

DRYING KINETICS AND LIPID COMPOSITION OF A MICROALGAE CONSORTIUM
GROWN IN A RACEWAY POND

by

THIRUVENKADAM VISWANATHAN

(Under the Direction of Sudhagar Mani)

ABSTRACT

Microalgae are promising feedstock for sustainable biofuel and bioenergy production. Preprocessing of harvested biomass involves several technical challenges that prevent the commercial success of the technology. In this study, thin layer drying kinetics of mixed species microalgae consortium suitable for biofuel production was studied at different drying temperature of 30, 50, 70 and 90°C. Effect of cell rupture pretreatments like French press, sonication and autoclave on the moisture removal rate, diffusivity and lipid properties of the consortium was also assessed. Page's model described the experimental moisture loss data of both ruptured and intact microalgal cells. It was also found that cell rupture had increased drying constant, effective diffusivity and the lipid extraction yield through solvent extraction and supercritical carbon dioxide extraction when compared to the intact cells.

INDEX WORDS: Microalgae, Biofuel, Drying Kinetics, Cell rupture, Supercritical Carbon dioxide extraction

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DEDICATION

To appa, amma and thambi.....

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CHAPTER 1

INTRODUCTION

Biofuels are rapidly gaining interest all over the world, due to issues on energy security, sustainability and greenhouse gas (GHG) emissions. Prolonged use of petroleum based fuels has resulted in higher concentration of CO₂ exceeding pre-industrial concentrations by almost 100 ppm. If the atmospheric CO₂ level does not stabilize at the 550 ppm and rise through 2100, the average temperatures could rise by more than 3°C, ocean levels could rise up to 3 feet, extreme weather events will occur more frequently, and disease transmission may become more prevalent. Biofuels, if produced and used appropriately, can avoid drastic climate changes and dependence on fossil fuels. In fact, not all the currently available biofuels from first and second generation biomass feedstock like corn, sugarcane, soy are beneficial when their environmental impacts are assessed on a life cycle basis (Scharlemann and Laurance, 2008). Hence novel biomass feedstock with higher potential and lesser impact on environment are needed. A new U.S. policy mandates the production of 36 billion gallons of renewable fuels by 2022 of which at least 21 billion gallons must be advanced biofuels (i.e., non-corn based fuel). Apart from the environmental benefits, the new biofuel infrastructure is expected to generate jobs that create significant socio-economic impacts (DOE Algae roadmap 2009).

Microalgae is one of the promising non-conventional, third generation biofuel feedstock. They are unicellular, photosynthetic microorganisms that are abundant in fresh water, brackish water, and marine environments. They utilize CO₂ and sunlight to generate the complex biomolecules necessary for their survival and these biomolecules are promising candidate for

large scale sustainable biofuel production (Chisti, 2007; Spolaore et al., 2006). The advantages of microalgae are,

- High photosynthetic efficiency (up to 6%) and carbon neutral.
- Does not compete with food source and grow in arable land and it can be grown in saline and waste water.
- Many species of algae can be induced to produce high concentrations of a chosen, commercially valuable compound, such as proteins, carbohydrates, lipids and pigments depending up on growth conditions.
- Lack of lignin enables easier thermo/bio chemical conversion to fuels and chemicals when compared to woody biomass.

Microalgae biomass is usually harvested from the growth system as slurry of about 20% solids. Usually the preprocessing steps like drying and cell rupturing are applied to harvested biomass before energy conversion. Drying removes both the intracellular and the bulk water which are problematic for biofuel applications. Bulk water can either promote the formation of emulsions in the presence of ruptured cells, or participate in side reactions. At the cellular level, it can be a barrier between the solvent and the solute. In this context, the issue of solvent access to the material being extracted is as important as the miscibility of the analyte in the solvent. Hence drying of microalgae slurry is a critical step in algal biomass production as it has the following advantages.

- In enhances lipid extraction efficiency by increasing the solvent accessibility to the lipids
- It prolongs shelf life
- It helps in better transportation and storage

- It is essential for the thermochemical energy conversion processes like pyrolysis, combustion, and extraction of pigments and other high value products

However, drying is energy intensive and it is expected to increase the cost of production by almost 75%. It is interesting to note that, extensive research has been carried out on the options available for algae harvest up to concentrations of 20% solids, like flocculation, centrifugation, but drying and cell rupturing has not been studied in detail. (Benemann et al., 1982; Benemann et al., 1977; Benemann and Oswald, 1994; Sheehan et al., 1998). A preliminary look at the energy balance of algal biomass production shows that any harvesting/drying scheme involving dry algae is energy prohibitive, requiring at least 60% of the energy content stored in algae. For example, an algae species with energy content of 5 Wh/g would require as much as 2.4 Wh/g of energy for harvesting/drying alone. But precise quantification of microalgae drying energy requirement is not available in the literature. Hence, it is critical to extensively study the drying aspect of the biomass production scheme to achieve considerable improvements in energy usage and to precisely determine the importance of drying in the algae biomass production chain.

Modeling of algal drying processes and systems would be a useful tool to assess the energy requirement and efficiency of current drying methods and to develop novel, low cost, eco-friendly drying systems. The most essential part of developing a new drying process for a specific material, involves determination of the drying kinetics of the material. Drying kinetics study describes the mechanism and the influence certain process variables on moisture removal processes (Kiranoudis et al., 1997). Studying the compositional changes, especially the lipid content change, with respect to drying would be useful to understand the effect of drying on composition and to further optimize the temperature of drying for desired biomass feedstock quality.

Cell rupturing is another important preprocessing step that helps in effective removal of analyte from the biomass matrix. The isolation of intracellular material requires the cells to be disintegrated by physical, chemical or enzymatic means to release their cytoplasmic content (Chisti and Moo-Young, 1986). Cell rupturing has been proven to increase the lipid extraction efficiency but its impact on the moisture removal rate has not been studied in detail. As mentioned earlier, the water in the algal slurry is essentially from both extracellular and intracellular water. Cell rupturing may expose the bound water inside the cell to the hot air which may increase the rate of drying. Hence the overall goal of this research is to study the drying characteristics of algal biomass suitable for biofuel application, improve the drying behavior by applying effective cell rupture and to further study the lipid property changes associated with drying and cell rupture.

CHAPTER 2

OBJECTIVES

1. To investigate the thin layer drying kinetics of mixed species microalgae consortium grown in raceway ponds at various drying temperatures.
2. To study the effect of cell rupture pretreatment methods (sonication, French press and autoclave) on the drying behavior of mixed microalgae species.
3. To study the effect of drying temperature and cell rupture on the lipid properties of the consortium using solvent extraction.
4. To evaluate the impact of cell rupture on the lipid extraction by supercritical CO₂ extraction.

CHAPTER 3

LITERATURE REVIEW

3.1 Microalgae Classification and Cell Structure

Microalgae were among the first life forms to appear on our planet (Falkowski and Raven, 1997). Microalgae make up to 0.2% of global photosynthetic biomass but account for approximately 50% of the global organic carbon fixation and contribute to approximately 40% to 50% of the oxygen in the atmosphere. The general structure of microalgae is given in figure 3.1. Some common classes of algae include the green algae (*Chlorophyceae*), diatoms (*Bacillariophyceae*), yellow-green algae (*Xanthophyceae*), golden algae (*Chrysophyceae*), red algae (*Rhodophyceae*), brown algae (*Phaeophyceae*), picoplankton (*Prasinophyceae* and *Eustigmatophyceae*) and cyanobacteria (*Cyanophyta*). Among these classes, green algae and diatoms are ideal candidates for biofuel application, where cyanobacteria are unsuitable as they do not store lipids. Generally, green algae have a cell wall made up of primarily cellulose and pectin and store food mainly as starch. Diatom cell walls are predominantly silica with some cellulose and cellular energy is stored as carbohydrates and lipids. Green algae like *Chlorella*, *Dunaliella* and diatoms like *Thalassiosira* and *Phaeodactylum* are extensively studied for biofuel applications (Thompson, 1996). It can be observed that the composition of microalgal cell wall varies not only with the species and but also between different stages of the growth cycle in the same species.

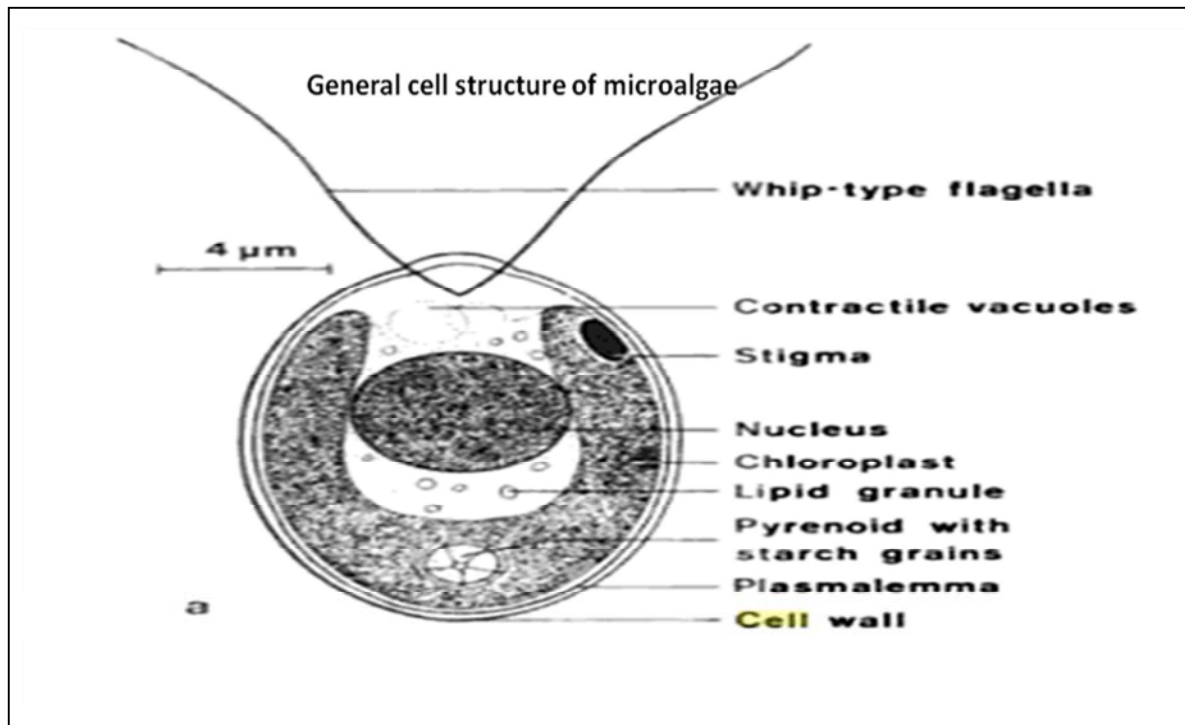


Fig 3.1. Cell structure of microalgae. (Adapted from Berner 1993)

3.2 Current Commercial Applications of Microalgae

The current market for microalgae is small and limited to a handful of products. The global microalgae biomass market produces 5000 t/year, most of which are produced in Asian countries (Pulz and Gross, 2004) and primarily used as pharmaceuticals, aquaculture feed and food additives. Microalgae species like *Arthrospira*, *Chlorella*, *Dunaliella salina* and *Aphanizomenon flos-aquae* are used as human feed supplement as tablets, capsules, and additives to food items like pasta, chips (Spolaore et al., 2006). On the other hand, *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, *Thalassiosira*, *Nannochloropsis oculata*, *Haematococcus pluvialis*, are cultivated for aquaculture feed and *Arthrospira*, *Nannochloropsis oculata* are grown for cosmetic applications (Apt and Behrens, 1999; Borowitzka, 1997; Muller-Feuga, 2000; Yamaguchi, 1996). High value polyunsaturated fatty acids like γ -Linolenic acid (*Arthrospira*), Arachidonic acid

(*Porphyridium*), Eicosapentaenoic acid (*Nannochloropsis*, *Phaeodactylum*, *Nitzschia*) and Docosahexaenoic acid (*Cryptothecodinium*, *Schizochytrium*) are produced at small scale. Additionally, carotenoids like β -carotene, astaxanthin, lutein, zeaxanthin, lycopene and bixin from *D. salina* or *H. pluvialis* (Del Campo et al., 2000; Vilchez et al., 1997) and phycoerythrin, phycocyanin from *Arthrospira* and the *Porphyridium* are also commercially available. Stable isotope biochemicals like B-phycoerythrin, R-phycoerythrin, Sensilight PBXL1: anti GST obtained from microbial source are helpful in structural determination and metabolic studies. Further commercial applications of algae are discussed elaborately in the literature (Radmer, 1996; Raja et al., 2008).

3.3 Biofuel Potential and Application

Microalgae are a potential feedstock for a wide range of energy conversion strategies in both thermochemical and biochemical platforms like gasification, combustion, liquefaction, pyrolysis, hydrogenation, anaerobic digestion and fermentation (Amin, 2009). Especially, its high lipid content attracts significant interest in liquid transportation fuel (Schenk et al., 2008). A recent projection from NREL suggests that the theoretical maximum production would be $354,000 \text{ L ha}^{-1} \text{ year}^{-1}$ of unrefined and the best cases projection indicates an yield $40,700\text{--}53,200 \text{ L ha}^{-1} \text{ year}^{-1}$ of unrefined oil. It should be noted that the lowest projection in the report, $40,700 \text{ L ha}^{-1} \text{ year}^{-1}$ is drastically higher than reported yields for corn, canola, or even oil palm (172 , 1190 , and $5,950 \text{ L}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ respectively) (Hu et al., 2008) (Table .1). Other than biodiesel application, lipids can also be used to produce biodistillates, commingled with crude petroleum or used as boiler fuels, in mixtures and in pure forms (Tyson, 2005). In spite of all the above mentioned advantages, microalgae biofuels are still not commercialized and the industry is considered still to be in infancy. But, more than 150 small companies have been formed (mostly

in the past two years) to participate in algal biofuel commercialization with an investment of about \$180 million for research and development (Pienkos and Darzins, 2009).

Table 3.1 Oil production potential of microalgae

Feedstock	Oil Yield (gallons/acre)
Corn	18
Cotton	35
Soybean	48
Mustard Seed	61
Sunflower	102
Rapeseed	127
Jatropha	202
Oil Palm	635
Algae (10 g/m ² /d)	635
Algae (25 g/m ² /d)	2637
Algae (50 g/m ² /d)	10549

Microalgae species with high carbohydrate content are preferred for bioethanol production where high lipid content is preferred for either biodiesel or biogas applications. In the promising microalgal classes (green algae and diatoms) for biofuel application, protein constitute for almost half of the cell composition followed by either carbohydrate or lipid depending upon the growth conditions and the growth phase at the harvest (Becker, 2007; Ben-Amotz et al., 1985). Selection of appropriate species for the desired end product determines the choice of growth system, growth conditions, nutrient requirements and harvest method for the biomass production.

Table 3.2 Cellular composition of different microalgae species

Species	Generation Time(hr)	Protein (%)	Carbohydrate (%)	Lipid (%)
<i>Chlorella vulgaris</i>	8.7	51-58	17	14-22
<i>Chlorella pyrenoidosa</i>	-	57	12	2
<i>Spirulina sp</i>	28.7	61.4	3	8.5
<i>Arthospora maxima</i>	24.3	60-71	13-16	7
<i>P. tricornutum</i>	16.5	35.2	32	13.3
<i>I. galbana</i>	8.7	37	11.7	7.1
<i>Anabena cylindrica</i>	-	43-56	25-30	7
<i>Tetraselmis suecica</i>	11.3	41-44	13	30-32
<i>C. calcitrans</i>	9.3	40.5	11.4	11.4
<i>Euglena gracilis</i>	11.6	39-61	14-18	14-20
<i>Porphyridium cruentum</i>	34.7	28-39	40-57	14
<i>Scenedesmus sp.</i>	13.9	50-56	17	14
<i>Botryococcus braunii</i>	13.3	22	14.1	44.5
<i>Dunaliella salina</i>	6.7	29.3	16.3	25.3
<i>Nanochloris sp</i>	6.7	33.1	13.2	20.8
<i>Aphnizomenon flosaquae</i>	-	56	25-30	7
<i>Chlamydomonas reinhardtii</i>	-	22	17	21
<i>Synechococcus sp</i>	27.7	63	15	11
<i>Thalassiosira pseudonana</i>	8.8	46	25	21

Extensive screening of potential microalgal species for biofuel application is one of the main accomplishments of aquatic species program. This program has screened more than 3000 species for high lipid content suitable for transportation fuels. Different species of microalgae had varied ability for oil production. Autotrophic microalgae, such as *Chlorella vulgaris*, *Botryococcus braunii*, *Navicula pelliculosa*, *Scenedesmus acutus*, *Cryptocodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochloris oleoabundans*, *Phaeodactylum*

tricornutum, and *Tetraselmis sueica* can accumulate oils (Chisti, 2007; Liang et al., 2006). Oleaginous algae like *Chrysophytes*, *Haptophytes*, *Eustigmatophytes*, *Dinophytes*, *Xanthophytes* or *Rhodophytes* (Hu and Gao, 2006) show an average total lipid content of 25.5% (in normal growth conditions) to 45.7% (in nutrient stress) (Hu et al., 2008). Unfortunately, the success of most of these species in outdoor mass production is not as promising as lab scale studies. Cellular compositions of different species are listed in table 3.2.

3.4 Algal Biomass Production

A typical algal biomass production chain consists of cultivation, harvest, and preprocessing and energy conversion steps. A system diagram of microalgal biomass production chain along with possible energy conversion options was given in figure 3.2. Microalgae are produced in the growth medium as dilute cultures of about 0.3-1 g/l. Through different stages of dewatering, 15-20% solid slurry can be obtained. Detailed review of each step is presented.

3.4.1 Growth Systems

Microalgae need sunlight, CO₂ (for photosynthetic growth) or carbon source (for heterotrophic growth), water, and land for efficient biomass production (Chisti, 2007; Chisti, 2008). Any arrangement that can supply the above mentioned requirements can be utilized as growth system for microalgae. The growth of microalgae, by itself is a complex process, sensitive to numerous parameters like light exposure, mixing, diffusion of gases that demands efficient system design to maximize the photosynthetic efficiency (Grobelaar, 2000). Different growth systems that were tested for algal biomass production have been discussed below.

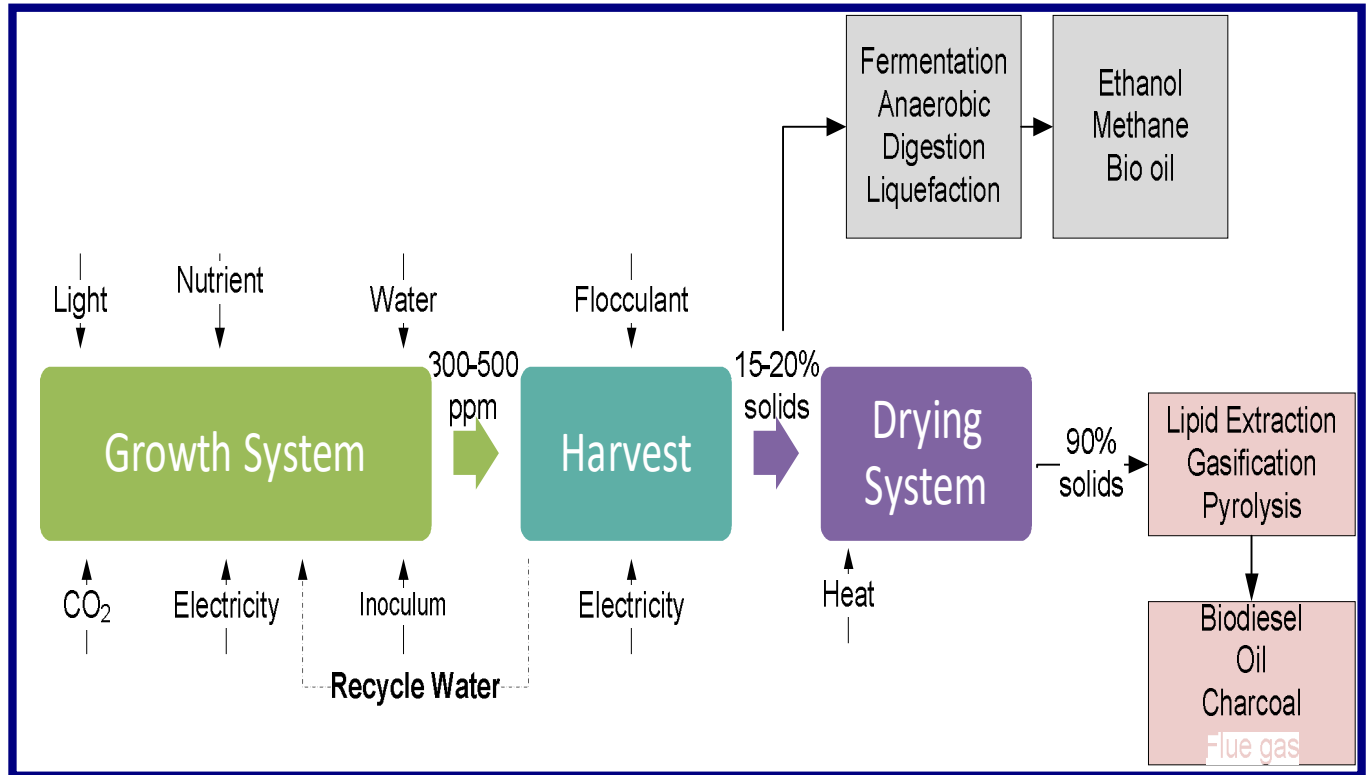


Fig 3.2. Microalgae biomass production and energy conversion scheme

3.4.1 Open Pond Systems

Open ponds are usually 80-90 cm deep ponds, with little or no mixing. High growth rates can be achieved, initially, due to lower cell density and higher dilution rate. Growth rate decreases progressively due to limited light exposure (Benemann and Oswald, 1994). Unmixed open ponds as large as 250 acre have been operated in Australia (Betatene Ltd) for *Dunaliella* production. Low Productivity ($1 \text{ g/m}^2/\text{d}$), high land requirement, and predator (zooplankton) dominance, makes this system unsuitable for intensive microalgae cultivation. Otherwise, unmixed open ponds are viable option for low quantity biomass production in places where land costs are minimum and sunlight is available all through the year (Borowitzka, 1999). In mixed open ponds, a central arm, spanning the entire pond, provides slight mixing that can increase the productivity to $8\text{-}21 \text{ g/m}^2/\text{d}$ (Benemann and Oswald 1994). Shallow ponds with diameter of 50 m

and land occupancy of 2000 m² were reported. The scale up is hindered by the strain of water resistance on the rotating motor (Yun et al., 1997).

Raceway ponds are the current option for intensive microalgae biomass production that are operated at industrial scale all over the world. It is the most productive, open pond system, primarily because of constant mixing by paddle wheels. Paddle wheels help in suspension of microalgae, effective carbon dioxide diffusion and light exposure. Cell concentration of about 0.5 g L⁻¹ can be maintained, and a productivity of about 25 g m⁻² d⁻¹ has been widely reported. Nevertheless, production of monoculture is limited only to extremophiles like *Dunaliella* (high salinity), *Spirulina* (high bicarbonate and pH) and *Chlorella* (high nutrition). The productivity variation with respect to the local environmental condition is a critical issue in generalization of results obtained from open pond based studies. Also, water loss due to evaporation, CO₂ escape to the atmosphere, threat of contamination, pond crash, and high volume processing at downstream due to dilute cultures are the major demerits in open pond systems. In spite of all these defects, raceway ponds are the choice of cheap biomass production for their low capital investment. Different raceway pond dimension designed were 10:1 ponds, 2:1 (length: width) (Putt, 2007) with an area occupancy ranging from 1 acre to 4 acre. The depth of the pond is usually maintained around 15-30 cm with an operational water flow velocity of 30 cm/s around the pond. The depth of the ponds is the most critical parameter in the design, as it decides the light exposure, mixing speed and hence the productivity (Benemann and Oswald 1994). Different configurations of the open pond systems are depicted in figure 3.3.

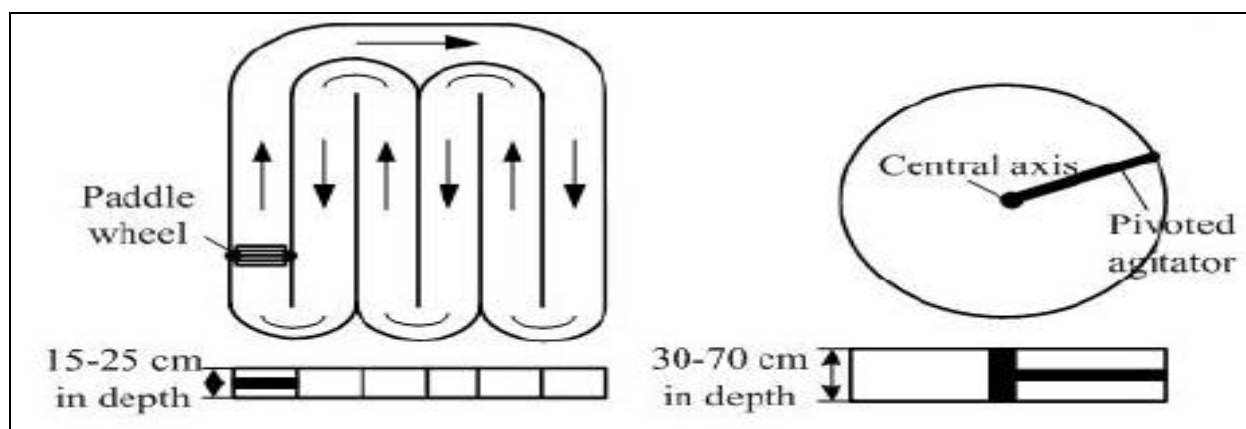


Fig 3.3. Raceway pond and open mixed pond system for algae biomass production

Another interesting system developed and operated in Czech Republic is expected to be competitive to raceway ponds. In this turf scrubber type thin-layer system, the culture circulated in shallow trays at a very low thickness of about 1 cm. The base material is either glass base (Livansky, 1997; Livansky and Doucha, 2000), other cheaper materials like geotextile foam that can achieve productivities as high as 31 g/m²/d (Doucha et al., 2005). Moreover similar cascade systems that are positioned at lower angle further reduce the culture depth to 0.6-0.8 cm that can enhance the productivity.

3.4.2 Closed photobioreactor System

Closed photobioreactors are suggested as alternative to open raceway ponds, and was extensively studied in Israel and Japan initially during 1970's the same time when U.S had started the aquatic species program to study algal production in raceway ponds. An extensive review of different photobioreactors and the design parameters is available in the literature (Carlozzi, 2008; Molina Grima et al., 1999; Pulz, 2001). A brief introduction to photobioreactors is presented in this report regarding the successful model and limitations for basic understanding.

High cell density and excellent species specificity can be obtained in this system making it attractive for the production of high value pharmaceutical products. Tubular photobioractor

and flat panel photobioreactors shown in figure 3.4 are the most successful designs that can be used in large scale applications. Other alternatives like horizontal straight tubes connected by U-bends, α -type photobioreactor with cross tubes arranged at an angle with the horizontal, flexible tubing coiled around a vertical cylindrical frame work are also available. The basic principle behind these system is to reduce the light path in the closed chamber so that the amount of light available for each cell can be maximized (Grima, 2004). Mixing was provided mechanically or through air lift to enhance gas transfer at gas-liquid interface and equal exposure of light to each cell. The optimum thickness of the algal culture in these reactors is between 2 and 4 cm (Borowitzka, 1999) but light paths up to 10 cm are reported. Temperature control is provided by evaporative cooling using cool water sprinklers or by immersing the system in water bath.

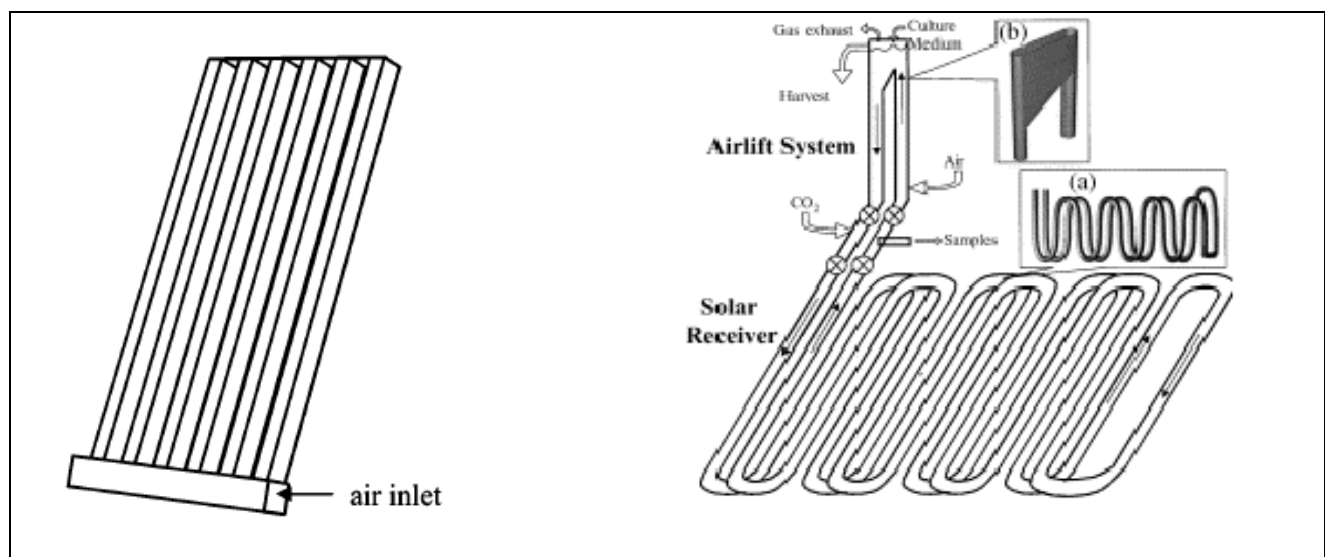


Fig 3.4. Flat panel photobioreactor and tubular photobioreactor configuration

In tubular photobioreactors different configurations like serpentine, long manifold and helical tube photobioreactors are available. Similarly for flat panel photobioreactors, alveolar (with internal partitions) and glass plate photobioreactors are available. Different construction materials like pyrex glass, PVC, LDPE film, and clear acrylic (polymethyl methacrylate,

PMMA(Burgess and Fernández-Velasco, 2007) also known by the trade names Plexiglas and Perspex were tested and the productivities were reported.

Apart from technically sophisticated photobioreactors, simple closed systems like plastic bag columns with air mixing alone is also widely studied. Accurate temperature controls, continuous mode of production are the main constraints in large scale application of such a bag-based system. Hydrodynamic parameters like mass transfer coefficient (K_La), gas hold up, aeration rate, mixing rate are critical for high cell concentration. In most of the designs, it is very difficult to maintain the same hydrodynamic characters during scale up resulting in low productivities in larger systems (Molina et al., 2000; Ugwu et al., 2008). Shut down of few models of photobioreactors Inc is a classical example for such scale up issues. Photo inhibition, temperature gradient, carbon dioxide gradient formation, pH variations, wall growth, settling are significant obstacles for scale up of this system.

3.5 Harvest and Processing

Harvesting is the process of removal of algal cell suspensions from the growth medium and it can contribute up to 20–30% to the total cost of biomass production (Gudin and Thepenier, 1986). This step offers the largest technical and economical challenges out of all steps considered in an algal biomass production process (Williams and Laurens 2010). In the past little consideration has been given to developing harvesting techniques that maximize the value of products (biorefining) obtained from the microalgae, since their focus has been on removal of algal cells from drinking water (Henderson et al., 2008) or aimed at a single product (Greenwell et al., 2010). Nevertheless, in the limited studies carried out on algal biomass production for biofuel applications, harvesting from raceway pond is usually carried out in two stages namely primary and secondary dewatering steps. Primary harvest methods include dissolved air

flotation, microstrainer, vacuum belt filter, autoflocculation, and bioflocculation and the secondary harvest is usually centrifugation. On the other hand, the microalgae grown in photobioreactor can be harvested by a single harvesting step, usually with a centrifuge, due to the higher cell density 1-3 g/l that can be achieved in photobioreactors. Detailed literature on principle of each dewatering technologies applicable to algae are available in the literature (Uduman et al. 2010).

3.6 Current Drying Systems for Algae

Drying is an essential part of algae biomass production scheme for most of the thermochemical energy conversion platforms, extraction of pigments and high value products. In fact a myriad of options are available for each stage of biomass production (fig A5) but the drying part has not been studied on par with either cultivation or harvest. This is because, most of microalgae studies in the past are concerned about methane production (1-2% solid slurry), and bio-oil extraction where drying is not necessary (Benemann et al., 1982; Benemann et al., 1977; Benemann and Oswald, 1994; Sheehan et al. 1998).

Spray Drying is the most commonly applied drying method for food additive and pharmaceutical preparations of *Spirulina*, *Chlorella* or *Dunaliella* at industrial level. Spray drying can yield powdered product that need no further size reduction with better quality than other drying methods for human feed purpose (Richmond, 1987; Shimamatsu, 2004; Soeder, 1980). It is suitable for high-value products (>1000 \$/ton) and its applicability for biomass production (300 \$/ton) seems uneconomical. Drum drying was recommended instead of spray drying because of better digestibility of the dried biomass, less energy requirements, and lower investments (Mohn, 1978). Electrically heated drum dryer with a surface area of 0.5 m² and evaporation capacity of 20 l/h/m² was tested to dry algae slurry of 30% solids at 120°C with a

power consumption of 52 kWh (Becker and Venkataraman, 1982). Besides, an energy input of 1.4 kWh was needed to run the dryer itself. Replacement of the electrically heated drum dryer by a steam heated drum dryer was proven to lower the cost of processing *Scenedesmus* by 6.8 times (Mohn, 1978). Lyophilization and vacuum drying have also been tried at lab scale, but considered to be cost prohibitive at industrial level. Different methods for cryopreservation of microalgae were reported in the literature (Taylor and Fletcher, 1998). Sun drying is probably the cheapest option but it depends heavily upon local temperature and relative humidity. In general, a drying rate of 100 g/sq.m/day can be achieved, which demands very large area for drying (Mohn, 1978). Hence drying needs to be done at extremely low cost either by designing of new efficient drying systems or by significantly improving the existing systems.

3.7 Theory of Drying and Drying Curves

Two fundamental processes that take place during drying are

- Heat Transfer - Transfer of heat to evaporate liquid from the biomass and
- Mass transfer - Transfer of mass as a liquid or vapor within the biomass and as a vapor from the surface of the biomass

In heat transfer aspect of convective drying, the transfer of heat from a moving gas phase (hot air) provides the heat for drying to a solid phase (biomass). The rate of heat transfer is described in terms of a heat transfer coefficient h :

$$Q = hA(T - T_s) \quad (3.1)$$

where Q is the heat flux, A is the area of solid exposed to hot air, T is the temperature of air and T_s is the temperature at the solid surface.

The mass transfer aspects of drying can be conveniently described by using a drying curve shown in figure 3.5.

Initially, the mass of water next to the gas moving across the wet biomass evaporates. The rate of movement of water from the inside to the surface is rapid enough that the surface remains saturated and the drying rate remains constant for a period called the constant drying rate drying period. During this period mass transfer is limited by a gas boundary layer at the surface of the solid. At critical moisture content, X_c , the internal rate of water movement is not enough to keep the surface saturated and hence the rate of drying begins to fall, as the rate is calculated still on the total surface area rather than wetted surface area. This drying period is called falling rate drying period, where the drying rate asymptotically approaches the equilibrium moisture content.

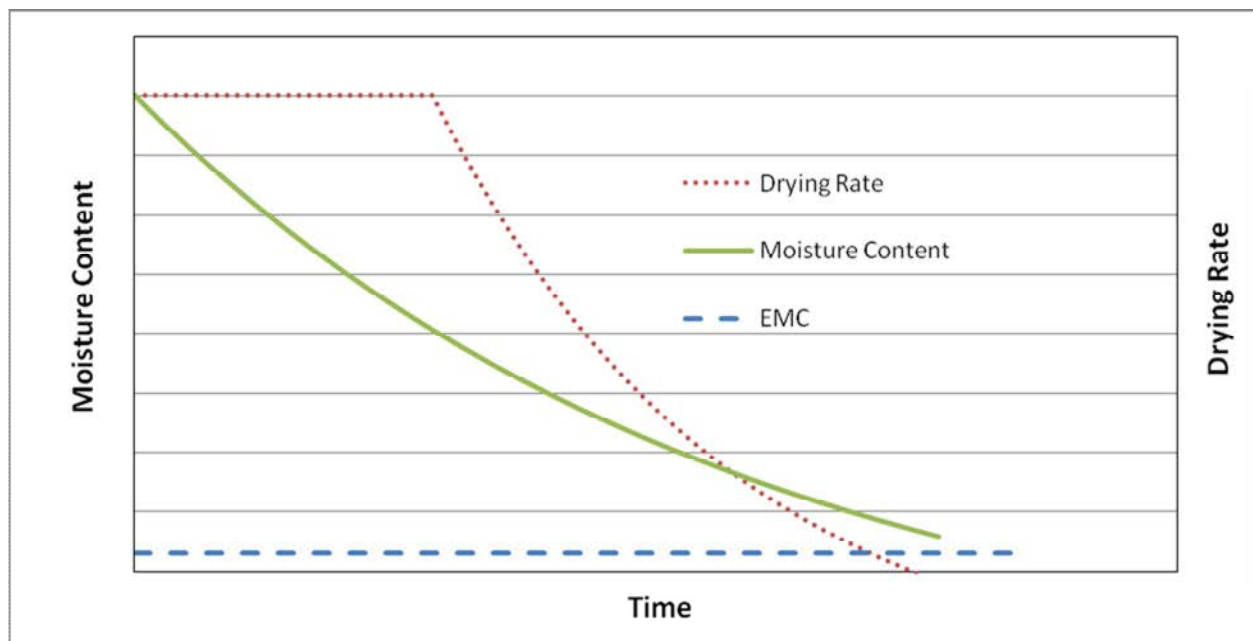


Fig. 3.5. Drying curve for biological materials

Usually, there is no constant rate drying period in completely hygroscopic biological solids or in any solid when the mass transfer is controlled completely by internal diffusion.

3.8 Theory of Drying Kinetics Studies

The most essential part of drying process development involves determination of the drying kinetics, which describe the mechanisms and the influence that certain process variables exert on moisture removal processes (Kiranoudis et al., 1997). The phenomenon underlying the drying process is a complex problem that involves simultaneous mass and energy transfer in a hygroscopic shrinking system (Ratti, 2001). A complete description of the actual mechanisms involved in drying is usually not obtainable, and would certainly be hopelessly complex (Carbonell et al., 1986; Hawlader et al., 1991; Mazza and LeMaguer, 2007). Hence thin layer drying kinetics equations are used to get insights into physical mechanism governing the drying process, in a less complicated and less time consuming fashion. The thin layer drying kinetics of agricultural products can be explained in either one of the three ways.

1. Theoretical Equations
2. Semi theoretical Equations
3. Empirical equations

Most of the theoretical equations have been derived from the diffusion model based on Fick's second Law for different geometries (Akpınar, 2006) or simultaneous heat and mass transfer equations. Fick's second law can be applied to explain the dehydration behavior of biological materials that normally follows a falling-rate drying period where the moisture migration is defined by diffusion. Assuming that the resistance to moisture migration is uniformly distributed within the homogenous isotropic material, this law can be stated as

$$\frac{\partial MI}{\partial t} = \nabla(D_{ef} \nabla MI) \quad (3.2)$$

where MI is the local moisture content, t is the drying time and D_{ef} is the effective diffusivity.

The solution to this equation is based on the geometry of the material. Crank (Crank, 1975) gave the analytical solutions to this equation for various regularly shaped bodies such as rectangular, cylindrical and spherical bodies. For infinite slab geometry, assuming that the moisture is initially uniformly distributed throughout the sample, that mass transfer is symmetric with respect to the centre, that the surface moisture content of the sample instantaneously reaches equilibrium with the conditions of the surrounding air and that shrinkage is negligible, the solution is

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n+1)^2} \exp(-(2n+1)^2 \pi^2 \frac{D_{ef} t}{4L^2}) \quad (3.3)$$

where moisture ratio MR is the unaccomplished moisture change defined as the ratio of the free water still to be removed at time t to the total free water initially available n is the number of terms considered in the expansion series, t is time, and L is the half-thickness of the slab if the evaporation takes place in both sides of the slab.

For the case of drying of a spherical body with constant radius r_o , if we assume constant values of D throughout the drying process, uniform initial moisture distribution, surface moisture equal to the equilibrium moisture content and symmetrical radial diffusion. The solution is given by the following relationship

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp(-n^2 \pi^2 \frac{D_{ef} t}{r_o^2}) \quad (3.4)$$

where r_o is the radius of the sphere.

In semi theoretical models, simplification of solution series to Fick's law is used to describe the thin layer drying. For slurry like algae, thin layer spread has been considered as one of the option for rapid drying and hence the solution of Fick's law for slab geometry becomes

relevant. For long dehydration periods, a limiting form of Eq. (3.3) is obtained by considering only the first term in the series expansion. Then, Eq. (3.3) can be written in the following form

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 D_{ef} t}{4L^2}\right) \quad (3.5)$$

The effective diffusivity, D_{ef} is a lumped parameter resulting from the contribution of all mass transport mechanisms like gas diffusion, capillary flow, and bound water migration occurring in different phases (Silva et al., 2000; Zogzas et al., 1994).

Although transport properties like liquid diffusivity, thermal conductivity are necessary to fully describe the drying kinetics of materials (Doymaz and Pala, 2002; Karathanos and Belessiotis, 1999; Panchariya et al., 2002; Sokhansanj, 1984), a single drying constant K , combining the effect of the various transport phenomena, is most commonly used in semi-theoretical models (Babalís and Belessiotis, 2004). Hence the first term of Eq. (3.5) can be approximated to the form

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = A \exp(-kt) \quad (3.6)$$

The above equation is called Henderson-Pabis model. This model has been used to predict the drying constant of corn (Henderson and Pabis, 1961), wheat (Watson and Bhargava, 1974) and peanut (Moss and Otten, 1989). The slope of this model, k is related to diffusivity when drying process takes place only in the falling rate period and liquid diffusion controls the process which is typical in drying of agricultural products (Madamba et al., 1996). It is important to note that the diffusivity value calculated from semi-theoretical models are called apparent diffusivity rather than effective diffusivity as this value represent the average of the diffusivity values measured across the entire drying process. On the other hand, the Lewis model (Lewis, 1921) is a special case of the Henderson-Pabis model where intercept is unity. By comparing the

drying phenomenon with Newton's law of cooling, the drying rate is proportional to the difference in moisture content between the material being dried and the equilibrium moisture content at the drying air condition. This law can be stated as

$$\frac{dM}{dt} = -k_o (M - M_{eq}) \quad (3.7)$$

This equation can be represented in an integrated form as

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \exp(-kt) \quad (3.8)$$

The semi-theoretical equations are simpler and take less computing time in comparison with theoretical equations and provide some understanding of the transfer process.

Empirical equations are developed from the satisfactory fit to experimental data. Various empirical equations simulate the kinetics of mass transfer (water) as a function of initial conditions such as temperature, pressure, air velocity, charge density, form and size, pretreatments, and others (Vega et al., 2007). These models neglect the fundamentals of the drying process and their parameters have no physical meaning. Therefore, they cannot give a clear view of the important processes occurring during drying. Among them the Thompson model (Thompson et al., 1968), Wang and Singh model (Wang and Singh, 1978), Page model and modified Page model are commonly used to predict the drying of agricultural products.

The effect of drying temperature on the model parameters like effective diffusivity or the drying rate can be expressed by an Arrhenius type equation (Karatas, 1997; Madamba et al., 1996; Maskan et al., 2002; Vaccarezza and Chirife, 1978)

$$\theta = \theta_o \exp(-E / RT) \quad (3.9)$$

where θ , denotes the drying rate or diffusivity, E denotes the activation energy (kJ mol^{-1}); R denotes the universal gas constant ($\text{kJ K}^{-1} \text{mol}^{-1}$); and T the absolute temperature (K). The value E can be calculated from the slope and the intercept of the linearized Arrhenius type equation (Barreiro et al., 1997).

3.9 Drying Kinetics of Microalgae:

Zepka et al (2007) investigated the dehydration characteristics of the cyanobacterium *Aphanothece microscopica Nageli* in a convective hot-air dryer at air temperatures of 40, 50 and 60°C and sample thicknesses of 3, 5 and 7 mm. The experimental dehydration data were fitted to the Henderson-Pabis model and the drying rate constant are situated among 0.005 and 0.024 min^{-1} . Effective diffusivity, calculated using Fick's second law was found to be between 8.1E-8 and 18.8E-8 $\text{m}^2 \text{s}^{-1}$. Similarly, the Henderson-Pabis model (Oliveira et al., 2009) was used to model the drying of *spirulina* in spherical shaped loads of 4 and 6 kg/m^2 . It was also found that Guggenheim, Anderson and de Boer (GAB) equation was more effective than Brunauer, Emmett and Teller (BET) correlations to model the isotherm data for spirulina. The drying rate constant k varied between 3.5E-4 and 4.2E-4 s^{-1} and the effective diffusivity changed between 2.86E-11 and 3.42E-11 for the air temperature of 50 and 60°C respectively in 4 kg/m^2 spheres. In another study on *spirulina* drying, (Desmorieux and Decaen, 2006) a long first drying period was observed for mild condition (40° C and air velocity below 2.5 m/s) where it was absent in more harsh drying conditions (60° C and air velocity 3.8 m/s). The drying rate was limited to 2.2 $\text{g}_w/\text{kg}_{db}/\text{s}$ in harsh conditions and it was a constant below 1 $\text{g}_w/\text{kg}_{db}/\text{s}$ for mild drying conditions. In an attempt to explore the possible shape transformation of final dried *spirulina* product, convective and infra red drying behavior of spirulina as small cylinders and thin layer was characterized. It was

observed that the spreading out in layers or in cylinders leads to comparable maximum rate of drying around $1.4 \text{ g}_w/\text{kg}_{bd}/\text{s}$ for a same specific area characterizing product-air exchange surface.

Drying kinetics of the red alga *Gracilaria* was studied (Lemus et al., 2008) at four temperatures (40, 50, 60 and 70°C) and it was observed that the Modified Page and Wang-Singh models were the best models for explaining the desorption characteristic of this algae. The authors have also proposed two new models that describe the experimental desorption curves. In another study on solar drying of the red algae *Gelidium sesquipedale*, the authors (Ait Mohamed et al., 2008) described the drying characteristics of this algae by a two term model in comparison with 13 other models. Vega-Galvez et al (2008) studied the drying kinetics of *Macrocystis pyrifera* at 50, 60, 70, and 80°C . The effective diffusivity increased from 5.56 to $10.22\text{E}-9 \text{ m}^2/\text{s}$ as temperature increased from 50 to 80°C . Midilli-Kukuc and logarithmic models obtained the best-fit quality for drying curves where GAB equation described the sorption isotherm. It is important to note that all of the above mentioned drying kinetics studies on algae have based their diffusivity value on the assumption that the shrinkage and porosity are not significant during drying. Interestingly, a recent study on the drying kinetics of *Spirulina* cylinders, the authors (Dissa et al., 2010) have stated that effective water diffusivity was influenced by shrinkage and its value would be over estimated by about 36.48% to 92.41% when shrinkage was not considered in diffusivity calculation. It was also found that *Spirulina* shrinkage was found to be weak and anisotropic. The glassy nature of the product during drying tended to reduce shrinkage and to facilitate pores and cracks formation. The dried product was very porous with a porosity approaching 80%.

3.10 Microalgae Cell Rupture Treatments:

A variety of disruption methods are available to disintegrate strong cellular walls and membranes and liberate the cell contents (Chisti and Moo-Young, 1986). The classification of cell rupture methods is depicted in figure 3.6. Microalgae cell disruption is carried out in concentrated cell preparations (50–200 kg m⁻³ dry weight) to minimize the cost and energy requirements.

Liquid shear methods involve either high pressure homogenizers or ultrasonication. The high-pressure homogenizer consists of a positive displacement piston pump with one or more plungers. The cell suspension is drawn through a check valve into the pump cylinder and, on the pressure stroke, it is forced through an adjustable discharge valve with restricted orifice. The pressure drop across the valve, not the operating pressure, was suggested as the main factor in cell disruption (Chisti and Moo-Young, 1986). High pressure homogenizers are widely used to disrupt *Haematococcus* cells for fish feed purpose (Molina Grima et al., 2003). Sound waves of frequency 15-20 kHz is known to cause inactivation and disruption of microbial cells. It was shown that the ultrasonication of suspended microalgal cells can be used to disrupt microalgal cells at small scale (Bermejo Román et al., 2002; Dunstan et al., 1993) but the application at large-scale is still a challenge.

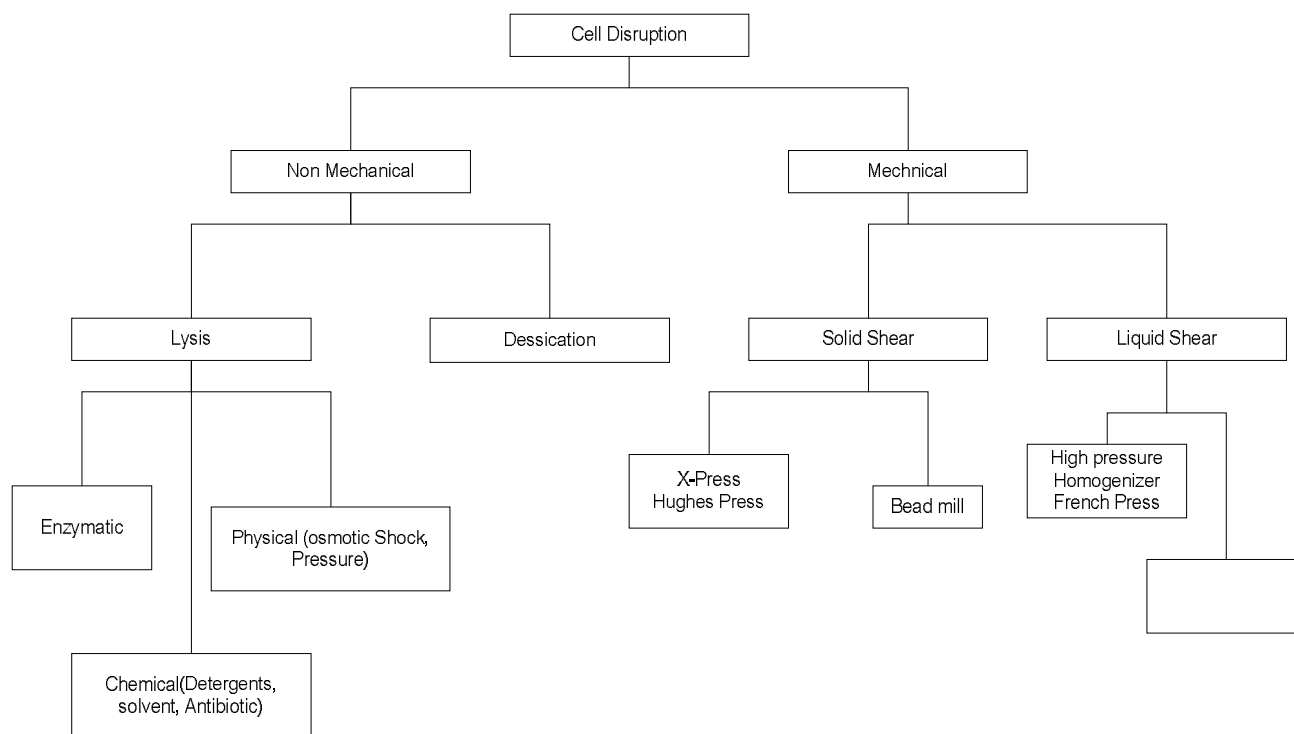


Fig. 3.6. Classifications of cell rupture methods

Cell disruption in bead mills is regarded as one of the most efficient techniques for solid shear based cell disruption. These mills consist of either a vertical or a horizontal cylindrical chamber with a motor-driven central shaft supporting a collection of off-centered discs or other agitating elements. The chamber is filled to the desired level with steel or glass beads which provide the grinding action on the algal cells. Agitation of microalgal biomass in presence of glass and ceramic beads (0.5 mm diameter) in bead mills (Chisti and Moo-Young, 1986) has been used to disrupt cells of *Scenedesmus obliquus*, *S.platensis* and *Monodus subterraneus* (Hedenskog et al., 1969).

Additionally, freeze-pressing of microbial cell suspensions can be used to disrupt the cells. A frozen paste of algal slurry is forced through a narrow slit or orifice, either in the presence of an abrasive at temperature just below zero or without the abrasive at temperatures of about -25°C (Chisti and Moo-Young, 1986). For the extraction of sensitive cell metabolites

enzymatic cell rupture using lysozyme was practiced but often expensive. Acids, alkalis, surfactants and solvents have been utilized for cell lysis. Combination of different rupture methods may also be used to rupture thick cell walled algae.

Lee et al (2010) compared different cell rupture method like, bead-beating (0.1 mm diameter, 2800 rpm for 5 min), microwave (about 100° C and 2450 MHz for 5 min), sonication (10 kHz for 5 min), osmotic shock using 10% NaCl solution and autoclave (125 C with 1.5 MPa) to break *Botryococcus sp*, *Chlorella vulgaris* and *Scenedesmus sp* cells and concluded that the microwave based rupture is simple, fast and effective. Similarly, for recovering astaxanthin from encysted cells of *Haematococcus pluvialis*, autoclave or mechanically disruption using high pressure homogenizer was more effective than hydrochloric acid, sodium hydroxide, enzyme and spray drying (Mendes-Pinto et al., 2001). Bubrick (1991) used cryogenic grinding of dried *Haematococcus* biomass to extract astaxanthin with butylated hydroxytoluene. This method is not realistic for large-scale commercial use. While treatment with alkali is an effective method of rupturing the cell wall, it is not generally suitable for sensitive products such as proteins (Molina Grima et al., 2003). In another study, four different cell disruption methods bead-beater (1 mm diameter for 2 min), French press, sonication and wet milling were studied for their effectiveness in solvent extraction of algal lipids from *S. dimorphus* and *C. protothecoides*. Wet milling was most effective for *S. dimorphus* lipid extraction, whereas bead-beater disruption was found to be the most effective method for *C. protothecoides* (Shen et al., 2009). In spite of large number of methods available for cell rupturing, only two process namely homogenization and bead milling are proved on large-scale non algae applications (Greenwell et al., 2010).

3.11 Compositional change of Algae Biomass with Drying:

The drying condition has been proven as an important factor that can affect the composition of algae. The protein content depends directly on the intensity and duration of the drying treatment that could result in some transformations in the physicochemical state of the biological matrix. A number of drying methods studied for the processing of *Spirulina* like cross flow drying, spray drying and oven drying resulted in approximately 50% loss of phycocyanin (Sarada et al., 1999). Similarly, it was observed (Esquivel et al., 1993) that the drying of two diatoms *Chaetoceros* sp. and *Phaeodactylum tricornutum* that the convective hot air drying at 30°C had caused 70% loss of total lipids, while freeze drying had left the proximate composition of the two diatoms unchanged (Esquivel et al., 1993). The reason for the high percentages of lipids lost initially was due to the fact that diatoms store at least part of their lipids as oil droplets. These oil droplets might be more volatile than other types of lipids, even at the low temperature. Leach et al (1998) concluded that the choice of appropriate drying conditions can affect the beta-carotene composition of *D.salina* and high outlet temperature in spray-drying should be practiced to produce a b-carotene rich powder from *D. salina*, without excessive degradation or change in isomer composition. Zepka et al (2007) have stated that the drying conditions can significantly affect the macronutrient composition of the biomass with respect to the protein, carbohydrate and lipid contents. In their study, they have demonstrated that the lipid content significantly changes with drying condition but it did not influence the polyunsaturated/saturated ratio of the biomass. Contrastingly, drying *Chlorella vulgaris* at 60°C have resulted in a slight decrease of triglyceride content where drying at 80°C or higher temperature have significantly decreased the triglyceride content when compared to freeze dried biomass (Widjaja et al., 2009).

3.12 Microalgae Lipid Extraction and Analysis

Microalgae synthesize fatty acids as building blocks for the formation of various types of lipids. Natural lipids generally comprise of mixtures of non-polar components such as triacylglycerol (TAG) and cholesterol, free fatty acids and more polar components like glycolipids, phospholipids. The most commonly synthesized fatty acids in algae have chain lengths that range from C16 to C18 similar to those of higher plants (Ohlrogge 1995). Fatty acids are either saturated or unsaturated, and unsaturated fatty acids may vary in the number and position of double bonds on the carbon chain backbone. In general, saturated and mono-unsaturated fatty acids are predominant in most algae examined (Borowitzka, 1988). During stress periods, especially in nutrient starvation condition, the algae stores TAG inside the cell.

Traditionally, the lipids are extracted from the dry algal biomass through a number of ways. The least expensive extraction is though cold pressing. Up to 70% of the oil contained within the algae can be extracted this way (Danielo, 2005). The use of organic solvents can increase the extraction efficiency. Several solvent based extraction procedures are reported. Folch et al (Folch et al., 1957) was one of the first to develop the chloroform/methanol/water phase system which was later modified by Bligh and Dyer (Bligh and Dyer, 1959). The concept of like dissolves like is the basis of co-solvent extraction procedure. The method exposes the lipid containing algae cell to a miscible co-solvent mixture comprised of an alcohol (methanol) and an organic solvent (chloroform). In this sense, methanol and chloroform combine to form a co-mixture solvent that favorably interacts with the lipids, thus leading to their dissolution into the co-solvent. The primary advantage of the Bligh and Dyer method is the reduction in the solvent/sample ratio (1 part sample to 3 parts of 1:2 chloroform/methanol followed by 1 or 2

parts chloroform) where the Folch method employs a ratio of 1 part sample to 20 parts of 2:1 chloroform/methanol, followed by several washings of the crude extract (Iverson et al., 2001).

It was found that the Bligh and Dyer method has underestimated the lipid content in samples of marine tissue that contained more than 2% lipids but worked well for samples that contained less than 2% lipids. Consequently, other combinations of co-solvents have been proposed for the extraction of lipids: hexane/isopropanol for tissue (Hara and Radin, 1978; Nagle and Lemke, 1990); DMSO/petroleum ether for yeast (Park et al., 2007); Hexane/ethanol (Cartens et al., 1996); and dodecane, NaOH-added methanol (Kang and Sim, 2007); heptane-ethanol-water-sodium dodecylsulfate (1:1:1:0.05 v/v/v/w); methylene chloride/methanol (2:1 v/v); hexane/acetone (1:1 v/v); dichloroethane/methanol (1:1 v/v) and acetone/dichloromethane (1:1 v/v) were few of them. In another interesting study, ethanol was used to extract the lipids from the lyophilized biomass and a biphasic system was formed by adding water and hexane to the extracted crude oil. In this way, most of the lipids were transferred to the hexane phase while most impurities remained in the hydroalcoholic phase (Fajardo et al., 2007). In general co-solvent should be designed in such a way the higher polar solvent breaks the cell wall, make the cells porous and extract the lipids from the cell wall, where the less polar co-solvent matches the polarity of the lipid to be extracted. It should also be noted when designing co-solvent systems to extract the entire range of lipids, one need to be aware that the use of more polar solvents will improve the range of lipids extracted but they may also decrease the carrying capacity of the solvent. This is because; solvents that extract polar lipids are not miscible with relatively high ratios of non-polar lipids. The sequence of solvent addition can also affect the extraction (Lewis et al., 2000) and the presence of water in algae would have significant effect in phase partitioning of the lipids in chloroform/methanol extraction (Akoh and Min, 2002). In spite of a numerous

co-solvent systems reported above, chloroform/methanol based system is widely used for total lipid extraction and hexane based system was for neutral lipids. The solvent fraction of chloroform usually consists of hydrocarbons, carotenoids, chlorophylls, sterols, triacylglycerols, wax esters, long-chain alcohols, aldehydes and free fatty acids. Methanol fraction consists of phospholipids and traces of glycolipids.

In algal lipid extraction studies, it can be observed that different solvent systems have been used and the optimal method varies from species to species. It has been stated that the methodology for lipid analysis has not been standardized and the literature values should be approached with healthy level of skepticism (Pienkos and Darzins, 2009). For *Botryococcus braunii* methanol chloroform extraction was more effective than hexane/isopropanol (3:2, v/v), dichloroethane/methanol (1:1 v/v), dichloroethane/ethanol (1:1 v/v), and cetone/dichloromethane (1:1 v/v) (Lee et al., 1998). The dichloroethane/methanol and dichloroethane/ethanol systems which were recommended (Nikolova-Damyanova et al., 1992) for the lipid extraction of the green alga *Cladofora*, but it was not effective for *B. braunii*. Grima and co-workers (Grima et al., 1994) have tested seven different solvent systems for *Isochrysis galbana* and concluded that Bligh and Dyer method rendered the highest yield of lipids (93.8%), followed by ethanol and hexane/ethanol (84.4 and 79.6%, respectively). Modifications to the Bligh and Dyer method have been adopted to improve the extraction efficiency specific to the sample. They included addition of water to the solvent mixture for dried samples, sonication of the samples (Dunstan et al., 1992), addition of water to freeze dried samples before solvent addition (Dunstan et al., 1993), increasing the methanol proportion in the solvent mixture.

Once the lipids are extracted from the biomass, they are usually derivatized to fatty acid methyl esters (FAME) with methanol and analyzed through gas chromatography. To reduce

time, and to combine the process of lipid extraction and FAME production, some direct transesterification reactions involve a mix of solvent, alcohol, and catalyst were attempted. Direct transesterification allows for a single step process that extracts and the algal oils and reacts them with methanol to result in FAME. Lepage and Roy (Lepage and Roy, 1984) have reported direct transesterification procedure with methanol-benzene 4:1 with acetyl chloride that is applicable for analysis of both simple (triglycerides) and complex lipids (cholesteryl esters, phospholipids; and sphingomyelin). The solvent works to extract the lipid as the alcohol and catalyst convert them into methyl esters. Others use heat combined with methanol and catalyst to remove and transform the fatty acids (Liu and Zhao, 2007). Lewis et al (2000) found that direct transesterification greatly increased the total amount of fatty acids extracted and that the extraction efficiency increased with time of the reaction. Direct transesterification has led to a higher recovery of medium chain and long chain fatty acids in human milk and adipose tissue (Lepage and Roy, 1984). Fatty acid profiles of numerous microalgae species were reported. (Fajardo et al., 2007; Hu et al., 2008; Jiang et al., 2004; Matsunaga et al., 2009; Zepka et al., 2008).

3.13 Theory of Supercritical CO₂ extraction

The primary disadvantage of solvent extraction is that the cellular materials are often denatured by the solvent, and it is very difficult to decontaminate such material as a result. The use of solvents at a large scale also requires additional costs owing to the very high standard of plant design criteria because of the risk of fire and explosion hazards (Greenwell et al., 2010). Supercritical carbon dioxide extraction (ScCO₂) has attracted significant interest as a promising alternative to conventional solvent extraction (Herrero et al., 2006). When carbon dioxide is compressed to its critical temperature (31.1°C) and pressure (72.9 atm), it becomes a dense gas

instead of becoming a liquid. Carbon dioxide at this state is called supercritical carbon dioxide (ScCO₂) and it behaves like a solvent. ScCO₂ recover non-polar neutral lipids that include esterified fatty acids, acylglycerols and unsaponifiable lipids. Solubility of seed lipids/fats and pure triglycerides can be found in the detailed studies of Stahl et al. (1988). Addition of methanol, ethanol or even water can increase the solubility of polar lipids in ScCO₂. In theory, each compound possesses a unique extractability under different conditions of supercritical CO₂ related to factors such as extraction temperature, pressure, and time. Theoretical considerations for designing supercritical extraction process were explained in detail by Rozzi and Singh (2002) and other reviews (Demirba 2001; Herrero et al., 2006). By appropriate control of the physical properties of the ScCO₂, each component of interest in a sample matrix can be extracted in an ordered manner, allowing for convenient fractionation of the extract (DOE 2009). This can reduce the cost and time involved in the separation compared to traditional solvent extraction for purifying specific components of interest from the extract (Xu and Godber, 2000).

3.14 Supercritical Carbon dioxide extraction of lipids:

ScCO₂ extraction have been applied for extraction of lipid from *Dilophus ligulatus* (Subra and Boissinot, 1991), red seaweed (Cheung, 1999), hydrocarbon from *Botryococcus braunii* (Mendes et al., 1994; Mendes et al., 2003), carotenoids from *Spirulina* (Mendes et al., 1995). It has been proved that ScCO₂ enhances the selectivity of extraction without any chlorophyll contamination. Compounds like gamma-linolenic acid from *Arthospira maxima* (Mendes et al., 2003; Mendes et al., 2006), beta-carotene from *Chlorella vulgaris*, *Dunaliella salina* (Mendes et al., 2003), bioactive lipids from *Nanochloropsis* species (Andrich et al., 2005), b-cryptoxanthin and zeaxanthin from *Spirulina Pacifica* (Careri et al., 2001) were selectively extracted.

The main process variables involved in the process are temperature, pressure, flow rate, flow direction, extraction time and the use of co-solvent. In general, pressure has a larger impact on the extraction efficiency than the temperature (Thana et al., 2008). It was also found that ruptured cells can yield better extraction probably due to higher accessibility of supercritical fluid to the analyte bound to the cell. (Mendes et al., 2003; Valderrama et al., 2003). It can be observed from the table 3.3. that the working condition for maximum extraction of the desired product varies with the species, system and the product itself. This technology is also preferable as the spent biomass is free of any solvent and can be utilized directly for methane, ethanol production without preprocessing.

Table 3.3. ScCO₂ condition for different products from microalgae

Species	Temperature (K)	Pressure (mPa)	Product	Reference
<i>Botryococcus braunii</i>	313.1	30.0	Hydrocarbons	Mendes et al., 1994
<i>Chlorella vulgaris</i>	328.1	35	Carotenoids	Mendes et al., 1995
<i>Spirulina maxima</i>	343.15	18	Total fatty acid	Canela et al., 2002
<i>Danaliella salina</i>	313.1	20	Beta carotene	Mendes et al., 2003
<i>Spirulina platensis</i>	313.15	20	Total fatty acid	Mendes et al., 2006
<i>Nanochloropsis</i>	328.15	70	Total fatty acid	Andrich et al., 2005
<i>chaetomorpha linum</i>	323.15	26	Total fatty acid	Aresta et al., 2005
<i>Haematococcus pluvialis</i>	333.15	30	Astaxanthin,	Valderrama et al., 2003
<i>Hypnea charoides</i>	323.15	37.9	omega 3 Fatty acids	Cheung, 1999

CHAPTER 4

MATERIALS AND METHODS

4.1 Biomass Production

Isolates of green algal species namely *Chlorella globosa*, *Chlamydomonas minutissima*, *Scenedesmus bijuga* were inoculated together in simulated raceway ponds of 1.52 m wide, 2.44 m long, and 0.15 m deep mixed by a paddle wheel. The working volume was 550 L. The individual cultures of these three species were initially maintained in 200 ml flasks. They were further scaled up to 4 L flask, then to 20 L carboy flasks and finally to the 150 L vertical tubes prior to cultivation in the raceway ponds. The microalgae species were allowed to grow at least for one week at each step of the above mentioned scale-up process. Nutrients were supplied with BG11 medium (Stanier et al., 1971) and 5-6% CO₂-air mixture was bubbled at a rate of 10 L min⁻¹ in the raceway ponds. The major components of the growth medium were NaNO₃ 1.5 g/l, K₂HPO₄ 0.04 g/l, MgSO₄ 0.075 g/l, CaCl₂ 0.036 g/l, Citric Acid 0.06 g/l, Ferric Ammonium Citrate 0.06 mg/l, EDTA 0.001 g/l and other trace elements like manganese, zinc and copper. Evaporative water loss was monitored and compensated by adding tap water. Biomass was determined periodically by filtering 10 mL of culture through a dried, pre-weighed 4.7 cm Whatman GF/C glass fiber filters and washing it with 10 mL of 0.65 M ammonium formate solution to remove excess salts and drying at 103°C. The biomass concentration was determined gravimetrically by weighing the vacuum cooled filter fibers. After 10 days of growth in the raceway ponds, the biomass was harvested using a centrifuge (AML Industries Inc. Model 12-413V). The centrifuge was operated with an inlet flow rate of 230 L h⁻¹ at 2250 g. These

microalgal species are used for the study as they have shown continued dominance for the local environment (Chinnasamy et al., 2010). The centrifuged slurry was frozen until further processing.

4.2 Biomass Compositional Analysis

The microalgal diversity and the extent of contamination in the raceway was determined using light microscopy. The total lipid content was determined by Bligh and Dyer (Bligh and Dyer 1959) with slight modifications (Kates and Volcani, 1966). An aliquot of 0.1 g of dried algal biomass was powdered and extracted with chloroform: methanol: water (2:2:1) solvent system. At the second step of extraction, 0.85% NaCl solution was used instead of water to remove any protein contamination in the extract. Further, the chloroform layer was filtered through the pinch of sodium sulfate placed over a filter paper to remove any traces of water and the algal cell in the chloroform layer. The lipid content was measured gravimetrically by evaporating the chloroform at 40°C in a clear glass vial under continuous stream of nitrogen. Carbohydrate content was estimated spectrophotometrically by phenol-sulfuric acid method (Dubois et al., 1956). This method had extracted sugars, oligosaccharides, polysaccharides and their derivatives. Protein content was determined from nitrogen percentage obtained from ultimate analysis (CHNS) using a factor of 6.25 (Boyd 1968). Proximate analyzer (TGA-701, LECO Corporation, Michigan, US) was used to investigate volatile matter, ash, fixed carbon. Higher heating value (HHV) was determined using an adiabatic oxygen bomb calorimeter according to the ASTM D 2015. In addition to the estimation of total lipids using chloroform/methanol solvent system, hexane-soluble lipid content was measured using Ankom extractor. A know weight (W_1) of powdered algae was placed in XT4 extraction bags and sealed with the impulse sealer. After drying, the extraction bags were kept in a re-sealable plastic bag

with desiccant material while each individual bag was removed and carefully weighed (W_2). The extraction bags were then placed into the extractor and the extraction was performed for 2 h at 90°C with hexane as solvent. After extraction, the bags were then transferred to the forced-air oven and dried at 60°C overnight and cooled in a desiccator. The bags were then weighed (W_3) and the hexane-soluble lipid content was estimated using the following equation

$$Lipid\% = (w_2 - w_3) * 100 / w_1 \quad (4.1)$$

4.3 Cell Rupture Treatment

An aliquot of 10 g of microalgal slurry was ruptured using three methods. 1. Autoclave at 121°C and 15 psi for a duration of 45 min. 2. Ultrasonication (Biologics Ultrasonic Homogenizer Model 150VT) at a frequency of 20 kHz and an output power of 60 W for 5 min. The sonication probe was immersed 3 cm into the sample. The sample container was placed inside an ice bath to prevent overheating. Hence a power density of 6 W/g was achieved. 3. French pressure cell at 10,000 psi for single pass. The exit valve of the French press was adjusted properly to counter the pressure drop due to slurry discharge. The above mentioned working conditions were selected on the basis of preliminary evaluation of cell rupture for different levels of process variables involved in each treatment. In case of autoclave, the working pressure and temperature were held constant at 121°C and 15 psi respectively and the only process variable was holding time (15, 30 and 45 min). Similarly for ultrasonication, a constant frequency of 20 kHz and an output power of 60 W were maintained and the only process variables tested was different time interval viz 3, 5 and 10 min of sonication.

4.3.1 Soluble COD and Nucleic acid Analysis

The effectiveness of rupture was measured using soluble chemical oxygen demand (SCOD) and nucleic acid content measurement of extracellular materials released due to the pores developed

in the cell wall. To evaluate the extent of rupture, an aliquot of 1 g of treated slurry was suspended in 10 ml of deionized water, vortexed and centrifuged at 5000 rpm for 10 min to separate the supernatant. It was hypothesized that any extracellular material released due to cell rupture would increase the COD and nucleic acid content of the supernatant. Similar supernatant collected by suspending intact cells was used as control. Soluble COD was determined calorimetrically using US EPA standard method 5220 D for wastewater analysis (HACH kit, Colorado USA). The nucleic acid content was measured by analyzing the absorbance at 260 nm ($A_{260\text{nm}}$) (Zhang et al., 2007) using a spectrophotometer. All the experiments were carried out in triplicates.

4.4 Drying Experiments

Initial moisture content of the slurry was determined gravimetrically by drying a known weight of microalgal slurry at 103°C for 24 hr in a convective hot air oven. To study the thin-layer drying kinetics, an aliquot of 10 g of algal slurry ruptured by various methods was spread in to a 3.15 mm thin-layer on a circular aluminum tray with uniform edges and subjected to drying in a dryer system with a constant parallel air flow velocity of 0.25 m/s at different ambient air temperatures 30°, 50°, 70° and 90°C. The drying kinetics experiments were carried out in the system shown in figure 4.1. The hot air from a heat source was channeled to pass over the circular sample holder mounted on an electronic balance (Sartorius CP series). The air flow was maintained streamlined and the velocity of the flow can be adjusted using inlet valve. The temperature of the hot air at the entry, over the sample holder and at the exit of the sample chamber was recorded using K-type thermocouples. The relative humidity of the hot air was not controlled externally and hence it varied with the daily changes in atmospheric humidity, especially at lower drying temperature. The biomass weight loss due to drying was recorded

automatically from the electronic balance every 30 S using software (Collect, Labtronics) till the equilibrium moisture content was reached. The equilibrium moisture was detected when there was negligible weight loss between consecutive weight readings of 30 min time interval. EMC was determined gravimetrically by drying a part of the biomass again in 103°C for 24 h. Each drying experiment was carried out in triplicates.

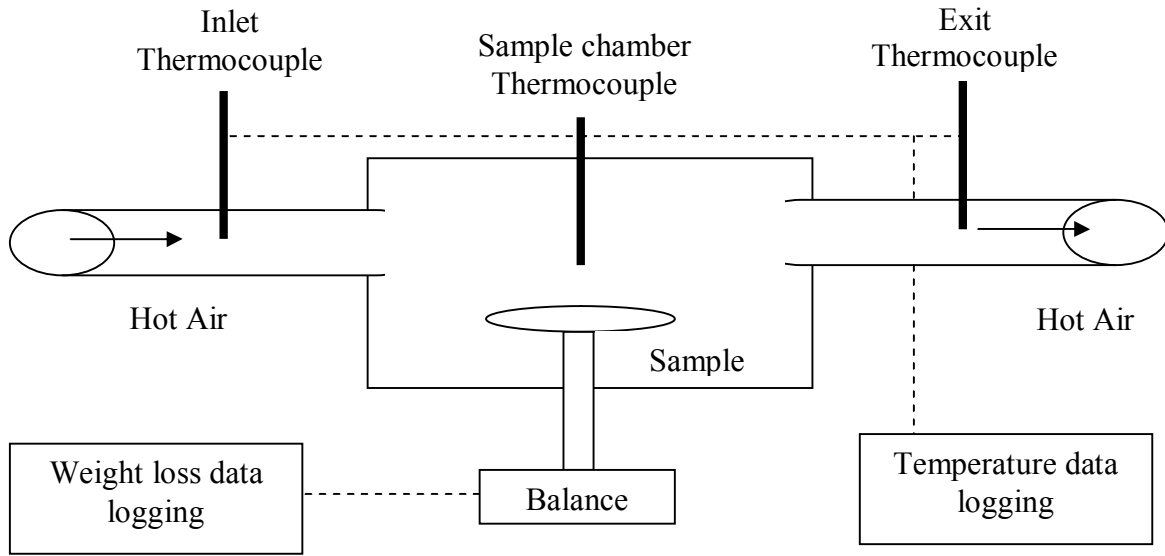


Fig.4.1 Automated drying system used in the study.

4.5 Modeling the Drying kinetics and Effective diffusivity Calculation

The kinetics of dehydration was modeled using three semi-theoretical models commonly applied for biological materials; Henderson-Pabis model (4.2), Newton model (4.3) and Page Model (4.4).

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = A \exp(-k_1 t) \quad (4.2)$$

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \exp(-k_2 t) \quad (4.3)$$

$$MR = \frac{M(t) - M_{eq}}{M_o - M_{eq}} = \exp(-k_3 t^n) \quad (4.4)$$

where MR, the dimensionless moisture ratio, is the unaccomplished moisture change defined as the ratio of free water still to be removed at any time t to the total free water initially available, $M(t)$ is the instantaneous moisture content, M_o is the initial moisture content and M_e is the equilibrium moisture content and k_1, k_2, k_3 is the drying constant. The constant A is related to the effective diffusivity of water from the sample and n is the product constant. Drying constant (k_1, k_2, k_3) varies with drying air temperature where n varies with the nature of the product.

The value of effective diffusivity was derived from the drying constant obtained in the Henderson-Pabis model (k_1) as

$$k_1 = \frac{D \pi^2}{4 L^2} \quad (4.5)$$

where L is the thickness of the thin slab when evaporation occurs in only one direction and n is the number of terms in the expansion series. The diffusivity, D is a lumped parameter resulting from the contribution of all mass transport mechanisms like gas diffusion, capillary flow, and bound water migration occurring in the different phases (Silva et al., 2000; Zogzas et al., 1994).

Effect of drying temperature on effective diffusivity or the drying rate can be expressed by an Arrhenius type equation (Karatas 1997; Madamba et al. 1996; Maskan et al. 2002; Vaccarezza and Chirife 1978)

$$\theta = \theta_o \exp(-E / RT) \quad (4.6)$$

where θ , denotes the drying rate or effective diffusivity, E denotes the activation energy (kJ mol^{-1}); R denotes the universal gas constant ($\text{kJ mol}^{-1} \text{K}^{-1}$); and T the absolute temperature (K).

The value E was calculated from the slope and k_0 from the intercept of the linearized Arrhenius type equation (Barreiro et al., 1997).

4.6 Supercritical Carbon dioxide Extraction

Dried microalgal biomass, both ruptured and the intact cells, were grind in a knife mill. The resulting powder (12.5 g) was mixed with glass beads (25 g) and loaded into the extraction thimble of a pilot-scale Sc-CO₂ extraction apparatus shown in figure 4.1. Glass beads were added to increase the porosity of the bed. Glass wool was placed at both ends of the thimble to prevent plugging of the cap top due to possible escape of biomass powder with the ScCO₂. Carbon dioxide from a siphon-tube tank was pumped through an air-driven non-lubricating gas booster pump (model AGD-62-C, Double Acting Single Stack, Haskel Inc., Burbank, CA). The extraction thimble was installed inside a temperature-controlled oven (model 3119-005, Instron, Canton, MA), and a 5 m length coil inside the oven preheated the CO₂ before entering the extraction thimble. Flow valves are properly adjusted to make the CO₂ to flow from top to the bottom. Upon exiting the thimble, the extract passed through a micrometering valve that reduced the pressure to atmospheric pressure, and finally, the extract entered a collection vessel. The collection vessel consisted of a glass test tube (200 mL) contained in a pressurized and temperature controlled cell maintained at room temperature. The extract from the extraction thimble entered the collection vessel via a tube that extended into the bottom of the vessel. The opening at the tip of the tube was welded shut and multiple 1 mm diameter holes were drilled on the side at 1 cm intervals up to 10 cm from the tip. This permitted the entry of extract into the collection vessel to be directed toward the wall. The extraction condition was as follows. The top and bottom coil pressure was 4950 psi and the temperature was 50 °C. The flow rate was

maintained at 2 l/min and the extraction time was 3 h. The extracted lipids were measured gravimetrically. The lipids were further tranesterified and analyzed for fatty acid composition.

4.7 Tranesterification and Fatty acid Profile

Fatty acid profile of the lipids extracted by both solvent extraction and supercritical extraction was studied. The total lipid was converted to fatty acid methyl esters (FAME) using AOAC Official method 996.01, Section E. Internal standard (50 μ L of C:17 at a concentration of 20 mg/ml) was added to each tube before tranesterification. Methanolic NaOH (0.5 N) was added to each tube and incubated at 100°C for 5 min followed by the addition of 2 ml of 14% BF₃. After incubation for 5 min at 100°C, 2 ml of hexane and 2 ml saturated NaCl solution were added and vortexed. The upper hexane layer was collected and analyzed with GC-FID. A PerkinElmer Inc. Clarus 600 GC-FID equipped with a Supelco SP 2340 fused silica column (Sigma–Aldrich Co.) was used to determine the fatty acids in FAME. The GC oven was heated to 150°C, ramped to 200°C at 1.3 °C min⁻¹ and held at 200°C for 20 min. The helium flow was 2.0 mL min⁻¹ at 1.6 psi and the FID temperature was 210°C. The fatty acids were identified by comparing the retention time of the standard mix (FAME 37, Sigma).

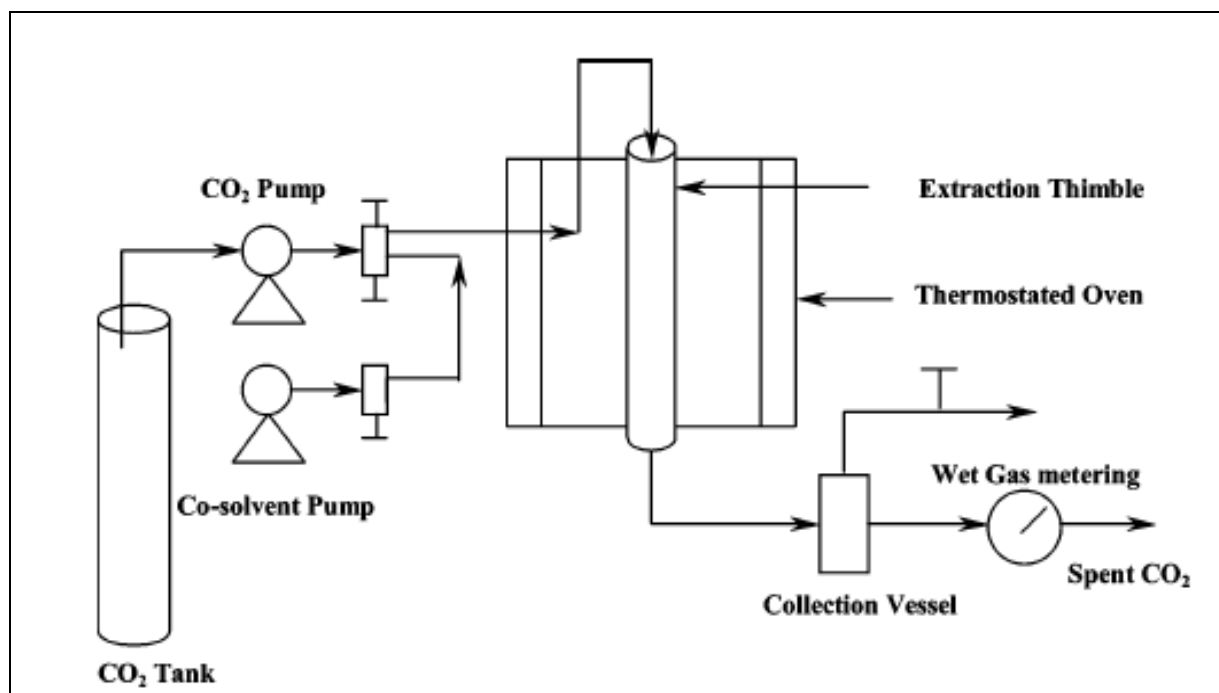


Fig.4.2. Supercritical Carbon dioxide extraction system (Yu et al., 2006)

4.8 Statistical Evaluation:

The experimental moisture loss data on was converted to dimensionless moisture ratio and plotted against drying time. The goodness of fit, for each model, was estimated by non-linear regression analysis using Curve Fitting tool available in MATLAB 7.6. The best model was selected based on high Coefficient of Determination (R^2), Root Mean Square Error (RMSE) and low Chi-Square and Standard Square of Error (SSE) values. Similarly, the significance of the impact of the variables like temperature and the cell rupture on the parameters like drying rate constant, diffusivity and lipid yield were determined using 2-way Analysis of Variance (ANOVA). Additionally, multiple comparisons among the levels of variables were calculated and grouped according to Duncan grouping procedure. All the statistical evaluations were conducted using SAS programming.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Biomass production

The biomass used in this study was produced in 550 L raceway pond during the April 2009 (Batch 1 and Batch 2) and once again in May 2010 (Batch 3). Batch 1 and 2 was harvested when the cell concentration was 0.272 g/l and 0.23 g/l respectively on the 9th day from inoculation. From light microscopy, it was found that *Scenedesmus bijuga* species dominated the harvested biomass with 40% (by cell count) followed by *Chlorella globosa* (35%) and *Chlamydomonas minutissima* (25%). Batch 1 and batch 2 biomass had differed only in their initial moisture content but the species diversity had almost remained the same. It was observed that the initial moisture content of the biomass can vary with the time of centrifugation during the harvest. For batch 3, the inoculum consisted of *Scenedesmus bijuga*, *Chlorella globosa* and *Chlorella sorokiniana*. The biomass was harvested on the 10th day when the concentration was 0.3 g/l. The biomass concentration achieved in our study was similar to earlier reports on raceway pond (Borowitzka, 1999; Chisti, 2007) biomass production suggesting that the fact the consortium has the potential to be grown as biofuel feedstock. The growth curves for batch 1 and batch 2 are given in figure 5.1.

5.2 Compositional Analysis

From the compositional analysis of freeze-dried biomass shown in table 5.1, it can be observed that the consortium was high in protein, moderate in lipids and carbohydrate. This is due to the fact that the consortium is dominated by the protein rich species, *Scenedesmus*. The

high protein content is also responsible for a low C/N ratio of 5.5 for the consortium in comparison to that of 20-25 which is considered ideal for high methane production (Angelidaki and Ahring, 1993).

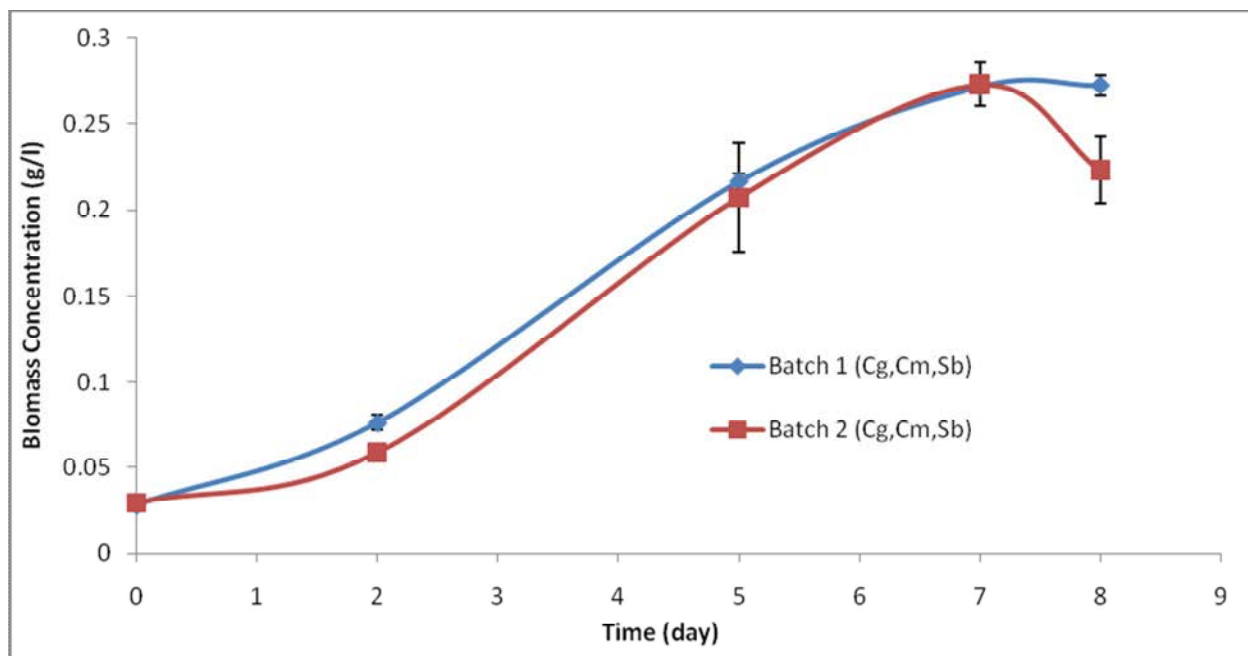


Fig 5.1. Growth curve for the algal consortium

Nevertheless, the consortium may be used as an efficient feedstock for anaerobic digestion, if preprocessing steps like chemical treatments, thermal treatment and ultrasonic treatments (Bougrier et al., 2006) that improve the disintegration of the recalcitrant cell walls of *Chlorella*, *Scenedesmus* species (Okuda, 2002) are applied. Total lipid content of the consortium is 8.93% which falls in the range reported for species like *Dunaliella salina*, *Scenedesmus obliquus*, *Scenedesmus dimorph*, *Isochrysis galbana* but less than the prominent lipid producers like *Botryococcus braunii*, *Nanochloropsis*, *Dunaliella* (Chisti, 2007; Chisti, 2008; Deng et al., 2009; Li et al., 2008; Mata et al., 2000). It should be noted that the above mentioned lipid value corresponds to the lipid fraction that includes both triacylglycerol content as well as the membrane bound polar lipids like phospholipids and glycolipids. On the other hand the hexane

soluble lipid fraction corresponding to approximately 2.93% represents the triacylglycerol which the ideal feedstock for the conventional alkali-based transesterification for biodiesel production. Hence the consortium can support biodiesel production only through non-conventional transesterification process that can handle feedstock with higher phospholipids and free fatty acids. High calorific value of the consortium 22.75 mJ/kg was similar to that of *Scendesmus* species (Matsunaga et al., 2009) *Chlorella* species (Illman et al., 2000) and the other values reported in the literature (Chinnasamy et al., 2010; Huntley and Redalje, 2007). The low carbohydrate content 12.77% makes the consortium less attractive for bioethanol fermentation in comparison to high starch strains like *Chlamydomonas reinhardtii*, *Dunaliella salina* (32%), *Porphyridium cruentum*(40-57%), *Spirogyra* sp. (33-64%) (Harun et al., 2009).

Table 5.1. Composition of algae consortium

Parameter	Values
<i>Biochemical Composition (% of dry weight)</i>	
Carbohydrate	12.77±1.30
Total lipid	8.93±0.66
Hexane soluble lipid	2.92±0.4
Protein	55.58±0.19
<i>Ultimate Analysis (% of dry weight)</i>	
Carbon	50.78±0.17
Hydrogen	7.96±0.07
Nitrogen	8.89±0.03
Sulfur	0.57±0.02
<i>Proximate Analysis (% of dry weight)</i>	
Ash	5.32±0.29
Volatile	80.09±0.81
Fixed Carbon	14.59±0.52
Heating value (MJ kg ⁻¹)	22.75±0.10

Values shown are mean ± standard deviation of three replications

5.3 Drying Characterization

The drying kinetics of biomass from the first batch was studied in a convective oven placed inside an air-conditioned room. The wet biomass used for drying and the dried flakes of microalgae was shown in Appendix Figure A1. Cell rupturing was not applied to the biomass from batch 1 as the primary objective was to get preliminary information on the drying parameters of intact microalgae cells in thin layer drying. The initial moisture content of the algal slurry from the first batch was 757.39% on a dry weight basis and the density was 0.99246 g/ml. The air velocity was maintained at 0.25 m/s. The relative humidity achieved at drying air temperature of 30, 50 70 and 90°C inside the convective oven was 23.9%, 15%, 6.5% and 3.9% respectively. Similar relative humidity was achieved for all the three replications for each temperature, as the convective oven had drawn the air from an air-conditioned environment. In other words, the effect of daily fluctuation in atmospheric relative humidity on the drying air inside the convective oven was minimal. The equilibrium moisture content (EMC), which determines the limit of moisture reduction in a material for a given ambient condition, decreased with increase in air temperature. In all the drying temperature, the EMC reached was less than 18% (wet basis) for the batch 1 biomass which proved that the dried biomass is commercially stable for storage (Vega-Galvez et al., 2008). The EMC achieved in the convective oven for drying air temperature of 30, 50, 70, 90°C were 13.01%, 10.15%, 1.52%, 1.54% respectively. The dehydration curves showed a clear exponential tendency (Figure 5.2) and it was observed that the drying time decreases with increase in the air temperature. The drying time for air temperature 30°, 50°, 70° and 90°C to achieve EMC was 1620, 1020, 690 and 440 min respectively. It must be mentioned that drying time is a function of both the drying material and

the drying system and hence this parameter cannot be used to compare among the drying rate.

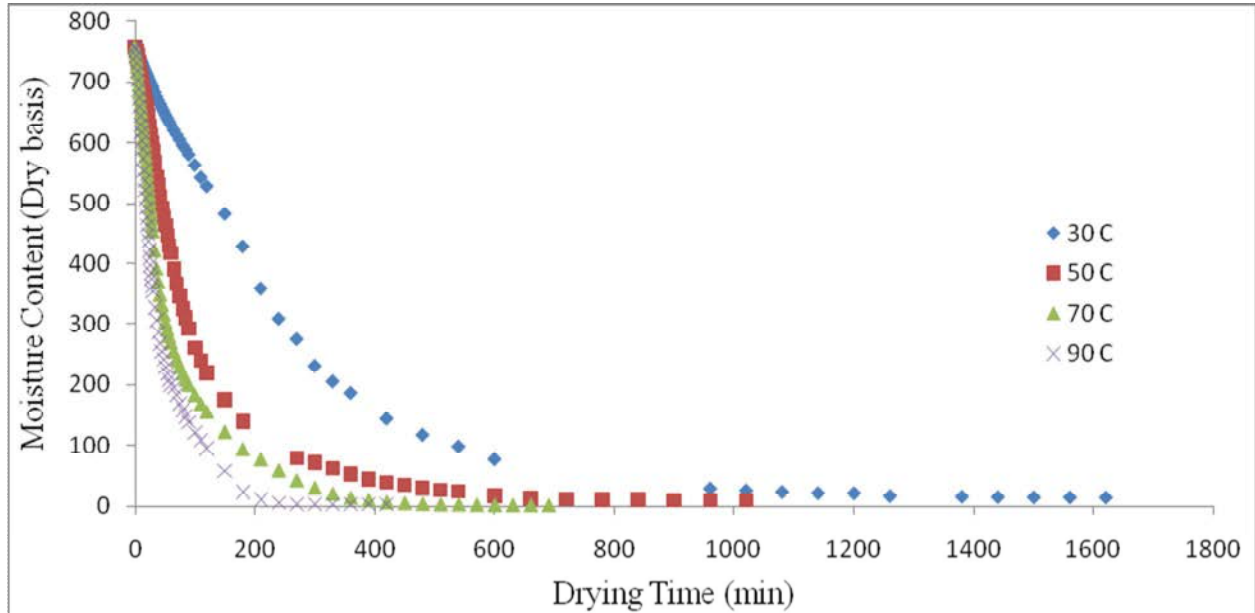


Fig 5.2. Drying curves of the consortium for different temperature

It can be observed that the constant rate drying period was present initially for a brief time and the predominant drying process took place in falling rate drying period even though the biomass had high initial moisture content. This may be attributed to the fact that the biomass has high protein content (Chirife, 1983) which may prevent any free water existence. This result also showed that liquid diffusion inside the biomass thin layer was the dominant physical mechanism responsible for moisture movement within the samples. Similar drying curves were obtained for species like *Gelidium sesquipedale* (Ait Mohamed et al., 2008), *Aphanothece microscopica Nageli* (Jacob et al., 2007), *Spirulina plantis* (Desmorieux and Decaen, 2006; Lemus et al., 2008; Oliveira et al., 2009) *Gracilaria* (Lemus et al., 2008) and *Macrocytis pyrifera* (Vega-Galvez et al., 2008) where almost no constant-rate drying period was observed. The above mentioned studies had also used microalgal biomass at its least possible moisture content. Hence it can be concluded that the drying behavior of the microalgae was species independent, and the entire

drying process takes place in falling rate drying period irrespective of their class (like green algae, red algae, brown algae etc).

5.4 Modeling of Drying Kinetics

The three exponential thin-layer drying kinetics models namely Newton's model, Henderson-Pabis model, and Page's model that are commonly used to describe dehydration characteristics of food materials were evaluated in this study. The instantaneous moisture content of the slurry for all drying experiments were converted to dimensionless moisture ratio and plotted against drying time. In general, all of the three models have shown good fit. The model parameters and statistical evaluation of all the models are given in the table 5.2. Among them, the Page's model best described the dehydration kinetics based on the high R^2 , RMSE and low SSE. The predicted moisture ratio using the parameters from page model have shown to fit the experimental moisture ratio in the figure 5.3. Page's equation has been reported to adequately predict the thin layer drying of bagasse (Vijayaraj et al., 2007), pistachio (Midilli and Kucuk, 2003), shell corn, peanuts, and rapeseeds (Pathak et al., 1991), sunflower seeds (Syarif et al., 1984). The drying constant k_3 , determined from page equation increased with increase in the drying temperature and it was found to be in the range of 0.004 to 0.031 min^{-1} . The page model had shown the best fit as it had an additional model constant n , the product constant. It can be observed that additional model constant can increase the effectiveness of prediction of the experimental data, but had no physical meaning in explaining the mechanism of drying.

The effective diffusivity calculated from the Henderson-Pabis model had also increased with increase in drying temperature obtaining values between 1.94E-10 and 1.74E-9 m^2/s for the experimental conditions studied.

Table 5.2 Model parameters and statistical evaluation of drying kinetics models

Model	30°C ^a	50°C ^a	70°C ^a	90°C ^a
Newton				
k ₂	0.0039±0.0005	0.0117±0.002	0.0163±0.001	0.0314±0.004
R ²	0.997	0.996	0.994	0.996
RMSE	0.021	0.022	0.027	0.019
SSE	0.0359	0.0347	0.0476	0.0218
Chi-Square	0.0004	0.0005	0.0007	0.0004
Handerson-pabis				
a	1.0176±0.005	1.014±0.021	1.007±0.007	1.005±0.014
k ₁	0.0048±0.0004	0.0120±0.001	0.0165±0.001	0.0316±0.004
R ²	0.998	0.997	0.994	0.996
RMSE	0.018	0.019	0.026	0.019
Chi-Square	0.0004	0.0004	0.0007	0.0003
SSE	0.0266	0.0274	0.0467	0.0209
Page Model				
k ₃	0.0021±0.001	0.0122±0.005	0.0197±0.002	0.0347±0.005
n	1.1283±0.039	1.0101±0.039	0.9505±0.102	0.9698±0.047
R ²	0.999	0.997	0.994	0.997
RMSE	0.010	0.019	0.025	0.018
Chi-Square	0.0001	0.0004	0.0007	0.0003
SSE	0.0070	0.0265	0.0434	0.0181
Diffusivity (m²/s)	1.94E-10	6.62E-10	9.11E-10	1.74E-09

^aValues shown are mean ± standard deviation of three replications

The increase in drying constant and effective diffusivity with respect to drying temperature was shown in figure 5.4. The temperature dependence of k and d follows an Arrhenius-type equation ($R^2 > 0.90$) as given below.

$$k = 46957.6 \exp \left(- 5060.5 / T \right) \quad (5.1)$$

$$D = 6.93 * 10^{-5} \exp(-3830.4 / T) \quad (5.2)$$

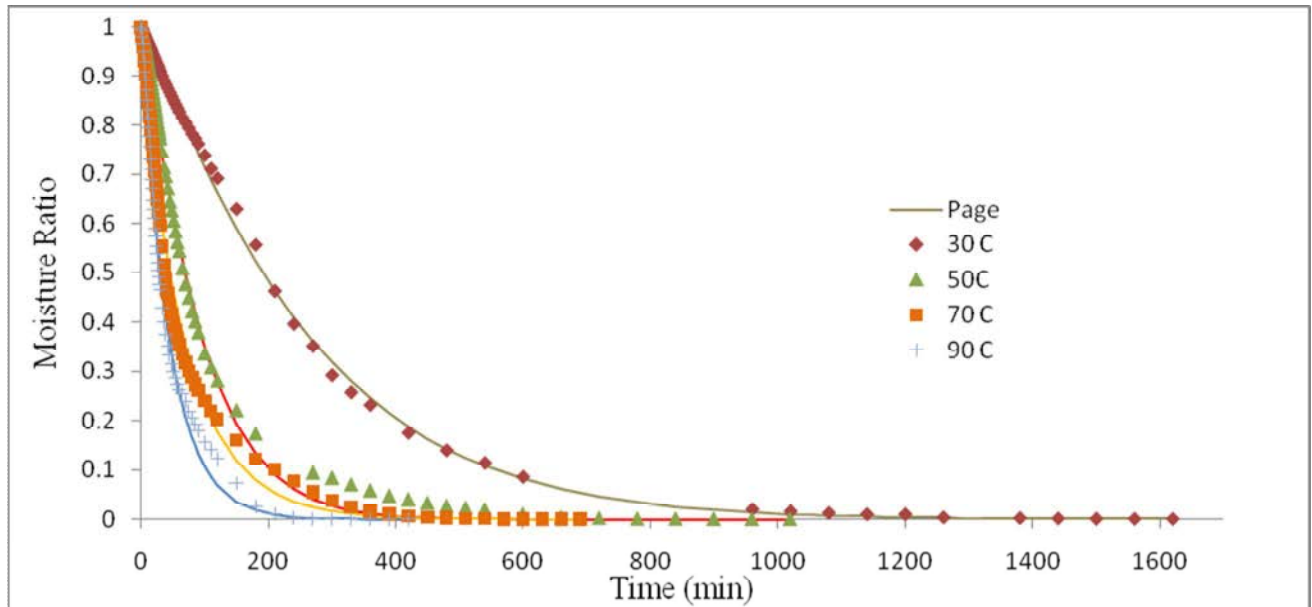


Fig 5.3. Experimental and predicted moisture ratio for different temperature

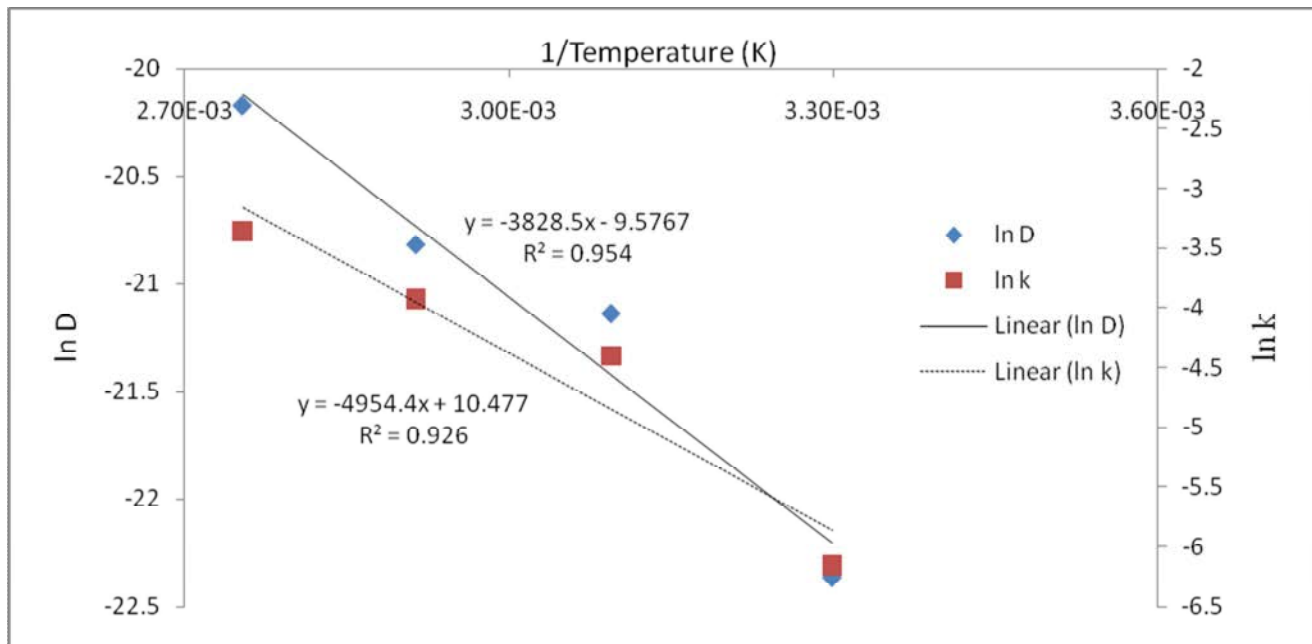


Fig 5.4. Arrhenius plot for drying rate constant and diffusivity

The activation energy for the drying constant k_3 was found to be 41.19 kJ/mol and the corresponding Arrhenius factor was $35489.78 \text{ min}^{-1}$. Similarly the activation energy for effective diffusivity was 31.83 kJ/mol and the corresponding Arrhenius factor was $6.93\text{E-}5$. In genral, the activation energy determines the sensitive of the reaction (diffusion) to the driving force

(temperature). Our results suggests that the activation energy for effective diffusivity of our consortium was higher than that of the brown algae *Macrocystis pyrifera* for which an activation energy of 19.87 kJ/mol and an Arrhenius factor of $9.35\text{E-}6 \text{ m}^2/\text{s}$ were obtained (Vega-Galvez et al., 2008). It can also be inferred that the consortium behaves like a foods of vegetal origin showing activation energy similar to figs (37.27 kJ/mol), kale (36.11 kJ/mol), red pepper (39.70 kJ/mol), aloevera (30.37 kJ/mol) and *Gracilaria chilensis* (39.92 kJ/mol).

5.5 Cell Rupture Treatments

The working conditions for each cell rupture treatment namely autoclaving, sonication and French press was selected based on soluble chemical oxygen demand (SCOD) and nucleic acid content of the supernatant. In fact, it is interesting to note that the cell rupture efficiency was often measured as a function of efficiency of lipid extraction (Lee et al., 2009) or by the quantification of soluble protein, or by counting the live cells by plating. Hence the methodology for selection of best cell rupture treatment was not universal and in most cases, depends directly upon the purpose for which the cell rupture is needed. It may be worthwhile to explore the possibilities of assigning an index to represent the effectiveness of rupture, taking all the above mentioned possible methodologies into consideration.

For autoclaving the working pressure and temperature was held constant at 121 °C and 15 psi respectively, the only variable was holding time (15, 30 and 45 min). Both SCOD and nucleic acid content had increased with increase in the holding time as shown in figure 5.5. Autoclaving for 15, 30 and 45 min had increased the nucleic acid content by 11.7%, 42.3% and 83.5% respectively. Similarly an increase of 38.6%, 40.7% and 64.5% increase was observed in the SCOD value for the different time of autoclaving. Hence it was obvious that autoclaving for 45 min at 121°C and 15 psi has the maximum cell rupturing for this method. Sonication was carried

out at a constant frequency of 20 kHz and an output power of 60 W for three different time intervals viz 3, 5 and 10 min. A power density of 6 W/g was achieved. The increase of either SCOD or nucleic acid with respect to time was not as pronounced as it was with autoclaving. Nucleic acid content increase with time of sonication was 22.5%, 26.4% and 25.3% and it was 11.2%, 15.9% and 11.4% for SCOD value. Autoclaving for 5 min at 20 kHz and 60 W was selected as the optimum condition for sonication based rupture.

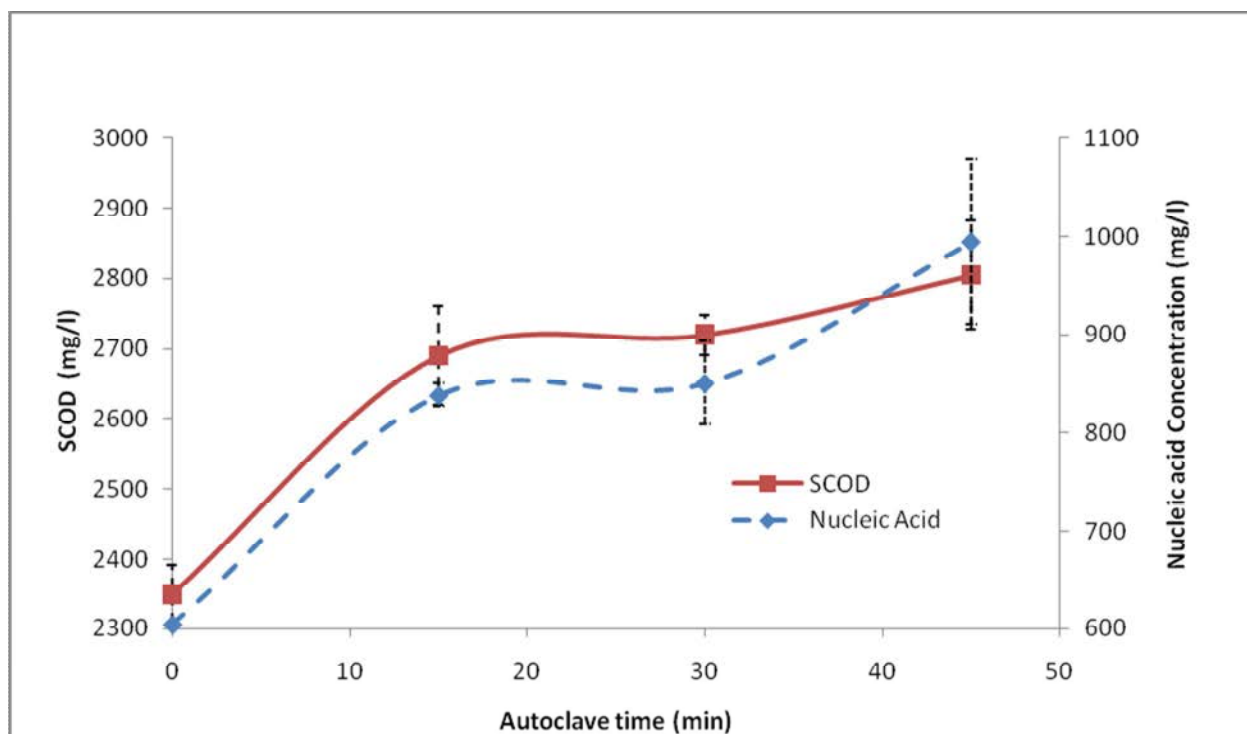


Fig 5.5. SCOD and Nucleic acid concentration for different autoclave time

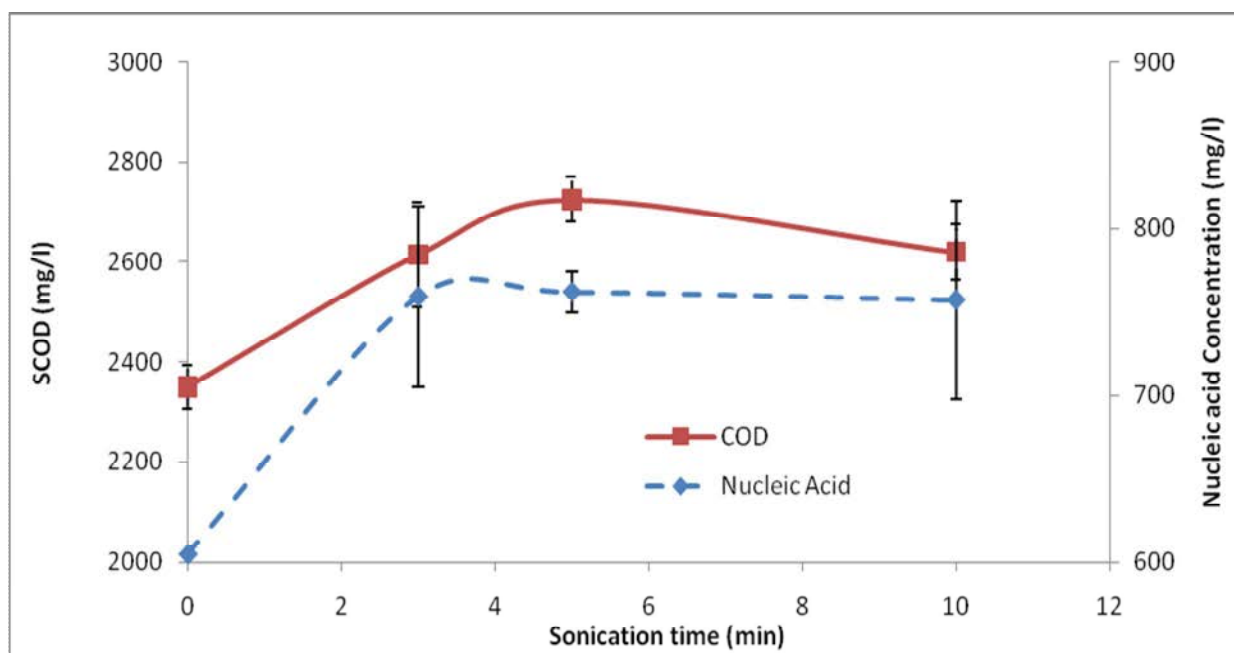


Fig 5.6. SCOD and Nucleic acid concentration for different sonication time

French press was carried out at 10,000 psi and different levels of either the operating pressure or the number of passes were not considered. Actually, French pressure cell used in this study can be operated at pressures as high as 20,000 psi but the pressure level was chosen as it was found to be the maximum possible pressure that can be achieved accounting for the pressure drop due to high concentration of the sludge. Higher pressure ranges may be applicable for more dilute sludge. A comparison of COD and nucleic acid values for the selected rupture conditions was given in the figure 5.7. The ruptured cells were also viewed under scanning electron microscopy to prove cell rupture as shown in appendix (Figure A2).

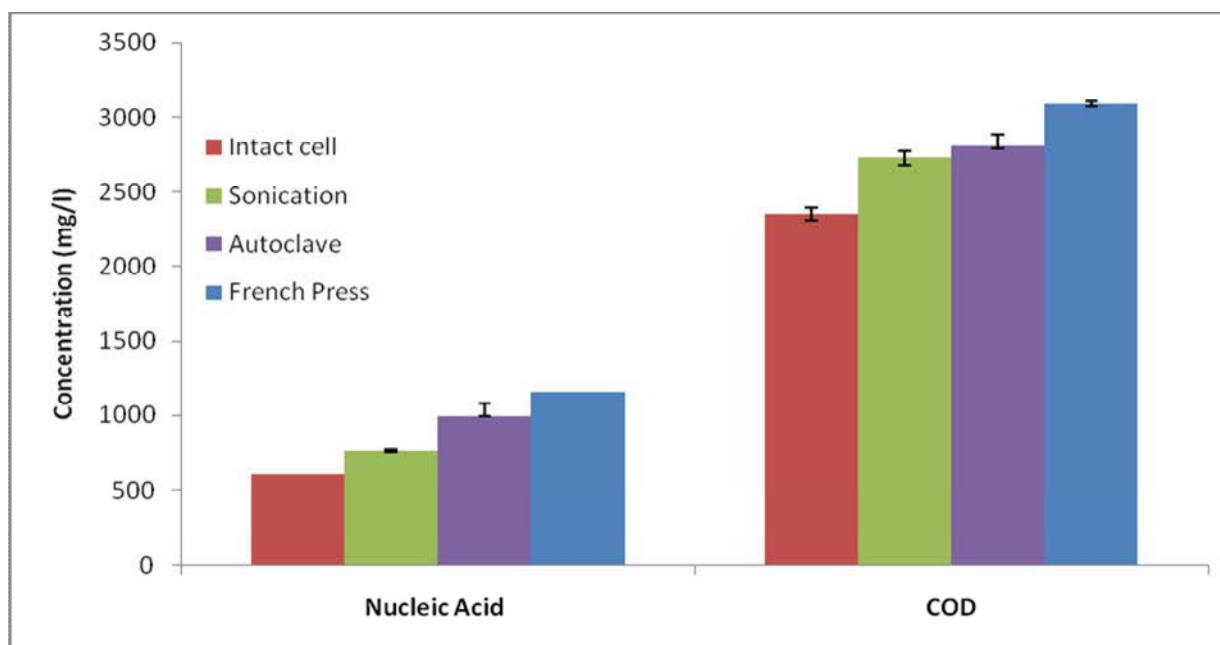


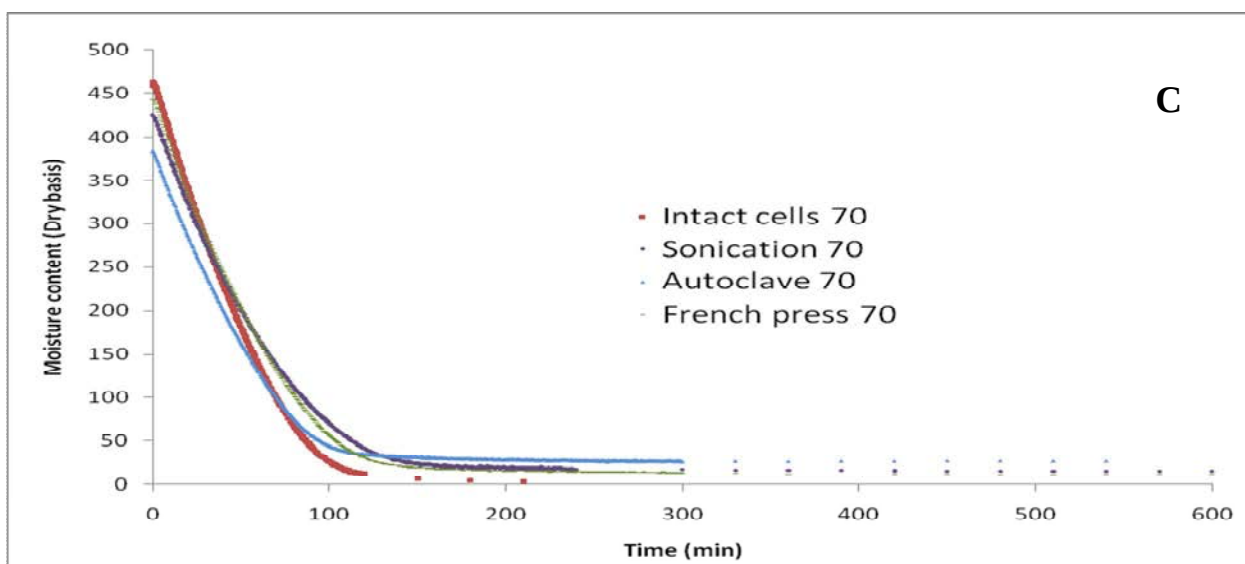
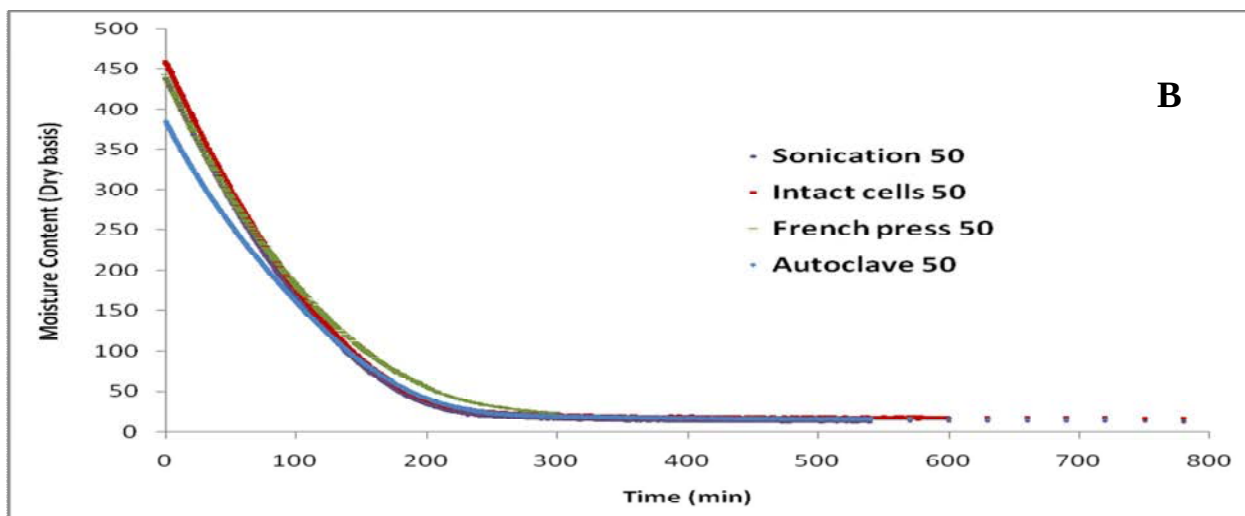
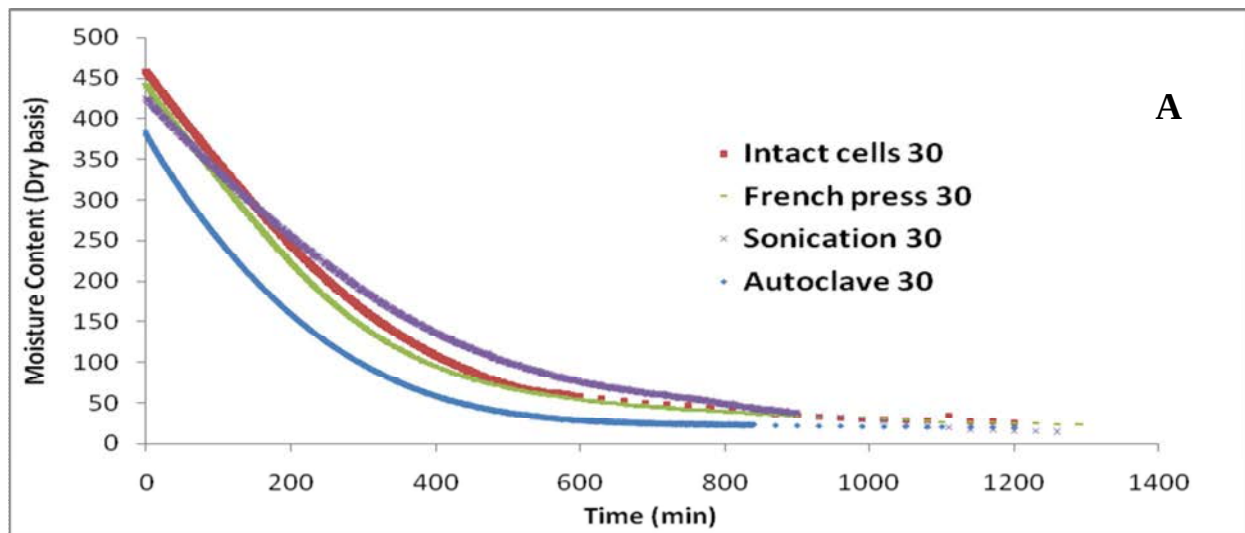
Fig.5.7. Comparison of COD and Nucleic acid values for selected treatment conditions

5.6 Effect of cell rupture on drying kinetics

The drying kinetics of the ruptured cells was studied in an automated drying system explained earlier. The weight loss data was recorded automatically in defined time interval. The system was more susceptible to the changes in atmospheric temperature and relative humidity as it draws air for heating from the atmosphere rather than air-conditioned room. Especially at lower drying temperatures (30° and 50°C) the relative humidity of the drying air depends directly on the atmospheric relative humidity. In addition to the variation in system, the biomass used to study drying kinetics of ruptured cells was from batch 2 harvest which varied in the initial moisture content. To account for these variations, drying kinetics of untreated biomass was carried out once again in the automated drying system along with that of the ruptured cells.

The initial moisture content of the untreated biomass was 419.73% on a dry weight basis and the density was 0.975 g/ml. The initial moisture content of the biomass varied slightly with the cell treatment methods and it was measured as 438.08% for ultrasonicated biomass, 384.73% for autoclaved biomass and 459.17% for French press treated biomass. These slight variations

are due to application of heat during the treatment (autoclave, sonication) and possibly due to periodic freezing and thawing the biomass. For all the treatment the biomass was spread to a thin layer of 0.31 mm and subjected to drying. For all the temperature and all the treatment the moisture loss was exponential without any significant constant-rate drying period. Hence it can be inferred that the drying rate was limited by the internal diffusion rather than external conditions. Comparison of drying curves for different treatment at each drying temperature has been shown in figure 5.8. It can be seen that the difference in initial moisture content of the treated samples had resulted in distinct drying curves for each treatment at low drying temperatures of 30°C and 50°C. On the other hand, for higher temperatures the drying curves for each treatment almost overlap one another. The equilibrium moisture content reached at different drying temperature for each treatment is given in the Appendix (Table A2). It must be mentioned that the EMC value reported in the table for lower temperature had shown high standard deviation mainly due to the fact that relative humidity of the heating air inside the system depended directly on atmospheric air humidity. But at higher temperature, consistent EMC values were reached for each replication. It can be observed that EMC was lesser for the ruptured cells when compared to the untreated biomass, except for French press treatment dried at 90 °C. It can also be observed that for all treatments the EMC decreased with increase in temperature, as expected.



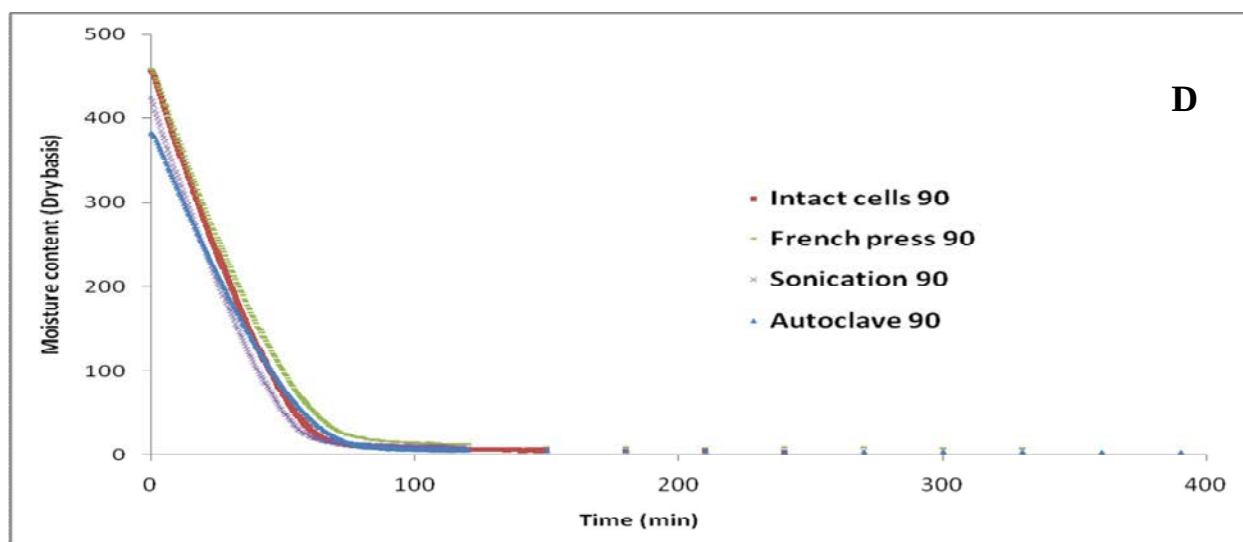


Fig 5.8. (A-D). Comparison of drying curves for cell rupture treatments at each temperature. (A- 30 °C, B- 50 °C, C- 70 °C, D- 90 °C)

5.7 Modeling of Drying Kinetics of the Ruptured cells

The drying kinetics of the ruptured cells was modeled by three mathematical models as explained earlier. Among them, Page model predicted the experimental moisture loss data better than the other two models based on the statistical evaluation. The fit of experimental moisture ratio and theoretical moisture ratio predicted by the pages model for French press treatment was given in the figure 5.9.

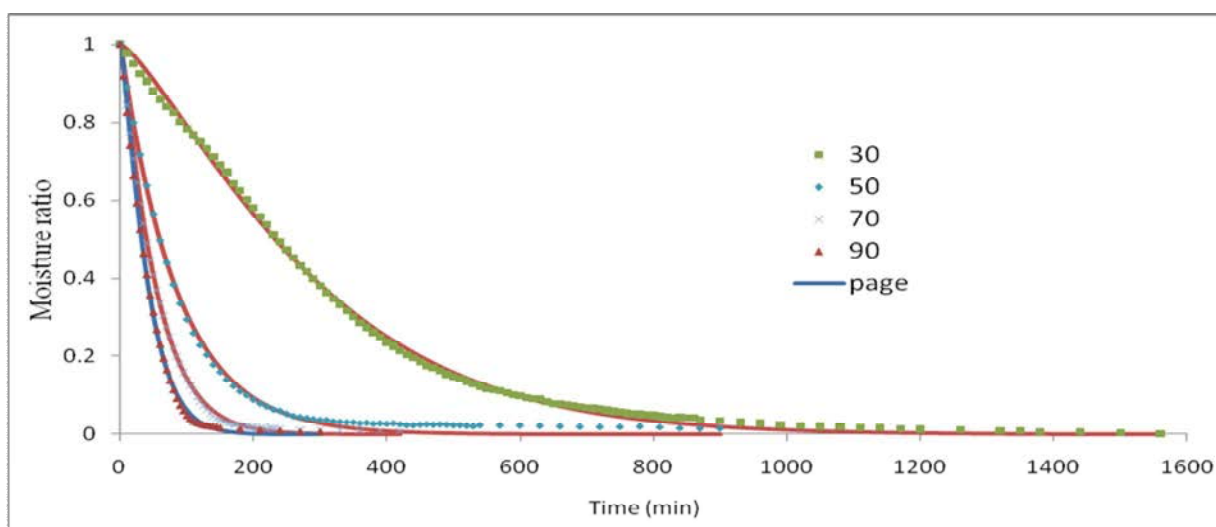


Fig 5.9. Experimental and predicted moisture ratio for French press ruptured cells

The page model parameters for other treatment are given in Appendix (Table A1). The k_3 value from the page model is a measure of rate of moisture transfer from the material. It predicts the moisture removal rate that can be experimentally calculated as the kilogram of water removed per kilogram of dry matter divided by the drying time (Dandamrongrak et al., 2002). As expected, increase in temperature had increased the drying constant k_3 for all treatments. This is due to the fact that higher drying temperature generates larger driving force for mass and heat transfer. Similarly, it can also be observed that the drying constant k_3 has increased with cell rupture treatment when compared to the untreated biomass at each temperature range as shown in figure 5.10. From our results, it is evident that rupturing the cell wall can increase the access of hot air to the intracellular moisture and increases the rate of drying. Hence the cell wall of microalgae has been a significant barrier for moisture release from the cells. Similar effect have been observed in fruit and vegetable dehydration studies in which the external skin of the fruit was made porous using different treatments. In fact, as there were limited reports on impact of cell rupture on drying kinetics of either microalgae or any other microbial cells, the specific details of our results were compared to the drying kinetics of food materials that were subjected to pretreatment methods.

The drying constant for each treatment has increased with increase in temperature except for French press ruptured cells. In fact, the increase in drying constant between 70°C and 90°C of French press treated cell was not linear. This result suggests that, if maximum cell rupturing was achieved (as in the case with French press treated cell), higher drying constant can be obtained at a relatively lower temperature. Further studies on elucidating the correlation of drying temperature on drying rate for a particular degree of cell rupture would be beneficial in terms of optimizing the cell rupture-drying process. A 2-way ANOVA (Table A3) analysis of the drying

constant data had shown that both drying temperature and cell rupture treatment have a significant effect on drying constant ($\alpha=0.05$) without any significant interaction.

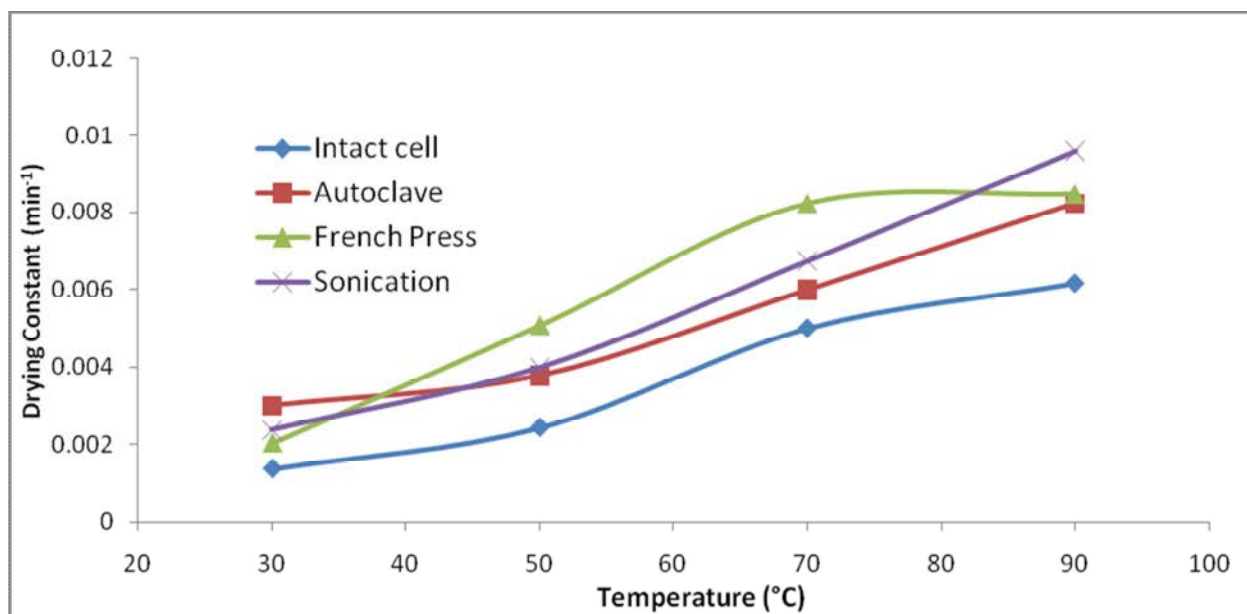


Fig.5.10. Comparison of drying rate constant for different cell rupture treatment

According to Duncan test, the average drying constant for each cell rupture treatment, across different drying temperature, has varied significantly from the average drying constant of the intact cells. At the same time, no significant difference was observed between average drying constant obtained for autoclave, sonication and French press treatments i.e., among cell rupture treatments. Hence it can be concluded that the drying rate constant can be improved through cell rupture. In a similar study on the effect of blanching on the drying of paprika (Ramesh et al., 2001) it was found that the drying rate can be improved if blanching is done for an appropriate period of time. Similarly, Yong et al., (Yong et al., 2006) studied the effect of pin holes, drill holes and blanching and freezing of potato, cassava, dragon fruit and red chill and concluded that increase in pin hole diameter and density increased the drying rate. The improvement of the drying rate was found to be dependent on the initial moisture content of the material too. (Verma and Gupta, 2004). It is evident from the earlier studies that any morphological change to the

outer membrane of the drying material can have an impact on drying rate if internal liquid diffusion is the rate-limiting factor of the drying. Our results also suggest that the drying rate can be increased as much as 57% by rupturing the microalgal cell wall and eliminating the barrier for moisture release. It would be interesting to explore in the future studies, if this increase in drying rate can reduce the drying energy requirement to the same extent through process modeling studies. As there was no significant difference between cell rupture methods, the cheapest method may be used at large scale. It can also be inferred from the ANOVA results that no significant synergistic interaction was detected between the drying temperature and the cell rupture.

The effective diffusivity value calculated from Henderson Pabis-model had increased with increase in temperature and cell rupture. From figure 5.11, a defined pattern for improvement of effective diffusivity was not observed. A 2-way ANOVA analysis (Table A4) had also shown that cell rupture method has a significant impact on the average moisture diffusivity. But the trend of increase of diffusivity values doesn't follow a similar trend that was seen in the case of drying constant. This anomaly was also found the study carried out by Dandamrongrak et al., (2002) on peeled banana. They have reported that 'freezing' and 'combined blanching and freezing' pre-treatment are very effective methods for improving the drying rate of peeled banana but similar trend was not observed in terms of theoretical effective diffusivity values. The effective diffusivity of the blanching treatment increased to nearly twice that of the control, while the 'freezing and the combined blanching' and 'freezing alone' treatments had diffusivities about three times that of the control.

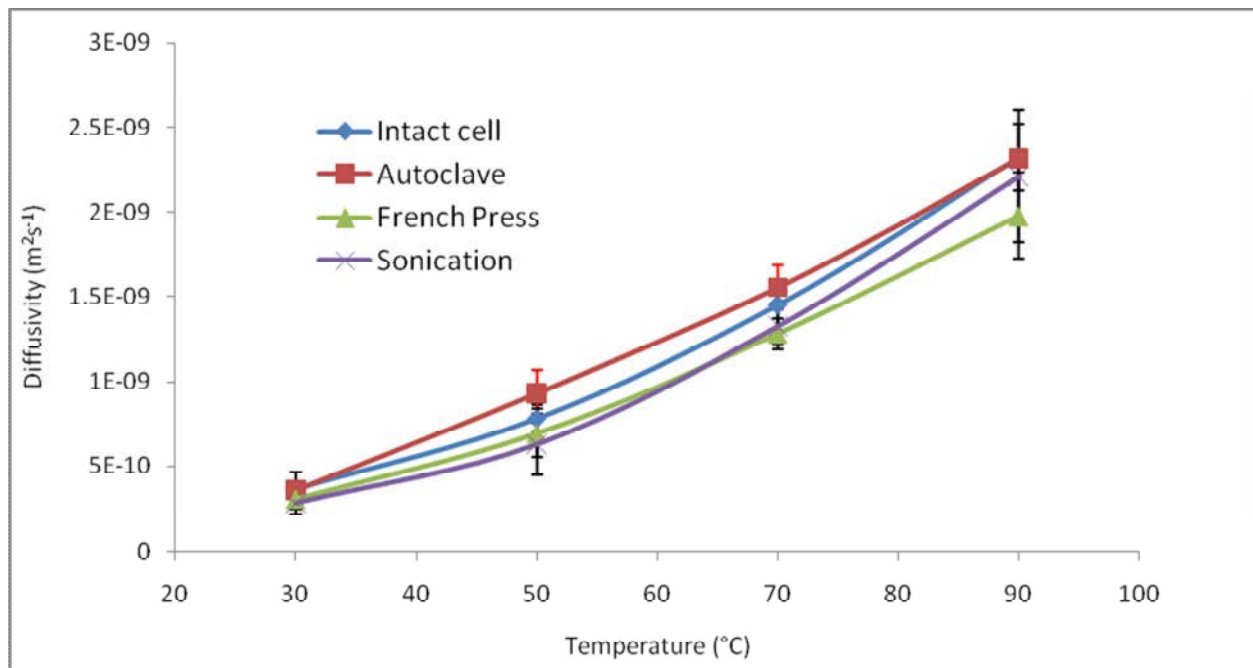


Fig.5.11. Comparison of effective diffusivity for different cell ruptures treatment

This anomaly may be due to the limitation of the semi theoretical models that represent the diffusivity as a lumped parameter. In fact effective diffusivity D is the average of the diffusivity values that took place during the entire drying process and sensitive to other physical phenomena like shrinkage and porosity. As the cells ruptured through different methodologies may behave differently in shrinkage and porosity, their effective diffusivity might not reproduce the same trend observed in the drying rate. The changes in the RH at low temperature may also have contributed to this anomaly.

5.8 Lipid Extraction and Analysis

The amount of total lipid extracted or lipid recovery, expressed as wt % of biomass, had increased with the cell rupture at all the temperature as given in the figure 5.12. The values were corrected for the residual moisture content of the biomass. It was observed from a 2-way ANOVA analysis that both the cell rupture and drying temperature had a significant impact on lipid extraction.

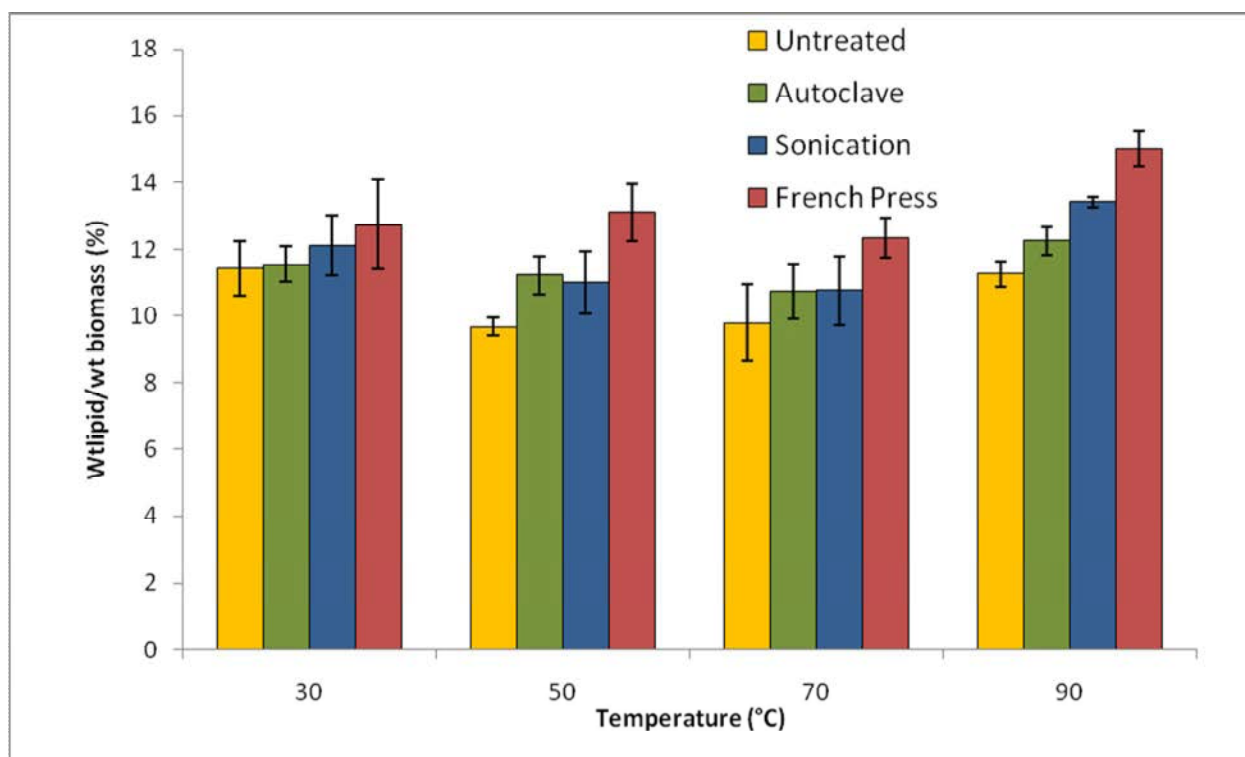


Fig 5.12. Comparison of total lipid content for different cell rupture treatments

Among the treatments, the French press cell rupture has shown the maximum increase followed by sonication and autoclave. On an average across all temperature range, French press, sonication and autoclave had increased the lipid recovery by 28.68%, 12.3% and 10.19% respectively. The increase in lipid recovery may be attributed to the fact of increased diffusion of the solvents through the pores/cracks on the algal cell walls. In a similar study it was shown that bead-beating had improved lipid recovery from *Botryococcus* sp by 28.1% and sonication had improved by 8.8% (Lee et al., 2009). For *Scenedesmus dimorphus* wet milling had increased the lipid recovery by 19% than the control followed by bead-beater. Unlike *S.dimorphus*, the highest lipid recovery was achieved from *C.protothecoides* by bead-beater with a 23% increase in lipid recovery followed by French press, wet milling and sonication (Shen et al., 2009). It is quite clear from the literature that the extent of improvement of lipid yield depends not only on the extent of cell rupture but also the species and its lipid content. In general it can be concluded that

cell rupturing can improve the lipid extraction yield as high as 30%. Based on Duncan's test, it was observed that French press treatment had improved the lipid recovery significantly different from the other treatments like sonication and autoclave supplementing the earlier observation on COD and nucleic acid values.

It is interesting to note that drying temperature had an impact on lipid recovery. According to Dunnet's grouping, drying at 90°C had a significantly higher lipid recovery when compared to other drying temperature. Our results suggest that the drying at 90 °C has improved the lipid recovery by almost 8%. Our results are similar to the study by Zepka and co-workers (Zepka et al., 2008) who reported an increase of lipid recovery from *aphanothece microscopic* by drying at 60°C instead of 40°C. On the other hand, our results contradict the report by Widjaja et al., (Widjaja et al., 2009) who observed significant reduction as much as 15% of lipids in *chlorella vulgaris* by drying at higher temperatures (80 and 90°C). The inconsistency of the effect of temperature on lipid recovery may be due to species difference and the duration of drying i.e the exposure of biomass to hot air. It may also be possible that the fatty acid profile, especially the level of unsaturation and amount of free fatty acids present in the species would contribute to increase or decrease of lipid recovery.

Fatty acid composition of the consortium subjected to different cell rupture treatments and temperature is given in table 5.3. The profile predominantly showed the presence of following fatty acids: palmitic (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic (C18:2), linolenic (18:3), archidic acid (C20:0), gadolic acid (C20:1). No change was observed with respect to any specific fatty acid either with the temperature or cell rupture. The differences in the values may be predominantly due to the differences in extraction of FAME into hexane layer during transesterification rather than the treatment itself. Out of the total fatty acids, palmitate

(28-36%), oleate (20-26%), linoleate (18-22%) and alpha linolenate (10-16 %) were the dominant fatty acids when compared to gamma-linolenate (0-1%), stearate (2-3%) and gadoliec acid (1-2%). The unsaturated fatty acid content of the consortium was between 56-67% similar to other microalgal species like *Spirulina maxima*, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Dunaliella tertiolecta*, *Nannochloropsis* sp. and *Neochloris oleabundans* (Gouveia and Oliveira, 2009). It is interesting to note that linolenic (C18:3) acid content (10-16%) was almost equal to the maximum limit (12%) recommended for good biodiesel quality by European Union (Chinnasamy et al., 2010). The degree of unsaturation was found vary between 0.97-1.47. It can also be observed from the table 5.3 that the degree of unsaturation decreased with increase in temperature for all the cell rupture treatment. This suggests that the increase in drying temperature may result in cleavage or oxidation of unsaturated bonds, resulting in lesser unsaturation. The degree of unsaturation observed in our study was lesser than that of *S.limacinum* (2.04-2.26) reported by Johnson (Johnson and Wen, 2009). As the unsaturation levels are higher in our consortium, it can be expected that biodiesel produced from the oil from our consortium will have a low cloud point, i.e., a superior cold flow property. On the other hand, if too high unsaturation results in an oxidation problem, a stabilizer (antioxidant like tocopherol) can be added to extend the storage life of the algal biodiesel (Knothe et al., 2005). It must be remembered that the fatty acid composition observed in this study may be specific to the neutral lipids present in the consortium. In spite of the fact that the total lipid was subjected to tranesterification, the alkali-based tranesterification method was selective only for neutral lipids fraction. The other fractions of the total lipid namely, free fatty acids and polar lipids were not converted to FAME, as they for soap during the tranesterification (Zhang et al., 2003). Hence complete fatty acid composition, including the fatty acids associated with polar moieties, may be

obtained by two a two step process that has an acid tranesterification followed by a base tranesterification.

Table 5.3. Fatty acid profile of the consortium subjected to different cell rupture and dried at different temperature*

Cell Rupture	Temp(°C)	16:00	16:01	18:00	18:01	18:02	18:03n-6	18:03n-3	20:00	20:01	Degree of unsaturation
Autoclave	30	28.4	6.48	2.4	19.8	22.5	0.92	17.2	1.26	1.05	1.20
	50	32.9	2.7	3.6	26	19.8	ND	10.3	ND	1.04	0.97
	70	27.9	6.14	2.4	20.1	21.8	0.85	15.6	0.99	1.03	1.14
	90	26.3	6.41	3.3	21.6	21.8	0.9	16.1	0.85	1.05	1.17
French Press	30	34.7	3.15	2.8	24.4	19.8	0.98	11.1	0.74	1.81	1.02
	50	34.3	2.93	3.3	25.1	19.6	1.03	10.7	0.74	1.79	1.01
	70	36.2	2.49	2.8	25.9	19.7	1.02	9.5	0.59	1.72	0.98
	90	36.2	2.94	3.3	24.8	18.6	0.99	10.2	0.64	1.71	0.97
Sonication	30	27.1	7.18	2.2	20.1	22.4	0.87	17.4	1.08	1.62	1.21
	50	29.2	4.99	2.9	23.2	21.6	0.88	14.7	0.84	1.62	1.14
	70	31.4	5.05	2.7	22.9	20.7	1.15	13.9	0.7	1.59	1.11
	90	28.4	6.25	3.1	21.5	20.7	0.94	15.6	1.08	1.44	1.13
No cell rupture	30	29.3	6.57	2.7	20.8	20.8	0.89	16.6	1.13	1.25	1.16
	50	35.5	3.51	2.9	23.5	19	0.83	12.6	0.77	1.34	1.03
	70	32.2	4.06	2.3	23.4	20.2	1.05	14.3	0.89	1.47	1.11
	90	35.8	ND	3.2	23.9	18.3	0.89	12.3	0.74	1.25	1.01

* Values expressed as weight % of total fatty acids

ND-not detected in GC-FID

5.9 Supercritical Carbon dioxide Extraction of Lipids from Biomass

As the supercritical extractor system was designed originally for pilot scale operations, optimization of the working conditions in terms of pressure and temperature ranges was not possible due to constraints in the amount of dried algal biomass required for one such study. It can be observed that the lipid extracted was sticking to the surface of the tubing and hence it could not be recovered properly at the collection chamber. Chloroform was used to wash the lipid from the tubing. It was also observed that the glass wool that was placed at the bottom of the bed was found to be pale yellow colored, suggesting a possible discharge of lipid at the sample holder itself. The oil extracted through this method was lesser green and more yellow when compared to the solvent based extraction (Figure A3). This may be due to decrease in extraction of chlorophyll and other pigments from the matrix. Cell rupture had significantly improved the lipid recovery by supercritical extraction when compared to the untreated biomass. Our result on improvement of lipid yield is in agreement with the report from Mendes et al., (2003). Their study had shown that 25% of lipids in *Chlorella vulgaris* were extracted from a partly crushed cell where 55% of lipids can be extracted from completely crushed cells. Improved mass transfer and accessibility of lipid globules to the supercritical fluid can be the reason for the increase. Comparison of the fatty acid profile of the lipids obtained from French press ruptured and unruptured cells were shown in Table 5.4. It was observed that the fatty acid composition of the extracted lipids showed predominance of unsaturated fatty acid to about 72-75% and the ruptured cells showed unusual presence of pentadecenoic acid (C15:1). The degree of unsaturation was in the range of 1.6 - 1.64. Hence our study showed that cell rupture can significantly improve the lipid recovery through supercritical carbon dioxide extraction and the lipids extracted through this method was highly unsaturated. The results obtained in this study

should be considered preliminary and detailed investigation of the effect of cell rupture on the supercritical extraction is warranted.

Table 5.4. Fatty acid composition of lipids from ScCO₂ extraction

Fatty acid	Common name	Intact cells*	French press ruptured*
C15:1	Pentadecenoic acid	0.00	12.44
C16:0	Palmitic acid	19.18	23.10
C18:0	Stearic acid	8.14	1.04
C18:1n	Oleic acid	0.00	5.25
C18:1n9c	Oleic acid	14.25	4.32
C18:2n6t	Linoleic acid	14.37	13.99
C18:2n6c	Linoleic acid	11.13	7.83
C18:3n3	Linolenic acid	0.00	0.84
c18 3n6	Linolenic acid	32.94	30.60
saturated		27.32	24.14
Unsaturated		72.68	75.27
Degree of unsaturation		1.64	1.60

* % of total fatty acid detected by GC-FID

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH

Microalgae biomass production and energy conversion have gained significant interest in alternate energy sector, and major research breakthroughs shall take the technology to industrial scale operation and economical success, within a decade or even earlier. Drying is one of the important processes in the microalgae production chain as it facilitates safe storage and transport in the future algae-based biofuels industry. The results from our research are very important, as it shed light on the drying behavior of microalgae feedstock suitable for biofuel application. Our research had shown that the microalgae behave like a vegetative material with long drying period mainly due high initial moisture content. The entire drying process took place in falling-rate drying period suggesting that liquid diffusion inside the biomass layer as the rate limiting mechanism. The drying kinetics was modeled with three semi-theoretical drying models. All the models predicted the experimental moisture loss well. Among them, Page's model best described the experimental moisture loss data based on R^2 , RMSE, and SSE values.

To our knowledge, this is the first study that has evaluated the effect of cell rupture on the complete downstream processing of microalgae like drying kinetics and lipid extraction. Cell rupture by autoclave, French press and sonication has improved the drying behavior of the microalgal slurry. It was found that Page's model can also explain the experimental moisture loss of the ruptured cells. The drying constant and the diffusivity increased with increase in temperature and the cell rupture. The drying temperature and the cell rupture method have shown a significant impact on both drying constant and diffusivity. As the rupture treatments can

increase the drying rate, it can be expected to reduce the drying time and hence the energy consumption of drying process. However, detailed energetic, economic and life cycle assessments are warranted to compare the ‘drying alone’ vs. ‘cell rupturing followed by drying’ approach. One such quantification would also be helpful in identifying the processes that need considerable improvements in terms of energy use. The drying rate constant values obtained in the study are also essential for further modeling of the drying processes and thereby evaluating the precise energy requirement of algal drying for biofuel production.

Our results also suggest that cell rupture can increase the efficiency of lipid extraction, in both solvent based extraction and supercritical carbon dioxide extraction. The fatty acid composition of the consortium had shown high level of unsaturation (56-67%) and was dominated by fatty acids like palmitate, oleate, linoleate and alpha-linolenate. For each cell rupture treatment, the degree of unsaturation had decreased with increase in temperature. In future, analyzing the rancidity of the lipids extracted from dried biomass may help in determining the feedstock quality of the dried product. It may also help in optimizing the drying process for a specific product quality in terms of biochemical composition of the end product. The supercritical carbon dioxide extraction studies carried out in this work were preliminary and extensive studies on optimization of working conditions are required to improve the efficiency of extraction. The possibility of extracting different compounds of interest in a sequential fashion using supercritical carbon dioxide extraction would enable multiple product stream approach to the biomass which can improve the overall economics. Detailed economic and life cycle assessment of the drying and cell rupturing processes tested in this study would be beneficial to evaluate the feasibility of these processes at the industrial scale.

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APPENDIX

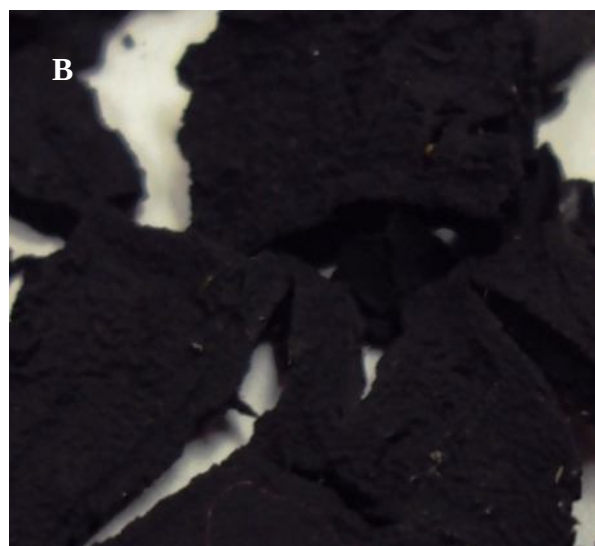
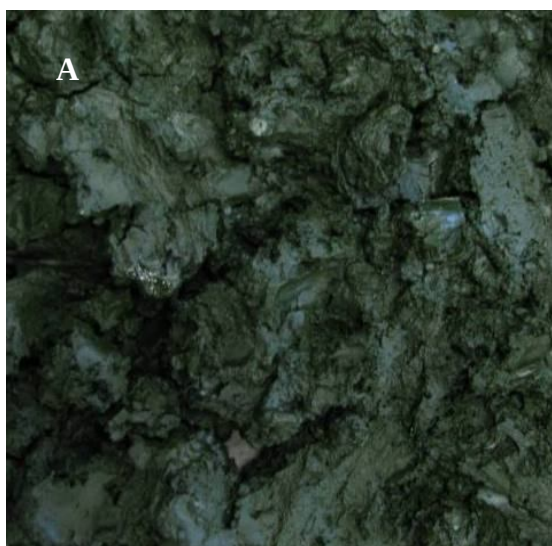


Fig A1. Wet and Dry algal consortium

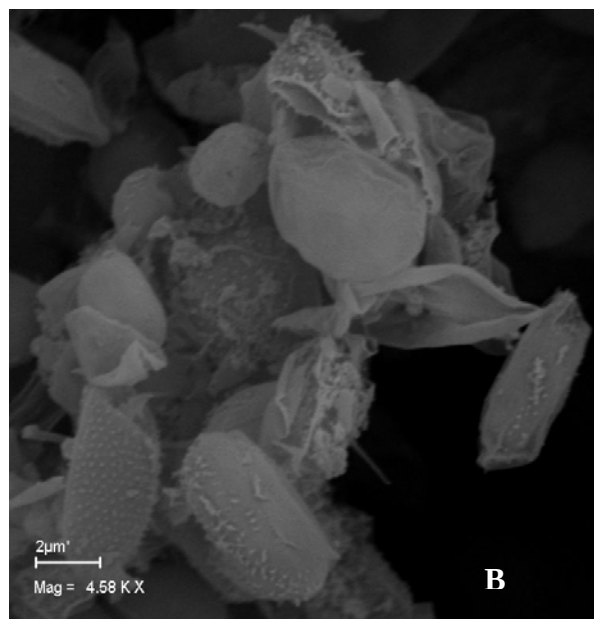
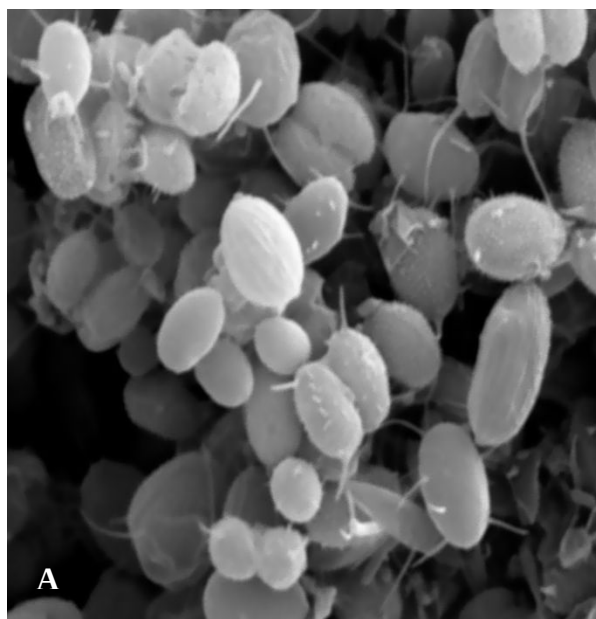


Fig A2. SEM images of intact algal consortium (A) and French press ruptured cells (B)



Fig A3. Supercritical lipids (A) and solvent extracted lipids (B) dissolved in chloroform

Table A1: Page model parameters and diffusivity for different treatments

		30°C	50°C	70°C	90°C
Untreated	k	0.0014±0.0002	0.0024±0.0002	0.0050±0.0009	0.0061±0.0004
	n	1.1905	1.319	1.2905	1.2925
	R ²	0.996	0.996	0.997	0.997
	D	3.66±0.96	7.38±0.77	14.5±1.48	23.2±1.93
Autoclave	k	0.0039±0.0015	0.0037±0.0001	0.0059±0.0011	0.0082±0.0025
	n	1.04375	1.245	1.2905	1.3255
	R ²	0.999	0.997	0.997	0.995
	D	3.58±0.39	9.32±1.33	15.6±1.35	23.2±3.72
Sonication	k	0.0040±0.0030	0.0053±0.0027	0.0067±0.0005	0.00960±0.0030
	n	0.9873	1.0865	1.2515	1.2655
	R ²	0.995	0.995	0.997	0.995
	D	2.8±6.08	6.29±1.8	13.2±1.06	22.1±3.89
French Press	k	0.0015±0.0009	0.0072±0.0039	0.0082±0.0037	0.0084±0.0023
	n	1.2065	1.025	1.1535	1.2445
	R ²	0.999	0.998	0.999	0.999
	D	3.06±6.34	6.96±1.44	12.8±9.29	19.8±2.51

Table A2: RH of heating air and EMC for different treatments

No treatment					Autoclave			
T	K (min ⁻¹)	RH(%)	EMC pred ^A (%)	Emc Expt(%)	K (min ⁻¹)	RH(%)	EMC pred ^A (%)	Emc Expt(%)
30R2	0.0016	48.2	13.7-15.1	14.91	0.0030	23.5	10.23-8.56	9.32
30R3	0.0011	56.3	17.47-17.14	16.91	0.0029	45.2	14.39-12.80	11.25
50R2	0.0023	18.1	8.9-7.9	9.83	0.0039	14.5	8.13-7.5	5.54
50R3	0.0027	20.6	9.3-8.2	13.67	0.0036	24.5	10.10-8.69	7.58
70R2	0.0053	7.8	5.58-6.91	3.71	0.0054	13.9	7.43-7.4	2.53
70R3	0.0039	8.9	5.98-7.00	4.35	0.0051	6.7	5.13-6.8	3.24
90R2	0.0066	6.1	4.22-6.77	1.47	0.0107	4.8	3.59-6.6	1.12
90R3	0.0062	4.8	3.59-6.66	1.99	0.0056	6.1	4.22-6.7	1.51

Sonication					French press			
T	K (min ⁻¹)	RH(%)	EMC pred ^A (%)	Emc Expt(%)	K (min ⁻¹)	RH(%)	EMC pred ^A (%)	Emc Expt(%)
30R2	0.0033	56.1	17.4-17.03	12.55	0.0006	56.2	17.44-17.05	23.52
30R3	0.0014	62.4	19.7-21.06	18.25	0.0016	56.1	17.41-17.03	22.82
50R2	0.0052	18.1	8.88-7.91	4.18	0.0116	14.3	8.08-7.51	9.44
50R3	0.0026	22.1	9.65-8.38	10.25	0.0040	21.9	9.61-8.36	11.58
70R2	0.0064	8.9	5.98-7.01	3.45	0.0125	11.6	6.82-7.25	4.06
70R3	0.0073	7.8	5.58-6.91	3.38	0.0057	8.9	5.98-7.01	4.43
90R2	0.0072	5.1	4.37-6.68	2.59	0.0108	3.6	2.92-6.57	3.67
90R3	0.0084	4.8	4.21-6.66	2.27	0.0062	4.8	3.59-6.66	3.50

^A EMC predicted by GAB equation. The value range was obtained from the model parameters reported by Ait Mohamed et al 2003 and Vega-Galvez et al 2008.

Table A3. ANOVA table for drying constant k_3

Source	DF	SS	Mean Square	F value	PR > F
Temperature	3	0.0023793	0.00007931	19.57	<0.0001
Cell Rupture	3	0.00004651	0.0000155	3.82	0.019
Interaction	9	0.00004084	0.00000454	1.12	0.3778

Duncan Grouping of k_3 for drying temperature and cell rupture

Drying temperature	Mean*
30	0.002123 ^C
50	0.004712 ^B
70	0.006493 ^A
90	0.008125 ^A

Cell rupture	Mean*
Untreated	0.003756 ^B
Autoclave	0.005477 ^A
Sonication	0.005477 ^A
French Press	0.006398 ^A

*Mean values with same alphabets are not statistically significantly different at $\alpha=0.05$

Table A4. ANOVA table for Diffusivity D

Source	DF	SS	Mean Square	F value	PR > F
Temperature	3	2.44E-17	8.13E-18	323.12	0.0001
Cell Rupture	3	3.69E-19	1.23E-19	3.82	0.0065
Interaction	9	1.65E-19	1.81E-20	1.12	0.6586

Duncan Grouping of D for drying temperature and cell rupture

Drying temperature	Mean*
30	3.11E-10 ^D
50	7.60E-10 ^C
70	1.40E-09 ^B
90	2.20E-09 ^A

Cell rupture	Mean*
Untreated	1.21E-09 ^{AB}
Autoclave	1.29E-09 ^A
Sonication	1.11E-09 ^{BC}
French Press	1.06E-09 ^C

*Mean values with same alphabets are not statistically significantly different at $\alpha=0.05$

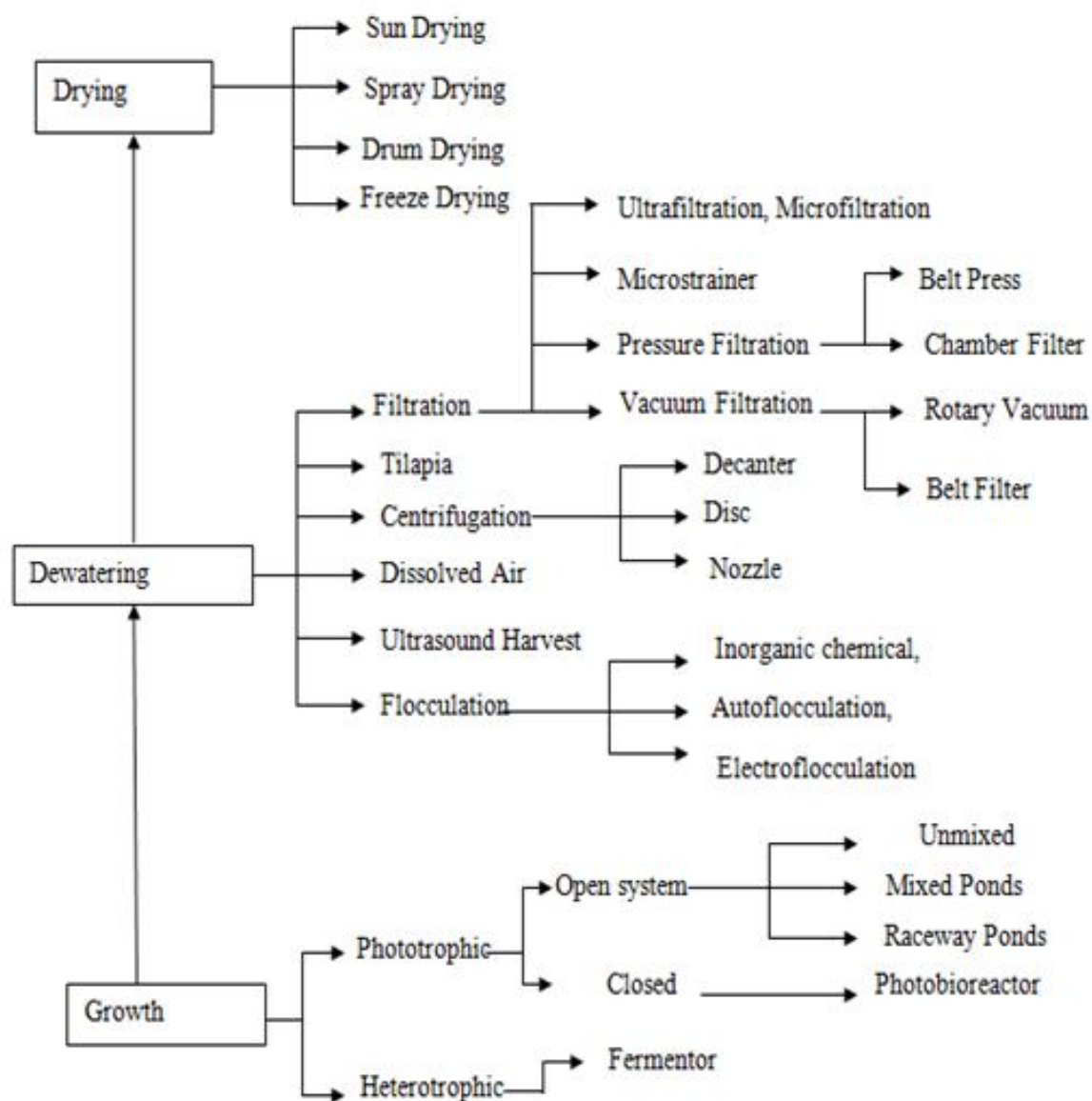


Fig. A4. Process options at each stage of microalgal biomass production