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Open Pond and Other Variants of Microalgae Cultivation, Crop Protection, Economics - A Critical Review and Evaluation

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Abstract

Current massive energy demand is challenging in combating climate change and global warming. One of the promising areas to tackle this issue is by cultivation of photosynthetic organisms like microalgae there by reducing the atmospheric carbon dioxide. Apart from biofuel, algal biomass can be used in the wide purposes like food, feed and serves as a raw material for chemical and pharmaceutical industry. Hence, for improved productivity of algal biomass, cultivation and harvesting system practices play a vital role. Sustainability of microalgal production depends on evaluation and optimization of different pond operation materials, energy inputs for cultivation systems to ensure scalability and robustness. Cultivation systems that are currently being practised are of two categories – open raceway ponds and indoor or closed systems. Compared to indoor cultivation, open pond systems are very challenging in terms of biotic and abiotic factors may directly or indirectly effect the algal growth leading to pond crash and economic losses. Algae have been successfully tested for large scale production of proteins, pigments, feed and fatty acids etc. But still many technological challenges, geopolitical wars, fluctuating crude oil prices in VUCA (Volatility, Uncertainty, Complexity and Ambiguity) world, significantly influenced the commercial viability of algae cultivation. Present review discusses about different cultivation practises widely used, for both indoor and outdoor systems and their advantages leading to better biomass and product formation. Various engineering and technological aspects related to instrumentation, sensors and artificial intelligence-based automation for cultivation systems will be discussed for economic viability.

Keywords: Indoor and Outdoor cultivation, Photo bio reactor (PBR), Raceway pond, Algal Economics, Automation, Artificial Intelligence

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1. INTRODUCTION

In current scenario one of the ways to combat climate change, global warming and to meet massive energy demand, is cultivation of microalgae and production of algal biomass. Microalgae are considered a promising feedstock for fuel, food and feed (Lin-Lan et al., 2020). Moreover, algal biomass can also serve as a sustainable raw material for chemical and pharmaceutical industry. Microalgae are fast growing organisms and are at least ten times more productive than any other crop. Furthermore, large-scale cultivation of algae does not require arable land and fresh water as microalgae are able to grow in saline and even in waste water (Abou-Shanab et al., 2013; Venkata Subhash et al., 2017). Hence, for economically viable production of algal biomass, selection of suitable cultivation system is very crucial and important aspect. Sustainability of cultivation systems for biomass production requires evaluation of different materials and energy inputs for cultivation systems to ensure scalable productivity, robustness, and less cost (Mendoza et al.,2013; Kuan Shiong et al., 2020; Doris Ying et al., 2020). The cultivation of microalgae requires a space and some important parameters responsible for optimal growth such as, nutrients quality and quantity, temperature, light, pH, salinity, aeration and agitation (Klinthong et al., 2015).

Broadly, cultivation systems that are currently being used for microalgae production are classified into two categories, open pond (raceway) systems and closed systems. In 1988, the first attempt to scale up for cultivation of microalgae was accomplished using open raceway ponds by Johnson et al. (Johnson et al., 1988). Subsequently, extensive research has been carried out to cultivate microalgae in raceway systems for production of biomass to be utilized for various products. Some of the foremost advantages of a raceway systems are nominal capital and operating costs, and a lesser energy requirement for agitation/mixing. On the downside, open cultivation systems require large areas for scaling up and are more prone to contamination with other microalgae, bacteria and grazers. Closed cultivation systems are primarily known as photobioreactors (PBRs), are cost-effective systems for algal biomass production in terms

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of quality and quantity. These can be operated at highly controlled conditions which are optimal for algal growth and, therefore, can easily overcome the drawbacks of an open cultivation system. PBRs can be designed and optimized according to the algal strain selected for a specific product. These are the closed system which relatively utilizes very less space, while increasing the light availability and significantly decreasing the contamination issues. However, PBRs also have some disadvantages, such as bio-fouling, overheating, cleaning issues and building up of high DO (dissolved oxygen) levels resulting in growth limitation and high capital cost in designing and operation. In future, for sustainable biomass production from microalgae, it's important to develop a large-scale closed cultivation system that can be operated under optimal growth conditions with capability for sterilization, and with negligible contamination risks. Although, it is difficult to compare both open and closed systems, but the general consensus advocates that open systems may predominate for mass cultivation of microalgae for biofuels, while photobioreactors will be more useful for production of high value products to be used in food, feed, cosmetics and therapeutics (Amaro et. al., 2011). This present literature review explains about the major cultivation systems for commercial production of microalgae biomass, also covers advantages and disadvantages of these systems while cultivating microalgae.

2. Cultivation of Microalgae:

2.1 Lab scale cultivation

2.1.1 Inoculum development

Generally, scale up of microalgae culture is done by successive transfers of algal cultures from smaller 250 ml flasks to 20 L carboys followed by the addition of culture media. As per published literature and common practice of algal cultivation includes different steps from inoculum development to open pond cultivation. First step is from the preservation tube to 250 ml conical flask system for culture activation in kuhner shaker under optimized conditions. Second stage is from the activation culture to 500-1000 ml shake flask the system to generate a pre-inoculum culture; thirdly the pre-inoculum culture is transferred to a system of 20 L carboys to obtain an inoculum for larger cultivation systems, followed by inoculation of the culture in multiple 20 L carboys called propagation cultivations to get required volume of culture to be inoculated in the photo-bioreactors and other cultivation systems. The four successive transfers, right from the preservation tube to shake flask step until the cultivation propagation, are run under sterile conditions to avoid any microbial contamination. The growth of microalgae in any cultivation system follows three main phases, lag phase or adaptation phase, log phase or exponential phase and stationary phase or death phase. The duration of each phase depends on type of algal species and the condition of cultivation. To understand the growth kinetics of each culture it is essential to monitor the cell count, dry weight and optical density of the algal samples on every day basis (Fig. 1).

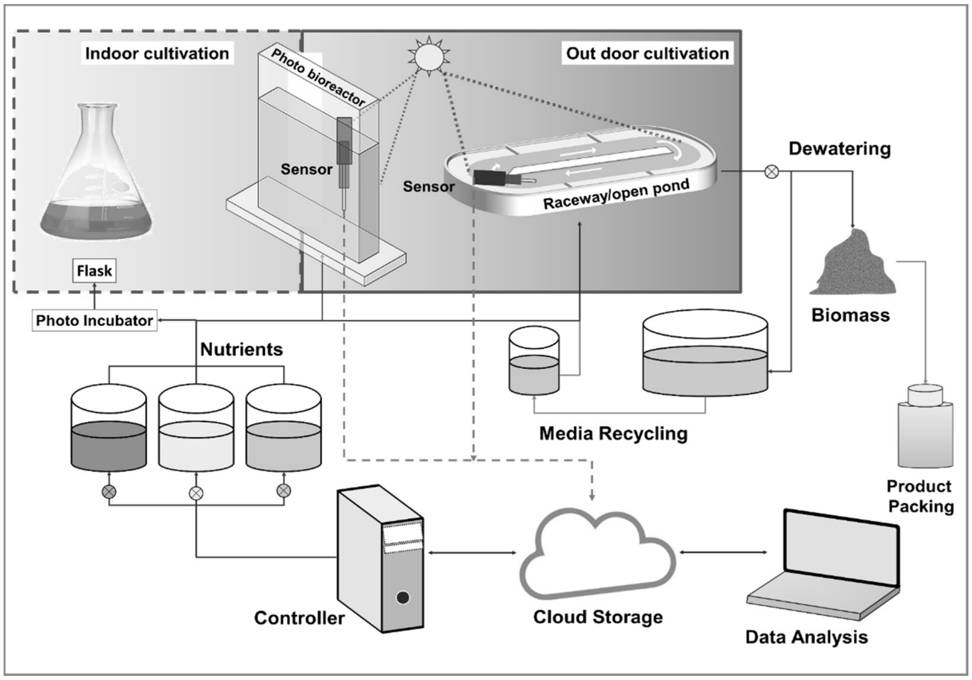

Figure 1: Schematic representation for microalgae cultivation methods and integration of technology

2.1.2 Indoor cultivation systems

Indoor cultivation consists of very sophisticated systems, where growth parameters can be optimized based on strain type and product formation, which are usually called as Photobioreactors (PBRs). In past few years, PBR's have been widely used to cultivate high density algal cultures and hence, large quantities of microalgae biomass (Chisti et al., 2007). These systems are usually made up of transparent materials, glass or plastics in shape of flat panels or cylindrical. There are different models of PBRs based on geometrical configuration and orientation such as: bubble column, conical, flat panel, inclined, horizontal, helicoidal, tubular and so on. The main objective of designing these photobioreactors is to achieve high volumetric productivity. These PBRs have many advantages over open cultivation systems, as in these systems it is possible to control cultivation conditions. The concentration of nutrients, pH, temperature and light can be adjusted depending on algal species to obtain higher productivity in shorter duration of time. In PBRs, light is not limited to surface of the system, but it also passes through the transparent walls to reach inside the culture to maximize the light availability to beneath cells (Richmond, 2004) and hence efficiency of photo-biological reactions increases. The increase in photosynthetic efficiency ultimately increases the biomass production.

a. Photobioreactors

i) **Plastic bag PBRs**

In recent years, plastic bag PBRs are an attractive low-cost option for commercial production of microalgae (Wang et al., 2012). Plastic bag photobioreactors can be easily prepared and installed with an aerator. These are portable, can be customized for different volume, shapes and hung in different patterns indoors as well as outdoors on metal stands based on sunlight availability. For scaling up of algal culture, multiple number of plastic PBRs with a definite volume can be arranged in series. For example, Chen et.al. in their research used plastic bag PBRs with a volume of 5 L and hung the bags vertically to produce *Chlorella sorokiniana* biomass on a large scale (Chen et al., 2013). In another research, a pilot scale cultivation system with 20 polyethylene bags (each bag being 20 cm in width and 2 m in length and having 0.2 mm material thickness and a volume of 16 L) arranged on a fixed metal stand, was found to be a promising cultivation system for *Scenedesmus obliquus* (Abomohra et al., 2014). To reduce the cost of algal cultivation at mass scale and to control temperature in summers, plastic bag PBRs with larger volumes can be immersed in a water pool. Though many of the researchers prefer these plastic bag PBRs, its main disadvantage includes photo-limitation and inadequate mixing. Furthermore, these PBR bags are intrinsically fragile, so chances of leakage are more than occasionally. Hence, it can be devastating for mass cultivation. In long run plastic PBRs are not found economical as these have a short lifespan and difficult to clean. Also, large scale disposal of plastic bags may create a potential problem in environmental ecosystem (Wang et. al., 2012).

ii) **Tubular PBRs**

Tubular PBRs comprise of a group of straight, looped or coiled, transparent tubes that are typically made of transparent glass or plastic (Fernandez et al., 2001, Hall, et al., 2003,). These transparent tubes aid in capturing of solar energy/light and are also attached to nutrient reservoir. The point where nutrient addition takes place, gas exchange also happens. The algae culture suspended in the PBRs circulates to and fro to nutrient reservoir through the tubes by a pump, or airlift technology (Molina et al., 2000). The reactors are generally cooled by spraying water to maintain an optimum temperature necessary for algae cultivation (Dogaris, 2015,). In some cases, the temperature control is reported to be controlled by submerging or floating the PBR tubes in a pool of water. Tubular photobioreactors are in fact, an attractive option for large-scale microalgae cultivation in an axenic form and probably, is one of the best appropriate types for outdoor cultivation of algae at mass scale (Molina, et al., 2001). The first 100 m2 tubular PBR made up of polyethylene tubes was developed by Gudin and Chaumont for the cultivation of Porphyridium cruentum which is a red alga. The mass cultivation of Spirulina platensis in tubular PBR outdoors was explored (Torzillo et al., 1986). Helical tubular PBR system comprises coiled polythene tubes arranged around an open circular framework laid vertically (Zhu et al., 2008). Conical helical tubular photobioreactor (CHTP) is another variant of tubular PBRs (Morita et al., 2001), which has been used for mass cultivation of Chlorella sp. with the productivity of 26.6 g (m^2 d) _1 per installation area in a single basic unit and 31.0 g (m^2/ d) in a four-unit system (Morita et al., 2001). Tube diameter in these PBRs are generally limited to 0.1 meter as increasing tube diameter may result in a decrease in the surface/volume ratio. The decrease in surface volume ratio has a significant impact on growth of algal culture. As the density of culture increase, the algal cells begin to shade one another, and this shading effect leads to reduction in volumetric biomass productivity per unit of incident light (Jimenez et al., 2003). Tube length of reactor also play a critical role as it influences the residence time or circulation of the nutrient medium inside a reactor (Converti et al., 2006). Hypothetically, higher productivity in tubular PBRs can be attained either by increasing the diameter or length of tube (Molina et al., 2001,), but both parameters have the limitations. Too lengthy tubes result in accumulation of dissolved oxygen levels inside a reactor thus, may inhibit photosynthesis (Taiz et al., 2006). Oxygen concentration above 35mg/ml has been reported toxic to most of microalgae. Decrease in CO_2 gradient is well-known in case of long tubes, long tubes lead to CO_2 depletion which further results in algae starvation for carbon. Also, change in CO_2 gradient lead to changes in CO_2 fixation rate which in turn alters the pH gradients. When there is high rate of fixation of CO_2, building up of hydroxide ion in culture medium takes place and results in increasing of pH as high as 11. On the other hand, poor rate of fixation of CO_2 in culture medium may result in increase in hydroxyl ions and hence, acidification from carbonic acid formation. A well designed photobioreactor needs to have a proportionate pH (Cuaresma et al.,2009).

iii) Flat-Panel PBRs

These PBRs are cuboidal in shape and made up of glass, plexiglass or polycarbonate. In these PBRs culture mixing is generally done by bubbling. Special feature of these flat panel PBRs is high surface to volume ratio with shorter light path, leading to higher density of culture and thus higher productivity. Flat panel PBRs have another design feature that don't allow accumulation of dissolved oxygen in culture. In these systems CO_2 dissolution is also higher and hence culture scale-up is also fast compared to other PBRs designs. However, the installation cost of such PBRs is very high. (San Pedro et al., 2016; Kandilian et al., 2014; Kumar, 2011, Pires et al., 2012). In comparison to tubular bioreactors, these PBRs have some advantages with respect to compactness and wall thickness. The narrow U-turns present in the flat panel reactors use less space than the coiled tubes in tubular PBRs. Additionally; reactor wall thickness can be thinner than the tubular PBRs. Moreover, in such PBRs the panels on one side are usually irradiated by direct sunlight and can also be positioned vertically at sun facing position. These flat panel bioreactors were first described by Samson and Leduy and reported to be illuminated by fluorescence lamps on both sides (Samson et al., 1985). Later, Tredici et al. further improved the design of PBRs by fabricating the panel from commercially available transparent sheets (Tredici et al., 1991). A tilted flat panel PBR also known as flat inclined modular photobioreactor (FIMP), was designed and placed in such a way that it provides the culture maximum solar irradiance as well as a significant temperature control. Such types of PBRs are made up of transparent glass material glued with silicone rubber such that the light path can be easily changed (Hu et al., 1996). Enhanced productivity of *Monodus subterraneus* which is eicosapentaenoic acid producing strain to 58.9 mg/L/day was achieved in FIMP by maintaining a high optimum culture density of 4g L/day (Hu et al., 1997). However, the tilt angle had to be adjusted four times a year to achieve the maximum productivity (Hu et al., 1998). The tilt design lead to high capital cost of these PBRs for mass cultivation of microalgae. Due to high capital cost and operational difficulty in flat inclined modular photobioreactor, horizontal and vertical flat plate photobioreactors were more frequently used for the cultivation of microalgae. Proper reactor orientation towards sun light is crucial in vertical system for maximum irradiance. Algal cultures also grew better in the north/south orientated system and in some studies placed the flat plate reactor in the east/west orientation. These apparently conflicting studies were recently explained by Sierra et al, who reported that the intensity of solar radiations increases with earth's latitude. There are some added advantages in case of vertical type flat panel photobioreactors in terms of amount of light per unit volume of culture. It is usually determined by the penetration depth of the incident light path, and it varies with species to species. Different algal culture seems to have an optimum incident light path. However, the light path is limited at higher biomass due to culture density leading to decreased penetration and thereby causing light saturation effect (Hu et al., 1996). Due to modular design and operational ease, flat panel photobioreactors are used in microalgae mass production in indoor as well as in outdoor culture systems. Moreover, these PBR's reported to have high illumination surface area and low accumulation of dissolved oxygen as compared to other PBR designs such as horizontal PBRs. A large scale commercially available flat panel photobioreactor has a reported capacity of 6000L.

iv) Vertical Column PBRs

Vertical column PBRs are cylindrical in shape and for mixing of algal culture these PBRs have a provision of keeping the spargers at bottom. Vertical column PBRs are further classified into bubble column reactors and airlift column reactors (Ding et al., 2016; Peng et al., 2016; Lopez-Rosales et al.,2016, Yen et.al., 2016). In these PBRs, mechanical agitation is not required (Kumar et al., 2011). Vertical PBRs can be kept indoors as well as outdoors. In case of indoor set-up, provision of artificial light source can be made and in outdoors there is abundance of natural light that can be utilized for biomass production (Eriksen et al, 2008, Suh and Lee, 2003). Recently, the vertical column PBRs have become popular for large scale algae cultivation due to their low cost, efficient mixing, higher rate of mass transfer and easy operation. (Bitog et al.,2011, Suali et al, 2012).

Airlift and bubble-column photobioreactors are compact, low cost and easy to operate. Also, these are widely used in bioprocessing and wastewater treatment industries. (Miron et al., 2002; Miron, 2000). In air lift photobioreactors, growth rate was found to increase by 33 to 50% in case of *Porphyridium* Sp. and *Undaria pinnatifida* (Zhang et al., 2002; Merchuk et al., 1998) due to homogenous flow pattern (Krichnavaruk et al., 2007; Kaewpintong et al., 2007). Contrary to this, in a bubble column photobioreactor flow pattern is random and culture gets exposed to high light intensities for a longer period of time which leads to productivity loss. Furthermore, in airlift bioreactors cells are uniformly suspended in medium, while culture sedimentation is reported in the bubble column (Kaewpintong et al., 2007). Both bubble column and airlift photobioreactors are also used in production of microalgal biomass for aquaculture (Silva-Aciares et al., 2008). Air lift bioreactor holds some advantages for pilot scale microalgal cultivation such as, rapid mixing, homogenous flow pattern, good suspension, low power consumption, vertical orientation and high mass transfer rate (Petersen, 2001). However, exponential decrease in penetration of light with increase in cell density may pose a problem for scale-up operation of these photobioreactors (Bosma.et al., 2007). As mentioned in case of tubular bioreactors, increase in column diameter is a limitation in these PBRs as well. Hence, in addition to considerations of space demands and ease of cleaning, an optimum column diameter must be determined as a compromise between productivity and capital cost (Loubiere et.al., 2009).

v) Stirred Tank PBRs

These photobioreactors are vertical type and cylindrical in shape. In stirred tank photobioreactors, there is provision of different size impellers for agitation and optical fibres as external light source. Stirred tank PBRs are the most conventional type of PBRs being used for microalgae cultivation at lab scale. The major drawback of these PBRs is low surface area to volume ratio and hence less light harvesting efficiency (Ogbonna et al., 1999). Like vertical PBRs, stirred tank photobioreactors cannot be scaled up for outdoor cultivation. However, multiple units can be integrated for scale up of these photobioreactors which may not be economical for algae cultivation.

b) Indoor Biomass Productivity

The biomass productivity is commonly reported in terms of aerial, volumetric and daily productivity. In aerial productivity algal biomass is reported as grams per square meter, tons per acre, tons per hectare, volumetric productivity is measured as grams per litre and daily productivity is expressed as grams per square meter (or per litre) per day. It has been reported that the biomass productivity in PBRs is affected by various factors such as shape and dimensions of PBRs, algal strain types, culture conditions (temperature, salinity, pH, location, indoor or outdoor, seasonal variations) mixing, light intensity, density of culture etc. The aerial productivity of plastic bag PBR systems has been claimed very high by Han et al., 2017. It can be as high as 40-50g/m^2/d, however, greatly varies depending on strain types, cultivation parameters and culture conditions. In a flat panel PBR cultivation system algal biomass productivity reported is ranging from 5 to 35 g/m^2/d (Acien, et al., 2017). On AFDW (ash free dry weight) basis, an annual average aerial productivity of 25 g/m^2/d is reported in flat panel PBRs (de Vree et al., 2015). In Netherlands, an average pilot scale aerial productivity for *Nannochloropsis sp*. in summer season was recorded 12.1g/m^2/d in horizontal PBR, 19.4 g/m^2/d in helical tubular PBR and 20.5g/m^2/d in flat panel PBR systems (de Vree et al., 2016). However, the maximum biomass productivity values reported in the mentioned PBR systems are 15.7, 24.4 and 27.5 g/m^2/d respectively (San Pedro et al., 2015). In Almería, Spain, *Nannochloropsis gaditana* cultivation experiments were conducted for two years under different operating conditions, the actual productivity recorded was 12.6 and 27.4 g/m^2/d in helical and flat panel tubular PBRs (San Pedro et al., 2015). Under optimal growth conditions and spacing the maximum biomass productivity for *Nannochloropsis* was found 30g/m^2/d in flat panel PBR (Huesemann, et al., 2016). A simulation model was designed in Netherlands for indoor cultivation of *P. tricornutum,* an average biomass productivity recorded was 11.5g/m^2/d in horizontal PBR, 15.6g/m^2/d in helical PBR and 29.7 g/m^2/d in flat panel PBR systems (Slegers et al., 2013). In Florida, the predicted biomass productivity based on simulation model in horizontal PBR for *P. tricornutum* was detected 24.1g/m^2/d followed by 38.5g/m^2/d in helical PBR and 39.2g/m^2/d in flat panel photobioreactor. Based on seasonal variations and solar irradiance, the target productivity values for PBRs thus may range from 25 g/m^2/day for horizontal tubes to 42.5 g/m^2/day for vertical/helical tubes to 52.5 g/m^2/day for flat panels/hanging bags. Theoretically, under controlled conditions, biomass productivities as high as 52.5 g/m^2/day were calculated (Tredici., 2010). The maximum theoretical photosynthetic efficiency recorded is 12.4% of total solar irradiation, but when accounting for photosaturation, photoinhibition, and other losses this number is further reduced to 5%. Thus, biomass productivity in range of 50–60 g/m^2/day depends on the location and corresponding total solar irradiance (Tredici, 2010).

According to Chen et al. (Chen et. al., 2016), maximum biomass and protein productivities claimed in *Chlorella vulgaris* was 268.1 and 155.4 mg/L/d, respectively, in 50 L outdoor vertical tubular PBR under optimum cultivation conditions. On the same study, they (Chen, et al., 2016) also compared the growth performance between indoor and outdoor cultivation using the same medium and aeration condition with the same PBR. The final biomass production (2.35–2.38 gm/L), protein content (54.2–52.4% per dry cell weight), protein productivity (110.1–114.5 mg/L/d) were similar for both indoor and outdoor growth. This performance similarity could be due to the photon flux density level (about 2000 μmol m^{-2}s^{-1}) in the outdoor culture, which allows the algal cells to grow quickly within the 8 hours of sunlight availability, as opposed to the constant 150 μmol m-^{2}s^{-1} in the indoor culture. In another study related to *Chlorella vulgaris* cultivation at a newly constructed flat panel airlift PBR with baffles was performed by Degen et al (Degen et al., 2001). In this flat panel reactor with uniform mixing, 1.7-fold increase in biomass productivity was recorded than in a chaotically mixed bubble column PBR of the same dimension. Further, 2.5-fold increase in productivity was also reported with a reduction of the light-path from 30 to 15 mm.

c) Advantages and Limitations in Indoor Cultivation

Indoor cultivation systems, contamination control is higher comparative to open cultivation systems, thereby leading to increase in biomass quality and quantity. In PBRs the evaporation loses are very low while CO_2 fixation levels are quite high. PBRs can operate at higher cell densities due to high surface to volume ratio. These systems are preferred systems for high value products as strain specific conditions can be optimized to get higher productivity (Amaro et al., 2011).

Algae culture scale-up and cultivation is challenging using PBRs because of their high manufacturing and operation costs (Carvalho et al., 2006). Use of glass to construct PBRs has many advantages but at the same time it has certain limitations like weight addition and safety risk. Some other operational difficulties are also associated with indoor cultivation systems, such as use of artificial light, culture mixing, cooling and gas exchange systems, mode of operation and most importantly cleaning of PBRs. There is a huge energy consumption to optimize algae cultivation parameters in PBRs to get the desired products (Gong and Jiang, 2011; Pires et al., 2012).

2.2. Outdoor cultivation methods

Algae can be cultivated in autotrophic (phototrophic), heterotrophic, mixotrophic (photoheterotrophic) (Ahmad et al., 2011, Amaro et al., 2011; Menghour et al., 2018; Tianzhong et al., 2013) modes. In phototrophic mode algae uses a sunlight and carbon dioxide (CO_2) as a source of energy and substrate to produce chemical energy through photosynthesis (Huang et al., 2010). The phototrophic cultivation is more economical and has environmental advantage. Since the atmospheric CO_2 is a principal greenhouse gas and this can be used for cultivation of algae to produce biomass and biofuels. To reduce the cost of cultivation and biomass production, there has been a continuous research in selecting robust algal strains and developing an efficient microalgal cultivation system. The algae are being cultivated at both laboratory and mass scales. The choice of algal cultivation system depends on capital cost, product type, nutrient sources, and CO_2 (Klinthong et al., 2015; Ángel et al., 2020). Microalgae can be cultivated either in open or in closed systems. In most the cases, the open systems are preferred for of mass multiplication owing to their easy operation and lower cost (capital and maintenance), freely available solar energy can be used and requires low energy for mixing and it is as low as $4Wm^{-3}$ (Mendoza et al., 2013; Jorquera et al 2010).

2.2.1. Types of open systems of cultivations

The open system of cultivation can be classified in to naturally found lagoons, ponds, lakes and man-made ponds, race ways, tanks and containers (Chun-Dan et al., 2016; Gupta et al., 2015). These are also termed as open-air cultivation systems. Based on mixing (agitated/inclined) strategies and shape, open systems are classified as stirred or unstirred open systems, circular, oval and race way ponds.

a) Unstirred open ponds

The natural pond/lake or constructed pond with a depth of 0-50cm (Hallmann et al., 2015) is used for this type of cultivation. In this type of cultivation, culture in the pond is not mixed, since there is no cost on water movement, these systems are considered as most economical. However, these ponds are characterized by lower productivity due to poor mixing and severe infections with bacteria, virus, protozoans and other cross algal contamination. The *Dunaliella salina* was cultivated for β-carotene production in unstirred ponds by Australian company Betatene Ltd (Borowitzka, 1999). The mixing in this pond was only due to natural wind convection (Borowitzka et al 1999). There are reports on the harvesting of microalgal biomass from natural lakes. In South East Asia more than 30 tons of biomass was harvested per year from natural lakes (Shen et al., 2009).

b) Stirred open ponds

i) *Circular ponds*

The circular open ponds have a provision of agitation with centrally mounted long arm agitator. The pivoting arm is a limitation in circular ponds, when the diameter of circular pond exceeds > 50 m, the mixing efficiency drops down. Because of this limitation, the circular ponds are constructed with a diameter of less than 45 m with 30-70 cm depth (Halliman, 2015; Shen, 2009). Various microalgal cultures are cultivated in this circular pond at different parts of the world (Japan, Taiwan, and Indonesia) (Lee, 2001). The *Botrycoccus braunii* was mass cultivated in outdoor circular ponds during various seasons (Ranga Rao et al., 2012).

ii) *Race way ponds*

Way back from 1950, the race way ponds are widely used for large scale microalgal cultivation. The race ways are simplest type of algal production systems (Jimenez et al., 2012; Shuwen et al., 2013) and superior to circular ponds. Race ways have closed loop circulation system (Jimenez et al., 2012). Typical depth of race ways varies from 15 to 30 cm (Lee, 1997). The race way ponds are constructed in oval shape with single channel or in race trach channel, where multiple race ways ponds are joined together. The material of construction is cement concrete or the dugged mud pond with various kinds of liners (Plastic or HDPE) to avoid the percolation losses (Bharathiraja, 2015). Race ways are constructed in open environment and may be covered using shade nets or by greenhouse structures to avoid the exposure to natural environment and to reduce the contamination level (Li et al., 2013). Media containing nutrient elements are added and inoculated with micro algae mixed by paddle wheel (Chisti, 2007; Radmann, 2007). The race way pond with paddle wheel mixing has been used in commercial scale algal cultivation from very long time. The paddle wheel creates vertical mixing and the typical velocity of water in race way operated paddle wheel varies from $0.15mS^{-1}$ to $0.30\ m\ S^{-1}$ (Craggs, 2014). The race way ponds are operated in batch, continuous, semi-contiguous and in fed batch mode (de Godos et al., 2014). Apart from paddle wheels, the supplementary agitation can be provided by using air lines (agitator) all along the race ways to ensure the better mixing (de Godos et al., 2014) and to avoid the culture settling. Compared to other open pond systems, race ways were considered as superior in terms of efficient operation, hence they are preferred for outdoor large-scale cultivation (Kumar et al., 2015). The worlds, >95 % of the algal production is based on race ways (Mendoza et al., 2013). The astaxanthin, a high value product is produced in race ways at capacity of 20000 L (Zhang et al., 2009). The largest race way facility for *Spirulina* biomass production is located at Calipa-tria, CA, USA and it occupies 440,000 m^2 area (Spolaore et al., 2006). The Australian company Betane Ltd. cultivate *Dunalialle salina* in large open pond race ways which are as big as 250 hectares and the system works without any mixing (Borowitzka, 1999). The race way ponds are meant to grow micro algae which can grow in environmental conditions only (Jimenez et al., 2003). Some of the micro algae reported previously in race way cultivation are *Scenedesmus* Sp., *Tetraselmis* Sp., *Arthrospiraplatensis, Chlorella* Sp., *Dunaliella salina*, *Anabaena* sp., *Haematococcus pluvialis*, *Nannochloropsis* Sp., *Actinas-trum* Sp., *Micractinium* Sp., *Phaeodactylum tricornotum* etc (Mendoza et al., 2013; Jorquera, 2010; Park et al., 2013).

2.2.2 Challenges in operating the open systems - biomass productivity

There are many limitations in achieving higher cell density in open pond systems which prevents the commercialization of open ponds for producing algal biomass (Shen et al., 2009; Perez Garcia et al., 2011).

i) Open ponds occupy nearly large areas

Generally, race ways are shallow, due to light limitation. Richmond et al., in their publication has stated that the shallow depth affects directly the scale up and productivities. To increase the biomass productivity, foot print area needs to be increased. As the pond becomes more shallower, CO_2 dissolution rate gets affected because of less contact time between gas and liquid. This would result in loss of sparged CO_2 (80-90%) (Richmond et al., 2004; Weissman et al., 1998). There are many other reports mentioning about the fixation efficiency of CO_2 in open ponds is in the range of 10-30% only (Li et al., 2013).

ii) *Geographical location:*

Geographical location will be primary factor that needs research for construction of open race way ponds. This is because of many local environmental elements determine the production and there by influencing the operations and cost feasibility. As Bennett suggests the main factors include, sunshine, rainfall, temperature, slope of the land, availability of water, cost of land and water. The geographical location receiving rainfall not more than one meter is ideally considered for constructing the race ways (Bennett et al., 2014). The rain fall factor is most important, as it reduces the salinity of marine algal cultures and thereby increasing the dilution of the culture. This dilution leads to increased downstream harvesting cost. Ideally the location of the ponds should be near to water source and most of the marine algal cultivation systems across the world are near to the sea shore. The minimum solar radiation required for cultivating micro algal culture in open pond is 4.65 KW h m^{-2} day^{-1} (Bennett et al., 2014). Operation of open ponds in temperate countries throughout the year will be difficult due to low sunshine and its effect is more in the winter seasons due to dropping temperatures. The slope of less than 5% is ideal for construction of open ponds with lower cost without levelling (Bennett et., 2014).

iii) *Operating depth of pond*:

Sutherland et al., reported that depth of the pond influences the engineering and biological parameters for algal growth. Depth affects productivity factors like mixing, temperature in the pond, power consumption of the paddle wheel, light availability, utilization and the frequency of algal cells exposure to light (Sutherland et., 2014). Areal productivity mainly depends on the pond depth and it increases with depth to certain extent. Sutherland et al, reported that, at 40 cm depth the aerial productivity was found 134-200% higher as compared to 20 cm depth (Sutherland et., 2014). The temperature also varies with depth, the shallower ponds have higher temperature which affects the growth of algae. Generally, the temperature variation in 10 cm pond will be 2 times higher than 50 cm pond depth. This is the reason why higher aerial productivity was observed in Algeria (a tropical country) with 50 cm depth as compared to 10 cm. Slegers et al., reported that productivity were found lower in Netherlands (temperate country) for 50 cm depth as compared to Algeria which is a tropical country. In contrary, some research work has reported the lower productivities for higher depth. At the depth of 5 cm pond operation Chiaramonti et al., reported the aerial productivities of *Tetraselmis suecica* F&M-M33 and *Nannochloropsis* sp. F&M-M24 were found 8.9 g m^{-2} day^{-1} and 10.44 g m^{-2} day^{-1} and these productivities were not significantly improved when the depth was increased to 10cm and it was 8.37 g m^{-2} day^{-1} and 14.1 g m^{-2} day^{-1} (Chiaramonti et al., 2013)

iv) *Mixing of culture:*

Hreiz et al., reported that the mixing of culture in race way pond is always inadequate due to large area, which accounts for approximately 69% of the total utility cost. In their studies they have observed that the biomass productivity can be increased by nearly 10 folds by optimum mixing (Hreiz et al., 2014; Emeka et al., 2020). Mixing ensures proper mass transfer, degas the produced oxygen by photosynthesis (Prussi et al., 2014), keep the cells in suspension, frequently expose the cells to light and improves the light utilization efficiency of algae (Chiaramonti et al., 2013). Rogers et al., reported that in the absence of light unnecessary mixing should be avoided, with mixing (during night time) the biomass losses were accounted up to 25%, hence it's advisable to slow down the mixing to avoid the biomass loses and reduce the cost (Rogers et al., 2014). Rogers et al., suggested that there have been several modifications and adoptions were done to improve the mixing pattern in race ways such as, mixing boards, mechanical pumps, Archimedes screws, airlift pumps etc. Other than these, modifying the islands, usage of flow deflectors, rectifiers, glided vans or bend designs were tried (29). All these were found costly and non-flexible during the operation (Rogers et al., 2014).

iv) *CO_2 sparging*

Algae utilize dissolved CO_2 as a substrate for growth and any limitation can affect its growth and productivity. The cost of CO_2 in algal medium accounts for 8-27% of total production cost (Li et al., 2013). It is also well reported by Keethesan et al., in 2011 that, the supply and transportation cost of CO_2, accounts for one third of cultivation cost (Keethesan et al., 2011). Weissman et al., in their study reported that the minimum CO_2 concentration of 65 µmol L-1 was found optimum for most of the micro algal growth, at pH 8.5 (32). Contrary to this, there are many studies where it has been reported about requirement of higher concentration of CO_2 for optimum growth of algae and this also depends on the strain of algae used (Weissman et al., 1988). Gonzalez lopes., 2012 confirmed in their report that the CO_2 addition depends on the depth of pond, pH, type of sparger, mixing velocity, gas liquid contact time. As the algae utilizes CO_2, the hydroxyl ions (OH) are formed, and pH goes down. The dissolution or absorption of CO_2 is higher at alkaline pH due to formation of bicarbonates from the formed OH group. However, Gonzalez lopes., 2012 stated that most of the algae grows best at 7-8 pH, therefore the absorption is limited at the optimum pH values of algal growth (Gonzalez lopes, 2012).

v) Light availability and utilization:

Being phototrophs, algae require light for photosynthesis. Kumar et al., observed that as the algal density increases in the pond, the increased pigmentation decreases the light availability (Kumar et al., 2013). It is also affected by other factors such as depth of pond and mixing rates. The biomass density and depth of pond determines the degree of light attenuation. While, mixing rate determines the frequency of cell exposure to light. The shallower pond receives higher amount of light as compared to deeper pond (Sutherland et al., 2014). However, decreasing the depth of pond can affect the biomass productivity. In the literature the depth of pond is reported as high as 50 cm (Brennan et al., 2010; Joana Monte et al., 2020). As per Mendoza et al 20cm depth of pond is ideal in terms of hydraulic performances and power consumption (Mendoza et al., 2013). Brennan et al., 2010 reported that the algal density of 0.66 g/L is not affecting the light penetration. To achieve higher cell density maintaining optimum mixing rate and depth of pond are essential.

vi) Oxygenation

Kumar et al., reported in their studies oxygen is a byproduct of photosynthesis of algae. For every gram of algal biosynthesis, 1.9 gram of oxygen is being produced (Kumar et al., 2014). Higher concentration of oxygen in the media causes photooxidation, which induces photorespiration and thereby leading to low algal productivity due to decreased photosynthesis. At higher partial pressure of oxygen, the oxygenase property of RUBISCO enzyme gets activated, which consumes the energy and causes the loss of around 50% fixed CO_2 from the biomass (Kumar et al., 2011; Zeng et al., 2011; Giordano et al., 2005). At mid-day of peak summer, the dissolved oxygen was found in the rage of 25-30 ppm for open race way ponds. It was reported that, oxygen concentration greater than 25 ppm had a negative effect on biomass productivity of algae. It's necessary to deoxygenate the ponds by proper mixing to avoid the loss of productivity.

vii) Salinity level

Sing et al., 2014, stated that the optimum salinity level in the media are required for the marine microalgal growth and maintenance and it changes with the rain fall and evaporation (Sing et al., 2014). The change in salinity inturn creates osmotic stress and affect the membrane permeability of algae which results in the poor growth (Sing et al., 2014). Optimum salinity can be maintained by adding fresh water or salts. The addition of fresh water increases the demand for potable water. According to Borowitzka 1999, there are many strains which can adopt to varying salinity levels in the media. Algal species such as *D. salina* and *Tetraselmis* sp. are known to grow in wider range of salinity (Borowitzka, 1999).

2.2.3 Outdoor biomass productivity

Algae can be grown in non-arable land using fresh/sea water (Chisti, 2007) in open ponds. The challenging task is to translate the high yields observed under laboratory conditions to outdoor productivities. The productivities vary with the cultivation methods and systems. However, compared to energy crops, microalgal biomass and oil yield were found higher than terrestrial crops. The energy crops such as switch grass, corn and soybean biomass productivity is 10-13Mt ha^{-1} $year^{-1}$, 9 Mt $ha^{-1}year^{-1}$, 3Mt $ha^{-1}year^{-1}$respectively. Adesanya et al., reported as the microalgal biomass productivity varies from 50-70 Mt ha^{-1} $year^{-1}$ in open ponds and it is 150 Mt ha-^{1}year^{-1} in closed systems such as photobioreactors. (Adesanya et al., 2014; José et al., 2020). The lipid content in algae varies from 10-40 % of their dry weight. Chisti, 2007 is stating that the oil yields from algae is 10-50 times higher than conventional oil seed crops (Chisti, 2007). For example, the oil production from rape seed cultivated in United Kingdom (UK) was accounted for 1.5 t ha^{-1} and the microalgae-based biodiesel obtained from large scale open ponds accounts for 40 t ha^{-1}. (Adesanya et al., 2014). The biodiesel production from micro algae can be commercialized if the cultivation cost is reduced to 0.25$ per kg of dry biomass with 40 % lipid content on dry weight basis. Besides these, there should be strategies like nutrient recycling, recovery of energy from the used biomass and sustainable supply of CO_2 which will substantially reduce the cost of production of biodiesel from algae (Chisti 2012; Alabi et al., 2009; Ze et al., 2020). The lower biomass productivity in raceway ponds is one of the limitations. The theoretical aerial biomass productivity in race ways cannot exceed 40 g m^2 d^{-1}, which is equivalent 146 t ha^{-1} $year^{-1}$ (Brennan et al., 2010). According to Chisti, under the most suitable climatic conditions, the micro algal maximum average annual biomass productivity in open ponds could attain 86.7 Mt ha^{-1} year $^{-1}$. Usually, the average annual productivity in outdoor ponds varies from 16-19 g m^{-2} day^{-1} algal dry cell weight. The average productivity of 13.2 g m^{-2} day^{-1} in *Chlorella* and 21 g m^{-2} day^{-1} in *Spirulina* were reported by 27 g m^{-2} day^{-1} and 26 g m^{-2} day^{-1} respectively .Handler et al (2012) reported that the biomass productivity in outdoor varies from 5-45 g m^{-2} day^{-1} based on foot print area of open pond and on the basis of power consumption it is 0.1-0.69 g W^{-1} day^{-1} (Ketheesan et al., 2011). The various microalgal productivities reported in outdoor open system of cultivation are shown in table 1.

Even though many efforts have been made to address the above challenges of open pond system (Fernandez et al., 1997), the biomass productivity remains considerably lower. To overcome the problems associated with open system, closed systems have been evaluated for cultivation of microalgae. The more focus has been given in developing economical and viable closed systems such as photobioreactors (PBRs), which are easy to operate and suitable for cultivation of all kinds of organisms. PBRs, offer better control over the process parameters, cross contamination and highest biomass is being produced (Richmond, 2004). There are many types of PBRs and are classified as, air lift, flat panel, horizontal tube, bubble column.

Table 1. A comparative biomass productivity analysis of various microalgae cultivated in open ponds.

Microalgae	Volume (L)	Depth (cm)	Mixing (ms-1)	Conditions	Aerial productivity (g m2 day-1)	volumetric productivity (mg/L/day)	References
Spirulina platensis	135000	30	0.3	Paddle wheel mixing	8.2	27	Jiménez et al. (2003)
Tetraselmis MUR 233	25000	20	0.2	Recycling of water, salinity tolerance	29.6 AFDW	Not reported	Sing et al. (2014)
Chlorella sp.	12	0.8	0.03	5 days growth study in batch	Not reported	350	Li et al. (2013)
Scenedesmus sp.	2000	20	0.22	Sump assisted race way pond	17	Not reported	de Godos et al. (2014)
Chlorella sp.	58890	12	0.3in race way and 0.5 in air lift	Hybrid -Race way and pond	Not reported	1670	Adesanya et al. (2014)
Scenedesmus sp.	20		0.08	Airlift driven, 4000lux	Not reported	160	Ketheesan et al. (2011)
Scenedesmus sp.	23		0.1	Airlift driven, 1% CO2, 110 μmol/m2/s;	Not reported	190	Ketheesan et al. (2012)
Scenedesmus sp.	23		0.1	Airlift driven, 1% CO2, 80 μmol/m2/s,	Not reported	85	Ketheesan et al. (2012)
Botryococcus bruanii Kutz AP103	2000		15	Manual mixing at 3ominutes interval	Not reported	114	Ashokkumar et al. (2012)
Botryococcus braunii LB-572	80		30	Twice a day manual mixing. 18 days batch	Not reported	100	Ranga et al. (2012)
Scenedesmus sp.	20000	20	0.22	Sump assisted race way pond, 5 days batch	Not reported	330	Dodd, 1986
Haematococcus pluvialis 26	100000	20	0.15-0.25	Circular pond - 15 days batch	Not reported	122	Zhang et al. (2009)
Haematococcus pluvialis Wz	20000	20	0.15-0.25	Circular pond - 15 days batch	Not reported	107	Zhang et al. (2009)
Nanochoropsis sp. F& M 24	20000	15-20	0.15-0.25	Batch mode -6 days	14.1		Chiaramonti et al. (2013)
Tetraselmissuecica F&MM33	20000	15-20	0.15-0.25	Batch mode -6 days	8.37		Chiaramonti et al. (2013)

2.2.4 Indoor vs Outdoor Cultivation Systems

Indoor systems (PBRs) are considered the best algal cultivation system to get higher biomass productivities from specific strains of algae and, the high value products with superior quality (Amaro et al., 2011). Moreover, in closed systems the chances of contamination are almost negligible. In case of contamination in PBRs, it’s limited to certain PBRs, hence, easier to opt a suitable crop control strategy to control the contamination. Major limitation of indoor cultivation system is that these are highly energy intensive, difficult to operate and hence, are not very economical. On the other hand, raceway pond cultivation systems are more economical, as energy usage is minimal in these systems in comparison to closed systems. The maintenance, construction, set-up, cleaning and operation is easier in open ponds. Moreover, these systems provide natural climatic conditions to algal strains like light, temperature and humidity. Some aspects of algae cultivation can be optimized depending on the algal strains selected for cultivation, like salinity, alkalinity and nutrient dosage.

Outdoor cultivation systems also have some technological challenges for sustainable cultivation of microalgae. These cultivation systems are vulnerable to contamination such as bacteria, fungi, zooplanktons and phytoplankton. In open systems, parameters crucial for cultivation like, temperature and light cannot be controlled. Significant amount of water losses in open pond systems can be seen due to evaporation process and there by leading to decrease in carbon dioxide efficiency usage by the algae. Contamination, less utilization of CO_2, fluctuations in light and temperature leads to low productivity in open cultivation systems.

2.3. Predators on algae cultivations

The open ponds are exposed to air current and wind. The monoculture of the target algae is least possible in open ponds due to exposure to external environment and due to cultivation practices. The culture in the pond is easily available to natural predators such as birds and aquatic insects. It also gets attacked by various microbes such as bacteria, fungi, virus, rotifers, ciliates, amoeba. This grazing reduces the productivity significantly. There is a constant search for robust strain which can tolerate and grow normally under the higher doses of biocides (Karuppasamy et al., 2018).

Due to inherent capacity of microalgae in producing biofuel or value-added products open pond algae cultivation is being extensively practiced worldwide (Wen et al., 2016; Hong et al., 2015; Rawat et al., 2011; Encarnacao, 2008). As per multiple studies and reports on microalgal cultivation and practices, open pond algae cultivation is very challenging in terms of abiotic and biotic factors. Abiotic factors such as temperature, light, salinity, pH, pond depth etc. and biotic factors such as zooplanktons, bacteria, diatoms, protozoans, other algae etc. directly or indirectly effect on the growth of algae which leads to pond crash and resulting in huge financial losses.

Karuppasamy et al., 2018, in their study observed that the biological sources are the major group of contaminants and usually responsible for crashing open algae pond in very less time. Biological contaminants include zooplanktons- copepods, amphipods, rotifers, ciliates, dinoflagellates etc. (Karuppasamy et al., 2018; Nalley et al., 2014; Kazamia et al., 2014), photosynthetic organisms or other alga like blue green algae, other plants etc., parasites e.g. amoebae, chytrids etc. (Lam et al., 2018; Gromov et al., 1999) and viruses (e.g. enteroviruses, hepatitis A, Norwalk, reoviruses, adenoviruses, rotaviruses, etc.,. Rowe et al., 2013 concluded in their study, that microalgal growth is depending on the seasons and accordingly the grazer diversity will also change, and thus other algal species are also can be a major threat to the open pond algae cultivation with targeted species. The unwanted algae or other species take over to the existing pond culture and supresses the proliferation of targeted strain. Algae phase could be an excellent example where it is being host specific it attaches to the targeted species and kill the algae (Rowe et al., 2013). As far as open pond algae cultivation is concerned the major question araises is that, what is the origin and different types of contaminations.

2.3.1 Origin of contaminations and types

There are many sources or origin of contaminations such as water, natural calamities, domestic animals, wind, birds, etc.

i) Water

Water is the primary requirement for algae cultivation and at the same time it is one of the crucial sources of contamination. Usually, open pond algae cultivation systems are located near to the water bodies such as sea, estuaries, lakes, canals, ponds, springs etc. The reason behind this is to avail the ample water supply for algae cultivation. However, these water bodies are the paradise for diversified organisms which later enters the algae pond as a biological contaminant. Some of the microorganisms can sustain in extreme conditions (Fenchel et al., 2012). Open algae pond may get complete crash in no time, if this contamination not taken into consideration.

ii) Natural calamities

By the observation from Bravo et al., 2014 reports on algal contamination, natural calamities such as earth quake, tsunamis, flood, heavy rain may destroy the pond cultures during disasters and after disasters too. Many known or unknown organisms may enter the pond and remain there in cyst form. Ciliates, dinoflagellates are the best examples which can rest in to the pond itself in cyst form during unfavourable conditions and throughout the seasons. (Bravo et al., 2014)

iii)Wind, domestic animals and birds

To control sources of contamination from birds and animals, Narala et al., 2016 mentioned in their literature that the selection of location is the major criteria for micro algae cultivation since all the biotic and abiotic factors are significantly responsible for pond crash. To minimize these issues, proper site selection is very crucial. Wind is the major factor where it brings all the contaminants in open pond such as dust, pollens, silica, leaves etc. Silica increases the diatom contamination very rapidly which may take over the targeted algal species. The diatom population compete for nutrients such as Nitrogen and Phosphorus there by targeted species becoming nutrient limited.

From the observations of Narala et al., 2016 studies, domestic animals such as dongs, cattle's etc. may become responsible to spread the contamination in pond, if site is not gated or enclosed boundary. Again, water bodies are the best feeding ground for domestic as well as migratory birds which are the major contamination sources or carriers. Birds keep on flying from one water body to another and likewise it spreads all types of contaminations in pond. Birds are the carrier of variety of biological pollutants (Narala et al., 2016) such as bacteria and viruses those contaminate algae culture. This may lead to significant reduction in biomass production or pond crash.

2.3.2 Type of contaminants

Through these all diversified sources different types contaminants may enter in open ponds. There are many types e.g. zooplanktons, copepods, rotifers, ciliates, dinoflagellates, protozoans, bacteria. viruses, fungi, crustaceans, fishes, larvae, parasites, amoebae, etc. These organisms might cause culture crashes in the whole pond in a short time, if not controlled without harming the targeted strain. These organisms based on certain chemotactic responses prefer to feed on specific type of algae (Verity, 1991). The commercial production of algae biomass faces significant hurdle when these above-mentioned organisms diffused to algae cultures.

Marine ciliates such as, *Scuticociliates*, *Uronema,* Hypotrichs, such as *Euplotes* sp. feed on bacteria, while copepods feed on ciliates which are then consumed by fish larvae. Pierce et al., reported the role of fish larvae and small fishes are being picked up by predatory birds (Pierce et al.,1992) or bigger fish in the food chain. Bacterial species such as, *Flavobacterium sp*., *Xanthus sp., Bacillus sp. and Pseudomonas sp*. can destruct mass algae cultivation (Wang et al. 2010a; Zhou et al., 2011; Imai et al., 1995; Shi et al., 2006; Wang et al., 2010a, b). From the published literature on grazer control in algal ponds is mandatory to save the targeted strains by applying some of the significant steps.

2.4. Predator controlling methods

Many researchers published information on achievement of sustainable and stable open pond algae cultivation successfully be accomplished by controlling the grazers. Contaminants may or may not be directly affect the algae however they may change the abiotic factors which may leads to the growth limitations. Integrated pest management strategies must be developed and practiced for maintaining appropriate algal densities (McBride et al., 2014). For successful algae cultivation, a huge challenge is the adoption of a specific strategy. Many researchers have developed such strategies successfully and being applied to control biological contaminants in open pond algae cultivation. (Karuppsamy et al., 2018, Venkat Subhash et al. 2019, Mendes et al., 2013, Lam et al., 2018). These all strategies can be categorised in different ways such as physical, chemical and biological and combinations of all the three.

i) Physical

This strategy is purely based on the physical stress effect on the organisms by different ways. Such as pH, salinity, temperature, electric shocks, UV- treatment, ultrasonication's, filtrations etc. The mechanisms behind this strategy is to damage the organisms by giving sudden physical stress by above mentioned ways to eliminate or minimize the contaminations from algae cultures. Ruttner-Kolisko, 1975 and Hoffmann, 1977 were tried temperature fluctuation to minimize the rotifer populations however individual parameter is not influencing the same dynamics. UV-radiations, high temperatures have also successfully tried to get rid of grazers in both laboratory and ballast water experiments however it was killing the algae as well (Munro et al., 1999; Tsolaki et al., 2010). Hence it is very important to optimize the same before applying. Ultrasonication is the common method for ballast water treatment to kill the unwanted species (Godi et al., 2010; Sawant et al., 2008). However, it is expensive but can be applied by optimising the methods. Subhash et al., 2019 have successfully come up with electric shock strategy to control the grazers in laboratory and open pond. Mostly all this physical stress strategy is been used to kill the organism or everything from ballast water or other things where they supposed to kill everything and not targeted algal species. However, these all physical strateges needs to be optimised with targeted algae and contaminants before implementations. This method is indeed very quick, crucial and safe if judiciously optimised and implemented.

ii) Chemical

Recent studies from Karuppasamy et al., 2018 and Montemezzani, et al., 2017 confirming that the chemical usages to control the pests is old being used rapidly throughout to control the grazers or pests in algal cultivation (Karuppasamy et al., 2018; Montemezzani, et al., 2017; Huang et al., 2014; Ke et al., 2009). All the chemicals are very much targeted to the particular thing such as membrane, nucleus etc. or it works as per the its mode of action and kill the organism. Hence this method is very popular in algal crop protection. Many published reports conform that the use of natural compounds, herbicides are effective in killing of grazers (Mendes et al., 2013; Macias et al., 2003; Sodaeizadeh et al., 2011). Apart from allelopathy compounds other fungicides, insecticides, hypochlorite also been used by many algologists to control the grazers in both laboratory and in open ponds (Karuppasamy et al., 2018; Weisman et al., 2010; Day et al., 2017)

iii) Biological

This strategy can be applied with keen observations of aquatic food chain or with the help of published literature. In aquatic food chain all organisms depend on each other or consume each other as a food. Hamilton et al., 2012, have used Daphnia to control the chytrids and hyper parasite to control the rotifers. This prey-predator relation or natural phenomenon can be used as a biological crop control strategy. However, it needs to be studied more extensively while applying in open pond algae cultivation. This could be one of the game-changing strategy in algae industry if get through succeeded at large scale.

vi) Combination of all three strategies

All the three strategies are unique and equally effective at their own places however it can be made more effective and economical than now by combining them together. If we use the half concentration of chemical or electric power e.g. Benzalkonium chloride works at 2 ppm and 5 mAP electric current on grazer respectively then half concentration of benzalkonium chloride i.e. 1 ppm and 2.5 mAp of electric power may reduce the cost of treatment and time of grazer killing as well. (Karuppasamy et al., 2018; Subhash et al., 2019). This both strategies work differently on single organism hence could be more effective in many ways, such as it reduces the cost of treatment and time of treatment with very effective results. The benefit of this combinations could be again chemical resistance in grazers may not develop due to dual effect of the strategies.

3. Economics

3.1 Indoor and outdoor cultivation

Although many algal strains have been successfully tested in outdoor and indoor for large scale production of protein, pigments, animal feed, fatty acids and anti-oxidants but technological challenges, geopolitical wars, fluctuating crude oil prices in VUCA (volatility, uncertainty, complexity and ambiguity) world greatly influenced the commercial viability of algal cultivation economics As per many algal TEA and LCA experts, microalgal biofuel production is quite a challenging task economically, because of many technical difficulties and efforts are to be made to be made to make the cost equivalent to fossil fuel economy (Davis et al., 2011). In techno economic model of algal biofuel, biomass production cost occupies 70-75% of the total cost. Outdoor cultivation is influenced by many parameters like selection of robust strain, land selection, abiotic stresses like fluctuating light, temperature, evaporation loss, heavy rainfall, biotic stresses like grazers and other contaminants. At indoor conditions all these above parameters can be controlled but it comes with heavy cost. Narala et al., 2016 has compared open pond, closed PBR, hybrid cultivation and recommended that the hybrid system gives contamination free exponential biomass production (15g/m2/day) with high lipid content and two-fold cultivation cycle of growth and harvesting. National Renewable Energy Laboratory report extensively covered 4 different PBR and open pond cultivation system and analysed many cultivation parameters which includes nutrition, inoculum supply, CO_2, nutrient, pumping, piping, dewatering of harvested biomass to 20 wt% solids) and other expenses and assessed the minimum biomass selling price (MBSP) based on $/ton of ash-free dry weight. As per their estimation, for open ponds MBSP is $494/ton and for four different types of PBR system its ranged from $639 to $1793/ton. In general, PBR cultivation cost is 2 to 2.5-fold higher than open pond cultivation (Clippinger and Davis, 2019). Hoffman et al 2017 opined that open pond cultivation yields biomass at $673/ tonne and fuel at $6.27 per gallon and compared with algal turf scrubber (ATS) growth which yields biomass at $510/ tonne because of simple harvesting system.

i)Value and volume

Based on their value and volume, algal biomass products can be broadly categorised into high volume low value products (HVLV) and low volume high values (LVHV) products. HVLV products like biodiesel and biocrude based products are preferred to grow at outdoor open pond systems. LVHV like, Polyunsaturated fatty acid, pigments astaxanthin and other value-added products are limited to closed indoor photobioreactors which needs stringent manufacturing practices and regulatory compliance with additional cost. However, researchers have demonstrated the open pond derived high value products, multi product bio refinery approaches, good cultivation practices and innovative cultivation techniques in both indoor and outdoor cultivations and valorised the algal biomass value and volume (Wang et al., 2017).

iii) Land

Right site identification and land cost for both indoor and outdoor cultivation has paramount importance which are included in capital expenditure (CAPEX). Site near to seashore reduces the cost of transport for seawater. Open pond occupies more land area which increases the land cost. Per unit of production of biomass product is less with reference to total land footprint and theatrical achievable limit us to 40g/m2/day.PBRs scores better in production and are space efficient (Clippinger and Davis 2019). Multisite evaluation under different climatic conditions, accessibility of the site, ecosystem, socio economic acceptance and livelihood of nearby inhabitants need to be considered for smell and proper outlet conveyance issue.

iv) Light

Open pond cultivation depends on time bound, fluctuating but cost-free sun light which greatly influences the productivity of algae. In closed system, investment cost, life time, PAR efficiency, and energy cost is a major concern, but it has a predictable productivity. Commonly different light sources like, fluorescent tubes, high intensity discharge lamps (HID), and light emitting diodes (LED) and wavelengths white, red and blue lights are use. It was predicted that LED lighting may increase PAR efficiency to 6% and there by decrease the capital expenditure.

vi) Net energy ratio

The net energy ratio (NER) is calculated based on input and output of energy of a system. NER is the ratio of the total energy produced by biomass and lipid (output) versus energy required (input) for material construction and all plant operations. Jorquera et al., 2010 predicted that horizontal tubular photobioreactors are not commercially viable because NER ratio is < 1. Flat-plate PBRs and raceway ponds is >1 and there is scope for increasing value if the lipid content increased to 60% dw/cwd (cellular dry weight).

v) ***Harvesting and dewatering***

Algal suspensions are harvested and dewatered by different energy intensive processes to desired concentration based on the business needs. Harvesting and dewatering occupies 18% of the total biomass production of CAPEX. (Bhujade et al 2017) Researchers have explored different possibilities to reduce the cost by physical, chemical, electrical, gravitational settling, bio-flocculation and genetic modifications of strains. Koley et al., 2017 tabulated the cost based on different harvesting techniques employed. For instances, pH based induction costs US$ 0.63-0.72, $FeCl_3$ based induction costs US$ 1.6 to 2.2, Dissolve Air Flotation costs US$ 5.4 to 6 and chitosan based US$16.2 to US$18.0 per kg of microalgal biomass.

vi) Contamination

Contamination of open pond algal cultivation through predators or non-desired organisms occurs seasonally which reduces the quality and quantity of algal biomass and can be controlled by physical, chemical, biological methods with additional cost (Karuppasamy 2018, Venkata Subhash et al 2019). Most of the occasions, open ponds are not operational because of rotifer, algal phages and grazers infestation and recovery which drastically increase the cost of biomass production.

vii) CO_2 capture, fixation efficiency and transport

Several cost-effective carbon capture mechanisms are available to concentrate atmospheric greenhouse gases and costs $40-45/metric ton but transport to algal site increases the cost additionally. For the production of 1.0 kg of algae biomass ~2.0 kg of CO_2 is utilised. In open pond, carbon fixation efficiency is 10 to 30% based on depth of the pond, whilst in PBR efficiency is ranging from 85 to 95%, assuming that the carbon content of the biomass is 50% (Zuang et al., 2016)

Viii) Energy consumption

Mixing of culture consumes more energy in PBRs than paddle wheel mixing in open pond cultivation system. NREL reported that power demand for open pond is 9,753 kW /5000 acres. And PBRs like horizontal tubular consume 16,681kW/5148acre, helical tubular consume 83155kW/3000acres, GWPII type consume 19,732kW/2428 acres and Leidos hanging bags need 12,967kW/2428 acres. (Clippinger and Davis et al. 2019). Cost of energy per unit is also based on economic status of the country and location of the site.

ix) Productivity

Year around average aerial productivity dictates the overall cost economics of algal cultivation. For open pond it is the range of 25g to30g /m2/day and in the case of PBR it is around 52.5g /m2/day (Clippinger and Davis, 2019). Productivity of strains are affected by many biotic, abiotic parameters and also by different cultivation practices. Attempts were made to achieve the maximum photosynthetic efficiency both in open and closed system by manipulating light and dark cycle.

x) Manpower

In the present days clean-in-place (CIP) of PBR and open pond, heavy rainfall and other natural and unnatural situations, malfunction of equipment restrict the operational days in a year and for productivity to be cost effective it needs to operate 300-330 days per year. Production and operational days are directly proportional and cost driver in open pond cultivation system. In manpower requirement, number of skilled and unskilled employee and their salary varies based on grade, skillsets and country.

3.2 Automation of the algal ponds

Microalgal growth and productivity, which is a complex photosynthetic process are influenced by multiple factors like temperature, pH, nutrient conditions, irradiation, contamination and fluid dynamics. Under certain conditions, either of these parameters could trigger critical situations, there by comprising the cultivation and biomass harvesting. Tracking of these situations, in large scale cultivation ponds can be challenging task due to complex microalgal biological changes, environment factors and human error. Industry 4.0, consisting of machine-to-machine communication systems, also known as “the Internet of Things” or IoT technologies can be widely applied in the microalgal biorefineries (Atzori et al., 2010). It consists of network of physical sensors and other technologies, that are interconnected for relaying of the data to end user through particular software over internet. Often these physical sensors are plug and play and can be widely used to monitor the algal growth and productivity in real time (Whitmore et al., 2015). Currently wide number sensors related to temperature, irradiance, water salinity, pH, optical density, turbidity, nutrient identification in the algal ponds are available in the market. These sensors are in turn monitored and can be controlled via Wi-Fi based systems through cloud connectivity. Based on the data provided by the IoT sensors, possible altercations can be made in the ponds or photo bio reactors to improve productivity or avoid any detrimental conditions. Eg. Nutrient content can be altered, based on the composition provided by sensors. Also, Decision Support Systems (DSS) can be built based on the sensor inputs and micro controller where automated choice can be made without any human intervention. Most of the high value products from the microalgae are grown either in indoor or photo bio reactors, where much of the cultivation parameters can be controlled. Hence, these IoT sensors are often used in the in PBRs so as maintain the productivity. *Spirulina* algae known for the best source of protein and antioxidants has be grown in alkaline water media with the stable temperature of around 86 °F. Ariawan . et.al., 2018 had established hardware and software architecture to grow these *Spirulina* using temperature, UV intensity, turbidity of the water flow and nutrient sensors which were able to monitor the culture condition in real-time (Fig 1).

i) Artificial neural network for microalgal contamination detection

Microalgal cultivation can be majorly done in two forms – one is of Open systems that include cultivation in natural or artificial open, shallow ponds and others are the closed systems consisting of traditional bioreactors. Each of these approaches has their own advantages and weakness both in terms of ease of working and productivity. For large scale biomass productivity and other product development open pond systems are economically feasible and scalable. Unfortunately, production in these systems are severely affected by external factors, such as physical, chemical and biological elements. Notably, biological contamination leads to reduced yields, culture crashes and poor control over the entire process. Biological contaminants in open ponds cover wide range of small and microscopic organisms that co-exist with the target algal species and compete for the available resources. Significant contaminants include grazers, fungi, bacteria and viruses. Detection limit of these contaminant in the huge sized ponds using microscopic techniques will be time consuming and not often error prone. Machine learning (ML) models can be quite handy for early detection of these contaminants and possible measures can be taken to prevent from pond crashing. Previously, many ANN models have been developed to identify the toxic algal blooms and their abundance in the coastal shores. (Recknagel. F. et. al., 1957) (Wei. B. et. al., 2000). Dawei. H had designed an ANN based model predictive control that can automatically learn dynamic performances of light algae bioreactor and there by regulate the light intensity illumination particularly for *Spirulina platensis* productivity. (Dawei. H et.al., 2008)

Artificial neural networks (ANN), support vector machines (SVM), Random forests (RF) are few of the major techniques that often used for algal bloom detection from the fresh water bodies (Li et al., 2014). Bioimage informatics combined with machine learning techniques can be used to handle extensive multidimensional data sets (Chessel, 2017). By using well understood and big training data sets, it is possible to apply machine learning to classify, cluster and quantify the contaminant population. ML Model development framework for contamination detection basically consist of four major steps –

- Image acquisition of the algal cells or pond cells
- Preprocessing and image segmentation
- Model training and development
- Prediction and classification.

Basic input for these machine learning tools include multi dimensional spectral images of algal cell or pond samples. For training the ML model various morphological features of different contaminants like grazers, rotifiers, amoeba, ciliates zoo planktons etc. as well as their chemical composition features can be extracted and trained for the model development. Also other variables like growth conditions, temperature, pH, season of detection and nutrient compositions which can alleviate the grazer development can be added as features for model training (Karuppasamy et al., 2018). Model prediction and evaluation can be done with three statistical analytics – precision, recall and F1-Score (Jungsu Park, 2019).

1. Conclusions and forward path

Sustainable and economic viability of microalgal productivity entirely depends on choosing the right cultivation systems and practices employed. Many important factors like geographical location, pond size and location, algal strain, etc. can play a vital role in the scalability. Combination of biological and engineering techniques can resolve many of the challenges that are currently being faced in the cultivation systems. With the advancement of complex biological editing technologies like CRISPR, algal strain engineering and performance can be improved by multi fold leading to better biomass or product formation. In addition, use of Industry 4.0 digital sensor technologies and artificial neural network based diagnostics, modelling will help in containing the algal pond crashes. This eventually will help in improved productivity and thereby reducing the carbon foot print in environment.

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