Chapter 6

SUMMARY AND CONCLUSION
Microbes constitute the invisible majority in the marine environment. Marine microorganisms include bacteria, fungi, yeasts, actinomycetes, viruses and protozoans. Eventhough there is considerable work on marine bacteria, study on marine yeasts is limited. Major properties of marine yeasts include mineralization of organic matter and mobilization of nutrients. Biotransformation of raw material into yeast biomass (single cell protein) is highly significant due to the nutritional quality of yeast and its possible utilization as animal or aquaculture feed supplement. They also have immunostimulatory property by virtue of their complex carbohydrate and nucleic acid components. Extra cellular metabolites from yeasts are also of considerable commercial importance.

The present work was focused on the occurrence and diversity of marine yeasts in the slope sediments of Arabian Sea and Bay of Bengal. Sediment samples were collected from 84 stations located at 200, 500 and 1000 m depths along the continental slope of Arabian Sea and Bay of Bengal. Hydrographical features and sediment characteristics were studied along besides the enumeration of the yeast population through plate count. The isolates were identified up to generic level based on morphological, physiological and biochemical characteristics. Growth of these isolates was examined at various pH, temperature and salinity to find out their optimal range. Hydrolytic enzyme production property of the isolates was also studied. Based on the very high lipolytic activity observed, the isolates were screened and subjected to crude oil degradation study. Black yeasts isolated during the study were subjected to detailed characterization focussing more on the bioactive compounds of commercial importance.

The salient findings of the study are as follows:

- In Arabian Sea at 200 m stations, the temperature of the sediment ranged from 14.4 to 16.1°C, at 500 m stations it ranged from 9.8 to 12.6°C and at 1000 m from 6.8 to 10.2°C. Salinity ranged from 34.9 to 35.8 ppt, and dissolved oxygen ranged from 0.95 to 2.01 ml/l in the study area.
In Bay of Bengal, the temperature of the sediment ranged from 12.1 to 16.8\(^\circ\)C along the 200 m depth stations, 8.6 to 11.3\(^\circ\)C at 500 m depth zones and 6.1 to 9.7\(^\circ\)C at 1000 m stations. The salinity ranged from 33.4 to 35.0 ppt and dissolved oxygen 0.04 to 1.34 ml/l in the sampling stations of Bay of Bengal.

In Arabian Sea the sediment texture was generally sandy at 200 m stations, silty at 500 m stations and clayey silt at 1000 m stations.

In Bay of Bengal the sediment texture was generally clayey silt at different depth stations except a few stations in the southern region of the Bay of Bengal.

In Arabian Sea the organic matter ranged from 0.83 to 12.8% at various stations. Maximum amount of organic matter was observed off Mumbai (12.8%). An increase in the organic matter content of sediment could be observed from 200 (2.23%) to 1000 m (7.1%) depth regions.

In Bay of Bengal, the percentage of organic matter was high along the northern region when compared to the southern region at all the three depth zones. The percentage organic matter ranged from 0.47% to 4.62% in the sediment samples at various stations which was found to be much less when compared to that of Arabian Sea. Even though the organic matter content was higher at 500 and 1000 m compared to 200 m depth regions, the increase was not significant.

In Arabian Sea (Cr. No. 228 & 233), the average yeast population was found to be maximum at 500 m (92.8 cfu/g dry weight of sediment) followed by 1000 m (35.2 cfu/g dry weight of sediment) and 200 m (12.5 cfu/g dry weight of sediment) depth regions. At 500 m depths, the northern transects (Off Coondapore to Porbander) showed comparatively higher population. However the population was negligible in the middle transects (Off Kannur to Ratnagiri).
The population in Bay of Bengal (Cr. No. 236) ranged from 0.19 cfu/g dry weight (Off Thammerapattanam) to 59.3 cfu/g dry weight of the sediment (Off Bheemuli). The population was very less off Karaikkal and Thammerapattanam in the estimated sample size. There was not much variation in average population between the various depth regions. Also the population was comparatively less than that of Arabian Sea.

Total population of yeasts in Bay of Bengal (Cr. No. 245) ranged from 0.18 cfu/g dry weight (Off Cuddallore) to 49.31 cfu/g dry weight of sediment (Off Kakinada). And generally the population was scanty in the northern transects. Yeast population varied considerably at different depth ranges and the population was maximum at 500 m depth followed by 200 and 1000 m depths.

Among the isolates obtained from Arabian Sea (Cr. No. 228 & 233), *Candida* (56.5%) was the predominant genus followed by *Lipomyces* (17.03%), *Rhodotorula* (11.8%), *Yarrowia* (9.5%), *Wingea* (1.7%), Black yeasts (1.3%), *Dekkera* (0.82%), *Debaryomyces* (0.67%) and *Pichia* (0.44%). Diverse genera were identified from 500 m stations.

Among the yeast isolates from Bay of Bengal (Cr. No. 236), *Candida* (46.4%) was the predominant genera identified followed by Black Yeasts (23.5%), *Wingea* (20.5%), *Rhodotorula* (3.38%), *Cryptococcus* (2.3%), *Bullera* (0.99%), *Yarrowia* (0.59%), *Lipomyces* (0.59%), *Dekkera* (0.39%), *Pichia* (0.39%), *Oosporidium* (0.39%) and *Trichosporon* (0.19%). Diverse genera could be recorded from 500 m stations and comparatively lesser from 200 and 1000 m depths. Black yeasts could be obtained only from 500 and 1000 m stations and formed a major group at these depths.

Among the Bay of Bengal isolates (Cr. No. 245), *Yarrowia* (42.2%) was the predominant genera identified followed by *Candida* (31.7%),
Summary and Conclusion

Cryptococcus (13.7%), Black yeasts (11.5%), *Debaryomyces* (1.33%), *Bullera* (0.88%) and *Lipomyces* (0.22%). Black yeasts were obtained from all the depths. Notably 39% of the isolates from 1000 m belonged to black yeasts.

- Among the isolates of Arabian Sea (Cr. No. 228 & 233), 92.5% were oxidative in nature and only 7.4% were fermentative. Genera wise analysis of the oxidative and fermentative forms showed that isolates belonging to the genera *Candida, Lipomyces, Yarrowia, Rhodotorula, Debaryomyces* and Black yeasts were cent percent oxidative. More than 95% of the *Wingea* spp. were oxidative and all the *Dekkera* spp. were fermentative.

- Among the isolates of Bay of Bengal (Cr. No. 236) 77% were oxidative and 23% fermentative. Genera wise analysis of the oxidative and fermentative forms showed that isolates belonging to the genera *Bullera, Oosporidium, Cryptococcus, Pichia, Lipomyces, Yarrowia, Trichosporon* and Black yeasts were cent percent oxidative in nature. *Wingea* and *Dekkera* were cent percent fermentative. Isolates belonging to *Candida* (63%) and *Rhodotorula* (93.3%) were mostly oxidative.

- Among the isolates of Bay of Bengal (Cr. No. 245) 58.4% were fermentative and 42.8% oxidative. Genera wise analysis of the oxidative and fermentative forms showed that isolates belonging to the genera *Bullera, Debaryomyces, Lipomyces* and Black yeasts were cent percent oxidative in nature whereas *Candida* and *Yarrowia* were cent percent fermentative. Isolates belonging to *Cryptococcus* (83.3%) were mostly oxidative.

- All the isolates of the Arabian Sea (Cr. No. 228 & 233) were lipolytic, followed by ligninolytic (15.8%), ureolytic (13.3%), proteolytic (8.9%), and amylolytic (4.4%) forms. None of the isolates produced aryl
sulfatase, DNAse, pectinase, cellulase and chitinase. Percentage of isolates producing protease, amylase and urease was more in 500 m depth zones. Black yeasts were cent percent positive for lipase, protease, amylase and ligninase. They were found to be the most potent isolates in enzyme production.

- All the isolates obtained from Bay of Bengal (Cr. No. 236) were lipolytic, followed by ligninolytic (63.7%), proteolytic (43.4%), ureolytic (36.2%), amylolytic (28.9%) and aryl sulfatase (1.45%) producing forms. None of the isolates produced DNAse, pectinase, cellulase and chitinase. Other than lipase production all other enzymes was found to be less in isolates from 200 m depth. The only isolate which produced aryl sulfatase was obtained from 500 m depth. Among the total isolates only one strain produced aryl sulfatase which belonged to the genus Cryptococcus isolated from 500 m depth station. Black yeasts were cent percent positive for lipase, protease, amylase, ligninase and 44.4% of them produced urease.

- All the isolates from Bay of Bengal (Cr. No. 245) were lipolytic, followed by proteolytic (28.5%), amylolytic (28.5%), ureolytic (18.1%) and ligninolytic (9.09%) forms. None of the isolates produced aryl sulfatase, DNAse, pectinase, cellulase and chitinase. Black yeasts were cent percent positive for lipase, protease and amylase. 40.9% were ureolytic and 31.8% ligninolytic.

- Most of the isolates preferred 30°C (69%) for maximum growth followed by 20°C (18.18%) and 40°C (12.72%). The isolates did not show growth at 10 and 50°C.

- Considerable growth could be noticed for all the isolates from 0 to 45 ppt salinity. However 15 to 25 ppt was found to be the most preferred range.
Most of the isolates showed maximum growth at pH 6 and 7. However, considerable growth could be recorded at a pH range 4-9.

Screening of lipolytic yeast isolates for oil degradation by visual observation showed that *Candida* sp. SD 302 and *Pichia* sp. SD 337 cause browning of the medium with dispersed tiny oil droplets.

These two potential isolates were identified by ITS sequencing as *Candida* sp. SD 302 and *Pichia guilliermondii* SD 337.

For *Candida* sp. SD 302, the growth was maximum at 30°C, 15 ppt salinity and pH 7. In the case of *Pichia guilliermondii* SD 337, the optimum growth conditions were 40°C, 25 ppt salinity and pH 7.

Suspended cells of *Candida* sp. SD 302 showed degradation of components C12 to C24 and also C28-C32. Immobilized *Candida* sp. SD 302 showed complete degradation of components C12 to C16, C18 to C24 and C28 to C32.

Suspended cells of *Pichia guilliermondii* SD 337 showed complete degradation of components C12 to C16 and C18 to C24. Complete degradation of components i.e. C12 to C14, C20 to C24, C28 to C36 and C32 to C36 was shown by immobilized *Pichia guilliermondii* SD 337.

The isolates, *Candida* sp. SD 302 and *Pichia guilliermondii* SD 337 were found to be potential degraders of n-alkanes both as free and immobilized cells.

The black yeast isolates showed radiating colonies on agar plates. The cells reproduced asexually by budding and fission. All the isolates showed filamentous growth. Molecular identification by ITS sequencing confirmed that the black yeast isolates belong to *Hortaea werneckii*.

All the black yeast isolates were able to produce lipase, protease and amylase. About 60% of the isolates were able to produce ligninase and
40% produced urease. All the isolates were oxidative in nature. They were found to be versatile agents of biodegradation by virtue of the extracellular enzyme production.

- The black yeast isolates had maximum growth at 30°C, 30 to 60 ppt salinity and pH 8.

- NMR spectroscopy of the melanins extracted from the 10 black yeast isolates showed that the melanins were of 1, 8-dihydroxynaphthalene (DHN) type.

- Black yeasts melanins exhibited inhibitory activity against fish / human pathogens tested.

- The black yeast extract (crude enzyme) was found to degrade the melanin extracted from the black yeasts.

The present study provides an account of the occurrence and diversity of marine yeasts in the slope sediments of Arabian Sea and Bay of Bengal. It also gives a clear idea about the role of yeasts in the benthic realm of marine ecosystem. The lipolytic potential of the organisms indicate the presence of rich lipid moieties in the study area. The isolates, *Candida* sp. SD 302 and *Pichia guilliermondii* SD 337 were proved to have potential oil degrading property and can be employed as bioremediators of oil spill after further characterization. The black yeasts isolated during the study area were found to have high commercial value by virtue of the by-products obtained from them. The melanin and the melanin degrading enzyme extracted from these organisms are potential bioactive materials for application in cosmetology.