *Syringodium isoetifolium* (488±12.09 shoots/m²) were recorded at Thondi in December-2011 and the maximum shoot density of *Halophila ovalis* (427.2±24.82 shoots/m²) was recorded in Manamelkudi during the month of December-2011 but the maximum shoot density of *Halodule pinifolia* (387.2±34.62 shoots/m²) was recorded at Manamelkudi in January-2012.
The minimum shoot density of *Cymodocea serrulata* (57.6±3.45 shoots/m²) was recorded at Manora and *Syringodium isoetifolium* (33.6±1.45 shoots/m²) was recorded at Mimisal and *Halophila ovalis* (56±3.73 shoots/m²) at Manamelkudi during the month of May-2012 and *Halodule pinifolia* (51.2±4.55 shoots/m²) was recorded at Kottaipattinam in April-2012.

5. The results of the canopy height suggested that, the maximum canopy height of *Cymodocea serrulata* (27.3±0.89 cm) was identified at Thondi in December-2011 and the maximum canopy height of *Syringodium isoetifolium* (34.7±1.94 cm) was recorded at S.P.Pattinam during the month of October-2011. The *Halodule pinifolia* canopy height was found maximum (9.4±0.61cm) at Kottaipattinam in December-2011. The canopy height of *Halophila ovalis* (7.1±1.2 cm) was found maximum at Manamelkudi during the month of March-2012.

The minimum canopy height of *Cymodocea serrulata* (10.6±0.82 cm) was recorded at Mallipattinam during the month of March-2012 and *Syringodium isoetifolium* (15.7±0.52 cm) was recorded at Manamelkudi in July and August-2012 and *Halodule pinifolia*
(9.4±0.61cm) at Kottaipattinam in December-2011 and *Halophila ovalis* (2.3±0.7 cm) at Manamelkudi during the month of September-2011.

6. The maximum above and below ground biomass ratio was noticed in *Cymodocea serrulata* (0.83±0.1) at Manora in January-2012 and *Syringodium isoetifolium* (0.81±0.08) was recorded at Manamelkudi in December-2011 and *Halodule pinifolia* (1±0.1) was recorded at Kottaipattinam in April-2012 and *Halophila ovalis* (0.91±0.09) was recorded at Thondi in March-2012.

   The minimum above and below ground biomass ratio was observed in *Cymodocea serrulata* (0.38±0.1) at Thondi in January 2012 and *Syringodium isoetifolium* (0.44±0.09) at Mimisal during the month of January-2012 and *Halodule pinifolia* (0.59±0.09) and *Halophila ovalis* (0.59±0.09) were recorded at Thondi during the month of January-2012.

7. The maximum percentage of *Cymodocea serrulata* (100%) was recorded at Mallipattinam throughout the year and *Syringodium isoetifolium* (52.76%) at S.P.Pattinam in September-2011 and *Halodule pinifolia* (48.94%) at Manora during the month of August-2012 and *Halophila ovalis* (21.98%) at Manamelkudi during the month of October-2011.
The minimum percentage of *Cymodocea serrulata* (27.69%) was recorded at Manamelkudi during the month of September-2011 and *Syringodium isoetifolium* (15.37%) was recorded at Thondi in January-2012 and *Halodule pinifolia* was recorded (9.74%) at Thondi during the month of February-2012 and *Halophila ovalis* (11.19%) was recorded at Thondi during the month of January-2012.

8. The maximum onshore deposition of *Cymodocea serrulata* (10.73±0.08 kg.drywt/m²) and *Syringodium isoetifolium* (7.93±0.08 kg.drywt/m²) was recorded at Thondi during the month of May-2012. But, the minimum deposition of *Cymodocea serrulata* (0.624±0.002 Kg.drywt/m²) was recorded at Mimisal in November-2011 and *Syringodium isoetifolium* (0.688±0.001 Kg.drywt/m²) was recorded at Thondi during the month of November-2011.

9. The coastal geomorphological features suggested that, the landform beach was observed in all the survey area. The sand dune was noticed in Kodiyakarai and Mimisal. The lanform spit was observed in Manamelkudi, Kottaipattinam and Mimisal. None of the survey area was not observed with beach ridges except Kodiyakarai. In addition, the land form lagoon was observed in Manamelkudi and S.P.Pattinam coasts. The heavy mineral deposition was observed in
Kodiyakarai, Mimisal and S.P.Pattinam. The river puthu aaru was observed in Kodiyakarai and Pambaru was recorded in Mimisal.

10. The maximum (433.5 mm) rain fall was recorded in November-2011 and minimum (0.00 mm) rainfall was recorded in January-2012 and June-2012. The maximum (12/06 KmPH/Knots) wind speed was recorded in the month of June-2012 and the minimum (01/00 KmPH/Knots) wind speed was recorded in November-2011.

11. A total of 11 yeasts strains were isolated based on the morphological characters from the semi-decayed seagrass leaves.

12. The ethanol tolerance test showed that, the isolates of YPD 1-6, 9 and 10 showed active growth in all the tested alcohol level and the isolate YPD 7, 8 and 11 showed less active growths in 100 and 130 ml.l⁻¹ but moderate growth was observed at 150 ml.l⁻¹.

13. The flocculation test indicates that, the isolate YPD 1-3, 5-7, 9 and 10 showed active settlement after vortexing and the isolates YPD-8 and YPD-11 showed less active settlement but no settlement was observed in the YPD-4 isolate.

14. All the isolates showed active growth in all the tested stress condition except YPD-11.
15. All the isolates showed active growth at the temperature ranged between 25°C-37°C. However, none of the isolates showed growth at 45°C.

16. The potential isolates having maximum bioethanol production were identified through molecular techniques. The most promising isolates of YPD-1, YPD-2 and YPD-4 showed the maximum similarity with Candida tropicalis and the isolate YPD-5 showed the maximum similarity with Candida sp.

17. The Syringodium isoetifolium showed maximum (45.33±0.53%) moisture content followed by Cymodocea serrulata (37±0.16%), Halophila ovalis (28.30±0.50%) and Halodule pinifolia (25.66±0.01%). The maximum (13.5±0.35%) ash content was observed in Halophila ovalis followed by Syringodium isoetifolium (11.5±0.40%) and the minimum level was observed in Cymodocea serrulata (9.3±0.15%). But, the level of cellulose, acid detergent fiber and neutral detergent fibre were increased after treatment. However, the hemicelluloses and lignin showed the minimum percentage in the treatment samples when compared with the control samples.
18. The FTIR spectrum of the untreated and pretreated seagrass samples reveals that, the minimum intensity was observed in the pretreated samples when compared with the untreated samples.

19. The XRD result indicates that, the crystallinity index values were found maximum in all the pretreated seagrass samples than the untreated samples.

20. The thermal stability of the pretreated samples was increased when compared with the untreated samples.

21. SEM analysis suggested that, numerous shrinkages and cracking were observed in all the pretreated samples when compared with the untreated samples.

22. The maximum reducing sugar was released from *Cymodocea serrulata* (452.79±0.62 mg.g\(^{-1}\)) followed by *Halodule pinifolia* (331.29±0.41 mg.g\(^{-1}\)), *Halophila ovalis* (265.14±0.27 mg.g\(^{-1}\)) but minimum was recorded in *Syringodium isoetifolium* (173.88±0.53 mg.g\(^{-1}\)) in the nitric acid pretreatment.

23. The maximum reducing sugar was released at the optimized temperature by 120°C and the time was optimized at 40 min.
24. The percentage of reducing sugar utilization suggested that, the *Saccharomyces cerevisiae* showed maximum by 89.9% in *Cymodocea serrulata* at 120 hrs, the YPD-11 showed the maximum sugar utilization percentage in *Syringodium isoetifolium* (85%) hydrolysate. In *Halophila ovalis*, the YPD-8 showed maximum percentage by 76.2% of sugar utilization. Similarly, the YPD-8 showed maximum percentage by 78.1% of sugar utilized in *Halodule pinifolia* hydrolysate fermentation.

The minimum sugar utilization percentage was recorded in *Cymodocea serrulata* hydrolysate (75.2%) by YPD-1 at 120 hrs. The *Syringodium isoetifolium* (75.9%) showed minimum sugar utilization by YPD-3 at 72 hrs. and the *Halophila ovalis* hydrolysate (62.8%) showed minimum by *Saccharomyces cerevisiae* at 96 hrs and the *Halodule pinifolia* hydrolyzate (63.7%) fermentation showed minimum by YPD-1 at 96 hrs. All the microbes showed maximum sugar utilization at 120 hrs for *Cymodocea serrulata*, 72 hrs for *Syringodium isoetifolium* and 96 hrs for *Halophila ovalis* and *Halodule pinifolia* hydrolysate fermentation.

25. Among the identified yeasts, the YPD-5 (*Candida sp.*) showed maximum production of ethanol by *Cymodocea serrulata* (2.7 g.l⁻¹),
Syringodium isoetifolium (1.5 g.l\(^{-1}\)), Halophila ovalis (2.2 g.l\(^{-1}\)) and Halodule pinifolia (2.6 g.l\(^{-1}\)) hydrolysates.

26. The cell density of the marine yeast and MTCC microbes were gradually increased when the fermentation period increased.

27. The maximum average detoxification percentage was recorded in Halophila ovalis (40.7%) and Halodule pinifolia (40.7%) hydrolysate. However, the detoxification percentage of Cymodocea serrulata (39.3%) and the Syringodium isoetifolium showed 38.8% of detoxification.

28. The Molisch’s test result 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 viz., glucose, fructose, galactose, lactose, maltose and xylose. However, the iodine test showed the absence of starch.

29. The morphometric and proximate composition values of the milk mushroom Calocybe indica was found maximum in paddy straw substrate than the other substrates. The elemental analysis of Calocybe indica reveals that, the level of nitrogen (N) was found maximum (2.9±0.9 mg.g\(^{-1}\)) in paddy straw and the maximum level of phosphorous (P) (2.5±0.4 mg.g\(^{-1}\)), potassium (K) (2.0±0.6 mg.g\(^{-1}\)) and
zinc (Zn) (2.0±0.7 mg.g\(^{-1}\)) were found in sterilised *Syringodium isoetifolium*. The manganese (Mn) level found maximum (6.3±0.8 mg.g\(^{-1}\)) in sterilized *Cymodocea serrulata*. The maximum level of ferrous (Fe) (1.9±0.7 mg.g\(^{-1}\)) and copper (Cu) (7.0±0.8 mg.g\(^{-1}\)) were found in pretreated *Syringodium isoetifolium* and the level of carbon (C) was found maximum (3.6±0.4 mg.g\(^{-1}\)) in pretreated *Cymodocea serrulata*.

It is concluded from the present study that, the seagrasses collected from Thondi coast could be used for the production of biofuel ethanol and the spent could also used as a potential substrate for the cultivation of milk mushroom *Calocybe indica*. 