Potential Aspects of Microalgae Cultivation–A Review

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ABSTRACT:*During the past few decades microalgae have received considerable amount of interest due to their potential use in several industrial sectors related to food, cosmetics and energy. For a better microalgal biomass generation it is important to know the rheological properties of algal suspensions as these properties directly affect the energy requirements. Both the biomass concentration and cell morphology are major factors affecting the rheology of cell suspensions. This implies that a much greater increase in the apparent viscosity of cell cultures can be expected with an increase in biomass concentration. This will eventually reduce the efficiency of cultivation process because the culture viscosity, mixing, mass transfer and culture behavior are interlinked. Hence, a comprehensive understanding of the approaches to reduce culture viscosity are needed to enhance productivity in large scale algal cultivation systems. The present paper reviews the conventional practices in algal cultivation, harvesting along with significance of mixing and flow behavior of algal suspensions. The purpose of this insight into rheology during microalgae production is to develop better engineering design of bioreactors for optimizing the growth of microalgae and to improve some downstream processing.*

KEYWORDS –*Microalgae, Bioreactor design, Photo bioreactors, Mixing, Rheology*

I. Introduction

1.1Algae

Algae are the first chlorophyll bearing thalloid organisms which can synthesize their own food by photosynthesis. They belong to kingdom Protista. The plant body of algae is thalloid having no differentiation into root, leaf and stem. The cell structure of algae is of two kinds: prokaryotic and eukaryotic. The term algae routinely is used toindicate polyphyletic, non-cohesive and artificial assemblage of oxygen evolving, photosynthetic organisms. Algae use similar defense strategies against predators and parasite to that of plants. They also exhibit similar morphological characteristics to that of plants [1,2].Microalgae are capable of performing photosynthesis and play a vital role in the life of earth. As they produce approximately half of the atmospheric oxygen and help in the removal of CO_2 by utilizing it in the photoautotrophic growth. Algae are the food source for almost all aquatic life and act as sources for a number of industrial and pharmaceutical products for humans [3,4,5]. Table 1 depicts the common types of algae along with their basic characteristics.

Table 1: Types of algae and characteristics

Type	Characteristic	Example
Cyanobacteria	Also known as blue green algae, are gram negative. They are well known for carbon fixation and nitrogen fixation. Most common habitats are marine, freshwater, terrestrial and symbiotic.	*Nostoc, Cyanothece, Anabaena*

Cryptophytes	Cryptophytes are often found in freshwater and marine habitats. They are photosynthetic, motile, unicellular creatures that have two flagella.	*Guillardia, Camplyomonas, Geminigera.*
Chlorophytes	Most common habitats are fresh water and terrestrial. They have similar features of terrestrial plants.	*Chlorella, Dunaliella, Haematococcus*
Rhodophytes	They are commonly referred as red algaedue to the presence of red pigments in chloroplast.	*Batrachospermum,Chroodactylon,Bangia.*
Euglenoids	These have freshwater as well as marine habituated species. They exhibit both plant and animal like characteristics.	*Euglena,Discoplastis,Phacus.*

1.2 Microalgal Suspension Applications

Micro-algae are a large and biologically diverse group of aquatic microorganisms with a relatively simple unicellular structure that can be found in various environments ranging from freshwater for some species to sea water for others. Microalgae along with cyanobacteria, collectively called as phytoplankton dominate in the process of marine photosynthesis. Depending on the species and on cultivation conditions the chemical composition of microalgae keeps on varying. As a result, the chemical composition of microalgae is not an intrinsic constant factor. Some have the capacity to accumulate to the environmental conditions by altering their chemical composition in response to environmental variability. Accumulation of desired products from microalgae, can be achieved by changing the environmental factors like temperature, illumination, pH, CO_2 supply, salt and nutrients [6,7].

The use of micro algal suspension has come into practice from centuries, some species like blue green algae, including *Nostoc, Arthrospiea* have been used for food industry for many years.Due to high protein content in algae; they are considered as source of proteins.Algal suspensions have the capability to enhance the nutritional content of conventional food that in turn gives positive result to the health of humans and animals [8,9].These algal suspensions are rich source of biological active components such as terpenoids, fatty acids, polysaccharides, carotenoids, phenolic compounds and phytohormones [10,11]. On further examinations conducted on algal suspension it was concluded that algal suspensions are a source of renewable energy in photosynthetic gas exchangers and waste water treatment technologies [12]. The metabolites and phytohormones present in algal suspension showed a potential interest in the use of algae in the field of agriculture [13,14,15,16]. Commonly found phytohormones are auxins, abscisic acid, cytokinins, ethylene and gibberellins. Each of these hormones have achieved regulation in plant growth and development, tolerance to stress levels such as salinity and nutrients scarcity[17,18,19,20]. Use of algal suspensions as bio stimulants is another wide application of algae. Application as bio stimulants improves soil fertility, crop protection and direct growth stimulation. Mode of action of bio stimulants in improving soil is through activities like nitrogen fixation, nutrient availability, improving the physical and chemical properties of soil [21,22,23]. Crop protection is carried out by antimicrobial activity, reduction in fungal disease severity, reduction in foliar diseases and increase in the biomass of plant. Wide applications of algal suspension are exhibited by the different properties of algae through different studies [24,25].

Microalgae are a renewable biomass source which in the last decades have found applications within very diverse sectors such as the production of biofuels, the food industry, pharma and cosmetics

[26,27,28,29,30,31].Technology for transforming algae into fuel offers a sustainable biofuel production, the study of the growth of algae on a large scale is still relatively undeveloped [32,33,34]. As a result, it faces a number of difficulties that prevent the commercialization of algal biofuel production. In view of rich potential of algae in the various sectors namely food, pharmaceutical, biofuels, this present review is aimed to highlight the significance of different cultivation aspects of microalgae along with their advantages and disadvantages. Through this review it is intended to provide an insight into the importance of rheological properties and energy requirement for mixing from the perspective of shifting lab scale cultivation into a commercial scale production. Overall this comprehensive review will help in understanding the issues and concerns associated with commercial cultivation, harvesting and development of products from them.

II. Cultivation Methods

Microalgal species are well known for their rapid growth. Numerous number of microalgal cultivation systems are developed by differing various parameters.An optimal microalgal growing system should include a sufficient light source, good material transfers across the liquid-gas barrier, low contamination rates, and high land efficiency. Cultivation systems are basically characterized into open and closed systems. Raceway ponds are an example of open pond system and photobioreactors are example of closed pond systems [35].

2.1 Production Systems

Different types of algal production systems are developed for suitable cultivation on large scale and industrial scale.

2.1.1 Open Pond System

This type of system is mostly adopted for large scale cultivation and is considered as one of easiest and oldest method of cultivation. Natural bodies of water like lakes and ponds, as well as man-made systems like raceway and circular ponds, are all examples of open pond systems.These systems are well known for their economic advantages such as less maintenance cost, ease in cleaning and high volume of production [36,37]. The major dis advantage of this system is that the culture conditions cannot be controlled and has risk of contamination. Agitators are provided in these systems to prevent sedimentation of the algal biomass and adequate mixing of the nutrients.Another type of open pond system used currentlyis raceway pond [38]. This system consists of series of closed loop channeland paddle wheel which helps in the process of recirculation of micro algal biomass[39].

2.1.2 Closed pond system

Photo bioreactors are an example for culturing phototrophs in closed pond system. This kind of arrangement forbids direct material exchange between the environment and culture. The size of photobio reactor is compact in design compared to open pond system. The main advantage of using a photo bioreactor is to achieve higher cell densities and productivity. Using a photo bioreactor, controlled culture conditions can be attained and efficient mixing can be provided. Photo bioreactors are categorized generally into three types horizontal, vertical and flat plate photo bioreactors[40,41,42,43,44]. Major disadvantages of this system are difficulty in scale up, expensive, complicated cleaning process and rapid change in pH and oxygen gradients.

III. Harvesting Techniques

One of the key steps in the processing of microalgae is microalgae harvesting. Every harvesting procedure aims in the separation of microalgae biomass from culture media. Due to the high capital expense and energy demand, it is estimated by a number of studies that it accounts for 20–30% of the entire manufacturing cost [45,46,47,48]. Biomass has been harvested using a variety of techniques, including filtering, centrifugation, flocculation, and flotation.

3.1 Filtration

Filtration is a physical separation process that separates solid matter and fluid from a mixture using a filter medium. Thus this technique can be used to extract high concentration of algal cells from the medium. However, due to its high propensity for fouling and clogging, this approach necessitates frequent replacement of fresh filters or membranes, which could greatly increase its processing costs [49,50,51,52].

3.2 Centrifugation

Centrifugation is a mechanical process that involves the use of the centrifugal force to separate particles from a solution according to their density, shape, size, medium viscosity and rotor speed. This technique helps to separate microalgae cells from the culture fluid according to each component's density and particle size. Although this method has a high effectiveness in cell harvesting, it requires a lot of time and energy. Different types of centrifugal systems used are, hydro-cyclones, imperforated basket centrifuges, perforated basket centrifuges, disc stack centrifuges and decanters [53,54,55,56].

3.3 Flocculation

Flocculation is a process by which a chemical coagulant added to the water acts to facilitate bonding between particles, creating larger aggregates which are easier to separate. By addition of flocculants, freefloating unicellular microalgae cells congregate to form a larger particle known as floc. Chemical flocculants and bio-flocculants are the two main categories of flocculating agents. Chemical flocculants with low costs and wide availability, including salts of iron and aluminum, have been extensively employed in industry [57,58,59]. In chemical flocculation the chemicals must be eliminated through additional treatment procedures, which raises the cost. These chemical flocculants are not eco-friendly due to their high toxicity.In comparison to chemical flocculants, bio flocculants are significantly safer and more environment friendly. Additionally, they are less expensive to use, and frequently no pretreatment is necessary [60,61].

3.4 Flotation

Flotation is a process for selectively separating hydrophobic and hydrophilic materials. Harvesting by flotation encourages floating of cells on the surface of culture fluid using tiny bubbles that stick to microalgae cells which can be utilized. Advantages of the flotation system include, its high harvesting efficiency, at low cost. Dispersed air flotation, dissolved air flotation systems, and electro-flotation are the three primary types of flotation systems that produce the necessary air bubbles via various mechanisms [62]. Dispersed air flotation creates air bubbles using a sparger, which has a lower energy requirement than dissolved air flotation. Electro-flotation is the process of trapping free-floating microalgae by applying electrolysis to create microbubbles from its electrode. When using alternating current, this method also enables simultaneous cell disruption operation. However, the system in question uses a lot of energy and requires frequent electrode replacement due to fouling, which could increase the cost of production [63].

IV. Mixing and Flow Behaviour of Algal Suspension

4.1 Significance of Mixing

Mixing plays a vital role in the field of bioprocess engineering. Mixing set-ups are widely used in many processes such as preparing farm chemical concentrations, balancing nutrient amounts, blending various different substances and processing farm products. Maintaining homogeneity in the process of mixing is a challenging task as the flow streams generated in the stirred vessels may be turbulent and chaotic. Different flow patterns are generated by mixing process. Flow pattern is usually the way in which the fluids move through a reactor. The overall flow pattern of fluid is controlled by the density of the gradients which is caused by the temperature or composition variations. Flow patterns are the primary considerations while designing a mixer because creating the right flow pattern is critical factor in achieving the desired result [64,65].

Most common flow patterns in mixing are axial and radial. Axial and radial flow impellers are rotating mixer components designed for various types of mixing. Horizontal flow is generated by radial impellers, having blades aligned parallel to the stirrer shaft. Liquid moves in from the impeller towards the walls of tank, where it divides into two streams. One moving up to the top of tank and other down to bottom, and therefore eventually reaching the central axis of the tank and then back to impeller. Circular type of flow is also generated by radial flow impellers. The circular flow generated is reduced by the application of baffles [66]. Axial flow is generated by impellers having pitched blades, which make an angle less than 90° with the plane of rotation. Axial flow helps in the generation of strong vertical currents. Fluids leaving from the impeller are driven downward until it is deflected from the floor of the vessel and then spreads over the floor of the vessel.

Impellers are used to transfer energy from the motor to the tank's substance as efficiently as possible. Flow patterns depend on how the impellers are organised, shape and dimension of impellers also[67]. Right type of impeller needs to be selected depending on the requirement, so that energy is not wasted. In order to improve the mass transfer efficiency, efficient mixing is required. As a result, mixing is also necessary in the case of algal cultivation for efficient distribution of nutrients. Stirrer assisted vessel helps in maintaining the concentration levels of substances. Such vessels prevent the uneven accumulation of substances [68,69].

4.2.Significance of Flow behavior

Rheology deals with deformation and flow of materials both in solids and liquids. Fluids are of two types, Newtonian and non-Newtonian fluids. Rheology generally accounts for the non-Newtonian fluids[70].Cellular cultures require an in-depth knowledge of biological and physical parameters to control and optimize the process. Among the physical parameters, viscosity and rheological behavior are of first importance.The efficiency of the cultivation process depends upon rheology[71].Algae slurries are complex fluids composed of a liquid phase, algae cells, and insoluble solids. Rheological characterization of algal suspension is significant mainly for commercial production of bio-products from algae. The rheological properties of algal suspension affect the mixing and power requirement of the system. Algal suspension below a certain concentration or at lower concentration exhibit Newtonian characteristics, whereas at higher concentration or above a certain concentration they exhibit non-Newtonian characteristics [72,73,74,75]. Therefore, biomass concentration is a major factor affecting the rheological characteristics of algal suspension.

V. Algal Production Systems and Challenges

Microalgae are mostly cultured due to their wide range of application in different fields such as food, agriculture and pharmaceuticals. They are cultured in laboratories or on a commercial scale by providing optimum conditions and appropriate nutrient medium (Bold Basal'smedia, NPK media), whereas it is still harder to ensure higher productivity on a commercial scale [76]. Microalgae can be cultivated as open cultivation system such as open ponds, tanks and raceway ponds and in controlled closed system such as by using different bioreactors. If the algal biomass density is low, it can damage the culture when excess radiation is given. This problem can be overcome by providing a dense culture and expose it to the light. Temperature is another factor to control on algal cultivation especially in open system. Change in temperature on a daily basis or seasonally will adversely affect the productivity of algae. An optimum temperature for microalgae cannot be achieved as they are species specific and hence difficult to be achieved in an open system cultivation. The problem with closed system is that of overheating. Thus high temperatures, high DO and low density culture may damage the algal cell. Mixing increases, the overall bioreactor performance, but mixing produces shear stress and it damages the algal cells. This problem can be overcome by avoiding the conventional use of centrifugal pump. Diaphragm and peristaltic pump are recommended as they are found to cause less damage than centrifugal pump [77,78].Nutrients are very essential for the growth of algae and nutritional requirements for different algal species varies. In order to attain the optimal growth, availability of nutrients play a major role [79]. For ideal culturing of microalgae proper light source, nutrients, appropriate temperature and mixing should be provided. Scaling up of microalgae cultivation from laboratory scale unit to commercial unit is a challenge due to the several factors affecting the cultivation. Large scale cultivation of algae has many environmental benefits as they act as valuable carbon capture source and feed on atmospheric nitrogen oxide and on other greenhouse gases [80,81,82].

5.1 Problems Associated with Mixing and Flow Behaviour of algal suspension

Algal suspension is a type of non-Newtonian fluidexhibiting high viscosities [83,84]. The viscosity offered by such fluids produces an effect of resistance in fluid motion. Potential problem associated with algal suspension is that a portion of the suspension remains in unmixed state due to inadequate movement of fluid. As the rate of mixing increases the power required for the mixing gets increased. In order to tackle this problem, the mixing system is modified by changing the dimensions of the impeller, using different types of impellers. In order to optimize the light regime, the mixing levels need to reach turbulent flow of the algal suspension, as light is an important factor for the algal growth[85]. Pumping is necessary for significant mixing of the algal suspension and optimization of mixing and pumping which may lead to cell damage, thereby affecting cell growth. Too much of dissolved oxygen and light supply with low levels of mixing leads to bleaching of algal suspension, which greatly inhibits cell reproduction. As a result, mixing is an important factor for the algal production.

Large scale culturing can be done in open ponds but they become easily contaminated, evaporated and control over pH, temperature, carbon dioxide and light is not possible. Whereas in a closed system their condition can be satisfied, but growing in a bioreactor is expensive and quantity or product produced will be low [86].The key parameters that influence the rheological properties for algal suspension are packing factor, pH,algae cell surface charge and extracellular polymeric substances. The packing factor is defined as the ratio of the volume of the solids to the total volume of the particulate slurry. An increase in the packing factor increases the viscosity of particulate suspensions. The packing factor depends upon the cell size and cell distribution [87]. On analysis of the numerical results dilute viscoelastic suspensions showed Newtonian rheological properties, while concentrated solution showed non-Newtonian shear thinning viscosity behavior. The viscous modulus will dominate, leading to the fluid-like behavior of the slurry. The cell surface charge, measured by the zeta potential, gives information on the attractive and repulsive forces between the suspended cells. The algal cells will flocculate when the pH is 4 (isoelectric point). The algal cells will not flocculate when the absolute magnitude of the cells is high. When flocculation happens, it affects the viscosity of the suspension. Extracellular polymeric substance is a group of macromolecules that is secreted outside the cell; giving the cell a slimy coating that acts as a protective barrier against the environmental stresses. In microalgae their synthesis takes place in golgi apparatus. The extracellular polymeric substance is known to increase the effective viscosity of the algal suspension.

5.2 Commercial applications

When compared to other crop plants, they have a quick time to double, making them the fastest growing organisms. Algae have a high biomass production rate in a variety of harsh settings, which makes them useful in a variety of contexts. These organisms are in high demand now because of their potential to produce food and fuel. Algal biofuels have become more popular in the fuel sector as a clean, environmentally beneficial, and economical alternative to traditional fuels. Algal fuels can include bioethanol, biogas, bio hydrogen, biodiesel, and bio-oil.For a variety of uses in the food industry, including the manufacture of single cell proteins, pigments, bioactive compounds, medicines, and cosmetics, algae have beenused. Algae contain a wide range of bioactive substances; however, they still need to be used. *Haematococcuspluvialis* is a rich source of astaxanthinpigment, *Dunaliella* and *Chlorella vulgaris* are used to produce carotene, and dietary supplements. Algae contain 50% of starch which can be converted into ethanol. By the process of pyrolysis algal biomass are converted into various cost effective and clean products such as organic liquids, acetic acid, acetone and methanol. *Arthrospira* and *Chlorella* are two microalgae species that are well-established in the skin care industry. Some cosmetic companies, such as Partulines, Exsymol S.A.M., and Monaco, are renowned for producing microalgal extracts of the *Arthrospira* and *Chlorella* species for the creation of skin care products. Table 2 depicts list of algal species and corresponding products obtained from them. Table 3 depicts the list of industries manufacturing products from algae.

Table2. List of algal species and corresponding products obtained

Algal Species	**Product**
Nostoc	Food and herbal ingredients
Haematococcussp.	Astaxanthin
Dunaliella sp.	β-carotene
Nannochloropsis sp.	Eicosapntaenoic acid (EPA)
Porphyratenera	Lutein
Palmariapalmata	Flavonoids
Undariapinnatifida	Alginic acid
Euglena gracilis	β-glucan
Hypneavalentiae and *Padinapavonia*	Methanolic extract
Chondrus crispus and *Sargassum sp.*	Ascorbic acid
Fucus sp. and *Laminaria sp.*	Tocopherol
Chlorella vulgaris	Dermochlorella-Cosmetic
Arthrospira (Spirulina)	Protulines
Laminariahyperborea	High-quality alginates

Table 3. List of few industries manufacturing products from algae

Name of Industry	**Algal Species**	**Product**
Algatech	Green algae	Supply Astaxanthin
Algenist	Green algae – *Dunaliellasalina, Haematococcuspluvialis*	Eye serum
Aubrey Organics	Blue green algae	Hair conditioning mask
Aquarev industries	Red algae	Liquid fertilizer
BlueBio Tech Int. GmbH, Germany	*Spirulina platensis, Haematococcuspluvialis*	Dietary supplements
Dove	Red algae	Regenerative repair shampoo
E.I.D. – Parry (India) Limited, Tamil Nadu	*Spirulina, chlorella*	Nutraceuticals
Earthrise Nutritionals, LLC, California	*Spirulina*production	Supply for biochemical production
Exsymol company of Monaco	Extract of *Arthrospira*	Cosmetics
Jenelt	Red algae- *Porphyraumbilicalis*	Sun screen
La Prairie	Snow algae –	Anti-aging cream

	Chlamydomonasnivalis	
L'Oreal Paris	Red algae	Face pack
Nykaa	Green algae and *Spirulina*	Body serum
Oilgae Chennai, India	*Spirulina, Chlorella*	Biofuel
Osea	Red algae – *Chondruscrispus*	Moisturizer for eyes and lips
Pentapharm Company of Switzerland	*Nannochloropsisoculata*	Cosmetics
Zhejiang Binmei Biotechnology Co., Ltd., China	*Spirulina*Extract	Food, health, cosmetics, pharmaceuticals

VI. Conclusions

Open-culture systems have almost always been located outdoors and rely on natural light for illumination. Although they are inexpensive to install and run, open systems suffer from many problems such as, cultures are not axenic so contaminants may out compete the desired algal species; predators like rotifers can decimate the algal culture, and vagaries of weather can make proper control of nutrients, light intensity, and CO_2 challenging factors that need to be considered. Closed photobioreactors, on the other hand, have been used to axenically grow photosynthetic microorganisms such as microalgae, cyanobacteria, plant cells, and photosynthetic bacteria for various research. The main disadvantage of open culture system is that commercial scale production of algal species is not possible and wild microbial predators possess a great threat in open culture systems.There will be significant loss of water through evaporation and water conservation will be a major issue.Carbon dioxide is not used efficiently anda large area of land is required, therefore only waste land can be used for growing culture.Lower biomass productivity than that in closed cultivation systems, higher algal biomass harvesting costs in open ponds, difficulty in temperature control, supply of appropriate nutrients, optimization of pond depth, carbon dioxideinjection systems are the challenges which need to be addressed.

To overcome these drawbacks closed culture systems are used for culturing. They have higher productivity, greater efficiency in the use and fixation of the carbon dioxide injected, maintaining of suitable conditions for the growth of a specific strain, also hindering the invasion by polluting organisms. Closed systems can maintain higher cell density values than the open systems, reaching higher productivity, although the construction, maintenance and operation costs of the closed systems are considerably high. Its use at industrial scale is justified by obtaining high value products, such as nutritional supplements and food for the aquaculture sector. Compared with open-air systems, there are distinct advantages to using closed systems, but technical challenges still remain. In view of potential applications, development of a more controllable, economical, and efficient closed culturing system is needed.

To optimize the algal growth in relevance of rheology, suitable cultivation systems need to be designed based on further researches on the algal fluid behavior. Similarly mixing is done to improve mass transfer efficiency, efficient distribution of gases and nutrients in the algal suspension. In order to achieve mixing on a large scale aeration rate is increased which improves the utilization of light by algal suspension and enhances the biomass production of algae. The type of agitator utilized for mixing plays a vital role. If appropriate type of agitator system is not adopted a large amount of input energy is wasted. The flow track of the particle depends on the type of impeller utilized. The shape and dimension of the impeller blade also affects the mixing. The main aim of the stirred vessel assisted mixing system is to maintain the concentration levels of different phases of the substances. The stirred vessel mixing system increases the interaction between the particles and avoids the uneven accumulations in the vessel. The type of equipment used for the mixing process should be analyzed in order to achieve an adequate turbulence and flow in the vessel. Performance of mixing process depends on the overall product of mixing time, type of impeller blades, blade size, angular speed and the configuration of the vessel used for mixing. For large scale, mixing process the entire mixing set up needs to be analyzed in order to achieve the homogeneous product by high turbulence setup.

It is well established that microalgae have tremendous potential as a source of biofuel, food and high value bio-compounds. However, the limitations in productivity of microalgae and the drawbacks of bioprocessing technologies render the full utilization of microalgae biomass to be impractical. Therefore, more work needs to be done to further improve the existing technology. For instance, more advanced culturing technique should be developed to increase the productivity of microalgae. It can be concluded that mixing and rheology plays a vital role in algae growth and production on large scale, and these two factors need to be investigated further.

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