Wastewater Treatment Using Microalgae: A Batch Study

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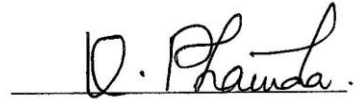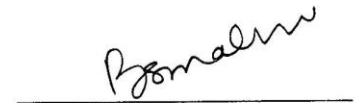
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Approval Sheet

This thesis entitled **"Wastewater treatment using microalgae: A batch study"** By Anjana Babu. B is approved for the degree of Master of Technology from IIT Hyderabad.

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Dedicated to

to my family...

Abstract

The main objective of this study is to examine the performance of mixed micro algal bioreactors in simultaneous treatment and production of biodiesel from three different types of wastewaters (Kitchen, Pharmaceuticals, Palm Oil Mill Effluent and BG 11 along with glucose used as control) in semi-continuous mode. For each type of wastewater 8 SRTs were used - 2, 4, 6, 8, 10, 12, 14 and 16 days. The studies were conducted in 500 ml wide mouth transparent glass bottles in sunlight with a 12:12 light-dark cycle. The nutrient uptake kinetic parameters were quantified using linearized Michaelis-Menten and Monod models at steady-state conditions. Lipid analysis and FAME analysis were performed after the end of the experiment. The nutrient removal efficiency and lipid production was found to be higher in Kitchen wastewater when compared with other wastewaters. The lipids produced consisted of over $60 - 70$ % of saturated fatty acid (C16: 0, C18: 0, C18:1) for all wastewaters which are considered suitable for synthesizing biodiesel.

Nomenclature

COD	chemical oxygen demand, mg/L
DOC	Dissolved Organic Carbon, mg/L
GC-MS	Gas chromatography-mass spectroscopy
h	hour
HRT	hydraulic retention time, d
IWW	Industrial (Pharmaceutical) Wastewater
KWW	Kitchen Wastewater
L	litre
mg	milligram
mg/L	Milligram per litre
MLD	Million litre per day
MUFA	Mono unsaturated fatty acid
POME	Palm Oil Mill Effluent
PUFA	Poly unsaturated fatty acid
SFA	Saturated fatty acid
SRT	Solid retention time, d
TN	Total nitrogen, mg/L
TOC	Total organic carbon, mg/L
TSS	Total suspended solids, mg/L
TVS	Total volatile solids, mg/L
VSS	Volatile Suspended Solids, mg/L

Contents

Chapter 1

Introduction

According to Central Pollution Control Board reported during 2010-2011, out of 38254 MLD of sewage generated by class I cities and class II towns, only 11787 MLD has been treated and thereby leaving a huge gap between sewage generation and sewage treatment. Central Pollution Control Board, reassessed sewage generation and treatment capacity for Urban Population of India for the year 2015. The sewage generation estimated to be 62000 MLD approximately and sewage treatment capacity developed so far is only 23277 MLD from 816 STPs (NPCB, 2015). The unavailability of an adequate system for sewage collection and its treatment in cities and the town's results in untreated wastewater being discharged into rivers or lakes or remains inundated on the land causing potential risk to the groundwater contamination. Disposal of untreated or partially treated wastewater into the water bodies affects the quality of water, so that water bodies become unfit for human consumption or for other uses. Therefore, efficient and cost effective method of treatment and disposal of sewages is necessary. According to the Central pollution Control Board, discharge limits of COD and Total nitrogen in water resources as well as for land disposal and for the new STPs Design are not more than 50 mg/1 and 10 mg/1 respectively.

Production of 1 kg of micro algal biodiesel needs to consume approximately 3,726 kg of freshwater, 0.33 kg of nitrogen, and 0.71 kg of phosphate (J Yang et. al, 2011). By using wastewater for the cultivation of algae, treatment of wastewater and simultaneous synthesis of biodiesel can be done. Microalgae are now the focus of

intensive research because of their potential as a renewable feedstock for biofuel production. High productivity, low land-area requirement, biodegradability, and capacity to satisfy the existing demand for petroleum fuels are brought attention to microalgae research. Theoretical algal biomass production for 1 L of medium strength domestic wastewater is 0.6 g. If we are assuming wastewater generated by class I cities and class II towns are undergoing a treatment by using micro-algae, and assume 90 % removal of carbon, nitrogen, and phosphorus, the total theoretical biomass production will be $33,480 \times 10^3$ kg/day. Assuming a biodiesel yield as 10%, then total biodiesel production per day is 3348×10^3 kg. The total volume of biodiesel production is 4.18 MLD. An estimated diesel consumption in per day in India is 57 MLD. So algal biodiesel will cut the petroleum diesel consumption by 7.3 %. Biomass left after extraction of biodiesel will be $18,591 \times 10^3$ kg. Assume methane yield of algal biomass is 0.3 L/g, then the theoretical methane yields for total algae residue is 3983×10^3 kg/day. That is Total energy yield per day will be 61.42×103 MWh. If we are assuming 30% efficiency of electricity generation, total electricity generation will be18426 MWh. So the percentage of methane electricity can be produced from algae-based wastewater treatment system in India is 0.7% of total electricity consumption in India during 2014. Cultivation of microalgae in wastewater is being extended to a process for nutrient removal as well as a solution of water and nutrients demanded in mass-cultivation of microalgae as a feedstock for biodiesel (Cho et al. 2011, Yun et al., 1997; Wang et al., 2010; Chinnassamy et al., 2010).

1.1 Research Objectives

- To study the performance of a mixed algal culture in treating wastewater and quantifying the nutrient uptake in terms of kinetic parameters at steady-states
- To study the content and speciation of lipid produced within the algal cells.

Chapter 2

Literature review

2.1 Micro algae

Algae is recognized as one of the oldest life-forms. They are primitive plants have no sterile covering of cells around the reproductive cells and have chlorophyll 'a' as their primary photosynthetic pigment. The most important classes of algae are green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta). Algae can be autotrophic, heterotrophic or mixotrophic. The autotrophic algae requires only inorganic compounds such as CO_2, salts and a light energy source for growth; while the heterotrophic algae are non-photosynthetic, therefore, require an external source of organic compounds as well as nutrients as an energy source ((Brennan et al. 2009). For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2, absorbed by chloroplasts, into adenosine triphosphate (ATP) and O_2, the usable energy currency at the cellular level, which is then used in respiration to produce energy to support growth (Rastogi et al. 2015). Algae-based biofuel production has a number of potential advantages such as

- Biofuels and byproducts can be synthesized from a large variety of algae.
- Comparing with plants, algae has a rapid growth rate,

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- Algae can be cultivated in brackish coastal water and seawater.

- Algae can take up high concentrations of nitrogen, silicon, phosphate, and sulfate nutrients from municipal, agricultural or industrial waste streams,

Algae can sequester carbon dioxide (CO_2) from industrial source

2.2 Growth parameters of algae

The factors affecting the algal growth are nutrient concentration, light, temperature, pH, aeration and mixing, predation by zooplankton, pathogens (including bacteria, fungi, and viruses) and invading species competition. All factors need to be considered for getting maximum algal productivity and water treatment efficiency. Abnormal changes in physical factors such as temperature, pH, and light intensity causes sudden death of algae.

2.2.1 Carbon source.

Algae is mostly autotrophic, which consumes light and carbon dioxide (CO_2) for normal growth during photosynthesis. Microalgae are photosynthetically more efficient than terrestrial plants to fix CO_2 and are well known to capture both atmosphere and industrial emissions (Brennan and Owende, 2010). Heterotrophic growth is an aerobic process where the assimilation of organic substrates generates energy through oxidative phosphorylation accompanied by oxygen consumption as the final electron acceptor. Mixotrophic cultivation is the growth mode where microalgae simultaneously use inorganic CO_2 and organic carbon sources in the presence of light (Kang et al. 2004); therefore, photo-autotrophy and heterotrophy occur

simultaneously (Wang et al. 2014). Several algal species are able to switch between photo-autotrophic and heterotrophic growth. The rate at which these algal cells take up specific nutrient depends on the difference between the concentration inside and outside the cell and also the diffusion rates through the cell walls. The thickness of the unstirred layer of water just outside the cell wall also plays a major role where thickness layer gives slower diffusion rates. The turbulence of water is highly essential for avoiding such situations. The basic photosynthesis process takes place in microalgae where it intakes the inorganic carbon and convert it into chemical energy with oxygen as a byproduct with the help of solar energy.

The inorganic carbon species generally used by the microalgae are in the form of carbon dioxide and bicarbonates. Apart from this, there are some microbes which oxidize carbon from organic sources and supply to algae. The heterotrophic metabolism is significant in waste loaded ponds. Atmospheric carbon dioxide may also be provided to algal culture by means of aeration. However, since the ambient atmospheric concentration is far below the optimum algal growth, the supply of external carbon may be necessary. The pH of media is one of the most dominating factors which determine the amount of dissolved CO2. At pH greater than 9 the inorganic carbon is in the form of carbonate, which cannot be taken up by the algae. Even though the decrease in availability of CO2 can limit algal growth, it is not that pronounced. In addition, mixing up of CO2 is one of the most costly processes.

2.2.2 Nitrogen and Phosphorus

An optimum C: N: P ratio for microalgae is 106:16:1 molar ratio or 56:9:1 C: N: P in weight ratio. (Hadiyanto et al., 2014). Nitrogen plays a major role in the growth of microalgae since it is comprised of a significant part of the

biomass. Algae mainly uses nitrogen in the form of ammonia (NH_4^+) and nitrate (NO_3^-). Ammonia is the most preferred among all forms if available. But the concentration of ammonia more than 20 mg per liter is toxic to algae. Other nitrogen sources are urea and nitrite. Algae takes up phosphorus as inorganic orthophosphate. The energy requirement for uptake of orthophosphate is also high. When there is a shortage of inorganic phosphate in the media, the formation of orthophosphates occurs at the cell surface. By the formation of orthophosphate, algae can reserve phosphorus for the prolonged growth in the absence of available phosphorus. So the growth rate of algae's does not get affected much even there is a change in external concentration of phosphorous. Usually, waste water would constitute the much higher amount of phosphorous than what is normally needed for the growth of algae. Even though nitrogen and phosphorus are the most predominant nutrients needed for algal growth there are many more nutrients which contribute to algae growth. For example, sulfur, potassium, calcium, and magnesium. Micronutrients which are required comparatively in smaller quantity are manganese, molybdenum, copper, iron, zinc, boron, chloride, and nickel. In addition, there are some other elements which are essential for certain specific algae like sodium, silicon, cobalt, iodine, vanadine and selenium.

2.2.3 Temperature

The increase in temperature can increase algal growth up to an optimal temperature; increase in temperature more than this leads to rapid decline in growth rate. In humid climates overheating of algae is a problem, whereas in countries like Sweden low temperature causes limitation for algal culture. At low temperature, microalgae are easily photo-inhibited by high light

intensities. Generally, temperature around 15-25 ^0C are suitable for algal growth. Green houses are constructed to form a solution for low temperature in high-latitude regions (D.L Sutherland et al.2015)

2.2.4 pH

The pH of culturing media is one of the most important factors for the growth of algae. During the time of photosynthesis pH value of the growth, median increases due to depletion of CO_2 and HCO_3. pH value above 10 is not uncommon when there is no carbon dioxide supplied and pH can reach 11 or more if carbon dioxide is limiting and bicarbonates are used as carbon source. pH is also increased because of nitrogen absorption by algae. Algae cannot survive if ammonia is used as a nitrogen source and the pH of the medium may decrease as low as to 3 causing too acidic state. Precipitation of phosphate in the medium by forming calcium phosphate occurs at high pH, but these may redissolve if pH drops, especially during night hours. The photosynthesis will be inhibited if the concentration of ammonia is higher at high pH. Flocculation may happen at high pH, which in turn lead to reduced nitrogen uptake and growth rate in some algae (D.L Sutherland et al.2015)

2.2.5 Aeration and mixing

To prevent sedimentation of the algae mixing is necessary. The algae cells should be in suspension to ensure that all cells of the population are equally exposed to the light and nutrients. For improving gas exchange between the culture medium and the air and to avoid thermal stratification especially in

outdoor conditions, mixing or aeration or both required. Aeration can provide a carbon source for photosynthesis in the form of carbon dioxide. CO_2 plays an important role in pH maintenance. The addition of CO_2 buffers the water against pH changes as a result of the CO_2/HCO_3^-. In open ponds mixing can be done by stirring daily by hand, aerating, or using paddle wheels and jet pumps depending on the scale of the culture system. Vigorous mixing may affect the growth of algae. A preliminary experiment is necessary to find the best algae growing conditions because the effect of the agitation speed is different on different microalgae. Too high agitation speed could damage the cells by causing the leakage of important chemicals from within the cell. A constant supply of air (or air plus CO_2) will only be in balance with growth at one point during the exponential phase of growth. Low cell density and too much CO_2 may lower the pH and depress growth. The CO2 limitation at high cell densities causes any further biomass increase to be more linear rather than exponential (with respect to time) and proportional to the input of CO

2.2.6 Light and Photoperiod

Most of the microalgae species are autotrophs, like plants which obtain energy from the light source for photosynthesis. Some algae is heterotrophs, by using organic compounds as energy and carbon source which are capable enough to survive in the dark. During the time of photosynthesis, a part of light gets converted to chemical energy and the major part is getting lost as heat energy. While designing a photobioreactor light period is an important factor to be considered. Excessive light exposure can lead to cell growth inhibition. With a photoperiod of 12h nearly doubling the microalgae biomass concentration in comparison with 3h, 6h, and 9h light periods (Lam

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and Lee, 2012). It has been found that growth rates and maximum cell density of microalgae increase proportional to light period duration, even at different light intensities (Amini Khoeyi et al., 2012). An exception to this was discovered with a 12:12h (light: dark) photoperiod, in which higher productivities and maximum cell density values were obtained, in comparison with the 14:10h (light: dark) (Jacob-Lopes et al., 2009). Microalgae get maximum light energy only if they remain in the surface layers of water and they adopt many strategies to remain there. Cyanobacteria floats in culture due to the presence of gas vacuoles. Since water absorbs photosynthetic active radiation (PAR), the algae in the deeper layer of culture vessels which cannot get enough light energy are normally light limited. Internal shading also causes light limitation in dense cultures that are algae themselves can decrease the light availability. Hence, the turbulence of water is necessary to avoid a light limiting condition in algae culture. So that all cells get exposed to light for at least short periods. In the case of an open pond, depth should be designed such a way that to prevent algae's from the light limited situation. Excess of light is also harmful to algae growth, which leads to photo-inhibition. Bhola.et al. (2010) reported C. vulgaris could withstand a light intensity ranging from 150 to 350 μmol photons $m^{-2}s^{-1}$ and further increase in light intensity resulted in a decline of the electron transport rate. Adequate mixing can prevent photoinhibition to an extent.

2.3 Wastewater treatment using microalgae

Combining treatment of wastewater and cultivation of microalgae is more interesting in the wastewater treatment field. Microalgae can use nutrient

present in the wastewater for growth and simultaneously treating of wastewater also happen. By using wastewater, large quantities of freshwater and nutrients required for algae growth could be saved. Using microalgae for treating wastewater is more sustainable and environmentally friendly way for the production of biofuel. The growth of algae mainly requires carbon, nitrogen and phosphorus. Different wastewater sources such as municipal wastewater, agricultural run-off, animal wastewaters and industrial water usually contain carbon, nitrogen and phosphorus sufficient for the efficient growth of algae. Algae can grow in various wastewaters as long as there are adequate amounts of carbon (organic or inorganic), N (urea, ammonium or nitrate), and P as well as other trace elements present (S. Mobin and F. Alam, 2014). Mass production of algal biomass for biofuel and other applications using wastewaters is bringing more attention for researches, because its efficiency and need. Algae can be periodically harvested from the treatment plant and use for making biofuel. So simultaneous treatment of wastewater and cultivation of algae can provide more economical end environmental friendly wastewater treatment compared to conventional wastewater treatment methods. It has been shown to be a more cost effective way to remove biochemical oxygen demand, pathogens, phosphorus and nitrogen than activated sludge (Green et al., 1996). The problems faced during treatment of wastewater using microalgae are

- Uncertainties and challenges, including variation of wastewater composition due to source, infrastructure, weather conditions
- Pretreatment methods and improper nutrient ratios (e.g., C/N and N/P),
- High turbidity due to the presence of pigments and suspended solid particles which affects light transmission, and
- The presence of competing micro flora and toxic compounds, and

- Accumulation of growth inhibiting compounds which are worsened if water is recycled and reused.

Table 2.1:Efficiency of algae in treating different wastewater

Algal Species	Type of wastewater	Removal efficiencies (%)			Retention time	Reference
		COD	N	P		
Mixed microalgae	Domestic wastewater	85.5	75	70	8 days	Venkata Mohan et al., 2014
Chlorella Vulgaris	Municipal wastewater	13.9	97.5	85.8	-	Nandini et al., 2013
Chlamydo monas sp.	Municipal wastewater	13.1	78.9	76	-	Devi et al., 2015
Chlorella pyrenoidosa	Settled domestic sewage	-	93.9	80	13 days	Tam et al., 1989
Cyanobacteria	Secondarily treated domestic effluent + settled swine water	-	95	62	1 day	Pontiot et al. 1989
Chlorella Vulgaris	Diluted pig slurry (suspended solids content to 0.2%)	BOD_5 98%	54-98	42-89	4.5 days	Fallowfield et al., 1985
Mixed culture of *Chlorella* and diatom species	Wood-based pulp and paper industry wastewater	58%	-	-	42 days	Tarlan et al., 2002

Chlorella sp.	Before primary settling	50.9%	68.4	83.2	9 days	Wang et al., 2010
Chlorella Vulgaris	Textile wastewater	38.3-62.3%	44.4-45.1	33.1-33.3		Lim et al., 2010
Chlorella sorokiniana and aerobic bacteria	Potato processing industry	84.8%	>95	80.7		Herandez et al., 2012

2.4 Factors inhibiting growth of algae

The growth of algae decline in cultures when either a specific requirement for cell division is limiting is an inhibiting reproduction. Substances like heavy metals, herbicides, pesticides, substances in detergents, household cleaning product, and personal care products can act as inhibitory compounds in photosynthesis if present in large concentration. At high pH and high-temperature, high concentration of ammonia act as inhibitory as its toxicity increases. Diffusion of free ammonia enters freely over membranes into the cells if ammonia concentration is high. Nutrient uptake of microalgae can also be affected by the exceptionally higher concentration of organic compounds. Auto inhibition can happen in microalgae by production and accumulation of toxic substance as a result of their own metabolism, which eventually accumulates to higher concentration causing inhibiting growth. Cyanobacteria (blue-green algae) can produce inhibitory substances to the growth of eukaryotic algae and some eukaryotic algae can produce an antibacterial substance. Culturing of pure monoculture is very difficult for

infection of parasites, predators or competing species are really harmful and protozoa and rotifers may enter into the culture. Avoiding infection completely is difficult, however we can keep pure culture by maintaining optimum conditions for the growth of algae. Once the algae dominated in a container, it will not any other species to grow in that. Applying biocides may spoil the quality of the product, expensive and also not environmentally sound.

2.5 Lipid Production

An important step in algae-based biodiesel production is selecting high lipid content and fast growing microalgae. Many microalgae can accumulate lipids due to excess photosynthesis, and some species can accumulate large amounts of lipids from heterotrophic or environmental stress factors, such as nutrient deficiency (Takagi et al. 2006). The concentration of nitrogen and phosphorous and other molecules plays a major role in the formation of lipid in microalgae. So that the level of the nutrient must be carefully monitored. Most researchers follow a standard protocol written by Bligh and Dyer which uses chloroform and methanol as the extraction materials. The lipid content of microalgae can be increased by environmental stress, such as limitation in some essential nutrients like nitrogen (Sheehan et al., 1998; Illman et al., 2000), phosphate (Reitan et al., 1994) and any metal components (Liu et al., 2008). The nitrogen source and concentration in the medium used are also known two of the most crucial factors affecting the lipid content of microalgae (Hsieh and Wu, 2009). Depletion of nitrogen changes cellular carbon flux to lipid synthesis from protein synthesis (Sheehan et al., 1998) and the lipid content of Chlorella Vulgaris can increase from 20% or less to up to 40% under nitrogen deprivation (Illman et al., 2000). Table 2.3 shows the lipid content of different algae adaptedd from H. Smith-baedorf.

Table 2.2 : Lipid content in algal species

Species	Lipid content (wt%)
Anabaena cylindrica	4-7
Botryococcus braunii	25-80
Chlamydomonas reinhardtii	21
Chlorella emersonii	25-63
Chlorella protothecoides	14-58
Chlorella Vulgaris	14-22
Dunaliella salina	6-25
Dunaliella tertiolecta	16-71
Hantzschia sp.	66
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-50
Phaeodactylum tricornutum	18-57
Scenedesmus dimorphus	16-40
Scenedesmus obliquus	11-55
Spirulina platensis	4-16
Synechococcus sp.	11

Commercial biodiesel is commonly extracted from soybean oil, palm oil, rapeseed oil, and waste cooking oil. Biodiesel production from these sources is, however, unsustainable due the limited availability of arable land for the cultivation of both energy and food crops (Liu *et al.*, 2007; Elshahed, 2010). The rapid growth of microalgae, which commonly double their biomass

within 24 hours, implies that a relatively small area of land would be required for its mass culture, making it more desirable as a fuel source relative to conventional oilseed crops (Chisti, 2007, 2008; Liu *et al.*, 2007). The table shows lipid percentage of algae obtained in different wastewater medium.

Table2.4: Lipid percentage of algae in different wastewater

Type of wastewater	Species	Total Lipid (%)	Cultivation time (days)	Reference
DigestedManure	Chlorella	14	21	Wang et al.
MSG Wastewater	Chlorella	14	5	Xue et al.
Artificial Wastewater	Chlorella	33	14	Yujie et al.
Muncipal(centra)	Chlamidomonas	25.2		Kong et al.(2010)
Industrial(Carpet mill,untreated)	B. braunii	13.2		Chinnasamy et al.(2010)
Artificial wastewater	Scenedesmus sp.	12.8		Voltolina et al. (1999)
Municipal	Chlorella	31	9	Cho et al.

2.6 Biodiesel production

As one of the outstanding alternative sources of renewable energy, biodiesel has attracted growing attention from researchers worldwide (Dharmendra

Kumar Singh and Nirupama Mallick 2014)TFA in the presence of an acid catalyst and methanol forms FAME (Chisti, 2007). Fatty acids produced from the extracted lipid of plants or animals can be converted into biodiesel, which can be used in any diesel engine with little to no modifications (National Biodiesel Board, 2007). Compared to petroleum diesel fuel, biodiesel tail pipe emissions cause less pollution to the environment, and also more readily biodegradable than petroleum diesel. Unsaturated fatty acids occur when there are double bonds of the cis configuration in specific positions. Fatty acids from microalgae have been found to contain combinations of zero to five cis double bonds (Thompson, 1996). The most typical lipid classes of plant and animal origin consist of fatty acids linked by an ester bond to the trihydric alcohol, glycerol, or to other alcohols such as cholesterol, or by amide bonds to long-chain bases, or on occasion to other amines (Christie, 2003). The quality and variety of lipids are important for biodiesel production because it will determine the need for pretreatment before it is converted to biodiesel. Different processing (i.e. Thermal depolymerization) may be required in the case of very poor quality lipids (high free fatty acid content, a high degree of unsaturation, etc.) to transform the lipids into transportation fuel. It is known that pure cultures of green algae contain primarily C16, C18 fatty acids with a high degree of unsaturation. Cultivation of microalgae requires less land compared to conventional crops which are giving biodiesel. So algae can be used for making biodiesel more efficiently. Hence, there is a larger potential for biomass production from algae with less land requirement. Previous studies on algae-based biodiesel production shown average algae biomass productivities of 30 dry tons/acre/year, which is 10 times more productive than wheat. Because of their higher photosynthetic efficiency, higher biomass production, and faster growth compared with other energy crops, microalgae

have been receiving attention as candidates for fuel production (Minowa et al., 1995)

Table 2.5 Comparison of oil yields from typical energy crops versus microalgae. Adopted from Mata et al. (2010).

Plant Source:	Oil yield a*	Area b*	Biodiesel c*
Corn/Maize (Zea mays L.)	172	66	152
Hemp (Cannabis sativa L.)	363	31	321
Soybean (Glycine max L.)	636	18	562
Jatropha (Jatropha curcas L.)	741	15	656
Camelina (Camelina sativa L.)	915	12	809
Canola/Rapeseed (Brassica napus L.)	974	12	862
Sunflower (Helianthus annuus L.)	1070	11	946
Castor (Ricinus communis)	1,307	9	1,156
Palm oil (Elaeis guineensis)	5,366	2	4,747
Microalgae (low oil content)	58,700	0.2	51,927
Microalgae (medium oil content)	97,800	0.1	86,515
Microalgae (high oil content)	136,900	0.1	121,104

a*-L oil/ha year

b*-m^2 year/kg biodiesel

c*-kg biodiesel/ha year

Commercial production of microalgae biofuel with waste water is possible only when we can obtain the conditions such as high biomass productivity, high lipid content and productivity and high tolerance to wastewater. The fact is creating nutrient starvation after reaching the exponential growth

stage enhance lipid accumulation. But higher lipid accompanies lower biomass productivity, consequently overall productivity decreases. In order to increase the sustainability of the overall process, the problems associated with the processing are to be overcome. The overall cost of production of biodiesel could still be minimized with sequestration of CO_2 from flue gas emissions, with wastewater treatment processes, and/or with the extraction of high-value compounds that has significant applications in other industries. Cultivation of algae requires large amounts water, so combining wastewater treatment and algae cultivation is the best solution. Harvesting and drying of algae is costly. So the cost of algal biodiesel production significantly reduced by using the most cost effective method for harvesting and drying. Use of mixed native algae species for cultivation in wastewater, can improve and promotes the biomass and lipid productivity. Wastewater, flue gas, and waste organic carbon resource supply microalgae with nutrients, inorganic and organic carbon resources. For achieving higher biomass and lipid for biofuel production, the combination of wastewater and different carbon resource can be used in different growth mode. For the sustainable, cost-effective production of microalga biodiesel, the potential of continuous production of bioethanol and methane from microalgae post-extracts can be considered. Table 2.2 Comparison of oil yields from typical energy crops versus microalgae

Chapter 3

Methods and methodology

3.1 Introduction

In this chapter, the methods followed in experiments are described. It includes the experimental design, material selection, description of kinetic models, and Protocols followed in analyzing various water quality parameters like the nutrient removal efficiency pH, COD, TS, VSS, TOC, TN, TP, Chlorophyll a'andd Chlorophyll 'b'.

3.2 Collection of Wastewaters and micro algae

3.2.1 Cultivation of Microalgae

The mixed culture of microalga *Chlorella Vulgaris, Scenedesmus quadricauda and Botriococcus brounii* are used for inoculum in this study. The mixed culture of algae was collected from Jawaharlal Nehru Technological University, Hyderabad (J.N.T.U.H) shown in the figure 3.1 (a). The batch culture consists of mixed algal cells in 1 L conical flask with BG11 media under sunlight (12:12 light-dark cycle) at atmospheric temperature for a period of 10 days as shown in figure 3.1 (b). The mixing was provided by using air spargers. After 10 days the culture was upscaled to 15 liter container as shown in figure 3.1 (c).
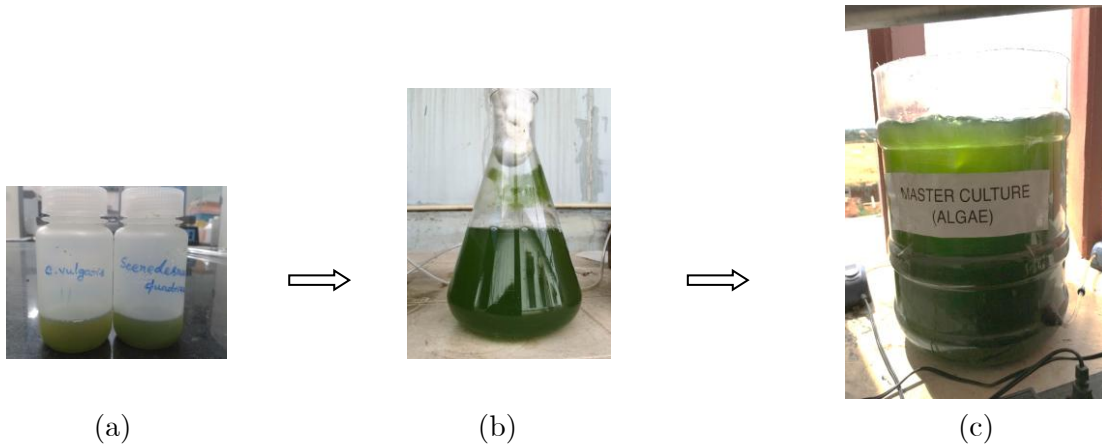
Figure 3.1: Cultivation of microalgae (a) Inoculum, (b) Seed culture, (c) Algae master culture reactor

3.2.2 Growth media

The formulation shown in Table 3.1, was used as the control. It contains macro and micro nutrients necessary for the growth of microalgae. The stock solutions were prepared as needed and stored in the fridge until final media preparation.

Table 3.1: BG 11 media composition

Composition	
$NaNO_3$	1. g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
Glucose	0.51g
Distilled water	1.0 L

3.2.3 Collection of wastewater

The kitchen wastewater was collected from the IIT Hyderabad mess (Figure 3.2), Palm Oil Mill Effluent was collected from Navbharat agro industries, Jagareddygudem, Hyderabad. The pharmaceutical wastewater was collected from a Pharmaceutical industry in Hyderabad. The wastewaters were stored in freezer at -20^0C.

Figure3.2: Collection of KWW

3.3 Experimental design

The studies were conducted in 500 mL wide mouth transparent glass bottles, equipped with cylindrical sandstone connected with plastic tubings to fish tank air-spargers as shown in the Figure. 3.3. The air spargers supply air to the test reactors and also induce mixing. Additional mixing was applied daily prior to sampling by hand using glass rods. The reactors were operated in sunlight with a 12:12 light-dark cycle at atmospheric temperature. In this experiment, three different types of

wastewaters and BG 11 with glucose (control) were used to study the performance of a mixed algal culture in treating wastewater. For each wastewater, 8 bottles were used for 8 different HRT/SRTs-2,4,6,8,10,12,14 and 16 days, which were operated on a draw-and-fill semi-continous mode. Initially the reactors have been inoculated with 100 ml of mixed microalgal culture from algae master culture reactor and the remaining portion was filled with wastewater(substrate). The experimental setup has been shown in the figure 3.4. The reactors were operated for a period of 3-SRTs. The reactors were fed with the substrate every day at the same time. Evaporation was filled up with deionized water accordingly before feeding.

Figure 0.3: Algae reactor

Figure 0.4: Experimental setup

3.4 Steady state kinetics Steady state kinetic parameters (Y, K_S, K_d, k, μ_m) were determined by fitting experimental data into linearized Michaelis-Menten and Monod models.

$$r_{su} = -\frac{kXS}{K_S + S} = \frac{-S_0 - S}{\theta}\ldots\ldots\ldots\ldots\ldots(1)$$

Where r_{su} = Rate of substrate utilization g/m^3-d

k = Maximum specific substrate utilization rate, g substrate/g of microorganism-d

K_S = Half velocity constant g/m^3

θ = Solids retention period, days

S   = Growth limiting substrate concentration, mg/l

S_0 = Initial substrate concentration, mg/l

X = biomass MLVSS mg/l

The linearized form obtained from Lineweaver burk model **from** the equation 6 is

$$\frac{\theta X}{S_0 - S} = \frac{K_S}{k} * \frac{1}{S} + \frac{1}{k} \dots\dots\dots\dots\dots\dots (2)$$

By using the above equation 7 we can find k and K_S Values by plotting $\frac{1}{S}$ On x-axis and $\frac{\theta X}{S_0 - S}$ On y-axis which gives slope $\frac{K_S}{K}$ and intercept $\frac{1}{K}$ As shown in the Figure 3.6.
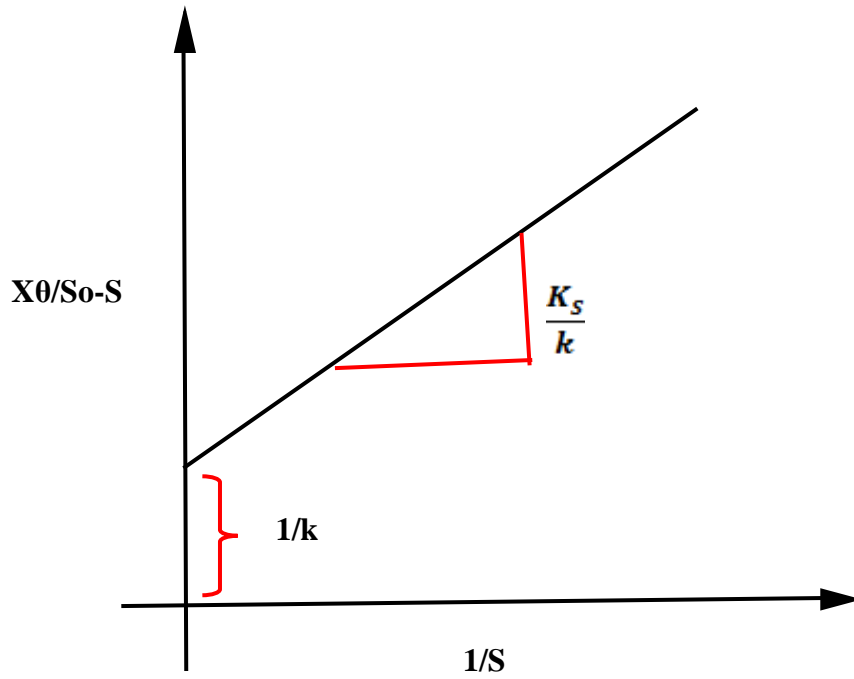
Figure 3.5: Lineweaver Burk plot 1/S vs Xϑ/So-S for determination of k and Ks

To find k_d,Y the relation used is

$$\frac{1}{\theta} = \frac{kYS}{K_S + S} - k_d \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Equation 8 is transformed to a linearized equation

$$\frac{1}{\theta} = -Y * \frac{S_0 - S}{\theta X} - k_d \ldots\ldots\ldots\ldots (4)$$

By plotting $\frac{1}{\theta}$ On y axis and $\frac{S_0 - S}{\theta X}$ On x-axis the slope gives the value

of Y and the negative intercept k_d As shown in the Figure 3.7.

Where Y = yields coefficient, g/g

k_d = decay coefficient (1/d)
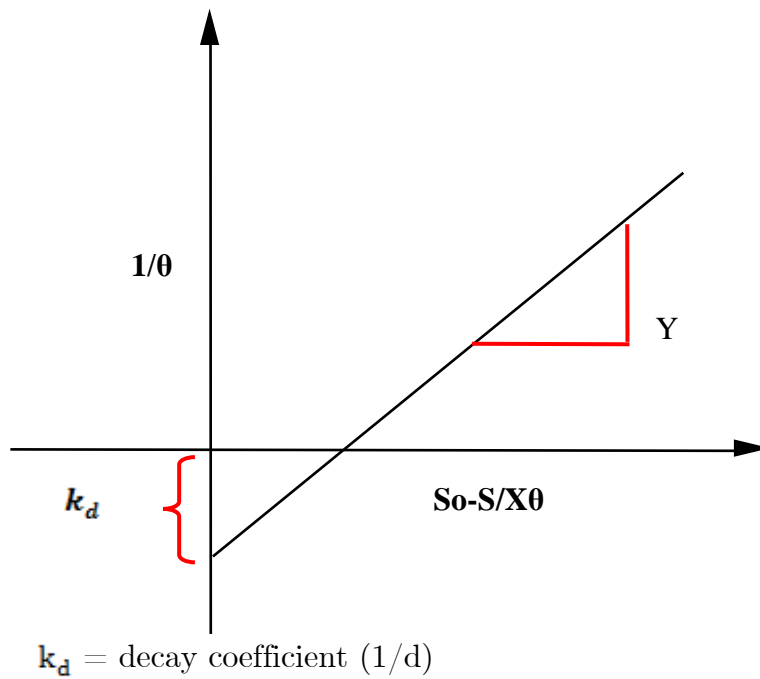
Figure 3.6: $1/\vartheta$ vs So-S/Xϑ for determination of kd and Y

25

3.5 Analytical methods

The influent and effluent samples were tested for chemical oxygen demand (COD), Solids, Volatile Suspended Solids, Total Phosphorous, Chlorophyll 'a' and chlorophyll 'b' following Standard Methods for Examination of Water and Wastewater (APHA)

TOC and TN were measured using TOC analyzer (make: Shimadzu) following procedure given in the operating manual.

3.5.1 Lipid extraction

At the end of experiment, algae from each reactors were filtered and dried at 60^0 C in oven as shown in figure 3.7(a). The dried algal biomass was finely powdered using a mortar and pestle as shown in figure 3.7 (b & c) . Lipid extraction was done following Modified Bligh and Dyer Method (Bligh,E.G. and Dyer,W.J. 1959). In this method 1.25 ml of chloroform and 2.5 ml of methanol (1:2 v/v) were added to each 100 mg of the dried, powdered algal biomass. The samples were shaken in a shaker for 15-20 min,1.25 ml of chloroform was added to each sample and then shaken on a vortex, which was followed by addition of 1.25 ml of water, and the samples were shaken again. The samples were centrifuged for 10 min at 600 x g to obtain two layers. The lower layer of each sample was transferred to pre-weighed aluminium dish and kept in oven at 80^0C for 1 hour as shown in figure 3.7(d). Lipid productivity (%) was calculated based on the ratio of total oil extracted to dry weight of algae as shown below

Lipid productivity = g of oil/dry weight of biomass

(a)

(b)

(c)

(d)

Figure 3.7: Lipid extraction (a) Drying algae, (b) Powdering algae by using mortar and pestle, (c) Algae powder, (d) Extracted lipid

3.5.2 Transesterification

Algae powder of 100 mg was taken in a vial and 2 ml of chloroform:methanol (2:1, v/v) and 3 ml of 0.6 M HCl:methanol was added. The vials were sealed and vortex well to mix the contents. Then the vials were placed in preheated block and heated at 85°C for 1 hour. After 1 hour vials were removed and cooled for at least 15 minutes, at room temperature.

After cooling to room temperature, 10 mL HPLC grade hexane was added to each of the vials vortexed well and left undisturbed at room temperature for 1 hour to allow phase separation. After phase separation the hexane layer was transferred to the GC vial for FAME analysis

3.5.3 Analysis of FAME

FAME composition was estimated by gas chromatography (GC-MS 400 series; Bruker) using flame ionization detector (FID), capillary column BR-5MS (FS 15 meter 0.25 mm ID, 0.25 µm df) and helium as carrier gas (flow rate, 1 mL/min). The temperature of the oven was initially maintained at 80^0 C for 2 min, later increased to 250 ^{0}C at a ramp for 55 min. The injector and detector temperatures were maintained at 230 and 300 C respectively, with a split ratio of 100:1. FAME composition obtained was compared with the Standard FAME mix C4–C24 (Supelco, USA).

Chapter 4

Results and Discussions

The test reactors were allowed to attain steady states at different SRTs before sample testing and further analyses were conducted. The different types of media used for this study were BG 11 medium with glucose, Domestic wastewater, Cow dung solution, Industrial wastewater from Chaithanya and Palm oil effluent. Bioreactors fed with cow dung slurry failed to grow algae despite same external environmental conditions of other reactors.

4.1 Characteristics of wastewater

The initial characteristics of the wastewaters , performed after centrifugation at 4000 rpm, are shown in Table 4.1. The COD of all wastewaters were brought to 500 mg/L by diluting and the pH of the wastewaters were made to 7.5 before feeding to the reactors.

Table 4.1: Initial characteristics of the wastewater

Wastewater type	pH	COD (mg/L)	TOC (mg/L)	Nitrogen (mg/ L)	Phosphate (mg/L)	C:N:P Ratio (in weight ratio)
BG11	7.1-7.5	24 -36	10.3-18	240	5.34	2:44:1
Kitchen WW	5-6	1000-1200	260	20-30	16	29:1:1
Pharmaceutical WW	7-8	2000-3600	800-1450	258-560	20-25	110:66:1
Palm oil effluent	7-7.5	7500-7800	2500-3000	210-360	105-150	13:2:1

4.1.1 Daily variations in COD, TN and TP of raw wastewaters

The wastewaters collected from different sources were subjected to centrifugation at 4000 RPM for 20 minutes, followed by filtration to remove suspended particles. Fig 4.1-4.3 shows the daily variation of influent concentrations of COD, TN and TP of different wastewaters used in this study.

4.1.1 Kitchen wastewater

Figure 4.1 shows the daily variations of influent concentrations of TN, TP, and COD added to the reactors. Kitchen wastewater was collected daily and diluted 2 times to adjust it to 500 mg/L COD. The daily influent concentration of COD, TN and TP were 500±88 mg/L, 50±5 mg/L and 8± 0.4 mg/L respectively.
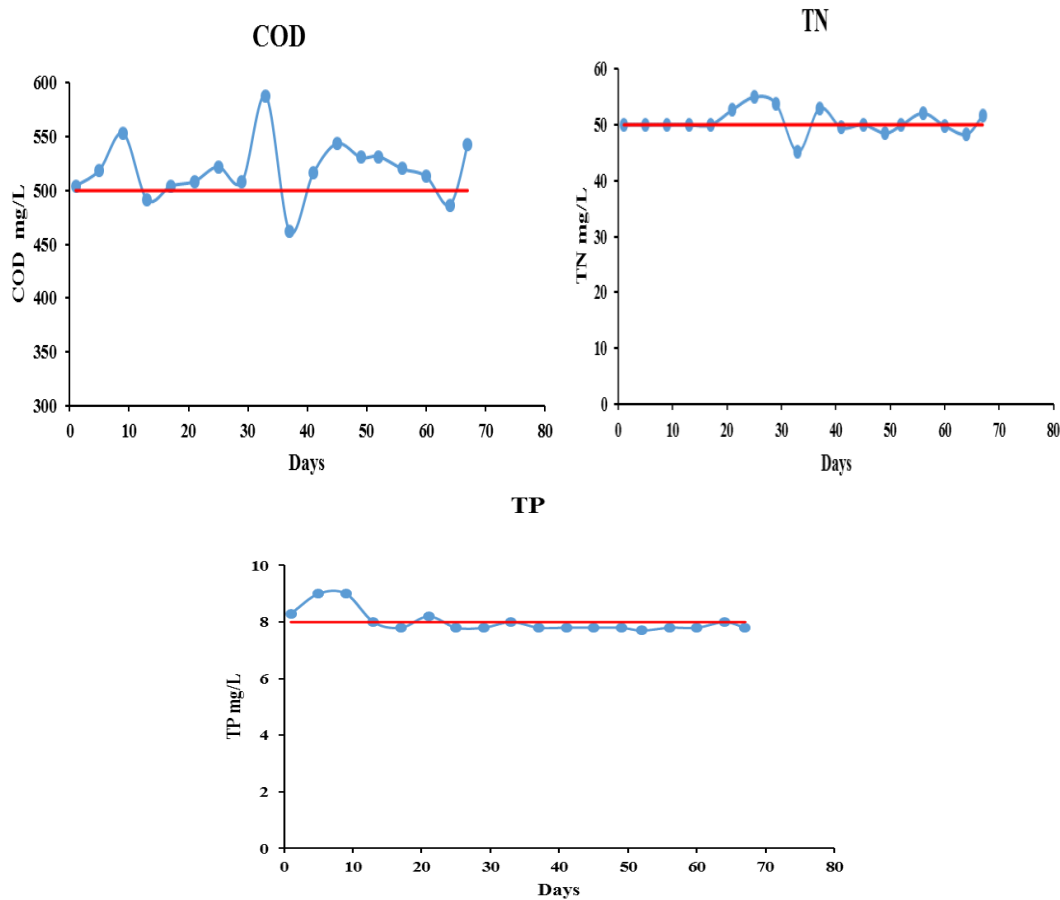
Figure 4.1: Daily variation of influent concentrations in KWW

4.1.2 Pharmaceutical wastewater

Figure 4.2 shows the daily profiles of inflow concentrations of TN, TP, and COD added to the reactors. Industrial wastewater collected prior to the starting of experiment stored in freezer at -20 ^{o} C after centrifugation and filtering. The daily influent concentration of COD, TN and TP were 500±52 mg/L, 120±7 mg/L and 5± 0.4 mg/L respectively.
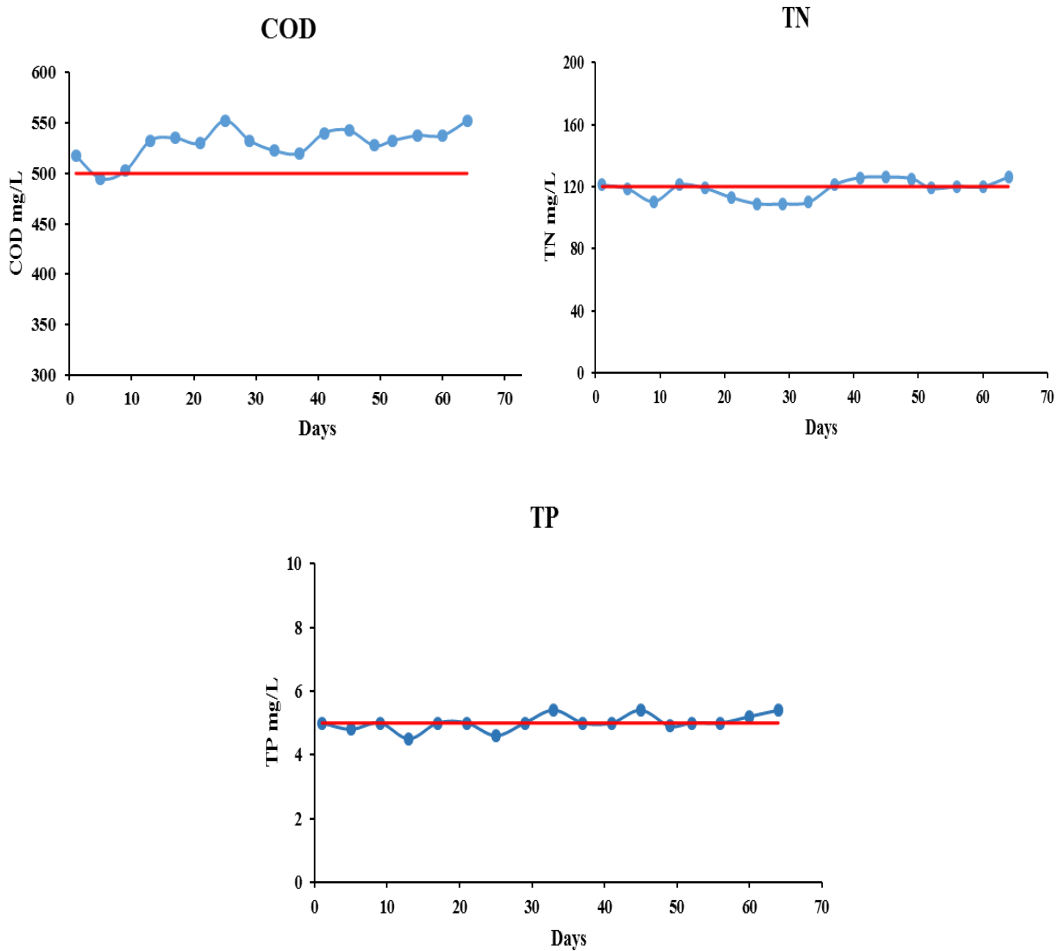
Figure 4.2: Daily variation of influent concentrations in IWW

4.1.3 Palm Oil Effluent

Figure 4.3 shows the daily profiles of inflow concentrations of TN, TP, and COD added to the reactors. After centrifugation and filtering of POME collected prior to the start of the experiment stored in freezer at -20 ^{o} C. The daily influent concentration of COD, TN and TP were 500 ± 30 mg/L, 38 ± 3 mg/L and 20 ± 4 mg/L respectively.
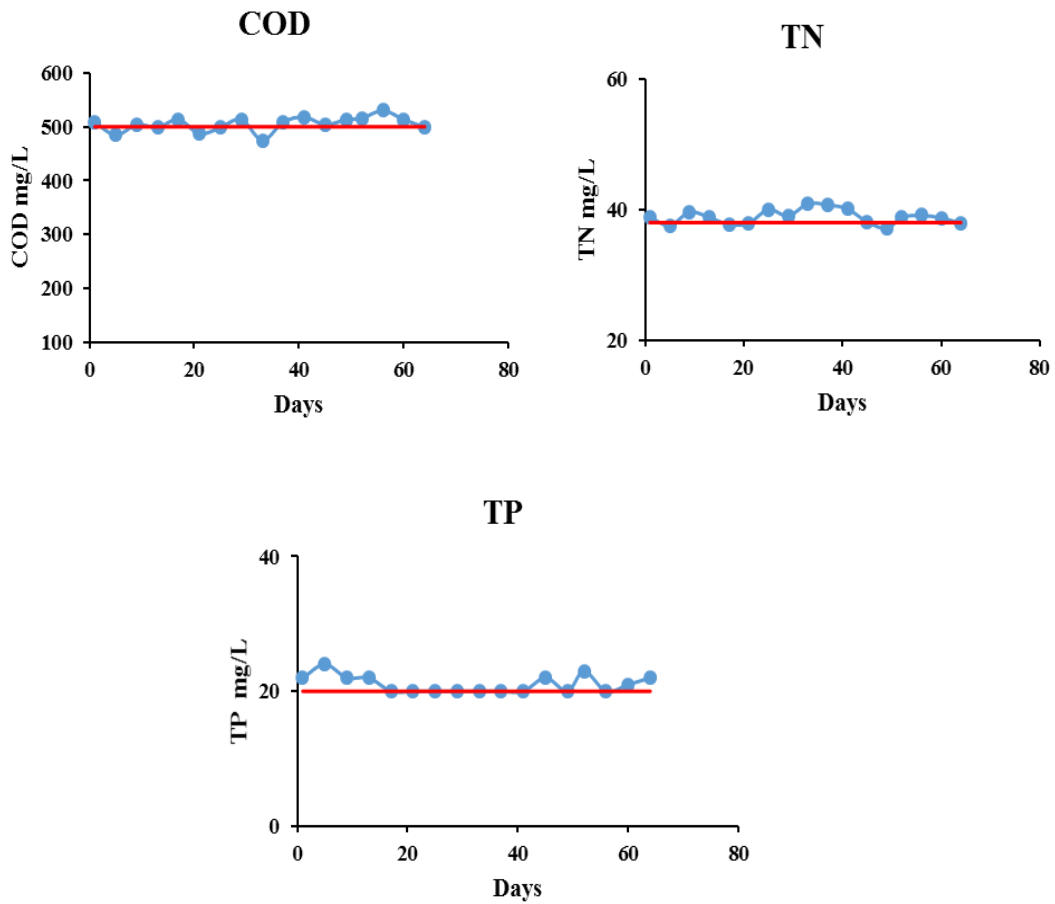
Figure 4.3: Daily variation of influent concentrations in POME

4.2 Characteristics of Algae

Before starting the experiment, the algae sample from master culture reactor was analyzed to find the parameters such as VSS, chlorophyll 'a', chlorophyll 'b' and OD at 650nm ,which were shown in table 4.2. The algae species present in the reactors were dominated by *Chlorella Vulgaris* and *Botriococcus Brounni*. Based on different dilutions the calibration curves relating OD,650 nm to chlorophyll 'a' and VSS were plotted and shown in figure 4.4(a) and 4.4 (b) repectively. A linear correlation was observed between chlorophyll 'a' vs OD ,650 nm and VSS vs OD,650 nm with coefficient of determination $R^2=0.99$.

Table 4.2: Characteristics of Algae

Parameters	Range
VSS (mg/L)	1050 – 1290
Chlorophyll a (µg/ml)	3.6 - 4.20
Chlorophyll b (µg/ml)	5.3 – 5.74
OD at 650 nm	2 – 2.4
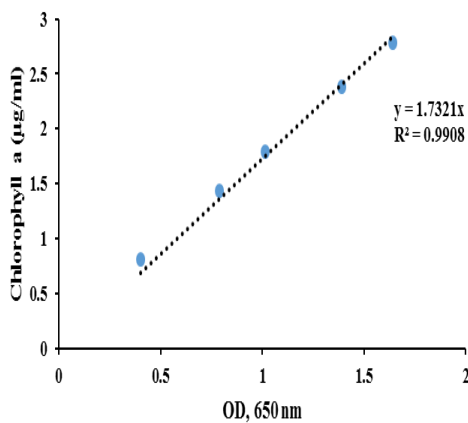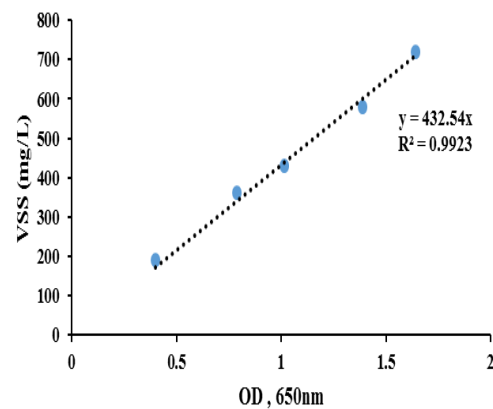
(a)   (b)

Figure 4.4 Correlation curves relating **(a)** chlorophyll'a' vs OD,650 nm, (b) VSS vs OD,650 nm

4.3 Nutrient Removal Semi-Continuous Experiment

Nutrient removal was determined by comparing the influent and effluent concentrations of COD, TOC, TN and phosphate throughout the experiment. The algae was initially cultivated in nutrient-rich growth media, BG11 and then transferred to reactors after reaching exponential growth phase. For the first 3 days, microalgae were allowed to grow in the reactor without wastewater replacement.

4.3.1 Kitchen wastewater

The reactors were fed with the Kitchen wastewater and experiments were conducted at different SRTs as mentioned previously. The amount of Total Nitrogen in the kitchenwastewater was 10 ppm after diluting to adjust the COD to 500 mg/L. Since algae previously cultivated in nutrient rich BG11 medium (TN-240 mg/L), the relatively small amount of Total nitrogen concentration in KWW (TN-10mg/L) progressively affected the algae growth in 2, 4, and 6 days SRTs during the experiment. It might be due to the sudden change in nutrient lavels. After noticing the declining of algae in bioreactors extra $NaNO_3$ corresponding to 40 mg/L of Total nitrogen was added daily to the influent wastewater which is corresponding to medium domestic wastewater Total nitrogen concentration(40 mg/L). The optimum C:N:P ratio for microalgae is 106:16:1 molar ratio or 56:9:1 C:N:P in weight ratio(Hadiyanto and M.M. Azimatun Nur, 2014). The level of Total nitrogen in the culture medium decreased slowly with time in all the reactors. After 50 days, the TN levels remained relatively constant till the end of the experiment (Fig. 4.5 a).
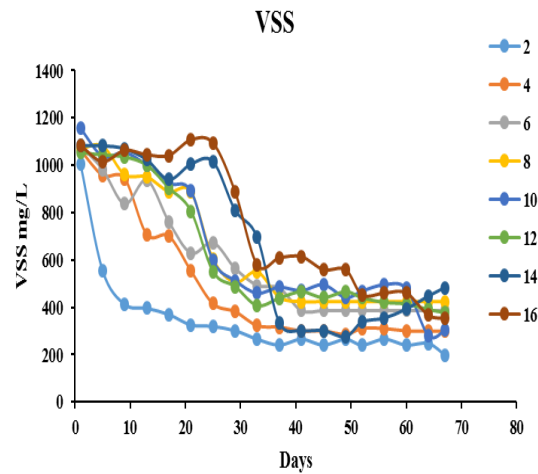
The amount of Phosphate in the Kitchen wastewater was 8 ppm after diluting it to 500 COD. Fig 4.5 (b) shows the variation in concentration of phosphorus in the

reactor for the entire period of the experiment. The concentration of phosphate decreased gradually and reached steady state within the first 50 days. There was an initial decrease in the biomass concentration followed by an increase before reaching steady state, as displayed in Fig 4.5(d).
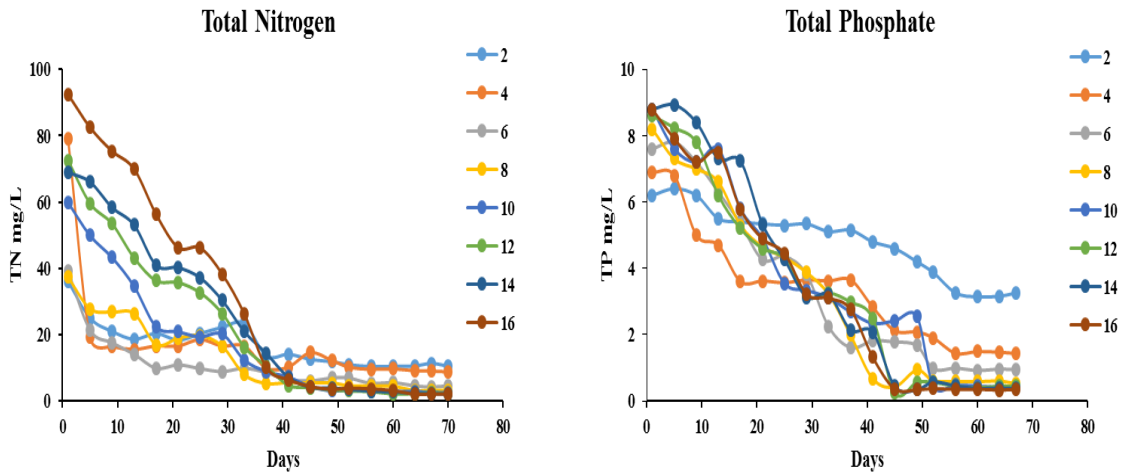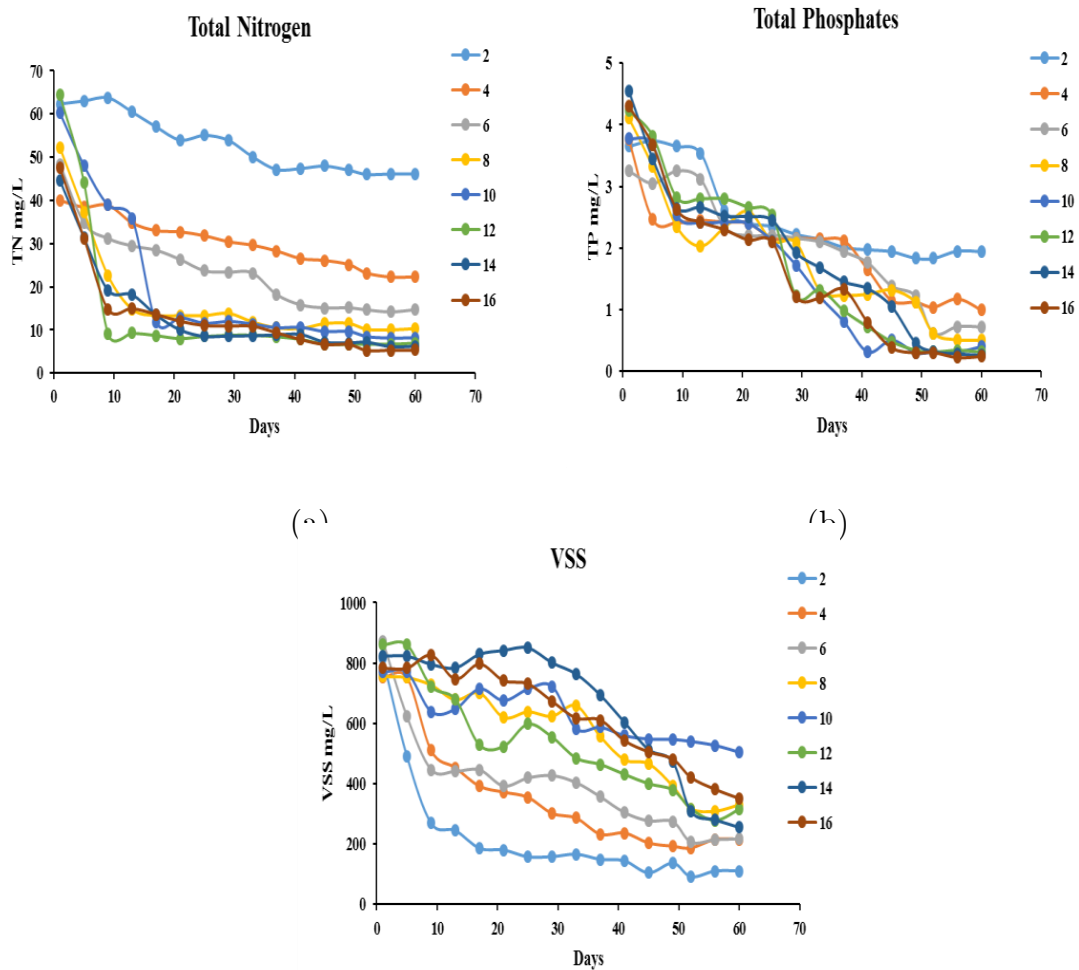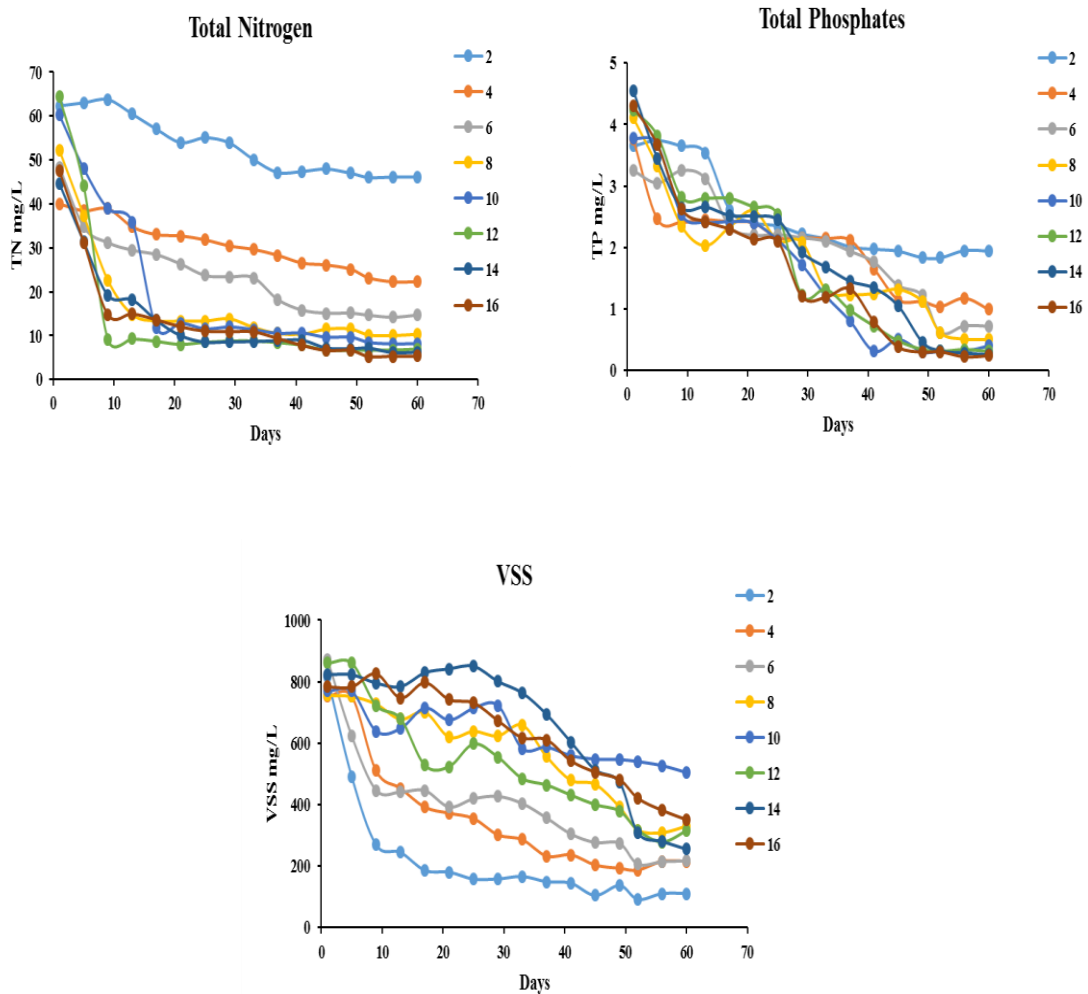

(a)

(b)

(c)

Figure 4.5: Daily variation of effluent parameters (a) Total Nitrogen, (b) Total Phosphate, (c) Volatile Suspended Solids in KWW

4.3.2 Industrial wastewater

The amount of Total nitrogen in the Industrial wastewater was 60 ppm after diluting it to 500 mg COD/L. The level of Total Nitrogen in the culture medium decreased slowly in all reactors with time. It reached a steady state after 40 days.

The concentration of Phosphate in the industrial wastewater was 5 ppm after diluting it to 500 mg COD/L. Fig. 4.6 shows the reduction pattern of phosphorus in the reactor for the entire period of the experiment. From the figure 4.6, it appeared that biomass growth declined in each reactor over the period of the first 40 days after that followed a steady state. The decline of VSS was greater in the reactors with the lower SRTs
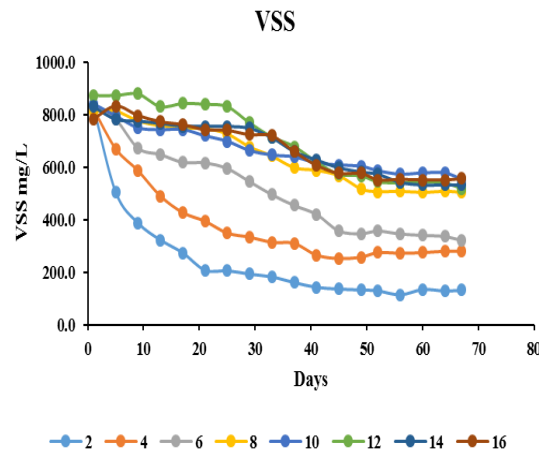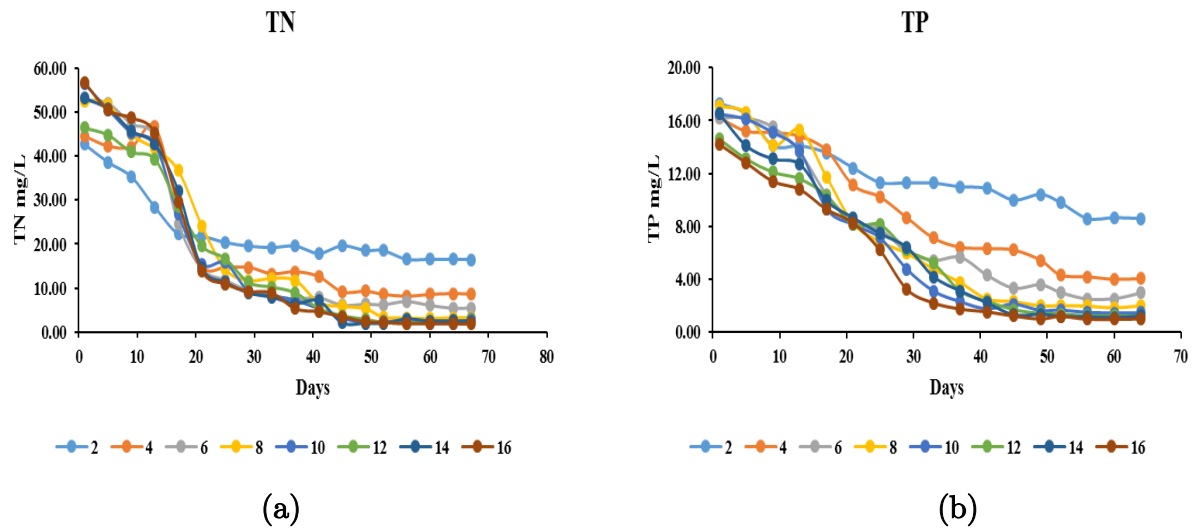

(a)

(b)

Figure 4.6: Daily variation of effluent parameters (a) Total Nitrogen, (b) Total Phosphate, (c) Volatile Suspended Solids in IWW

4.3.3 Palm Oil Effluent

Total amount of the nitrogen in the Palm Oil Effluent was 38 ppm after diluting it to 500 mgCOD/L. The level of Total Nitrogen in the culture medium decreased slowly in all reactors with time. It reached a steady state after 50 days.

The amount of Phosphate in the POME was 20 ppm after diluting it to 500 mgCOD/L. Fig 4.7 shows the reduction pattern of phosphorus in the reactor for the entire period of the experiment. The concentration of phosphate decreased gradually within the first 40 days and then reached a steady state condition.

Figure 4.7: Daily variation of effluent parameters (a) Total Nitrogen, (b) Total Phosphate, (c) Volatile Suspended Solids in POME.

4.3.4 Control

Total amount of the nitrogen in the Control was 240 ppm after diluting it to 200 mg COD/L. The level of Total Nitrogen in the culture medium decreased slowly in all reactors with time. It reached a steady state after 50 days.

The amount of Phosphate in the Control was 5.3 ppm after diluting it to 200 mg COD/L. Fig 4.8 shows the reduction pattern of phosphorus in the reactor for the entire period of the experiment. The concentration of phosphate decreased gradually within the first 50 days and then reached a steady state condition.

(a)

(b)

(c)

Figure 4.8: Daily variation of effluent parameters (a) Total Nitrogen, (b) Total Phosphate, (c) Volatile Suspended Solids in control

4.4 Nutrient removal efficiencies

The nutrient removal efficiencies of different wastewaters were plotted with SRTs as shown in figure 4.9. It was observed that kitchen wastewater showed highest nutrient removal compared to IWW and POME. The removal efficiency progressively increased with increasing SRTs for POME,IWW and control. But in the case of KWW though the nutrient removal percentage increased with SRT surprisingly, it was observed that after 12-d SRT the nutrient removal percentage decreased for 14-d and 16-d SRTs. It might be due to the decrease in algal concentration. in 14-d and 16-d SRTs. The highest removal efficiency of TN for KWW, IWW, POME and control were 50%, 33%, 24% and 35% respectively. The highest removal efficiency of TP for KWW, IWW, POME and control were 47%, 35%, 30% & 26% respectively.
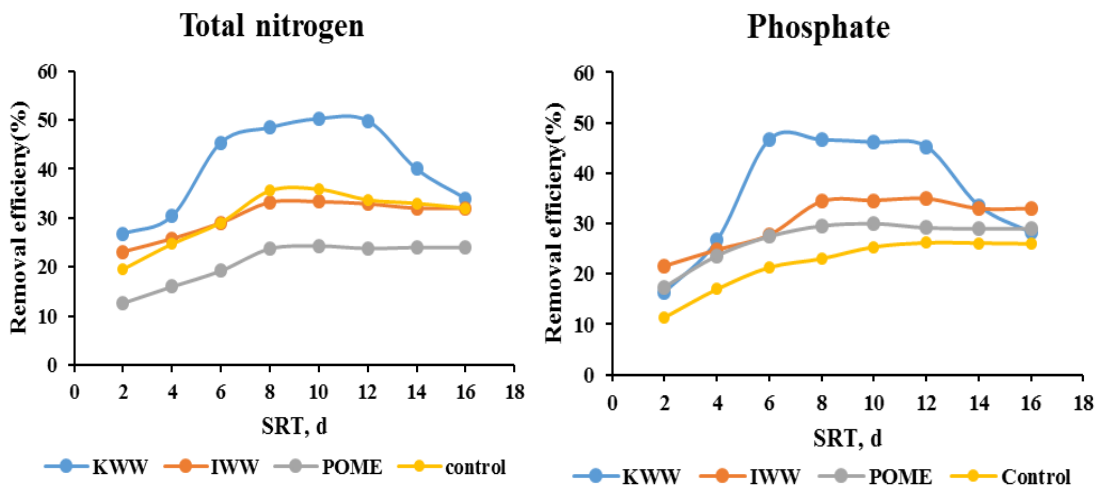

Figure 4.9: Total Nitrogen and phosphate removal efficiencies

4.5 Kinetic evaluation

The kinetic parameters of Nitrogen and Phosphorus uptake for different types of wastewater were calculated Based on method described in chapter 3

4.5.1 Nitrogen uptake kinetics

The bio-kinetic parameters of nitrogen uptake for different wastewaters were plotted as discussed in chapter 3 were shown in figure: 4.10. The kinetic parameters , maximum specific substrate utilization rate k, g substrate/g of microalgae-d, Half velocity constant Ks g/m^3, Yield coefficient Y, Decay coefficient Kd (d^{-1}), and specific growth rate (μ_{max}) were determined for nitrogen uptake were shown in Table 4.3.
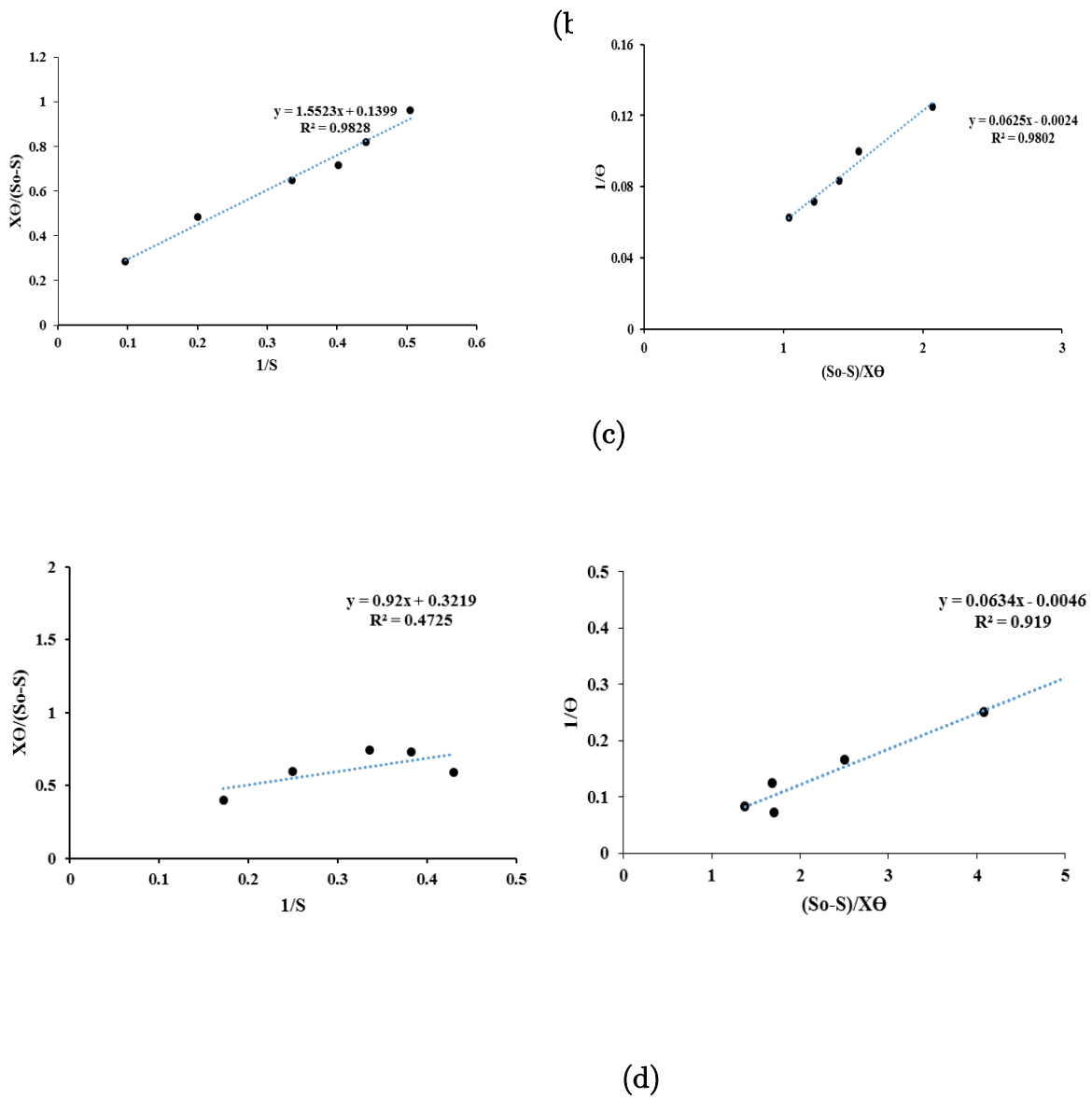

a)

(b)

(c)

(d)
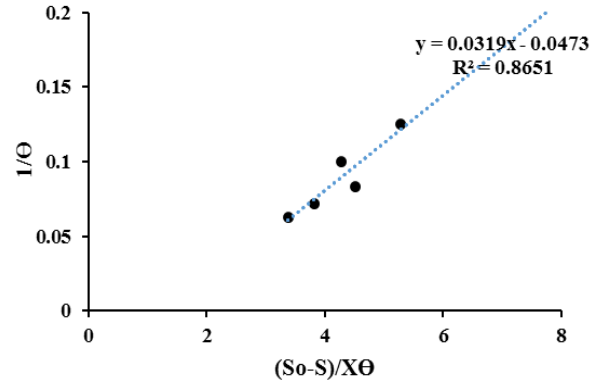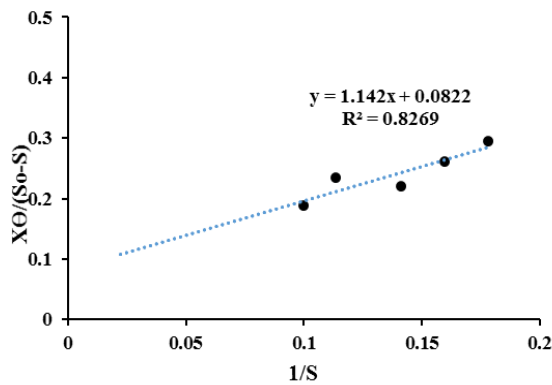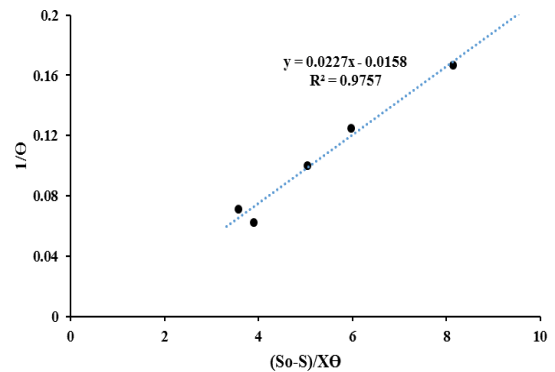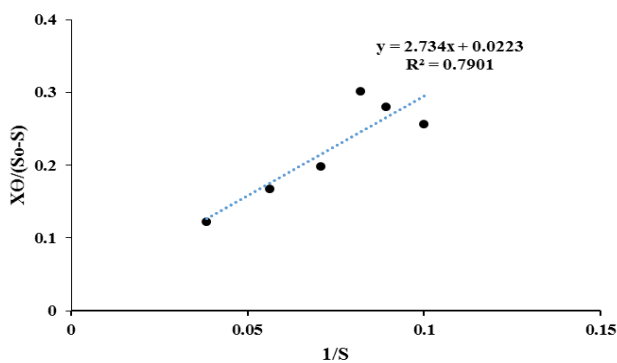
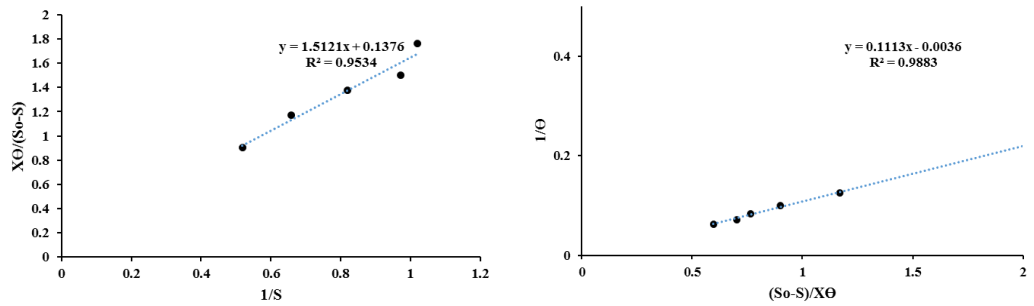**Figure 4.10**: Nitrogen uptake kinetics for (a) IWW, (b) KWW,(c)POME &(d) control

Table 4.3: Nitrogen uptake kinetics parameters

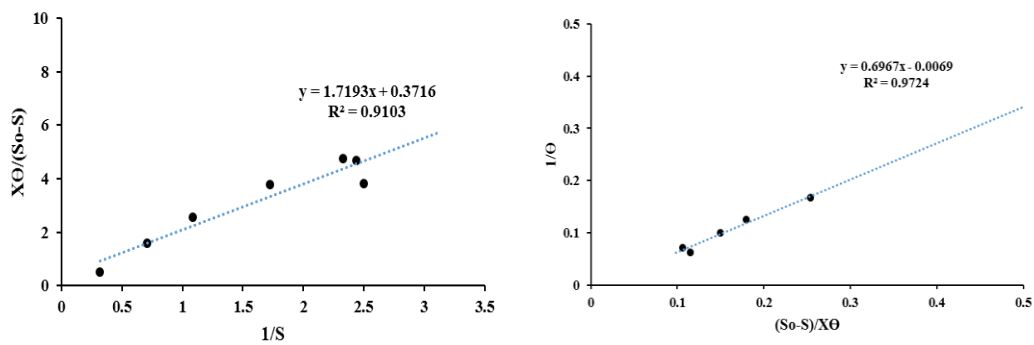| Algal Species	Type of WW	k (1/day)	Ks (mg N/L)	Y	Kd (1/day)	μm (1/day)	Reference
Mixed microalgae	KWW	3.1	0.28	0.06	0.004	0.19	This Study
	IWW	7.5	6.1	0.03	0.017	0.38	
	POME	7.14	11.09	0.09	0.037	0.68	
	Control	36.43	1.44	0.02	0.012	0.87	
Chlorella vulgaris	Synthetic ww	1.5	31.5	0.15		0.225	Aslan et. al(2006)
Chlorella vulgaris	BBM	8.47	19.4	0.0119			Rowley(2010)
Aerobic bacteria	Domestic ww	1-30	0.2-0.5	0.3-1	0.03-0.06		Metcalf Eddy

The kinetic parameters obtained in this study were found to be similar with literature as shown in table 4.3. Increased values of Ks were observed in IWW, POME and control compared with aerobic bacteria which shows the decrease in specific growth rate. It reveals that there is only limited amount of substrate in the wastewater. The Y value obtained for different wastewater were less compared with aerobic bacteria. The rate of the substrate utilization by algae was found to be in range of aerobic bacteria. The improved value of k in control appeals that the utilization rate of substrate by algae was higher with the desired concentration of the biomass and increased concentration of the substrate.

4.5.2 Phosphorus removal kinetics

The maximum specific substrate utilization rate k, g substrate/g of microalgae-d, Half velocity constant Ks g/m^3, Yield coefficient Y, Decay coefficient Kd (d^{-1}), and specific growth rate (μ_{max}) were determined for nitrogen removal are shown in Table 4.4. The amount of biomass produced by the growth during the removal of phosphorus was low in Industrial wastewater compared to the other wastewater. The graphs plotted for calculating kinetic parameters are shown in fig 4.11
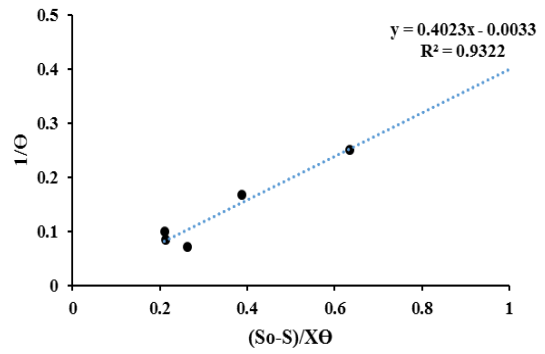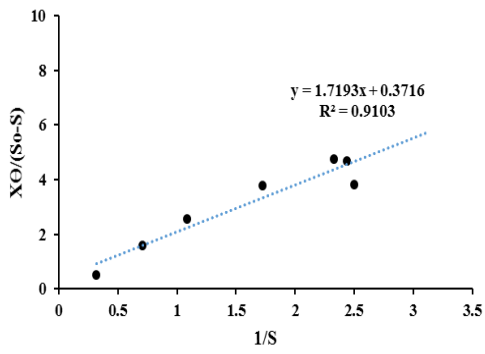

(a)

(b)
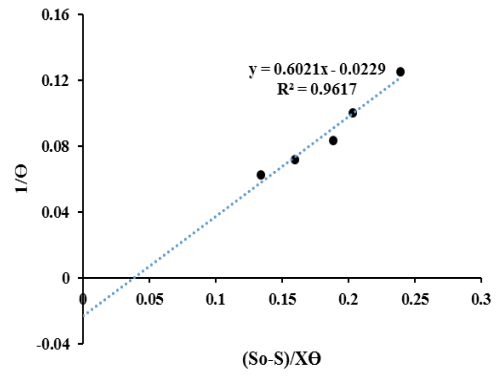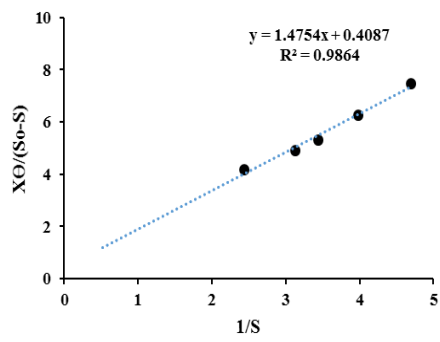
(c)

(d)

Figure 4.11: Phosphorus uptake kinetics for (a) IWW, (b) KWW, (c)POME & (d) control

<div align="center">Table 4.4: Phosphorus uptake kinetics parameters</div>

| Algal Species	Type of WW	k(1/day)	Ks(mg PO_4^{2-} /L)	Y	k_d (1/day)	μ_m (1/day)	Reference
Mixed microalgae	KWW	4.62	2.69	0.33	0.004	0.901	This Study
	IWW	3.61	2.44	0.60	0.022	1.47	
	POME	10.98	7.26	0.11	0.003	0.80	
	Glucose	4.6	2.69	0.69	0.006	1.87	
Chlorella vulgaris	Synthetic ww	0.5	10.5	0.14		0.07	Aslan et. al(2006)
Chlorella vulgaris	BBM	2.05	1.61	0.037		0.07	Rowley (2010)
Aerobic bacteria	Domestic ww	2-10	15-70	0.3-0.7	0.05-0.12		Metcalf Eddy

The kinetic parameters obtained in this study were found to be similar with literature as shown in table 4.4. Decreased values of Ks were observed in all wastewaters compared with aerobic bacteria which shows the increase in specific growth rate. The Y value obtained for different wastewater were within the range of compared with aerobic bacteria. The rate of the substrate utilization by algae was found to be in range of aerobic bacteria.

## 4.6 Batch study to validate the increase in TOC using BG - 11 medium

During the experimental period, the effluent TOC values for all wastewaters in all SRTs were found to be higher than influent wastewater TOC values which were shown in the figure 4.12. Since there was no removal seen in test reactors in order to validate the experimental results, a batch study was

conducted in duplicates without any carbon source in BG-11 media for a period of 27 days. The reactors were operated in sunlight with a 12:12 light-dark cycle at atmospheric temperature The air spargers were used to supply air to the test reactors and also induce mixing. The daily variation of TOC, TN, Phosphate and VSS for the batch study were shown in Figure 4.13. During the test period, an increment in the biomass was observed in both reactors with significant reduction in Total nitrogen and phosphate. The total organic carbon was increased from 17 mg/L to 50 mg/L, in proportion to biomass growth. The increase in TOC might be due to the release of some extracellular organic carbon compounds from algae. Similar results were reported in M Melkonian (1979) and S. Cho et al.(2011) where the Dissolved Organic Carbon was increased with biomass. During lag and stationary growth phase release of DOC was higher (10-20 % of dry weight) than during log phase growth (1% of dry weight). It was suggested that the observed differences in amount and nature of dissolved organic substances were related to different metabolic pathways which were involved in the transition of several cell types during growth of alga. The presence of this organic compiunds would affect the performance of treatment processes as well as the quality of water (reduction in coagulation efficiency, membrane fouling, presursors of disinfection by-products, etc.) (Henderson et al., 2008).
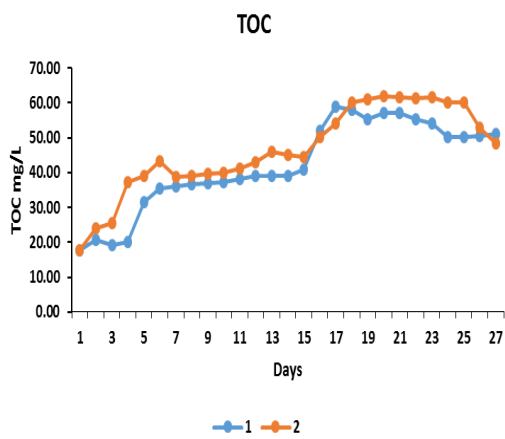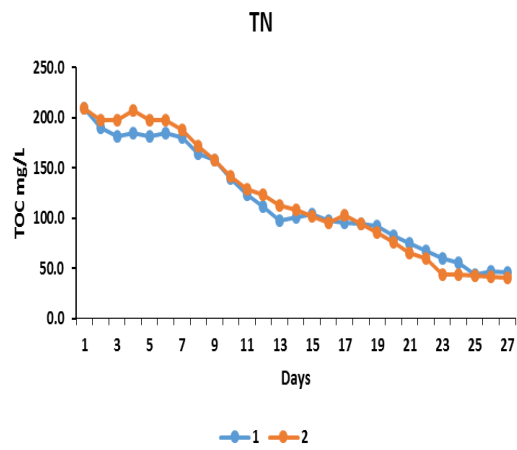

(a)   (b)

46

TOC-KWW

TOC-Control
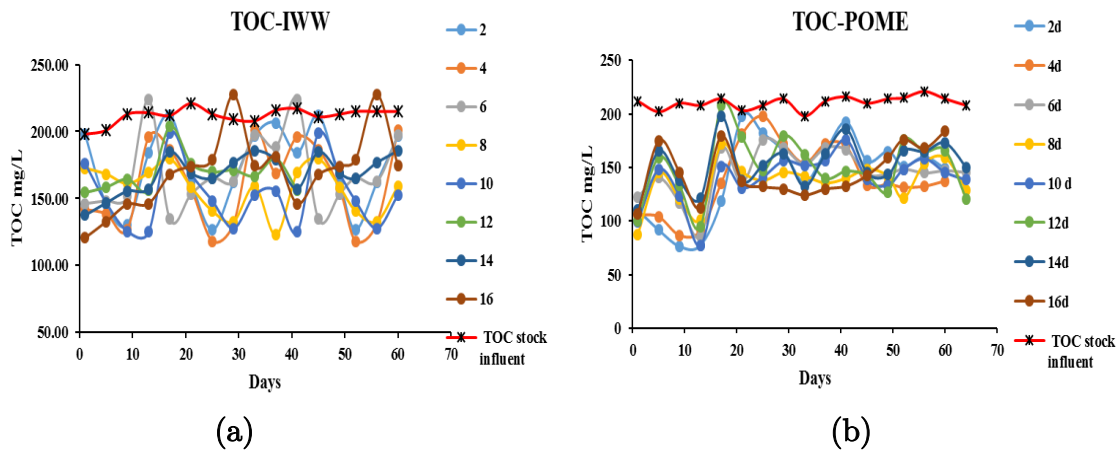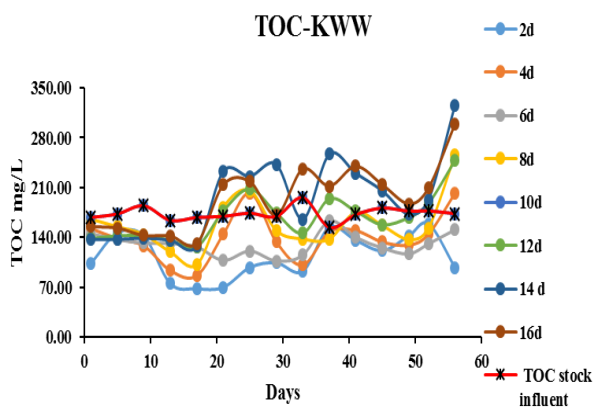
(c)
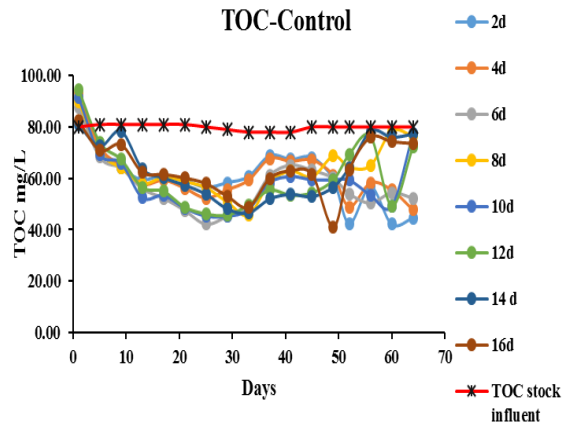
(d)

Figure 4.13: Daily variation of TOC in effluent of wastewaters (a)IWW, (b) POME,(c) KWW, (d) Control.
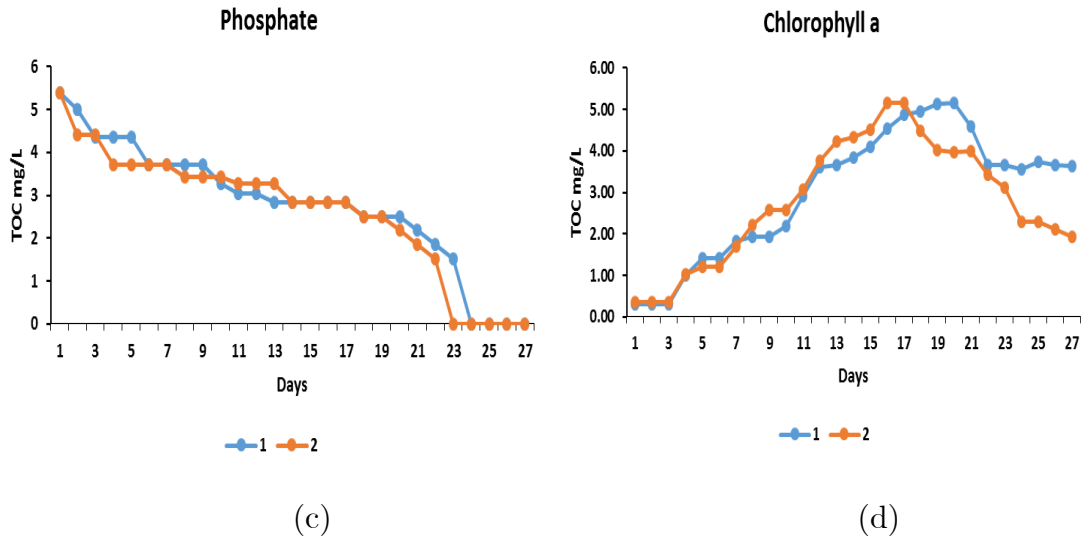

TOC

TN

(a)

(b)

Figure 4.13: Daily variation of parameters in two batch reactors with BG11 (a) TOC, (b) TN, (c) Phosphate, (d) Chlorophyll a

4.7 Lipid production

Lipids were extracted using the modified Bligh and Dyer method. The lipid production (%) of the cultures was calculated based on the ratio of total oil extracted to dry weight of algae (g oil/g algal-biomass dry weight). Total lipid for different wastewaters as shown in table 4.5. It was observed that the lipid content of algae grown in control was very less when compared with other wastewaters., it might be due to excess amount of total nitrogen presented in BG -11 medium. The lipid content of algae grown in IWW and POME were found to increased with increase in SRTs, whearas lipid content of algae grown in KWW were increased with SRTs for 2-10 d, and decreased for 12,14, and 16d SRTs.The algae cultivated in Kitchen wastewater showed lipid concentration increased with increase in SRTs. The two batch reactors with BG-11 medium (without NaCO$_3$) also showed 24% lipid content which was equal to maximum lipid content of algae grown in

wastewater in semi-continous mode, this might be due to nutrient starvation condition.

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Table 4.5: Total Lipid content of Different wastewater with respect toSRT

Type of wastewater	Total Lipid (%)							
	SRT							
	2	4	6	8	10	12	14	16
Control (Glucose)	4.89	2.94	2.46	2.41	3.44	3.46	4.6	3.34
POME	10.52	12	11.8	22.2	20.2	21.70	23.60	24.20
IWW	4.33	9.6	10.1	10.4	11.8	16.2	16.8	17.4
KWW	6.6	12.50	26.8	29.3	28.8	19.6	16.9	17.2
BG 11				24				

4.8 FAME analysis

Fatty acid composition (after transesterification) was studied at the end of the experiment, to study the speciation of fatty acids. Not all lipids can be converted to FAME, therefore, measurement of FAME in algal biomass is a direct indication of the amount of lipids suitable for biodiesel production (Y. Li et al. 2011). Thirty five fatty acids, included in the range C4:0-C24:1 and exceeding a minimum of 0.1% of the total FAs, were identified.

The abundant fatty acid component obtained from different wastewater are Palmitic acid (C16:0), Stearic acid (C18:0), Oleic Acid (C18:1n9c) and Elaidic Acid (C18:1n9t) as shown in figure 4.13. The FAME yield for the algae cultivated in Kitchen wastewater, is 0.05 to 0.13 g-biodiesel/g-dried algae, with citric acid (C18:0) as the most abundant fatty acid derived from algae bodies; Palmitic acid (C16:0) as the second. For algae cultivated pharmaceutical wastewater, the FAME

yield is 0.01 to 0.09 g-biodiesel/g-dried biomass. The most abundant fatty acids obtained from algae were also Palmitic acid (C16:0) and Stearic acid (C18:0). For algae cultivated in Palm Oil effluent wastewater, the FAME yield is 0.01 to 0.11 g-biodiesel/g-dried biomass. The most abundant fatty acids obtained from algae were also Palmitic acid (C16:0) and Stearic acid (C18:0). For algae cultivated in BG 11 medium, the FAME yield is 0.01 to 0.03 g-biodiesel/g-dried biomass. The most abundant fatty acids obtained from algae is Palmitic acid (C16:0). Analysis shows that there was no significant difference in FAME content for algae cultivated in different SRT.
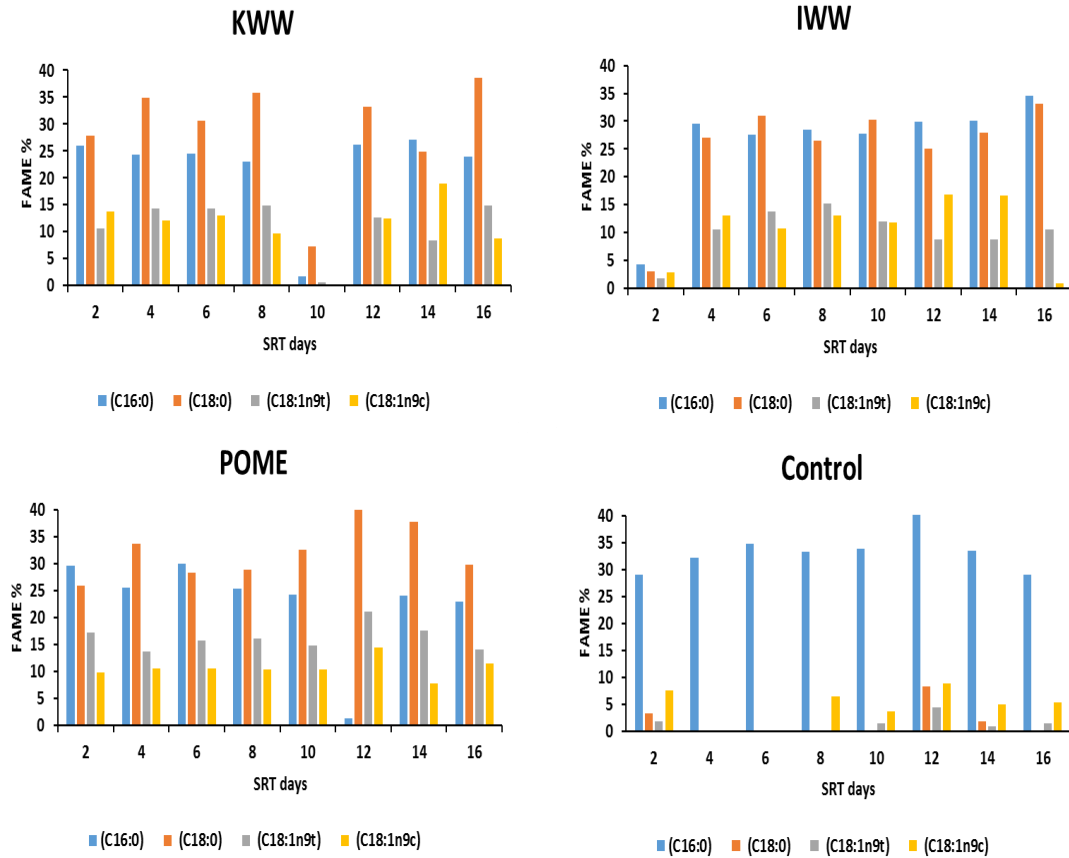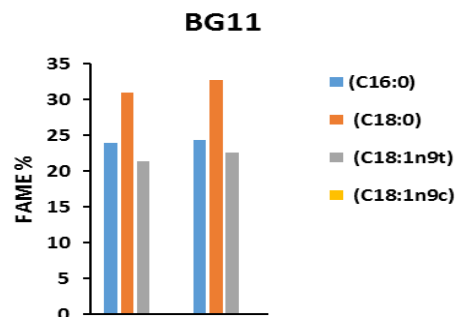
Figure 4.13: Main fatty acid component obtained from different wastewater

The percentage of SFA, MUFA and PUFA obtained for all waste water is as shown in table 4.6. It reveals the high relative percentage of saturated fatty acids than monounsaturated fatty acids and polyunsaturated fatty acids. Lower USFAs induces less risk on the combustion characteristics and ignition delay (Benjumea et al., 2011). It can be clearly observed that SFA is 50% to 70% in all reactors, which is desirable for good quality biodiesel as these offers oxidative stability to the fuel. Oxidative stability is one of the major issues affecting the use of biodiesel because of the presence of polyunsaturated methyl esters in algal oil (Res et al. 2014).

Table 4.6: SFA, MUFA and PUFA profiles of different wastewater with SRTs

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SRT	KWW			IWW			POME			Glucose		
days	SFA %	MUFA %	PUFA %	SFA %	MUFA %	PUFA %	SFA %	MUFA%	PUFA %	SFA %	MUFA %	PUFA %
2	62.69	28.96	8.35	70.31	13.86	15.83	65.80	30.92	3.28	52.69	14.14	33.17
4	66.41	30.22	3.37	64.97	27.41	7.62	**68.54**	28.08	3.38	**61.85**	4.10	34.05
6	63.62	31.00	5.38	68.01	27.76	4.24	64.51	30.22	5.28	56.78	5.83	37.39
8	66.32	28.12	5.57	63.57	32.23	4.65	65.76	30.42	3.82	58.66	10.69	30.65
10	20.72	4.39	74.90	66.65	27.74	5.61	67.68	28.31	4.01	58.55	12.94	28.51
12	66.88	28.51	4.61	62.48	29.56	7.96	52.08	41.07	6.85	61.76	18.47	19.77
14	60.87	31.99	7.14	64.11	29.38	6.51	67.54	29.25	3.22	53.43	10.54	36.03
16	**69.49**	27.21	3.30	**76.89**	16.63	6.48	63.84	29.98	6.18	50.90	15.50	33.60

Chapter 5

Conclusion

KWW showed higher nutrient removal efficiency when compared with the POME, IWW and control. It was observed that the nutrient removal efficiency and lipid productivity increased with increase in SRT for POME,IWW and control and decreased after 12 d SRT for KWW. The lipid content of algae in control was less when compared with other wastewaters might be due to over supplement of nutrients. In this study, the lipids produced from all wastewaters contained over 60 – 70 % of saturated fatty acid (C16:0, C18:0, C18:1) which were considered to be suitable for synthesize of biodiesel. The organic carbon level in the 2 batch reactors were found to increased with days, whereas the nutrient levels (TN, Phosphate) showed more than 80 % removal. The increase in TOC might be due to the release of some extracellular organic carbon compounds from algae which mainly contains proteins, amino acids and carbohydrates. The presence of this organic compiunds would affect the performance of treatment processes as well as the quality of water

References

Amini Khoeyi, Z., J.S. and Z.R., 2012. Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris. *Aquacult. Int.*, (20), pp.41–49.

Astorga-España, M.S. & Mansilla, A., 2013. Sub-Antarctic macroalgae: opportunities for gastronomic tourism and local fisheries in the Region of Magallanes and Chilean Antarctic Territory. *Journal of Applied Phycology*, pp.1–6.

Benjumea, P., Agudelo, J.R. & Agudelo, A.F., 2011. Effect of the degree of unsaturation of biodiesel fuels on engine performance, combustion characteristics, and emissions. *Energy and Fuels*, 25(1), pp.77–85.

Brennan, L. & Owende, P., 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2), pp.557–577. Available at: http://dx.doi.org/10.1016/j.rser.2009.10.009.

Chandra, R. et al., 2014. Bioresource Technology Regulatory function of organic carbon supplementation on biodiesel production during growth and nutrient stress phases of mixotrophic microalgae cultivation. *BIORESOURCE TECHNOLOGY*. Available at: http://dx.doi.org/10.1016/j.biortech.2014.02.102.

Chinnassamy, S., Bhatnagar, A., Hunt, R.W., Das, K.., 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Technol*, (101), pp.3097–3105.

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, 26(3), pp.126–131. Available at: http://dx.doi.org/10.1016/j.biotechadv.2007.02.001.

Cho, S. et al., 2013. Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources. *Bioresource Technology*, 131, pp.515–520. Available at: http://dx.doi.org/10.1016/j.biortech.2012.12.176.

Cho, S. et al., 2011. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. *Bioresource Technology*, 102(18), pp.8639–8645. Available at: http://dx.doi.org/10.1016/j.biortech.2011.03.037.

D. Hernandez., B. Ria~no., M. Coca., and M.C.G.-G., 2012. Treatment of agro - industrial wastewater using microalgaebacteria consortium combined with anaerobic digestion of the produced biomass. *Bioresource Technology*, 135,

pp.598– 603.

Fallowfield, H. D., & Barret, M.K., 1985. The photosynthetic treatment of pig slurry in temperate climatic conditions: A pilot plant study. *Agricultural Wastes*, (12), pp.111–136.

Jacob-Lopes, E., C.H.G. Scoparo, L.M.C.F.L. and T.T.F., 2009. Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photo- bioreactors. *Chemical Engineering and Processing: Process Intensification.*, (48), pp.306–310.

Joshi RM, P.M., 2007. Flow properties of biodiesel fuel blends at low temperatures. *Fuel*, (86), pp.143–51.

Kang R, Wang J, Shi D, Cong W, Cai Z, O.F., 2004. Interactions between organic and inor- ganic carbon sources during mixotrophic cultivation of Synechococcus sp. *Biotechnol Lett*, (26), pp.1429–1432.

Kong, W., H. Song, Y. Cao, H. Yang, S.H. and C.X., 2011. The characteristics of biomass production, lipid accumulation and chloropyll biosynthesis of Chlorella vulgaris under mixotrophic cultivation. *African J. Bio.*, 10, pp.11620–11630.

Lam, M.K. and K.T.L., 2012. Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production,. *Appl. Energy*, (94), pp.303–308.

Mata, T.M., Martins, A.A., Caetano, N.., 2010. Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews*, (14), pp.217–232.

Melkonian, M., 1979. Release of Dissolved Organic Substances by the Green Alga Fritschiella tuberosaIyeng. (Chaetophorineae) During Different Growth Phases. *Zeitschrift für Pflanzenphysiologie*, 94(2), pp.133–125. Available at: http://dx.doi.org/10.1016/S0044-328X(79)80126-9.

Minowa, T., Yokoyama, S.-Y., Kishimoto, M. & Okakura, T., 1995. Oil production from algal cells of dunaliella tertiolecta by direct thermochemical liquefaction. *FUEL*, (74), pp.1735–1738.

Mobin, S. & Alam, F., 2014. Biofuel Production from Algae Utilizing Wastewater. , (December).

N. Nandini., M. Kumar., S. Sivasakthivel., M.V.K., 2013. Efficacy of Microalgae on the Removal of Pollutants from Wastewater. *IJETCAS*, pp.82–86.

Nur, M.M.A., 2014. Lipid Extraction of Microalga Chlorella sp . Cultivated in Palm Oil Mill Effluent (POME) Medium. , 31(5), pp.959–967.

Oswald, W.J., Green, F.B., Bernstone, L.S., Lundquist, I.., 1996. Advanced integrated wastewater pond systems for nitrogen removal. *Water Sci. Technol*, (119), pp.115– 122.

Production, L. & Green, C.O., 2014. Lipid Production by a CO 2 -Tolerant Green

Microalga, Chlorella sp. MRA-1. , 24(5), pp.683–689.

Rastogi, R., Agarwal, S. & Mishra, S.K., 2015. Microalgae : A Promising Energy Source for Sustainable Development. , 2, pp.92–98.

Report, T. & Roorkee, T., 2016. Algae based waste water treatment. , (January 2015).

Res, J.M.B. et al., 2014. Accumulation potential of lipids and analysis of fatty acid profile of few microalgal species for biodiesel feedstock. , 4(1), pp.37–44.

S. Devi, A.M.& R.R.K., 2015. Textile dye wastewater treatment using freshwater algae in packed-bed reactor: modeling, Desalination and Water Treatment,.

Smith-baedorf, H.D., Microalgae for the biochemical conversion of CO 2 and production of biodiesel.

Sutherland, D.L. et al., 2015. Bioresource Technology Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, 184, pp.222–229. Available at: http://dx.doi.org/10.1016/j.biortech.2014.10.074.

Takagi, M., 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglycerides in marine microalgae Dunaliella cells. *BioSci. Bioeng.*, (101), pp.223–226.

Tam, N.F.Y., Wong, Y.S., 1989. Wastewater nutrient removal by Chlorella pyrenoidosa and Scenedesmus sp. *Environ. Pollut.*, (58), pp.19–34.

Tarlan, E., Dilek, F. B., & Yetis, U., 2002. Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. *Bioresource Technology*, (84), pp.1–5.

Thompson, G.A., 1996. Review: Lipids and membrane function in green algae. *Biochimica et Biophysica Acta*, (1302), pp.17–45.

Tobergte, D.R. & Curtis, S., 2013. The high light- inducible polypeptides stabilize trimeric photosystem I complex under high light conditions in Synechocystis PCC 6803. *Journal of Chemical Information and Modeling*, 53(9), pp.1689–1699.

Wang, S.-K., Wang, F., Hu, Y.-R., Stiles, A.R., Guo, C., Liu, C.-Z., 2014. Magnetic flocculant for high efficiency harvesting of microalgal cells. *ACS Appl. Mater. Interfaces*, (6), pp.109–115.

Wang, S.-K., Wang, F., Stiles, A.R., Guo, C., Liu, C.-Z., 2014. Botryococcus braunii cells: ultrasound-intensified outdoor cultivation integrated with in situ magnetic separation. *Bioresour. Technol.*, (167), pp.376–382.

Wang, L. et al., 2010. Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, 162(4), pp.1174–1186.

Xue, F., J. Miao, X.Z. and T.T., New strategy for lipid production by mix cultivation of Spirulina plantensis and Rhodotorula glutinis. *Appl. Biochem. Biotechnol.,*, 160, pp.498–503.

Yang, J. et al., 2011. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. *Bioresource Technology*, 102(1), pp.159–165. Available at: http://dx.doi.org/10.1016/j.biortech.2010.07.017.

Yun, Y., Lee, S., Park, J., Lee, C., Yang, J., 1997. Carbon dioxide fixation by algal cultivation using wastewater nutrients. *Chem. Technol. Biotechnol*, (69), pp.451– 455.