Treatment of Sewage Water by using Microalgae coupled with Membrane Bioreactor (MBR) System

Thesis Submitted to AcSIR for the Award of the Degree of
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In Biological Sciences

BY

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Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled ***"Treatment of Sewage Water by using Microalgae coupled with Membrane Bioreactor (MBR) System"*** submitted by ***Ms. Gayatri S. Gera*** to Academy of Scientific and Innovative Research (AcSIR) in fulfillment of the requirements for the award of the Degree of ***Doctor of Philosophy in Biological Sciences***, embodies original research work under my/our supervision/guidance.

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I hereby declare that the thesis ***"Treatment of Sewage Water by using Microalgae coupled with Membrane Bioreactor (MBR) System"*** submitted for the degree of Doctor of Philosophy to the AcSIR has been carried out by me at the Chemical Engineering and Process Development Division, CSIR-National Chemical Laboratory, Pune – 411 008, India, under the supervision of ***Dr. Sanjay Kamble***. Research material obtained from other sources has been duly acknowledged in the thesis. I declare that the present work has not been submitted to any other University for the award of any other degree or diploma.

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Gayatri S. Gera

Dedicated to my daughter Siya

Abstract

"Microalgae in combination with membrane technology is an emerging process to combat the ever increasing pollution in the water bodies (rivers, lakes and sea) along with CO_2 sequestration in a eco-friendly way without using the chemicals. As a microalga cultivates under four different conditions (photoautotrophic, heterotrophic, mixotrophic and photohetrotrophic), it can uptake nutrients, organic compounds, inorganic carbon (in the form of CO_2)in the presence of bacteria and uses natural sunlight as a energy source for their growth. In short, microalgae are versatile unicellular species which not only prevents the eutrophication of the water bodies but also helps to increase the dissolved oxygen concentration thereby, helping the aquatic habitat to flourish in a natural way. As, microalgae were generally dispersed and suspended in the water, its harvesting is one of the bottleneck issue of the microalgal industries to grow forits mass production for various applications like fertilizers, biofuel, animal feed etc. Recently membrane technology shows sustainable solution for the harvesting of microalgae from water. Membrane filtration in combination with microalgal treatment for sewage water not only reduces the water footprints but also reduce the energy requirement as it does not require extensive oxygen like the conventional sewage water treatment plants. Therefore, microalgae in combination with membrane technology will be the futuristic technology for the treatment of sewage water.

Conventional sewage water treatment plant required huge amount of air for aeration which is costly and also space required for plant is very large. The main objective of this dissertation is to (1) Screen and isolate microalgae from its natural habitat having potential to remove nutrients from sewage water.(2) Sewage water treatment using starved isolated microalgae species for the efficient removal of nutrients (TN and TP) (3)Optimization of process parameters for improving growth rate of microalgae and effective removal of nutrients.(4) Study the kinetics of nutrients uptake from the sewage water by immobilizing microalgae as well as to make effort for the reduction in the residence time. (5) Harvesting of microalgal biomass by applying membrane technology using different types of membranes. (6) Study the effects of various operational parameters such as Tran-membrane pressure (TMP), fluxes and membranes physicochemical properties parameters to get higher fluxes with maximum biomass recovery. (7) Economical analysis of the whole process for the treatment of sewage water using microalgae in combination with membrane technology for a small village.

The microalgae-membrane based technology has a huge potential for the treatment of sewage as well as industrial wastewater in the near future. However, efficient design of photo-bioreactors or raceway pond using artificial radiation or solar radiation is essential. The commercial viability for the treatment of sewage water/industrial wastewater using microalgae-membrane based process will be depend upon the efficiency of microalgae for uptake of nutrients, design of photo-bioreactor for growth of microalgae and its harvesting using suitable membrane technology.”

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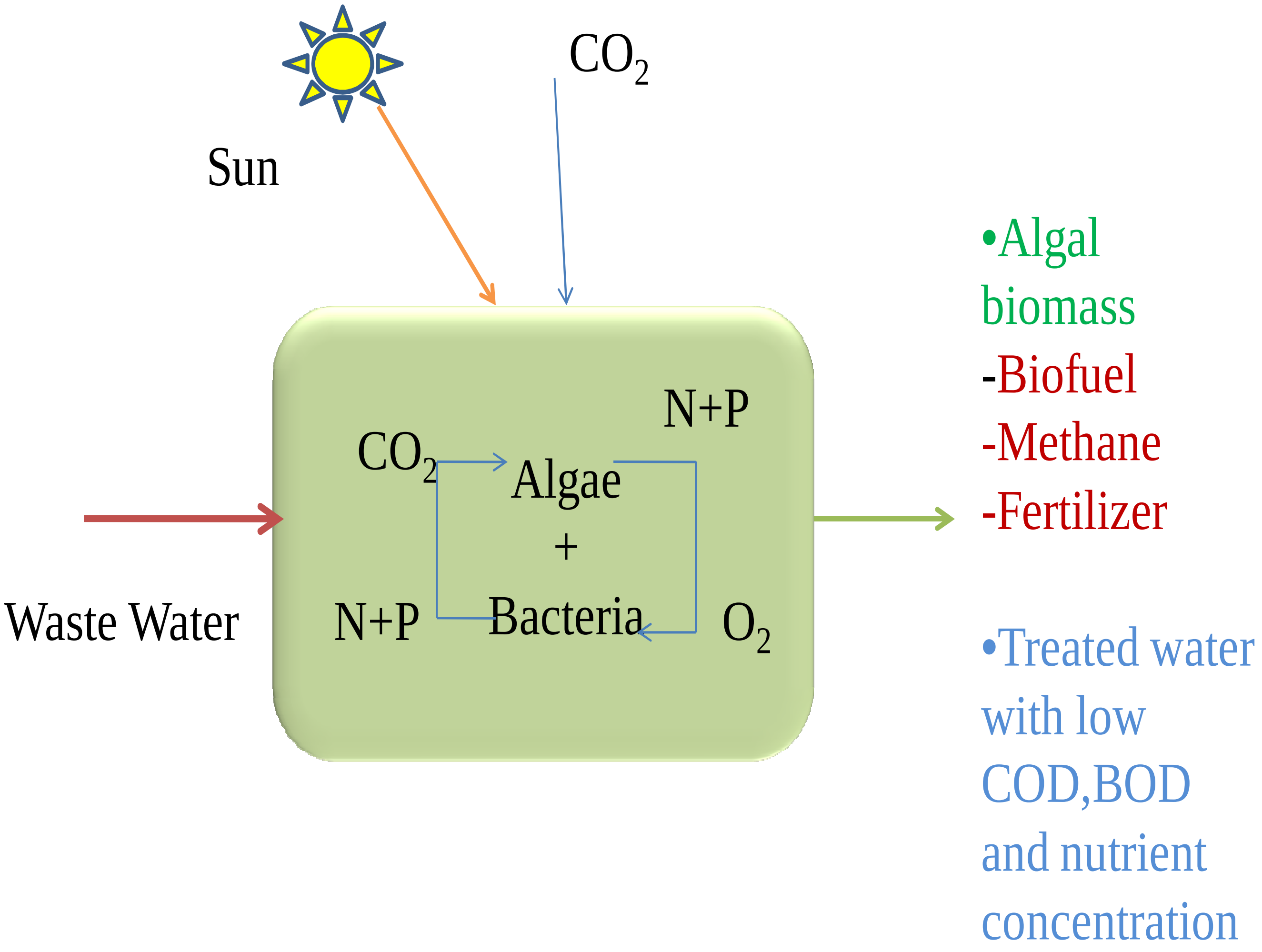
Introduction



1. Introduction [1]

General Introduction

Government of India (GOI) , International water management institute (IWMI) and United Nations (UN), has reported that India's water demand is set to increase to 1047 billion cubic meter (BCM) by 2050. Consumable water available in India is 1123 BCM whereas; the total water consumption was 829 BCM in 2006 (CPCB, 2013). This is an alarming situation for India as it will reach its limit for water consumption in few years. Out of this total usable water, India consumes maximum water (83%) for agriculture or irrigation purpose and only 5% of it is used for household purpose. In order to overcome this challenging task of water supply, sewage water generation and its treatment to produce reusable water needs attention. According to Central pollution control board (CPCB) 29000 million liter per day (MLD) of sewage water is generated from the cities and towns of India. When in fact, only 6000 MLD of sewage water treatment facilities exists. This commands for the development of eco-friendly sewage water treatment ability, so that the nutrients present in sewage water gets utilized thereby, providing fresh water for irrigation and preventing the eutrophication (nutrient pollution) of water bodies. Prevention and Control of Pollution Act 1974 highlights the use of treated sewage water for irrigation. However, from the past few decades conventional chemical methods were used for the treatment of sewage water which, in turn causes pollution in the form of precipitated coagulants (Grima et al., 2003).

The main objective of this dissertation is to study the feasibility for the treatment of sewage water by using microalgae in combination with membrane bioreactor (MBR).Microalgae also known as microphyte are unicellular photosynthetic organisms which utilizes sunlight, nutrients and carbon dioxide to form algal biomass. Nutrients viz. nitrogen and phosphate present in the sewage water in dissolved form may cause eutrophication of water bodies if discharged without nutrients removal. Although algae has immense potential for nutrient removal in addition with CO_2 sequestration thereby reducing the GHG emissions, but the capital and operational cost required for its commercialization are presently restrained(Chen et al., 2015, Ruiz et al., 2013,Kumar et al., 2010). Algae respirate the oxygen for the bacterias to grow in sewage water and which in turn helps to reduce the organic and inorganic matter in the form of COD, BOD and TOC. This symbiotic relationship between algae and bacteria support the pollutant removal from sewage water in an eco-friendly way without use of any chemicals (Sriram and Sreenivasan, 2012, Rawat et al., 2011). This grown algal biomass in sewage water can be converted into biofuel for viable displacement of fossil fuel such as petroleum based transport fuel. But, harvesting of the algal biomass grown in sewage water is currently the bottle neck issue for the algal industries to flourish on commercial scale. In order to address the harvesting problem membrane technology was studied in detail.

In order to overcome the challenges associated with algal biomass harvesting many key aspects needs attention such as: genetically engineered algae with big size, their density, recycling of inorganic nutrients, selecting the algal species with high oil content ≥40%, selection of membrane with less fouling and high flux (Bhave et al., 2012, Uduman et al., 2010). Integrated method of sewage water treatment along with algae harvesting is gaining lots of significance nowadays. Table 1.1 gives a brief idea about the various membrane technologies used along with microalgae for the treatment of sewage water. Lots of research has been carried out in National Aeronautics and Space Administration (NASA) for treating the domestic wastewater discharged along the seashore by growing algae inside the membrane enclosures which serve the dual purpose of pollutant removal as well as filtration of water rich in oxygen which will otherwise create a marine deadzone (Wiley et al., 2013). On the same lines nutrients removal of 90-92% was reported by Honda et al. by using polyvinyldifluoride (PVDF) membranes. It is therefore very clear that several investigations have already proven the effectiveness of membrane filtration in a single step microalgae harvesting.

Research objectives

The main objective of the proposed work is to evaluate the effectiveness of proposed technology in a quantitative manner so that it can be projected as a viable tool for sewage water treatment. It can be further divided as:

1) Investigations with microalgae:

- Optimization of process parameters for improving growth-rate.
- Improving potential to remove nutrients effectively from sewage water

- Investigate seasonal effects of growth of chosen algae

2) Investigations with polymeric membranes:

- Improvement in the fluxes

-Improvement in the antifouling properties: -Material selection: charge vs. other property balance (mechanical strength, spin ability, etc.)

3) Operational parameters:

- Evaluations with Flux vs TMP balance (has direct bearing with cost)

-Evaluations with other operational parameters: TMP, geometries and types of membrane modules), tanks, etc.

4) Economic analysis of the process for the treatment of sewage water using microalgae in combination with membrane bioreactor by considering a small village.

Scope of the Thesis

Chapter 1 reports the current water availability and its future requirement in India along with the general introduction about microalgae and membrane technology for the treatment of sewage water. It also throws light on the current sewage water generation and treatment methodology. In addition to this research objectives of the present work were discussed.

Chapter 2 gives a brief insight about the past, and present research studies carried out for the treatment of domestic sewage water and industrial wastewater by using microalgae. It also explains the importance of microbial selection, photobioreactor design and influence of various environmental parameters for the treatment of sewage water. In addition to this, literature review for sewage water treatment using microalgae coupled with membrane technology was thoroughly addressed. A future prospect for the potential uses of algal-bacterial biomass for wastewater treatment with CO_2 sequestration was also reviewed.

Chapter 3 reports the performance of standard algal cultures viz. *Chlorella protothecoides*, *Scenedesmus obliquus* and their combination for the treatment of sewage water in a closed photobioreactor illuminated with white LED lights. Then the process was studied by coupling a sidestream ceramic membrane module in a fed batch mode for algal cell recycle so, that the sewage water can be treated continuously for the days.

Chapter 4 describes the isolation and identification of microalgae cultures from the local sewage contaminated water bodies, from suburban of Pune city. One of the isolate viz. *Scenedesmus sp.* was studied for the effect of phosphate starvation on the treatment of sewage water. FTIR studies were carried out for comparing the performance of phosphate starved and supplemented algal cells for sewage water treatment.

Chapter 5 addresses the issue of reducing the residence time for the treatment of sewage water by using immobilized *Scenedesmus obliquus* on polyurethane foam cubes packed in a transparent column. Kinetic studies in terms of pore and film diffusion was reported along with the development of model equation for predicting the nutrients uptake from sewage water based on various operating parameters. The performance of algal biofilm reactor for the treatment of sewage waterwas studied in continuous mode of operation for 90 days.

Chapter 6 accounts for the treatment of sewage water coupled with membrane technology using solar radiation. Here, three different algal isolates were studied for the treatment of sewage water along with the three different membrane modules. In this chapter more emphasis was given on the harvesting of microalgae in terms of microalgae size, membrane material, transmembrane pressure and membrane flux. This study reveals the possibility of side-streamed microfiltration MBR as a low cost biomass harvesting process.

Chapter 7 provides the techno-economic analysis for the treatment of sewage water using microalgae in combination with side-stream membrane assembly as a case study for the small village. It gives a detail process economics based on raceway pond design, membrane assembly, process costing and cost of sewage water treatment including the algal biomass production.

Chapter 8 and 9accounts for the general conclusions of the thesis and future recommendations for implementing this technology on a pilot scale for the treatment of sewage water using microalgae coupled membrane system.

Chapter 2

Literature Review

Once-barren forest lands next to the Thane-Belapur industrial area in Navi Mumbai were given a fresh lease of life by an afforestation drive using sewage water (Ref: Times of India,9th June 2017)

2. Literature Review[2]

[2]"A version of this chapter has been published.

Gera G., Yewalkar S., Kamble S., Nene S. Remediation of domestic and industrial effluents using algae, Published, Springer publication ISBN no. 978-93-81891-23-0 (2015).

2.1 Introduction

In the environment, microbial activity is considered as one of the most important mechanisms for the abatement of water borne pollutants. In the natural and anthropogenic environment the wide range of contaminates are not eliminated by single specie but by the complex interaction of a mixed microbial population performing complementary reactions. This principle is very much applicable for the treatment of the industrial and domestic wastewater, which has excess of nitrogenous compounds (N) and phosphates (P). N and P along with various organic pollutants if not properly treated would create a devastating impact on natural aquatic ecosystems. Among the many other disturbing impacts, most prevalent is the phenomenon of eutrophication, which is the accumulation of high levels of organic matter and the decomposing organisms, which deplete the water of available oxygen, and causing the death of other organisms, such as fish. The excess nutrients in the aquatic ecosystem support the growth of various phytoplanktons. It not only spoils the water quality but also adversely affects the whole aquatic ecosystem. This chapter will address the possibility of effectively utilizing a natural microbial flora / consortium, enriched with the rapidly growing algae, for the remediation of polluted water bodies

2.2Potential of microalgae for the treating domestic and industrial effluents

Nutrient removal is becoming an important priority for wastewater treatment plants due to its detrimental impact on water bodies receiving this effluent. Dairy wastewater, swine manure or wastewater from piggery, food processing industries (fish processing, slaughter house waste), agro-industrial waste, are rich in nitrates, phosphate and other organic content. The current biological methods of nutrient removal make use of aerobic, anaerobic /anoxic methods that are inadequate for effective removal of these nutrients (Singh and Thomas, 2012). These constituents are often the nutrients required for the growth of green microalgae. Thus soluble nitrates and phosphate get removed from the waste when treated with green microalgae. At this point it is important to make a distinction between algal blooms and microalgae. Algal blooms are multicellular phytoplankton known to naturally produce bio toxins and grow as visible patches in water bodies. They are known to harm the health of plants, animals and adversely affect the environment.Periodic mechanical removal of these algal blooms is one of the solutions to avoid the dangerous side effect on the ecosystem. The other option is maximum removal of nutrients from the waters. Microalgae, on the contrary, are typically found in freshwater and marine systems. They are unicellular species which exist individually, or in chains or groups, are known to fix light photosynthetically and act as feed for rotifers, fish and other aquatic life forms. This biological method of removal of the phosphates and nitrates from the wastewater has many advantages over waste treatment process currently used in terms of

1.Effective uptake of N and P if sufficient solar energy is available

2.Simultaneous production of $O_2$and consumption ofCO_2in presence of light

3. No requirement for the supply of extra organic or inorganic nutrients.

4.Providing oxygenated water due to activity of algae.

5. Less sludge accumulation

6.Absence of generation of secondary pollutants; Ecologically, a safe technique

7. Generation of microalgal biomass which can be utilized for feedstock, fertilizer, biogas and biofuel (Xin et al., 2010, Wang et al., 2013, Woertz et al., 2009).

The treatment of sewage water/wastewater by using microalgae is a sustainable as well as eco-friendly approach and the resultant biomass helps for reducing energy, nutrients,fresh water cost as well as reducing green house gas emission (Olguin, 2012).

2.2.1 Direct use of algae

This algal system has more potential for the waste treatment in tropical and subtropical regions.Table 2.1 summarizes the various algae used for the treatment of the domestic and industrial effluents and their effectiveness in removal of phosphate and nitrates.

Table 2.1: Algae growing in the wastewater by exploiting the nutrients present in the wastewater

Wastewater source	**Algae used**	**%N**	**%P**	**Time (Days)**	**Reference**
Secondary treated sewage	*Phormidium laminosum*	48.7	99.7	2	*Sawayama et al., (1998)*
Municipal wastewater	*Chlorella* sp.	58.85	97.1	24	*Wang et al., 2013*
Domestic wastewater	*Chlorella* Sp.	36	12.5	3	*Singh and Thomas, 2012*
	C. vulgaris	47.35	18.8	3	
	Scenedesmus quadricauda	42.8	31.3	3	
	S. dimorphus	45.1	25		
Agroindustrial waste	*C. vulgaris*	60	100	9	*Gonzfilez et al., 1997*
Piggery waste	*Euglena viridis*	56.77	60	8	*Godos et al., 2010*
	C.sorokiniana	78.57	45	8	
Municipal wastewater	*C. vulgaris.*	81.6	92.8	10	*Lau et al., 1995*
Simulated wastewater	*C. vulgaris* immobilized	100%	93.9	1	*Tam and Wong, 2000*
Municipal wastewater	MixedalgalSp.	96%	99%	15	*Woertz et al., 2009*
Piggery wastewater	*S. quadricauda*	92.2 %	75%	19	*Gantar et al., 1991*

Growth of bacteria along with these algae must be acknowledged for their symbiotic relation and enhancing the remediation of the wastewater (Jia and Yuan, 2016, Sriram and Sreenivasan, 2012). These symbiotic bacteria utilize complex organic matter present in the wastewater as a carbon source and oxygen produced during algal photosynthesis. The bacterial growth enhances COD reduction of the wastewater. Though some algae are reported to degradesome toxic phenolic compounds, the bacterial symbiotic partner has the lion's share in degrading the toxicants present in industrial wastewater (Pinto et al., 2003).

2.2.2 Photosynthetic aeration

Mechanical agitation system or aerators are generally employed in the wastewater treatment process for providing sufficient aeration required for bacterial degradation of biological matter. However, it is the most energy intensive step as compared to any other process parameter. Brandi et al., (2001) and Sánchez-Monedero et al., (2008) have suggested the need for a submerged oxygenation system instead of mechanical aeration as the later system was found to be less environment friendly, due to a high rate of aerosol dispersion around the tanks containing microorganisms. This was likely to promote airborne transmission of pathogenic bacteria and fungi with an adverse bearing on human health (Li et al., 2013). The algal growth in the wastewater treatment tank provides a significant part of the oxygen(by photosynthesis)required by aerobic bacteria to breakdown the organic matter present in the wastewater and lowering the BOD level. Bacteria would, on the other hand, generate CO_2 required by algae as carbon source for growth thereby establishing a symbiotic relationship between the two. Oxygen generated by microalgae promotes the bacterial biodegradation of recalcitrant pollutants like phenolics, organic solvents, and polycyclic aromatic hydrocarbons (PAH's). Therefore, it is especially advantageous as the recalcitrant and toxic compounds can be easily degraded aerobically than the anaerobic process (Munoz and Guieysse, 2006). In eutrophic lakes dissolved oxygen concentration and pH increase is attributed to algae and these results in the inactivation of the faecal coliforms as well as *E.coli*. This is particularly beneficial to communities in developing countries who use raw untreated water from lakes and other freshwater sources (Ansa et al.,2011). The algae *Chlorella sorokinianain* combination with various pollutant specific bacteria e.g. *Ralstonia basilensis* for salicylate, *Acinetobacter haemolyticus* for phenol, *Pseudomonas migulae* and *Sphingomonas yanoikuyae* for phenanthrene were able to degrade the specified pollutants completely by photosynthetic aeration without any external aeration mechanism (Borde et al., 2003). The same consortium of *R.basilensis* and *C.sorokiniana* was reported to remove sodium salicylate at 87 $mgL^{-1}h^{-1}$ in a continuous closed photobioreactor with the 77 mg

$O_2L^{-1}h^{-1}$ of oxygenation capacity which is very much close to that of the mechanical aerator (Munoz et al., 2004). In this manner, the synergistic relationship between algae and bacteria is efficient for the treatment of industrial waste by minimizing the cost of aeration, which is a major cost centre in the wastewater treatment by conventional methods.

2.3 Characterization of wastewater

The ever increasing urban population with fewer sewage treatment plants is posing a threat to water resources. This in turn will create risk for sanitation and hygiene of public health. According to Central Pollution Control Board (CPCB) studies (2013), only 11,786 mega liter per day (MLD) of sewage is getting treated which is about 31 % of the total sewage generated. Whereas, the projected water demand for irrigation only is set to increase upto 1072 billion cubic meters (BCM) by 2050.Therefore, there is a need to adapt new strategies and technologies for the treatment of sewage water so that it can be reused for irrigation, industries and household purposes.Sewage water is generally composed of organic and inorganic matter, nutrients such as nitrate, ammonia, phosphorus etc., solids (settleable and non-settleable), oil, grease and many diseases causing pathogens. This sewage water is generated from personal washing, laundry, food preparation and the cleaning of kitchen utensils. Many disease-causing viruses, parasites and bacteria are present in sewage water which affects the community health. Hence, treatment of sewage water is an important issue of concern to public health. Table 2.2 gives the typical composition of untreated sewage water.

2.4 Microbial selection

The first step in wastewater treatment is to ‘know the wastewater’ and characterization physico-chemical parameters of waste water. The selection of the proper algal specie is another important step for the wastewater treatment. Growth of genera *Scenedesmus and Chlorella* is commonly observed in the water contaminated with domestic sewage in warmer climates (Jalal et al., 2011). Water site rich in silicates supports growth of diatoms **(**Brzezinski et al., 1998).Analysis of metal polluted rivers, receiving waste from a paper mill, containing 20-80 ppm chromium showed growth of *Oscillatoria, Phormidium, Scenedesmus and Pandorina* (Cervanteset al., 2001). In short, remediation of water occurs naturally by selective algae growing in the wastewaters; their growth being controlled by the nutrients present in the water and environmental factors like temperature and light.

Table 2.2: Typical composition of untreated sewage water (Source: CPCB 2013).

Constituents	**Unit**	**Weak**	**Medium**	**Strong**
Alkalinity (as $CaCO_3$)	mg/L	50	100	200
BOD	mg/L	100	200	300
COD	mg/L	250	500	1000
Total suspended solids (TSS)	mg/L	100	200	350
Settleable solids	mg/L	5	10	20
Total dissolved solids (TSS)	mg/L	200	500	1000
Total Kjeldahl nitrogen (TKN)	mg/L	20	40	80
Total organic carbon (TOC)	mg/L	75	150	300
Total phosphorus	mg/L	5	10	20
Total coliform	No./100ml	106-108	107-109	107-1010
Fecal coliform	No./100ml	103-105	104-106	105-108
Cryptosporidum oocysts	No./100ml	10-1-100	10-1-101	10-1-102
Giardia lamblia cysts	No./100ml	10-1-101	10-1-102	10-1-103

Considering this point, one should select the algae /mixture of the algae for the treatment, based on the organic and inorganic components present in the wastewater, ambient temperature and light conditions of the natural climatic ecosphere. Proper selection of algae will help in faster (less retention time), effective (maximum removal of the nutrients, toxic components) treatment of the wastewater and generate more biomass. This will make the process cost effective. Isolating the algae/ consortia growing in the various wastewaters naturally is very relevant field of research to select the proper algae for the wastewater remediation.

2.4.1 *Microbial tolerance*

Though wastewater nutrients to support algal growth, it also has several compounds that are potentially toxic to the micro algae, such as heavy metals and other recalcitrant compounds, especially in the industrial wastewater. Heavy metals like Cr are potential inhibitors of photosynthesis; at higher concentration they affect the cell machinery responsible for oxygen evolution notably the electron transport chain of the resistant algal culture (Yewalkar et al., 2013). This definitely hampers the growth rate of the algae. Phenolics compounds are one of the major industrial water pollutants that adversely affect the growth of algae and bacteria.

Piggery wastewater and swine manure supports the growth of algae after dilution. The growth rate of *Scenedesmus* sp. was increased by 3 fold after supplementing the medium with 3% (v/v) fermented swine urine (Kim et al., 2007). Gogdo et al., (2010) found that *C. sorokiniana* and *E. viridis* were found to grow well in the four to eight times diluted piggery wastewater and were able to remove P and N(*E. viridis* removed approximately 60% N and P. *C. sorokiniana* was able to remove 80 % N and 50%.P). However *S. obliquus* was unable to sustain continuous growth while *S. platensis* growth was completely inhibited in the eight times diluted swine manure. This growth inhibition was due to its sensitivity towards the high concentration of NH_3 found in the piggery wastewater. The microalgae species studied by them showed different degrees of intolerance to NH_3. The use of NH_3 tolerant microalgae may improve the biodegradation of piggery wastewater.

The tolerant algal species can be obtained by genetic manipulation, cell acclimation to progressively higher pollutant concentration or isolating the algae form the site of contamination where the prevailing microorganisms have already been exposed to the contaminates.

2.4.2 *Microbial interaction*

Heterotrophic bacteria play a significant role in aquatic ecosystems. They are not only decomposers in the aqueous phase but also convert dissolved organic carbon into particulate organic carbon (in the form of microalgae) (White et al., 1991). These bacteria grow symbiotically in wastewater. Microalgae provide O_2 essential for the aerobic bacteria, which aerobically degrade the organic pollutants. The algae consume the CO_2 generated by bacterial respiration. However due to algae growth the pH and dissolved oxygen concentration (DOC) of the wastewater was found to increase. This may be allowing selective bacteria to grow. The exo-polysaccharides synthesized by the algae were found to support the growth of microorganisms.

This microbial interaction is an essential requirement in the efficient treatment of wastewater treatment.

2.4.3 Microbial growth rate

The microbial growth rate in the wastewater depends on the concentration of nutrient, and inhibitory substances. Bacterial growth rate is several orders of magnitude higher than that of algal. This difference in growth rate may lead to an imbalance in populations of bacteria and algae; however the availability of oxygen to bacteria and carbon- dioxide to algae often acts as a rate-limiting step. . Mouget et al., (1995) found increased growth rate of *S. bicellularis* and *Chlorella* when co cultured with *Pseudomonas diminuta* and *P. vesicularis*. As the wastewater is highly complex and variable with respect to the chemical composition as compare to the synthetic media, growth rate of the algae may differ accordingly. It is also clear that bacteria enhance the algal growth by utilizing the photosynthetic oxygen evolved by algae, which would otherwise have caused them some deleterious effects due to photoxidation.

2.4.4 Microalgae predominance

Those algae which have a higher adaptability to the environment in terms of faster uptake rates of P and N assimilation, shifts in high pH and elevated levels of DOC, will have a faster growth rate in wastewater. The faster growing algae only predominate in wastewater. In uncontrolled environments it is difficult to maintain specific microalgal cultures, especially in case of raw sewage it is difficult in both open ponds and closed photo-bioreactor to maintain any selectivity in the algal flora because the sewage being treated may have some of its own algal flora that will grow along with the inoculated algal culture. It is our observation that many a times inoculating the domestic sewage with a selected algal specie, is often completely displaced by filamentous blue green algae or *Chlorella* at the end of the treatment.

2.4.5 Inoculation and selection

The rate of removal of the nitrates and phosphates form the wastewater depends on the speed of algal growth. The rate of removal of phosphates and nitrates can be accelerated by increasing the initial cell density of inoculum (Lau et al., 1995). Inoculation with immobilized algal for an improved degree of removal of a pollutant is another approach (Tam and Wong et al., 2000). However both these approaches are difficult to implement at a large scale. At larger scale preparation of an algal inoculum can be done using either fresh water for development of the algae or raw sewage or activated sludge (Munoz et al. 2006).

2.5 Waste Stabilization ponds (WSP)

For the wastewater treatment stabilization ponds are widely employed in which wastewater is treated by natural processes using consortium of algae and bacteria (figure 2.1). These waste stabilization ponds have proven to be one of the most stable, cost-effective and easy to operate process for the treatment of domestic and industrial wastewater treatment (Kayombo et al., 2000). These ponds can be effectively employed in the tropical and sub-tropical countries having moderate to high temperature, as energy from the sun is the sole requirement for its operation. Apart from the low energy requirement, it is easily manageable and requires simple maintenance in terms of cleaning and surveillance. Removal of pathogens is one of the key advantages of using waste stabilization ponds (Mara et al., 1987). Contrary to this, closed photobioreactor systems are more energy intensive, complex to operate and require sophisticated materials of construction. However compared to the open pond systems, PBR's are more flexible to operate, being more amenable to changes in physiological and biological nature of algal species and the improved mass and heat transfer, mixing and reduction in photo-inhibition and photo-oxidation. The closed photobioreactor are found as flat plate, tubular, vertical column or helical photobioreactor systems. They have lower footprint and better control over some parameters like the circulation speed, agitation, temperature, dissolved O_2 and CO_2 etc.which make them more beneficial for the treatment of wastewater using algae.

Stabilization ponds are the shallow man-made basins for the treatment of wastewater by using a natural process. They are also known as oxidation ponds or sewage lagoons or redox ponds and are widely used for secondary wastewater treatment. The first reported stabilization pond for the treatment of wastewater was Mitchell lake of an average depth of about 1.4 m in the city of San Antonio, Tex., in 1901 (Earnest. Gloyna WHO report, 1971). These are widely used in the tropical and sub-tropical countries where the sunlight is available in natural abundance.WSP are very suitable for the developing countries like India where solar radiation is available abundantly and operating cost is very low. Removal of pathogens by the combined treatment with algae and bacteria makes it more attractive for treating wastewater. Temperature, evaporation rate, waste flow and receiving BOD are the important considerations while designing the WSP. West Bengal is the only state in India where the WSP are installed, as Calcutta is one of the biggest wastewater fed-fisheries in the world. The four places in West Bengal where these WSP are located at Titagarh, Panihati, Ballay North Howrah and on the outskirts of Nabadwip. The history of Calcutta East wastewater fed-fisheries is80 years old and is still in place, providing employment to some 4000 people (Mara, 1997). Stabilization ponds can be classified as anaerobic pond, facultative pond and maturation pond which are employed in series or in parallel, depending upon the type of waste effluent, for effective wastewater treatment.

In the waste stabilization ponds helminthic eggs and cysts are removed during the process of sedimentation whereas *V.cholerae* is reported to be killed by the presence of low sulphide concentration in anaerobic pond (Mara, 1987). Waste Stabilization Ponds are more reliable and cost effective as they can be constructed using the relatively cheap local materials. If designed correctly it gives consistent and high quality treated effluent, which can be reused, in the aquaculture and for crop irrigation. A poor design may lead to odor emission in case of anaerobic ponds. Therefore, there is a need for expert supervision for periodical removal of sludge and its disposal (figure 2.2).

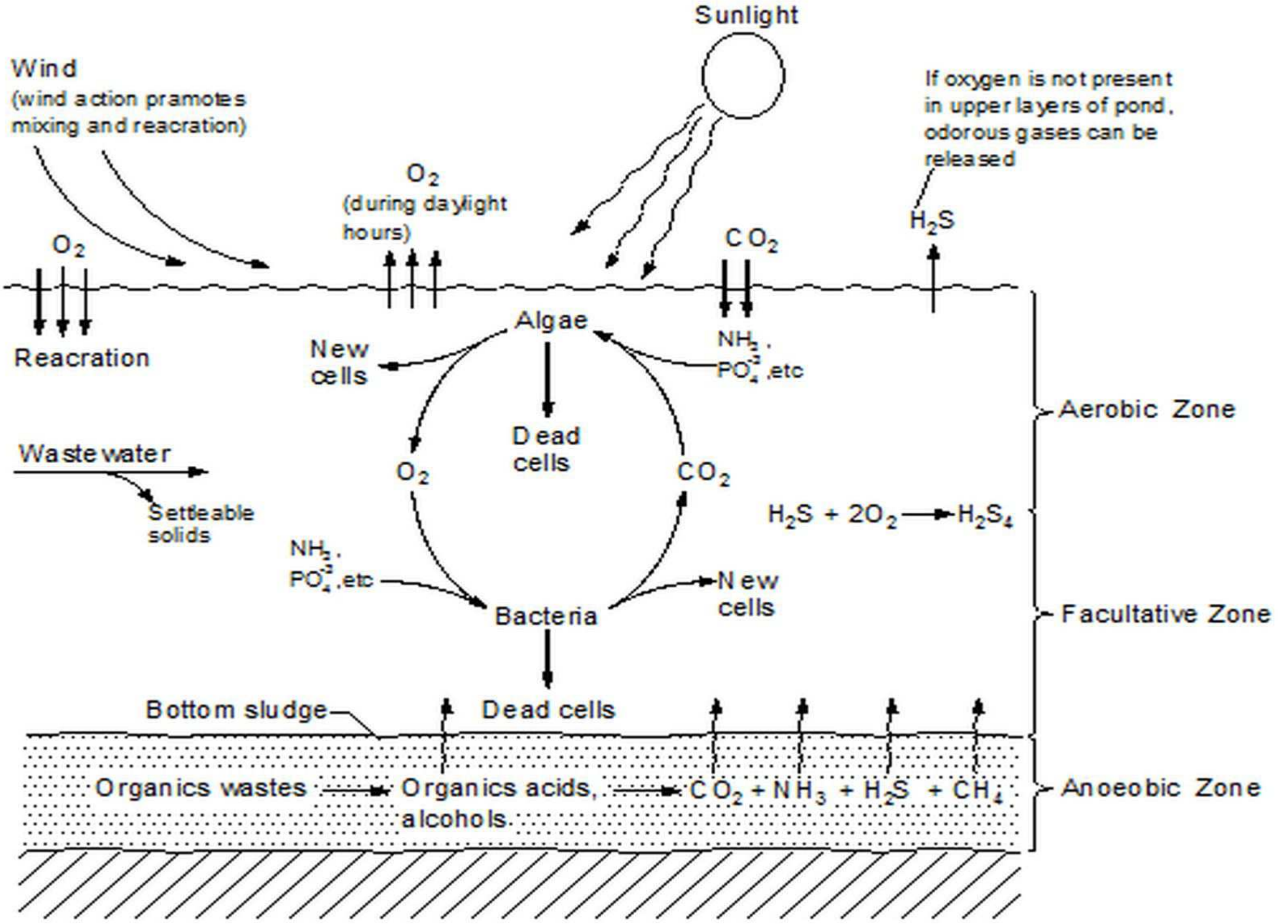


Figure 2.1: Waste Stabilization Pond (Ref:http://www.appropedia.org)

2.5.1 Anaerobic pond

Anaerobic sewage ponds are generally designed on the basis of volumetric BOD loading rates (g/m^3d). In order to maintain anaerobic conditions and prevent odour emissions the value of volumetric BOD loading rate should lie between 100-400 g/m^3d. The performance of anaerobic pond significantly depends upon its temperature as the BOD volumetric rate increases with the rise in temperature (Mara, 1997). They generally have negligible dissolved oxygen and contain algae. Anaerobic ponds are commonly 1-5 m deep and the bacterial consortium in the pond is very sensitive to the $pH < 6$. BOD removal up to 80% can be achieved by using anaerobic ponds in series with the HRT of 1-2 days (Rao, 1972). The main advantage of an anaerobic pond is that it not only stabilizes the organic matter of wastewater but also has a lower land area requirement as compared to facultative ponds (figure 2.1).

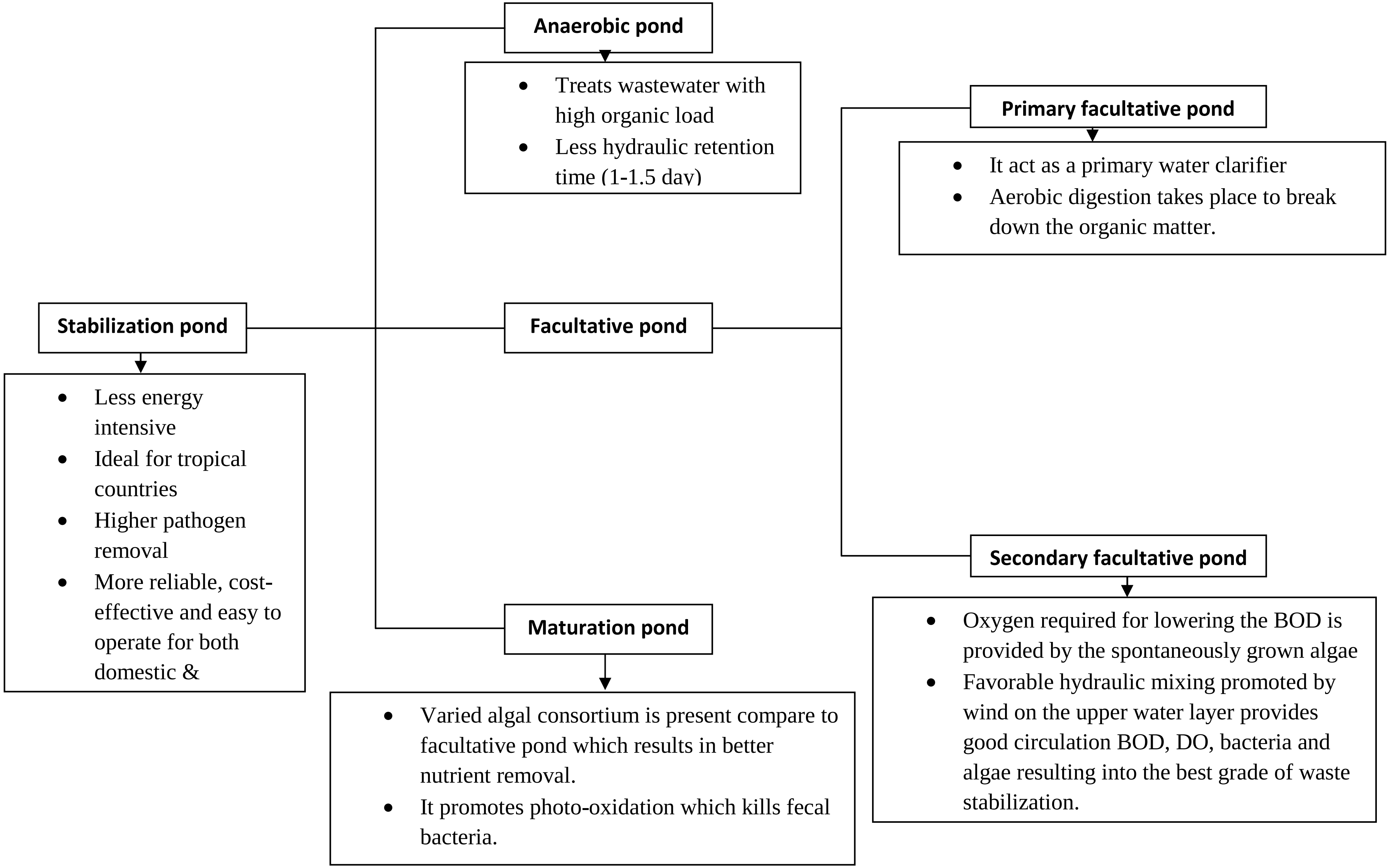
2.5.2 Facultative Pond

Facultative pond or lagoons (1.5-2 m deep) are designed based on the surface BOD loading (kg/ha d) and act as primary as well as secondary facultative ponds. A primary facultative pond operates as a primary clarifier for the wastewater received from the anaerobic pond. Aerobic degradation of organic matter by the bacteria is prominent in the primary facultative pond. The secondary facultative pond receives this particle free wastewater. Raw wastewater is simultaneously treated in anaerobic and primary facultative ponds along with the secondary facultative pond (figure 2.2). Facultative ponds need clear particle free water for deep light penetration to promote algal photosynthesis and decomposition of organic matter. A facultative pond consists of three layers during its operation namely aerobic zone, facultative zone and anaerobic zone. In the uppermost aerobic zone mixing is promoted by wind and photosynthetic oxidation by microalgae takes place increasing the dissolved oxygen, which prevents release of odorous gases like H_2S. Organic wastes are decomposed to acids and alcohols which are further degraded into CO_2, NH_3, H_2S and CH_4 in the facultative zone (figure 2.1). The algal bacteria consortia present in the facultative zone help in nutrient and BOD depletion respectively.

In the secondary facultative pond the remaining BOD is lowered by heterotrophic bacteria namely *Pseudomonas, Flavobacterium, Archromobacter and Alcaligenes* sp. (Raouf et al., 2012). The bacterial and microalgal growth is supported by the symbiotic relationship that exists between the two viz. oxidation of BOD by bacteria using the O_2 released by the photosynthetic growth of algae which in turn uses the CO_2 released by the bacteria. The dissolved oxygen concentration in the facultative pond increases after sunrise as a result of photosynthetic activity of algal culture and gets depleted at sunset. Increase in CO_2 released by the bacteria often leads to increase in the pH of the waste upto 8-9. At such an alkaline pH fecal coliform bacteria are killed thereby improving the quality of wastewater treatment. Several weeks (2-3 weeks) are required for the treatment of waste in the facultative pond which makes it less efficient, moreover the growth of rotifers and protozoa’s which feeds on algae may lead to depletion in dissolved oxygen level giving rise to bad odour due to anaerobic decomposition of organic matter. Cyanobacterial growth in the pond too reduces the light penetration thereby leading to the death of selective algal strain and inhibiting the organic matter decomposition.

2.5.3 Maturation pond

Pathogen removal is the most important task performed by maturation ponds from the wastewater received from facultative ponds and are known as polishing ponds. Maturation ponds are generally 0.8-1.5 m deep where a pond depth of 1.0 m is common. Their main purpose is to provide high quality effluent by getting rid of pathogens from the facultative pond. For designing the maturation ponds residence time, temperature, pH and light intensity are the major important parameters. A light intensity over wavelengths of 425-700 nm are reported to cause death of most faecal bacteria, with the exception of*Vibrio cholerea*(Mara and Pearson, 1998). Maturation ponds are not designed to lower BOD significantly as compared to facultative and anaerobic ponds, where 90% lowering of BOD takes place. In short, due to lower initial organic loading concentration BOD removal is depressed. Effective nutrient removal can also be achieved if operated in conjugation with the algae and/or fish breeding (Tilley, 2014).Maturation ponds are strictly used when treated wastewater is required for irrigation and should contain faecal coliform bacteria <1000 per 100 ml according to WHO guidelines.



Stabilization pond

- Less energy intensive
- Ideal for tropical countries
- Higher pathogen removal
- More reliable, cost-effective and easy to operate for both domestic &

Anaerobic pond

- Treats wastewater with high organic load
- Less hydraulic retention time (1-1.5 day)

Facultative pond

Primary facultative pond

- It act as a primary water clarifier
- Aerobic digestion takes place to break down the organic matter.

Secondary facultative pond

- Oxygen required for lowering the BOD is provided by the spontaneously grown algae
- Favorable hydraulic mixing promoted by wind on the upper water layer provides good circulation BOD, DO, bacteria and algae resulting into the best grade of waste stabilization.

Maturation pond

- Varied algal consortium is present compare to facultative pond which results in better nutrient removal.
- It promotes photo-oxidation which kills fecal bacteria.

Figure 2.2:Advantages of the waste stabilization ponds

2.6 Photobioreactor Design

Microalgae rich in lipid and carbohydrate were one of the organisms selected to provide an alternative source of renewable energy to meet the increasing fuel demands all over the world. This biological source of energy was largely independent of expensive raw materials and uses solar energy as the primary source of energy for conversion of CO_2 to Carbohydrate and lipid by photosynthesis (Schenk et al., 2008, Mata et al., 2009,Tanget al., 2012). Design of photobioreactors for cultivation of algae has received much attention in the past two decades, with the realization that more engineering efforts are needed for designing these low-energy consuming and high throughput photobioreactor systems for the microalgae cultivation. Currently for the microalgae cultivation, open and closed photobioreactor systems are employed.

2.6.1 Open pond system

Open systems comprise of the open raceway pond (figure 2.3) and waste stabilization pond. Open raceway ponds were in use from the late 1950's and was studied extensively for growing algae (David et al.,1953). Open raceway ponds are the shallow ponds fabricated from cheap materials including plastic cladding, designed for the cultivation of algae. They are generally 0.3-0.6 m deep and 0.8-1 m wide having a paddle wheel for effective water circulation and mixing. Open raceway ponds are also termed as high rate algal ponds because of their effectiveness in treating the wastewater effluent at a HRT of 8-10 days. 90-95% of nutrient and 80% of COD removal is reported from the fish farm waste and domestic wastewater by using high rate algal pond in 10 day HRT (Posados et al., 2014). These systems are relatively economical and are used commercially for growing algae as a food, feed and fuel source. Some of the disadvantages associated with open raceway pond include less control over algal culture conditions, bigger footprints, lower light penetration and poor productivity. Much research is needed to minimize the water evaporation losses by optimizing the operating conditions especially turbulence in the raceway pond.

High rate algal ponds can treat upto 35 $g.BOD.m^{-2}d^{-1}$(175g $BOD.m^{-3}d^{-1}$ in a 0.2m deep pond) compared to 5-10 $g.BOD.m^{-2}.d^{-1}$ (5-10g $BOD.m^{-3}d^{-1}$ in a 1m deep pond) in a waste stabilization pond(Munoz and Guieysse, 2012).Stringent water discharge norms into the water bodies led to polishing of the treated effluent by intermittent use of sand filters in combination with phytoremediation using wetlands (Racault and Boutin, 2005).

Figure 2.3: Open raceway pond (Courtesy: CSIR-National Chemical Laboratory, Pune)

2.6.2 Closed Photobioreactor

Contrary to the open pond system a closed photobioreactor is not open to the environment, but is enclosed in a loop where the section exposed to light could be tubes, cylinders, channels or flat plates made of transparent materials like glass or polymers (polycarbonate, acrylates etc.) (figure 2.4). It provides a controlled environment for the growth of microalgae in terms of pH, mixing, light intensity, culture density and temperature. As it often promotes the monoculture of microalgae, higher biomass productivity can be achieved for a longer period of time as compared to an open pond reactor; besides the contaminants (rotifers and protozoans) are restricted because of the controlled environment. Tubular photobioreactors are the most commonly used system for the microalgal cultivation.. It has been reported that trading of algal biomass is expensive (up to5000€/t) and is used mostly for animal feed and not for production of biofuel (Lehr and Posten, 2009). Cultivating microalgae in wastewater for lipid production provides an alternative for cost reduction of biomass along with concomitant phycoremediation atno extra cost for nutrient supplementation. However, using closed photobioreactor for wastewater treatment makes it relatively expensive as they are difficult to operate and scale up, compare to open raceway pond. Also supplying air rich in carbon dioxide is not economical unless a suitable source (e.g. Stack gases) is available in the immediate vicinity. In order to achieve high microalgal biomass productivity along with the metabolites with a by-product value, Habiba et al.,(2012) suggested the need for innovative research strategies for designing and developing the photobioreactors in combination with the genetically engineered strains. The following Table 2.3 gives an overview of the advantages and disadvantages associated with the different designs of closed photobioreactor.

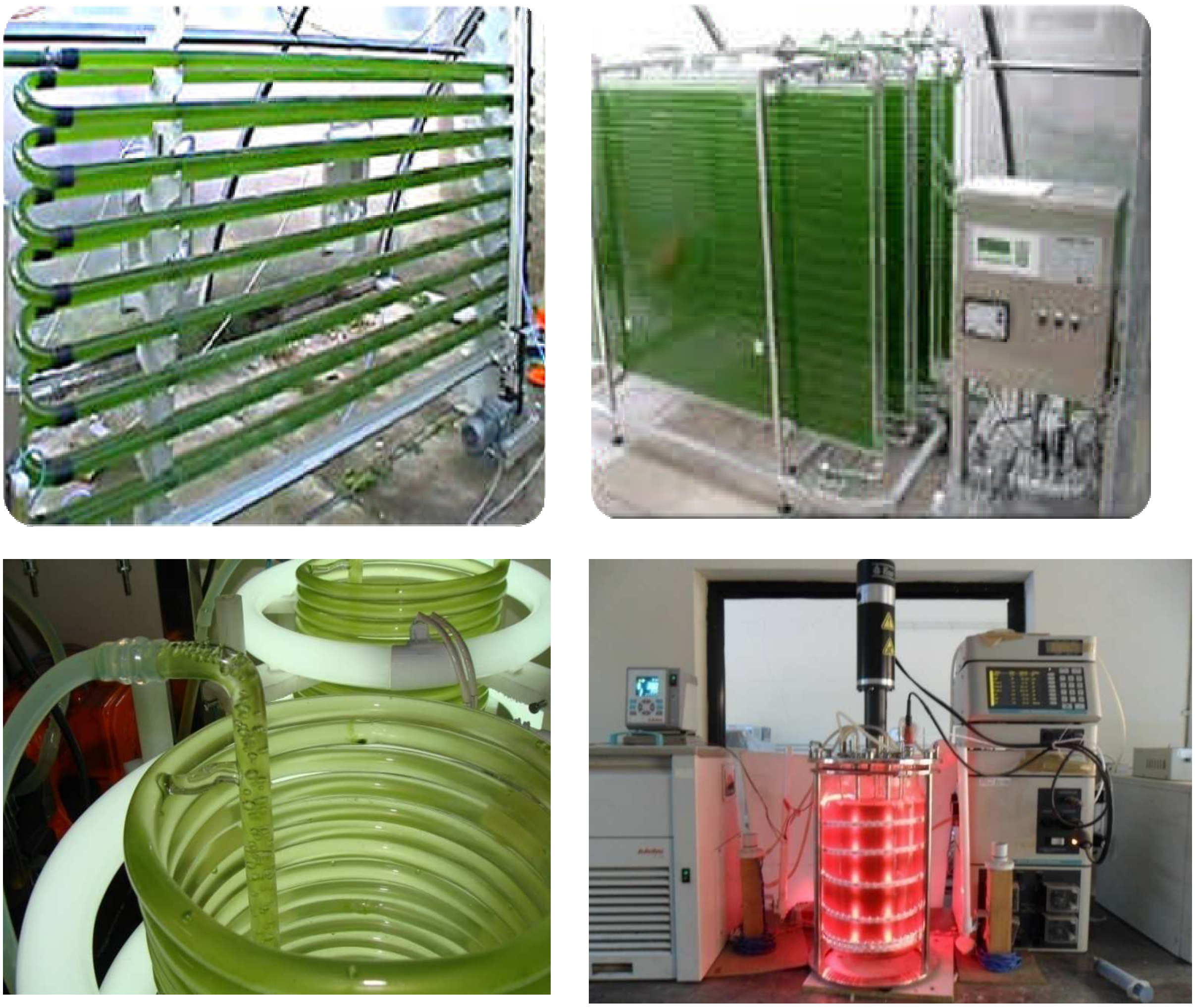


Figure 2.4: Closed photobioreactors 3A: Tubular photobioreactor, 3B: Flat plate photobioreactor, (Source courtesy for A and B photobioreactors, www. Chempuretech.com) 3C: Helical photobioreactor, 3D: LED photobioreactor.

Table 2.3: Comparison of different closed photobioreactors

Type of closed photobioreactor	Advantages	Disadvantages
Flat plate PBR	Provides large surface area, good light path, good biomass productivities.	Scale-up requires many compartments and support materials.
	Low oxygen buildup	Difficult to control temperature.
		Hydrodynamic stress to algal strain with some degree of wall growth.
Tubular PBR	Low shear stress on tubes with good biomass productivities, good mass transfer and good mixing.	Gradients of pH, dissolved oxygen and CO_2 build up.
		Larger foot prints.
		Wall growth which affects light penetration.
		Decrease in illumination surface area on scale-up.
		Requires sophisticated material.
LED PBR	Low shear stress because of good mixing.	Comparatively expensive.
	Rapid light utilization rate	Low illumination surface area.
		Heat generation and wall growth.

2.6.3 Mixing

A favorable degree of mixing plays a pivotal role for the growth of microalgae and subsequent reduction of nutrient level, good mass transfer and sufficient light exposure for the photosynthetic activity. Waste stabilization ponds generally do not require a mixing mechanism as the wind (surface aeration) plays a major role, however at times it may lead to the formation of anaerobic zones and increases the mass transfer limitations. Optimal mixing is one of the abiotic factor that greatly affects the pollutant removal, as too much of mixing may lead to shear stress of the algal cells leading to their damage and thereby increasing the operational cost (Su et al., 2011). It has been also reported that the highest productivity of the algal culture of *Chlorella sp.* was achieved in a Plexiglas bowl at 300 rpm due to the centrifugal action of a rapid mixing device named as Algraton. Three such Algratons having 1 m in diameter are claimed to sustain a human diet of1600 calories per day from the algal biomass produced (Oswald, 2003).Paddle wheels are the most commonly used mixing device in the open raceway ponds designated as one of the low cost instrument for efficient mixing (Oswald and Gotaas, 1957). However, many sophisticated designs in the form of swirl vanes were developed even for the smaller tubular photobioreactor, which provides mixing by causing helical flow for circulating the culture (Wiley et al., 2013). Such type of mixing has reduced settling of the microalgae by 86% of the total biomass. But such kind of mixing instruments add up to the cost of the closed photobioreactor, whereas paddle wheels are the most efficient mechanism in open ponds and high rate algal ponds (HRAP) as they are profitable in terms of mixing as well as energy cost.

2.6.4 Biomass harvesting

Algal biomass harvesting is proving to be the barrier for the algal technology to proliferate economically for energy production compared to the conventional methods (Rawat et al., 2011). Commercial production of algae for human consumption on an industrial scale started in the early 1960’s in Japan and extensive research was conducted in the US, Germany and other countries for use as a food supplement. Growing *Chlorella sp.* on a commercial scale as a food source was considered seriously as early as 1947-48 (Burlew, 1953, Borowitzka, 1999). Unfortunately, most of the algal species, which grow at faster rates, are unicellular and hence, pose problem for harvesting. Some of the desirable characteristics of algae for the cost effective harvesting are discussed by Borowitzka, (1999) is given in the following Table 2.4

Sedimentation, filtration, centrifugation, flocculation, sonication and flotation are various methods are used for algal biomass harvesting and can be broadly classified into two important steps viz. dewatering and drying. The most critical engineering features related to reduction of the cost of the whole process will be covered in the area of biomass harvesting. In general practice, flocculation followed by gravity sedimentation is the most commonly used method for wastewater treatment as there are large volumes to treat and low biomass concentration (Grima et al., 2003). Multivalent cations and cationic polymers are generally added as a flocculent in the broth to reduce or neutralize the negative surface charge in order to agglomerate the microalgal cells in suspension. The flocculent of choice should be inexpensive, non-toxic and effective in low concentration (as observed for chitosan).

Table 2.4: Characteristics of algal biomass for efficient harvesting

Desirable characteristics	**Advantages**	**Disadvantages**
Growth in extreme environment	Reduces problems with competing species and predators.	Only limited number of species are available and some extreme environments are difficult to maintain on a large scale (e.g. cold)
Rapid growth rate	Provides competitive advantage over competing species and predators; reduces pond area required	Growth rate is usually inversely proportional to the cell size; i.e. fast growing cells are usually very small
Large cell size, colonial or filamentous morphology	Reduces harvesting costs	Large cells usually grow slower than small cells.
Wide tolerance of environmental conditions	Less control of culture conditions required for reliable culture	-
Tolerance of shear force	Allows cheaper pumping and mixing methods to be used	-
High cell content of the product	Higher value of biomass	Products are usually secondary metabolites, high concentrations mean slower growth

Alum is the most widely used multivalent metal salt in wastewater treatment process especially for flocculating *Scenedesmus* and *Chlorella sp*.(Gouleke and Oswald, 1965).According to

Jorquera et al., (2010) the energy requirement for algal biomass harvesting can be significantly reduced if microalgae can be concentrated about 30-50 times by coagulation-flocculation followed by gravity sedimentation prior to the dewatering stage. Centrifugation provides good results by compact thickening of the algal slurry but it is one of the most energy intensive processes compared to three other techniques studied by Sim et al., (1998) for harvesting microalgae namely chemical flocculation followed by floatation and continuous filtration with a fine weave belt filter. Recently Evodos has come up with an improved centrifuge with spiral internals that has been extensively tested on harvesting algae. Separation of algae using electrical energy is also a very effective process as algae carry a net negative surface charge and can be concentrated in an electric field. The major advantage of this process is that there is no addition of chemicals but its high power requirement and electrode cost makes it unsuitable for large scale harvesting of algae (Uduman et al., 2010).Commercial mechanical harvesters are in practice to yield algal biomass designed to separate algae from a continuous moving belt through vacuum before it passes through the culture broth (Shepherd, 2010). One more continuous belt harvester system is used by Algaventure systems; Inc. is based on a capillary extraction mechanism. In this technique the primary belt is in contact with a secondary belt made of superabsorbent polymer material on which the water is absorbed and the dried biomass is collected on the primary belt while water is drained out from secondary belt by compression before it comes in contact with the primary belt (Young and Cook 2010,Christenson and Sims, 2011). In a patent by Mendez et al., (2009) for Sapphire Energy it describes a genetically modified algae to facilitate controlled flocculation and easier harvesting. The algae are modified to express a ligand or receptor for encouraging flocculation. Such genetically modified algal species including cyanobacteria have been used for production of ethanol, where conventional cell harvesting is avoided, by enclosing the growing culture in a greenhouse where the water vapor and ethanol condense on the ceiling and gets collected in a channel (Woods et al., 2010). Preference of algal harvesting method largely depends upon the nature of the product. For instance, if algal biomass is meant to be used as food or feed supplement then flocculants to be used should be compatible with the food standards and should not cause toxic effects. Whereas, certain applications do not require harvesting as, in aquaculture where the mussels, cladocerans, rotifiers, shell fish or fish are directly fed with algae. This process is termed as biological filtration and the feeders are termed as filter feeders which also help in effluent removal along with biomass generation (Etnier and Guterstam, 1991). A company, Live Fuel Inc. utilizes fish as a means of harvesting algae by the planktivorous fish tilapia for oil and fishmeal (Wu et al., 2010a).Immobilized algae are considered to be the best option for continuous production and harvesting of algal biomass, however, very little information is available on its use on large scale (Christenson and Sim, 2011).However harvesting of algae using membrane technology is emerging as one of the promising approach to algal industries. Generally, micro- and ultra-filtration in cross flow configuration as one of the effective way for biomass harvesting (Zhang et al., 2010). There are certain disadvantages associated with membrane technology such as fouling, low transmembrane pressure etc., but they can be resolved with more focus on membrane material and operating conditions. Extensive research is needed in the area of biomass harvesting which can reduce the overall cost of algal production.

2.6.5 Biomass Concentration

Production of algal biomass using wastewater effluent has been in use since the late 1950's and can be defined as the energy stored as new biomass per unit of light absorbed. In addition to the light intensity, oxygen accumulation and shear stress are the limiting factors for the microalgae productivity in the photobioreactor design (Janssen et al., 2003). Due to the abundance of nutrients like nitrogen, phosphorus and carbon found in wastewater it is an enriched media for biomass production along with the phycoremediation. Although unicellular algae grow at a faster rate with high accumulation of nutrients but they are difficult to harvest and increases the biomass harvesting cost (Pittman, 2010). HRAP, are best suited for the algal biomass production using wastewater with minimum environmental impact, but the algal species needs to have tolerances to seasonal and diurnal variations in outdoor conditions. At times they form aggregates which increase the ease of harvesting especially if they have high accumulation of lipids and other valuable products (Park et al., 2010).However, shading effects occur when the algal biomass concentration increases resulting in reduction of available oxygen required by bacteria as the algae enter the dark respiration phase. The amount of algal biomass concentration obtained in open photobioreactors is almost the same as that in closed photobioreactors e.g. 20 g/m^2d of maximum biomass concentration is reported in high rate algal pond treating domestic wastewater as well as in tubular photobioreactor using the secondary treated wastewater. Also, the algal turf scrubber raceway pond gives 11-14 gdw/m^2d of algal biomass by using the dairy manure effluent (Mulbry et al., 2008). In high rate algal ponds, algae are susceptible to consumption by herbivorous protozoa and zooplankton (e.g. rotifers and cladocerans) which can reduce the biomass concentration and cause the pond to crash in a matter of days (Park et al.,2011).

Sun dried, spray dried or the compressed tablets are the common form of microalgal biomass product available in the market from the microalgal biotechnology. *Chlorella* and *Spirulina* dominate the microalgal market with an average production of 2,000 and 3,000 tonnes/year respectively and other products are limited to very few taxa viz. *Dunaliella*,*Nostoc* and *Aphanizomenon* (Pulz and Gross, 2004). Genetically transformed microalgae are providing a new ray of hope for the mass production of high value products as the microalgae lacks cell differentiation and exhibit a much simpler system for genetic manipulation as compared to higher plants.

2.6.6 Surface/volume ratio

For obtaining superlative algal growth, maximum sunlight exposed, surface to volume ratio is one of the key parameter for designing the photobioreactor. A comparison of surface to volume ratios in different types of photobioreactor is given by Carlozzi, (2003) in which he reports helical type of photobioreactor to have maximum surface to volume ratio. Large surface to volume ratio photobioreactor offers maximum light utilization and greatly improves the productivity. Higher illuminated surface to volume ratios of horizontal or inclined tubular and flat plate photobioreactor makes them ideally suited to wastewater bio-remediation, however they cannot be used for axenic culture as sterilizing the reactor medium is difficult (Lee, 2001).

Immobilizing algae for better algal productivity has gained much attention recently as immobilized algal surface provides higher surface to volume ratio for light illumination (Burke, 2013, Ozkanet al., 2012).Rotating photobioreactors with the algal culture immobilized on the surface is emerging as an improved method for the nutrient removal from wastewaterand prevention of eutrophication. These rotating photobioreactors are capable of providing high surface to volume ratios thereby increasing the gas transfer, light illumination, biomass productivity and making harvesting inexpensive (Burke 2013). Maximum biomass yield of 24.94±2.07 g/m^2 of *B.braunii* on dry basis is obtained in algal biofilm photobioreactor with significant reduction in energy and wastewater requirement for cultivation (Ozkan et al., 2012).Higher biomass productivity can be achieved by immobilizing algae as compare to suspended culture if surface area is large (Middlebrooks et al., 1974).Tubular photobioreactors provide maximum surface area to sunlight exposure, hence, it is more suitable to outdoor mass cultivation (Brennan and Owende, 2009). Munoz and Guiysse (2006) demonstrated the action of algal biofilm on the pollutant removal in the vertical flat bed and horizontal algal turf reactor in terms of surface to volume ratio. Here, a vertical flat bed photobioreactor with biofilm attached to the reactor wall was illuminated from the side where the most active algae were not directly exposed to the pollutant bulk liquid. Bacteria consumed the pollutant and its concentration decreased through the biofilm. The most active algae was rejuvenated and the depleted oxygen concentration in the biofilm restored. In contrast a horizontal algal turf reactor containing microalgae when directly illuminated by light and exposed to pollutant water resulted in pollutant toxicity to the biofilm. A maximum degradation rate of 295 mg BOD $l^{-1}h^{-1}$ was achieved in an algal turf reactor (Munoz et al., 2009).

In order to get maximum surface to volume ratio for higher illuminated surface area, wide land area is required especially for wastewater treatment using microalgae. In this scenario, waste stabilization ponds are more economical and easy for maintenance. Algal-bacterial process is suitable for treatment of 60-4000 m^3/d of wastewater with the load of 30-1800 kg/d can be established depending on the local land prices (Munoz and Guieysse, 2006). As the artificial lighting adds load to the cost of wastewater treatment in algal-bacterial photobioreactor, solar radiation is the sole and freely available power source for the oxygenation. Hence, surface to volume ratio plays a key role in designing the photobioreactor, which incorporates the factors such as the biomass concentration, the hydrodynamic regime and the incident light intensity. Along these lines, open raceway ponds and waste stabilization pond provides maximum surface exposure and are best suited for phycoremediation.

2.6.7 Hydraulic retention time (HRT)

One of the most important factor that governs the successful and cost efficient wastewater treatment process is the hydraulic retention time. It was always suggested to keep the HRT long enough such that dilution rate should not exceed the maximum algal growth rate μ_{max} and thereby prevents wash out effects during wastewater treatment. However, longer HRT’s are not preferred as it may result into slower growth rate due to nutrient limitation and increased internal shading (Lund, 2006). HRT varies greatly with the seasonal changes, being higher in winter as compared to summers, especially, in case of outdoor waste effluent treatment using open raceway ponds and high rate algal ponds. Nutrients removal was very much affected by the seasonal factor as reported by Villa et al., (2005), where 45% reduction in phosphorus was achieved in winter as compared to 73% reduction obtained during summer with the *Scenedesmus obliquus* cultivated outdoors in artificial wastewater. Dissolved nitrogen concentrations of 53% and 21% of their initial values in winter and summer respectively, were used. Usually 2-6 days of HRT is recommended for the outdoor wastewater treatment system comprising of waste stabilization ponds and raceway ponds (Mara and Pearson, 1986). However, similar HRT’s are observed in case of closed photobioreactor process (Singh and Thomas, 2012). At a shorter HRT of 98 h and

183 h with 98% elimination of phosphorus and almost 100% removal of ammonium was achieved in a stirred tank photobioreactor at 25˚C for urban wastewater using *Scenedesmus obliquus* (Martinez etal., 2000). Almost quantitative removal of the pollutant was achieved with shorter HRT's if the algal photobioreactor system was combined with a membrane bioreactor for polishing and safe disposal of treated effluent (Singh and Thomas 2012, Wiley et al., 2013).

2.7 Influence of environmental parameters

Algal growth and nutrient uptake in the wastewater is dependent on abiotic and biotic factors. Abiotic factors include physical parameters like pH, light intensity, temperature, color /opacity of the wastewater, and chemical parameters like concentration of macro and micro nutrients in the wastewater. Biotic factors include the initial density of the algal cells, presence of zooplanktons, algal pathogens etc. (Lau et al., 1995, Park et al., 2011, Grobbelaar, 2009).

2.7.1 Chemical abiotic factor/ Nutrients

Micro algae require energy source in the form of light energy for autotrophic growth or organic carbon for the heterotrophic growth. The mixotrophic algae growing in the microbial consortia of the wastewater may outcompete the auxotrophs, as they are able to withstand the fluctuations in the type and concentration of the nutrients. The growth of algae in the wastewater is also determined by the concentration of the micro and macronutrients present in the wastewater. The common nutrients present in the wastewater are phosphorus (that occurs as organic phosphates), nitrogen (that occurs as ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and urea ($CO(NH_2)_2$) and carbon.

Phosphorus

Phosphorus is one of the important macronutrient required by algae to grow as it participates in formation of vital organic molecules. RNA is the most abundant phosphorus-containing molecule followed by other nucleic acids (DNA), phospholipids and adenosine tri phosphate (ATP). The concentration of nitrogen and phosphorous present in the water is considered to be a fundamental factor that directly influences algal growth kinetics. Total phosphorus concentration in weak untreated domestic wastewater is around 4mgL^{-1}, medium untreated domestic wastewater it is around 8 $mgL^{-1}$and strong untreated domestic wastewater 15mgL^{-1}total phosphorous (Rawat et al., 2011).

The micro alga have tendency to store the phosphorus in the cell in the form of polyphosphate (PPB) granules when they are growing in the environment with excess phosphate concentration. These reserves can be sufficient to prolong the growth in phosphate deficiency in the surrounding medium (Shivkumar et al., 2012, Larsdotter, 2006, Markou et al., 2012).

Nitrogen

Nitrogen is among the most important macro nutrient and growth limiting nutrient for microalgae. The concentration of nitrogen at which cell growth get inhibited depends upon the culture conditions and the algal species (Arumugu et al., 2013).Nitrogen can be utilized as NO_3, NO_2 or NH_4 and also as N_2. Some of the nitrogen fixing cyanobacteria like *Anabaena*, *Spirulina*, *Oscilatoria* can use N_2 diazotrophically. They fix the atmospheric nitrogen into ammonia by the enzyme nitrogenase (Sawayama et al., 1998). Total nitrogen concentration in the weak untreated domestic wastewater is approximately 20mgL^{-1}, medium untreated domestic wastewater around 40 mgL^{-1}and strong untreated domestic wastewater shows 85mgL^{-1}total nitrogen (Rawat et al., 2011).

Algae remove nitrogen from environment less efficiently then phosphate. It is observed that complete phosphate removal from the growth medium is possible at any N/P ratio, however, removal of nitrogen from the waste is dependent on N/P ratio. *Scenedesmus* sp. required N/P ratio in the range of 5:1 to 20:1 for maximum nitrogen removal efficiency reported by Xin et al., (2010).Kapdan and Alsan, (2008) reported the optimum N/P ratio for *Chlorella vulgaris* was 8:1.Nitrogen starvation negatively affects the PSII photosynthetic system, causing decrease in chlorophyll and carotenoid content. The flow of photosynthetically fixed carbon is diverted to an accumulation of lipids or carbohydrates rather than for the synthesis of proteins.

Carbon

Carbon is an essential requirement for growth and can be taken up in either organic or inorganic form. Dissolved CO_2 provided carbon to the algae for the biomass production when algae grow phototrophically. Most algae utilize dissolved inorganic and organic carbon in wastewater, while heterotrophic or mixotrophic algae tend to use only organic carbon.

Inorganic carbon species utilized by algae are CO_2 and HCO_3. Intracellular carbon is in the form of HCO_3, which get converted to CO_2 by enzyme carbonic anhydrase (Larsdotter, 2006). CO_2dissolved in water forms a weak acid/base buffer system, namely bicarbonate/carbonate buffer system. This is one of the most important buffer systems present in natural water.

Cyanobacteria and *Chlorophyceae* can grow with upto 18% dissolved CO_2 in the cultivation medium (Markou and Georgakakis, 2011). Beside this some algae utilize organic carbon present in the wastewater in the form of sugar, organic acids, glycerol, acetate heterotrophically. However the ability to grow heterotrophically or mixotropically on one or more carbon substrates is species dependent (Muhling et al., 2005).Total organic carbon (TOC) concentration in the weak untreated domestic wastewater is approximately 80 mgL^{-1}, medium untreated domestic wastewater it is around 160 $mgL^{-1}$and strong untreated domestic wastewater shows 290 mgL^{-1} total TOC (Rawat et al., 2011).

Carbon: Nitrogen(C: N) and carbon: phosphorus(C: P) ratios in domestic sewage (C:N 3.5:1; C:P 20:1) and dairy lagoon water (C:N 3:1; C:P 10:1) are low compared to typical ratios required by rapidly growing algal biomass(C:N 6:1; C:P 48:1). This dearth of carbon limits growth of the algae and results in incomplete removal of the nutrients from the wastewater (Woertz et al., 2009). CO_2 is most costly nutrient required for the cultivation of the microalgae (Borowitzaka, 1992). Hence the system that couples a waste CO_2 source can reduce the cultivation cost and mitigate the CO_2 (Yewalkar et al., 2011)

2.7.2 Physical abiotic factors

The physical abiotic factors, which control the growth of algae in the wastewater, are temperature, pH and opacity (for light penetration) of the wastewater.

Temperature

Temperature of the wastewater is an important parameter. It determines or control, gas solubility (O_2 and CO_2), pH, ionic equilibrium of the wastewater. Most of the algal species are able to carry out photosynthesis and growth over a wide range of temperature from 15°C- 30°C. Increase in temperature in this range increases the growth rate of the algae till it reaches an optimum value (35˚C), beyond which it causes cell growth inhibition. At lower temperatures algae show photo-inhibition at higher light intensity. The warmer climates of tropical and sub tropical countries support the outdoor cultivation of the algae in wastewater. In these regions growth of algae in the outdoor photobioreactors are controlled by the seasonal fluctuations of temperature. In temperate zones and countries situated above 40°N latitude (with the exclusion of France, Italy, Belgium, Russia, Germany, Ukraine, Turkmenistan), climatic conditions are unsuitable for outdoor algal cultivation (Zittelli et al., 1996).In such conditions closed photobioreactor with temperature control remain the only possible option.

Light

Different algal species respond show a variable response to increase and decrease in light intensity in outdoor open ponds. Algae show photo-acclimatization by synthesizing or degrading the active components of its photosynthetic machinery. Whereas at sub-saturating light intensity, chlorophyll pigments and the photosynthetic reaction center proteins D1 and D2 increase, an over-saturated light intensity causes photoxidation in algal cells, leading to degradation of the photosynthetic pigments and protein.

Algae use many techniques to remain at the surface of the water and there by catch the maximum light intensity. These include synthesis of gas vacuoles, accumulation of the fat and synthesis of the mucilage, which help reduce the density of the algal cell and prevent it from sinking. However not all algae are able to float. Many of them sink and are unable to get light. To avoid this, one simple option is to keep the depth of the photobioreactor as low as possible.

Light shielding occurs when cell density increases considerably. If the wastewater has many suspended particles, they also cause shielding. The color and transparency of the wastewater also controls light penetration (Larsdotter et al., 2006, Shivkumar et al., 2012).

pH

pH of the wastewater affects many biochemical process which control algal growth and metabolism. In the photosynthetic algae, CO_2 assimilation causes pH of the wastewater to rise above 10. pH of the wastewater also decides which inorganic species of the carbon will get fixed during the photosynthesis. pH can increase to 11 if CO_2 is limiting and inorganic carbonate has been used as the source of carbon. Nitrogen assimilation by algae is also affected by pH. If ammonia is used as nitrogen source, then the pH of the medium turns acidic (as low as 3), however use of nitrates raises pH of the medium to an alkaline range. Hence pH regulates not only algal growth but also the nitrogen removal efficiency. Optimal pH for the algal growth is 8. However many algae can grow in more alkaline conditions (Park et al., 2011).

2.7.3 Biotic factors

A number of biotic factors also determine the mode of algal growth, extent of algal growth and alga cell density.

Cell density

The nutrients removal efficiency from the wastewater with algal system is directly related to the cell mass or number of active cells. More the number of active algal cells, rate of nutrients removal will be faster and retention time of the wastewater will be reduced. However, very dense algal cell culture (more than 1x 10^7 cells/ml)results in self-shielding. Because of self-shielding the algal cells may shift to a mixotropic or heterotrophic mode of nutrition. This can be avoided by providing proper mixing (Lau et al., 1995). Immobilizing algae (Tam and Wong 2000, Burke, 2013 and Ozkan et al., 2012) can also provide a higher cell density.

Zooplanktons grazes and /Predators

Treatment of wastewater in open pond reactor by algal, one cannot avoid grazers. In this ‘artificial uncontrolled ecosystem’, grazers form the primary consumers of algae. The herbivorous protozoa and zooplanktons (like rotifers and cladocerans) can reduce algal concentration to a very low level within just a few days, causing ponds to crash. If cell density of the rotifers and cladocerans exceeds 10^2/L they can reduce the algal population by 90% within 2 days. Daphnia was responsible for 99% reduction of chlorophyll in open pond (Park et al., 2011). Zooplanktons grazing may be controlled through physical treatments like- filtration, centrifugation and low dissolved oxygen/ high carbon loading. Chemical treatments to control the grazers are by application of chemicals, invertebrate hormone mimics, increased pH, and free ammonia addition.

The parasitic fungi *Chytidium* and some algae attacking virus may grow along with the algal culture and spoil the food chain completely. Control of such organisms is vital for effective waste treatment. Fungal parasites and grazers are the most ubiquitous biotic drivers of decimation of the algal community. Some of the most effective methods to control the growth of zooplanktons is by reducing aeration, reducing retention time and adjustment of pH of wastewater to 11.

2.7.4 Dissolved oxygen concentration (DOC)

Dissolved oxygen in the raw wastewater is very low. Many conventional systems used energy intensive aerations systems to increase the dissolved oxygen for the oxidation of the organic matter. In case of algal wastewater remediation, algae generate the oxygen during the photosynthesis. This photosynthetic generated oxygen is responsible for the oxidation/biodegradation of organic material. Algae like *C. sorokiniana, E. viridis* proved their potential to treat the piggery waste efficiently with these photosynthetic oxygenation (Godos et al., 2010). In high rate oxidation ponds, intense photosynthesis increased DOC by 200% during daytime. High dissolved oxygen levels in water have negative impact on algal growth (Park et al., 2011). Fortunately O_2 saturation cannot cause harm during the biodegradation process. As the oxygen generated by the algae is utilized by the heterotrophic, symbiotic bacteria. The DOC levels remain low during the degradation of the all organic pollutants; however after depletion of the organic pollutants the DOC levels raised rapidly (Munoz and Gueysse, 2006).

2.8 Sewage water treatment coupled with membrane technology

The economy of any country predominantly depends upon its energy consumption and resources and any shortage in them may hamper the future progress. Energy is not only required for the production purposes but also to reduce the pollution created during the manufacturing process. Various new technologies are emerging to treat polluted water resources with a view to recycle & reuse water. Current advanced technologies for the treatment of sewage water are energy intensive. Membrane filtration in combination with biological treatment (using microalgae) may prove to be the futuristic technology for the treatment of sewage water. The treated water can be reused for irrigation, industries, and household purposes.Sewage water treatment is both the earliest and largest application of MBR, and it is predicted that this will continue to be its primary use. Due to its small footprint and potential for reuse of high-quality effluent, MBR is capable of coping with population growth and limited space. For industrial applications where more stringent regulations are imposed, it provides an effluent that can be safely discharged into the environment. The main applications of membrane technology reported in the industry are for treatments of heavily loaded wastewaters such as oily wastewaters, or discharges from tanneries and textile industries. Promising applications also exist in treating landfill leachate, chlorinated solvents in manufacturing and for groundwater remediation.

Treatment of sewage water is one of the major issues in India. For instance, only about 26% of the domestic and 60% of the industrial wastewater is treated in our country. In 423 Class-I cities (i.e. cities with a population of > 100,000), only 29% of the wastewater is treated. The situation is even worse in Class-II towns (i.e. towns with population between 50,000 and 100,000), where wastewater treatment is only around 4%. Rural areas do not have any wastewater treatment facilities since, conventional treatment processes are expensive, requires complex operations and maintenance.

Shortage of clean water will be another significant driver for MBR’s in India. Sewage water reuse and recycle is an important problem and MBR technology is more attractive, since it is the only system that can provide consistently good quality effluent for reuse. The treated water can be used for gardening, toilet flushing, civil construction, industrial applications etc. The process of treatment of sewage water by using microalgae in combination with membrane system serves the dual purpose of bioremediation and as a raw material source for biofuel production which has gained enormous research interest globally (Bilad et al., 2014).Table 2.5 gives summary about the current processes used for the treatment of sewage water by using microalgae coupled with membrane system. Removal of nutrients i.e. TN and TP were found in the ranges of 25-98% and 57-100% respectively by using microalgae alone. The algal species primarily used for the treatment of municipal wastewater, piggery wastewater to electronic factory wastewater were *Chlorella* and *Scenedesmus* species (Yadavalli et al., 2014, Nwoba et al., 2016, Posadas et al., 2015, De Godos et al., 2010). Membrane filtration was applied either as a one step or two step microalgae harvesting process. From Table 2.4, it is evident that, most studies on microalgae harvesting were carried out in batch or semi-continuous mode of operation. Batch operation is only practical for small volumes whereas commercial large scale filtration requires continuous operation. Gao et al., (2015) and Boonchai et al., (2015) has treated secondry waste effluent in a continuous mode of operation by using microfiltration membrane. Also, semi-continuous pilot scale based membrane filtration were also reported for the treatment of municipal as well as anaerobically digested piggery wastewater (Nwoba et al., 2016). Normally, filtration was carried at a constant transmembrane pressure ranging from 0.1-3 bar, but as the filtration progresses the flux was decreased over the period of time. In order to maintain the flux and to restore the cross-flow velocity cleaning cycles were also incorporated and applied at different time intervals for different studies (Bhave et al., 2012, Min et al., 2014, Su et al., 2012, Hwang et al., 2013). These membrane studies will prove to be effective at different stages of microalgal cultivation.

Table 2.5: Treatment of different types of wastewater by using microalgae in combination with membrane system.

S.No.	**Type of wastewater**	**Volume to be treated (L)**	**Microalgae species**	**Process (Type of reactor, Continuous/batch process**	**Initial nutrients value (TN,TP mg/L)**	**Type of MBR (membrane material, pore size, flux, biomass retention)**	**Mode of operation (filtration time)**	**Nutrient value in the permeate (TN,TP mg/L)**	**Time**	**Ref.**
1.	Domestic secondary effluent	4 L	*C. vulgaris*	Continuous flow mode	14.12, 0.78	PVDF (0.1µm)	Submerged	0.95, 0.11	35 days	Gao et.al., (2016)
2.	Artificial media	NA	*Nannochloropsis sp*	One step harvesting	-	PVDF (0.1, 0.2 µm, 35–684 L/m^2h)	Batch, cross flow	NA	NA	Bhave et., al., (2012)
3.	Wastewater	NA	*C. vulgaris*	Batch process	-	PET (4 µm), PVDF (0.45 µm) PVA coating	Cross flow	NA	NA	Hwang et. al., (2013)
4.	Domestic wastewater	5 L	*C. vulgaris, S. quadriquda,S.obliquus*	Batch process	25-30, 8-12	PES	Submerged	0.7-1.4, 10-20	3 days	Singh and Thomas (2012)
5.	Municipal wastewater	-	*C. vulgaris*	Continuous operation	16.30, 1.83	0.2 µm membrane	-	0.41, 0.065	24 days	Wang et. al. ,(2013)
6.	Raw leachate	NA	*Microalgae and bacterial culture*	Batch operation	Removal rate 9.18 mg/L.day	-	-	NA	NA	Sniffen et. al .,(2015)
8.	Municipal wastewater	160 L	*C. pyrenoidosa*	Batch lab scale	Removal rate TP-96.87%, TN- 98.17	NA	NA	NA	96 h	Yadavalli et. al. ,(2014)
9.	Anaerobic digestion piggery wastewater	160L	*Chlorella sp., Scenedesmus sp.*	Semi continuous lab scale	TN- +- 25.9%	NA	NA	NA	NA	Nwoba .et. al., (2016)

10.	Municipal wastewater	160 L	*Scenedesmus sp.*	Semi-continuous, pilot scale	TN- 79, TP- 57 %	NA	NA	NA	10 days	Posadas et. al., (2015)
11.	Piggery wastewater	160L	*Scenedesmus sp.*	Semi-continuous, pilot-scale	TN-98%	NA	NA	NA	8 days	Godos et. al. ,(2010)
12.	Municipal wastewater	130 L	*C. vulgaris*	Batch	TP-86.9, TN-96%	Biofilm Membrane	NA	NA	NA	Gao et. al., (2016)
13.	Synthetic wastewater	0.5L	*Spirulina platensis*	Continuous lab scale	TN- 68 to 82%	Glass Reactor with Hollow fiber membrane	NA	NA	20-40 days	Kumar et. al., (2010)
15.	Sewage water	10 L	*Chlorella vulgaris*	Batch, lab scale	TN- 95, TP-96%	Plexi glass tank MBR with immersed hollow-fiber membrane	NA	NA	10-20 days	Gao et. al., (2014)
16.	Secondary sewage effluent	10 L	*Chlorella sp. ADE4, C. vulgaris*	Continuous	TN-66.5, TP-94.5%	Double column-type MBR with hollow fiber microfiltration membrane	NA	NA	2 days	Boonchai et. al., (2015)
17.	Municipal wastewater	NA	*Chlorella, Cryptomonas, Scenedesmus*	Continuous, large scale	TN-75, TP 93%	NA	NA	NA	NA	Novoves káet. al., (2016)
18.	Swine manure wastewater	NA	*Chlorella sp.*	Batch, pilot scale	TN-86.6, TP-91.4%	NA	NA	NA	4 days	Min et. al., (2014)

NA- Not Available

2.9 Future prospects

Wastewater treatment becomes economical with the introduction of algal biomass in the consortium of waste utilizing organisms. This sustainable approach can further influence the lowering of cost of treating wastewater. Practically, if suitable methods of algal biomass separation/ harvesting are developed, algal biomass can provide byproduct credit. The algal biomass generated during wastewater treatment can be utilized for energy production or as a bio-fertilizer, but may not be suitable for food, animal feed, nor nutritional components as it is grown on wastewater. The algal biomass generated on the industrial wastewater may not be useful as a bio-fertilizer in case of accumulation of heavy metal in the algal biomass (Munoz and Guieysse, 2006)

2.9.1Potential uses of algal-bacterial biomass

The best use or algal-bacterial biomass generated from the wastewater treatment is for the energy production in various ways as listed below-

Biodiesel- If the algae grown on the waste has high amount of lipids, then lipids can be extracted. The extracted algal oil (raw microalgal lipid) after transesterification gets converted to renewable, non toxic, biodegradable biodiesel.

Thermal decomposition of the algal biomass can give different types of energy fuels depending on temperature used during the conversion process.

Gasification - Partial oxidation of the biomass at 800-1000 °C produces syngas (combustible mixture of carbon dioxide, hydrogen gas, nitrogen and methane). Syngas can be directly used as fuel.

Thermo chemical liquefaction- The wet biomass is subjected to a thermal treatment (300-350°C)at high pressure (50-200atm) in presence of catalyst to produce a bio oil.

Pyrolysis- Is a thermal conversion in the presence or absence of catalyst and in absence of air/ oxygen. Pyrolysis of biomass produces charcoal, condensable organic liquids (acetic acid, acetone, and methanol) and non-condensable gases. Pyrolysis of alga biomass was found to produce higher quality bio-oil than lignocellulosic compounds.

Combustion – Direct combustion or burning of biomass in presence of air for conversion to energy in the form of hot gases has been practiced. The conversion efficiency of the algal biomass to energy is more favorable than that of coal, however it requires dry biomass containing low amount of water (<50%) (Rawat et al., 2011, Olguin, 2012).

2.9.2 Combining wastewater treatment with CO_2 mitigation

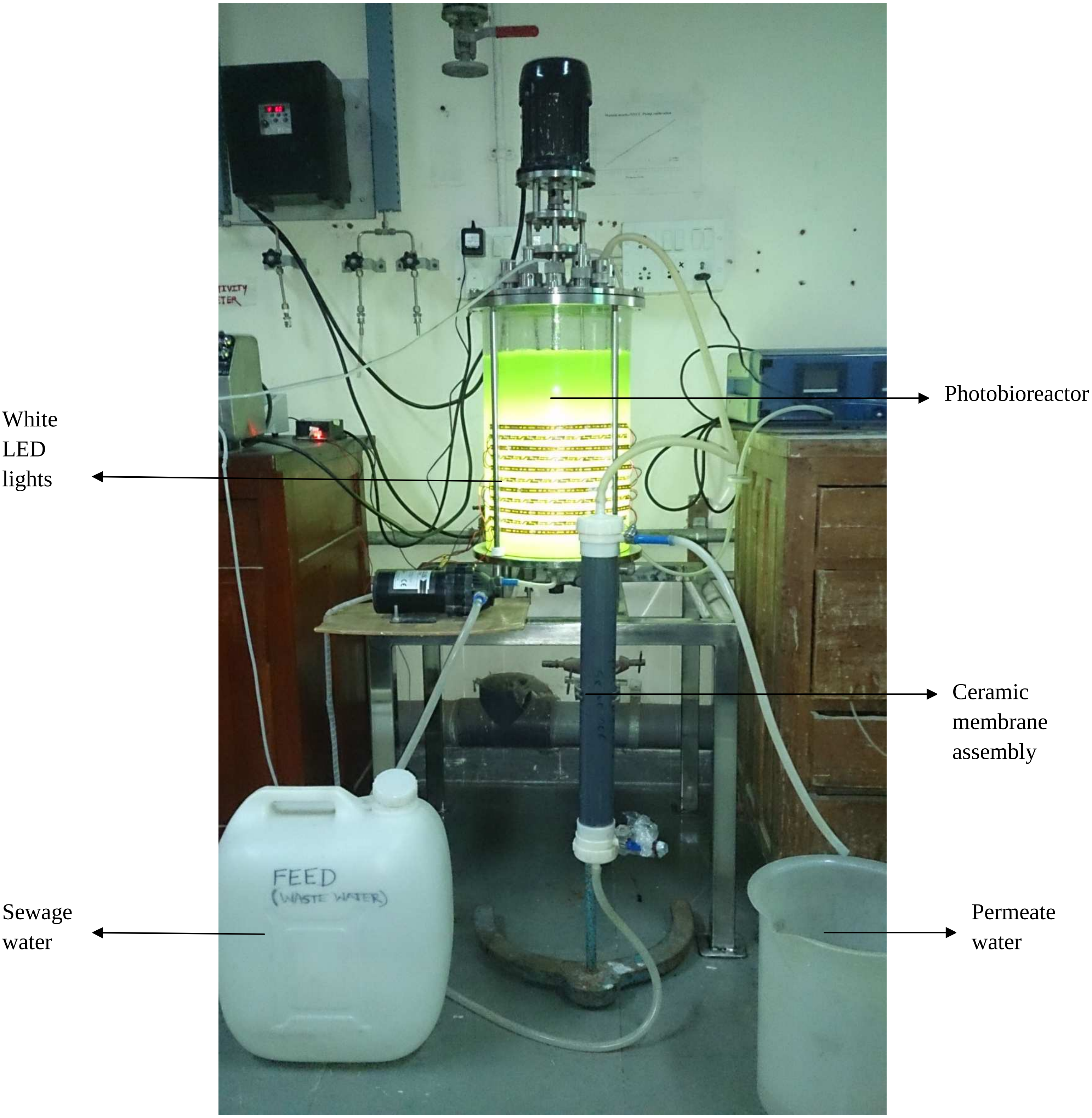
Algae can utilize the P and N in the wastewater and generate biomass. On the same lines CO_2 from the flue gas generated from heavy industries (Cement, petroleum, power plants and oil industries) can be utilized to improve the growth rate and lipid content of algae. This approach is also known as "CO_2 mitigation". In large-scale cultivation of algae, to avoid the CO_2 limitation, concentrated CO_2 sparging is essential to improve the growth rate and lipid accumulation. The CO_2 coming from flue gas is an ideal source of C for algal photosynthesis. However the flue gas in addition to CO_2 also has SO_2 and NOx, which inhibit the growth of algae drastically. This inhibition could be overcome by buffering the water with $CaCO_3$. It may be necessary to pre-treat the flue gas for desulphurization or for removal of inhibitory substances. The other limitations of CO_2 mitigation using algal biomass is the low specific rate of consumption of $CO_2$and the land requirement for constructing very large raceway ponds. Several scores of hectares of land would be required for building algal photobioreactors/ cultivation pond sand pretreatment of flue gas (McGinn et al., 2011, Yewalkar et al., 2011). The problem can be partially solved by separating CO_2 the from the flue gas by membrane separation followed by compression and transportation to the site of cultivation or piping the flue gas directly from the stacks to the site of cultivation. However this would substantially add to variable costs and add to the complexity of algal biomass production.

3.0 Conclusions

The treatment of the wastewater with algae-bacteria system is efficient for removal of phosphorous and nitrogen to an acceptable level. It generates algal biomass as a byproduct which can be used for various applications like as a fertilizer, biofuel, animal feed etc. However, harvesting of algal biomass remains a major challenge. Prevention of predators, especially in wastewater streams will be major cause of worry. This sustainable approach needs further development and refining of existing techniques and processes for cost effective production of algal biomass and its separation and harvesting. It is clear, however, that algae are here to stay in the bioremediation of wastewater.

Chapter 3

Phycoremediation of sewage water by using combination of microalgae photobioreactor and membrane cell recycle bioreactor in presence of artificial radiation



3. Phycoremediation of sewage water by using combination of microalgae photobioreactor and membrane cell recycle bioreactor in presence of artificial radiation[3]

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[3]"A version of this chapter is under review in Biocatalysis and Agricultural Biotechnology

Gera G., Swati N. Yewalkar., Nene S., Kamble S., Phycoremediation of sewage water by using combination of microalgae photobioreactor and membrane cell recycle bioreactor in presence of artificial radiation

3.1 Introduction

Recently, algal membrane bioreactor is regarded as one of the emerging technique for the treatment of wastewater along with the CO_2 sequestration (Chen et al., 2015, Ruiz et al., 2013, Kumar et al., 2010). This combination not only helps in removal of pollutants but also helps in fixing CO_2 emission thereby, paving a path for a new greenhouse gas mitigation strategy. Innovative strategies for biological nutrient removal in anaerobic, anoxic and aerobic tanks in combination with a membrane bioreactor (MBR) for treating municipal wastewater are gaining importance (Monclús et al., 2010, Sun et al., 2010). More recently, National Aeronautics and Space Administration (NASA) has developed a technique for treating wastewater by microalgal cultivation by using offshore membrane enclosures for growing algae (OMEGA) contained in a floating photobioreactor. The main motive of this study is to treat the wastewater outfalls near the coastal cities along with the CO_2 utilization from CO_2 rich flue gas on shore (Wiley et al., 2013). MBR results in high effluent quality compared to the conventional sewage water treatment process with smaller footprints and low sludge production which makes it versatile and suitable for sewage water treatment (Judd, 2010).

Several studies have shown the ability of algal cultures to remove nutrients from the sewage water with nitrogenous and phosphorus compounds. Bioremediation with the aid of microalgae and MBR may help to reduce the operating cost by eliminating the energy intensive methods for treatment sewage water. Fast growing microalgae species with high biomass production and simple nutrient requirements would be preferred for sewage water treatment. Usually *Chlorella sp.* and *Scenedesmus sp.* were used for the treatment of sewage water as they showed high endurance to difficult effluents with 90-95% nutrient removal rate and could sustain themselves in a wide range of temperature and pH conditions which makes them suitable for sewage water treatment (González et al., 2008, Wang et al., 2010). Compared to conventional sewage water treatment process which involves the formation of activated sludge or a biological floc, to degrade the organic matter into CO_2 whereas, microalgae can assimilate the organic pollutant into the cellular constituents such as lipids and carbohydrates, thus accomplishing the pollutant removal in a more environment-friendly way (Wang et al., 2010). Most of the sewage water treatment processes for denitrification of ammonia requires the external addition of carbon sources such as acetate, methanol, ethanol or volatile fatty acids. This increases the cost of treatment in terms of energy, chemical consumption, and sludge disposal. Microalgae alone are incapable of pollutant abatement and require the participation of bacteria present in the sewage water for breaking down the organic matter (Al-Hadabi et al., 2012, Godos et al., 2012). The symbiotic relationship between algae and bacteria enhances the phycoremediation process, as bacteria provide the required carbon dioxide for the growth of microalgae and, in turn, consume the oxygen produced by microalgae by photosynthetic respiration (Sriram and Sreenivasan, 2012, Rawat et al., 2011).This also helps to reduce the cost of aeration, which is a major energy intensive method for traditional sewage water treatment plants. Oxygen generated by microalgae also promotes bacteria to biodegrade the heavy pollutant materials like phenolics, organic solvents, and polycyclic aromatic hydrocarbons (PAH’s). An additional benefit is that recalcitrant and toxic compounds can be preferentially degraded aerobically than by an anaerobic process (Munoz et al., 2006).

The work by Singh and Thomas (2012) and also by Marbelia et al., (2014) evaluates the nutrient removal by microalgae from the aerobic membrane module permeate at very low initial nutrient concentrations. These studies are mainly focused on nutrient removal rate and biomass productivity without considering the other pollutant removal. Wang et al., (2013) have suggested that the nutrient removal with the aid of microalgae species to be the most promising approach for sewage water treatment along with the production of biomass and renewable energy as the additional benefit from the sewage water treatment. According to the recent work by Gao et al., (2016) demonstrates the performance of microalgae photobioreactor in which the submerged membrane bioreactor along with the microalgae *Chlorella vulgaris* gives 87.7% of nitrogen and 76.7% of phosphorus removal from diluted wastewater. It also claims the effective metal ions removal efficiency.

For the current study, the sewage water treatment plant from where the effluent was collected uses a sequential batch reactor (SBR) process for sewage water treatment. The SBR process requires a large aeration system which limits their cost effectiveness along with the high maintenance required during the decanting mechanism (Mahvi, 2008). The objective of present studies is to evaluate the rate of removal of nutrient by using a microalgae PBR treatment and eventually side stream MBR was integrated as a polishing step for evaluating nutrient as well as organic compound removal efficiency. The advantage of this process is that it minimizes the use of aeration system thereby reducing the operating cost. To our knowledge, a side stream MBR combined with a microalgae photobioreactor has not been used for evaluating the efficiency of removal of nutrients and organic compounds from the sewage water. The ultimate aim of the experiment is that the treated sewage water can be directly discharged into the water bodies thereby, restricting the eutrophication problem. The two microalgal species namely *C. protothecoides*, *S. obliquus* and mixed culture were grown and used for nutrient removal using a PBR in batch mode of operation. Subsequently, the sewage water was also treated by using a microalgae photobioreactor (PBR) and a membrane bioreactor (MBR) in the fed-batch mode of operation.

3.2 Materials and Methods

3.2.1 Inoculum preparation

The green algae *C. protothecoides* and *S. obliquus* were employed because of their capacity to assimilate the nutrient present in the sewage water. Conventionally *Chlorella* was widely employed for nutrient removal from the municipal wastewater effluent stream (Wang et al., 2010, Mamun et al., 2012). The two microalgae species were obtained from experimental phycology and culture collection of algae at the university of Göttingen (SAG- 211-7b, SAG 276.3d).The inoculum of *C. protothecoides* was cultured on polytoma medium with glucose (PolGlu) and Bold’s Basal Medium was used to cultivate *S. obliquus* as shown in tables 3.1 and 3.2 respectively (Heredia-Arroya and Wei Wei Hu 2010, Bischoff and Bold, 2003). The stock solutions were autoclaved separately and prepared under aseptic conditions. Algal strains were maintained separately at 26˚C under the continuous illumination of white fluorescent lamps of light intensity 8000±200 lux (Equinox T176544 lux meter) at 120 rpm for efficient mixing on a rotary shaker. The algal strains were filtered and washed several times with distilled water to remove media components before being inoculated into sewage water. The sewage water was collected from the Municipal wastewater treatment plant located at Sangvi, Pune (India) having the capacity to treat 20 mega liters per day (MLD). Sewage water was coarsely filtered through a mesh (size=2mm) for the removal of any debris present, before using it for the experimental runs.

The algal strains were grown in shake flasks using sewage water as the sole growth media before exposing them to the sewage water contained in the photobioreactor. The initial cell density for the treatment of sewage water in a photobioreactor was maintained around 2.5×10^6 cells/mL for all the algal cultures in the preinoculum. For the mixed culture of *C. protothecoides* and *S. obliquus* initial cell count is maintained as stated above in 1:1 ratio.

Table 3.1: Composition of Polytoma medium with Glucose (PolGlu) (*C.protothecoides)*

Composition	**mg/L**
Glucose	1000
Yeast extract	1000
Bacto-tryptone	1000
Soil extract	30 ml/L
Trace metal solution	1 ml/L

Table 3.2: Composition of Bold's Basal Medium (*S.obliquus*)

Composition	**mg/L**
KH_2PO_4	175
$CaCl_2.2H_2O$	25
$MgSO_4.7H_2O$	75
$NaNO_3$	250
K_2HPO_4	75
Na_2EDTA	10
KOH	0.62
$FeSO_4.7H_2O$	4.98
H_2SO_4	1ml/L
H_3BO_3	8.05
Trace Metal Solutions (g/L)	
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.22
$NaMoO_4.5H_2O$	0.39
$CuSO_4.5H_2O$	0.08
$Co(NO_3)_2.6H_2O$	0.05

3.2.2 Microalgae PBR and membrane bioreactor

The schematic diagram of microalgae PBR and MBR is shown in figure 3.1. It shows a typical batch photobioreactor made up of transparent glass of 20 L of working volume. The glass tank was cylindrical in shape with a height of 1.2 m and 0.164 m internal diameter. The reactor represents a typical aerobic system where the air is fed at a rate of 0.2 L/min to the reactor by an aquarium aerator, through a diffuser located at the bottom of the reactor containing microalgae. The glass reactor was fed with 19 L of coarsely filtered raw sewage water that was made up to 20 L by adding 1 L (5% v/v) of inoculum. The sewage water was continuously stirred at a speed of 120 rpm using mechanical stirrer located at the center of a microalgae PBR. The microalgae PBR was continuously illuminated with the white LED lights wrapped outside the reactor and average light intensity was found to be 8000±200 lux. All experiments were conducted at the room temperature (30-35˚C). Initially, a microalgae PBR was operated in the batch mode of operation for 6 days. The residence time of a microalgae batch PBR was set at 6 days throughout the experiment. The initial concentrations of nutrient and organic compounds in the PBR were monitored after each day during the fed-batch mode of operation.

Since most sewage water treatment plants are operated continuously and the growth of algae is a time consuming process, we thought of periodically passing the contents of the photobioreactor through a ceramic membrane of pore size 1.5μm and an effective area of 0.1 m^2 (BHEL, Bangalore) using a peristaltic pump (Watson Marlow 313S) so that there was a selective recycle of algae-rich retentate into the PBR and concomitant removal of treated sewage water in the permeate. The procedure of intermittent removal of algal free sewage water was as follows. The membrane bioreactor was connected to further polish the treated sewage water from microalgae PBR. A 5 L of treated sewage water from microalgae PBR was passed through the ceramic membrane and at the same time 5 L of fresh domestic sewage water was added in the PBR for further nutrient removal. An average permeates flux of 7.2 L/m^2 h was observed for the 5 L of permeate. The microalgae PBR was then operated for another 24 h so that the algae cells proliferate by depleting the nutrient concentrations and again connected to the ceramic membrane for further polishing of sewage water and this process was repeated for 6-15 days depending on culture used. The initial concentrations of nutrient and organic compounds in the PBR and as well as that in MBR permeate was monitored periodically for the fed-batch mode of operation.

3.2.3 Analytical methods

All water samples unless stated otherwise were centrifuged at 6000 rpm and filtered through a 0.45μm cellulose acetate filter paper (Millipore) before analysis. 50 ml of sample was withdrawn directly from the PBR for batch studies and permeate from MBR in fed-batch studies for analysis of its constitutes in triplicate. Total Kjeldahl nitrogen (TKN) was analyzed by using KjelTron Nitrogen/Protein digestion system (KDIGB 6M).Total phosphates were estimated by using the vanadomolybdophosphoric acid colorimetric method (APHA 1998). Chemical oxygen demand

(COD), biochemical oxygen demand (BOD), turbidity, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) were measured, in accordance with the standard methods for the examination of water and wastewater (APHA, 1998). Total organic carbon (TOC) was measured using TOC analyzer (Model: TOC-L CPH/CPN E200 Shimadzu, Japan). The microalgae cell count was estimated by using hemocytometer.

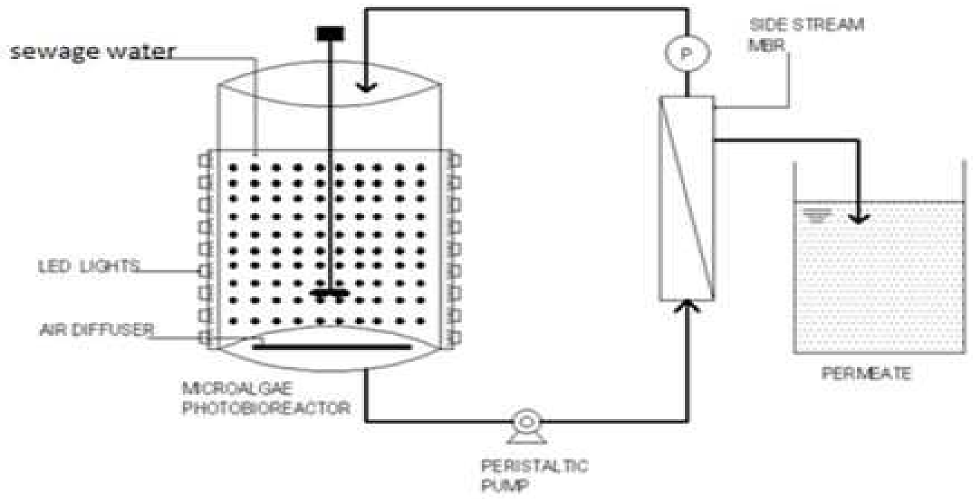


Figure 3.1: Schematic representation of microalgae PBR and MBR fed-batch process

3.3 Results and Discussion

3.3.1Microalgae growth in raw sewage water

Initial inoculum namely of *C. protothecoides, S. obliquus,* and mixed culture were cultivated in shake flask using sewage water. Subsequently, microalgae growth was monitored in batch PBR using raw sewage water at a time of interval of 24 h over a period of 6 days. The population of *C. protothecoides* and mixed culture were 3 and 2.5 times increased in 48 h in a batch reactor (figure 3.2). Microalgal growth rates are highly dependent on the type of species and the batch operating conditions viz. light intensity, nutrient concentrations, pH, temperature and aeration (Wang et al., 2013, Xin et al., 2010). Nevertheless, all the microalgae concentration increased over a period of 4 days indicating that the sewage water is a suitable growth medium. After 4 days of accelerated growth rate, all the microalgae species showed decline due to nutrient depletion in the sewage water (figure 3.2).

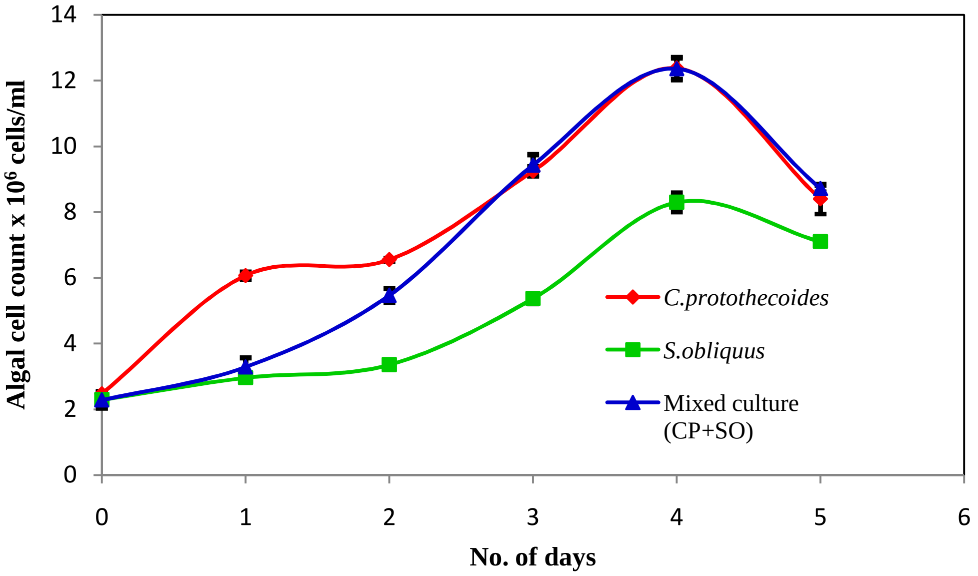


Figure 3.2: Algal growth in batch PBR

3.3.2 Performance of microalgae PBR for the removal of nutrient and organic compounds in batch mode of operation

The performance of microalgae namely *C. protothecoides*, *S. obliquus* and mixed culture were studied for the removal of nutrient in batch PBR. The figures3.3a, 3.3b, 3.3c, and 3.3d shows the percent removal of nutrient and organic compounds (TKN, TP, and TOC) from the sewage water over a period of 5 days. The concentrations of these nutrients, COD, BOD, and TOC are depleting over the 5 days of operations.

Figure 3.3a shows the depletion of TKN, it was observed that > 90% of TKN was depleted within 3 days of operation.Fig.3b shows the uptake of TP by these microalgae's; it was found that the uptake of TP using *S. obliquus* is 6.5% and 17.5% higher than that of *C. protothecoides* and mixed culture respectively. However at the end, 5 days batch PBR operation about 74%, 80% and 62 % of TP were removed by using *C. protothecoides*, *S. obliquus*, and mixed culture respectively. It is also evident that the initial concentrations of nutrient in the sewage water are low. In the present work > 90% of TKN was depleted within 3 days of operation which is higher than those reported by Wang et al., (2010) as they have achieved 82.8% of total nitrogen removal. Both the cultures tested showed that they were able to consume the nutrient by approximately similar amounts. The higher TP removal (figure 3.3b) was observed by using S. obliquus as compared to TKN in spite of slower growth rate of 0.18 h^{-1} as compared to that of C. *protothecoides* (0.25 h^{-1}) and mixed culture (0.28 h^{-1}). Previous studies with *Chlorella* and *Scenedesmus* species have shown the removal rates of NO_3, $NO_2$and TP between 43-54%, 83-

95%, and 70-92% respectively from sewage water after 3 days of operation in the batch PBR (Singh and Thomas, 2012). Thus, it can be concluded that microalgae are capable of removing the nutrient from sewage water. However, the kinetics of nutrient removal was very complex and is highly dependent upon numerous factors such as sewage water composition, operating conditions, and environmental conditions viz. light intensity, initial nutrient concentrations, mixing and inoculum concentration. The nutrient removal increases with the increase in the days of operation; however, longer residence times are not favorable for the treatment of the sewage water and more research is needed to further reduce the residence time for the microalgae application in traditional wastewater treatment plants.

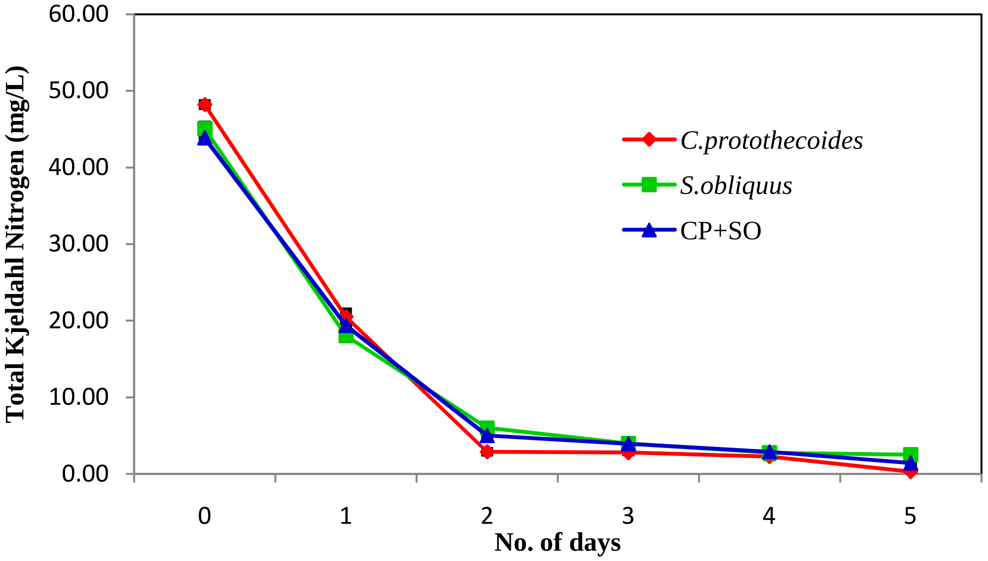


Figure 3.3a: TKN reduction by using algae-bacteria consortia in PBR

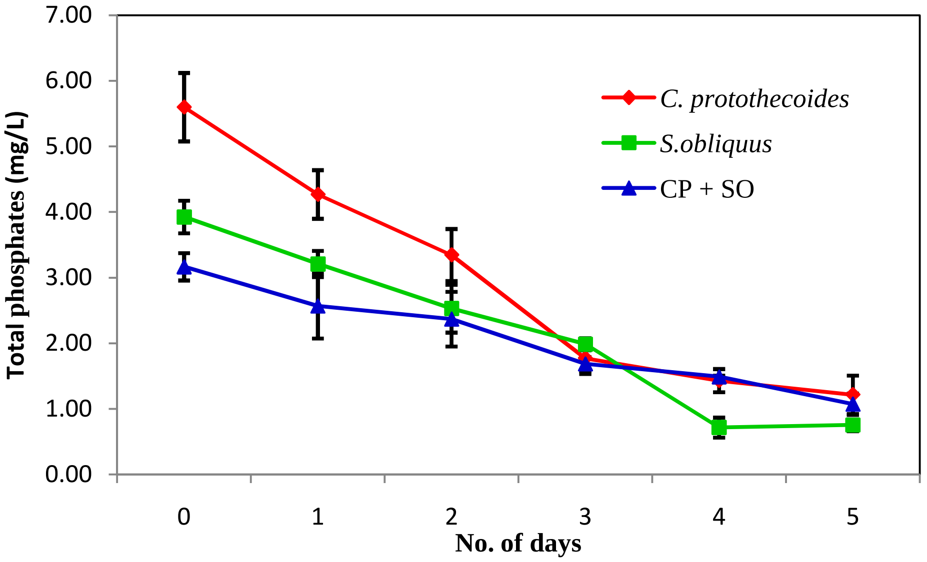


Figure3.3b: TP reduction by using algae-bacteria consortia in PBR

TOC estimation was done to assess the bacterial breakdown of organic matter present in sewage water. At the end of 5 days of batch PBR operation about 75%, 90% and 82 % of TOC were reduced by using *C. protothecoides*, *S. obliquus*, and mixed culture respectively (figure 3.3c). This is because, the microalgae provide the required oxygen for the bacterial species which are in the present sewage water to proliferate and, in turn, give CO_2 for photosynthesis to the algal species (Munoz et al., 2006). Thus, it can be concluded that the microalgae are able to remove the nutrient effectively along with the CO_2 fixation, thereby reducing the greenhouse gas emissions.

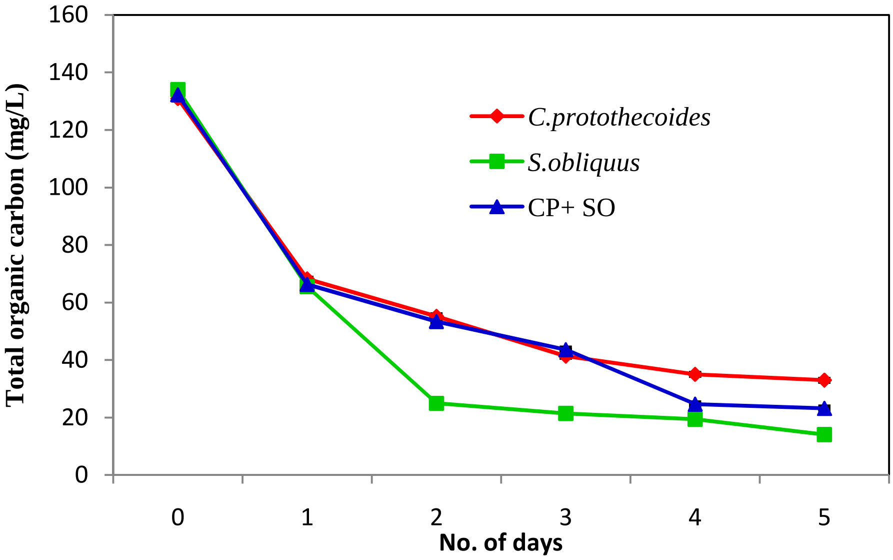


Figure 3.3c: TOC reduction by using algae-bacteria consortia in PBR

In the present study, almost 83-85% reduction in the COD levels was achieved by using these microalgae species in a batch mode of operation (figure 3.3d) in 5 days. In the first 2 days of operation, *S. obliquus*, *C. protothecoides*, and the mixed culture shows 46%, 68 % and 38% reduction in the COD value. Fig. 3e shows the reduction in BOD levels over 5 days of operation indicating the increase in the dissolved oxygen concentration in the sewage water. It was found that the BOD was significantly reduced (88±5%) by using these microalgae species in a batch mode of operation in PBR.

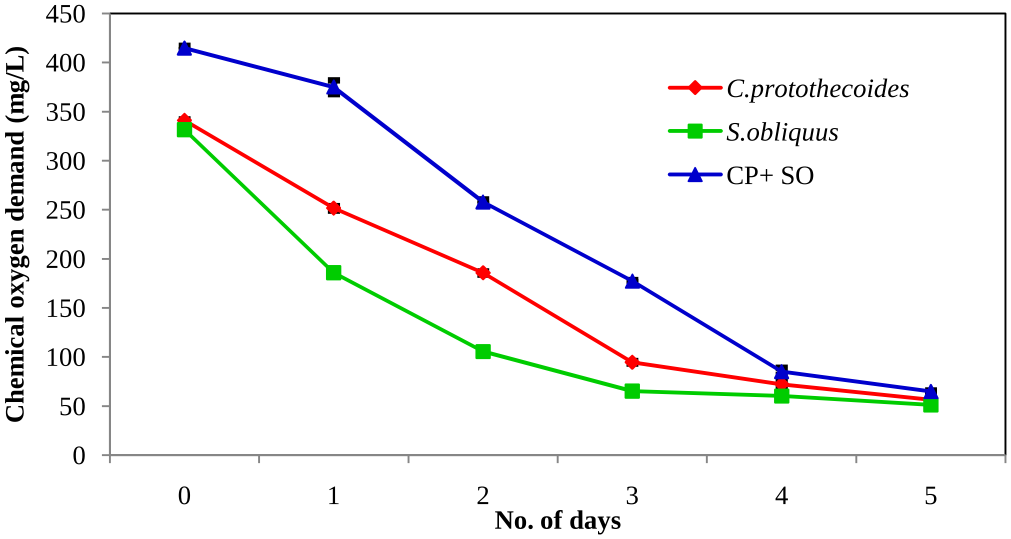


Figure 3.3d: COD reduction by using algae-bacteria consortia in PBR

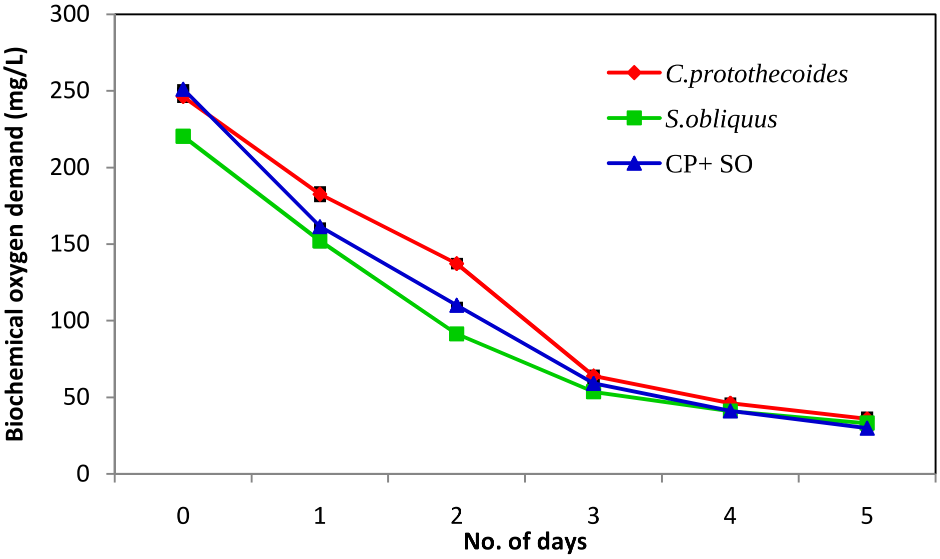


Figure3.3e: BOD reduction by algae-bacteria consortia in PBR

3.3.3 Nutrient and organic compound reduction by using a microalgae PBR followed by MBR in a fed-batch mode of operation

The membrane bioreactor was connected to further polish the treated sewage water from microalgae PBR. 5 L of treated sewage water from microalgae photobioreactor was passed through the ceramic membrane and at the same time 5 L of fresh domestic sewage water was added in the PBR for further nutrient removal. The microalgae PBR was then operated for another 24 h so that the algae cells proliferate by depleting the nutrient concentrations and again connected to the ceramic membrane for further polishing of sewage water and this process was repeated for 6-15 days depending on culture used. The initial concentrations of nutrient and organic compounds in the PBR and that in MBR permeate was monitored periodically for the fed-batch mode of operation.

Initially, the microalgae PBR and an MBR were operated for 15 days in a fed-batch mode using *C. protothecoides*. The algal cell count of around $10\pm2\times10^6$ cells/mL was maintained without any addition of growth media externally, during the days of operation. The main objective of the fed-batch operation was to make the technique viable for the treatment of sewage water by reducing residence time.

Figures 3.4a and 3.4b shows the performance of a microalgae PBR followed by a MBR in a fed-batch mode of operation for the uptake of nutrient and organic compounds from sewage water. From figure 3.4a, it was found that the TKN value depleted from 14-11 mg/L to <5 mg/L in the MBR permeate after every 24 h of operation. In a similar way, TP was reduced to <2 mg/L for the entire days of operation as shown in figure 3.4a. Figure3.4b shows initial concentration of TOC in the PBR and the final concentration of TOC in MBR permeate. The TOC concentrations in MBR permeate were found in the range of 8-10 mg/L (figure 3.4b).

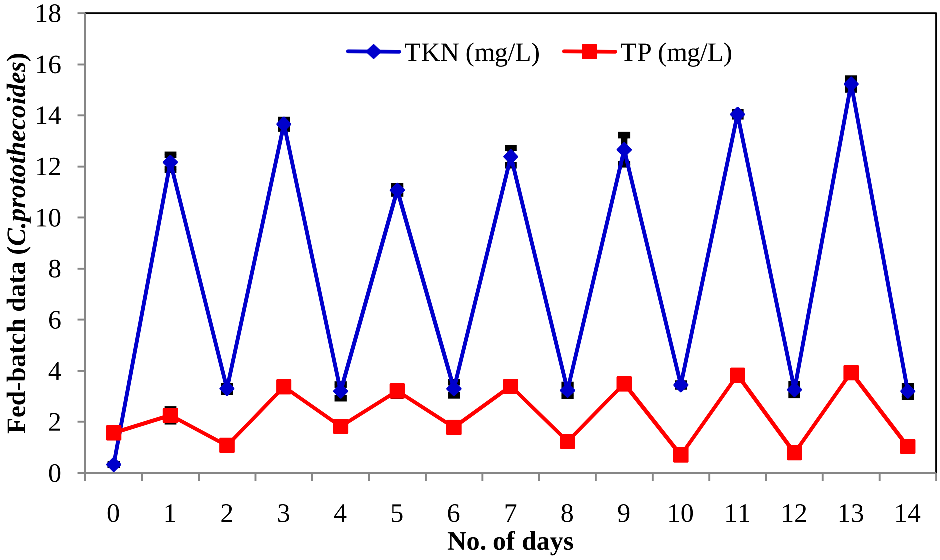


Figure 3.4a: Nutrient reduction by using *C.protothecoides* in fed-batch mode of operation

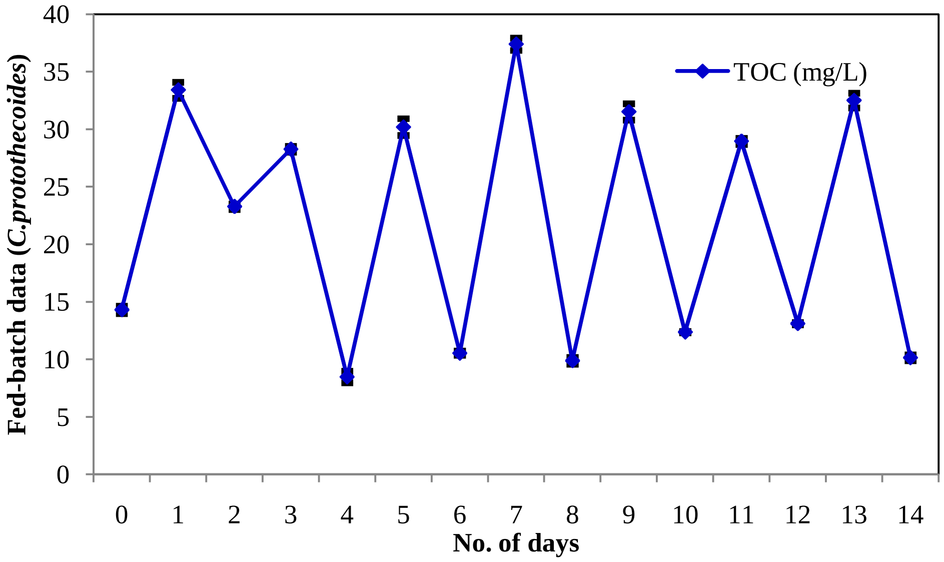


Figure 3.4b: TOC reduction by using *C.protothecoides* in fed-batch mode of operation

Figure 3.4c shows initial levels of COD and BOD in the PBR and final concentration of COD and BOD in MBR permeate. It was found that COD and BOD concentrations in MBR permeate were in the range of 58-50 mg/L and 38-26 mg/L respectively. It shows that the characteristics of treated water are reasonably complied with the discharge norms stipulated by the central pollution board of India.

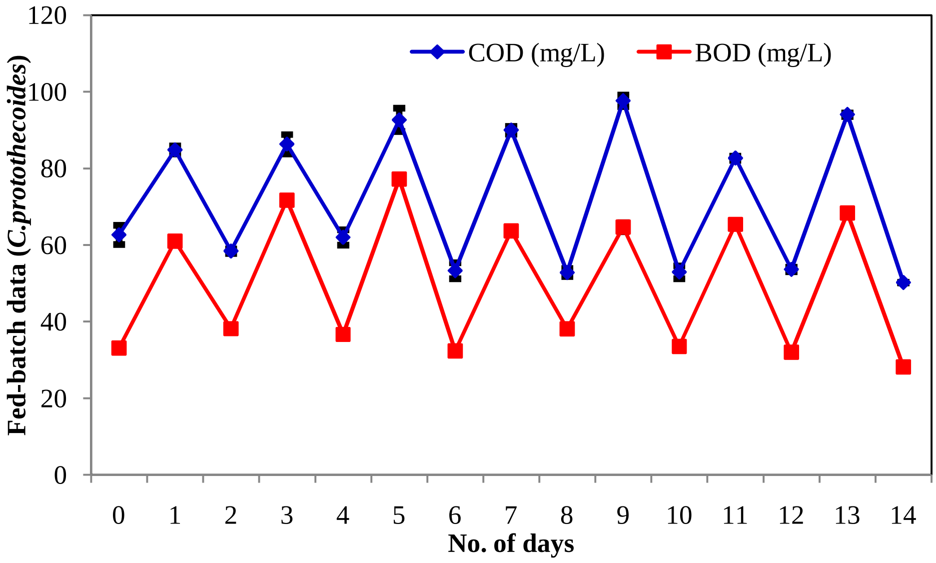


Figure 3.4c: COD and BOD reduction by using *C.protothecoides* in fed-batch mode of operation

A similar process was repeated for *S. obliquus* and mixed culture by using microalgae PBR followed by a MBR in a fed-batch mode of operation as the microalgae species have shown almost the same efficiency for the uptake of nutrient and organic compounds from sewage water. One of the observations made during this process is the algal shading caused by the growth of blue-green algae on the sides of the walls of glass reactor which causes algal dark respiration, reducing the amount of oxygen available to the bacteria (Grobbelaar and Soeder, 1985). Along with this, the zooplanktons were also observed thereby, causing the lowering of the algal cell count. Because of these reasons the microalgae PBR along with an MBR was operated for 6 days for *S. obliquus*, which is almost one third as compared to *C. protothecoides*. However, in case of *S. obliquus* the TKN and TP concentrations in the MBR permeate was found to be much lower <3 and <1 mg/L respectively (figure 3.5a). It was also found that the TOC was reduced in the range of 10-5 mg/L (figure 3.5b). Similarly, the COD and BOD level was reduced and was in the range of 62-55 mg/L and 30-40 mg/L respectively (figure 3.5c).

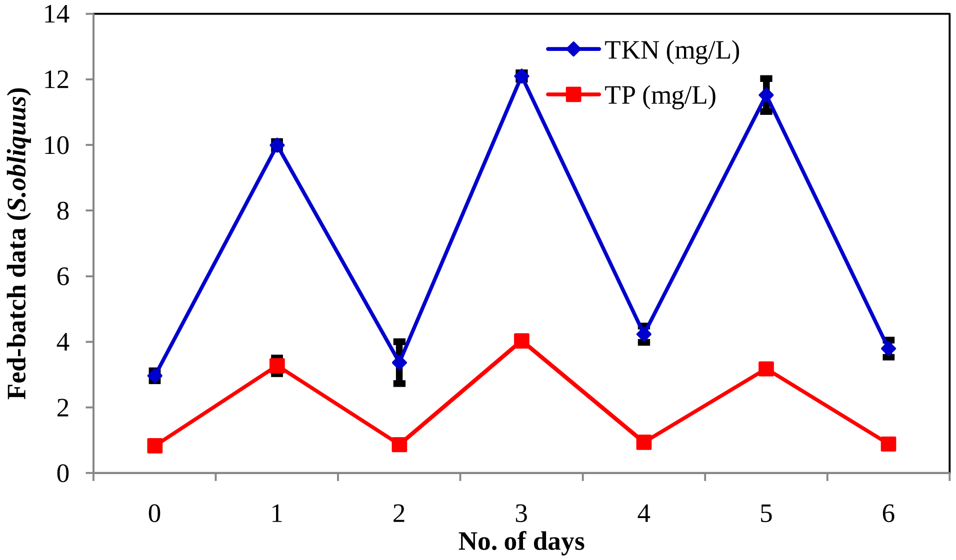


Figure 3.5a: Nutrient reduction by using *S.obliquus* in fed-batch mode of operation

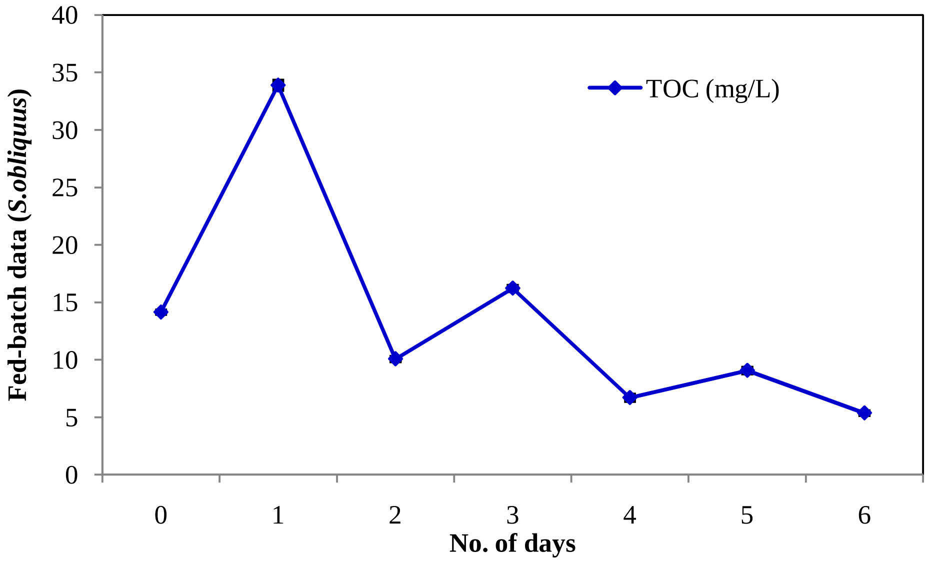


Figure 3.5b: TOC reduction by using *S.obliquus* in fed-batch mode of operation

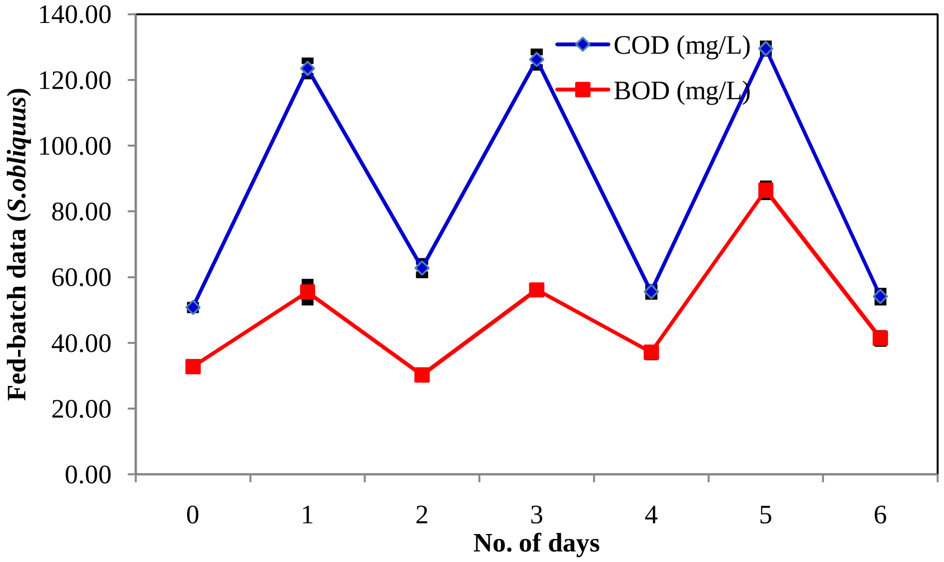


Figure 3.5c: COD and BOD reduction by using *S.obliquus* in fed-batch mode of operation

The microalgae PBR followed by a MBR was also operated for 7 days in fed-batch mode operation using mixed culture. Figures 3.6a, 3.6b, & 3.6c show that almost same nutrient and organic compound removal efficiency can be achieved as was observed with *S. obliquus* species. One of the interesting observations was that the nutrient values obtained in permeate of MBR were lower than those in the effluent in a batch mode microalgae PBR at a lower residence time. Table 3.3 illustrates the initial and final characteristics of sewage water from the MBR permeate. From the data it can be deduced that the filtration effect of the membrane module attached in a external loop to the photobioreactor prevented the microalgae cells from being washed out and enabled the reactor to operate in a efficient way.

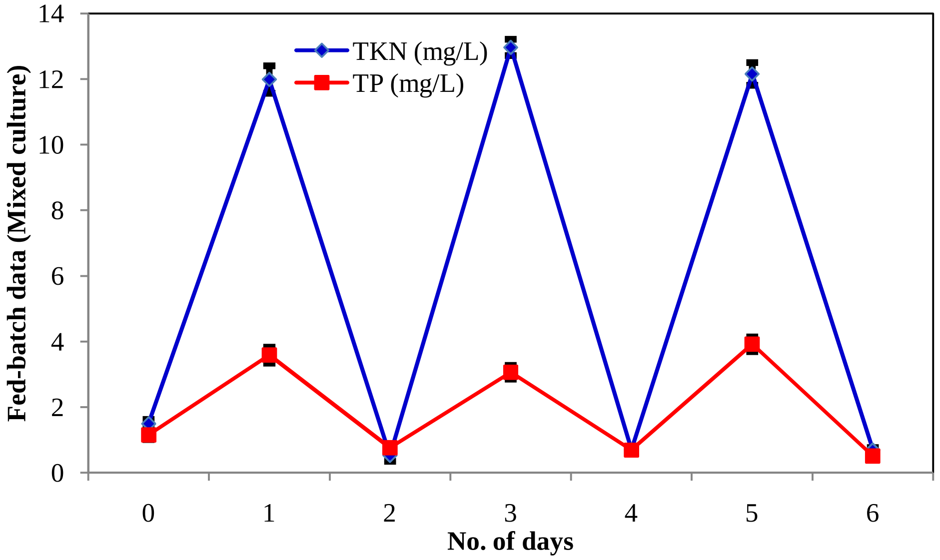


Figure 3.6a: Nutrient reduction by using mixed culture in fed-batch mode of operation

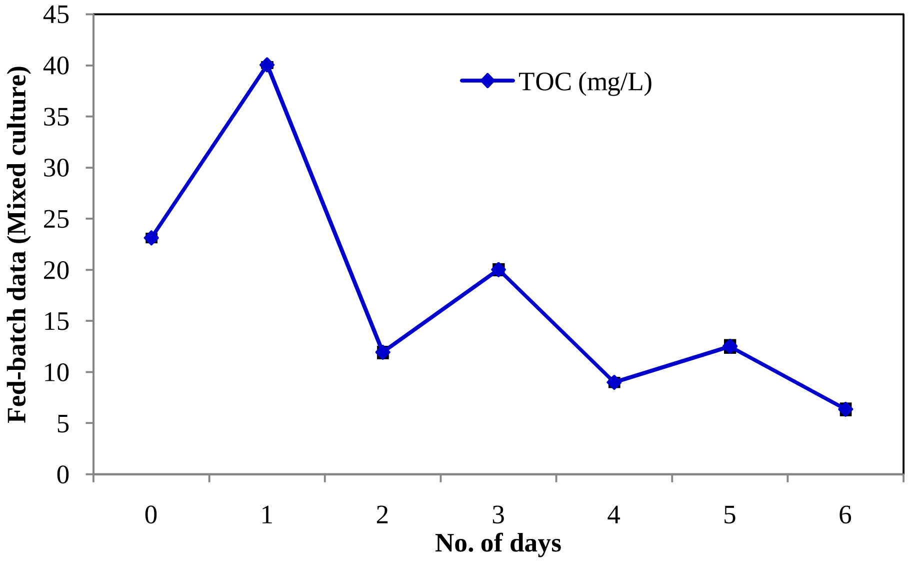


Figure 3.6b: TOC reduction by using mixed culture in fed-batch mode of operation

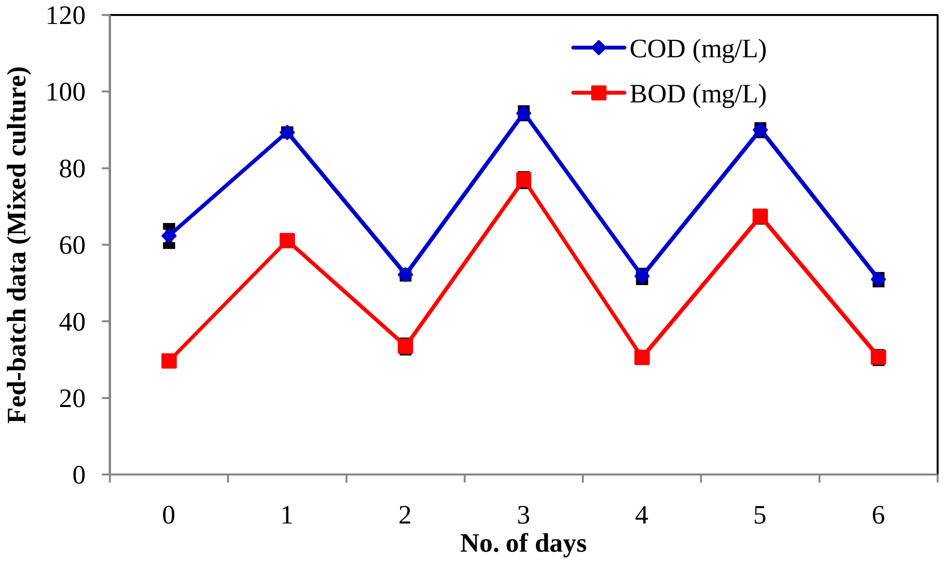


Figure 3.6c: COD and BOD reduction by using mixed culture in fed-batch mode of operation

Table 3.3: Characteristics of sewage water and MBR permeate

Parameters	**Sewage water** **(initial characteristics)**	**MBR Permeate** **(Treated outlet characteristics)**
pH	6.5-7	7-7.5
TKN (mg/L)	40-50	<5

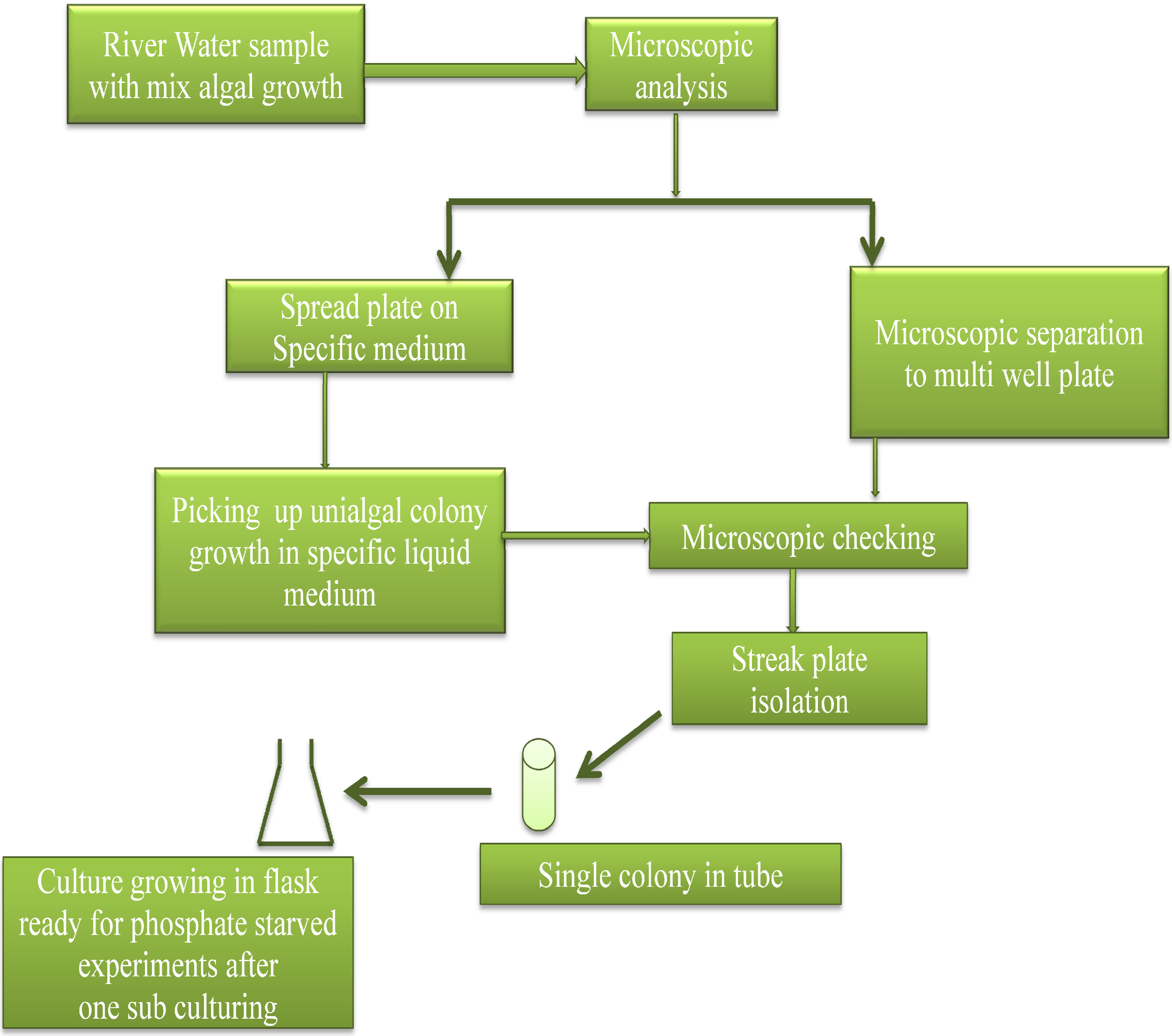
TP (mg/L)	10-12	<5
TOC (mg/L)	130-150	5-9
COD (mg/L)	400-450	50-52
BOD (mg/L)	200-250	30-31

3.4 Conclusions

The performance of microalgae namely *Chlorella protothecoides, Scenedesmus obliquus* and mixed culture was studied for the uptake of nutrient and organic compounds by using a microalgae PBR in a batch mode of operation. It was observed that individual microalgae species and their combination were able to remove the total Kjeldahl nitrogen (TKN) and TP within 5 days of batch operation. Subsequently, microalgae PBR along with a membrane bioreactor (MBR) system were operated for 6-15 days in the fed-batch mode of operation. It was found that the microalgae PBR in combination with an MBR were able to reduce the nutrient as well as organic compounds consistently from the sewage water. It shows that the characteristics of treated water are reasonably complied with the discharge norms stipulated by the central pollution control board of India. It can be concluded that the microalgae PBR has a great phycoremediation potential for the treatment of sewage water in combination with a membrane bioreactor. It was also observed that the long residence time is one of the biggest challenges for microalgae MBR based process and, therefore, further research work is needed to optimize and reduce the residence time. Varying the initial inoculum concentration of microalgae in the batch PBR and testing different membrane bioreactor configurations for effective biomass concentration can resolve the higher residence time issue. The presence of blue-green algae and zooplanktons is inimical for the cultivation of the algal MBR in sewage water and hence, more research needs to be conducted on methods of control of these organisms.

Chapter 4

Isolation and identification of microalgae from sewage disposal sites: Exploiting phosphate-starved cells of *Scenedesmus* sp. for the treatment of raw sewage water



4. Isolation and identification of microalgae from sewage disposal sites: Exploiting phosphate-starved cells of *Scenedesmus* sp. for the treatment of raw sewage water[4]

[4]**"A version of this chapter has been published.**

Swati Yewalkar-Kulkarni, Gayatri Gera, Kiran Pandare, Sanjay Nene, Sanjay Kamble, Insight studies on using phosphate starved cells of Scenedesmus sp. for the treatment of raw sewage, Indian Journal of Microbiology, *DOI 10.1007/s12088-016-0626-0*(2016).

4.1 Introduction

Phosphorous is second most important nutrient in the domestic waste water. It is not removed very efficiently. This nutrient along with nitrogen is responsible for eutrophication. There are many chemical methods to remove the soluble phosphate from the waste water by precipitation (de-Bashan and Bashan, 2004). Disposal of the sludge generated in this chemical precipitation is a big question mark and these methods are not very cost effective (Powell et. al., 2008). Removing phosphates and nitrates from the wastewater biologically with algal system has many advantages over waste treatment process currently used in terms of- (a) Effective uptake of N and P, if sufficient solar energy is available. (b)Simultaneous production of O_2 and consumption of CO_2 in presence of light. (c) No requirement for the supply of extra organic or inorganic nutrients. (d) Providing oxygenated water due to activity of algae. (e) Less sludge accumulation. (f) Absence of generation of secondary pollutants; (g) ecologically safe (h) Generation of microalgal biomass which can be utilized for feedstock, fertilizer, biogas and biofuel (Xin et al., 2010, Wang et al., 2013, Woertz et al., 2009, Olguin, 2012). However it is very essential to enhance the nutrient uptake so that the process of swage cleaning will be faster. The nutrient removal efficiency of the algal cell could be increased by intrinsic and extrinsic factors which include culture density, appropriate algal species, and environmental factors like temperature, pH, CO_2 concentration and starvation of algae, etc.

Higher algal inoculum density of *Chlorella vulgaris* was found efficient in the phosphate and nitrate removal from the domestic waste (Lau et al., 1995).The algal culture density can be increased with immobilized algae. Several authors utilized immobilized algal cells for the faster nutrient removal (de-Bashan and Bashan, 2004, Hernandez et al., 2006, Zhang et al., 2008). The immobilized algae offer more flexibility in the reactor design, accelerate the reaction rate due to increased cell density, better operation stability for the waste water treatment. Immobilized algae also simplifies harvesting problem. (Mallick, 2002). However it may also introduced complexity in the operations at larger scale. Ruiz-Marin et al., (2010) found immobilized algal cells did not offer advantage over free cells for removing nutrients from the waste water.

The phosphate starvation leads to reduction in the total cellular phosphate content (Prieto et al., 1997). It is also known that the P-starved algal cells showed faster nutrient uptake rate than the saturated algal cells (Yao et al., 2011, Hernandez et al., 2006, Zhang et al., 2008, Zhang et. al.,2012).Phosphate starvation causes increase in the lipid and carbohydrate content (Devi et. al., 2012, Beardall et. al., 2001).

The changes in the macromolecular pool can be monitored by FTIR on the basis of infrared absorption of vibrationally active functional group (including O-H, N-H, C=O, =C-H, -C-H, C-O-C and >P=O) in the biological sample. FTIR was utilized by Jebsen et. al., (2012), to study effect of the variation in light intensity, nutrient stress, on physiology of bule green algae *Microcystis aeruginosa*, and marine dino flagellate *Protoceratiun reticulatum*. Duygu et. al.,(2012) utilized FTIR techniques to identify the algal cells. D’souza et al.,(2008) was able to find the physiological and compositional changes induced by cadmium (Cd) in *Pandina tetrastromatica* with FTIR.

As algae isolated from the domestic wastewater, can adapt and execute better for treatment of municipal wastewater. Two different algae were isolated from the wastewater and identified as *Scenedesmus* sp. and *Ankistrodesmus* sp. Both the isolates were evaluated for phosphate and nitrogen uptake from the sewage. The objective of this study was to develop practically applicable, simple, economical primary treatment of the domestic wastewater using algal cells having accelerated nutrient uptake with minimum footprints. An effort was made to understand the underlying rationale behind this improved cellular mechanism. The depletion of the internal phosphate during starvation and other compositional changes were monitored by FTIR.

4.2 Materials and Methods

4.2.1Algal cultures and growth conditions

The unialgal cultures of *Scenedesmus* sp. and *Ankistrodesmus* sp. were isolated from local sewage contaminated water body, from suburban of Pune, India. The isolation was done by agar plating method. They were identified using 18S rDNA and 23S rDNA sequencing,18S rDNA sequencing was done with NS1 and NS4 universal primer. 23S rDNA sequencing was done with rvf 1/rvR universal primers. These two species were identified as *Scenedesmus obliquus* and *Ankistrodesmus stipitatus*.

Once algae are obtained in pure form, they were cultivated under aseptic conditions and preserved for further experiments. The algae were grown on Bold's basal medium (BBM) containing following chemicals $NaNO_3$ (0.25 g/L), K_2HPO_4 (0.075 g/L), KH_2PO_4 (0.175 g/L), NaCl (0.025 g/L), $MgSO_4$ (0.075 g/L), $CaCl_2$ (0.025 g/L),and trace metals $ZnSO_4$ (5 x10^{-6} g/L), $MnSO_4$. $4H_2O$(1 x10^{-5}g/L), H_3O_3 (5 x10^{-5} g/L), $Co(NO_3)_2.6H_2O$ (5x10^{-6} g/L), $Na_2MoO_4.2H_2O$ (5x10^{-6}g/L), $CuSO_4.5H_2O$ (0.025 x 10^{-6}g/L), $FeSO_4.7H_2O$(3.5 x 10^{-3}g/L), Na.EDTA (4 x 10^{-3} g/L) (Singh and Thomas,2012). The cultures were incubated in the average light intensity of 7 W/m^2 at 30 ˚C temperature.

4.2.2 Algal growth in raw sewage water

The sewage was collected from a wastewater treatment plant located in Pune (India). Untreated 200 mL sewage was taken in 500 mL Erlenmeyer flasks with 10% algal inoculums. The flasks were incubated in the average light intensity 7 W/m^2 at 30 ˚C temperature. Sewage water then monitored for nutrients (nitrogen and phosphate) concentration, COD and number of algal cells for 3 days. The samples were centrifuged at 3000xg for 5 min before analysis. Total Kjeldahl nitrogen was analyzed by using KjelTron Nitrogen/Protein digestion system (KDIGB 6 M) by the standard procedure mentioned in American Public Health Association (APHA) 4500-NH3 A,B,C. Total phosphate were estimated by using the vanado-molybdophosphoric acid calorimetric method as mentioned in APHA 4500 . Chemical oxygen demand (COD) was analyzed by using standard methods of APHA 5220B (Clescir et al., 1999).The number of algal cells was determined by counting in the hemocytometer. The experiment was done in triplicate.

4.2.3 Phosphate starvation

The *Scenedesmus* and *Ankistrodesmus* growing in the early logarithmic phase were harvested by centrifugation, washed three times with sterile BBM medium without phosphate and nitrate, to remove the media impurity. Part of the washed cells was re-suspended in fresh 200 mL phosphate free BBM and remaining part suspended in phosphate containing BBM (regular medium), such that every flask has approximately 1 x 10^6 cells/ml. To determine intracellular phosphate, 10 mL sample was taken from each flask daily. The cells were harvested by centrifugation at the speed of 30009g for 5 min and resuspended in phosphate and nitrate free BBM. The cells were digested by boiling in an autoclave for 1 h with5:1(v/v) alkaline potassium persulfate (Maher et al., 2002). Intracellular phosphate was estimated by the phosphor molybdate-blue method as described in APHA 4500 E (Clescir et al., 1999). The number of cells was monitored by counting in the hemocytometer. All experiments were done in triplicate.

4.2.4 FTIR analysis of the phosphate-starved and supplemented cells

The *Scenedesmus* cells were grown in BBM with and without phosphate for 120 h. The cells were harvested, washed and dried in the vacuumed oven at 100˚C. This dried cell mass was utilized for FTIR analysis. For FTIR analysis, sample preparation was carried out as described earlier (D'Souza et al., 2008). Dry algal sample, 2.5 mg was mixed with150 mg potassium bromide (KBr) using mortar pestle. The mixture was filled in high press 13 mm diameter die to get the pellet. The IR of KBr –algae pellet was recorded at 23 ± 1˚C temperature in the mid-infrared range (4000–450 cm^{-1}) using FTIR (Perkin Elmer, Spectrum One). Thirty scans were single averaged for single spectrum. Each spectrum was displayed in terms of transmission. Analysis, of peak area estimation was done by Spectrum One software. The carbohydrate-to-protein band ratio was given by the ratio of an area of the carbohydrate region (900–1200 cm^{-1}) and that of amide II band (1300–1500 cm^{-1}).

4.2.5 Utilization of phosphate-starved cells for sewage water treatment

The *Scenedesmus* culture was grown in 2 L flask containing800 ml BBM. The culture was harvested by centrifugation in the early logarithmic phase. Cells were washed three times with sterile phosphate and nitrate free BBM medium to remove the media impurity and excess phosphate. Half of the cells were inoculated in phosphate free BBM and incubated in light for 48 h for phosphate starvation. The remaining half (untreated) were inoculated in three, 500 mL conical flasks containing 200 ml raw sewage at three initial inoculums sizes were 1 x10^{6}, 5 x 10^{6} and 10 x 10^{6}cells/ml. Further, the sewage was monitored for its nutrient (nitrate and phosphate) concentrations and COD. A similar procedure was adopted for the treated cells .Sewage was monitored further for the nutrients and COD. All experiments were conducted in triplicate.

4.3 Results and Discussion

4.3.1 Growth of algae isolates in sewage water

The algae isolated were checked for their growth rate in raw sewage water after isolation. In the first 24 h, the *Scenedesmus* was able to remove 72–76% phosphate; 93% phosphate removal occurred in 72 h. *Scenedesmus* was able to remove 84.5% of nitrogen in 24 h and 98% nitrogen removal after 72 h. The COD was decreased by 87% during 72 h of incubation with *Scenedesmus* (figure 4.1a). *Ankistrodesmus* was able to remove 72% of phosphate in first 24 h, and up to 83 % of phosphate after 72 h. *Ankistrodesmus* consumed 75% of nitrogen in first 24 h while100% nitrogen depletion occurred in 72 h. Also, 79% of COD was reduced by *Ankistrodesmus* culture in 72 h (figure 4.1b). However further incubation of *Ankistrodesmus* did not reduce the phosphate concentration in the raw waste-water, as nitrogen was completely utilized in 72 h. Values for phosphate, nitrogen, and COD in the control sewage are shown in figure 4.1c. In all these experiments the initial cell density for both of the culture was 1 x 10^{6}

cells/ml. In previous studies, it took 24 days for 97%removal of phosphate and 59% removal of nitrogen, when *Chlorella* was grown on filtered and autoclaved municipal waste-water (Wang et al., 2013). Singh and Thomas, (2012) used membrane reactor permeated sewage water to grow four different local algal isolates viz. *Chlorella*, *C. vulgaris*, *Scenedesmus quadricauda*, and *S. dimorphus* and found that 66% PO_4 could be removed by the microalgae in first 24 h with an initial cell density of 1.2 x10^6cell/ml. *Scenedesmus obliquus* with the initial cell density around 2 x 10^6cells/ml was able to remove 100% nitrogen and 83% PO_4 after 48 h from urban waste-water (Ruiz-Marin et al., 2010).To make the process more viable for practical application, raw, unsterile sewage was used in the present study, with inoculum of minimum initial cell density. However as it was unsterile, after 24 h, when algae reached their peak growth, zooplanktons (grazers) appeared and reduced the algal cell number. Depletion of nutrient and combating grazers reduced the number of algal cells after 24 h of incubation in the case of *Scenedesmus* (figure 4.1a). However, cells of *Ankistrodesmus* were exceptional to this phenomenon of reduction in the cell number, indicating an ability of growth in low concentration of nutrients like phosphate and nitrogen. It may possess some defense mechanism against grazers as then number of algal cells did not reduce like cells of *Scenedesmus* (figure 4.1b).

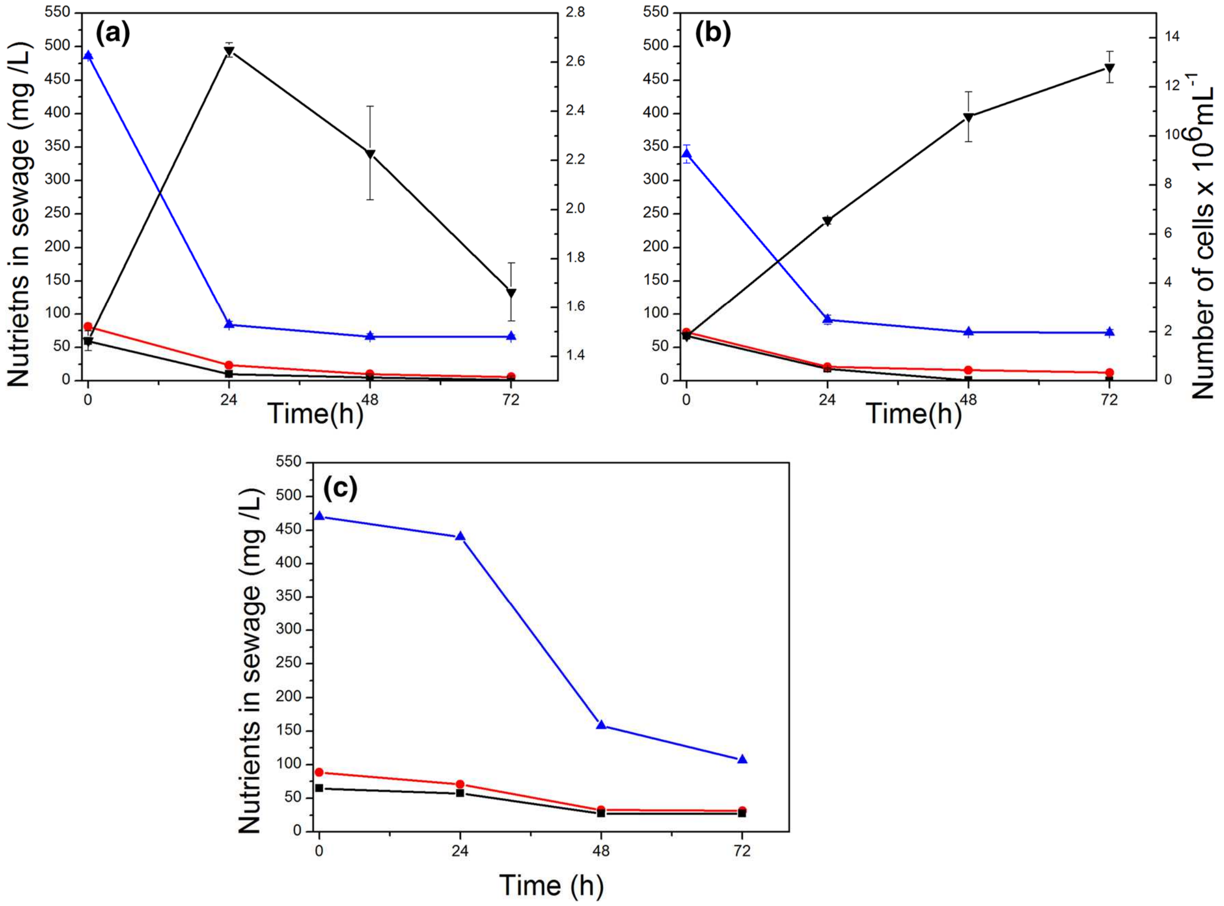


Figure 4.1: Growth of algal isolates in sewage: Growth of algal isolates and concomitant reduction of total phosphate and nitrogen from sewage. (a) Growth of *Scenedesmus*, (b) growth of *Ankistrodesmus*, (c) control or un-inoculated sewage. X axis shows time in h. Y axis shows filled square Kjeldahl nitrogen in sewage (mg/L), filled circle total phosphate sewage (mg/L), filled triangle COD of sewage (mg /L), filled inverted triangle number of algal cells/ml x 10^6.

4.3.2 Effect of phosphate starvation on algal cells

When the cells of *Scenedesmus* were inoculated in phosphate supplemented and phosphate free BBM, the growth of *Scenedesmus* in phosphate-free BBM is hampered considerable only after 48 h of incubation. In this duration, the cell density was raised to approximately around, 5 x 10^6 cells/ml , from the initial 1 x10^6cells/ml in both types of BBM. The cells growing in phosphate-free medium utilized their internally stored phosphate and able to multiply for first two generations in a similar way as that of normal growing cells. However after 48 h, the growth rate of the phosphate-starved cells was reduced as compared to the algal cells growing in the normal medium. At the end of 96 h incubation, the phosphate supplemented cells were able to multiply for more than four generation while the phosphate-starved cells showed only 2.5 generations.

In other words, the phosphate-starved *Scenedesmus* cells showed the stationary phase of the growth cycle after48 h (figure 4.2b). In case of *Ankistrodesmus*, no reduction in the growth rate was not observed after giving phosphate starvation (data not shown). These algal cells were also observed growing in sewage when nitrogen was almost depleted and with 12 ppm of total phosphate indicating heterotrophic growth (figure 4.1a). Hence, cells of *Ankistrodesmus* were not selected for the treatment of phosphate starvation and utilizing further. The internal phosphate

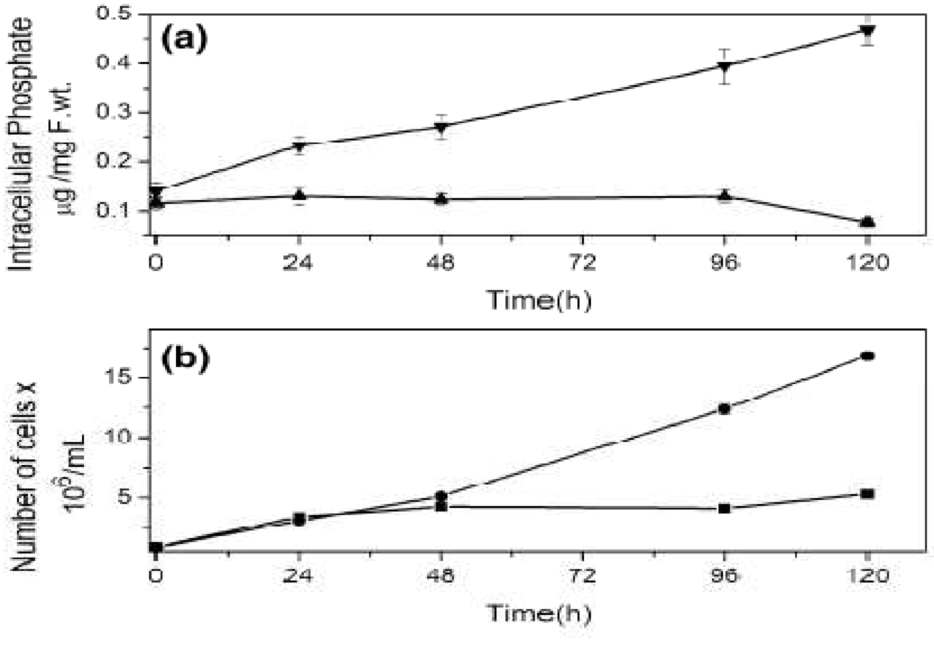


Figure 4.2: Phosphate starvation effect on *Scenedesmus*. a Intracellular phosphate concentration in *Scenedesmus*. (b) Growth of *Scenedesmus* in phosphate free BBM and Phosphate supplemented BBM. X-axis shows time in h for (a) and (b). For a Y axis shows filled triangle intracellular phosphate concentration in the phosphate-starved cells, filled inverted triangle intracellular phosphate concentration in the phosphate supplemented cells (l g/mg of fresh weight of the cells).In (b) Y axis have filled square number of phosphate-starved cells/ml x 10^6, filled circle number phosphate supplemented cells/ml x 10^6.

concentration in the phosphate supplemented *Scenedesmus* cells showed an increasing trend indicating the phosphate accumulation in the daughter cells while growing in the phosphate-rich medium. After 120 h the internal phosphate concentration in the phosphate supplemented *Scenedesmus* cells was raised up to 0.41 l g/mg of fresh weight (figure 4.2a). The cells growing in phosphate free BBM though did not showed significant reduction in the internal total phosphate content for first 96 h of incubation as the stored phosphate might be getting distributed to the daughter cells. However at the end of 120 h of incubation internal phosphate was reduced from 0.12 to 0.078 l g/mg of fresh weight in the phosphate-starved cells (figure 4.2a). Cells of *Phormidium, Sphaerocystis* and *Scenedesmus* when grown in phosphate supplemented medium showed an increase in the intracellular phosphate content (Beardall et al., 2001). When grown in phosphate-starved medium algae able to utilize the internal phosphate and could sustain for 3–4 generations under the starvation conditions (Yao et al., 2011).

4.3.3 FTIR analysis of phosphate-starved and supplemented Scenedesmus cell

Effect of the phosphate starvation was further studied with FTIR. To visualize the effect of phosphate starvation stress, the integrated FTIR band of different spectral were studied. The dried cell mass showed transmission peaks over wave number 450–4000 cm^{-1}, are given in figure 4.3. The peaks were tentatively identified on the basis of published FTIR spectra in relation to the specific molecular groups. The FTIR spectra were compared by taking the ratios of areas, of the respective IR bands in the absorbance mode. The region from 900 to 1200 cm^{-1} is characteristics ofC–C, C-O, C–O–C, C–O–P of polysaccharides stretching vibrations of polysaccharides (Duygu et al., 2012, D’souza et al., 2008). The cells grown in phosphate free BBM showed the peak at 1022, 1077, 1156 cm^{-1} (figure 4.3a). These peaks are due to various polysaccharides. Carbohydrate pool containing various polysaccharides increased during the phosphate-starved conditions. The increase in polysaccharides is another indicator of the stationary phase of the algae growth cycle. Cells were grown in phosphate supplemented BBM showed the peak at only at 1085, 1152 cm^{-1} (figure 4.3b).However, algae growing in phosphate supplemented BBM did not show the peak at 1022 cm^{-1}. Similar findings were reported in *Sphaerocystis* and

Phormidium, where these algae showed strong bonds at 1024, 1080 and 1150 cm^{-1} under phosphate starvation; however, these bonds were disappeared within 24 h after supplementing cells with phosphate (Beardall et al., 2001). Phospho diester bond stretching generates peak at 1245and 1240 cm^{-1} in phosphate- starved and supplemented algal cells respectively. We found an increase in intensity and broadening of the aromatic phosphate bond (P=O) at1240 cm^{-1} and symmetric aliphatic phosphate bond (C–O–P) at 1085 cm^{-1} indicating phosphate storage in the phosphate supplemented *Scenedesmus* cells. This results of FTIR got confirmed when the intracellular phosphate was quantified with the phosphomolybdate-blue method, the phosphate supplemented cells showed 5.7 times more intracellular phosphate than the phosphate-starved cell(figure 4.2a). Collective effect of phosphate starvation and supplementation on the macromolecular pool is given in (figure 4.4). The phosphate starved *Scenedesmus* cells showed significantly increased in carbohydrate/phosphor (C/P), carbohydrate/lipid (C/L) and carbohydrate/protein (C/AII) ratio. When cells of *Microcystis aeruginosa* and *Phaeodactylum tricornutum* were grown in phosphate-limited conditions, they showed decreased growth rate, and increase in (C/AII) ratio (Jebsen et al., 2012, Stehfest et al., 2005, Ponnuswamy et al., 2012). *Phaeodactylum tricornutum* showed increased in lipid/phosphate after 3 weeks of phosphate starvation (Stehfest et al., 2005). In this study L/P ratio of phosphate supplemented and starved *Scenedesmus* cell did not differ significantly. This may be due to a short starving period of 120 h. The Lipid/amide II (L/AII) and amide/phosphor (AII/P) did not show a significant difference after phosphate starvation. FTIR study confirms the stationary phase in phosphate-starved *Scenedesmus* cells as observed in growth curve study (figure 4.2). These starved, stationary algal cells might be able to consume nutrients at faster rate compare to the phosphate supplemented cells which might prove helpful for the faster treatment of raw sewage.

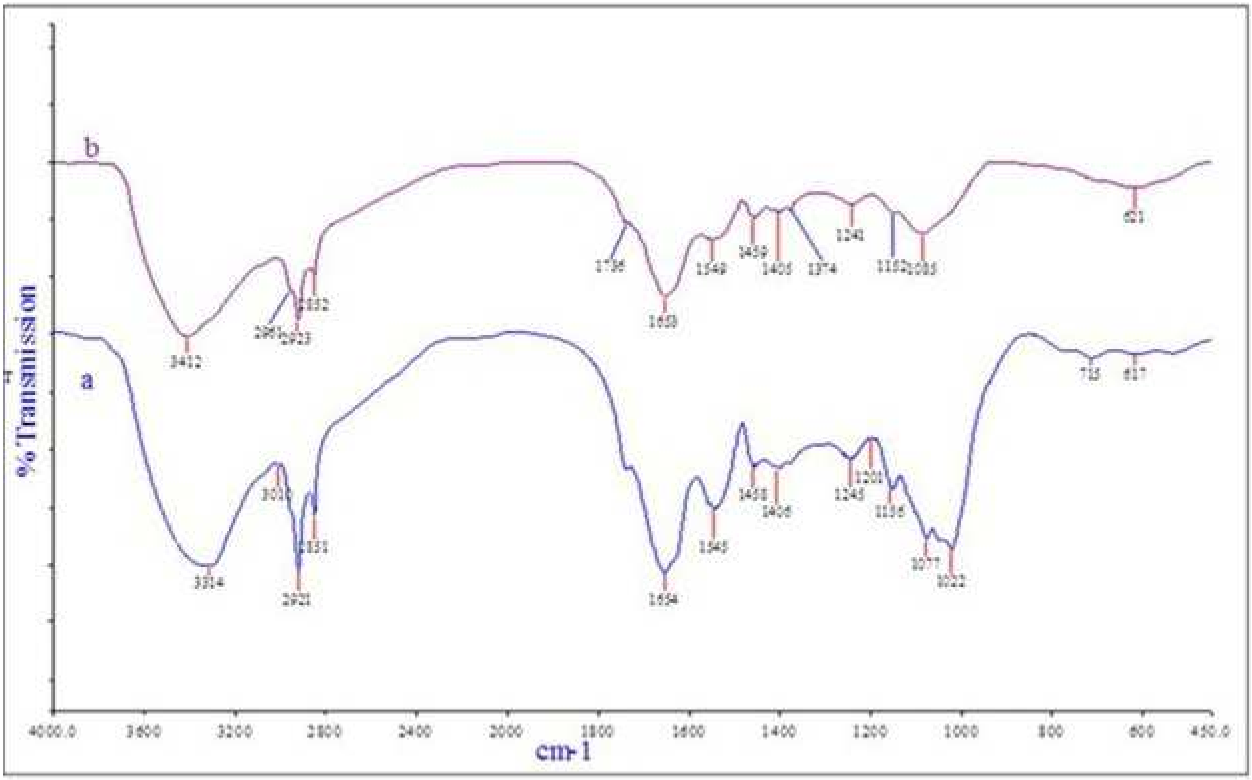


Figure 4.3: FTIR spectrum: FTIR peak of phosphate (a) starved and (b) supplemented cells of *Scenedesmus*. At X axis—wavelength (cm^{-1}), Y axis represents % transmission every spectrum is average of thirty scans. The phosphate starved cells showing peaks at 1022 cm^{-1}. This polysaccharide peak is absent in phosphate supplemented cells, however these cells showed broad peak at 1240and 1085 cm^{-1}.

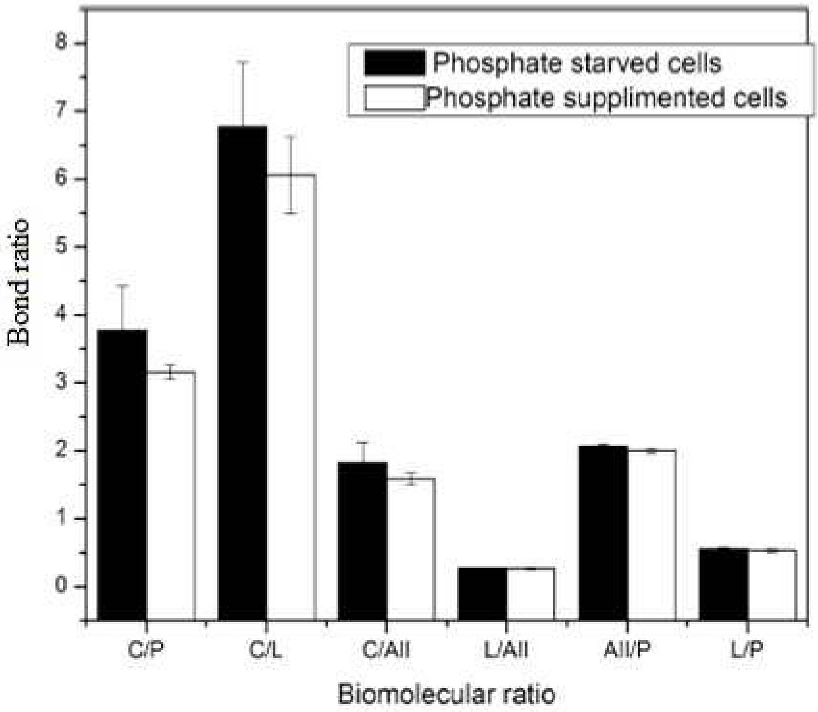


Figure 4.4: Effect of phosphate starvation on macromolecular pool: The bond ratios of the various bio-molecules of phosphate-starved (gray bars) and supplemented (white bars) *Scenedesmus* cells are plotted on X axis. Carbohydrate/phosphor (C/P), carbohydrate/lipid (C/L) and carbohydrate/protein (C/AII) ratio is increased in phosphate starved cells.

4.3.4 Comparison of phosphate-starved and supplemented Scenedesmus for sewage water treatment

The phosphate-starved and phosphate supplemented *Scenedesmus* cells were used for the treatment of sewage. The sewage water with initial phosphate concentration 124 mg/L when treated with phosphate-starved1 x 10^6cells/ml, the phosphate was reduced to 17.6 mg/L (86%) at the end of 24 h (figure 4.5a). Similar cell density of phosphate supplemented cells was able to reduce the phosphate concentration to 27 mg/L (76%) (figures 4.5a and 4.5b). Previously three days phosphate-starved and immobilized cells of *Chlorella sorokiniana* able to reduce the 23%phosphate from synthetic waste water in 48 h. This reduction was further enhanced by co-immobilizing *C. sorokiniana* with bacteria *Azospirillum brasilense* (Hernandez et al., 2006). Zhang et al., (2008) used two days starved cells of *Scenedesmus*, immobilized 2 x 10^8cells/ml (cell intensity in the bead) and found 100% removal of PO_4 in just 135 min from filtered, sterilized secondary domestic waste water. Similar experimentation was done with 2 days phosphate-starved *Chlorella*, with immobilized 1.4 x10^8cells/ml (cell intensity n the bead), took 4 h for the complete removal of phosphate from filtered, sterilized secondary domestic wastewater (Zhang et al., 2012).In this study raw sewage water without any prior treatment like filtration and sterilization is used. Hence, it is more viable to the practical application. This unsterilized, un inoculated sewage has its own natural microbial flora which is responsible for reduction in phosphate, nitrogen and COD with time (figures 4.5, 4.6, and 4.7).The phosphate-starved *Scenedesmus* cells, with initial cell density 1 x 10^6cells/ml, were able to utilize 100%nitrogen within 24 h (figure 4.6a) however the phosphate supplemented cells were able to reduce the nitrogen level up to 24 mg/L (52%) within 24 h (figure 4.6b). The initial total nitrogen concentration in the sewage water was 50 mg L^{-1} indicating fast uptake of nitrogen by the starved, stationary, *Scenedesmus* cells. Zhang et al., (2008) report 99.1% NH_4 removal by two days phosphate-starved *Scenedesmus* from filtered, sterilized secondary domestic waste water. When 2 x 10^8cells/ml immobilized (cell intensity in the bead) were used for the treatment. Two days phosphate-starved *Chlorella*, with immobilized 1.4 x 10^8cells/ml (cell intensity in the bead), took 4 h for the 98.8% removal of NH_4 from filtered, sterilized secondary domestic wastewater (Zhang et al., 2012). In the present study P-starved *Scenedesmus* isolate showed no significant increase in phosphate and nitrogen reduction with the increase in a number of free cells (1 x 10^6

to 10 x 10^6cells/ml) in the sewage water (figures 4.5a and 4.6a). However, the phosphate supplemented cells at higher cell density showed faster nitrogen reduction with increasing the number of free cells in the sewage (figures 4.5b, and 4.6b). Reduction of the COD was similar for both phosphate-starved and supplemented cells (figures 4.7a, and 4.7b).

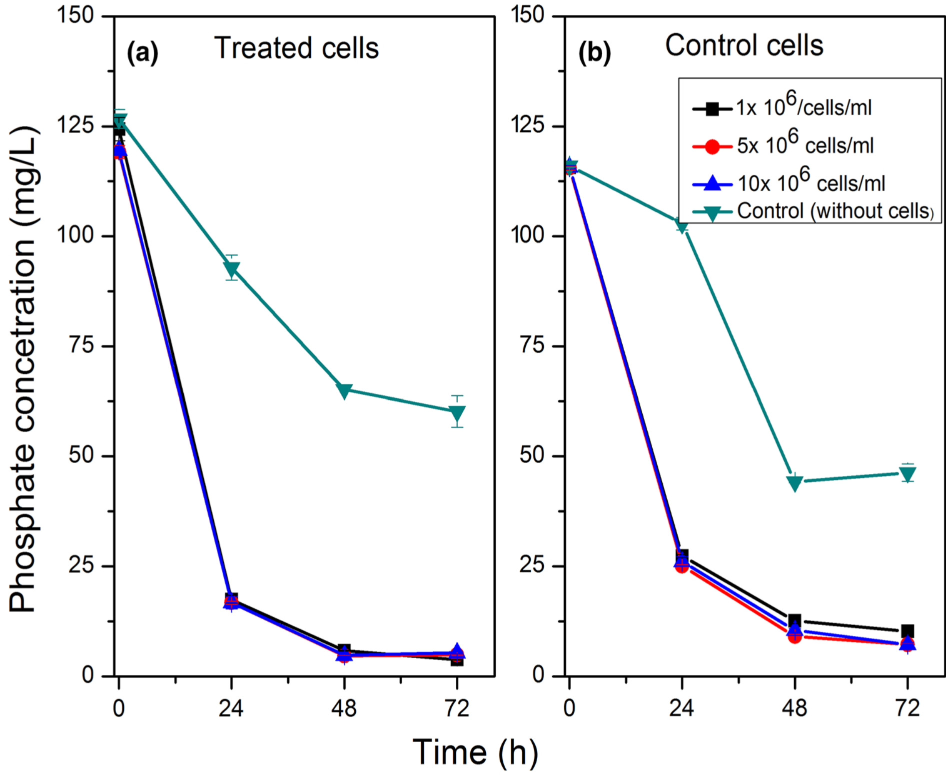


Figure 4.5: Effect of algal phosphate starvation on phosphate uptake: the phosphate-starved (a) and phosphate supplemented (b) *Scenedesmus* cells inoculated in sewage. The X-axis represents time in h. Y-axis shows phosphate concentration in mg/L utilized by filled square1 x10^6cells/ml, filled circle 5 x 10^6cells/ml, filled triangle 10 x 10^6cells/ml, filled inverted triangle un inoculated sewage.

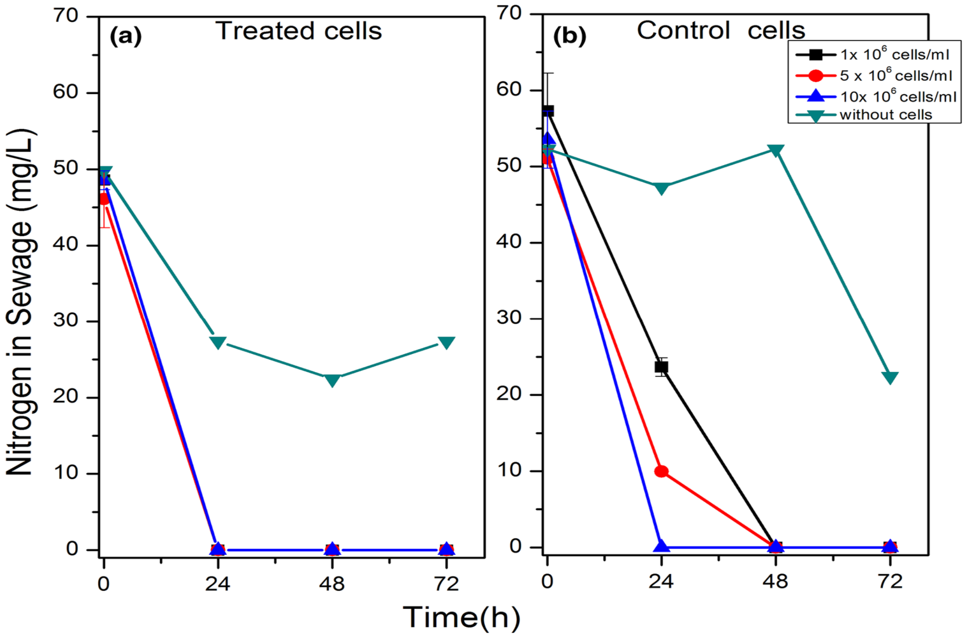


Figure4.6: Effect of algal phosphate starvation on nitrogen uptake: The phosphate-starved(a) and phosphate supplemented (b) *Scenedesmus* cells inoculated in sewage. The X axis represents time in h. Y axis shows nitrogen concentration in mg l-1 utilized by filled square1 x 10^6cells/ml, filled circle 5 x10^6cells/ml, filled triangle10 x 10^6cells/ml, filled inverted triangle un inoculated sewage.

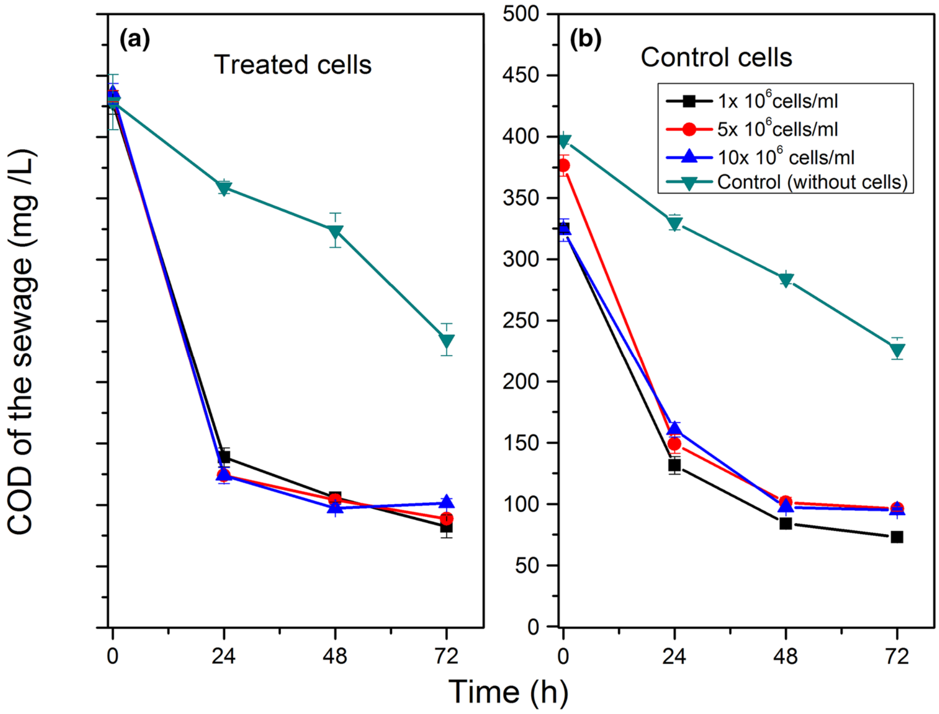


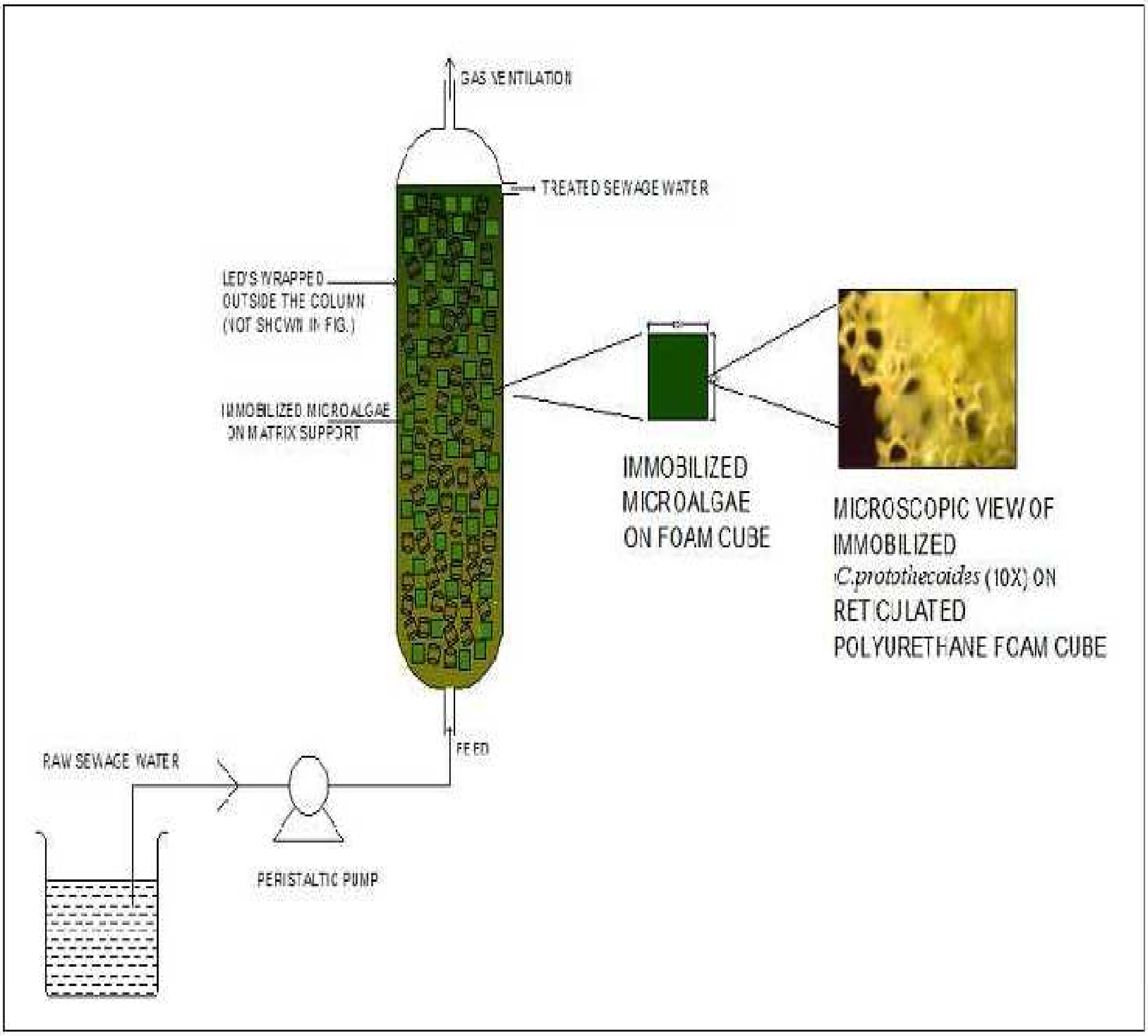
Figure4.7: Effect of algal phosphate starvation on COD reduction: The phosphate starved (a) and phosphate supplemented (b) *Scenedesmus* cells inoculated in sewage. The X axis represents time in h. Y axis shows COD mg/L utilized by filled square1 x 10^6cells/ml, filled circle 5 x 10^6 cells/ml, filled triangle10 x 10^6cells/ml, filled inverted triangle un inoculated sewage.

4.4 Conclusions

The local algal isolate *Scenedesmus* was entering in the stationary phase of life cycle due to the phosphate starvation. These stationary phase cells of *Scenedesmus* showed effective phosphate and nitrogen utilization from the untreated sewage water. However, the phosphate starvation did not induce any stationary phase in the cells of *Ankistrodesmus*. Phosphate starvation of *Scenedesmus* for 120 h showed a reduction in the internal phosphate and rise in carbohydrate pool. Phosphate starvation of algal cultures appears to be a good technique for an enhanced rate of removal of the total phosphorus and nitrogen contents in sewage. Additional detailed studies need to be conducted under steady state conditions in a chemostat to determine how phosphate starvation triggers an enhanced rate of nitrogen and phosphate.

Chapter 5

Kinetic studies on an algal biofilm reactor for raw sewage water treatment



5. Kinetic studies on an algal biofilm reactor for raw sewage water treatment[5]

[5]“A version of this chapter has been published.

Gera G., Swati N. Yewalkar., Nene S., Kamble S., Kinetic studies of algal biofilm reactor for raw sewage treatment, Chemical Engineering Technology, 39 (9), 1629–1635 *DOI:10.1002/ceat.201500447* (2016).

5.1 Introduction

Packed bed columns are widely employed in biochemical treatment processes due to their simplicity and cost effectiveness. Biofilm formation is a natural process where microalgal cells can be anchored to the support without the use of chemicals and form thick layers of cells known as a biofilm. The advantage of biofilm reactors is that they provide very high cell densities and hence can be effectively utilized for the biochemical conversions. This will also avoid expensive immobilization protocols and use of harsh chemicals during immobilization processes. In the sewage treatment process, the symbiotic relationship between bacteria and microalgae plays a significant role especially in the process of biofilm formation. As, investigated by Holmes, (1986) and Hodoki,(2005), attached algal colony increases notably when more extent of bacteria was present. Wastewater treatment based on large scale biofilm process has already become a part of the industry. It was also suggested that if sufficiently large surface area is provided the larger extent of algal biofilm can grow as compared to the free algal cells. This can help in overcoming the major challenges to production and harvesting of microalgae (Wuertz et al., 2003).

In reality, algae immobilization for the treatment of sewage water has gained more attention as it proves to be a cheap source of nutrients for algal growth and a less energy intensive method for biomass production with the additional benefit of pollutant removal from the environment. Immobilizing microalgae can represent an alternative for solving the sewage treatment problem by providing advantages such as lower residence time with maximum biomass retention, less foot print, and higher metabolic activities. In the past, few articles were published on the use of suspended algae for nutrients removal from sewage in shallow artificial ponds (Christenson and

Sims, 2011).However, in recent years, much research is focused on immobilizing uni-algal cultures on the flat plate, fiber bundle or in between twin layers of micro porous ultra-thin sheet for nutrients removal from sewage water to overcome the difficulty of harvesting and thereby, developing a robust viable process (Tercero et al., 2014, He and Xue, 2011, Shi et al., 2007). Gil and Serra, (1993) have reported nearly 90% nitrate removal by immobilizing *cyanobacterium* species on the polyvinyl foam pieces in a packed bed reactor with just 3-4 h of residence time. Immobilized algae on loofa sponge have proved to be very effective in removal of heavy metals like cadmium, chromium from wastewater (Akhtar et al., 2008, Saeed and Iqbal, 2006) .Algae captured in alginate beads was extensively studied for nickel and copper removal from solution and it was found that immobilized algae are more tolerant to Cd, Zn, and Cr than the suspended algal cells, hence suggesting their more possibilities for wastewater treatment processes (Abu Al-Rub et al., 2004, Tam et al., 2000, Mallick, 2002).Rotating photobioreactor with the algal culture immobilized on the surface is emerging as an improved method for the nutrients removal from wastewater to prevent eutrophication. These rotating photobioreactors are capable of providing a high surface to volume ratios thereby increasing the gas transfer, light illumination, biomass productivity and making harvesting inexpensive (Burke, 2014, Christenson and Sims, 2012, Bove et al., 2015).The algal biofilm-based processes for sewage treatment need further improvements in biofilm design to optimize the process so as to integrate it with the conventional treatment plants.

In the present study, we have used dual packing, namely, glass raschig rings to facilitate gas ventilation from the packed bed column and polyurethane foam cubes used to support the algal biofilm. By adjusting these two supports in a particular ratio across the packed bed, biofilm formation, and its density can be controlled. The reaction kinetics studied was based on the initial total nitrogen (TN) and total phosphate (TP) content in the raw sewage along with the different $\frac{L}{D}$ ratios, the size of foam cubes and sewage water feed rate. Based on all these parameters, Thiele modulus and effectiveness factor was calculated to study the mass transfer phenomena in terms of film and pore diffusion in heterogeneous catalysis. A model equation was developed to predict the performance packed bed algal biofilm reactor. In order to study the feasibility of packed bed algal biofilm reactor for the treatment of sewage water, the reactor was continuously operated for 90 days. Considering, all the approaches investigated, algae biofilm-based dynamic studies in terms of mass transfer are the least understood, hence there is a need to develop a correlation between different operating variables of algae biofilm packed bed reactor for nutrients depletion in sewage treatment. Hence, kinetics of removal of nutrients from sewage water using algal biofilm reactor has been studied. From the pseudo reaction rate constant, Thiele Modulus and effectiveness factor were calculated and a kinetic model equation for fractional nutrients uptake was developed in terms of operating variables.

5.2 Materials and methods

5.2.1Microalgae strain and cultivation

The microalgae *Chlorella protothecoides* (SAG- 211-7b) obtained from experimental phycology and culture collection of algae at the University of Göttingen Germany has been used in the present investigation. The culture was maintained on polytoma medium agar slant at 4˚C and was periodically sub-cultured.

5.2.2Polytoma media (PolGlu)

The microalgae culture was grown in the medium consisting of 1000mg/L glucose, 1000 mg/L yeast extract, 1000 mg/L bacto-tryptone, 30 ml/L soil extract and 1ml/L of trace metal solution. The stock solution was autoclaved separately and prepared under aseptic conditions. Algal strains were maintained separately at 26˚C under the illumination of white fluorescent lamps at 120 rpm for efficient mixing.

The algal strains were filtered and washed several times with distilled water to remove media components before being inoculated into sewage water. Raw sewage water was collected from the Sangvi Municipal wastewater treatment plant located in Pune (India) having the capacity to treat 20 MLD of sewage water. Sewage water was coarsely filtered through the mesh to remove debris and no sterilization treatment was employed before being used for the experimental runs.

5.2.3 Immobilization protocol

Figure 5.1 (a) and (b) shows the schematic diagram and actual photograph of the packed bed algae biofilm reactor system. Reticulated foam cubes (0.01×0.01m) and glass raschig rings (0.01×0.01m) were used for immobilizing microalgae. Foam cubes and raschig rings were washed and sterilized before using it for immobilization. Glass raschig rings were used to avoid compression of the foam cubes as well as for channeling the gas liberated during the course of operation. The known number of foam cubes and glass raschig rings were filled in a column of volume 0.142 L (L=0.25 m, D=0.025 m) in 1:1 ratio. The column was made of transparent polycarbonate material and was wrapped outside with white LED lights for continuous light illumination. The reactor was fed with coarsely filtered raw sewage water by using the peristaltic pump (Watson-Marlow, 312S) connected at the bottom of the column and treated water was collected from the top of the column. The culture of *C. protothecoides* at exponential phase (0.25 L) in polytoma medium was recirculated through the column continuously for 2 days (0.05 L/h).After 2 days; the freshly prepared polytoma medium was recirculated through the column till a good algal biofilm was visible on the foam cubes. Microalgae also form a biofilm on the interiors of raschig rings. The same immobilization protocol was used for the subsequent experiments. The effect of the film diffusion and the $\frac{L}{D}$ ratios of the packed bed column was studied by keeping the weight of the biocatalyst same. Whereas, to study the pore diffusion, the size of the foam cube was changed by keeping the volume of the reactor constant. At each flow rate, the reactor was operated for a period of 24 h to ensure steady state conditions. All the experiments were carried out at room temperature (30-34 ˚C).The effect of various parameters such as the size of the cube (0.005-0.02 m), diameter of the reactor (0.02-0.035 m), height of packed bed (0.16-0.35 m) and feed rate (5.17×10^{-10}-5.83×10^{-9} m^3/s) on the removal of nutrients from the sewage water was studied and optimum values were established.

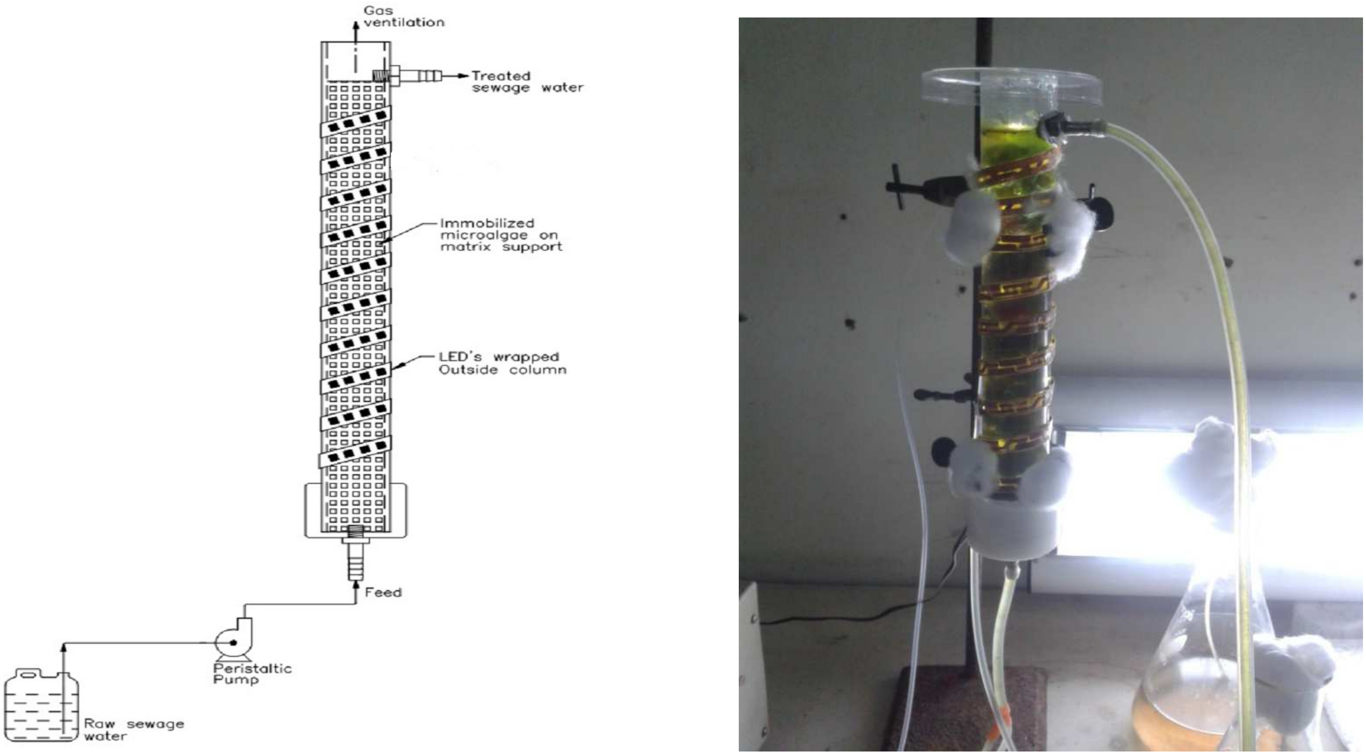


Figure 5.1: (a) Schematic diagram and **(b)** actual photograph of packed bed algae biofilm reactor for sewage water treatment

5.2.4Analysis

All water samples unless stated otherwise were centrifuged at 6000 rpm and filtered through a 0.45µm cellulose acetate filter paper (Millipore) before analysis in triplicates. Total nitrogen (TN) was analyzed by using TN analyzer (Shimadzu,Japan). Total phosphates (TP) were estimated by using vanadomolybdophosphoric acid colorimetric method (APHA 4500 P-C) (APHA, 1998).

5.3 Theory

Most of the kinetic models use a continuum approach in biofilm research for simulation and modeling of complex wastewater systems (Wuertz et al., 2003).Detailed kinetics is often found to be essential in complex processes with an approximately high number of intermediates, which may or may not participate in reactions. In the present investigations, we have used classical chemical reaction engineering approach to predict the order of the reaction by using the following equation for a packed bed reactor (Levenspiel, 1999).

$$\int_{C_{A0}}^{C_A} \frac{-dC_A}{dt} = (-r)\int_0^t \frac{d\tau}{dt} \qquad (5.1)$$

After solving the equation (5.1) we get

$$t = (\tau)\alpha - \ln(1-X) \qquad (5.2)$$

A straight line plot of τ vs. $-\ln(1-X)$ ensures that the nutrients uptake follows first order behavior. When the algal cells are settled and proliferated on the polyurethane foam cubes they are assumed to be uniform on all sides of the cube and inside. In a packed bed reactor, there are zones very near to the biocatalyst surface where the velocity approaches zero. In the stagnant fluid region, the reactants must cross the fluid film by means of molecular diffusion only. The expression for film diffusion in terms of mass transfer coefficient can be defined as

$$r = k_m\, a_m\, (C_A - C_{AP}) \qquad (5.3)$$

Under steady state conditions, it can be expressed as,

$$r = \eta k a_m C_{AP} \qquad (5.4)$$

From equation (5.3) and (5.4),

$$C_{AP} = \frac{k_m C_A}{(\eta k + k_m)} \qquad (5.5)$$

Substituting equation (5.5) in equation (5.4) and integrating it gives-

$$-\ln\left(\frac{C_A}{C_{A0}}\right) = [\eta k_m a_m k/(\eta k + k_m)]T \qquad (5.6) \qquad \text{Or}$$

$$-\ln(1 - X) = K'T \qquad (5.7)$$

Where,

$$K' = [\eta k_m a_m k/(\eta k + k_m)]$$

A straight line plot of $-\ln(1 - X)$ vs. T will give the values of pseudo-first-order reaction rate constants for a different foam size. Thiele modulus is defined as a ratio of the diffusion time to the reaction time. However, in order to generalize this term many interpretations are used in the literature. According to Aris, (1957), Thiele modulus for the first order reaction kinetics obeys following equations (5.8) for different catalyst shape.

$$\phi = X_0 \sqrt{\frac{k}{D_0}} \qquad (5.8)$$

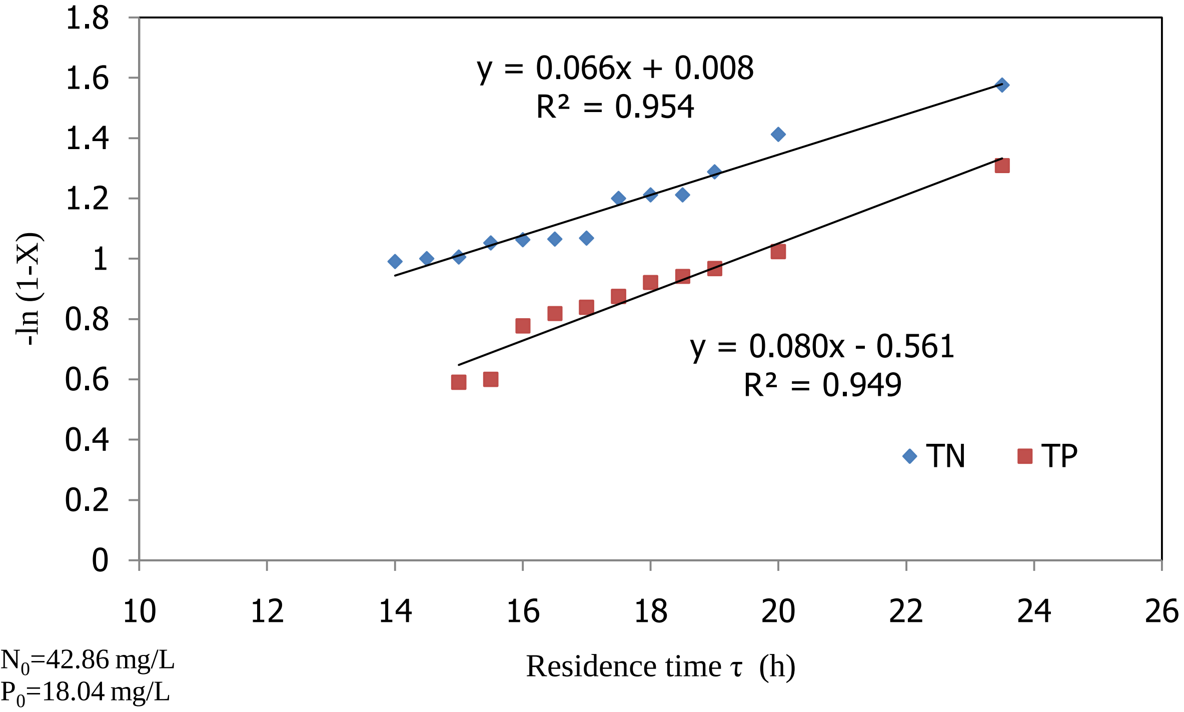
Effectiveness factor explains the interaction between pore diffusion and reactions on the surface of the porous catalyst particle. For a first order reaction in a cube, effectiveness factor can be written as

$$\eta = \frac{1}{3\phi}\left[\frac{1}{\tanh\phi} - \frac{1}{\phi}\right] \qquad (5.9)$$

Equation (5.8) and (5.9) can be solved for obtaining the values of ϕ and η .

5.4 Results and Discussion

The effect of various parameters such as the size of the cube (0.005-0.02 m), the diameter of the reactor (0.02-0.035 m), height of packed bed (0.16-0.35 m) and feed rate (5.17 x 10^{-10}-5.83 x 10^{-9} m^3s^{-1}) on the removal of nutrients from the sewage water was studied. To confirm the reaction kinetics and film diffusion, sets of experiments were conducted with different dimensions of packed bed reactor $\left(\frac{L}{D}=8,9,10\&11.6\right)$ with the equivalent amount of biocatalyst (algae immobilized foam cubes of 0.01x0.01 m size and 25 gm of wet weight of biocatalyst). Figure 5.2 shows the uptake of TN and TP at different residence time while other parameters are kept constant $\left(\frac{L}{D}=11.6\right)$, size of foam cube (0.01 m). A straight line plot (figure 5.2) of $-\ln(1-X)$ vs. different residence time indicates that the nutrients uptake from sewage water follows the first order kinetics.



TN_0=42.86 mg/L
TP_0=18.04 mg/L

Figure 5.2: Confirmation of first-order kinetics for the uptake of nutrients from sewage water

5.4.1Effect of film diffusion

Figure 5.3 represents the uptake of TN and TP as a function of space time for different $\frac{L}{D}$ ratios ranging from 8-11.6 while biocatalyst loading kept constant. At lower flow rates, (higher space time value) the effect of film diffusion was significant whereas, at higher flow rates (low space time value) the effect of film diffusion is marginal. This can be explained as follows: at higher flow rates, since the particles are cubical in shape, significant eddy flow was observed around the cube with back mixing (Tseng, 2006).Because of back mixing, high turbulence occurred hence the film diffusion could not be observed. Whereas, at very low flow rates, the liquid around the cube flows slowly and film diffusion effect was observed. Figure 5.3 shows highest uptake of nutrients in packed bed column having the high $\frac{L}{D}$ ratio=11.6 as compared to other column reactor $\frac{L}{D}$ ratios (8, 9 &10) with the same space time value. This clearly indicates the effect of film diffusion. This behavior is expected in film diffusion where the linear velocity of feed increases and film thickness decreases for the same value of space time. The same behavior was observed by Ramakrishna et al.,(1991) for ethanol fermentation in continuous reactor packed with immobilized yeast cells in alginate beads. Nevertheless, attempts for nutrients removal (TN and TP) from sewage water by using the algal biofilm have not been reported and hence there is a need to study the kinetics of nutrients uptake w. r. t. nutrients loading for scale up.

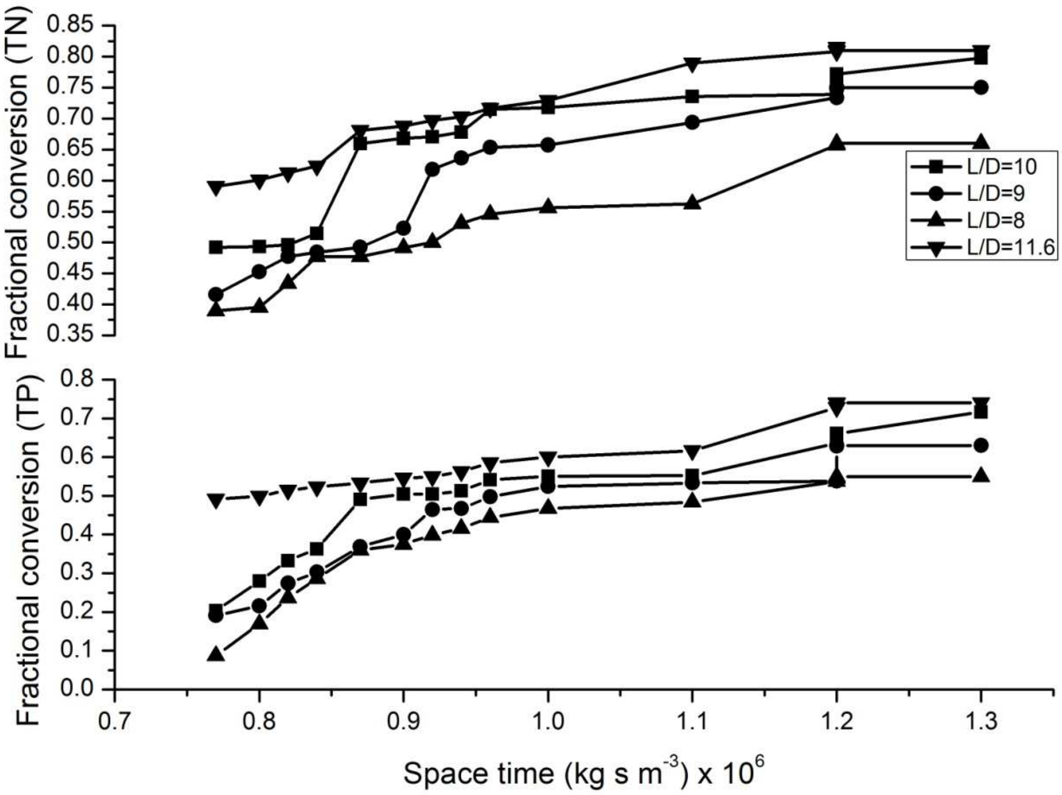


Figure 5.3: Effect of film diffusion on the uptake of TP and TN with space time

5.4.2 Effect of pore diffusion

To study pore diffusion, the size of the foam cube was varied between 0.005-0.02 m while other parameters such as $\frac{L}{D}$ =11.6 and biocatalyst loading of $\approx$ 25 gm on wet basis were kept constant. Figure 5.4 reveals the fractional uptake of TN and TP with respect to space time. It can be noticed that as the foam size decreases the nutrients uptake increases because of the increase in surface area. It is evident that nutrients uptake take place when sewage water is exposed to the surface of a biocatalyst. For a foam size of 0.005 m maximum uptake rate is achieved as compared to that

of 0.02 m which indicates pore diffusion limits the uptake of nutrients. The pseudo first order nutrients uptake rate constants for different foam size are calculated and shown in figures 5.5a and 5.5b for TN and TP uptake respectively.

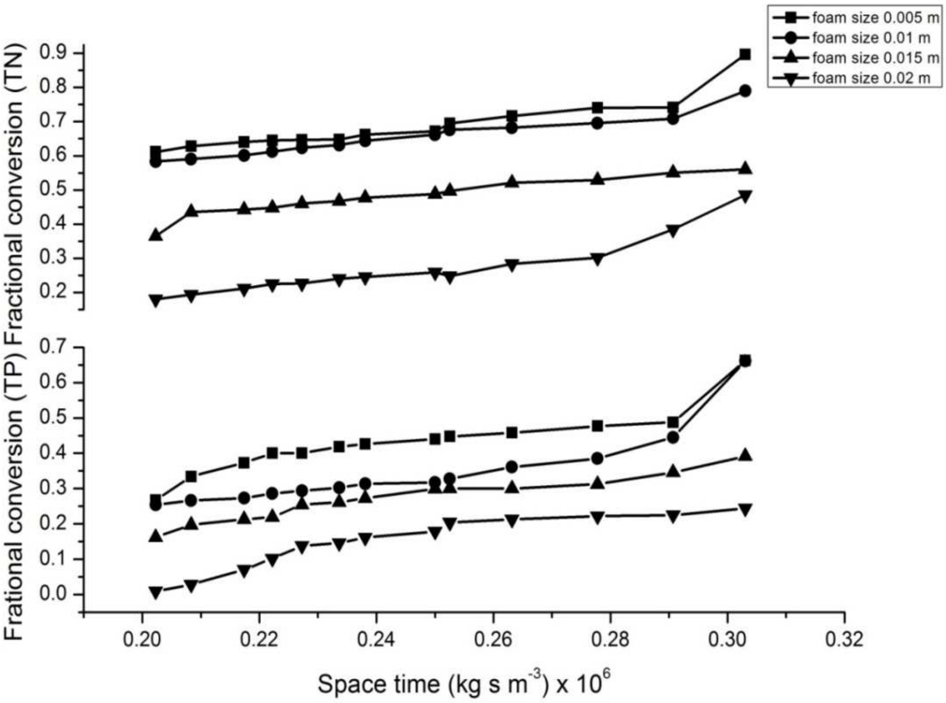


Figure 5.4: Effect of pore diffusion on uptake of TN and TP uptake at different space time

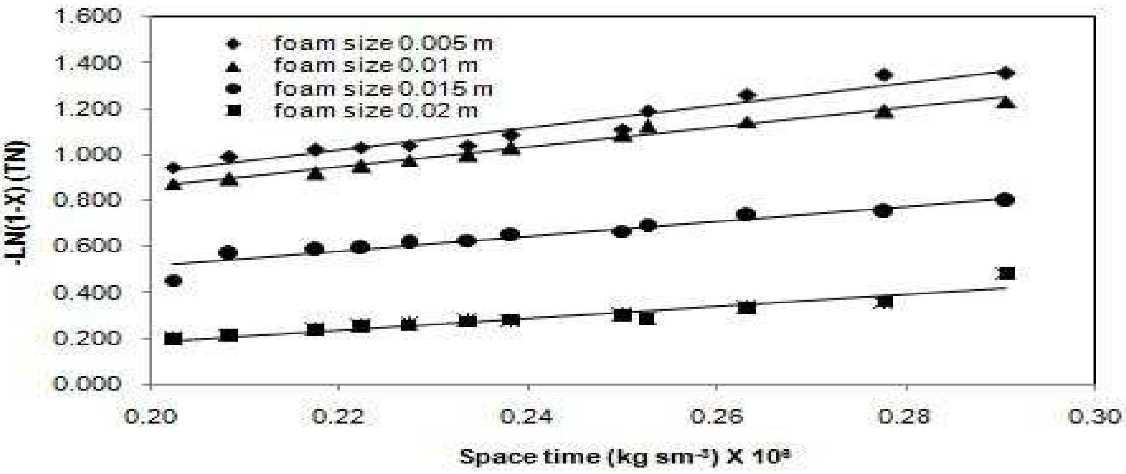


Figure 5.5a: Pseudo-first order rate constants for TN uptake at different foam size packing

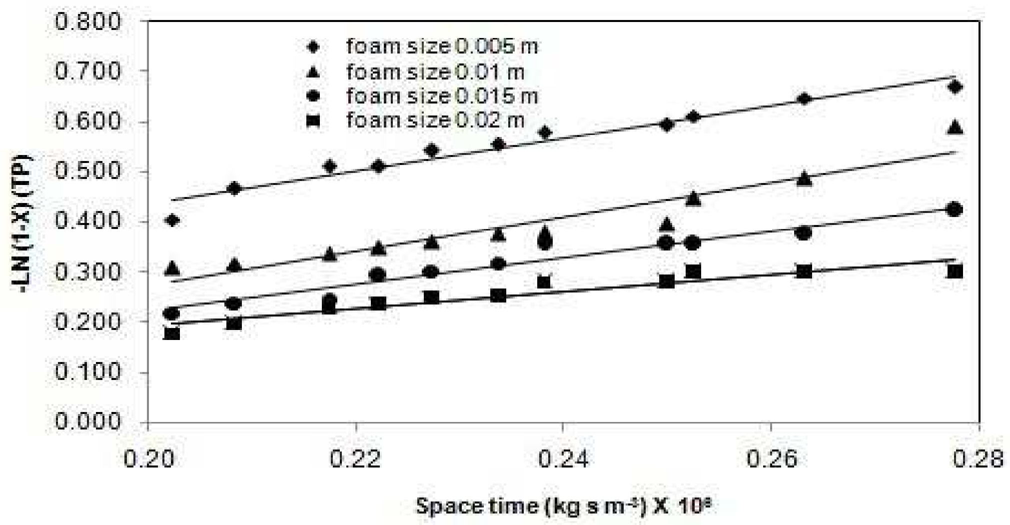


Figure 5.5b: Pseudo-first order rate constants for TP uptake at different foam size packing

Table 5.1 shows the values of Thiele modulus and effectiveness factor calculated by using equation (8-9) and from the experimental values for the uptake of TN and TP from sewage water. Thiele modulus is regarded as a measure of the ratio of the rate of reaction to the rate of diffusion. Thiele modulus is inversely proportional to the effectiveness factor which takes into account the overall reaction rate in the foam piece to the reaction rate at the external surface of the foam piece. From the data, it was observed that, as the size of the foam piece decreases, Thiele modulus decreases with the increase in the effectiveness factor. For the large value of Thiele modulus, the rate of reaction is much greater than the rate of diffusion; the effectiveness factor is much less than unity and thus, it can be concluded that the diffusion rate in the cube is limited and vice-versa. This indicates that at the smaller size of foam cube surface reaction is rate limiting and at the larger size of foam cube film diffusion control the overall reaction. Whereas, in the case of effectiveness factor which is the ratio of the average of the rate of reaction by diffusion to the average of the reaction rate in the bulk stream, and it approaches unity as the catalyst size become small (Adagiri et al., 2012).Thiele modulus gives the relation between catalytic activity and size of a particle with no mass transfer limitations. Thus, Thiele modulus alone is not sufficient to define the catalytic reaction and a value of effectiveness factor is also essential.

Table 5.1: Pseudo rate constants, Thiele modulus and effectiveness factor for various sizes of the cube

Size of cube $\times 10^{-2}$ (m)	Pseudo rate constant $\times 10^{6}$ (m^3 $kg^{-1}s^{-1}$)		Thiele modulus ϕ		Effectiveness factor η (exp.)	
	TN	TP	TN	TP	TN	TP
0.5	4.909	3.257	0.2	0.17	0.98	0.98
1	5.027	3.155	0.42	0.33	0.91	0.94
1.5	4.138	3.789	0.56	0.54	0.85	0.86
2	3.867	4.709	0.72	0.79	0.78	0.75

In this case, it can be supposed that the nutrients concentration on the external surface is the same as that of the bulk liquid and the system is diffusion limited for nutrients concentration only, although virtually liberation of gasses will also be influenced by the effect of diffusion. It is assumed that at the external surface of the cube the substrate concentration is uniform and that of bulk liquid. The results presented in the present study sufficiently describe the effect of film and pore diffusion, but could not waive out one form to observe the other as in the case of continuous stirred tank reactor.

5.5 Continuous treatment of sewage water using packed bed biofilm reactor

Algae biofilm packed bed column used in the present investigation cannot strictly follow the plug flow conditions because of the coproduction of gasses during the course of sewage treatment. Following assumptions were considered while developing a model equation for TN and TP uptake in algae biofilm reactor-

- The reactor studied was considered to be ideal plug flow reactor with no back-mixing.
- The amount of catalyst, i.e. biomass concentration, was kept sufficiently high and was assumed to have no effect on the kinetics.
- During sewage treatment, there is an evolution of carbon dioxide and oxygen which makes the system three phase. But the effect of gas evolved was neglected and the reactor was assumed to be a two-phase system.

While, studying the diffusion effect, the role of gas evolved was neglected. However, it is not precise to neglect the effect of the gas. In the present study, the results obtained indicate that the gas ventilation with the help of raschig rings is adequate and no gas hold up in the column was observed.

The fractional uptakes of TN and TP under various experimental conditions were analyzed by the multivariable regression analysis method using Matlab (version 7.6.0.324-R2008a). Here, column dimensions $\left(\frac{L}{D} ratios\right)$, residence time, initial concentrations of TN & TP and catalyst size from experiments were considered as inputs for finding the final uptake of nutrients as an outcome. These predicted values were compared with the experimental values to validate the model. The following two equations (5.10 and 5.11) can predict the TN and TP uptake with ±5% deviation from the experimental values (figure 5.6).

$$X_N = -0.3569 + 0.0320\left(\frac{D^2L}{Q}\right) + 0.0079\,CN_0 + 0.0628(d) \tag{5.10}$$

$$X_P = -0.2124 + 0.0414\left(\frac{D^2L}{Q}\right) + 0.0093\,CP_0 + 0.123(d) \tag{5.11}$$

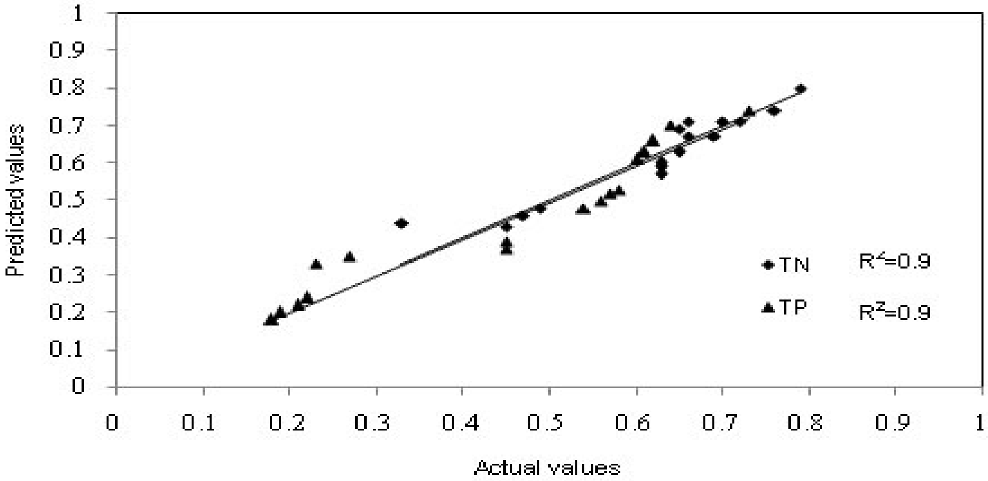


Figure 5.6: Comparison of actual and predicted values for TN and TP uptake

Packed bed algae biofilm reactor of having $\frac{L}{D}$ =11.6 with the residence time of 24 h was continuously operated for 90 days for nutrients removal from sewage water. Consistently for 90 days approximate 76-83% of TN and 70-76% of TP removal was achieved. Figure 5.7 shows the performance of continuous algae biofilm packed bed reactor for % uptake of nutrients removal from sewage water. The disruption shown around 30-35 days is due to seasonal variation in the characteristics of the sewage water used during the experiment.

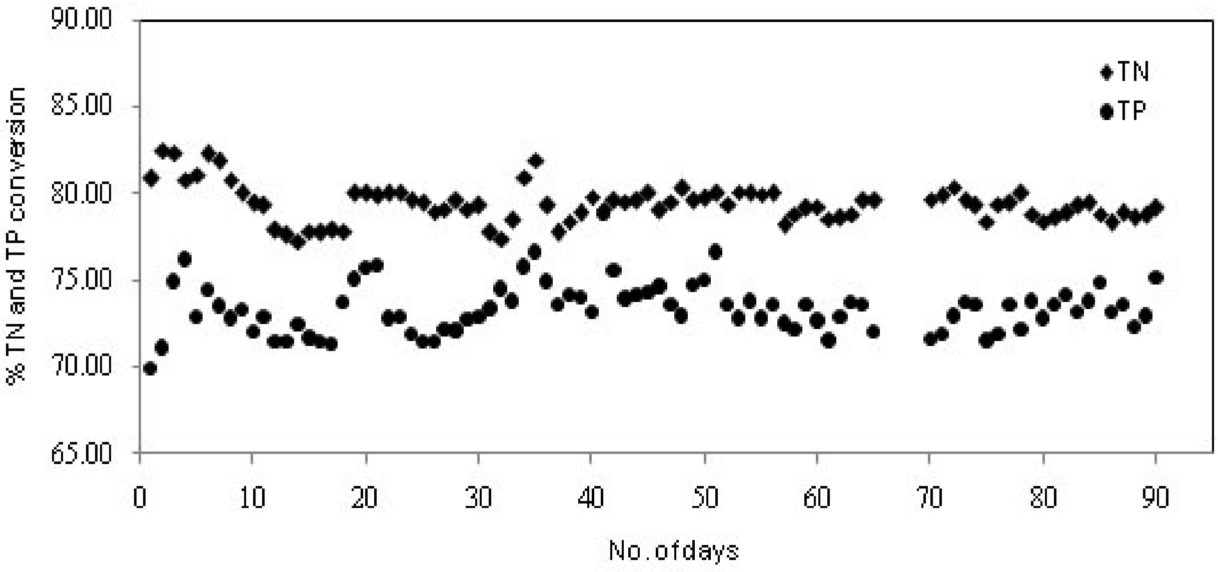


Figure 5.7: Performance of continuous algae biofilm packed bed reactor for sewage treatment

TN and TP concentration after treatment remains in the range of 10.4-8.1 and 4.6-3.5 mg L^{-1} respectively, which clearly indicates the potential of packed bed algae biofilm reactor for the raw sewage water treatment. Table 5.2 depicts the characteristics of sewage water before and after treatment.

Table 5.2: Characteristics of sewage water before and after treatment using packed bed algae biofilm reactor

Parameters	**Before treatment (mg/L)**	**After treatment (mg/L)**
Total Nitrogen	40-50	10.38-8.06
Total Phosphate	16-20	4.57-3.5
pH	6.8-7	9-8

5.6 Conclusions

Reticulated polyurethane foam has been found to be an effective support for the growth of mixed algal-bacterial biofilm in sewage water. The algae biofilm packed bed column studies showed that the significant reduction in TN and TP levels from raw sewage in a relatively short residence time of 24 h. The uptake of TN and TP follows pseudo-first order kinetics. Both, film and pore diffusion effects were observed as the rate controlling steps for the algae biofilm packed bed reactor for sewage treatment. A correlation of the fractional uptake for TN and TP w.r.t. various operating parameters like algae biofilm packed bed column dimensions, residence time, raw sewage water feed rate and foam cube dimensions has been developed and validated. The multivariable regression model can be used to predict nutrients uptake efficiency and will help in scale-up of the packed bed biofilm reactor to design strategies for efficient operations. Thus, the present study demonstrates the use of cheap, re-usable, inert, autoclavable matrices having high-porosity, as support with for biofilm-based sewage treatment.

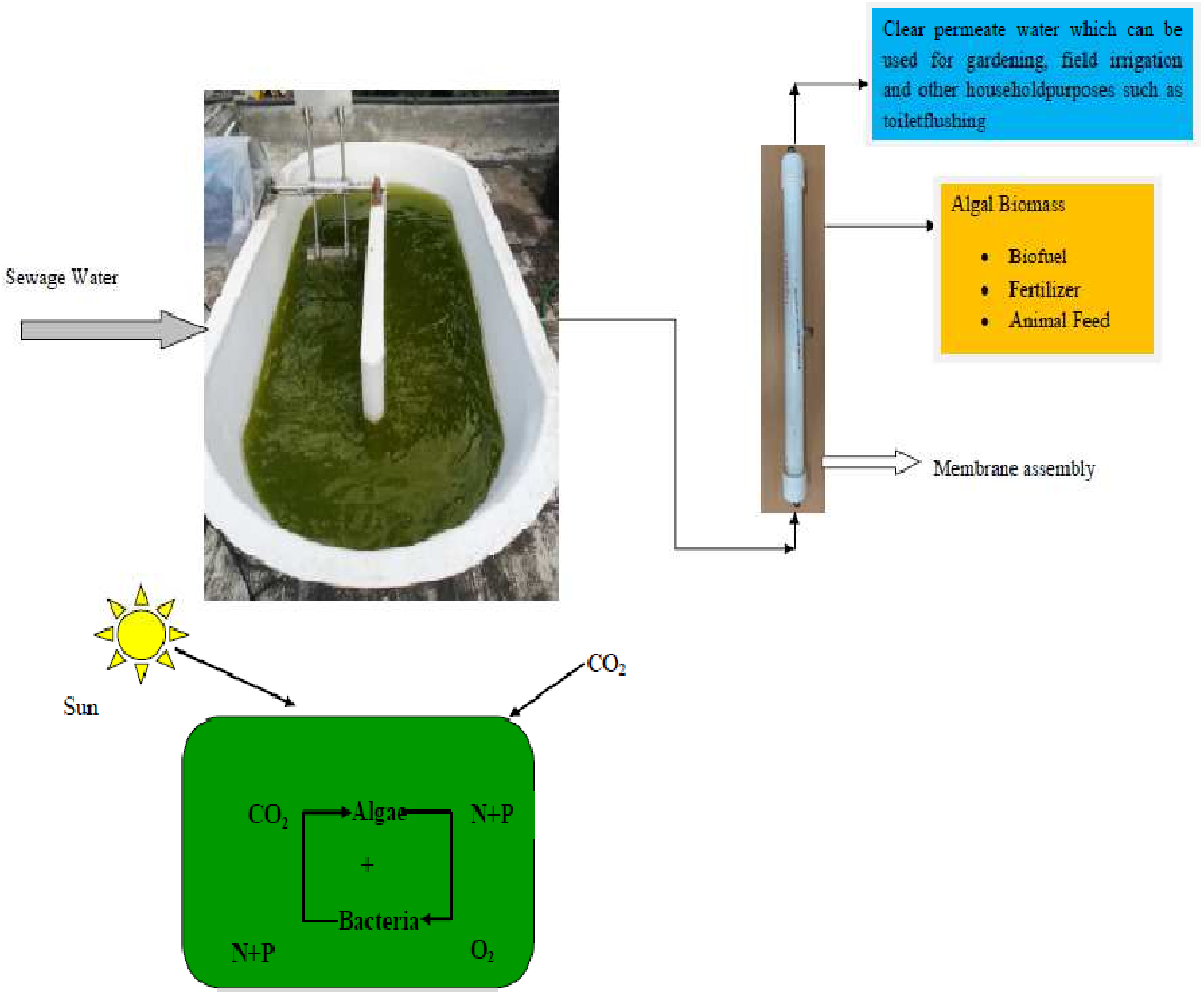
Abbreviations

a_m	$[m^2kg^{-1}]$	Surface area per unit mass of catalyst particle
C_A	$[mg\ L^{-1}]$	Nutrient concentration at any time
C_{A0}	$[mg\ L^{-1}]$	Initial nutrient concentration
C_{AP}	$[mg\ L^{-1}]$	Nutrient concentration on the foam cube surface
C_{N0}	$[mg\ L^{-1}]$	Initial nitrogen concentration
C_{P0}	$[mg\ L^{-1}]$	Initial phosphate concentration
D	[m]	Diameter of reactor
d	[m]	Catalyst particle size
De	$[m^2s^{-1}]$	Effective diffusivity
k	$[m\ s^{-1}]$	First order reaction rate constant
K’	$[m^3kg^{-1}s^{-1}]$	Pseudo first order reaction

		rate constant
k_m	[m s^{-1}]	Mass transfer coefficient
L	[m]	Length of reactor
Q	[m^3s^{-1}]	Flow rate of feed
r	[kmol $kg^{-1}s^{-1}$]	Reaction rate
t	[s]	Reaction time
T	[kg s m^{-3}]	Space time (weight of biocatalyst/flow rate of feed)
V_{pfr}	[m^3]	Volume of the packed bed reactor
X	$\left[1-\left(\frac{C_A}{C_{A0}}\right)\right]$	Fraction of nutrient consumed
X_0	$\frac{Volumeofthecatalystparticle}{Areaofthecatalystparticle}$	Characteristic dimension
X_N	$\left[1-\left(\frac{C_A}{C_{N0}}\right)\right]$	Fraction of TN consumed
X_P	$\left[1-\left(\frac{C_A}{C_{P0}}\right)\right]$	Fraction of TP consumed
τ	[h]	Residence time
Greek symbols		
ε		Voidage
η		Effectiveness factor
φ		Thiele modulus

Chapter 6

Treatment of Sewage Water using Microalgae in Combination with Membrane Bioreactor: An Eco-Friendly Approach


Clear permeate water which can be used for gardening, field irrigation and other householdpurposes such as toiletflushing

Algal broth grown in sewage water

6.Treatment of Sewage Water using Microalgae in Combination with Membrane Bioreactor: An Eco-Friendly Approach[6]

[6]**"A version of this chapter has been published.**

Gera G., Salunkhe V., Kharul U., Jadhav S., Bhattacharjee T., Kamble S., Treatment of Sewage Water using Microalgae in Combination with Membrane Bioreactor: An Eco-Friendly Approach, Current Environmental Engineering,5, *DOI:10.2174/2212717805666180124153612* ,(2018).

6.1 Introduction

Microalgae for the uptake of nutrients viz. nitrogen and phosphate from wastewater have gained significance because of increase in greenhouse gas (GHG) emissions in the atmosphere as it provides a green alternative by fixing carbon dioxide which leads to lipid accumulation in a large amount of biomass (Maity et al., 2014, Honda et al., 2012). This biomass can be used for the production of third generation biofuels in abundance.

Harvesting of microalgae biomass is still considered to be the bottle neck for the biofuel industry to flourish. Till date, no technology has been developed and demonstrated for the cost-effective production and harvesting of algae (Christenson and Sims, 2011). Although, lots of review on different approaches for the production of algae as well as benefits and disadvantages of algae harvesting paths, including chemical, mechanical, biological and electrical methods are reviewed in the literature (Bilad et al., 2014, Rawat et al., 2011, Uduman et al., 2010). The integrated method for the microalgae biomass production using wastewater and subsequent harvesting is gaining plenty of importance as it offers an advantage in terms of less expensive and ecologically safer method. Now a day's attached algal biofilm cultures were studied as one of the promising approach for controlled algae production and harvesting for wastewater treatment (Gera et al., 2016, Burke, 2014, Christenson and Sims, 2012). The smaller size of microalgae in water makes it difficult to harvest and leads to huge operational cost during dewatering. Uduman et al., (2010) had described various dewatering methods for the microalgae biomass production and suggested that the auto flocculating and large cell size will be beneficial for the effective algae harvesting. But, according to Bhave et al., (2012), the small sized alga in suspended form for the cross flow or tangential filtration is more effective compare to dead end type of filtration using membrane.

In order to achieve high biomass concentration flocculation, centrifugation and cross-flow filtration are the most suitable downstream processes. There is a lot of scope to explore various hybrid techniques for the production and harvesting of algal biomass on large scale. One of the advanced methods developing for the dewatering of algae is the integrated membrane-based system. The major barrier in using membrane-based separation for algae is the fouling, of the membranes which increases the operating costs. In order to limit the membrane fouling much focus is needed on the understanding of the fouling mechanism. According to Kimura et al., (2005), the food-microorganism ratio and membrane filtration flux were the important operating parameters that greatly control the membrane fouling in membrane bioreactors (MBR's) (Kimura et al., 2005). Many of the studies involving MBR focus on the removal of nutrients, organic matter and other effluents from domestic wastewater with the membrane module submerged in the photobioreactor (Gao et al., 2016, Marbelia et al., 2014, Bilad et al., 2014). However, due to slow growth rate cultivation of microalgae in closed photobioreactors is not very ambitious for the treatment of domestic wastewater because of algae's slow growth rate (Cai et al., 2013, Bilad et al., 2014). Therefore, high rate algal ponds (HRAP) or raceway ponds are the best systems to treat domestic wastewater in presence of sunlight especially in a tropical country like Indian subcontinent where sunlight is available throughout the year and they are relatively inexpensive to build and operate. In order to advance the wastewater treatment methods, various membrane configuration needs to be explored for the efficient harvesting and reuse of microalgae (Bilad et al., 2014, Bhave et al., 2012).

In the present work, the treatment of sewage water was studied for three different algal species viz. *Scenedesmus obliquus*, *Chlorella vulgaris* and *Ankistrodesmus stipitatus* for the uptake of nutrients and biomass production in an open raceway pond. In order to study the effect of initial concentration of microalgae on the uptake of nutrients and other organic matter, the initial microalgae concentration was varied from 1×10^6 to 5×10^6 cell/ml in an open raceway pond. The microalgae biomass grown in open raceway pond was harvested using three different types of sidestream membranes. The membranes used were polyacrylamide nitrile (PAN), Polysulfone and ceramic membrane. The membranes were evaluated for their performances in terms of membrane flux at four different transmembrane pressures (TMP) and the phenomenon of membrane fouling was studied. An optimal TMP along with the suitable membrane was suggested for the effective dewatering of microalgae from the treated sewage water.

6.2 Materials and Methods

6.2.1 Algal cultures and growth conditions

The isolation of the *Scenedesmus obliquus*, *Chlorella vulgaris,* and *Ankistrodesmus stipitatus* was done from the regional sewage contaminated water body (Mutha river), from suburban of Pune, India. The isolation was done by agar plating method. The isolated microalgae were identified by using 18S rDNA and 23S rDNA sequencing was done with NS1 and NS4 universal primer (Yewalkar-Kulkarni et al., 2016). The unialgal cultures obtained in pure form were cultivated under aseptic conditions for these experiments. Bold's basal medium (BBM) was used to grow the algae with the following chemical composition $NaNO_3$ (0.25 g/L), K_2HPO_4 (0.075 g/L), KH_2PO_4 (0.175 g/L), NaCl (0.025 g/L), $MgSO_4$ (0.075 g/L), $CaCl_2$ (0.025 g/L), and trace metals ZnSO4 ($5x10^{-6}$ g/L), $MnSO_4.4H_2O$ ($1x10^{-5}$ g/L), H_3O_3 ($5x10^{-5}$ g/L) , $Co(No_3)_{2-}.6H_2O$ ($5x10^{-6}$ g/L), $Na_2MoO_4.2H_2O$ ($5x10^{-6}$ g/L), $CuSO_4.5H_2O$ ($0.025x10^{-6}$ g/L), $FeSO_4.7H_2O$ ($3.5x10^{-3}$ g/L), Na.EDTA ($4.9x10^{-3}$ g/L). Algal strains were maintained separately at 26˚C under the continuous illumination of white fluorescent lamps of light intensity 8000±200 lux (Equinox T176544 lux meter) at 120 rpm for efficient mixing on a rotary shaker.

6.2.2 Algae cultivation in open raceway pond

Open raceway pond having a working volume capacity of 30 L was used to grow algae in the sewage water in presence of 12:12 h light (sun light)-dark cycle, with temperatures of 30˚C and 18˚C during sun light and dark hours. The depth of the pond was 100 mm in order get efficient light penetration at the bottom of raceway pond (figure 6.1a). The pond was made up of polycarbonate material with white paint coated inside the surface along with the paddle wheel rotating at a constant speed of 20 rpm. Air was supplied at a rate of 0.2 L/min through the ring sparger located at the bottom of raceway pond placed inside the pond to enhance the rate of circulation of sewage water and keep microalgae in suspension form. The sewage water was collected from community sewage treatment plant located in the CSIR-National Chemical Laboratory campus, Pune, India. The sewage water was coarsely filtered through a mesh (size=2mm) for the removal of any big solid materials present, before using it for the experimental work. Before inoculating into the sewage water the algal strains were filtered and washed several times with distilled water to remove media components. The initial inoculum size (1 L of volume) of all the three algal cultures viz. *S.obliquus*, *C.vulgaris,* and *A.striptus* was varied from1, 2 and $5x10^6$ cells/ml in order to study its effect on nutrients and organic matter uptake.

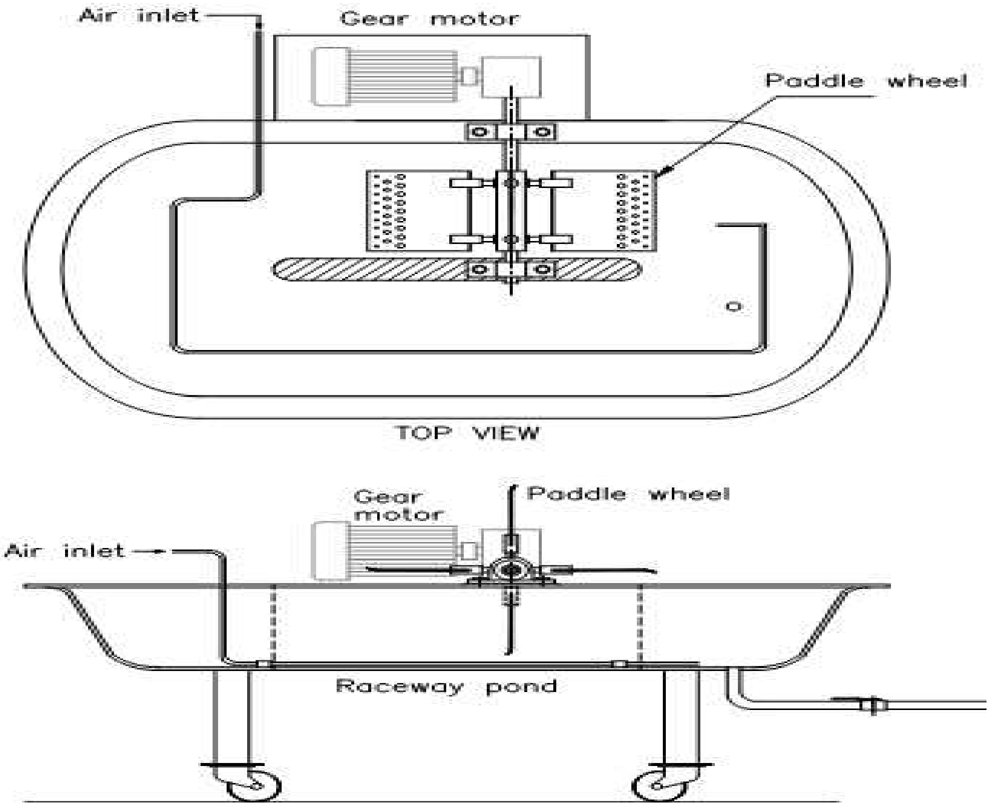


Figure6.1a: Open raceway pond for algae cultivation

6.2.3 Cross flow filtration

Hollow fiber membrane composed of polyacrylamide nitrile (PAN) and polysulfone (UNHF 2021) were evaluated in the tangential configuration. The average pore size of these hollow fiber membranes was found in the range of 0.1-0.3 μm. The PAN membrane (250 mm long) was fabricated indigenously having 120 hollow fibers with the membrane area of 0.08 m^2.Polysulfone membrane (300 mm long) was purchased from Uniflux Membranes LLP, Pune, India having a membrane area of 1 m^2 with 80 hollow fibers. The tubular ceramic membrane was purchased from BHEL India, having an effective area of 0.1 m^2 was also evaluated to study the inorganic membranes having pore diameters ranging from 0.1 to 0.8 μm (table 6.1).

The main objective behind using the membrane of pore size 0.1-0.8 μm is that it ensures minimal pore fouling with effective backpulsing especially at high biomass concentration, as well as it retente the microalgae from the treated sewage water, normally the size of microalgae >1 μm (Bhave et al., 2012).

Table 6.1: Characteristics of membrane module

Membrane module	**Membrane area (m^2)**	**Pore size(µ)**	**Water flux(L/m^2h)**
Polyacryloamide nitrile (PAN)	0.08	0.1	200-250
Ceramic	0.1	0.1-0.8	60-70
Polysulfone	0.23	0.1	70

A cross flow filtration assembly with hollow and tubular membrane module was evaluated for dewatering of microalgae from treated sewage water and the schematic experimental setup is shown in figure 6.1b. The tangential flow was provided to the membrane module by a piston pump. The system was designed to hold a single membrane module at a time. It contains a feed tank and permeate tank each having 10 L capacity to collect the algal broth and clear water respectively. The system was equipped with back pulse device along with air flushing mechanism to minimize membrane fouling and flux reduction as the biomass gradually increases with increase in time. The backpulse operation, tangential flow rates, and pressures were controlled and monitored with automated valves and the values were recorded manually. The membrane flux (F) for the different sidestream membranes was calculated by using following equation (6.1).

$$F = \frac{V}{at} \quad (L/m^2.h) \qquad (6.1)$$

Where, V is the volume (L), t time (h), a effective filtration area (m^2) Before each experimental run, hollow fiber membrane modules (PAN and polysulfone),as well as tubular ceramic membrane was washed with 0.1 N alkali solution in order to remove any layer of biomass cake on the surface and ultimately scrubbed with tap water for another 30 min. Membrane modules were given cleaning before each experimental run and were tested for flux retention with water.

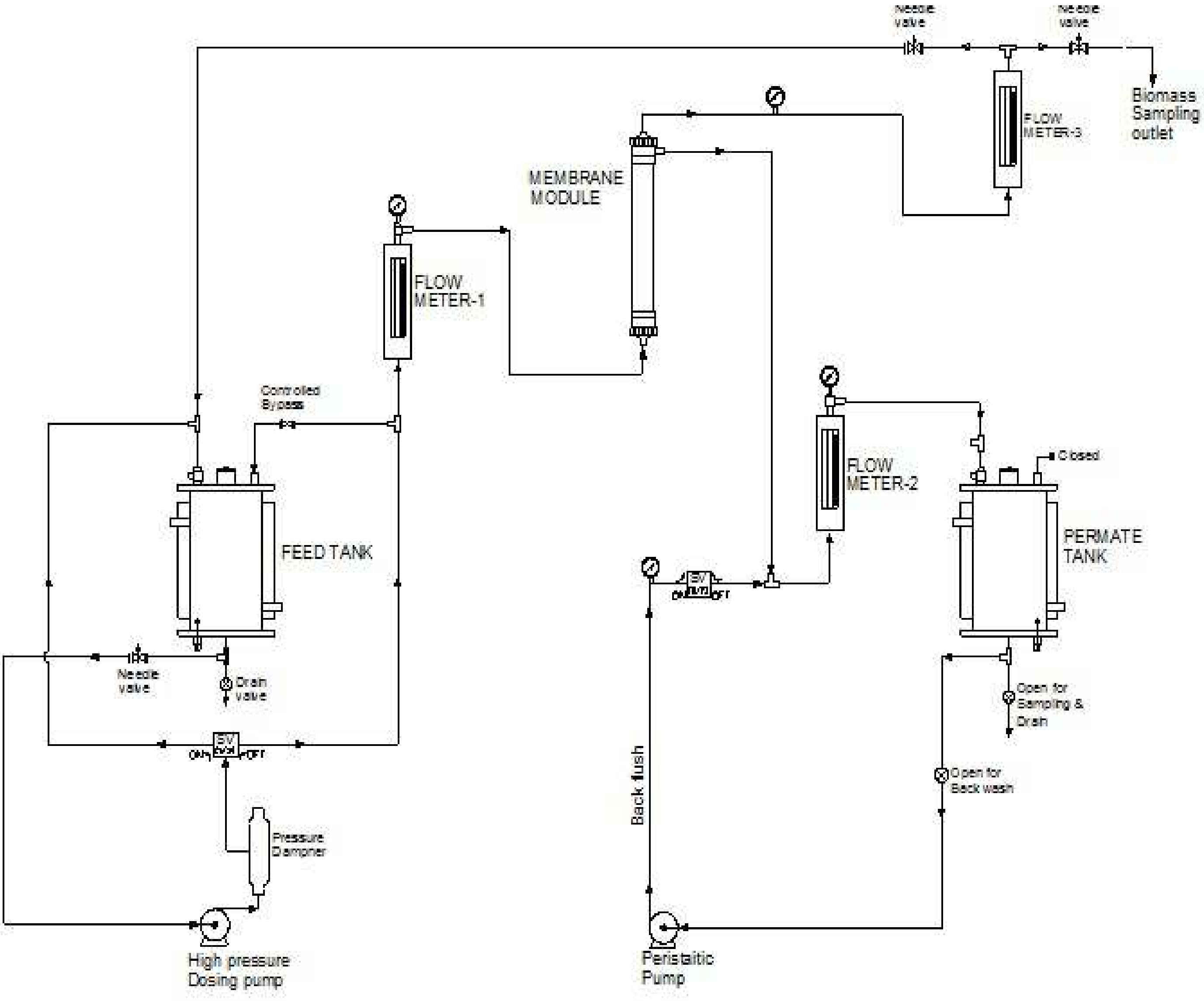


Figure 6.1b: Process flow diagram of cross flow filtration membrane assembly for harvesting of microalgae

6.2.4 Analytical methods

All water samples unless stated otherwise were centrifuged at 6000 rpm and filtered through a 0.45µm cellulose acetate filter paper (Millipore) before analysis. 100 ml of sample was withdrawn from open raceway pond after every 24 h of interval for analysis of its constituents in triplicates. Total nitrogen (TN) and Total organic carbon (TOC) was analyzed by using TN/TOC analyzer (Model: TOC-L CPH/CPN E200 Shimadzu, Japan). Total phosphates (TP) were estimated by using vanadomolybdophosphoric acid colorimetric method (APHA,1998). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were measured, in accordance with the standard methods for the examination of water and wastewater (APHA,1998). The microalgae cell count was examined by using hemocytometer. To determine the biomass concentration, 50 ml sample was centrifuged at 6000 rpm for 10 min and the pellet was dried under vacuum at 60˚C for 24 h. The rate constants for the uptake of nutrients was calculated according to

$$\ln\left(\frac{C_0}{C}\right) = kt \qquad (6.2)$$

Where C_0 and C were the initial and final concentration of nutrients in mg/L and t is the time in h.

6.3 Results and Discussion

6.3.1 Algal growth with nutrients uptake from the sewage water

As discussed in previous section (Materials and methods 2.4) the initial inoculum concentration of all the three microalgae viz. *S.obliquus*, *C.vulgaris,* and *A.stipitatus* was varied as 1, 2 and 5x10^6 cells/ml to study the effect of initial microalgae concentration on the uptake of nutrients from the sewage water as well as the growth of individual algal species. The sewage water was treated with a specific concentration of microalgae for 5 days using sunlight. During experiment, the growth of microalgae, as well as nutrients and organic matter present in the water, was monitored every day.

It is evident from figure 6.2 at higher initial cell concentration (5x10^6cells/ml) growth of *S.obliquus* was less as compare to *C.vulgaris* and *A.stipitatus* cultures. Maximum cell count for the *S.obliquus* species after 4 days was 5.99x10^6cells/ml compare to *C.vulgaris* and *A.stipitatus* as 13.2 and 12.1x10^6 cells/ml respectively. This clearly indicates that these microalgae species were comparatively got adapted in the natural sewage water environment. Moreover, it can be found that algal growth was significantly enhanced during the first 4 days but as the nutrients depletion starts then it enters into the lag phase. Thus, algal ponds with high inoculum might be more beneficial as high nutrient level tends to promote rather than prohibit algal growth, which helps as the basis for applying microalgae for the treatment of sewage water to deplete the nutrients load and prevent eutrophication of water bodies. Another advantage of the higher concentration of microalgae is that the rate of uptake of nutrients is rapid i.e. treatment time required for sewage water will be lower.

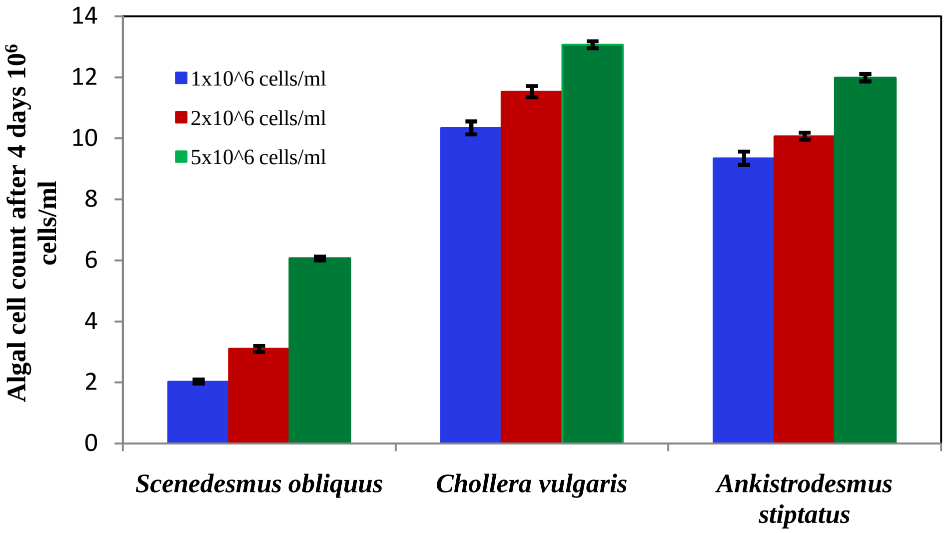


Figure 6.2: Algal cell count after 4 days at different initial cell concentrations

The TN and TP uptake by microalgae after 4 days of cultivation in sewage water at different initial cell concentration is depicted in figures 6.3 and 6.4. The dynamics of nutrients depletion depends highly on the characteristics of sewage and operating conditions of the open raceway pond. During the experiments, neither the nutrients addition nor the sewage water was diluted. Figure 6.3 clearly reflects the effect of initial algal concentration on the TN uptake, as it can be deduced that TN depletion at5x10^6 cells/ml for all the three cultures was higher than that of 1 x10^6 and 2x10^6 cells/ml. Initial TN in the range of 41-48±7 mg/L has been reduced to 0.67-0.43 0.55±0.2 mg/L after 4 days of cultivation of *S.obliquus*, *C.vulgaris* and *A.stipitatus* at a concentration of 5x10^6 cells/ml initial inoculum concentration. These microalgae species are capable to uptake about 98-99% of TN from the sewage water after 4 days of operation. It seems very obvious, as the initial cell concentration increases the rate of nutrients uptake will increase. These results are in agreement with Gupta *et al.*, (2016) which claims *S.obliquus* and *C. vulgaris* species for better nutrients removal and adaptability to physiological stresses.

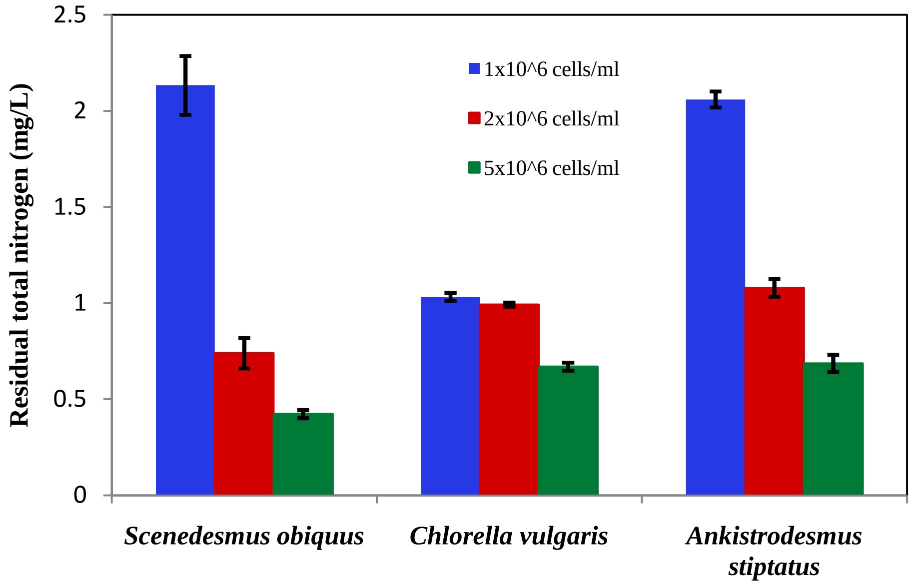


Figure 6.3: TN uptake at different initial algae cell concentration

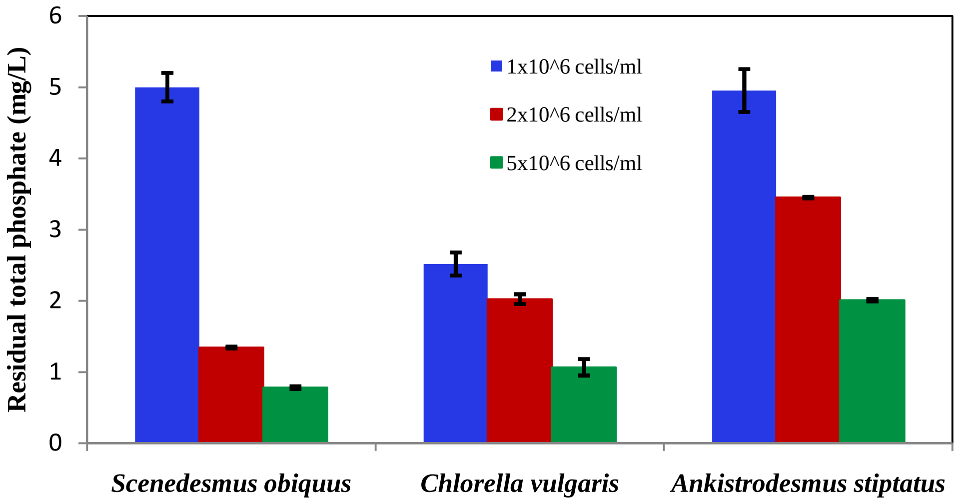


Figure 6.4: TP uptake at different initial algae cell concentration

Figure 6.4 shows the uptake of TP from the sewage water by using *S.obliquus*, *C.vulgaris*, and *A.stipitatus* during 4 days of operation. It was observed that these microalgae species are capable of uptake 97-99% of TP resulting to give a high quality treated effluent. The initial TP was in the range of 70-88 mg/L at 5x10^6 cells/ml as initial cell concentration was reduced in the range of 2-0.8 ± 0.2 mg/L and it is highest for *S.obliquus* compare to the other two algae species (figure 6.4). This indicates the daily average elimination of 15.7-16.8 ±1.1 mg/L/day of TP for all the three algae at 5x10^6 cells/ml of initial microalgae concentration and it was higher than as reported by Baky *et al.*, (2012). In the present study, it was found that *S.obliquus,* in particular, shows highest nutrients uptake compare to the other two algal species.

Rate constants for the uptake of nutrients by these microalgae was also calculated are shown in Table 6.2 at different initial microalgae concentration. If we plot a graph of $\ln\left(\frac{C_0}{C}\right)$ vs. t, straight line was obtained and according to the equation 6.2, the uptake of nutrients by algae in a constant volume open raceway pond follows first order reaction. Average rate constant values for the $2x10^6$ cells/ml and $5x10^6$cells/ml are almost the same for all three cultures. From this data, it can be concluded that $2x10^6$ cells/ml as the initial cell concentration in open raceway pond is sufficient for the effective nutrients removal from the sewage water. It can be concluded that these microalgae species could adapt well in the sewage water and the nutrients uptake rate are also nearly same at the end of 4 days of operation. Similar kind of observation was made by Xin et al., (2010) and Ruiz-Marin et al., (2010) were *S.obliquus* showed higher uptake of nutrients from urban wastewater than *C.vulgaris* (Voltolina, 2005).

Table 6.2: Rate constants for the uptake of nutrients by algae at different cell concentration

Cell count (cells/ml)	***Scenedesmus obliquus***		***Chlorella vulgaris***		***Ankistrodesmus stipitatus***	
	Rate constant (h^{-1})					
	TN	**TP**	**TN**	**TP**	**TN**	**TP**
$1x10^6$	0.016	0.02	0.025	0.019	0.028	0.025
$2x10^6$	0.029	0.024	0.028	0.020	0.030	0.027
$5x10^6$	0.035	0.034	0.035	0.032	0.035	0.030

6.3.2 COD, BOD and TOC reduction in open raceway pond

Figures 6.5 and 6.6 shows the residual COD and BOD values of treated sewage water at different initial concentration for all the three microalgae species. The initial COD & BOD values of sewage water were in the range of 200-288 ±88 mg/L and 145-200 ±55 mg/L respectively. Reduction in the COD values for all the three cultures was found in the range of 80-88% whereas; 81-86% of BOD depletion took place over the period of 4 days. These values of COD and BOD suggest that the bacterial consortia with the help of algal species used in this study can speedily utilize different organic compounds as carbon sources besides CO_2 (Li et al., 2011). The residual BOD value of treated water was found 21, 33.6, 23.38 mg/L for *S.obliquus*, *C.vulgaris*, and *A.stipitatus* respectively at an initial cell concentration of microalgae of 5x10^6 cells/ml. This shows that *S.obliquus* shows better performance as compared to *C.vulgaris*, and *A.stipitatus*. Also the residual COD value of treated water was found 30, 48, 33.4 mg/L for *S.obliquus*, *C.vulgaris*, and *A.stipitatus* respectively at an initial cell concentration of microalgae of 5x10^6 cells/ml. This is because the cells of *S.obliquus* were furnished with spines and bristles, which makes the colony more resilient and allowed higher light and nutrients uptake (Cai et al., 2013).

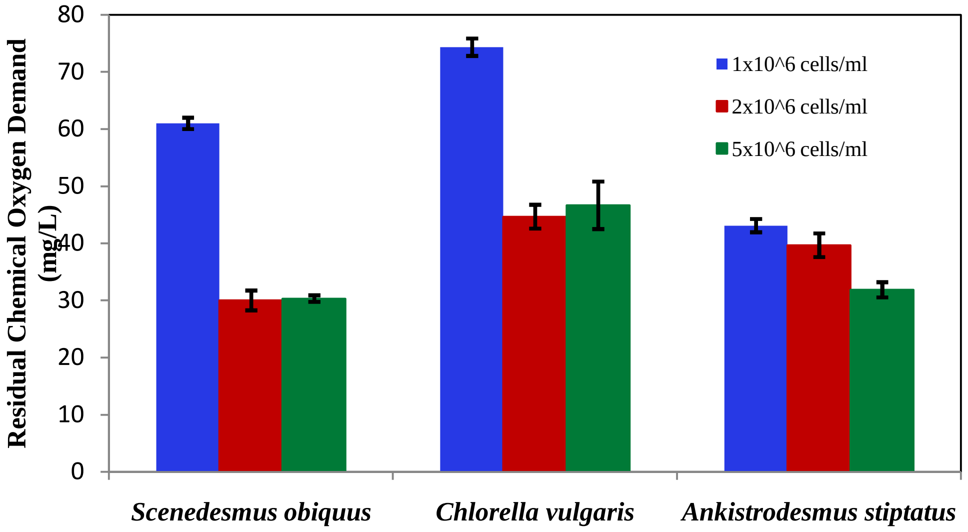


Figure 6.5: COD removal at different initial algae cell concentration

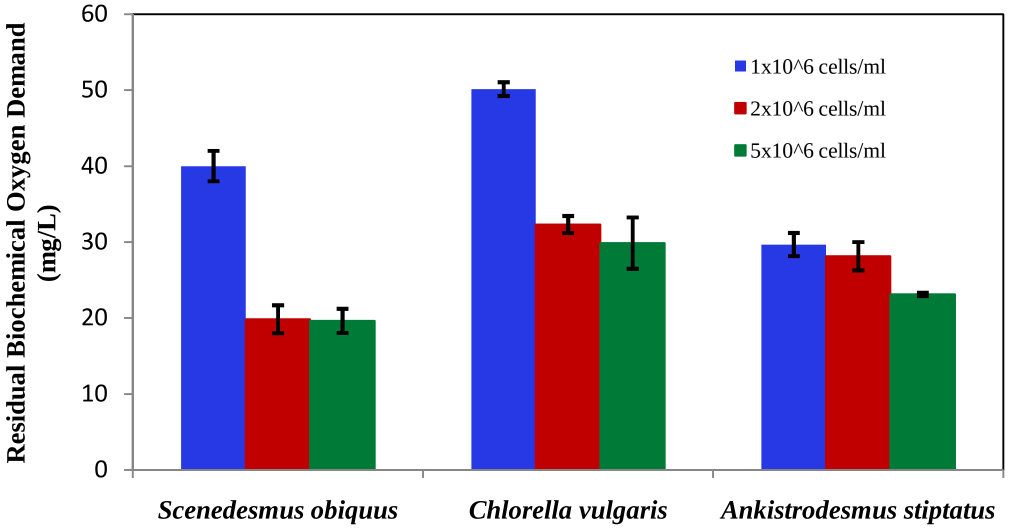


Figure 6.6: BOD removal at different initial algae cell concentration

Depletion in the TOC values was also observed for these algal species which reflects the total organic carbon utilization for the faster growth under photoautotrophic or mixotropic mode in light or dark conditions (figure 6.7). TOC values for the algal cultures were decreased from 146-183±37 mg/L to 7-4±3 mg/L for all the three species. *A.stipitatus* shows higher uptake of organic matter from sewage water compare to the other two microalgae species which depicts it as a potential mixotropic culture. COD, BOD and TOC concentrations remains almost stationary after 4 days of cultivation which probably resulted in the decline of algal growth as a result of shortage of nutrients and organic carbon which is agreement with the results reported by Zhou et al., (2012). Even though, uptake of nutrients and organic matter was convincing at the end of primary stage without addition of any supplementary algal inoculum or conditions, the algal biomass obtained needs to be harvested to make the whole process effective. Thus, further studies for efficient algae dewatering needs attention.

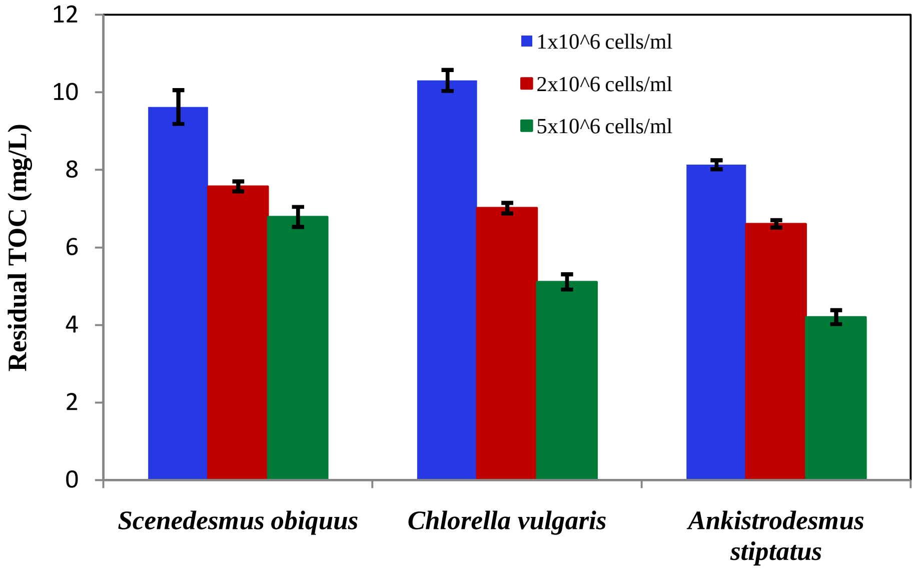


Figure 6.7: TOC removal at different initial algae cell concentration

6.3.3 Membrane flux and biomass concentration

From the previously studied results for nutrients and organic matter uptake *S.obliquus* shows promising water quality parameters as compared to *C.vulgaris* and *A.stipitatus* species as also the size of *S.obliquus* was 2-5 μm which is bigger than the other two microalgal species and it can be retained easily by microfiltration. Hence, for further study pertaining the harvesting the microalgae using different membranes, *S.obliquus* microalgal species was selected. 30 L of the *S.obliquus* culture having different initial concentration have been grown in sewage water and subsequently used to study the membrane flux and biomass concentration for different initial concentrations of *S.obliquus* cell as well as for different three membrane viz. PAN, polysulfone and ceramic membrane where studied. Here, fouling mechanism was studied in terms of flux reduction as the biomass was progressively concentrated to achieve economical harvesting process. Membrane performance as a function of transmembrane pressure (TMP) and biomass concentration was evaluated for the hollow fiber (PAN, Polysulfone) and tubular membranes (Ceramic). The results at 2 bar of TMP for different initial *S.obliquus* concentration was depicted in figures 6.8 (a), (b), and (c). Although the experimental runs at 0.5,1 and 1.5 bar were also carried out (results not shown), however, in order to better understand the flux profile maximum TMP (2 bar) was considered as the membrane tends to start fouling rapidly at high pressures. For the PAN membrane flux was highest, 224.2 L/m^{2}h for initial concentration of microalgae of 1x10^6 cells/ml in the beginning as subsequently after 135 min. of filtration it reduces to 74.7 L/m^{2}h with 45 sec of back pulsing after every 5 minutes of interval (figure 6.8a). The nature of graph for the two other membranes was also same as they showed maximum 43.2 L/m^{2}hfor polysulfone and 56.3 L/m^{2}h for ceramic membrane modules at the lower initial concentration of *S.obliquus*(1x10^6 cells/ml). As the filtration progresses with the backpulsing mechanism the fluxes had reduced upto 9.1 and 2 L/m^{2}h for polysulfone and ceramic which is much lower as compare to PAN membrane module. From figures 6.8 (b), and (c), as the *S.obliquus* concentration increases, the membrane starts fouling and the initial flux reduces to 147.3, 46.7 and 44.2 L/m^{2}h for PAN, polysulfone and ceramic membrane respectively inspite of backpulsing mechanism. This clearly reflects the impact of initial concentration *S.obliquus* on filtration. Here, without addition of any coagulant the algal biomass was concentrated. These results provided a good basis to correlate hollow fiber dewatering rate with tubular membrane under uniform conditions. It can be seen from figures 6.8 (a), (b), and (c) that although all the three membrane module showed higher flux at dilute biomass concentration, flux decreases considerably at higher concentrations due to concentration polarization and less effective backpulse at higher solid concentrations. In spite of that, PAN hollow fiber membrane shows very high flux (224.2-147.3 L/m^{2}h) which is 5 times higher than hollow fiber polysulfone and tubular ceramic membrane. Such higher fluxes for dewatering of microalgae by using side streamed hollow fiber (PAN) membrane were not reported in the literature (Bhave et al., 2012, Rossignol et al., 1999).

Figures 6.8 (d), (e), and (f) illustrates the biomass concentration profile of the retented streams with respect to the 2 bar TMP at different initial *S.obliquus* concentration. Figures 6.8 (d), (e), and (f) shows the concentration of biomass for three different membranes at different initial

S.obliquus concentration. As, it can be seen for the PAN membrane at dilute *S.obliquus* concentrations (1x10^6 cells/ml) gives higher biomass of 4.1 g/L which was 22.8 times of the initial 0.18 g/L of biomass on dry basis. On the similar lines for polysulfone and ceramic membranes it was 3.5 and 2.8 g/L which is 19.4-13.3 times concentrated of the initial biomass content of 0.175-0.21 g/L. Whereas, for the higher initial *S.obliquus* concentrations viz. 2 and 5x10^6 cells/ml, during the start of filtration process it was 6.8-11.1 times concentrated which is lower than that of initial concentration of *S.obliquus* 1x10^6 cells/ml. This may be because, as the filtration starts with higher initial biomass content (0.32-0.52 g/L) the permeate flow drags the algal cells onto the membrane surface, which then starts fouling the membrane pores and start creating kind of algal cake layer as reported by Bilad et al., (2014) and it's a common feature seen in microfiltration membranes.

Table 6.3 shows the comparison of performance of various polymeric membranes w.r.t. flux for the harvesting of microalgal. The fluxes reported by Bhave et al., (2012) was high in the range of 52-329 L/m^2 h, however, it was achieved by using two step membrane filtration system and coagulant was added to improve the fluxes. Whereas, Rossignol et al., (1999) got 120 L/m^2 h of fluxes by using PAN membrane which is low as compare to the results obtained in the present work. Whereas, coagulant based one step harvesting process was reported by Lee et al., (2012) in details by varying the coagulant dosage in combination with polytetrafluoroethyelene (PTFE) membrane has achieved maximum flux of 180 L/m^2h (Hwang et al., 2013, Singh and Thomas, 2012, Lee[a] et al., 2012). All these studies were carried out by growing algae in synthetic media and hence, limited with respect to the actual domestic wastewater grown biomass and the fluxes resulted from that. Also, some lab scale studies by using polyethersulfone (PES) and chlorinated polyethylene (PE) were reported out by Singh and Thomas, (2012) and Marbelia et al., (2014) respectively for polishing the domestic wastewater and concentrating the microalgal biomass showed very low fluxes (8 and 2.6-13 L/m^2h) (Lee[b] et al., 2012). However, no precise development in the membrane material for a particular microalgae species harvesting was reported in the literature. According to Hwang et al., (2013), hydrophilic membranes are more resistant to membrane fouling. Most of the research was carried out using polyvinyl di-fluoride (PVDF) membranes as it also offers better flux (50-329 L/m^2h) and can be used as a hydrophilic membrane (Lee[c] et al.,2012).

Table 6.3: Summary of studies on harvesting of microalgae using hollow fibre membrane module

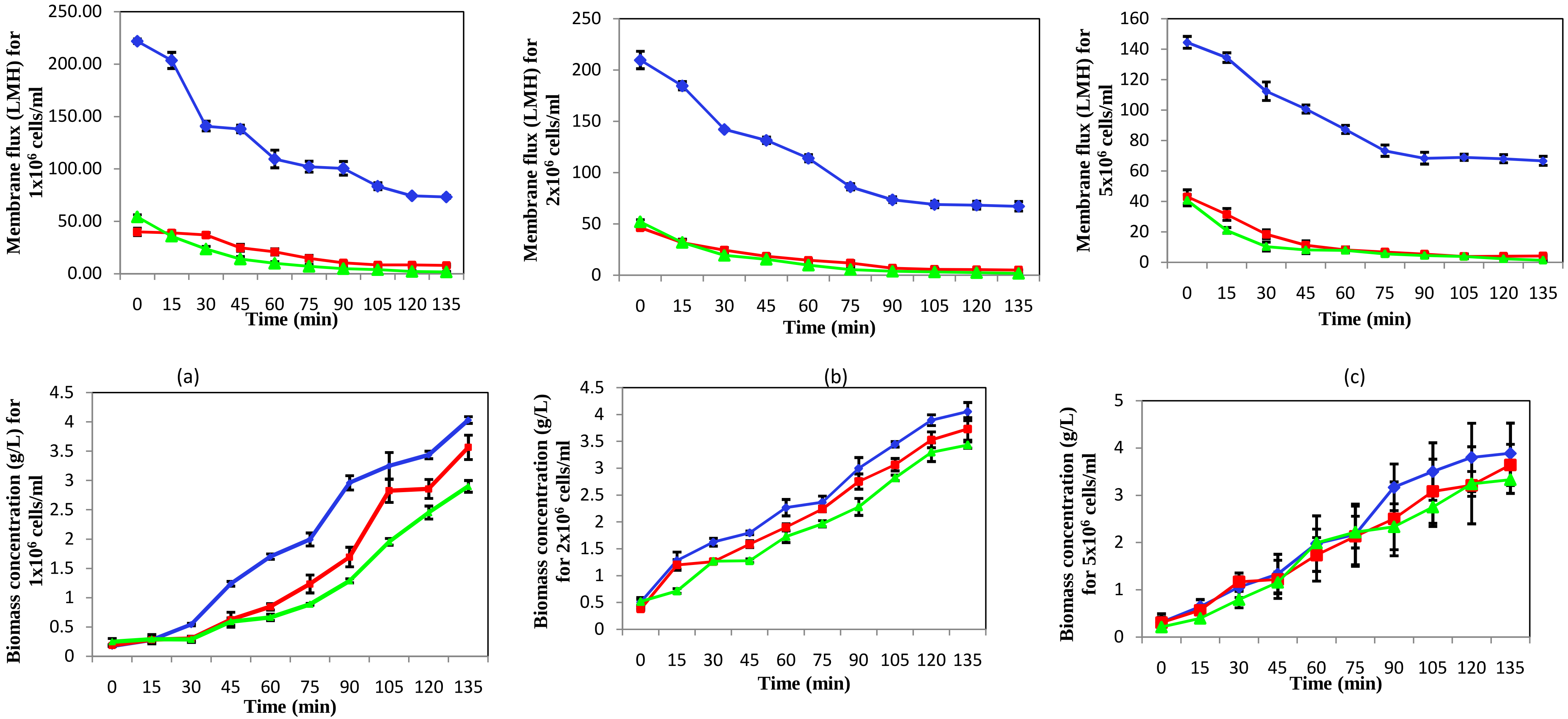
Sr. No.	**Algal Species**	**Growth Media**	**Membrane Physio-chemical characteristics and configuration**	**Membrane flux (L/m^2 h)**	**References**
1.	*Chlorella vulgaris*	Simulated domestic wastewater	Hollow fiber chlorinated polyethylene (PE) Pore size=0.2 µm Surface area=NA Submerged	2.6-13	Marbelia et al.,(2014)
2.	*Chlorella vulgaris*	Synthetic media	Polyethylene terephathalate (PET) Pore size=4 µm Surface area= 0.0014m^2 Cross flow configuration	25-75	Hwang et al., (2013)
3.	*Chlorella vulgaris*	Synthetic media	Polyvinylidene fluoride (PVDF) Pore size=0.45 µm Surface area= 0.0014m^2 Cross flow configuration	50-120	Hwang et al., (2013)
4.	*Chlorella, C. Vulgaris, S.quadricuada, S.dimorphus*	Domestic wastewater	Hydrophilic polyethersulfone (PES) Pore size= 0.45 µm Effective surface area= 0.1m^2 Submerged configuration	8	Rossignol et al., (1999)
5.	*Chlorella vulgaris*	Artificial media	Polytetrafluoroethylene (PTFE) Pore size=0.91 µm Cross flow configuration	30	Lee[a] et al., (2012)
6.	*Chlorella vulgaris*	Artificial media	Polytetrafluoroethylene (PTFE) Pore size=0.91 µm Cross flow configuration	30-180	Lee[b] et al., (2012)
7.	*Chlorella vulgaris*	Artificial media	Polytetrafluoroethylene (PTFE) Pore size=0.91 µm Cross flow configuration	30-180	Lee[c] et al., (2012)
8.	*Nanochloropsis*	Sea water media	Hydrophobic Polyvinylidene fluoride (PVDF) Pore size= 0.1-0.2 µm Surface area= 0.08-0.12 m^2	52-329	Bhave et al., (2012)
9.	*Chlorella vulgaris & Phaeodactylumtricornut*	Synthetic media	Polyvinylidene fluoride (PVDF) Pore size=0.008-0.013 µm Surface area= 0.016 m^2	38-42.5	Bilad et al., (2014)

	um		Submerged configuration		
10.	*Scenedesmus quadricauda*	Artificial media	Polyvinyl chloride (PVC) Pore size= 50 KDa Cross flow configuration	45	Zhang et al., (2010)
11.	*Tetraselmiss uecica*	Synthetic media	Polyvinylidene fluoride (PVDF) Pore size=0.22 µm Cross flow configuration	20	Danquah et al., (2009)
12.	*Hasleaostrearia & Skeletonema costatum*	Synthetic media	Polyacrylonitrile (PAN) Pore size= 40 KDa Effective surface area= 0.01 m^2 Cross flow configuration	120	Rossignol et al., (1999)
13.	*S.obliquus*	Sewage water	Polyacrylonitrile (PAN) Polysulfone Ceramic Pore size=0.1-0.8 µm Surface area= 0.08-0.23 m^2 Cross flow configuration	PAN=224 Polysulfone=43.2 Ceramic= 56.3	Present study

The permeate water characteristics were also analyzed as shown in Table 6.4. The values of the water quality parameters viz. TN, TP, TOC, COD and BOD were much below than those specified by the Central pollution control board (CPCB), India in the permeate water to be discharged in the water bodies or it can be used for gardening purpose. The algal cake filtered can be further used as a biofertilizer as it is very rich in nutrients content, or for lipid extraction as a potential biofuel. Figure 6.9 shows the appearance of sewage water, with algae grown as well as the permeate water from the membrane module. Approximately 80% of water recovery was achieved by applying hollow fiber membrane and tubular membrane modules.

Table 6.4: Initial sewage water and membrane permeate characteristics for the membrane module

Parameters	Sewage water (initial characteristics)	MBR Permeate (Treated water outlet characteristics)		
		PAN	Polysulfone	Ceramic
pH	6.5-7.3	7.5-8	7.6-8.1	7.2-8.4
TN (mg/L)	41-48	0.2-0.3	0.34-0.4	0.2-0.4
TP (mg/L)	75-88	0.6-0.4	0.7-0.8	0.6-0.7
TOC (mg/L)	183-132	3-4	2-3	1-3
COD (mg/L)	200-288	20-15	18-20	20-21
BOD (mg/L)	145-200	10-8	6-8	10-8



(a) (b) (c)

(d) (e) (f)

Figure 6.8: Membrane flux (a), (b), (c) and biomass concentration (d), (e), (f) profile for *S.obliquus* at different initial cell concentration (♦ PAN membrane, ■ Polysulfone membrane, ▲ Ceramic membrane).

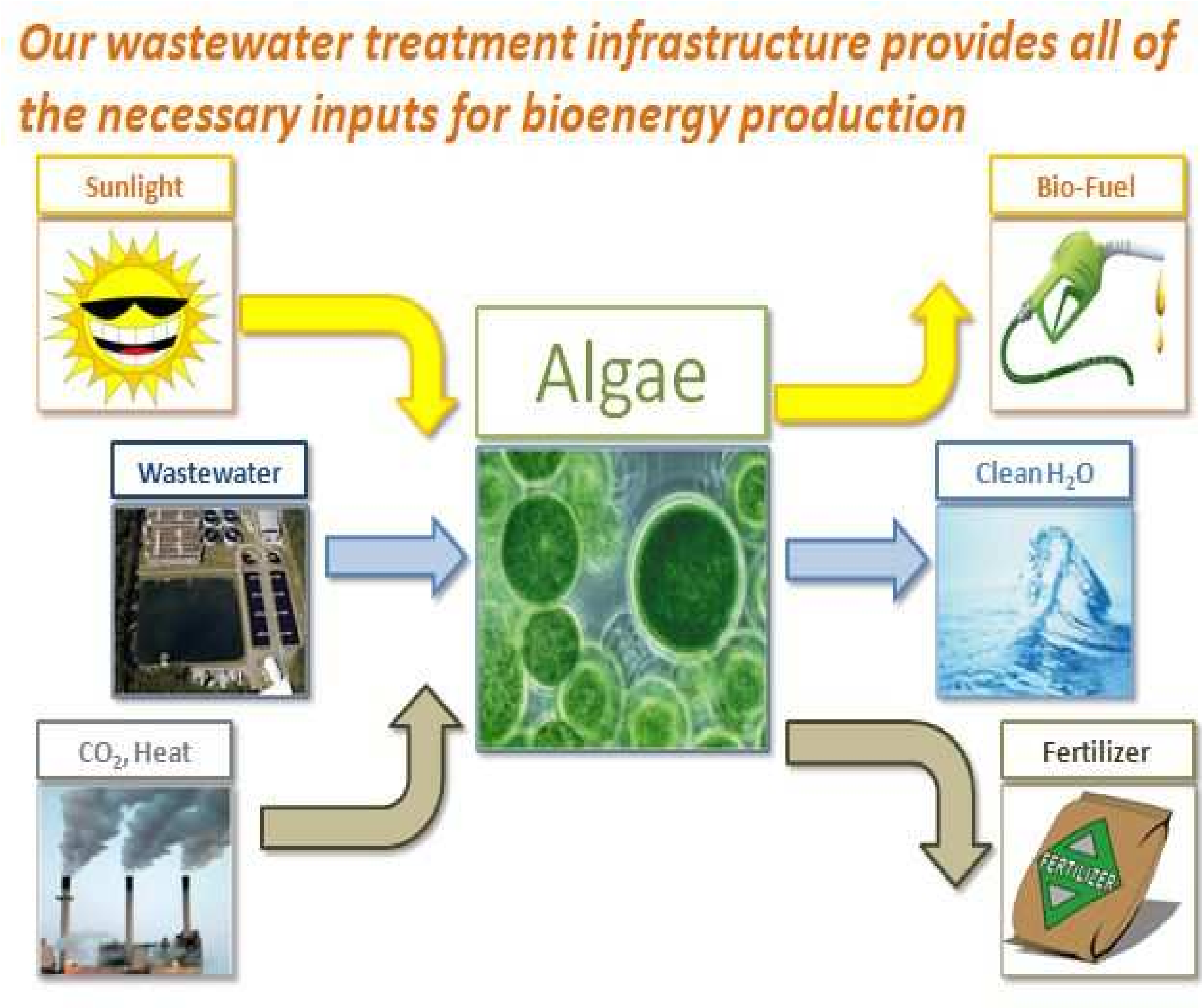
Figure 6.9: Appearance of sewage water before and after treatment

6.4 Conclusions

The treatment of sewage water was attempted by using three different microalgae cultures namely *Scenedesmus obliquus, Chlorella vulgaris* and *Ankistrodesmus stipitatus* in combination with the membrane bioreactor (MBR). The effect of various operating parameters such as the concentration of microalgae, different types of membranes, and transmembrane pressure, on the quality of treated water, has been investigated in details. It was found that at high initial biomass concentration ($5x10^6$ cells/ml) the rate of the nutrients uptake was maximum for all the three cultures and PAN membrane gave the highest permeate flux (224 L/m^2h) with less fouling compared to ceramic, polysulfone membranes. High-quality effluent in terms of nutrients uptake and organic matter reduction were achieved together by the combination of microalgae and membrane bioreactor. The approximately 70-85% yield of biomass was achieved after the treatment of sewage water in a combination of the membrane system. PAN membrane shows better performance in terms of membrane fluxes and biomass concentration as compare to polysulfone and ceramic membranes. This study reveals the possibility of side-streamed microfiltration MBR as a low cost biomass harvesting process. Thus it can conclude that cultivation of microalgae in combination with the membrane is one of the potential advanced techniques for treatment sewage water. This needs to be explored on larger scale as it helps to reduce water foot prints by recirculating permeate water as a feed for the growth of microlage.

Chapter 7

Economic analysis for the treatment of sewage water using microalgae in combination with side-stream membrane assembly: A case study



7.Economic analysis for the treatment of sewage water using microalgae in combination with side-stream membrane assembly: A case study[7]

7.1 Introduction

From the past few decades efforts were made to make the algal biomass production/harvesting economical so that microalgae derived biodiesel can replace the biofuel extracted from food grains to make the process sustainable (Cai et al., 2013, Christenson and Sims, 2012, Danquah et al., 2009). The substitute to the petroleum driven transport is offered by microalgae which provides carbon neutral renewable fuel which will also help in reducing the global warming. Microalgae are a photoautotrophic organism which not only fix the atmospheric carbon dioxide but also takes up the nutrients (nitrogen and phosphate) which causes eutrophication of the water bodies. In addition to this it also helps bacteria to degrade organic pollutants present in the sewage water by giving out the oxygen needed for their growth thereby, treating the sewage water. The microalgae in combination with bacteria grows symbiotically in the sewage water producing the algal biomass which serves as a raw material for the biofuel production as well as can be used as fertilizer or animal feed (Benemann, 2013, Sriram and Sreenivasan, 2012, Rawat et al., 2011) . Treatment of sewage water with microalgae and bacteria serves dual purpose of pollutant removal and algal biomass production were accomplished. This whole process becomes more efficient and viable when membrane were used for the harvesting of algal biomass as the water in the permeate is free of any bacteria and pollutant and can be directly used for the gardening as well as for toilet flushing purpose.

According to Chisti et al., (2008) unlike any other crops microalgae can double there biomass within 24 h which makes them potential source of biodiesel. By using sewage water as the growth media for algae eliminates the additional cost of inorganic nutrients required for their growth. Also, in tropical country like India where sunlight is abundant and available throughout the year makes it most viable microalgae cultivation technology applicable for algal biomass production and pollutant removal from sewage water (Honda et al., 2012). In a nut shell, microalgae cultivation in sewage water has numerous benefits, combining carbon dioxide mitigation, biofuel production and wastewater treatment. Harvesting the microalgae is one of the major bottleneck for the algal industries till date. There are several methods suggested for harvesting the algal biomass which typically includes filtration, sedimentation, centrifugation or flocculation which is economically more challenging when larger scale of production is considered (Uduman et al., 2010). The choice of harvesting the algal biomass and drying will largely decides the economics of biofuels which are low valued. Also, the carbon foot print of algal biofuel is relatively low than that of the petroleum derived fuel on an equal energy basis which makes its production beneficial (Chisti, 2013).

As far as global demand for the fuel is concerned it is expected to grow 40% by 2025 (Hirsch et al., 2006) .The production cost of algal biomass is highly dependent upon on the yield of algal biomass from the culture system, harvesting cost, and oil content of the biomass. Yield and cost analysis reported in the literature clearly indicates that the algal cultivation solely for the production of biofuel is not cost competitive (Chisti, 2013, Haas et al., 2006). The only possibility to make algal biofuel production economical if any breakthrough was achieved in increasing the photosynthetic efficiency of algae, improved penetration of light in a dense algal culture, inducing the cells to extract oil and to accomplish the auto flocculation of biomass to facilitate the harvesting of algal biomass (Day et al. 2012). All these targets can be achieved by using genetic and metabolic engineering which will allow algae to grow to new efficiency. In this chapter sewage water was used as a growth media for microalgae and therefore, external addition of nutrients was shaved so as to make the process of producing biomass more cost effective. However, by using membrane assembly for the harvesting of biomass adds to the cost of production of biomass but it can be compensated as the clear water permeate can be used and recycled on industrial/large scale thereby, reducing the water foot print as well as the biomass paste can be simply sundried as only 10% water content in it. Without development of microalgal genetic and metabolic engineering for the improved production and harvesting of algal biomass (self flocculating algal species) and subsequent recovery of algal fuels, economics of algal technology will be hindered compare to the petroleum fuels (Chisti, 2013, Day et al. 2012) . On the same lines membrane technology needs to be developed for less expensive and fouling resistant membranes.

7.2 Process economics: A case study for the treatment of sewage water by using microalgae

In this section, we discuss the economic aspect for the treatment of sewage water in a village by using microalgae and subsequent harvesting of algal biomass with the aid of polyacrylonitrile (PAN) membrane assembly which will also help in polishing the treated effluent. Potential bottlenecks on commercializing the treatment of sewage water by using microalgae in combination with membrane assembly will be analyzed. The tentative cost required for the treatment of sewage water by using microalgae and membrane technology has been calculated.

7.3 Techno-economical aspects for design of sewage water treatment plant by using microalgae and MBR technology for small village having a population of 3000

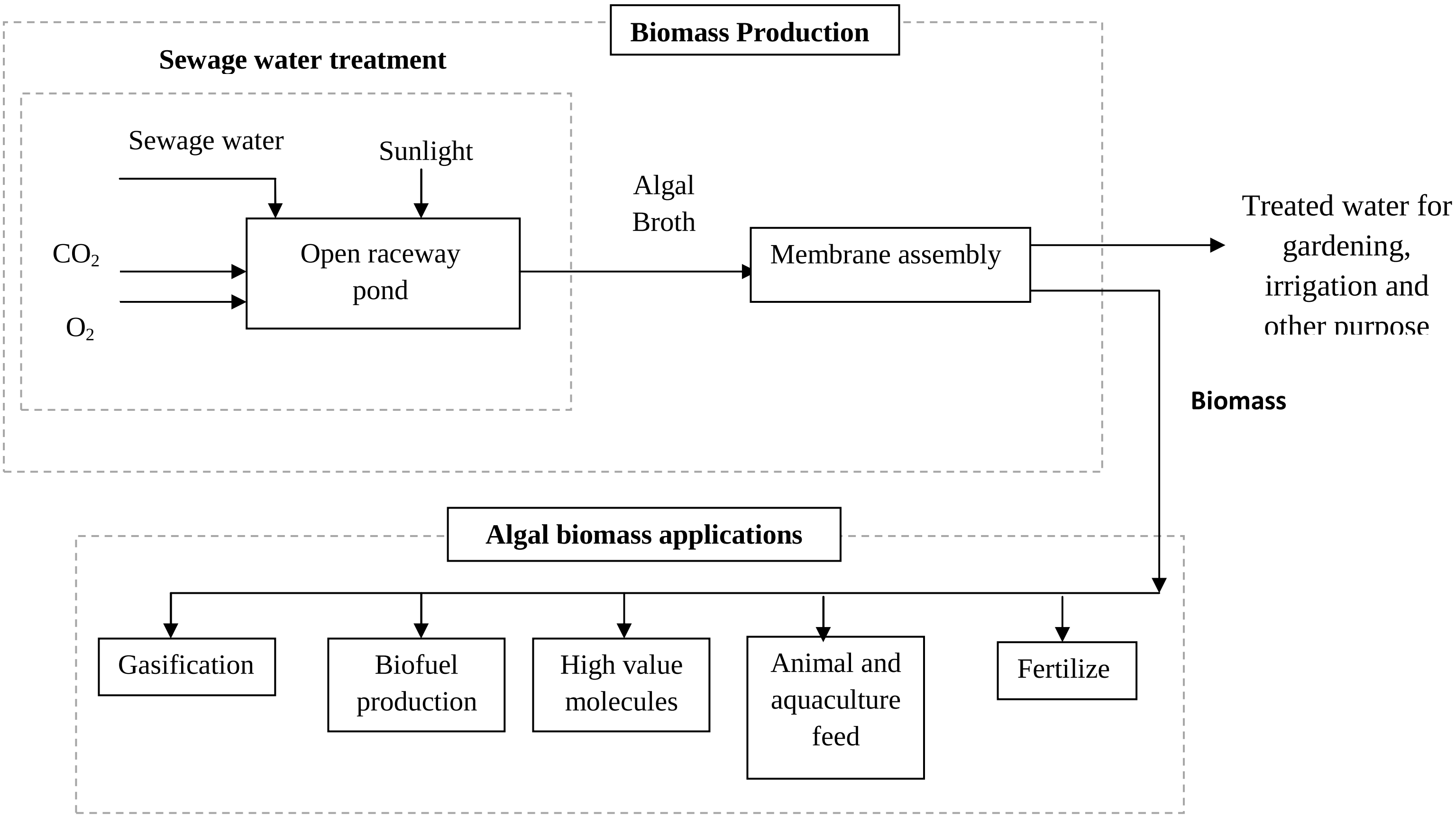


Figure 7.1: Treatment of sewage water by using microalgae with subsequent harvesting of algal biomass using membrane assembly.

The process of treatment of sewage water by using microalgae along with the algal biomass harvesting with the help of sidestream membrane assembly was shown in figure 7.1. This completed technology was developed at CSIR- National Chemical Laboratory, Pune, India. All conditions of the process have been demonstrated at the bench scale. The entire process can be divided into two parts:

1. Treatment of sewage water by using microalgae along with bacteria for the removal of nutrients (Total nitrogen (TN) and Total phosphate (TP)) and organic matter in a open raceway pond with subsequent production of algal biomass without any addition of external growth medium.

2. One-step concentration of algal biomass by using polyacrylonitrile (PAN) membrane assembly (figure 7.1).

The optimal processing conditions and harvesting method at the bench scale was demonstrated by Gera et al., (2017).The residence time used is one-fifth of the reactor volume per day during the day night hours. The *Scenedesmus obliquus* biomass concentration in the broth from the open raceway pond is 5 g/L (5 kg/m^3) on average. The algal broth produced was concentrated by using one step membrane filtration process with 90% of water removal as a permeate with no pollutants. This treated water can be used for field irrigation as well as for toilet flushing. The wet biomass paste can be sundried of which about 80-85% is recoverable. This one-step harvesting process removes several intermediate processes such as centrifugation, coagulation etc. that was required for the algal biomass concentration (Grima et al., 2003). The sundried *S.obliquus* biomass is a good source of animal feed as well as it can be directly used in fertilizer formulation.

Also, the produced algal biomass can be directly used for the extraction of biofuel. According to Chisti et al., (2008), microalgal biomass contains 20% (w/w) oil, with a biomass concentration of 1 kg/m^3 having a productivity of 0.025 kg/m^2/day which is high enough to produce a total energy yield of 1,444 GJ/ha/year. This algal biomass residue after oil extraction can be used for the production of biogas by using anaerobic digestion. This is a ample amount of energy and it should make the whole sewage water treatment process profitable.

The basis of this economic analysis was a village having population of 3000 people. Each family was considered having on an average 4 members. 300 L of sewage water will be generated from each family per day. Considering the following assumption-

Total population of a village- 3000
No. of members in each family-4
No. of families in a village-750
Sewage water generation from each family- 300 L/day
Total amount of sewage water generated- 225000 L/day or 225 m^3/day

This integrated sewage treatment and algal biomass production facility can be installed near the community sewage disposal site which will provide ready acess to sewage water and the sunlight required for the biomass productivity in an open land. On an average 1 kg/m^3/day of dry algal biomass productivity can be achieved in continuous system of operation. The processes for the treatment of sewage water along with algal biomass production and membrane based harvesting process were accessed separately to gain detailed understanding into the major contributors to the final price of sewage treatment and algal biomass production. For the economical assessment the cost of the primary equipments required for the treatment of sewage water , algal biomass production and for harvesting were included as a charges related to capital investment as well as all operating expenses.

By considering the above mentioned sewage water generation, around 225 m^3 or 225000 L of sewage water/day will be generated. Outdoor open raceway ponds or high rate algal ponds were generally preferred for such a large scale treatment of sewage water by using microalgae. Open raceway ponds are elongated in shape with round corners having shallow depths as shown in figure 7.2. Rotating paddle wheels are installed to enhance mixing and flow in the pond.

7.4 Raceway pond design

In order to treat 225 m^3 of sewage water, 3 ponds each having a working volume of 75 m^3 connected to each other were considered (figure 7.2). A typical open raceway pond (figure 7.2) consist a depth of 0.25-0.3 m for efficient light penetration. According to Chisti et al. 2013, surface area of a raceway pond should not exceed more than 5000 m^2. From the working volume capacity (75 m^3) the surface area of the raceway pond can be calculated by using eq. 7.1.

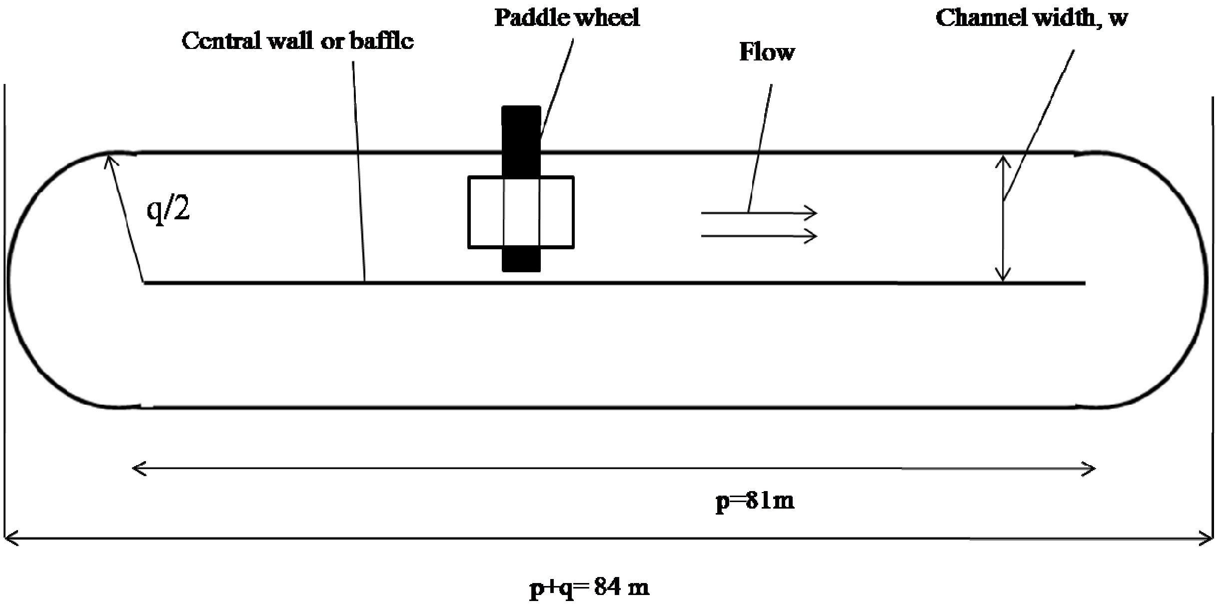


Figure 7.2: Top view of a typical open raceway pond

$$V_L = A \times h \tag{7.1}$$

$$75 = A*0.3$$

$$\therefore A = 250 m^2$$

Where, V_L– Pond’s working volume (m^3)

A-Area of raceway pond (m^2)

h- Depth of the pond (m)

Total area required for 3 ponds is 750 m^2 or 0.075 ha or 0.19 acre

From the area of the pond, the length and width of the pond [q (m)] can be determined by using equation 7.2 (Chisti, 2013).

Surface area of the pond $$A = \frac{\pi q^2}{4} + pq \qquad (7.2)$$

Where $\frac{p}{q} > 10$. This ratio should not be too small, as it affects the flow in the straight channel because of the disruption originated by the bends at the end of the channel.

Let’s consider, q= 3m

$$250 = \frac{3.14 \times 3 \times 3}{4} + p \times 3$$

$$\therefore\ p = 81m$$

Where, p- Length of the pond (m)

q- Width of the pond (m)

A solid concrete construction with the above mentioned design criteria are relatively cheap set up for the treatment of sewage water. The surface area to volume ratio is always equal to 1/h (Chisti, 2013). A lower depth always helps to increase the exposed surface area and thereby better light penetration. Usually depths lower than 0.25 m is not preferable for large ponds.

For pumping 100 m^3/h of sewage water into the open raceway pond, 0.5 hp of pump will be required (eq.7.3 and 7.4)

$$\text{Hp (Kw)} = \frac{Q \times d_h \times \rho}{3.6 \times 102000 \times e} \qquad (7.3)$$

$$= \frac{100 \times 1 \times 1000}{3.6 \times 102000 \times 0.7}$$

$$= 0.39\ Kw$$

$$\text{Hp of pump} = \frac{Hp(Kw)}{0.746} \qquad (7.4)$$

$$= \frac{0.39}{0.746}$$

$= 0.52$ Hp

Here, 70% of the pump efficiency was considered.

Q-Flow rate (100 m^3/h)

d_h- Differential head (1m)

ρ- Density of water (1000 kg/m^3)

e- Pump efficiency (0.7)

The investment capital was assumed to be 100% government venture capital and, therefore, no debt charges were included.

7.5 Membrane assembly

Membrane filtration is one of the integral part of sewage water treatment especially for separation of algal biomass grown in sewage water. In this process, polyacrylmide nitrile (PAN) membranes having an average pore size of 0.1-0.3 μm will be used for dewatering of algae. These membranes were chosen due to their high flux (224 $L/m^2/h$) in tangential flow configuration as reported by Gera et al., (2017).These membranes will be fabricated indigenously.

Total volume of grown algal biomass in sewage water for filtration- 225000 L/day

Minimum 4 days of residence time is required for the treatment of sewage water by using microalgae and for the growth of algal biomass. Therefore, there will be 4 storage tanks of 300 m^3 volumes and one additional raceway pond of same treatment capacity (225 m^3).

In order to filter out 225 m^3 of grown algal biomass following calculations will help to estimate the number of membranes required-

$$F = \frac{Q}{A_{Membrane}} \tag{7.5}$$

Where, F- Membrane flux (5376 $L/m^2/day$)

Q- Volume to be treated (225000 L/day)

$A_{Membrane}$ - Total surface area of membrane (m^2)

$$\therefore 5376 = \frac{225000}{A_{Membrane}}$$

$$A_{Membrane} = 42\ m^2$$

5 membranes having an effective surface area of 10 m^2 each will be required for processing the entire sewage water containing biomass. The surface area of the membrane can be estimated by using

$$A_{Membrane} = A_{fiber} \times N_{fiber} \tag{7.6}$$

$$10 = 1 \times N_{fiber}$$

$$\therefore N_{fiber} = 10$$

Where,

A_{fiber} - Area of each fiber (m^2)

N_{fiber} - No. of fibers

Each membrane will have 10 fibers having a total area of 10 m^2. Whereas, the length of each membrane will be 1 m and diameter of 0.3 m. A cross flow filtration assembly with hollow membrane module will be installed for dewatering of microalgae from treated sewage water. The tangential flow was provided to the membrane module by a piston pump. The system was designed to hold a single membrane module at a time. It contains a feed tank and permeate tank each having 300 m^3capacity to collect the algal broth and clear water respectively. The system was equipped with back pulse device along with air flushing mechanism to minimize membrane fouling and flux reduction as the biomass gradually increases with increase in time. The backpulse operation, tangential flow rates, and pressures will be controlled and monitored with automated valve. Figure 7.3 shows the possible implementation of open raceway ponds for the sewage water treatment using microalgae and the subsequent dewatering of grown algal biomass using side stream membrane assembly. Here, storage tank of 300 m^3 capacity for sewage water collection along with the inoculum preparation tank were shown.

Table 7.1: Primary equipment cost (PEC) for the treatment of sewage water and algal biomass production

Items	Delivered cost (Rs.)	No. of units	Total costs (Rs.)	% (PEC)
Open raceway ponds (225 m^3)	200000	3	200000	13.9
Medium feed pumps 0.5 hp (100 m^3/h)	5000	3	15000	1
Inoculum preparation tank (25 m^3)	10000	1	10000	0.7
Harvested broth storage tank (300 m^3)	50000	1	50000	3.5
Weighing machines	20000	1	20000	1.4
Membrane cost (10 m^2 area)	30000	5	150000	10.4

Membrane filtration unit	1000000	1	1000000	69.4
Total PEC (Rs.)			**1445000**	

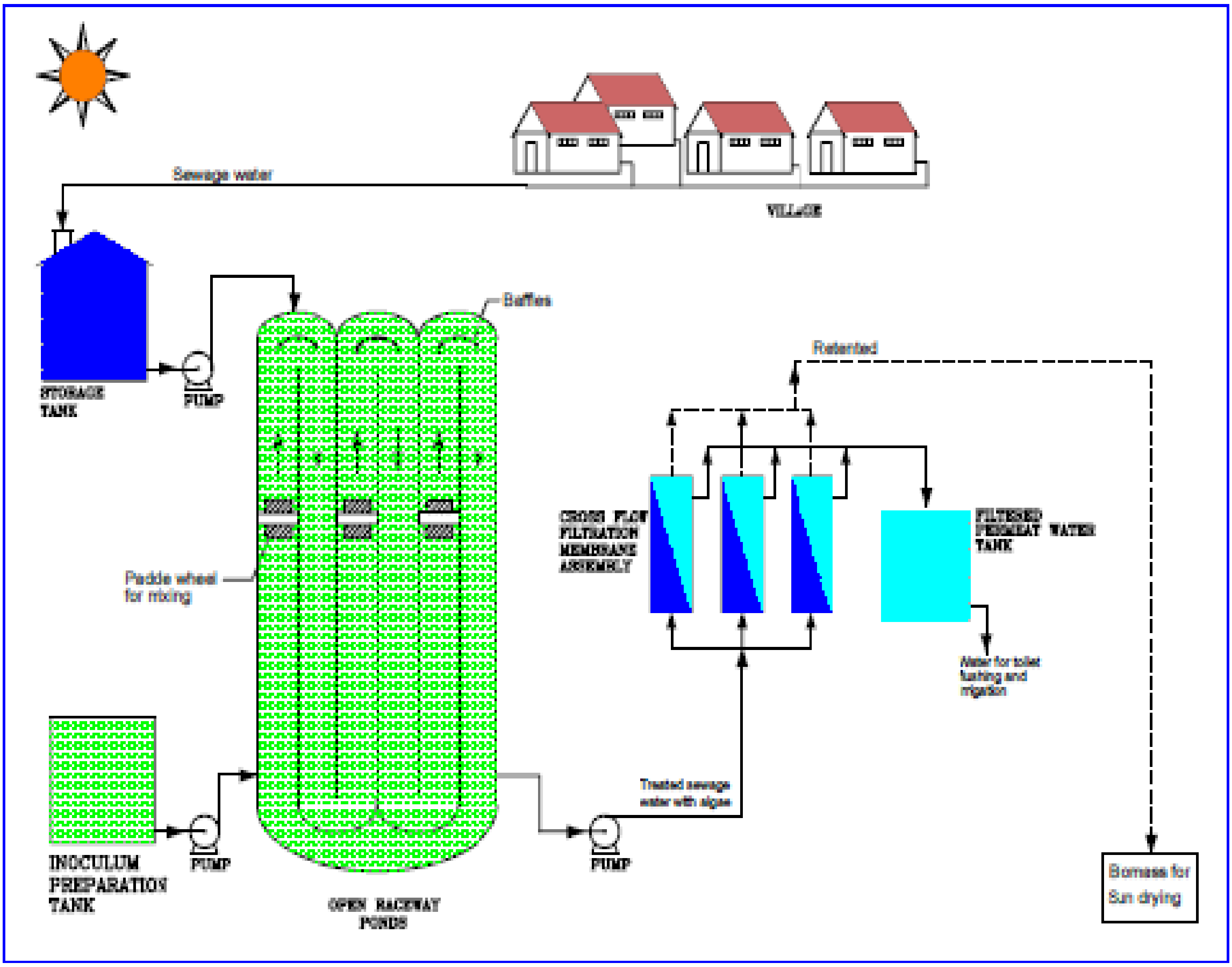


Figure7.3: Proposed sewage water treatment facility by using microalgae coupled with membrane technology in a small village

Table 7.2: Total and annual fixed capital cost for the treatment of sewage water and algal biomass production (Grima et al., 2003)

S. No.	Item	Cost (Rs.)	% F
1	Primary equipment cost (PEC)	1445000	52
2	Installation cost	10000	5
3	Instrumentation and control (at 0.1 PEC)	144500	1
4	Piping	20000	10
5	Electrical	5000	3
6	Service facilities	10000	5
7	Land (at 0.05 PEC)	72250	3
8	Engineering and supervision	50000	2
9	Construction expenses (at 0.2 ∑ items 1-8)	341350	13
10	Contractors fees (at 0.04 ∑ items 1-8)	68270	3
11	Contingency (at 0.04 of total fixed cost)	108400	4
Total Fixed capital investment F (Rs.)		2710008	

Item	Cost (Rs.)	% C
Depreciation (at(∑1-7, 9-12)/15 years)	146835	98.5
Property tax (at 0.01 depreciation)	1468	1.0
Insurance (at 0.005 depreciation)	734	0.5
Total fixed capital per year, C (Rs.)	149037	

Table 7.3: Direct cost of sewage water treatment and biomass production

S.No.		Total quantity	Cost (Rs.)	% E
1.	Labor (at Rs. 20/h, 2 shifts)	4	1401600	98
2.	Electric supplies (Rs. 6.35/ Kw h)	10,000 kw h	63500	2
Total (E)			1465100	
Total Production Cost P (C+E)			1614137	
Unit cost of treatment of sewage water (Rs./L)			7	

7.6 Cost of sewage water treatment and algal biomass production

For the treatment of 225 m^3 of sewage water 3 open raceway ponds each having a working volume of 75 m^3were required. It was estimated that 225 kg of algal dry biomass can be produced from these open raceway ponds. The cost of open raceway ponds is based on the cost estimated from the local vendor. The biomass was harvested by using side-stream membrane filtration assembly consisting of 5 PAN membranes each having an area of 10 m^2. The primary equipment cost required for the treatment of sewage water and algal biomass production was listed in table 7.1. The open raceway ponds and membrane assembly cost is based on the quotations received from the local vendors. The other costs have been estimated using standard process cost engineering information (Westney, 1997; Haas et al., 2006) or from the quotations received from local vendors. The fixed capital costs are generally based upon the primary equipment costs according to the standard process engineering costing (Westney, 1997; Haas et al., 2006). The fixed capital costing for the treatment of sewage water and algal biomass production is listed in table 7.2. For this process membrane costs and membrane filtration unit costs contributes 6% and 82% of the primary equipment cost which forms a big share in the fixed capital cost. The direct cost for the treatment of sewage water and algal biomass production includes labor, maintenance, utilities, and general overheads as tabulated in table 7.3. The cost of treatment of sewage water was estimated to be 7 Rs./L. The labor and general plant overheads contribute ≈ 98% of the total cost directly involved for the treatment of sewage water and algal biomass production. The whole process for the treatment of sewage water along with algal biomass production can be made economically feasible with the help of genetically engineered stable algae to give maximum nutrient uptake with high oil content. On the same lines rigorous economic assessment is needed for the effective reuse and recycle of treated sewage water to make the process more eco-friendly. The main objective behind using microalgae for the treatment of sewage water is to make the whole process eco-friendly without any use of chemicals as well as protect the natural habitat of the rural India.

7.7 Conclusions

In order to make the process economics for the algal biomass production feasible, sewage water was employed as the growth medium without externally adding nutrients which serves as the technique for pollution control. Thus, the energy required for growing the algae was considerably reduced. Still the process economics for the membrane has space for improvement in terms of membrane cost, fouling and longer lifespan. Design of the operating ponds and efficient membrane harvesting will play a major role in making the process cost-effective and viable in the near future. In conclusion, microalgae sewage water treatment systems in combination with membrane systems with CO_2mitigation for the algal biomass production is highly recommended as a near term R&D objective as a starting point for commercial operation.

Chapter 8

Conclusions

This thesis investigates the treatment of sewage water by using microalgae coupled with the membrane technology. Membrane technology was used to serve dual purpose of sewage water treatment as well as to study the harvesting of microalgae. However, harvesting of algal biomass remains a major challenge for the industrial researchers. The present study provides an evidence that backwashing which is currently being practiced worldwide can be a productive way to reduce fouling or blockage of the membrane and increase its harvesting potential, still no ultimate solution for the complete prevention of biofouling of membranes have been developed. In the present research, study was carried out to reduce the residence time for the treatment of sewage water using microalgae by using local microalgae isolates and starving them for the nutrients. Immobilization of standard culture was studied for the sewage water treatment to make the process feasible on large scale. Studies on different membrane materials as well as operating parameters were investigated to choose the best membrane for harvesting the grown algal biomass. Economic analysis for the whole process was examined by considering a village as a case study. The summary of each chapter from the thesis is explained as-

Chapter 1 and 2 gives an overall view of the possibility of effectively utilizing a natural microbial flora / consortium, enriched with the rapidly growing algae, for the remediation of polluted water bodies. Potential of microalgae for the treatment of domestic and industrial effluents was studied from the literature. It also throws light on the membrane technology along with microalgae for bioremediation and harvesting purpose. This literature survey not only helps to establish the fact that focused research on algal biology and membrane engineering technology is necessary but also algal biomass generated is having potential to replace significant fraction of petroleum consumption in the form of algal biofuels in the near future.

Chapter 3 deals with the treatment of sewage water using (i) a microalgae photobioreactor (PBR) and (ii) the combination of microalgae followed by a membrane bioreactor (MBR) process studied. The performance of microalgae namely *Chlorella protothecoides*, *Scenedesmus obliquus* and mixed culture were studied for the treatment of sewage water. Individual microalgae species and their combination were able to completely remove the total Kjeldahl nitrogen (TKN) within 4 days of operation. The removal rates of TKN, TP, COD, BOD, and TOC were 94-99%, 63-80%, 83-85%, 85-88%, and 75-90% respectively achieved after 5 days of batch mode of operation using a microalgae PBR. Subsequently, microalgae PBR along with a membrane bioreactor (MBR) system were operated for 6-15 days in fed-batch mode. It was found that the microalgae PBR in combination with MBR were able to remove on average 80% of TKN, 81% of TP, 57-60% of TOC, 44-57% of COD and 51.60% BOD consistently from the sewage water.

Chapter 4 demonstrates that the phosphate depletion is one of the favorable ways to enhance the sewage water treatment with the algae; however, detailed information is essential with respect to internal phosphate concentration and physiology of the algae. The growth rate of the phosphate-starved *Scenedesmus* cells was reduced drastically after 48 h. Indicating cells entered in the stationary phase of the growth cycle. Fourier Transform Infrared analysis of phosphate-starved *Scenedesmus* cells showed the reduction in internal phosphate concentration and an increase in carbohydrate/phosphate and carbohydrate/lipid ratio. The phosphate-starved *Scenedesmus* cells, with an initial cell density of,1x 10^6cells/ml shows 87% phosphate and 100 %nitrogen removal in 24 h. The normal *Scenedesmus* cells need approximately 48 h to trim down the nutrients from wastewater up to this extent. Other microalgae, *Ankistrodesmus*, growth pattern was not affected due to phosphate starvation. The cells of *Ankistrodesmus* were able to reduce 71% phosphate and 73% nitrogen within 24 h, with an initial cell density of 1 x 10^6cells/ml.

In chapter 5, a packed bed algae biofilm reactor was developed using porous and non-porous dual packings. The biofilm was cultivated on reticulated polyurethane foam cubes of 0.01 m dimension. The non-porous glass raschigs were used as bed support that helps the removal of generated gas from the system. The effect of variables such as column $\frac{L}{D}$ ratio, catalyst cube dimension and feed flow rate on the treatment of sewage water was studied. The reaction kinetics indicates that the nutrients uptake rate is dependent on both pore and film diffusion. The kinetics of uptake of nutrients follows a pseudo-first order reaction. From the pseudo reaction rate constant, Thiele Modulus and effectiveness factor were calculated and a kinetic model equation for fractional nutrients uptake was developed in terms of operating variables. It was observed that the model can predict the reaction rate with ±5% deviation. The packed bed column was operated continuously for 90 days with 76-83% of TN and 70-76% TP removal in 24 h of residence time and the results obtained may be useful for large-scale treatment of sewage water.

Chapter 6 deals with the treatment of sewage water by using three different microalgae cultures namely Scenedesmus obliquus, *Chlorella vulgaris* and *Ankistrodesmus stipitatus* in combination with the membrane bioreactor (MBR). The effect of various operating parameters such as the concentration of microalgae, different types of membranes (viz. ceramic, polysulfone and polyacrylonitrile (PAN)), and transmembrane pressure, on the quality of treated water, has been investigated in details. During the operation, various process parameters such as nutrients uptake [viz. total nitrogen (TN), total phosphate (TP)], pH, TOC, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were monitored. It was found that at high initial biomass concentration ($5x10^6$ cells/ml) the rate of the nutrients uptake was maximum for all the three cultures and PAN membrane gave the highest permeate flux (224 L/m^2 h) with less fouling compared to ceramic, polysulfone membranes. High-quality effluent in terms of nutrients uptake and organic matter reduction were achieved by using microalgae and membrane bioreactor. The approximately 70-85% yield of biomass was achieved after the treatment of sewage water in combination of a membrane system. Thus it can conclude that cultivation of microalgae in combination with the membrane is one of the potential advanced techniques for the treatment sewage water.

Detailed economic analysis for the sewage water treatment using algae and membrane technology in a village was studied in chapter 7.Capital and operating cost required for the treatment of sewage water by using microalgae and membrane technology was estimated. Although, the cost analysis clearly indicates the limitation for the commercialization of sewage water treatment by using algae and membrane technology, the primary objective of pollutant free water can be accomplished in a green way. India’s water demand is expected to rise to 1047 billion cubic meters by 2050, which places India on second position after United States of America. Out of the most usable water, agriculture consumes around 83% of water and only 5% was consumed for the household purpose. Villages in India still suffers from water shortage especially lack of safe potable water. Water borne diseases affect number of people in villages because of poor sanitation and don’t have proper sewage treatment facility. The sewage water generated in villages was directly disposed off in the open area or pits which give rise to various water borne diseases. In the present study, possible solution for the treatment of sewage water in a eco-friendly way without use of any chemicals was suggested. Microalgae not only help to reduce the pollutants in sewage water but also provide biomass in the form of fertilizer for the fields thereby, maintaining the eco-system in a sustainable way. As well as, the clear permeate water from the membrane assembly can be directly used for the irrigation of the land field or for toilet flushing.

Chapter 9

Future Recommendations

The following recommendations are proposed for the future work-

1. In this work, artificial white LED lights were used for growing standard algal cultures in sewage water in a chemo stat. More experiments are needed with different color lights to examine effects of different wavelengths of light on microalgae growth and nutrient uptake. Also, focus is needed on design of the closed photobioreactor to prevent algal shading effects and growth of the zooplanktons.
2. Microbial interaction of algae and bacterial consortia can be studied in detail to establish an optimized ration for efficient pollutant removal with lower residence time. Study of some standard bacterial culture for a particular pollutant removal along with microalgae will be helpful for the further treatment of industrial wastewater with high COD and BOD levels.
3. Scale-up of the immobilized packed bed column will be beneficial in terms of reducing residence time for the treatment of sewage water. Different type of packing material like pieces of bricks, metal waste etc. can be tried for the immobilization of microalgae along with the wipering to remove the excess grown algal biomass from the column. The treated sewage water can be directly fed to the membrane assembly to get the clear permeate. As the algal leach out in the treated effluent will be less, membrane will have longer lifespan with reduced biofouling.
4. In terms of membrane system, membrane material with less fouling properties and high flux needs to be explored. Extensive research is needed in the area of biomass harvesting which can reduce the overall cost of algal production. Filter press suitable along with autofloculating algal species needs to be examined to improve the harvesting algal biomass.
5. Algal productivity depends on the geographical latitude i.e. where the facility is located, and we live in a tropical hot country where solar radiation is available in abundance throughout the year. With such a highly favorable environment, efficient open raceway pond design, genetic and metabolic engineering of microalgae will help to increase the productivity of algal biomass. The CO_2 gas released from the industries can be directly fed to the algal ponds required for their growth. This will also reduce the carbon

footprints thereby lowering the greenhouse gas emission. Prevention of predators, especially in wastewater streams will be major cause of worry.

6. Economics as this are always not trustworthy because of the insufficiently developed photobioreactor or open raceway pond design engineering. Also, membrane engineering along with other harvesting technique needs a breakthrough in research and development. Extensive efforts are needed to achieve commercial scale treatment of sewage water and algal biomass production. This will open a new gateway for the microalgae derived biofuels to completely evict petroleum derived liquid transport fuel in the near future.

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11.List of Publications

I. **Gera G**., Swati N. Yewalkar., Nene S., Kamble S., Kinetic studies of algal biofilm reactor for raw sewage treatment, Chemical Engineering Technology, 39 (9), 1629–1635 *DOI:10.1002/ceat.201500447* (2016).

II. **Gera G**., Salunkhe V., Kharul U., Jadhav S., Bhattacharjee T., Kamble S., Treatment of Sewage Water using Microalgae in Combination with Membrane Bioreactor: An Eco-Friendly Approach, Current Environmental Engineering,5, DOI:10.2174/2212717805666180124153612 (2018).

III. **Gera G**., Swati N. Yewalkar., Nene S., Kamble S., Treatment of sewage water by using microalgae and membrane bioreactor, Chemical Industry Digest, 29(6),77-84 (2016).

IV. Swati Yewalkar-Kulkarni, **Gayatri Gera**, Kiran Pandare, Sanjay Nene, Sanjay Kamble, Insight studies on using phosphate starved cells of Scenedesmus sp. for the treatment of raw sewage, Indian Journal of Microbiology,57(2),241-249,*DOI 10.1007/s12088-016-0626-0*(2016).

V. **Gera G**., Swati N. Yewalkar., Nene S., Kamble S., Phycoremediation of sewage water by using combination of microalgae photobioreactor and membrane cell recycle bioreactor in presence of artificial radiation, Under review, Biocatalysis and Agricultural Biotechnology, (2017).

Book Chapter

I. **Gera G.,** Yewalkar S., Kamble S., Nene S. Remediation of domestic and industrial effluents using algae, Published, Springer publication ISBN no. 978-93-81891-23-0 (2015).

Conferences

I. International conference on algal biorefinery, IIT Kharagpur (West Bengal), India, 2013.Oral presentation – “Remediation of Domestic and Industrial Effluents Using Algae”.

II. International conference on “Sustainable Development for Energy and Environment (ICSDEE-2017)”, National Chemical Laboratory, Pune.

Oral presentation-“Treatment of sewage water using microalgae in combination with membrane bioreactor: An eco-friendly cost effective approach”.

Curriculum Vitae

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Educational Qualification

- Ph.D. , CSIR-NCL,Pune (2011-2016)
- M.Tech (Chemical Engineering, Dr. Babasaheb Ambedkar Technological University,Lonere, India, 2007) (C.G.P.A.- 8.0)
- B.Tech (Chemical Engineering, College of Engineering and Technology, Akola, India, 2005) (66%)

Professional Experience

- Worked in Research and Development division of Matrix-Praj Industries as a Technical Research associate (Oct. 2007- Mar.2011).

Research Experience

- Worked as a project trainee during M.tech at **Bhabha Atomic Research Centre, Mumbai**on a project “Micro granulation of fine silicon carbide powder centrifugal pressure swing processor”. (June 2006- June 2007)

  Research Supervisor- Dr. D. Sathiyamoorthy, Head, Dept. of Powder Metallurgy
- B Tech. Industrial Training at **Shobha Chem (Mumbai):** “Synthesis of Chemical dyes”. (April-June, 2004)
- Worked as a research associate in **Matrix-Praj Industries** on transesterification and biotransformation using enzymes. (August 2007-August 2009)

  Research head- Dr. Alankar Vaidya, Scientist, Matrix-Praj Industries.

- Worked as a technical research associate in **Matrix-Praj Industries** on immobilization of yeast for rapid ethanol fermentation, algal harvesting, and fermentation techniques. Research Head- Dr. S.V. Ramakrishna, Head, Matrix-Praj Industries. (Sep.2009-March 2011)

Contribution in the field of research

- Ph.D. in the field of biological as well as by using membrane technology for the treatment of sewage water, thesis title is "**Treatment of Sewage Water by using Microalgae coupled with Membrane Bioreactor (MBR) System".** (2011-2016)
- Successfully developed the technology for the project titled "**Performance of Packed Bed Biofilm Reactor for Continuous Fermentation of Alcohol"** for Matrix-Praj Industries (Sep. 2009- March 2011).
- Developed technology for **"Evaluation and optimization of immobilized lipase for esterification of fatty acid and monohydric alcohol"** for Matrix-Praj Industries (Aug.2007-Aug.2009)

Awards/Fellowships

- Technical Research Associate, Matrix-Praj Industries, India, (2009)
- CSIR- Senior Research Fellowship, India, (2011)
- Certificate of appreciation for the paper presentation at International Conference on Algal Biorefinery held at IIT Kharagpur, India, (2016)
- Awarded for the best paper presentation at a International conference on sustainable development for energy and environment, held at CSIR-National Chemical Laboratory,Pune,India, (2017)

Publications

- **Gera G.**, Swati N. Yewalkar., Nene S., Kamble S., Kinetic studies of algal biofilm reactor for raw sewage treatment, Chemical Engineering Technology, 39 (9), 1629–1635 *DOI:10.1002/ceat.201500447* (2016).
- **GeraG.**, SalunkheV., Kharul U., Jadhav S., BhattacharjeeT., Kamble S., Treatment of Sewage Water using Microalgae in Combination with Membrane Bioreactor: An Eco-Friendly Approach, Current Environmental Engineering (2017). (Accepted, in press)
- **Gera G.**, Swati N. Yewalkar., Nene S., Kamble S., Treatment of sewage water by using microalgae and membrane bioreactor, Chemical Industry Digest, 29(6),77-84 (2016).
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raw sewage, Indian Journal of Microbiology,57(2),241-249, *DOI: 10.1007/s12088-016-0626-0*(2016).

- **Gera G**., Swati N. Yewalkar., Nene S., Kamble S., Phycoremediation of Sewage by combining microalgae and MBR, Under submission stage (2017).
- **Vaidya A**., Gera G., Ramakrishna S., Evaluation and optimization of immobilized lipase for esterification of fatty acid and monohydric alcohol, World Journal of Microbiology and Biotechnology, 24, 2987-2995, *DOI: 10.1007/s11274-008-9842-z*(2008).

Book Chapter

- **Gera G.,** Yewalkar S., Kamble S., Nene S. Remediation of domestic and industrial effluents using algae, Published, Springer publication ISBN no. 978-93-81891-23-0 (2015).

Conferences

- International conference on algal biorefinery, IIT Kharagpur (West Bengal), India, 2013. Oral presentation – "Remediation of Domestic and Industrial Effluents Using Algae".

- International conference on "Sustainable Development for Energy and Environment (ICSDEE-2017)" , National Chemical Laboratory, Pune.

  Oral presentation-"Treatment of sewage water using microalgae in combination with membrane bioreactor: An eco-friendly cost effective approach".

References

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