Chapter 8

SUMMARY AND CONCLUSION
SUMMARY

Fatty acids are composed of a long hydrocarbon chain and a terminal carboxylate group. A great variety of fatty acids exist in nature. Fatty acids can be broadly categorized into three types: saturated, monounsaturated and polyunsaturated fatty acids. Saturated and monounsaturated fatty acids are not necessary in the diet as they can be synthesized in the human body. Certain polyunsaturated fatty acids are, however essential in the human diet, as the human body cannot synthesize them and hence known as essential fatty acids (EFAs). Long-chain polyunsaturated fatty acids (PUFAs) have more than one double bond and 18 or more carbon atoms. They are classified according to the position of the first double bond as counted from the methyl terminus. A ω-3 PUFA has its first double bound at position 3 as counted from the methyl terminus whereas a ω-6 PUFA has its first double bond at position 6 as counted from the methyl terminus.

Docosahexaenoic acid (DHA, 22:6), a long chain polyunsaturated fatty acid of the ω-3 family, is widely recognized to be an important dietary constituent. DHA is considered essential fatty acid because it cannot be synthesized denovo by humans. Although α-Linolenic acid is elongated and desaturated to DHA in mammals, it is believed that this conversion is very slow, particularly in the presence of large amounts of linolenic acid, as the two substrates compete for the same enzyme system and that DHA found in human tissues originates from dietary DHA.

The DHA is primary structural fatty acid of the highly active neural tissues and make up about 60% of the structural lipid in the grey matter of the brain. Since the retina, cerebral cortex, testes, and sperms of mammals are particularly rich in DHA, it has perceived functions in the nervous and reproductive systems!

The current commercial source of DHA is marine fish and its oil. Levels of DHA in fish oil are somewhat variable and can range from 8 to 20%. The composition and content of ω-3 fatty acids in fish oils from each source varies depending upon species of fish, season and geographical location of catching sites. The application

Dr. H. S. Gour University, Sagar and Indian Institute of Integrative Medicine, Jammu. 2008
of fish oil PUFAs in foods, for inclusion in infant formulas, or for pharmaceutical applications, may have some disadvantages because of contamination of the fish oil by environmental pollution and problems associated with the typical fishy smell and unpleasant taste. Furthermore, as marine fish oil is a complex mixture of fatty acids with varying lengths and degrees of unsaturation, expensive DHA purification may be required before application. As a result, alternative sources of high-quality PUFAs have been sought. Like humans, fish receive much of their LC-PUFAs from dietary sources, which in this case are the primary producers in the oceanic environment: the microorganisms.

A group of marine protists, *Schizochytrium*, are now considered potential sources for the production of DHA (Lewis et al., 1999). These organisms can be cultured in controlled bioreactors and thus can offer an alternative and unlimited source of DHA. The genus, *Schizochytrium*, consists of unicellular, zoospore producing, cosmopolitan, osmoheterotrophic organism found in saline lake, marine and estuarine waters. They are important sources for the production of polyunsaturated fatty acids, some of which are essential fatty acids such as docosahexaenoic acid (DHA, 22:6), docosapentaenoic acid (DPA, 22:5), eicosapentaenoic acid (EPA, 20:5).

This thesis focuses on the production of docosahexaenoic acid (DHA; 22:6), an ω-3 polyunsaturated fatty acid with applications in foods and pharmaceuticals, using *Schizochytrium* sp. PV-1. This heterotrophic marine protist has been studied since the end of the nineteenth century and has been identified as a good producer of DHA. *Schizochytrium* sp. PV-1 can accumulate lipid to over 20% of its biomass dry weight, with a high content of DHA (over 25% of the total lipid). The other polyunsaturated fatty acids represent less than 1% of the *Schizochytrium* sp. PV-1-derived oil. The aim of undertaking this research was to identify relevant process parameters for the large-scale production of docosahexaenoic acid (DHA). Several cultivation protocols for *Schizochytrium* sp. PV-1 were developed, analyzed and optimized with respect to the production of biomass, lipid and DHA. The solutions were sought for newly identified bottlenecks for industrial DHA production.
In chapter 2 extensive and comprehensive recent up to date review of the literature has been cited, and which describes in detail the basic structure of the fatty acids, lipids and various PUFA's giving emphasis on DHA. A brief overview of the other microorganisms being used for DHA production has been introduced.

The heterotrophic protist Schizochytrium sp. PV-1 was batch cultivated in media containing glucose, yeast extract, peptone; dihydrogen potassium phosphate, sea salt prepared in distilled water. Increasing amounts of yeast extract stimulated growth but influenced lipid accumulation negatively. Sea salt concentrations above half the average seawater salinity were required for good growth and lipid accumulation. Schizochytrium sp. PV-1 was able to grow on a glucose concentration as high as 60 g/l, concentrations above 60 g/l resulted in reduced yields of biomass as well as lipid. Although the culture in study effectively utilized certain simple and complex nitrogen sources lipid yield was more in complex nitrogen source yeast extract and peptone than in simple nitrogen source. Comparison of growth at 20 and 28 °C showed that the higher incubation temperature was more favorable for growth. The optimum pH was optimized as 7 with a rotation speed of 220 RPM. Incubation for 120 h was required for the maximum production of biomass and lipid and DHA in lipid. In this period, the percentage of docosahexaenoic acid of the lipid was found to be 20.0 % and 4.0 g/l respectively.

In chapter 4 fed-batch cultivation was studied as an alternative fermentation strategy for DHA production. All the fermentation parameters as well as media constituents were optimized for maximum growth and lipid production by Schizochytrium sp. PV-1. The fed batch cultivation resulted in higher biomass as well as lipid yields than batch cultivations. Glucose when used at an initial concentration of 20 g/l with a feeding of 10 g/l after every 24 h was found to produce maximum biomass (56 g/l) and lipid (5.4 g/l). The initial pH of the medium was optimized at 7. An incubation temperature of 28°C and 220 rpm was found to be optimum conditions for growth of the Schizochytrium sp. PV-1 under
fed batch cultivations. The fed batch cultivation of the culture resulted in increase in DHA content of the lipid (24.5 %) which was earlier 20 % in batch cultivation. In chapter 5, alcoholic beverage distillery waste water was used as a medium component. Alcoholic beverage distillery wastewater is one of the major pollutants which are concerning the environmental scientists worldwide. The distillery wastewater is known to contain many organic, inorganic solids and protein which can be utilized by *Schizochytrium* sp. PV-1 for production of DHA. The distillery wastewater was found to have an BOD value of 13,000 mg/ml. Total protein in the distillery wastewater was 0.51%. *Schizochytrium* sp. PV-1 utilized very effectively distillery wastewater as a source for nitrogen and a maximum biomass 59 g/l was observed when medium was prepared in 100% distillery waste water. 30 % of the lipid produced was found to be DHA. Further optimization studies helped in improving the yield of biomass as well as DHA yield.

So far, DHA production has been studied with glucose and other mono, di and polysaccharides as carbon sources. In chapter 6 studies was under taken to investigate the potential of sodium acetate, acetic acid and ethanol as an alternative carbon source for DHA production by *Schizochytrium* sp. PV-1. In shake flask cultures, the protest was able to grow on media consisting of yeast extract, peptone, KH₂PO₄, sea salt and sodium acetate/ethanol. Acetic acid was found to inhibit the growth as well as lipid production. Sodium acetate when used as carbon source produced a biomass of 43 g/l with a lipid content of 4.5 g/l in fed batch cultivation. Sodium sulphate when used as a replacement of sea salt for providing marine conditions, found to be promising and results obtained for biomass, lipid and DHA was comparable to that found in M4 medium. A DHA of 28.2% was obtained with sodium sulphate as a medium ingredient. Sodium sulphate when used in combination with distillery wastewater facilitated the growth and lipid production by *Schizochytrium* sp. PV-1.

To investigate the effect of scaling up on the production of biomass, lipid and DHA by *Schizochytrium* sp. PV-1, studies were undertaken in lab scale fermentors. Three different fermentor volumes were used viz 5-L, 15-L and 50-L.

---

Dr. H. S. Gour University, Sagar and Indian Institute of Integrative Medicine, Jammu. 2008
The *Schizochytrium* sp. PV-1 adjusted well to the high production volume as well the mechanical stress caused within the fermentor. Productivity increased in a linear manner from 5-L to 50-L fermentor. At 5-L scale 62 and 5.9 g/l biomass and lipid yields were produced. This production yield increased to 70 g/l and 6.4 g/l biomass and lipid respectively in 50-L fermentor. Maximum DHA content was observed in 50-L fermentor which accounted for 32% of the lipid produced. Experiments with distillery wastewater and sodium sulphate resulted in promising results which can be explored for commercial production of DHA.
CONCLUSION

- Docosahexaenoic acid (DHA) is an essential fatty acid. This LC-PUFA consists of 22 carbons with six double bonds, which belongs to ω-3 family of PUFAs.
- DHA recently attracted much attention because of its various physiological functions in the human body. It has perceived functions in the nervous, reproductive systems and development of the brain.
- The current commercial source of DHA is fish and fish oils, which also contain EPA and other more saturated fatty acids. Because of the relatively low proportion of DHA in fish oil and the problems encountered in extraction and purification of omega-3 fatty acids, large-scale production of DHA is difficult.
- In this study a marine protist Schizochytrium sp. PV-1 was used for the production of biomass and DHA rich lipid.
- In batch cultivation the organism under study produced (g/l) 47 and 4 biomass and lipid respectively. The DHA content of the lipid was 20%.
- The fed-batch cultivation resulted in increase in both biomass as well as DHA content and a maximum biomass, lipid (56 and 5.4 (g/l) was achieved. The DHA content was found to be 24.5% of the total lipid.
- The Schizochytrium sp. PV-1 effectively utilized the alcoholic beverage distillery waste water as a nitrogen source and produced 30% of the lipid as DHA. A maximum biomass and lipid of 59 and 5.8 g/l was obtained with distillery waste water as a medium component.
- Growth of the organism was not so effective on C₂ compounds and resulted in low lipid and DHA production. Sodium acetate and ethanol resulted in the production of 4.3 and 2.7 g/l lipid respectively. Sodium sulphate when used as an replacement for sea salt produced a maximum biomass of 56.7 g/l. The DHA content of the lipid was found to be 28.2 %
- The high production volume as well as the mechanical stress caused within the fermentor did not showed any negative effect on the growth of
the organism and DHA production. A maximum biomass and lipid of 62.5 and 5.9 g/l respectively was obtained in 5-L fermentor. When further scaling up was done in 50-L fermentor a biomass of 70 g/l was obtained. The DHA content (32 %) in the total lipid (6.4 g/l) was produced.

- It can be concluded that the organism in study *Schizochytrium* sp. PV-1 is a good candidate for the production of biomass and lipid rich in DHA. By carefully manipulating the cultivation conditions DHA yields were increased. The organism in study can be a good source for commercial production of DHA and therefore can help in overcoming barrier between the demand and supply of this essential fatty acid (DHA).