# AGENT BASED MODELING AND EXPERIMENTAL STUDIES ON A $3^{RD}$ GENERATION CLOSED CYCLE FOR IMPROVED BIOGAS PRODUCTION

*By*

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**In the partial fulfillment of the requirement of the Degree of Doctor of Philosophy**

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# Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

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# Executive Summary

Continued use of fossil fuels is now widely recognized as unsustainable because of diminishing supplies and the contribution of these fuels to the increased carbon dioxide concentration in the environment. Microalgae are considered as a most capable feedstock for the generation of biofuels due to its several advantages, compared to the feedstock of first- (food feedstock) and second- (non-food feedstock) generation biofuels. It is a photosynthetic organisms that convert carbon dioxide to potential biofuels, foods, feeds and high-values bioactives. When comparing microalgae as feedstock for biofuel production with conventional raw materials, several advantages are observed: much faster growth rate (doubling time of hours), greenhouse gas fixation ability (net zero emission balance), higher area yields (potentially 15- 300 times more), possibility of growing in non-arable land, no competition with food crops, less land requirement, cheaper raw materials, lower water consumption, possibility to grow in saline water and non-potable water, etc. However, one major challenge of microalgal biofuels lies in algal biomass harvesting and nutritional cost for production. Therefore, work described in thesis was designed, development an integrated process of microalgal production using anaerobic digestion under outdoor conditions. This decreases the production cost of microalgae as well as biofuel. The main objective of this thesis was to develop a closed loop for biological wastewater treatment system of AD slurry that could be utilized for algal growth and simultaneously produce the renewable energy in the form of biomethane and remove polluting nutrients as well as reduce the greenhouse gases. The integration of microalgae growth with anaerobic digestion could significantly improve the economics and energy balance of biofuels production. Nutrient removal, in particular nitrogen and phosphorus; and COD reduction from wastewater is a developing regulatory need and the use of algae cultivation could create a unique integration between waste treatment and biofuel production. Both the quantitative and qualitative aspect of the mass (substrate and biomass) and biogas production was studied and modelled. This integrated process of *Chlorella pyrenoidosa* cultivation coupled with anaerobic digestion (under outdoor conditions) in order to properly design a

microalgae growth-anaerobic digestion process to minimize the overall biofuel production costs. The process also reduces the need for fresh water by reusing AD slurry water.

The feasibility for the microalgal based waste water treatment was also tested experimentally using anaerobic digester slurry as a nutrient. *Chlorella pyrenoidosa* was cultivated in biogas digester wastewater as a nutrient source. The growth kinetics of the algae as well as the bioremediation effect on the waste water was studied at different temperatures, optimum pH and waste water characteristics. The microalgae, *Chlorella pyrenoidosa* utilized the nitrate nitrogen, total ammonia and phosphorus content present in biogas digester wastewater as a substrate for its growth. The growth of *Chlorella pyrenoidosa* based on the experintal values of Nitrogen nitrate was simulated on NetLogo software of Agent based modeling. The growth of microalgae was found to follow the same Monod growth model pattern as followed in experiments satisfactorily. This model helps to predict the behaviour of microalgae species based on the specific growth rate value and saturation constant value of nitrogen utilization. The growth of microalgae single and double species based on the $CO_2$ utilization values in the literature was also simulated using NetLogo software. The effect of saturation constant for $CO_2$ utilization had large effect in the yield of species in both single species and different species combination model. Overall, the proposed model has been able to simulate the pattern of biomass concentration change in a nutrient limited culture.

The substrate utilization and cell concentration in agent based model validates the experimental process for the development of water saving closed cycle. This model helps to identify the species also on the basics of substrate utilization and specific growth rate or doubling period.

Microalgae, *Chlorella pyrenoidosa* was found to be the best for biomass production with >75 % and >80 % of nitrogen nitrate and total ammonical nitrogen removal and >60 % of COD reduction. The net specific growth rate of *Chlorella pyrenoidosa* was found to be 0.367 $D^{-1}$. The growth kinetics of microalgae was found to follow the Monod growth model satisfactorily. Produced algal biomass was co-digested with cattle dung for biogas

production. Interestingly, 87 g/L of algal biomass was obtained under ambient algae cultivation conditions, and with that amount of biomass, 1032 mL $CH_4$ (with C:N=18.1:1) was produce through anaerobic co-digestion process. Agent based simulation for $CO_2$ and nitrogen utilization was also studied using NetLogo software based on experimental results. Validation of the growth model was performed by quantitatively and qualitatively by comparing modelled results with experimental results. The simulation and experimental results suggested that the cultivation of *Chlorella pyrenoidosa* in biogas wastewater may be considered as efficient, water saving as well as digestible biomass producing method.

# Abbreviations

| | | |
|---|---|---|
| 1 | AD | Anaerobic Digestion |
| 2 | ABM | Agent based Modeling |
| 3 | ATP | Adenosine Triphosphate |
| 4 | BOD | Biological Oxygen Demand |
| 5 | $H_3BO_3$ | Boric acid |
| 6 | C | Carbon |
| 7 | $CaCl_2 2H_2O$ | Calcium chloride dihydrate |
| 8 | CNG | Compressed Natural Gas |
| 9 | COD | Chemically Oxygen Demand |
| 10 | DNA | Deoxyribo Nucleic Acid |
| 11 | DW | Domestic waste |
| 12 | ER | Endoplasmic Reticulum |
| 13 | EDTA | Ethylene Diamine Tetra Acetic acid |
| 14 | GHGs | Green House Gases |

| | | |
|---|---|---|
| 15 | $FeSO_4 7H_2O$ | Iron sulfate heptahydrate |
| 16 | $MnCl_2 4H_2O$ | Manganese chloride tetrahydrate |
| 17 | $MgSO_4 7H_2O$ | Magnesium sulfate heptahydrate |
| 18 | N | Nitrogen |
| 19 | NREL | National Renewable Energy Laboratory |
| 20 | OLR | Organic loading rate |
| 21 | OD | Optical Density |
| 22 | P | Phosphorus |
| 23 | PBR | Photobioreactor |
| 24 | PSI | Photosystem I |
| 25 | $K_2HPO_4$ | Potassium phosphate |
| 26 | $Na_2MoO_4 2H_2O$ | Sodium molybdate dehydrate |
| 27 | $NaNO_3$ | Sodium nitrate |
| 28 | TAN | Total ammonia nitrogen |
| 29 | UV | Ultra Violet |

| | | |
|---|---|---|
| 30 | VS | Volatile solids |
| 31 | $ZnSO_4 7H_2O$ | Zinc sulfate heptahydrate |

# CHAPTER 1

# Introduction

## 1.1 Biogas

Biogas basically refers to a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen. The process for biogas production is known as Anaerobic Digestion (AD). It is a natural process in which microorganisms decomposes biomass or organic matter (also known as feedstock) in airtight digester tanks to produce biogas as well as digestate. The convertion behind biodegradable organic materials into biogas in AD uses four different stages- hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methane and carbon dioxide are the primary gaseous end products of this process. Table 1.1 lists the typical composition of biogas.

Table 1.1: Composition of Biogas (Volumetric Percent) Radlof (2011)

| **Biogas Component** | **Composition of Biogas (%)** |
|---|---|
| Methane ($CH_4$) | 45-65 |
| Carbon dioxide ($CO_2$) | **30-40** |
| Hydrogen sulfide ($H_2S$) | 0.3-3 |
| Ammonia ($NH_3$) | 0-1 |
| Moisture ($H_2O$) | 0-10 |
| Nitrogen ($N_2$) | 8 0-5 |
| Oxygen ($O_2$) | 0-2 |
| Hydrogen ($H_2$) | 0- 1 |

**STEP 1: HYDROLYSIS:** In anaerobic digestion, hydrolysis is the essential first step, as biomass is normally comprised of very large organic polymers, which are otherwise unusable. Through hydrolysis, these large polymers, namely proteins, fats, and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars.

**STEP 2- FERMENTATION OR ACIDOGENESIS:** This is the next step of anaerobic digestion in which acidogenic microorganisms further break down the biomass products after hydrolysis. These fermentative bacteria produce an acidic environment in the digester while creating ammonia, $H_2$, $CO_2$, $H_2S$, shorter volatile fatty acids, carbonic acids, alcohols, as well as trace amounts of other byproducts. Although acidogenic bacteria further breaks down the organic matter, it is still too large and unusable for the ultimate goal of methane production, so the biomass must next undergo the process of acetogenesis.

**STEP 3- ACETOGENESIS:** It is the creation of acetate, a derivative of acetic acid, from carbon and energy sources by acetogens. Acetogens catabolize many of the products created in acidogenesis into acetic acid, $CO_2$ and $H_2$, which are used by methanogens to create methane.

**STEP 4- METHANOGENESIS:** This constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis, as well as from some of the intermediate products from hydrolysis and acidogenesis.

Methane production continues to occur as long as conditions favorable to the survival of fermenting bacteria and methanogens are maintained in the digester.

### 1.1.1 Feedstock for generating biogas

A great variety of organic material can be used as feedstocks for generating biogas. These feed-stocks or raw materials may be obtained from the variety of sources like livestock and poultry wastes, food-processing and paper wastes, crop residues, and materials such as aquatic weeds, water hyacinth, microalgae, and seaweed. Cellulose, hemicelluloses and lignin are the chief components of all types of biomass. Various problems are encountered with these wastes with regard to collection, transportation, processing, storage, residue utilization, and ultimate use. Residues from the agricultural sector such as spent straw, hay, cane trash, corn and plant stubble, and bagasse need to be shredded in order to facilitate their flow into the digester reactor as well as to increase the efficiency of bacterial

action. Livestock manures are low-energy feedstocks because they are predigested in the gastrointestinal tracts of livestock Divya *et al.* (2015). Organic rich raw material normally contains elements such as carbon, hydrogen, oxygen, nitrogen and sulphur. The composition of each element is based on the nature of the biomass or raw material. Studies explored that carbon/nitrogen ratio of the biomass is a principle factor which critically affects the entire production process. The feedstock should also be balanced with respect to the ratio of carbon and nitrogen (C:N = 20:30) Mandalam and Palsson (1998), since the microorganisms use carbon and nitrogen at this ratio range. Substrates possess optimum C/N ratio only can meet the probable nutritional requirement of the microbes involved in the process, where as a decrease or increase in carbon to nitrogen ratio perhaps process instability.

Physical and chemical characteristics of the feedstock such as moisture content, volatile solids, nutrient contents, particle size and biodegradability could exceedingly affect the process stability and biogas production.

**Examples of Feedstock**

Few examples of the feedstocks that could be use for the production of biogas are listed below:

- Waste feed
- Food processing wastes
- Fats, oils, and grease
- Slaughterhouse wastes
- Corn silage (energy crop)
- Syrup from ethanol production
- Glycerin from biodiesel production
- Milk-house wash-water
- Fresh produce waste
- Cafeteria waste

### 1.1.2 Advantages

Biogas plants are slowly becoming popular due to the many benefits associated with them. They are already being used for public transport (Example:The Baltic Biogas Bus project in Sweden: This project summed up its results and achievments in October 2012, concluding that biogas is the best choice available to lower emissions of greenhouse gases from public transports while also improving inner city air quality), industrial heating and many more applications. Some of the advanges of Biogas are as follows:

**1. Renewable Source of Energy:** Biogas is considered as a renewable source of energy. Since it often produced from materials that form sewage and waste products, the only time it will be depleted is when we stop producing any waste.

**2. Non-Polluting:** It is also considered to be non-polluting in nature. The production of biogas does not require oxygen, which means that resources are conserved by not using any further fuel.

**3. Reduces Landfills:** It also uses up waste material found in landfills, dump sites and even farms across the country, allowing for decreased soil and water pollution.

**4. Cheaper Technology:** Applications for biogas are increasing as the technology to utilize it gets better. It can be used to produce electricity and for the purpose of heating as well. Compressed Natural Gas (CNG) is biogas that has been compressed and can be used as a fuel for vehicles. Production can be carried out through many small plants or one large plant.

**5. Fertilizer:** The slurry generated for biogas plant can be recycled in the farm to increase C: N ratio along with N.P.K. contents of the soil which will enhance the yield of crop.

### 1.1.3 Limitations

**Logistics:** - As with all agricultural commodities, energy crops and residues all require appropriate supply chain infrastructure. Cost of transportation increases the production cost of biofuels.

**Resource and environmental issues:** - Biomass feedstock production can have both positive and negative effects on the environment. Climate changes is the serious problem with biogas production. Temperature variations normally disturbs the metabolic pathway of methanogenic bacteria and hence disturbs the stainability of biogas production.

$CO_2$**: Greenhouse gas emision:** Biogas normally contains 25-45 % of $CO_2$ which can harms not only the environment but also having corrosion activity. Biogas enriched with $CO_2$ cannot be directly connected to genset systems or any other energy translator. The usage of biogas as vehicle fuel has also significantly increased in the last years. For an effective use of biogas as vehicle fuel it has to be enriched in methane. This is primarily achieved by carbon dioxide removal which then enhances the energy value of the gas. Moreover, Due to the presence of carbon dioxide ($CO_2$) and hydrogen sulphide ($H_2S$) in biogas, it has become extremely difficult to transport and store it effectively especially where 2 $it^s$ produced in commercial quantities.

**Sustainability of Biogas:** Availability of quality biomass and water in whole of the year is a major challenge for the sustainability of biogas production in India.

### 1.1.4 Alternatives to overcome these limitations

**Integration of biogas plants with other technolologies**

Few integrated process makes the biomass energy efficient are as follows:

**1. Integration with power plants**

The advantages of anaerobic digestion coupled with Combined Heat and Power Plants

(CHP) to generate energy are numerous. As noted by Wiser, Schettler, and Willis (2011), these advantages include the following:

• Biogas generated from anaerobic digestion is a valuable source of fuel for CHP systems.

• Electricity generated from biogas is reliable and available for immediate use. This reduces the cost of transportation from the digester to the user.

• In some cases, biogas-generated electricity can be made available for export and sale to power utilities.

• Generated electricity is a product of biogenic carbon and is carbon neutral. The generated power displaces largely fossil-fuel-derived, electric-utility-produced power.

But this integration cannot require biogas upgradation for the removal of $CO_2$ and $H_S$ from biogas due to corrosive nature of these gases.

**2. Use of waste water treatment**

Anaerobic digester and wastewater treatment systems represent a proven sustainable technology for a wide range of very different industrial effluents, including those containing toxic/inhibitory compounds. The process is also feasible for treatment of domestic wastewater with temperatures as low as $14 - 16$ and likely even lower. In comparision to conventional aerobic treatment systems the anaerobic treatment process merely offers advantages like:

• It can be employed at very low costs, because technically plain and relatively inexpensive reactors are used, and anaerobic treatment systems generally can be operated with little if any consumptive use of high grade energy.

• Instead of consuming energy, useful energy in the form of biogas is produced.

• It can be applied at practically any place and at any scale.

• Very high space loading rates can be applied in modern anaerobic wastewater treatment systems, so that the space requirements of the system are relatively small.

Anaerobic digestion of municipal wastewater sludge was studied by Corrala *et al.* (2008). According to them, Overall, the process converts about 40% to 60% of the organic solids to methane ($CH_4$) and carbon dioxide ($CO_2$). The chemical composition of the gas is 60-65% methane, 30-35% carbon dioxide, plus small quantities of $H_2$, $N_2$, $H_2S$ and $H_2O$. Similar studies was done by Usman and Ekwenchi (2013) and Malik (2007). They used agriculture waste water and use of water hyacinth as alternate substrate together with cattle waste for biogas digesters.

**3. Integration with water water treatment system**

Integration of waste water treatment process with anaerobic digester led to the treatment of outlet slurry and reutilization of extracted water. This integration process reduces the use of fresh water for algae cultivation and makes low cost production of biogas. In this context, cultivation of microalgae have emerged as a promising process for the treatment of waste water. Moreover, biofuels derived from microalgae are seen as one of the most promising solutions to mitigate climate change and as alternative to the production of fuels and specially chemicals fast depleting of fossil fuels and oil reserves. Microalgae and macroalgae underwent an intense academic and industrial research, due to their capability to overcome the drawbacks related to the first and second generations of biomass resources. Major advantages of algae are: no competition with food crops for arable land, high growth rates, low fractions of lignin which reduces the need for energy-intensive pretreatment and compatibility with biorefinery approach implementation.

## 1.2 Algae: $3^{rd}$ generation biofuel

The first generation biofuels are produced from edible feed stock like corn, soybean, sugarcane, and rapeseed. The use of these resources for energy production was blamed for a rise of food prices. Second generation of biofuels was produced from waste and dedicated lignocellulosic feedstocks, and having more advantages over those of first generation. The major benefits are higher stock yields and lower land requirements in terms of quality and

quantity. The main problem associated with lignocellulose conversion to biofuels is its strong resistance to degradation. Thus, second generation biofuels still lack of economic viability at large scale. Third generation biofuels feedstock is represented by micro-and macro-algae, which present further advantages over the previous two.

Algae differ from unicellular species with a number of micrometers in diameter to huge seaweed growing over 50 meters long. They are the most ancient organisms which have had deep effects on Earth and its biota for billions of years. Microalgae are microscopic algae with a micrometer-ranged size. Unlike higher and terrestrial plants, they do not have roots, stems and leaves. Microalgae make use of water, sunlight and $CO_2$ to perform photosynthesis. They can grow in freshwater, saline/brackish seawater and wastewater with a fast growth rate. Microalgae are normally found in aquatic systems, but some algal species can live in a symbiotic relationship with various organisms on the surface of soil, such as lichen which consists of microalgae, fungi and bacteria.

In recent years, the interest in microalgae biomass has increased in both fundamental and applied research fields aimed at producing biofuels and biochemical (Uggetti *et al.*, 2014). Microalgae are typically composed of proteins, carbohydrates, lipids, and other valuable components (e.g. pigments, anti-oxidants, fatty acids, and vitamins). Wastewater containing the biological wastes of animals impregnated with abundant inorganic nitrogen and phosphorus is one of the most significant causes of eutrophication in water bodies. The use of microalgae has also attracted attention because microalgae have the ability to remove $CO_2$ and NOx during their growth Park *et al.* (2013).

The advantages of microalgae as a biodiesel feedstock are many, but the most amazing one is that it has the potential to integrate microalgae cultivation in wastewater with microalgae-based biodiesel production Zhu *et al.* (2013). The experiment on harvest water recycling times demonstrated that 100% of the harvest water could be recycled twice with the addition of sufficient nutrients (Zhu *et al.*, 2013).

### 1.2.1 Reproduction and growth in batch systems

A homogenous microalgal culture in a batch type photobioreactor shows an ideal growth pattern with the following sequence: lag phase; accelerating growth phase; exponential growth phase; decreasing log phase; stationary phase and accelerated death phase Becker (1994). In batch cultures, the nutrient supply is limited and nothing is added or removed during algal growth. Whereas, a fresh medium is supplied to the homogenous culture, and the algal culture is harvested continuously in continuous culture systems. Growth Kinetics of Pure microorganisms Culture in a Batch System contains the different phases (Yang *et al.*, 2011). These are as follows:

1. Lag phase
2. Log (exponential growth) phase
3. Stationary phase
4. Death or declining death phase

These phases are explained below Richmond (2004), Pereda and Zamarreno (2011):

1) Lag phase: In this phase a delay in growth initially happens due to the presence of nonviable cells in the inoculums or physiological adjustments to change in nutrient concentration or culture conditions;

2) Exponential phase: It is also called as Log phase, where cells grow and divide as an exponential function of time, as long as mineral substrates and light intensity are saturated.

3) Stationary growth phase: where the growth rate remains constant. However, increase of nutrient concentration may lead to luxury storage of nutrients by algae during this phase; and

4) Decline or death phase: In this phase the decrease in the concentration of nutrients and/or accumulation of toxic waste products leads to microorganisms death. Production of secondary metabolites occurred in this phase.

Microalgae are able to grow under three cultivation conditions, namely photoautotrophic, heterotrophic and mixtrophic Kleinova *et al.* (2012). In photoautotrophic conditions, microalgae utilize energy from light to perform metabolic processes and fix $CO_2$. In heterotrophic conditions, algae consume organic carbon for growth and a light supply is non-essential. The combination of these two conditions is called the mixtrophic conditions.

### 1.2.2 Factors influencing microalgal growth

The growth of microalgae, including photosynthesis, growth pattern, cellular metabolism and cell composition, is influenced by many cultivation parameters, particularly the light supply, temperature, pH and mixing. These parameters are considered for the experiments.

#### Nutrients

Microalgae have two major possible ways for nourishment, namely autotrophy using light and/or heterotrophy using a chemical compound as energy source, respectively Molina (2001). Microalgae obtain various nutrients from the culture medium. In general, the culture medium must contain nitrogen, phosphorus, essential inorganic salts and other micro-nutrients. The alga, *Chlorella pyrenoidosa*, cannot grow without a nitrogen source and its growth is directly proportional to the concentration of nitrate in the medium. As nitrate source is increased in the medium, enhancement in biomass concentration was recorded Nigam *et al.* (2011).

Carbon dioxide can be taken up and utilized by microalgae in two forms, namely $HCO^{3-}$ and $CO_2$ Razzak *et al.* (2013). $CO_2$ is the only form of carbon with electric neutrality that can cross the membrane passively. When $HCO_{3-}$ is utilized as carbon source it occurs via active uptake or by extracellular conversion from $HCO^{3-}$ to $CO_2$ by enzymatic activity Razzak *et al.* (2013).

The ability to use $CO_2$ directly from industrial emissions as a resource of carbon for the growth of microalgae is a promising feature for fluegas mitigation Collet *et al.* (2011). The $CO_2$ is supplied by three ways: as $CO_2$ recovered from the purification which is dissolved in water, as dissolved $CO_2$ in the anaerobic digestion output flow, or as compressed gas injected in the ponds Collet *et al.* (2011).

**Carbon:** The carbon sources for microalgal growth are categorized as inorganic carbon sources and organic carbon sources. In autotrophic algal cultivation, $CO_2$ enriched air is sparged into the algal culture and the effects of $CO_2$ concentration on the growth of various algal species and accumulation of metabolites have been extensively studied Li *et al.* (2013).

**Nitrogen:** Apart from carbon, nitrogen is also the most important element required for algal growth. It is the essential constituent of all proteins in microalgal cells. Nitrogen is usually provided in the form of nitrate or ammonium salts, such as $NaNO_3$ or $NH_4$Cl, in the media. Microalgae can make use of photosynthetically fixed carbon to synthesize lipids or carbohydrates, rather than proteins, under nitrogen-limiting conditions Al-balushi *et al.* (2012).

### Temperature

Generally, the temperature range for microalgal growing is from 20 to 40 ℃. Temperature, as one of the most important cultivation parameters, regulates the photosynthetic and metabolic processes of microalgae. Franco *et al.* (2012) cultured Chlorella sorokiniana under suboptimal temperatures (20 ℃) and found that the specific growth rate of C. sorokiniana significantly decreased, and the photosynthetic efficiency and productivity were much lower than C. sorokiniana cultured under its optimal growth temperature (38 ℃). Temperature has a strong correlation to biochemical reactions and therefore affects microalgal growth Posten (2009). Microalgae have different temperature optima for growth, usually somewhere between 20 to 30 ℃Mandalam and Palsson (1998). Maximal productivity is obtained when the nutritional needs are fulfilled and the cultivation tem-

perature is optimal Mandalam and Palsson (1998). Temperatures lower than 16 ℃decrease normally the growth rate of microalgae; however, and over 35 ℃most of them could not be survive Mandalam and Palsson (1998). There is a relationship between temperature and light intensity since lamps and sunlight emits heat Posten (2009) Ogbonna and Tanaka (1996).

### pH

The optimal pH value for most microalgal species is around 7 (neutral) while some of them favor higher or lower pH conditions. For instance, *Spirulina platensis* can keep growing under pH 9 Hu *et al.* (1998). The pH affects the growth of microalgae, and different species and strains which have different optima at which the fastest growth is achieved (Roleda *et al.*, 2013). The pH optimum for *Chlorella pyrenoidosa* is around 7.5 and this species was growing fastest around this pH (Roleda *et al.*, 2013). Control of pH can be achieved by adding an acidic or buffer solution. Effects of pH on the growth of *Chlorella vulgaris* was investigated under heterotrophic conditions Yeh *et al.* (2010). The change of pH from pH 8.5 to pH 7 resulted in the increases of biomass yield, lipid content and lipid productivity.

### Light

The light supply shows the most important role in microalgal growth, especially for autotrophic growth. Light can be generally derived from two types of light sources: the natural light source and the artificial light sources Yang *et al.* (2006). Sunlight, being natural light, is absorbed by microalgae under outdoor conditions. Direct sunlight utilization depends on the geographic location of the outdoor cultivation systems: areas with abundant sunlight radiation and a warm climate would be preferable for culturing microalgae outdoors Yeh *et al.* (2010). The effect of uptake of carbon sources in presence of light on biomass, lipid productivity and fatty acid profiles of this alga was studied and compared Rai *et al.* (2013). This was based on growth and lipid quality of

*Chlorella pyrenoidosa* under acetate and glycerol supported medium. They reported that the cultures grown in the presence of organic substrate (sodium acetate and glycerol) and light had higher biomass, oil content and better fatty acid profile than photoautotrophic cultures Rai *et al.* (2013).

## 1.3 Algae mass cultivation systems

Microalgae make use of water, sunlight and $CO_2$ to perform photosynthesis. They can grow in freshwater, saline/brackish seawater and wastewater with a fast growth rate. Microalgae are normally found in aquatic systems, but some algal species can live in a symbiotic relationship with various organisms on the surface of soil, such as lichen which consists of microalgae, fungi and bacteria. Microalgae are eukaryotic organisms which have a membrane-bound nucleus. Microalgal cultivation systems can be generally classified as two types, the open system and the closed system. Open pond is representative of the open culture system, which is currently the most feasible culture system for large scale production of algal biomass Grobbelaar *et al.* (1990). The closed culture system includes different kinds of PBRs with different cultivation scales. The high cost of microalgal cultivation is one of the major obstacles for making the process commercial viable. Two major types of algae growth systems are in use at different scales: photobioreactors and open ponds.

### 1.3.1 Closed Photo-bioreactors

Photobioreactors (PBRs) are used for phototrophs and are closed systems where the light does not fall directly on the cell suspension surface (Posten, 2009). PBRs are closed systems which permit the exchange of light and energy but exclude material exchange with the surroundings. The light has to pass through some kind of transparent wall before it reaches the cells. PBRs were developed to increase the productivity and maintain a monoculture, the risk for contamination is therefore low. There are different PBRs. Some

of them are tubular bioreactors, plate reactors or bubble column reactors (Molina, 2001). They are expensive to run, hard to clean and fouling can occur (Molina, 2001). However, there are various advantages associated with PBR as well. The contamination risk is low, the space required is low, and the water and the $CO_2$ losses are minimized.The main problems in the large-scale cultivation of microalgae outdoors in open ponds are low productivity and contamination. To overcome these problems a closed system consisting of polyethylenes sleeves was developed by Cohen *et al.* (1991). A process control is possible, no weather dependency exists and a high biomass production can be achieved.

A closed system was designed by covering a normal open raceway pond with a transparent cover, which directly touched the surface of microalgal culture media Li *et al.* (2013). This special system helps to prevent the supply $CO_2$ escaping into atmosphere and also increase the retention time of $CO_2$ Li *et al.* (2013).

### 1.3.2 Open raceways

The common methods of open pond cultivation systems comprise natural or artificial ponds, race-track ponds, and cascades James (2010). Such systems involve large surface areas open to the surroundings, allowing for natural light and gaseous transfer between the algal culture and the environment. These systems are constructed from clay or concrete and are usually lined with polyvinyl chloride to avoid loss of media and nutrients. These systems are the most commonly used system of algae cultivation to produce nutritional products and treat wastewater. Compared to photobioreactors, open systems popularly known as raceways are relatively easier to construct and maintain and, also economical for mass cultivation of algae Mendoza *et al.* (2013). Open algae reactors or raceways have most of the drawbacks, as compared to closed photobioreators which can limit their performance. These includes the high possibility of culture contamination and low final biomass concentrations incurring high harvesting costs, Richmond (2004) the lack of temperature control Richmond (1992), and the poor gas/liquid mass transfer Jorquera *et al.* (2010). It is also very hard to keep algae monocultures in such open pond systems due to

contamination from other algal strains and bacteria.

## 1.4 Biofuel production from microalgae

Fuels and chemicals derived from biomass are considered as an ecologically sociable alternate to petroleum based products. It signifies a vital sector within renewable energy and plays a critical role in overcoming the challenges modelled by fossil transport fuels, primarily diesel and gasoline. The most wide-ranging research into the development of biofuels from algae was performed by the NREL from 1978 to 1996 Sheehan *et al.* (1998). NREL concluded that a more practical approach for near term production of algae biodiesel is to utilize wastewater treatment for algae cultivation Sheehan *et al.* (1998).

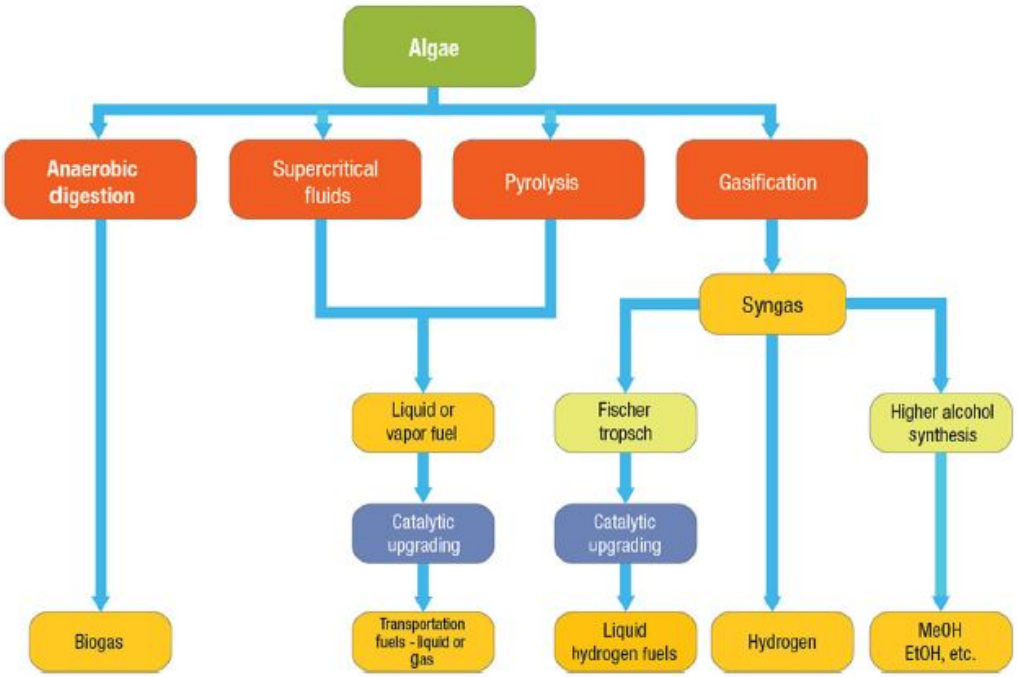


Figure 1.1: Biofuel production from microalgae biomass Schneider *et al.* (2014)

The microalgae biomass can produce biodiesel (through transesterification process), bioethanol (from residual biomass), biogas (through anaerobic digestion), biohydrogen and bio-oils, as shown in Figure 1.1.

## 1.5 Microalgae species of the study

*Chlorella pyrenoidosa*, or synonymously called *Chlorella vulgaris*, (see Figure 1.2) belongs to the division Chlorophyta and the cells are close to spherical Uggetti *et al.* (2014). The width of the cells is 1.5-10 $\mu_m$. The pyrenoid is ellipsoidal or spherical and has 2-4 starch grains surrounding it. The autospores are spherical and each sporangium contains 2, 4, 8 or even 16 cells (Han *et al.*, 2013). The same species and strain has been used for flue gas conditions, with a bit lower concentrations of $CO_2$ of 10 %, NO of 57 ppm and $SO_2$ of 0.8 ppm (Han *et al.*, 2013). A high carbon fixation capacity exists for this species. Some strains of *Chlorella vulgaris* have shown a capability of producing a starch content of 37 % DW (Mandalam and Palsson, 1998). The species has the potential to grow in a heterotrophic way with an organic carbon source. The strain of *Chlorella pyrenoidosa* is mentioned as unchanged, no genotypic or phenotypic changes, even after being cultivated under different environmental conditions for many years (Han *et al.*, 2013).

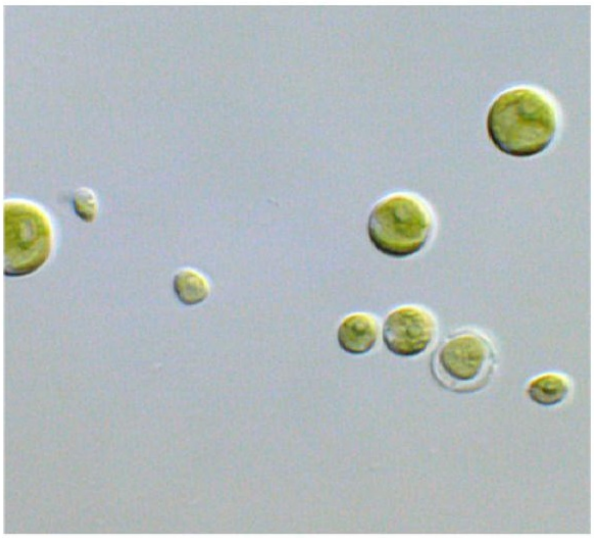

Figure 1.2: Chlorella pyrenoidosa, $www.ccap.ac.uk/strain_info$

### 1.5.1 Role of *Chlorella pyrenoidosa* for different waste water treatment and biogas production

*Chlorella pyrenoidosa* was found to have the highest value of theoretical and stoichiometric methane potential Prajapati *et al.* (2014*b*). A study was done for the biogas production potential through BMP protocols using *Chlorella pyrenoidosa* as a feedstock Prajapati *et al.* (2014*b*), Prajapati *et al.* (2014*a*). Relatively higher biogas yield was found 0.464 ± 0.066 $m^3$ biogas $kg^{-1}$ VS with 57% (v/v) $CH_4$ during 30 day digestion Prajapati *et al.* (2014*b*). Summary of waste water treatment from different sources by *Chlorella pyrenoidosa* is shown in Table 1.2.

Table 1.2: Examples of different wastes

| Source of waste water | Culture time (h) | Removal % | | | Biomass ($gL^{-1}$) | Ref. |
|---|---|---|---|---|---|---|
| | | NH4+ -N | TP | COD | | |
| Soybean Processing Wastewater | 120 | 89.1 | 70.3 | 77.8 | 0.64 | Hongyang *et al.* (2011) |
| Settled Sewage | 136 | 81.4 | 57 | - | 0.5 | Tam and Wong (1990) |
| Activated Sewage | 136 | 83.5 | 66 | | 0.29 | Tam and Wong (1990) |
| Anaerobic sludge for starch processing waste water | 624 | 83.06 | 96.97 | 65.99 | 0.37 | Tan *et al.* (2014) |
| piggery wastewater | 240 | 91.2 | 77.7 | 55.4 | 0.04 | Wang *et al.* (2012) |

## 1.6 Modeling based on Algal Systems

Microalgae growth kinetic models tell the growth rate of algae to the substrate concentration in a culture media. Kinetic models deliver an idea of biomass production and nutrient consumption rate with respect to time. It is essential for designing efficient culture systems or photobioreactors for the purpose of nutrient removal as well as predicting process performance, and optimizing operating conditions. The most famous kinetic models are the Monod and Droop models. Many studies have been conducted for finding those two model's parameters for different species of algae Zwietering *et al.* (1990), Pereda and

Zamarreno (2011), Yang *et al.* (2011), Rosch *et al.* (2012).

## Motivation for modeling

Current cost for production of micro-algal biomass range of $8 - 15 /kg for ash-free organic dry biomass James (2010). It was asserted that the capital and operating cost of the paddle wheel-mixed high-rate pond system for microalgae biomass production would be above $ 100,000/ha for biofuels production alone. So, the main concern in algae drived biofuels is the lipid extraction process from microalage which is very expensive. The main purpose is to show the production cost of microalgae drived oil which is very high as compare to microalgae drived biogas. The cost of microalgae cutivation and harvesting could be reduced, if it uses waste water as a medium and biomethane as a output. For algal biomass to be cost competitive with petroleum-derived fuel stocks, the cost of production needs to be reduced James (2010). So, there is also a need to optimize the trade-off between biomass growth and lipid production.

### 1.6.1 Mathematical Model

*Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions was done Yang (2011). The growth processes of *Chlorella minutissima* were same fitted to Monod & Logistic equations. The equation is:

$$\mu = \mu_{max} \frac{s}{k_s + s} \tag{1.1}$$

where s is the substrate concentration, $\mu_{max}$ is the maximum specific growth rate achieved at high, non-limiting nutrient concentrations and $K_s$ is the half-saturation constant (the nutrient concentration at which the specific growth rate is half of the maximum).

The Monod expression is developed as an acceptable mathematical description of

experiments conducted with pure microbial cultures growing on single substrates. In wastewater treatment practice, the biomass concentration is substituted with non-specific parameters like BOD (biological oxygen demand) or COD (chemical oxygen demand). Although they are mathematically treated as single substrate components, these parameters include a great variety of organic compounds with different biodegradation characteristics. The influent wastewater also contains artificially manufactured chemical compounds and toxic materials, to which various organisms respond differently. Furthermore, conditions like the dissolved oxygen (DO) concentration and the pH may vary within the treatment plant. Consequently, in the biological reactors used for the removal of the mixture of organic compounds in wastewaters, there is no way to select a given microbial species, since a mixed microbial community develops as an enriched culture, resulting from natural selection.

Some modifications to the Monod growth kinetics model have been proposed. The modified models relate the specific growth rate of algae to any of the following: nitrogen concentration Pereda and Zamarreno (2011), carbon concentration Yang (2011), Grobbelaar *et al.* (1990) or light intensity Talbot *et al.* (1991).

Agent based modeling of an activated sludge process in a batch reactor Netlogo software was done by Pereda and Zamarreno (2011). This work was done to study the feasibility of microalgae growth using agent based modeling to study the activated sludge process Pereda and Zamarreno (2011). The concentrations of substrate and algal biomass in an activated sludge batch reactor is shown in Figure 1.3

**Existing kinetic growth models related to carbon concentration** $CO_2$ and $HCO_3$ are the most important resources of carbon supply for the autotrophic growth of microalgae. The bicarbonate-carbonate buffer system ($CO_2 - H_2CO_3 - HCO_3^{-|} - CO_3^{-2}$) present in freshwater provides enough $CO_2$ through chemical reactions and maintains a specific pH that is optimal for cultivated species Richmond (2004). The injected air, which contains a specific amount of $CO_2$, provides the main resource of inorganic carbon for the cultivation of algae in photobioreactors. The modified Monod growth kinetics was used

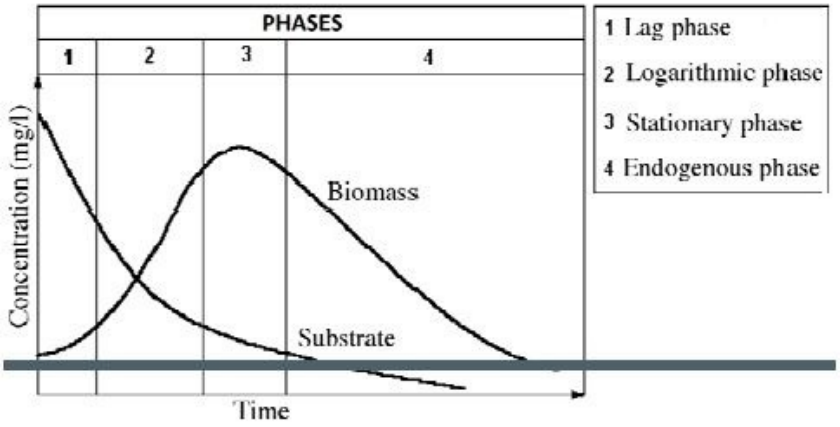


Figure 1.3: Concentrations of substrate and algal biomass in batch process Pereda and Zamarreno (2011)

by Tang *et al.* (2011) as shown in below equation:

$$\mu = \mu_{max} \frac{S_c}{k_{s,c} + S_c} \tag{1.2}$$

where, $S_c$ is Carbon concentration,

**Existing kinetic growth models related to Nitrogen concentration**

Numerous studies have used the Monod growth kinetics model for a nitrogen limiting culture Tam and Wong (1990), Sultan *et al.* (2011), Rosch *et al.* (2012); most of them aimed at estimating the optimum values of Monod's parameters for different species of algae Tam and Wong (1989).

Modified Monod growth model for nitrogen utilization was done by Aslan and Kapdan (2006). Which is:

$$\mu = \mu_{max} \frac{S_N}{K_{s,N} + S_N} \tag{1.3}$$

where, $\mu_{max}$ is Maximum specific growth rate, $K_{s,N}$ is Half-saturation constant and $S_N$ is Nitrogen concentration.

### 1.6.2 Agent based model

Agent-based Modelling (ABM) is an exciting field of work. It is a modelling tool where components of a system are implemented as autonomous interacting nodes, or "agents". According to Macal and North (2006), the high level Agent-based Modelling process starts from a hypothesis, in which a researcher would develop a theoretical model, then proceed to implement it. Finally, the researcher would validate the model against the actual phenomena of interest, and then analyse the model quantitatively and qualitatively before the process begins again with a new hypothesis.

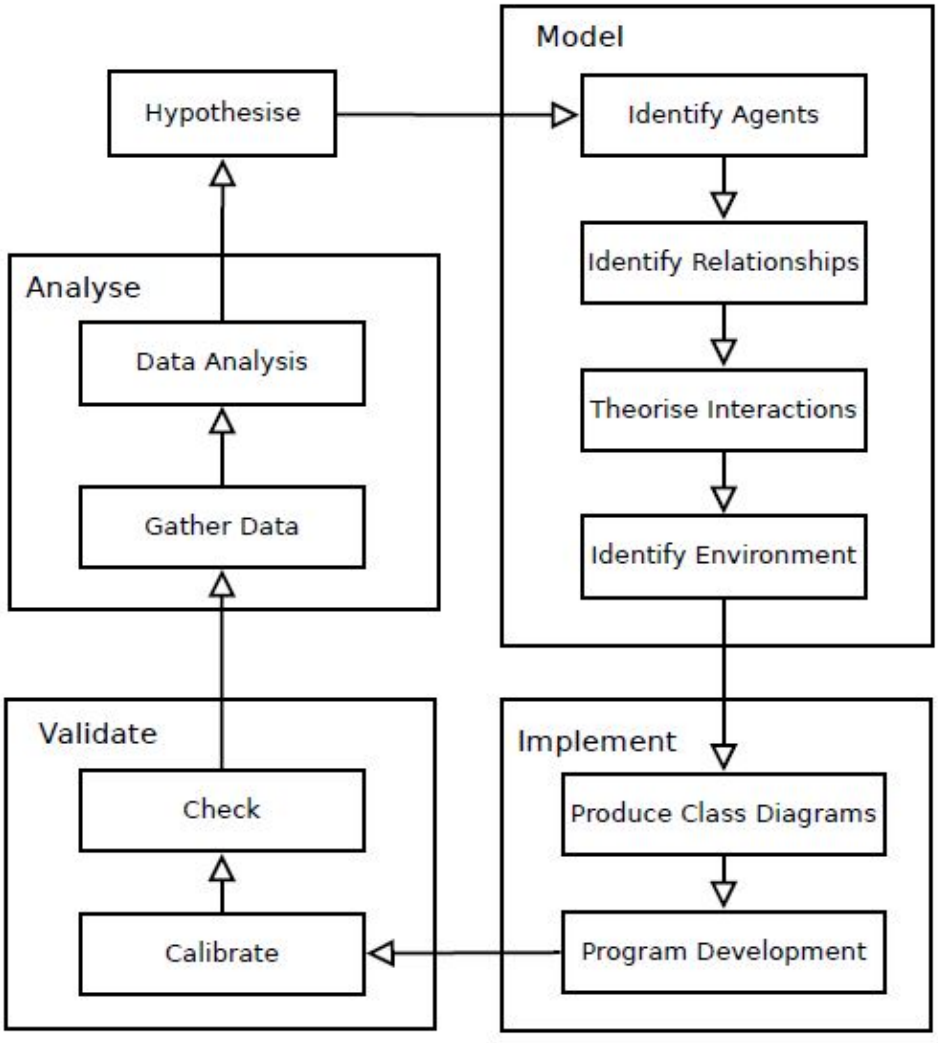


Figure 1.4: Agent based Modelling process, Macal and North (2006)

The use of agent-based simulation models (ABMs) for research and management is growing very fastly Railsback *et al.* (2006).Therefore Agent-based simulation model using

Netlogo software was used for conducting simulation study in present work. Pereda and Zamarreno (2011) has studied the Agent-based modeling of an activated sludge process in batch reactor using Netlogo software. The technique of ABM has got a landmark in recent years as a well-developed research methodology in relations of practice and theory. The recent work of Macal and North identifies five characteristics that seem to be the most common Macal and North (2006) in recent literature:

1. Identity - Agents must be identifiable, discrete individuals
2. Situation - Agents are situated in some fashion
3. Goal-oriented - Agents have goals to achieve
4. Autonomy - Agents are autonomous and may operate independently
5. Learning - Agents could potentially learn and adapt

Agents in ABM are normally represented by a set of commands or rules for their behaviour, or a decision tree, or a finite state machine. In agent-based modeling (ABM), a system is modelled as a collection of autonomous decision-making entities called agents. It is one of the most exciting practical developments in modeling for simulating the actions and interactions of autonomous agents with a view to assessing their effects on the system as a whole. Table 1.3 shows the comparison of some of the popular ABM tools reported by Railsback *et al.* (2006).

SWARM is one of the oldest and most stable ABM's that is able to support complex models, but has weak error-handling capability. Repast is one of the powerful agent-based modeling platforms. However, it requires an extensive knowledge of Java and is suitable for computationally intensive models. Mason is also a good simulation tool and is a good choice for an experienced programmer but is computationally intensive. NetLogo has a user friendly environment and has its own programming language that is simpler than Java or Objective-C. It also has good documentation and visualization abilities. From the above comparison, NetLogo was found to be a suitable platform for modeling since it is able to handle a large number of agents.

Table 1.3: Comparison between various agent-based platforms Railsback *et al.* (2006)

| **ABM platforms** | **User base** | **Modeling Language** | **Speed of execution** | **Ease of learning and programming** | **User materials** |
|---|---|---|---|---|---|
| **Ascape** | Diminishing | Java | Moderate | Moderate | Good documentation |
| **Mason** | Increasing | Java | Fastest | Moderate | Limited documentation |
| **Repast** | Large | Java, python | Fast | Moderate | Limited documentation |
| **NetLogo** | Large | NetLogo | Moderate | Good | Extensive |
| **SWARM** | Diminishing | Objective C,Java | Moderate | Poor | Good documentation |

**Agents and their interactions with the environment**

The first process in the Agent Based Modelling is to identify the agent types along with their characteristics. The agent types are the different species of individuals (entities in general) playing an important role in the model. The agents are characterized by their qualities.

**According to Tesfatsion (2002), Attributes can be grouped into:**

1. Spatial location: identifying the position of the agents in the environment. Sometimes it is not used; for example, if the topology of the agents is a network, or if the spatial position of the agents is not relevant.

2. Appearance characteristics: for visualization purposes. Agents can have different visualization properties, such as: shape, colour, visibility, label, trail, etc.

3. Custom attributes, depending on the system to be modelled, such as: age, temperature, etc.

The agents can have two types of attributes, static ones (also called parameters), and

dynamic ones that change over time (also known as agent states).

#### NetLogo: ABM tool

**NetLogo** is an open foundation programmable modelling environment for simulating natural and social phenomena. It was authored by Uri Wilensky in 1999 and has been in unbroken development ever since at the Center for Connected Learning and Computer-Based Modeling. It comes with an extensive models library including models in a variety of domains, such as economics, biology, physics, chemistry, psychology, system dynamics Wilensky (1999). NetLogo permits investigation by modifying switches, sliders, choosers, inputs, and other interface elements. Beyond investigation, NetLogo allows authoring of new models and modification of existing ones.

## 1.7 Preface

The remainder of the thesis is organized as follows. Chapter 2 discusses the various techniques for culturing microalgae, economics of microalgae biofuels system, selection of suitable microalgae species to our research and modeling based on microalgae systems. Chapter 3 addresses the material and methodology to achieve the research objectives, namely designing of outdoor conditions for our experiments. This involves culturing of microalgae in AD waste water in the month of June, October and February. The achieved targets are discussed in Chapter 4 wherein we will explain how we implemented outdoor conditions in the selected microalgae for biomethane production and bioremediations. We will also discuss the results for the Agent based modelling and simulation based on nitrogen and $CO_2$ as a nutrients from microalgae growth with suitable software (NetLogo) in Chapter 4. We will finish the thesis in Chapter 5, where we finds the final conclusion and answer the research questions and problem statement, as well as give recommendations for future research.

# CHAPTER 2

# Literature Review

## 2.1 Introduction

The worldwide usage of the fossil fuels cannot stay at its current rate, because of accumulation of carbon dioxide in the atmosphere drastically increases global temperature. Man must explore **clean** and renewable sources of energy that could minimize dependence on fossil fuels. To minimize the requirement for purchasing fossil fuels, a lot of processes being developed. These process developed the first, second and third generation biofuels. This has two advantages; it improves the lifecycle greenhouse gas emissions for the process and lowers the operating costs by avoiding fossil fuel purchases.

First generation biofuels, which are generally accepted to be ethanol produced from sugar or starch crops and biodiesel (methyl esters) made from vegetable oils and animal fats, have been introduced and used commercially as transportation fuels in a number of countries around the world. These first generation biofuels do provide some environmental benefits, have supported agriculture and rural economic development, and have diversified the transportation fuel supply system in many countries. These fuels have generally required some financial support from governments, adjustments to the fuel distribution system to allow their introduction, and in many regions there has been some resistance from the existing market participants to adopt these fuels.

There are other biofuel productions processes that are being developed and promoted that may offer some advantages over the existing biofuels. These fuels have been called $2^{nd}$ **generation biofuels**. The $2^{nd}$ generation biofuels are the biofuels that are produced from lignocellulosic feedstocks such as straw, wood and grass through either a biochemical production process or a thermochemical production process. It is claimed that these $2^{nd}$

generation biofuels may offer even greater benefits in terms of environmental performance, better overall energy efficiency, the ability to use lower cost and more widely available feedstocks, and be more easily integrated into the existing fuel supply and distribution system. The main drawbacks of the second generation biofuel production are the demand of large area for cultivation and that woody part of plants that do not compete with the food production. Moreover, According to International Energy Agency (IEA) 2006, many of the $2^{nd}$ generation biofuels are facing the same market barriers as the $1^{st}$ generation biofuel.

The disadvantages related with first and second generation biofuels can be overcome with the use of microalgae. This can produce large volumes of biomass, and subsequently biofuels, on much smaller areas, as viable alternative energy resource. Moreover, microalgae involves in different and diverse activity of ecological balance for pollution control in the environment Subashchandrabose *et al.* (2011). They act not only as remover of greenhouse gases from the atmosphere but can also be used for wastewater treatment ($NH^{4+}$, $NO^{3-}$, $PO_4^{3-}$) and environmental pollution control.

One of the main concerns in algal-derived biofuels is the microalgae harvesting process and high amount of chemical fertilizers that are required Clarens *et al.* (2010), Chinnasamy *et al.* (2010). This increases the final production cost of biofuel and the discharge of unconverted nutrients that can cause the eutrophication and greenhouse gases emissions. The utilization of wastewater as nutrient source and the reuse of the spent biomass as well as water could help to solve that problem, reducing also the fresh water consumption. Lim *et al.* (2013) successfully used synthetic urban organic waste streams to culture microalgae (*C. sorokiniana*), obtaining consistent algal productivities and very high nutrient removal. Other studies have proven that agricultural wastewaters can also support the growth of algae for biofuel production, proving also the potential of algae for tertiary or quaternary wastewater treatment Lee and Choul-gyun (2002). Moreover, several authors have studied the utilization of the residual algal biomass after the oil extraction as a way to reduce the environmental impact and to improve the economy of the process. Gao *et al.* (2012) concluded that algal biomass residue after oil extraction is also a potential

source for biofuel production. Models and experiments linking biodiesel production with simultaneous biogas yielded from oil-spent algal biomass have been studied Wang *et al.* (2010). Moreover, it has been proved that large-scale growth of algae can be potentially used to fix $CO_2$ rich flue industrial gases by direct injection on airlift photobioreactors (PBRs) Tan *et al.* (2014).

## 2.2 Bioremediation of waste water as well as $CO_2$ removal by microalgae

The potential of microalgae due to its various applications has made them the subject of considerable research effort in the past Brandenberger *et al.* (2013). These applications involves renewable energy source, source of high value chemicals for the pharmaceutical industry, source of proteins for animal feedstock and fertilizer.

Cultivation of Chlorella seems to be one of the feasible methods to reduce the amount of nitrogen andphosphorus entering the nearby coastal water, thus preventing the eutrophication problem Tam and Wong (1989). As algae started to grow and multiply, both nitrogen and phosphorus content in wastewater decreased significantly Tam and Wong (1989). Similarly, growth of Chlorella on wastewaters sampled from four different points of the treatment process flow of a local municipal wastewater treatment plant (MWTP) was done by Wang *et al.* (2009). They investigate how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the waste waters. Moreover, domestic wastewater samples from sewage wastewater treatment plant of Bopodi, Pune city was used to study the role of microalgae in wastewater treatment. Chlorella species showed the best removal capacity of nitrate and phosphate reduction Kshirsagar (2013).

$CO_2$, a green house gas is a part of medium for the culturing of microalgae. $CO_2$ is also a major component of biogas from anaerobic digestion. Algal systems are capable of utilizing the $CO_2$ from biogas. Emissions of $CO_2$ to the atmosphere can be reduced

through biological $CO_2$ mitigation which can further lead to the extensive uses of biofuel Kumar *et al.* (2010). Algal systems can also utilize the waste or toxic contaminants like nitrate nitrogen, nitrite content present in biogas digester outlet slurry as a substrate for its growth. Number of marine microalgae species have been tested for $CO_2$ sequestration applications Mann (2009) got positive results. They showed that algae contains the pigment chlorophyll and use the Calvin Cycle to fix carbon autotrophically.

The purification and treatment of the biogas remains an area for further improvement. Since, traditional biogas purification technologies rely on chemical and physical processes (e.g.: PSA) for the purification of biogas generated during fermentation. Furthermore, Large amount of $CO_2$ generation during fermentation is another critical issue associated with it. However, biogas purification could be effectivly combined with biogas production to reduce both the $CO_2$ emmision and economic of algae cultivation.

This may be possible as the algae, especially micro algae, have eminently desirable composition of bio-molecules such as polysaccharides, lipids, proteins etc., in their cell organizations, which can easily be converted into methane rich biogas under anaerobic digestion. The produced biogas can be utilized as a $CO_2$ source for growth of algal cell. This will enable both the purification of the biogas and improved economics for the algae growth and also the anaerobic digestion of that algae biomass for biogas production.

The advantages of using microalgae over conventional methods as summarized by Zhu (2015) are:

(a) Nutrients can be removed more efficiently;

(b) No generation of toxic by-product (sludge);

(c) Biofuels can be produced from biomass harvested (energy efficient);

(d) Cost-effective;

Several microalgae species have been studied as useful for nutrient removal including *Botryococcus braunii*, *Chlamydomonas*, *Scenedesmus* and *Chlorella*. Ambati *et al.* (2010) successfully cultured *Botryococcus braunii* in secondary effluent in both batch and contin-

uous experiments, with the removal efficiency of 99 % for nitrate and 93 % for phosphate. Tam and Wong (1996) reported removal of nitrate nitrogen by cultivating *Chlorella vulgaris* in wastewater. The removal efficiency increased corresponding to decreased initial nitrogen concentration: 100 % nitrogen removal was achieved with initial nitrogen concentration lower than 20 mg/L and 95 % nitrogen removal corresponding to 40-80 mg/L initial concentration, and 50 % removal with initial concentration higher than 80 mg/L.

Morover, Algae have been proposed as a method to fix atmospheric carbon dioxide. Vunjak-Novakovic *et al.* (2005) used a pilot-scale microalgae photo-bioreactor and found that $CO_2$ removal efficiency was 50.1 % on cloudy days and 82.3 % on sunny days from flue gas with a $CO_2$ concentration of 8 %. Processes that produce $CO_2$ can use algal biomass to fix carbon and to avoid air pollution. The carbon fixation occurs by the accumulation of fatty acids and hydrocarbons in algae biomass, which can be converted to bio-oil or biogas.

Atmospheric air contains 0.03 % of carbon dioxide, which can sustain algae growth, but below the maximum potential growth rate. Therefore, additional carbon dioxide can be supplied to increase the algae growth rate if sufficient light and nutrients are available Becker (2008).

Microalgae, *Chlorella vulgaris* consumed 38.7 % of an enriched $CO_2$ stream (6-8 % by volume) and produced 1 kg of algae biomass from 1.74 kg of $CO_2$ Doucha *et al.* (2005). The algae fixed 4.4 g $CO_2$ in 24 h with the enriched air stream compared to 3.0 g for atmospheric air. This microalgae is one example of an algae that can shift between an organic and inorganic carbon source according to the light availability Becker (2008). The presence of organic carbon is an alternative resource to the algae that may reduce the biomass loss during the dark period. Organic carbon could take the form of sugars that are supplied to algae during heterotrophic fermentation to increase the biomass and oil yield. Using animal manure as a nutrient source could also provide an organic carbon source to limit respiration losses during dark periods

## 2.3 Biomethane production potential of microalgae

In order to get an idea of microalgae bio-methane potential, it is advisable to look at their biochemical composition. A study conducted by Brown et al. for the nutritive characteristics of microalgae. The overall composition of microalgae differs between the species. In their study, they report protein content of 6-52 %, carbohydrates 5-23 % and lipids 7-23 % Brown *et al.* (1997). Angelidaki and Sanders (2004) compiled specific methane yields for carbohydrates, lipids and proteins shown in Table 2.1. According to them, when the composition of the organic matter is known, it is possible to evaluate the theoretical methane and ammonium yields that can be expected from the anaerobic digestion.

| **Substrate** | **Composition** | **L** $CH_4$ gmV/S |
|---|---|---|
| **Proteins** | $C_6H_{13.1}O_1N_{0.6}$ | 0.851 |
| **Lipids** | $C_{57}H_{104}O_6$ | 1.014 |
| **Carbohydrates** | $(C_6H_{10}O_5)n$ | 0.415 |

Table 2.1: Specific methane yield from three types of organic compounds

Sialve *et al.* (2009) studied the algae's theoretical methane potentials by using organic compounds as a basis of their calculations. Table 2.2 shows the composition and theoretical methane potential of different microalgae species calculated. These yields were calculated by using the following formula modified by Symons and Buswell (1993). It shows that the methane yield varies from 0.09 to 0.95 L gmV/S depending on the species and culture conditions. Based on the analysis given in the table, microalgae *Chlorella pyrenoidosa* could produce more biomethane as compare to other species and also having high carbohydrate content as compared to microalgae *Euglena gracilis* which is also having high biomethane potential.

$$C_aH_bO_cN_d + (\frac{4a-b-2c+3d}{4})H_2O = (\frac{4a+b-2c-3d}{8})CH_4 + (\frac{4a-b+2c+3d}{8})CO_2 + dNH_3 \quad (2.1)$$

In this equation, the organic matter is stoichiometrically converted to methane, carbon dioxide and ammonia. The specific methane yield expressed in litres of $CH_4$ per gram of volatile solids (VS) can thus be calculated as:

$$B_0 = \frac{4a + b - 2c - 3d}{12a + b + 16c + 14d} \times V_m \tag{2.2}$$

where $V_m$ is the normal molar volume of methane.

Table 2.2: Certain algae species composition and theoretical methane potential Sialve *et al.* (2009)

| Species | Proteins (%) | Lipids (%) | Carbohydrates (%) | $CH_4$ (ml CH4/g VS) |
|---|---|---|---|---|
| *Euglena gracilis* | 61 | 20 | 18 | 800 |
| *Chlamydomonas reinhardtil* | 48 | 21 | 17 | 690 |
| *Chlorella pyrenoidosa* | 57 | 2 | 26 | 800 |
| *Chlorella vulgaris* | 58 | 22 | 17 | 630 |
| *Dunallella salina* | 57 | 6 | 32 | 680 |
| *Spirulina maxima* | 71 | 7 | 16 | 749 |
| *Spirulina platensis* | 63 | 9 | 14 | 690 |
| *Scenedesmus obliquus* | 56 | 14 | 17 | 690 |

Brune and Yen (2007) and Kaosol and Sohgrathok (2012) have studied the effects of the carbon nitrogen ratio in microalgae to efficacy anaerobic digestion. Algal biomass normally having C:N ratio of 6:1, which means too high nitrogen content. When the biomass has high nitrogen content, it leads to an increase in ammonia in anaerobic digestion by ammonification and can become an inhibitory factor in methane output. The studies have concluded that optimal ratio between carbon and nitrogen varies around 12-20:1. Caporgno *et al.* (2015) have written that the functional ratio is somewhere between 16:1 and 25:1. To improve the C/N ratio extra cellulose, or other carbon sources, needs to be added. This is called as co-digestion process. In addition, the biomass can be mixed with

wastes having higher carbon content, such as animal manures, sludges or waste paper Brune and Yen (2007).

Ehimen *et al.* (2011) concluded that in semi-continuous reactors, Hydro Retention Time is a major single factor when digesting algal residues from biodiesel production. Methane yields improved when microalgae were digested for periods over 5 days. They suggested that C/N ratio of 12.44:1 was optimum for biogasification of algal residues when HRT was 15 days. Initially, C/N ratio was 5.4 and the methane yields were significantly low. They co-digested residual algal biomass with different amounts of glycerol and temperature being at 35 $\pm$ 0.5 ℃.

Mussgnug et al. studied the bio-methane potential of six different microalgae species by digesting them anaerobically for a period of 32 days. They concluded that the yield of biogas was noticeably dependent on the species and should be tested separately. The methane yields of those species that had a robust cell wall structure were lower than of those that had an easily degradable cell wall or no cell wall at all Mussgnug *et al.* (2010).

## 2.4 Integrated processes for biofuel production

With the integration of microalgae cultivation and application of Anaerobic Digestion (AD), several advantages can be identified. First of all, biogas is produced, which represents a source of renewable energy. Furthermore, during AD, nutrients such as phosphorus and nitrogen are released, in the form of phosphates and ammonia respectively. These can then be recovered and reused as substrate for microalgae cultivation, contributing to the economical balance of the process Alcantara *et al.* (2013). Harun *et al.* (2011) or Sialve *et al.* (2009) have already pointed out the real need of anaerobically digesting algal residues to make microalgal biodiesel a feasible alternative.

Most researches have also concentrated on the suspended microalgae growing in suspension termed high rate algal pond. The result of such effort is that some commercial technologies and processes are already available in the market such as the Advanced In-

tegrated Wastewater Pond Systems (AIWPS) technology commercialized by Oswald and Green, LLC, in the United States Olguin (2003). One of the main limitations of this technology is that it is difficult to harvest or separate the suspended microalgae biomass from the treated water discharge. None of the harvesting approaches have proved to be simple, inexpensive and suitable enough for a large-scale outdoor treatment Richmond (1992).

Sialve *et al.* (2009) reviewed and concluded that explored about fifty years ago, the promising integration process coupling anaerobic digestion and microalgal culture deserves sustained research and development efforts. This will probably re-emerge in the coming years either as a mandatory step to support large scale microalgal cultures or as a separate bioenergy producing process.

Similarly, the integration of microalgae growth with anaerobic digestion can significantly improve the economic and energy balance of such a promising platform technology Alcantara *et al.* (2013). The mass (carbon, nitrogen and phosphorus) and energy balances in the integrated process of *Chlorella sorokiniana* cultivation (under photoautotrophic and mixotrophic conditions) coupled with anaerobic digestion in batch mode was done. This designed process reduced the overall microalgae cultivation costs was done Alcantara *et al.* (2013). The proposed closed cycle according to Alcantara *et al.* (2013) is shown in Figure 2.1.

Similarly, Ichsan et al. used Palm Mill Oil Effluent (POME) to cultivate microalgae, Spirulina species and Chlorella species. The high COD value in POME can be converted into biogas production. Based on the findings, it was also possible to integrate the biogas system with microalgae cultivation. The microalgae could grow in different POME concentrations. This integration of biogas microalgae from POME already given additional benefits for the palm industry, local community, and environment Ichsana *et al.* (2014).

Moreover, waste water from agricultural processes also can be converted into biogas Usman and Ekwenchi (2013). This will reduce COD level from the waste water and reduce contamination from the evaporated methane and other Green House Gases (GHGs). The

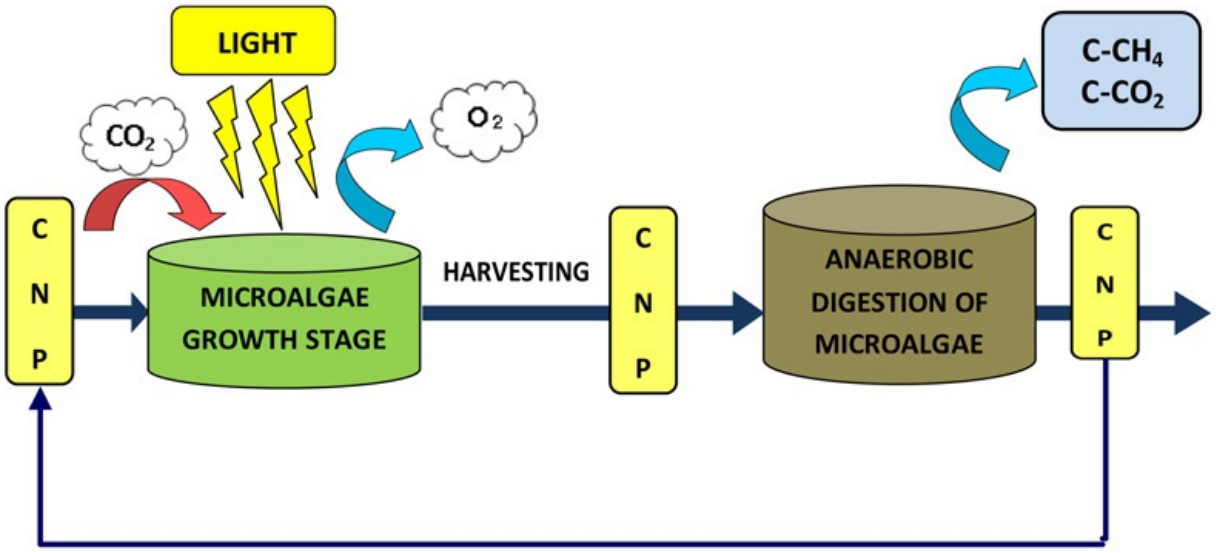


Figure 2.1: Integrated microalgae growth with anaerobic digestion process Alcantara *et al.* (2013)

biogas production can be cleaned and expected can be used for electricity generation, heating supply, and biofuel production (e.g. compressed biogas, cooking stove).

## 2.5 Problem formulation

An issue in the production of biofuels is finding usable water sources to dilute the biomass. On the other hand excess water is an issue in extracting liquid biofuels from algae. These two problems therefore, complement each other. Further wastewater derived from municipal, agricultural and industrial activities may also act as a source of nutrients for microalgae cultivation that could significantly reduce the operational costs of algal production systems. The use of wastewater could reduce nutrient addition for nitrogen and phosphorous by approximately 55 %. For high-lipid algal biomass production the use of waste nutrients could be problematic because the selective enrichment of high-oil algae species could be hampered by the contamination with native algae species and bacteria that are abundant in untreated wastewater and more competitive than the target oil producing algae. Figure 2.2 shows a flow diagram for algae waste water treatment for $CO_2$ mitigation and biofuel production Razzak *et al.* (2013).

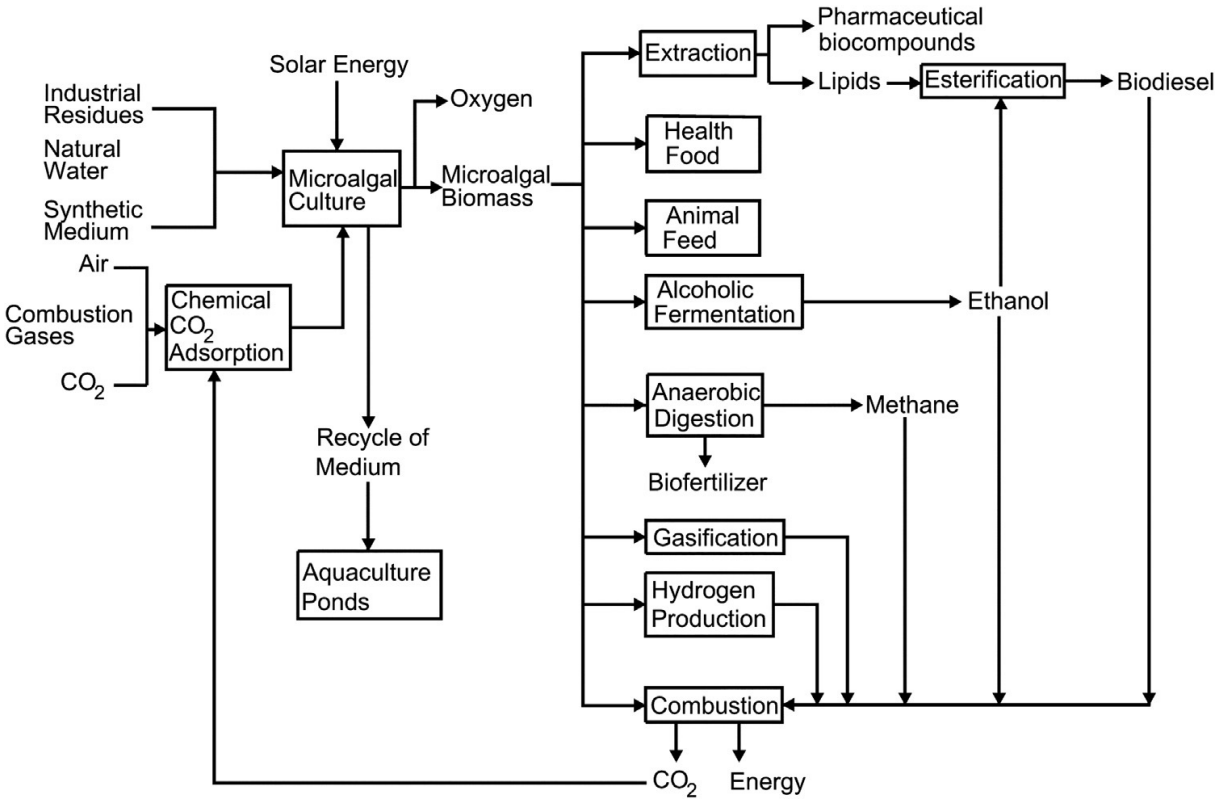


Figure 2.2: Flow diagram for algae waste water treatment with $CO_2$ mitigation and biofuel production

Another sustainable option to decrease the demand of nitrogen and phosphorus for

microalgae cultivation is nutrient recycling by the reuse of nutrients in the residual algal biomass after oil extraction. The release of industrial and municipal wastewater poses serious environmental challenges to the receiving water bodies. The major effect of releasing wastewater rich in organic compounds and inorganic chemicals such as phosphates and nitrates is mainly eutrophication. This is a global problem that can be solved by the use of microalgae whereby the wastewater is used as feed for microalgal growth. The advantage is that while the microalgae will be removing excess nutrients in the wastewater, there will be concomitant accumulation of biomass for downstream processing. The use of a wide range of microalgae such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* for treating domestic wastewater has been reported and efficacy of this method is promising.

Algae also can be used as feed biomass to produce biogas through anaerobic digestion. Anaerobic digestion is a process involving the degradation of organic matter by bacteria in the absence of molecular oxygen. Methanogenic archea present in anaerobic digesters convert algal carbon and other organic matter into carbon dioxide ($CO_2$), methane gas ($CH_4$), and organic acids. Anaerobic digesters require few energy inputs and can tolerate solids with high water content.

## 2.6 Aims and Objectives

If the work executed properly, it may effect the various issues like:

1. **Economic:** Cost effective & economic method for the production of $3^{rd}$ generation feedstock.
2. **Feedstock:** Eliminating the problem of external feeding for biogas production.
3. **Fresh Water Saving:** Treatment of biogas tank outlet slurry by microalgae culturing and reuse it further for anaerobic digestion.

### 2.6.1 Broad Objectives

• Development of economic cost effective closed cycle for improved biogas production using $3^{rd}$ generation biomass.

• Design and testing of anaerobic digestion process for production of biogas from algal biomass.

• To determine the rate of nitrate, phosphorus, total ammonia and COD removal by *Chlorella pyrenoidosa* from anaerobic digester waste water in outdoor conditions.

• Agent based modeling using NetLogo software for the culturing of microallgae using Nitrogen Nitrate and Carbon dioxide.

### 2.6.2 Specific Objectives

**1. Production of $3^{rd}$ generation biomass:**

Development of process for the cultivation of microalgae with the filtered anaerobic digester outlet tank slurry as a source of nutrients and biogas as $CO_2$ fixation. This involves two processes:

**1.1 Bioremedation**

To monitor the system and study the kinetics of phosphate-P, nitrate-N, ammonium-N and Chemical Oxygen Demand (COD) elimination.

**1.2 $CO_2$ mitigation**

Use of biogas as a $CO_2$ source for microalgae culturing and biogas scrubbing system.

**2. Anaerobic digestion**

• Study of biogas production from *Chlorella pyrenoidosa*, cow dung and their co-digestion with treated water in outdoor conditions.

• To invetigate the biogas production quantitatively and qualitatively.

**2. Design of Integrated process (microalgae cultivation and anaerobic digestion)**

This mainly focuses on providing a technology that is cost effective, water saving, requires low maintenance, and has minimal operator requirements for treating industrial wastewater containing high amounts of nitrates, phosphates and heavy metals. This involves:

• Design a closed loop for the continuous production of biomethane.

• To investigate the C/N ratio for anaerobic digestion; and concentration of phosphate-P, nitrate-N, ammonium-N and Chemical Oxygen Demand for microalge cultivation.

**3. Modeling**

Here we have done Agent based modeling for the improved biogas production from $3^{rd}$ generation biomass feedstock using two different feedstock that includes biogas as a $CO_2$ source and anaerobic digester outlet slurry as nutrient source. This Invloves:

• To Design a NetLogo tool based on experimental results for Nirogen consuptiom. This is to validate and compare the experimental results using NetLoge.

• To Design a NetLogo tool based on literature values for carbon dioxide consuptiom from biogas.

• To use the developed ABM to have a better understanding of the system and to generate new insights and conclusions that could facilitate the control of the system.

# CHAPTER 3

# Material & Methodology

## 3.1 Chemical and Reagents

$MgSO_4.7H_2O$, $K_2HPO_4$, $CaCl_2.H_2O$, Agar(Difco), $H_3BO_3$, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$, $Na_2EDTA$, $FeSO_4.7H_2O$, $K_2Cr_2O_7$, $NaNo_3$, $(NH_4)_2Fe(SO_4)$ and ferroin indicator. All chemicals were purchased from Sigma-Aldrich in Laboratory Reagent grade.

## 3.2 Experimental Design

### 3.2.1 Microalgae

*Chlorella pyrenoidosa* was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Dr. Homi Bhabha Road, Pune- 411 008, India. *Chlorella pyrenoidosa* was maintained in Fog's Medium as per National Chemical Laboratory, Pune at room temperature with aseptic conditions.

### 3.2.2 Culture medium for *Chlorella pyrenoidosa*

The medium used for *Chlorella pyrenoidosa* cultivation was Fog's medium. The Fog medium contained macromolecules, 1 ml of micronutrient and 5ml of Fe-EDTA sloution. Macromolecules in gm/L : $MgSO_4.7H_2O$, 0.2; $K_2HPO_4$, 0.2; $CaCl_2.H_2O$, 0.1; Agar(Difco), 12.0. Micronutrient solution contained in mg/L: $H_3BO_3$, 286.0; $MnCl_2.4H_2O$, 181.0; $ZnSO_4.7H_2O$, 22.0; $Na_2MoO_4.2H_2O$, 39.0; $CuSO_4.5H_2O$, 8.0; Distilled water 100.0 ml.

Fe-EDTA solution was prepared by dissolving 745.0 mg of $Na_2EDTA$ in hot water and then adding 557.0 mg of $FeSO_4.7H_2O$, then boiling it for few minutes and on cooling making up it to 100 ml. pH was adjusted to 6.5-6.6. It was maintained at room temperature in aseptic conditions in the photobioreactor. The temperature inside it was 19 ℃. All chemicals were purchased from Sigma-Aldrich in Laboratory Reagent grade.

### 3.2.3 Wastewater collection and processing

Anaerobic Digester (AD) waste water was collected from the cow dung based biogas plant located in UPES, Dehradun (India) as shown in Figure . The collected AD waste water was filtered through muslin cloth (pore size - 0.5-1.5 mm) in order to remove the large particles and debris and stored in cold storage (<4.0 ℃) until its use in the experiments. The filtered AD waste water was analyzed for determination of nitrate-nitrogen ($NO_3$ -N), total ammoniacal nitrogen(TAN) and chemical oxygen demand (COD). Methods used for determining TSS, ($NO_3$ -N), TAN COD is discussed in Section 3.4.


Figure 3.1: Cow dung based Anaerobic digeater plant in UPES, Dehradun

## 3.3 Outdoor cultivation of *Chlorella pyrenoidosa*

To ensure microalgae growth and bioremediation, *Chlorella pyrenoidosa* was cultured in 1000 ml flask in the month of June and October. After inoculation, flasks were incubated under natural day: night cycle. Initial *Chlorella pyrenoidosa* inoculum density was adjusted at 1 gm/L. During the experiments, the range of the temperature was $18 - 34$℃ in the month of October and $25 - 40$℃ in the month of June. Light intensities were <1.0 Klux (sunrise and sunset) and >80.00 Klux (mid-day time). At every $3^{rd}$ day, a homogenized aliquot (15 ml) was withdrawn from each flask for determination of algal growth and nutrient removal.

Figure 3.2: Photobioreactor of 10 lit capacity using Anaerobic digester as nutrient

Based on the growth kinetics and nutrients utilization in outdoor conditions, *Chlorella pyrenoidosa* was then cultivated in 10 L (pilot-scale bubble columns with diameter of 0.1 m; height of 1.014 m) closed photobioreactor (Figure 3.2) in the month of February. Initial *Chlorella pyrenoidosa* inoculum density was 0.1 gm/L. Exteral source of light was provided through the use of Linear fluorescent tube. Light intensity was measured by using the Lux meter which is of Digital Instruments Manufacturer. This meter uses one silicon photo diode with filter as a Photo detector.

## 3.4 Anaerobic digestion of algal biomass with cattle dung

Freshly collected dung and the algal biomass were processed for determination of elemental composition. Elemental composition (Carbon, nitrogen and hydrogen) was determined using CHN analyzer (Thermo 2000 series, Thermo Scientific) that uses highly sensitive thermal conductivity detector (TCD).

Batch anaerobic digestion was performed in 2L capacity of digester (Figure 3.3) for selected algal biomass; cattle dung and their mixture (1:1 ratio). Biomass was subjected with RPM 200, temperature 37 ℃, pH 6.5, HRT 15 days) for biogas production.

The data for both cumulative gas generation and total solids reduction were obtained during the digestion. Gas production was measured daily by inserting a needle that was attached to a frictionless syringe through the septum. The compositions of the gas were analysed via Gas Chromatogram.

Figure 3.3: Anaerobic digester of 2 L capacity with water displacement method

## 3.5 Analytical methods

### 3.5.1 Data Collection and Analysis

UV-VIS spectrophotometer of Systronics (Model No. 117) was used to analyse the nitrate and ammonia content reduction as well as for the concentration of microalgae. In order to study the growth of Chlorella pyrenoidosa in the biogas wastewater, absorbance of sample was recorded at 680 nm. For biomass estimation, sample containing grown algae (10 mL) was centrifuged at 8000 rpm for 10 min. The supernatant was collected for analyses of residual nutrients and COD. As per Monod growth kinetics model, the substrate nitrate nitrogen ($NO_3$ N) content consumption kinetics may be expressed as substrate conversion to product.

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} \tag{3.1}$$

Where - dS/dt is the total consumption rate of nitrate nitrogen; t is total time for microalgae growth and substrate consumption; $Y_{x/s}$ is the maximum microalgae Yield coefficient; x is the cell concentration and S is nitrate nitrogen concentration.

Microalgae growth process can be explained using Monod equation. This is based on mass balance. There are various terms associated with Monod kinetics with the contribution of biomass and substrate to the environment. S represents the substrate concentration and X represents the biomass concentration. The rate of substrate and biomass growth is ds/dt and dx/dt. Rate equation for algal growth is

$$-\frac{dX}{dt} = \frac{\mu_{max}}{k_s + S}X \tag{3.2}$$

$\mu_{max}$ is the maximum specific growth rate of the microalgae; X is the concentration of microalgae in the medium; S is the substrate concentration.

### 3.5.2 Wastewater analysis and pollutant removal efficiencies

Wastewater samples (prior and after treatment collected from biomass estimation step) were analyzed. Total Suspension solids, Nitrate Nitrogen (N-$NO_3$), Total Ammonical nitrogen and COD were analysed. % reduction was calculated as:

$$Removal(\%) = (1 - \frac{s_t}{s_0}) \times 100 \tag{3.3}$$

Whereas, $s_0$ and $s_t$ concentrations of pollutants (mg/L) in wastewater samples before and after the algal treatment.

#### Total Suspension Solids

For Total Suspension Solids estimation, sample containing grown algae (10 mL) was collected and centrifuged at 8000 rpm for 10 min. The supernatant was collected for analyses of residual nutrients and COD whereas solid base material was weighed and called as Total Suspension Solids.

#### Measurement of residual nitrate content

The nitrate concentration in the culture was determined according to the modified method reported by Sultan *et al.* (2011). A liquid sample from the photobioreactor was filtered with a 0.22 lm pore size filter, and then diluted 20-fold with DI water. The samples were collected and residual nitrate content was determined according to optical density at wavelength of 220 nm (i.e., $OD_{220}$) using a UV/VIS spectrophotometer (model 117, Systronics).

#### Measurement of Chemical Oxygen Demand

Chemically Oxygen Demand (COD) of anaerobic digester outlet slurry was determined before and after microalgae culture. It is an indirect determination of organic compounds that exist in water by estimating water quality (mg/L). The sample is refluxed with a known amount of potassium dichromate ($K_2Cr_2O_7$) in the sulphuric acid medium and the excess potassium dichromate ($K_2Cr_2O_7$) was estimated by titration it against ferrous ammonium sulphate, using ferroin as an indicator. COD was measured by using the given formula and procedure for measuring COD is shown in flow chart 3.4.

$$= \frac{A - B \times N \times 8 \times 1000}{v} \tag{3.4}$$

where as, A is the Volume of Ferrous Ammonium sulphate for blank, B is volume of Ferrous Ammonium sulphate for Sample, N is Normality of Ferrous Ammonium sulphate and v is volume of sample taken.

### 3.5.3 Biogas volume and composition analysis

Biogas volume was measured through acidic water (pH = 2.0) displacement (Angelidaki et al., 2009) after every 24 h. Composition of the biogas was determined using Gas Chromatograph equipped with stainless steel column packed with Porapak-Q 80/100 mesh

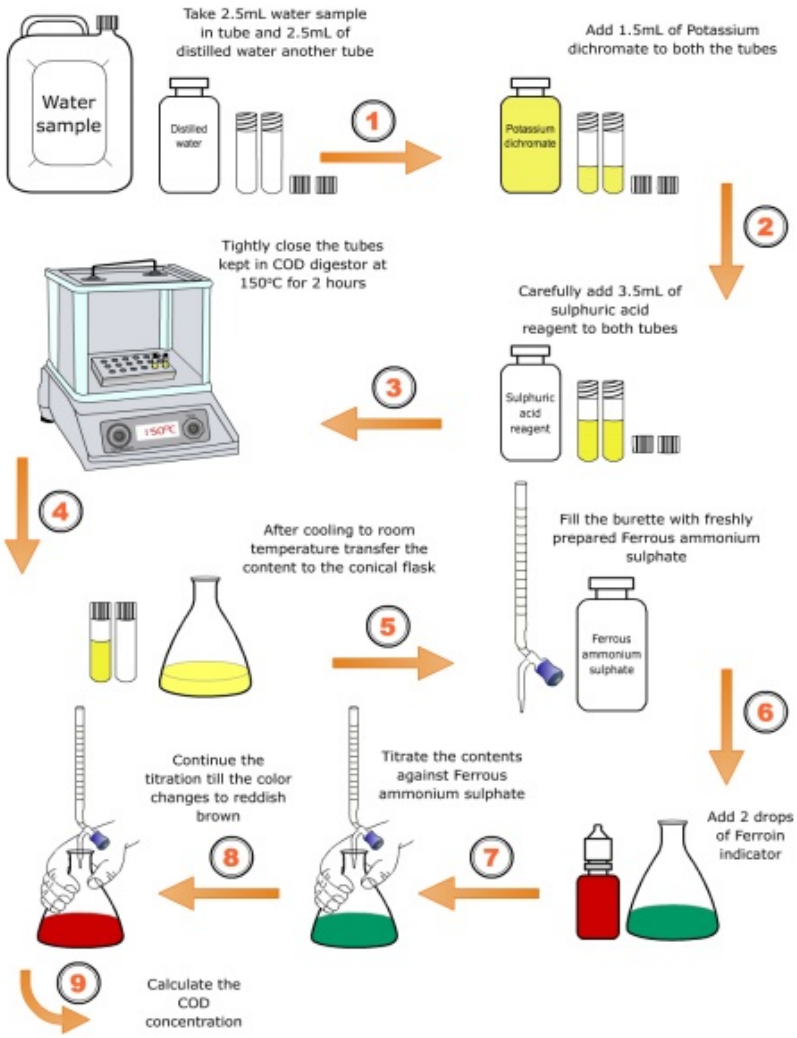


Figure 3.4: COD procedure flow chart (source: http://nitttrc.ac.in)

(Supelco), Argon as a carrier gas and thermal conductivity detector (TCD).

## 3.6 Agent Based Model

In agent-based modelling (ABM), a system is modelled as a collection of autonomous decision making entities called agents. It is one of the most exciting practical developments in modelling for simulating the actions and interactions of autonomous agents with a view to assessing their effects on the system as a whole. NetLogo is a freely available programmable modelling environment for simulating natural and social phenomena. It is used to create and open simulations and play with agents, exploring their behaviour under

various conditions. In this software, agent generally behaves like a being that can follow instructions. Sliders are used in this software for changing the values of variables. Mobile agents or microalgae are treated as turtles which are moved over a grid of stationary agents or the unit of area which is called as patches. The system modelled in this work was based on the experimental data of substrate utilization and cell growth.

This work has been carried out using the modeling environment Netlogo. It is particularly well suited for modeling complex systems developing over time. Modelers can give instructions to hundreds or thousands of independent **agents** all operating concurrently. This makes it possible to explore the connection between the micro-level behavior of individuals and the macro-level patterns that emerge from the interaction of many individuals. This section gives explanation about the environment and how to model the agents.

### 3.6.1 Description of NetLogo platform

The NetLogo window has three tabs: interface tab, the information tab, and procedures tab as shown in the top left of Figure 3.5. Only one tab is visible at a time but one can switch between different tabs by clicking on the tabs at the top of the window. The interface tab is used to visualize the output of the simulation and to control it. The information tab provides text-based documentation about the simulation and expected results. The procedure tab is the workspace where the code of the model is stored.

In NetLogo, two-or three-dimensional models can be created. In the 2D version of NetLogo, the interface tab includes a black square called view which is made up of patches. A patch is a spatial environment on which the agents move. The simulation program instructs the agents to move and act from patch to patch. The results can be seen in the view (Figure 3.5). The interface tab is a visual editor in which one can edit graphical elements such as buttons, sliders, switches, monitor, and output (Figure 3.5). Interface tab also allows changing the world dimension, and the dimension of the overall setup.

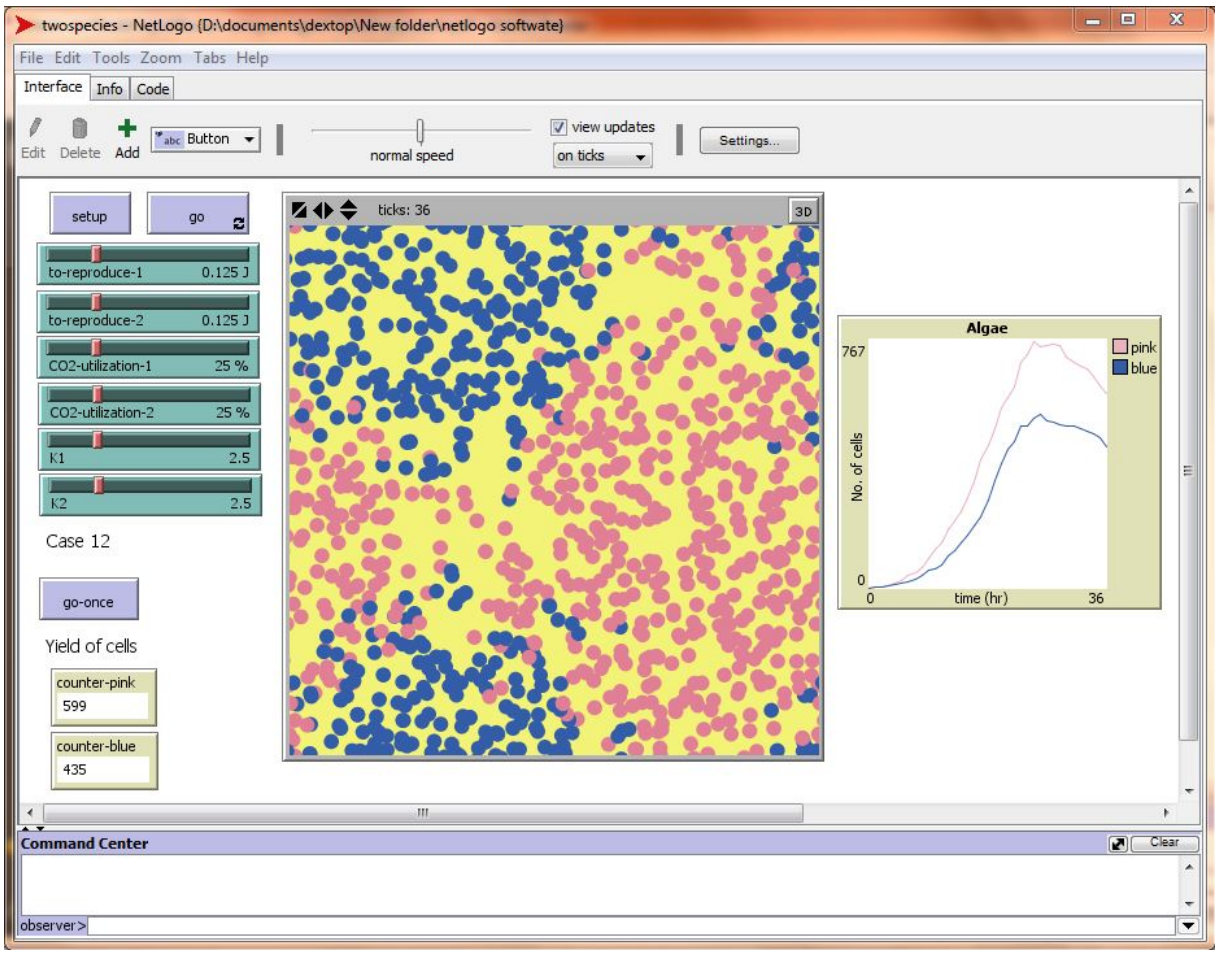


Figure 3.5: Screen shot of interface tab. The yellow box is the view

In the procedure tab commands are written in a specific format as shown in Figure 3.6. (The code used in this study is given in Appendices) The program has three parts: first, the global variables are defined; second, setup procedure is written to initialize the simulation; and third, go procedure is written that is repeatedly executed by the system. The go procedure tells each agent to carry out the given instruction independently. Agents in NetLogo model are referred as turtles. Typically, a population of turtles is initialized and procedures are written that control the behavior of the turtles. The turtles represent physical entities whose behaviors result in movements around the two dimensional world.

### 3.6.2 Agents in NetLogo

NetLogo has four different types of agents and each agent can follow instructions and carry out its own activity. These agents are turtles, patches, links, and observer. Turtles

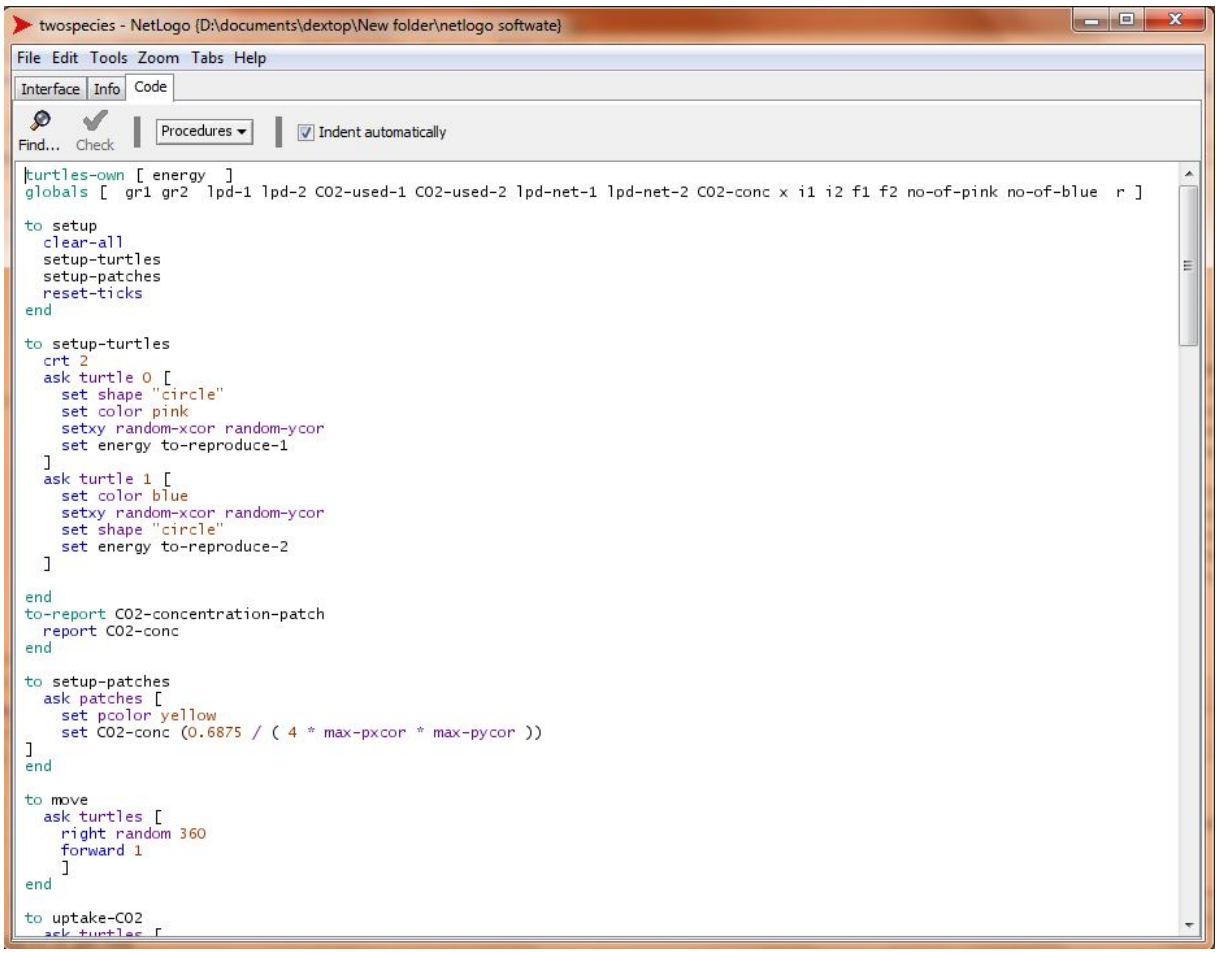


Figure 3.6: Screen shot of procedure tab

are the functional agents and the patch is a square ground over which the turtles move. Initially, the world is empty and the turtles are created by the observer. In addition, the patches can create turtles, too. Patches and turtles have their coordinates determined by the variables xcor and ycor for turtles, and pxcor and pycor for patches. The patch with the coordinate (0, 0) is the origin which can be placed anywhere in the view box based on the model requirement. The total number of patches is determined by the parameters min-pxcor, max-pxcor, min-pycor, and max-pycor.

By default, NetLogo has fixed patch coordinate values, when the model starts that can be changed accordingly. For example, when NetLogo starts, min-pxcor, max-pxcor, min-pycor, and max-pycor values are -16, 16, -16, and 16 respectively (Figure 3.7). This means pxcor and pycor both range from -16 to 16, so there are 33 times 33, or 1089 patches. The patches coordinates are only integers whereas the turtles coordinates are

whole numbers and fractions. This means the turtles can move anywhere on the patch. A link is an agent that connects two turtles if there is relation between them.

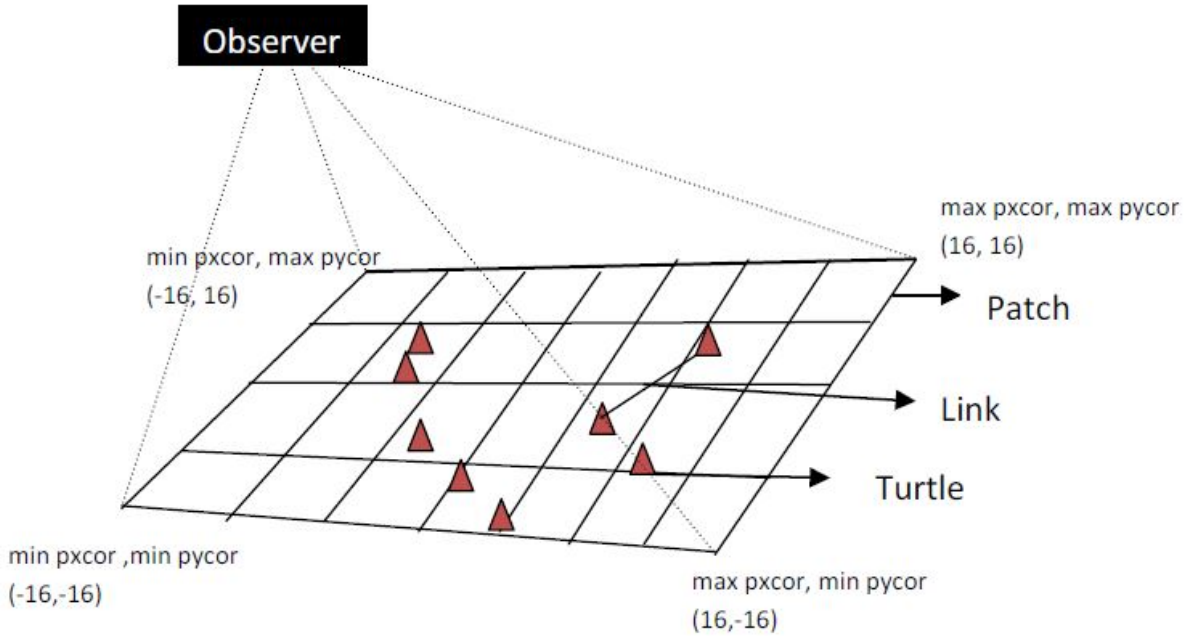


Figure 3.7: NetLogo world, Xin *et al.* (2008)

In NetLogo world, the patches can wrap around indicating that when a turtle moves to the edge of the world it disappears and then reappears on the opposite end. If this approach is used, a one dimensional simulation can be run using a two dimensional view box. Time is discrete in NetLogo and turtles act on each tick. A tick represents one step of the model, i.e., one cycle of the main loop. Ticks can be either seconds, hours, days based on the simulation requirement. If the daily events are simulated, then the time scale is one tick per day. If hourly events are simulated, then one tick is an hour. The real time the model takes to run is different than the model time. For example, if time scale is one tick per year, and the model only takes one second to run, then 100 years simulation can be simulated in 100 seconds.

### 3.6.3 Input/ Output

Once the code is finalized, input parameters are provided via various buttons in the interface tab. Output from the simulation is viewed in the interface tab without changing

the window. Basic buttons required for NetLogo are setup and go buttons which are created by the user. Other buttons such as sliders, switches, and monitors are created by the user based on the requirement. Output plots can easily be exported to an excel sheet for further analysis.

### 3.6.4 Simulation based on biogas as a $CO_2$ source

#### Algae conditions

When analyzing the algal growth kinetics, Optimal Conditions for *Chlorella pyrenoidosa* are as follows:

- *Chlorella pyrenoidosa* could grow well under $CO_2$ concentrations ranging from 5% to 35% Makareviciene *et al.* (2011).

- The removal efficiency of N and P of *Chlorella pyrenoidosa* reached relatively high values: TN - 91% Makareviciene *et al.* (2011).

- The pH value ranged from 6.42 to 7.08 under the $CO_2$ concentration of 24% Makareviciene *et al.* (2011).

- Max lipid productivity (gm/L/day) was found to be 0.62 $\pm$0.01, Max lipid content (% of DW) was 56.3 $\pm$ 1.4 and Max DW biomass (gm/L) was 2.83 $\pm$ 0.04 Pribyl *et al.* (2012).

- Optimum Temperature for *Chlorella pyrenoidosa* was 20-25 ℃.

#### Model of $CO_2$ Utilization

The system modeled in this work is a batch bioreactor of 1 lit volume. The vessel is initially filled with biogas at atmosphere (correspond to 0.6875 gm of $CO_2$).Algae consume the carbon dioxide content present in the biogas for its growth thus providing us with purer form of biogas which can be easily utilized for various purposes.

The relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. The kinetics is usually described by the Monod equation.

The dissolved $CO_2$ concentration in the biomass system was 0.6857 gm/L. As per Calvin cycle in the photosynthesis process, 6 molecules of carbon dioxide lead to the formation of 1 molecule of glucose through the fixation of carbon dioxide to 12 molecules of triose phosphate. This molecule of glucose then undergoes glycolysis that converts glucose to pyruvate. In glycolysis overall 7 ATP are produced per glucose, which traces back to 6 molecules of carbon dioxide. We know that one mole of ATP releases 45.6 kJ of energy. So for ATP per 6 molecule of $CO_2$ a species can get 1.21 kJ of energy.

Different species of algae have different capacities for carbon fixation. Carbon dioxide concentration of each patch decreases with every tick as the algae utilize dissolved carbon dioxide from the medium for their movement and growth.

To achieve the targets of the study, various parameters were consider and some data were assumed to tune the simulation:

**1.** As in biogas, the percentage of $CO_2$ is 35%. So, $CO_2$ concentration is assumed 35% and was set as 0.6875 gm/L.

**2.** $CO_2$ is assumed as a sole sole source of energy of carbon.

In this work, the effect of three parameters i.e. $CO_2$ utilization and energy for doubling and saturation constant for $CO_2$ utilization explain the algae growth rate.

For two species and three species combination, three variables were studied with two levels of these variables. High (H) is 75% of the maximum value, where as low (L) is 25% of the maximum value. Three cases for each species shows the effects of these variables on the growth or yield of algal species. The simulation was run by Plackett-Burman design, which shows the summary of the different cases with three variables and two levels for three species and two species combination. Each horizontal row represents a trial and each

vertical column represents the H (high) and L (low) values of one variable in each trials. This design requires that the frequency of each level of a variable in a given column is equal and that in each test (horizontal row) the number of high and low variables should be equal.

Modeling eqautions for $CO_2$ uptake used in Netlogo software are:

$$CO_2 - conc = \frac{((CO_2conc * 4 * max - pxcor * max - pycor) - CO_2used)}{(4 * max - pxcor * max - pycor)} \tag{3.5}$$

$CO_2$ concentration is based on the $CO_2$ used by the microalge in all the four corners.

$$CO_2used = (CO_2utilization/100 * CO_2conc) \tag{3.6}$$

$CO_2$ used is calculated on the basis of $CO_2$-utilization which is set by the sliders and $CO_2$ conentration.

$$energy = energy + (1210 * CO_2used) \tag{3.7}$$

Energy gained by the microalgae is based on the $CO_2$ used by the microalgae.

### 3.6.5 Simulation based on N-$No_3$ using AD as a nitrogen source for microalgae

Various parameters which were consider from literature and some data were assumed to tune the simulation are as follows:

**1.** As in biogas digester waste water, the percentage of $NO_3 - N$ is 87 mg/L (experimental value).

**2.** It is assumed that $NO_3 - N$ is the sole source of energy for the system and no additional energy is supplied.

**3.** The system is operating in batch type reactor.

**4.** The substrate for biogas production is algal biomass only.

**5.** Temperature for the algae cultivation and biogas production is assumed to be the optimum value.

**6.** $\mu_{max}$(Maximum Specific growth rate) is assumed to be 3 $\times 10^{-2}$ $Hr^{-1}$ (Quinn *et al.* (2011)).

**7.** Ks Saturation constant 0.005 gm/L (Quinn *et al.* (2011)).

Microalgae is treated as a agents or turtles. Microalgae species can differentiate on the basics of specific grow rate value, saturation constant value and nutrient utilization. These values in modeling is used from experimental data. Sliders are used for assigning the values and can be change depending on the microalgae species. Interface tab for nitrogen utilization is shown in Figure 3.8. This shows the cell kinetics, biogas production and nitrogen concentration during the batch reactors of 25 days. Value of Nitrogen utilization or reduction can be change using slider as shown in Figure 3.8. After pressing the go button, microalgae in green colour starts consuming nutrient from the surrounding atmosphere and reproduce itself. Simultaneously, the rate of nutrient and cell is shown in the graph on the same screenshot.

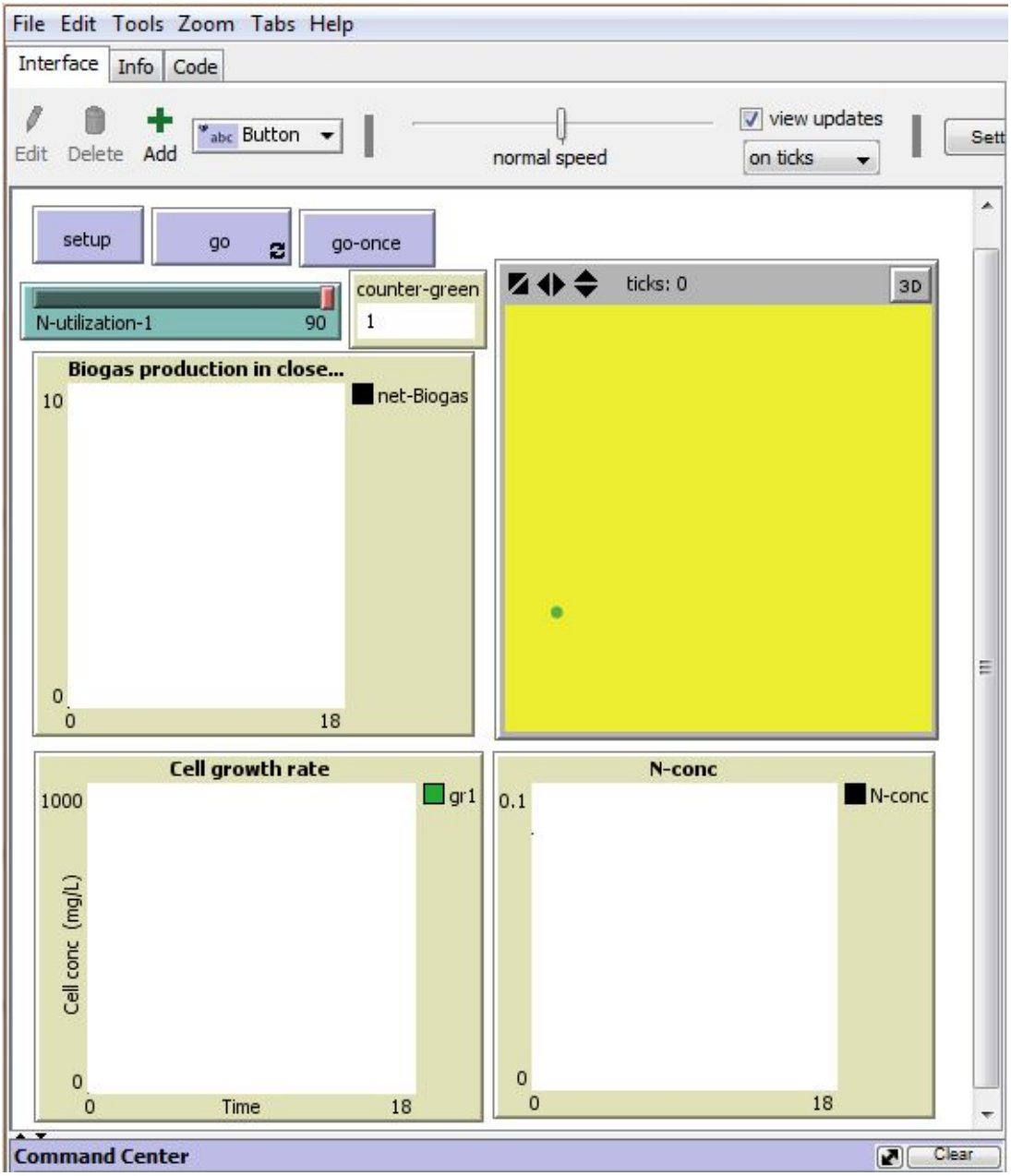


Figure 3.8: Screen shot of interface tab for nitrogen utilization

Equations for growth kinetics of cell and nitrogen utilzation based on Monod kinetics is shown below.

$$setN - used - 1(N - utilization - 1/100 \times N - conc) \tag{3.8}$$

Nitrogen used by the microalgae is based on the N-Utilization which is set by the slider and the Nitogen concentration remaining in the medium.

$$setgrowth - rate((growth - rate - max \times N - used - 1) \times no - of - pink/K1 + N - used - 1) \quad (3.9)$$

As per the Monod growth kinetics, growth rate is based on the substrate concentration which is nitrogen in this case and saturation constant value.

$$setN - conc(((N - conc \times 4 \times max - pxcor * max - pycor) - N - used - 1)/(4 \times max - pxcor \times max - pycor)) \quad (3.10)$$

Nitrogen concentation is calculated by the nirogen used by the microalgae from all the four corners of the surrounding area.

# CHAPTER 4

# Results & Discussion

## 4.1 Introduction

In this chapter, modeling and experimental studies on the growth kinetics of *Chlorella pyrenoidosa*, its potential to treat waste water and biomethane production will be discussed. Section 4.2, 4.3 and 4.4 discusses about the Characterization of AD waste water and growth of *Chlorella pyrenoidosa* in it at the outdoor conditions. Simulation based on nitrogen and carbon utilization using Agent based modeling will be described in this Section 4.11.

To be able to use biogas as carbon source and anaerobic digester outlet slurry as source for nutritional requirements and water in efficient cultivation of microalgae physical factors like temperature, pH, nutrient concentration and C:N ratio of process water on the algal growth and cellular composition are needed to be understood.

## 4.2 Characterization of AD waste water

The collected anaerobic digester waste water was light brown in color. The physiochemical properties of anaerobic digester waste water listed in Table 4.1 shows that it was rich in with the average of COD (5532.00 mg/L), Nitrate Nitrogen (88.03 mg/L), TAN (146.41 mg/L), respectively. Results were in agreement with the literature reports on high COD and nutrients levels in anaerobic digester waste water. For instance, Cumby *et al.* (1999) reported average COD (probably total COD) and TAN values (for LSW collected from 20 different dairy farms) in the range of 6550- 17,300 and 310-580 mg/L, respectively. Since, the COD and nutrient levels in the anaerobic digester waste water are significantly higher

than the recommended limits for wastewater discharge or reuse in irrigation (Table 4.1), proper treatment needs to be done before its discharge to the environment.

Table 4.1: Physiochemical properties of anaerobic digester waste water

| **Parameter** | **Concentration** | | |
|---|---|---|---|
| | **Experiment 1 (June)** | **Experiment 2 (October)** | **Experiment 3 (February)** |
| Volume | 1 L | 1 L | 10 L |
| pH | 6.9 | 6.5 | 6.8 |
| $N - NO_3$ | 84 mg/L | 87 mg/L | 93.1 mg/L |
| TAN | 145.07 mg/L | 139 mg/L | 155.18 mg/L |
| COD | 5596 mg/L | 5487 mg/L | 5513 mg/L |

## 4.3 Growth of *Chlorella pyrenoidosa* in AD waste water: Month of June

The possibility of microalgal nitrogen treatment was tested in biogas digester wastewater. In this work, *Chlorella pyrenoidosa* was cultivated in biogas digester wastewater as a nutrient source. The growth kinetics of the algae as well as the bio-remediation effect on the waste water was studied. The microalgae, *Chlorella pyrenoidosa* can utilize the nitrogen content present in biogas digester wastewater as a substrate for its growth. The growth of microalgae was found to follow the Monod growth model satisfactorily.

Under the optimal condition in biogas waste water medium of microalgae, a maximum biomass of 2.71 gm/L was obtained in fifteen days during summers when room temperature was 35 $\pm$ 4 ℃. The net specific growth rate of microalgae *Chlorella pyrenoidosa* was found to be 0.1 $D^{-1}$. The growing algae also removed 76 % of nitrate nitrogen ($NO_3 - N$) from the biogas wastewater. The growth kinetics of C. pyrenoidosa is shown Figure 4.1.

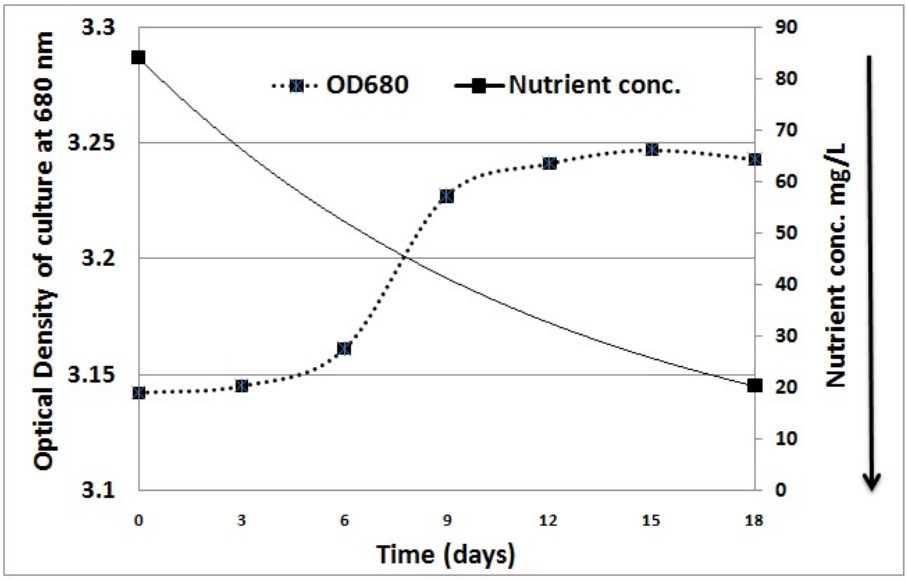


Figure 4.1: Growth Kinetics of *Chlorella pyrenoidosa* in biogas digester outlet slurry

## 4.4 Growth of *Chlorella Pyrenoidosa* in AD waste water: Month of October

One more experiment conducted during winter season when room temperature was 19 ± 4 ℃. In this study microalga biomass was found to be 3.9 gm/L. This system removed 92.8 % of nitrate nitrogen from the medium. Algal growths in terms of optical density $OD_{680}$ in the Biogas wastewaters under axenic condition were plotted in Figure 4.2.

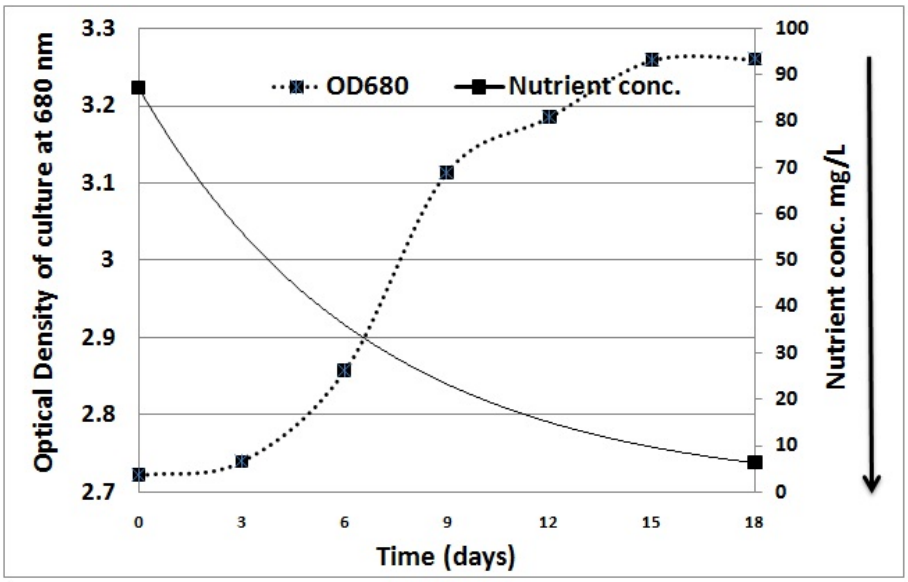


Figure 4.2: Growth kinetics of *Chlorella pyrenoidosa*

## 4.5 Growth of C. Pyrenoidosa in AD waste water: Month of February

Batch experiments were conducted to study the growth of *Chlorella pyrenoidosa* in biogas digester wastewater or nitrified effluent. In this experiment, *Chlorella pyrenoidosa* was cultured in a tubular shape acrylic body phtobioreactor with a source of light.

Batch reactor of 10 L capacity is used to cultivate *Chlorella pyrenoidosa* in anaerobic digester waste water. Wet 87.1 gm/L was *Chlorella pyrenoidosa* cultivated in the fifteen days of batch cultivation period in biogas waste water at room temperature. This study demonstrated that the microalgae *Chlorella pyrenoidosa* could grow well in biogas wastewater and could be used for the treatment of the wastewater.

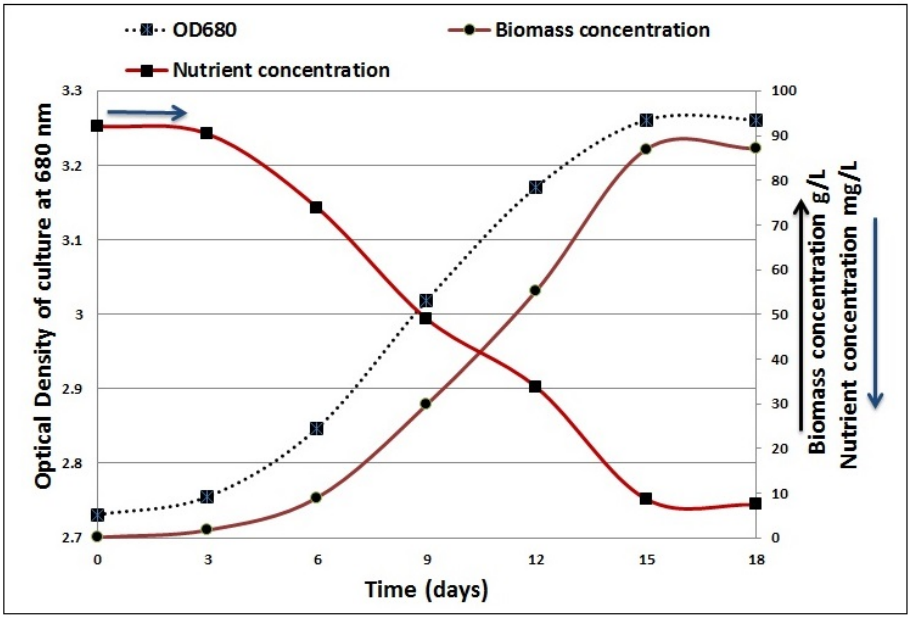


Figure 4.3: Growth and nutrient removal profiles of Chlorella sp. under outdoor conditions: Variation of biomass concentration and $OD_{680}$

## 4.6 Correlation of biomass concentration to $OD_{680}$

*Chlorella pyrenoidosa* could grow well in the biogas wastewater. The monod growth model equation was used in this study as reported by Perez *et al.* (2004). Number of growth

models are found in the literature, such as the models of Gompertz, Richards, Stannard, Schnute, and the logistic model. These models describe only the number of organisms and do not include the consumption of substrate as a model based on the Monod equation would do Zwietering *et al.* (1990).

The corresponding algal culture densities ($OD_{680}$) were 3.24 (June), 3.261 (October) and 3.26 (February), respectively (Figure 4.1, 4.2 and 4.3). The final biomass concentration at the end of the exponential phase ($15^{th}$ day) was found to be 87.1 gm/L (10 L batch; month February), 2.71 gm/L (1 L batch; month June) and 3.9 gm/L (1 L batch in October) respectively and all the results are shown in Table 4.2. Monod growth kinetic parameters specific growth rate ($\mu_g$); initial and final concentration of biomass ($X_o$, $X_t$) and substrate ($S_o$, $S_t$); and doubling time ($\tau_d$) at different room temperature is shown in Table 4.2. Doubling time of Chlorella pyrenoidosa is almost similar in different outdoor conditions. Moreover, *Chlorella pyrenoidosa* was grown well in the biogas wastewater and followed Monod growth kinetics satisfactorily. The observed higher biomass productivity could be due to significantly higher nutrient concentration in anaerobic digester outlet slurry.

Table 4.2: Kinetic growth parameters of batch cultivation from Monod model at outdoor conditions

| **Temperature** | $X_o$ **(gm/L)** | $X_f$ **(gm/L)** | $S_o$ **(mg/L)** | $S_f$ **(mg/L)** | $\tau_d$ **(d)** | $\mu_g(d^{-1})$ |
|---|---|---|---|---|---|---|
| 17-22 ℃ in autumn (October) | 0.1 | 3.9 | 87 | 6.19 | 3.3 | 0.205 |
| 26-31 ℃ in summer (June) | 0.1 | 2.71 | 84 | 20.16 | 3.85 | 0.18 |
| 15-21 ℃ in winter (February) | 1 | 87.1 | 93.1 | 14 | 2.79 | 0.248 |

## 4.7 Bioremdiation by microalgae: Nitrate consumption and COD analysis

A wide variety of nitrogen sources, such as ammonia, nitrate, nitrite and urea, can be used for growing microalgae Makareviciene *et al.* (2011). In the experimental data, $NaNO_3$ were used to investigate the effect of the source of N and concentration of $NaNO_3$ on the growth of biomass. The removal efficiency of N of *Chlorella pyrenoidosa* at different room temperatures reached relatively high values: 92.8%, 75.4 % and 93.7 % respectively. During the algae cultivation period major part of organic pollutants was consumed. Reduction of COD also led to the conclusion that Chlorella species is suitable for the bioremediation process. Bioremediation effect in outdoor conditions (specify by month) is shown in Table 4.3.

Table 4.3: % Reduction of pollutants

| **Month** | **Before culture (mg/L)** | | | **After culture (mg/L)** | | | **% reduction** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **COD** | $N-No_3$ | **TAN** | **COD** | $N-No_3$ | **TAN** | **COD** | $N-No_3$ | **TAN** |
| June | 5596 | 84 | 145.07 | 2331 | 20.16 | 18.44 | 58.34 | 76 | 87.28 |
| October | 5487 | 87 | 139 | 2179 | 6.19 | 12.05 | 60.28 | 92.8 | 91.33 |
| February | 5513 | 93.1 | 155.18 | 2047 | 7.4 | 10.08 | 62.86 | 92.05 | 93.5 |

## 4.8 CHN analysis

The CHN analysis was performed for the cultivated species at the end of the runs. The characteristics of Chlorella pyrenoidosa biomass and fresh cattle dung are summarized in Table 4.4. For this species the carbon content content was 57 %, the hydrogen was 6.8 % and the nitrogen was 7.1 % . The sample weight was 32 gm. CHN of the species was analysed using CHN Thermo FLASH 2000 Series CHN Analyzer. C:N ratio was 8.02, which is very low for the anaerobic digestion. To maintain the C:N 20 Mandalam and Palsson (1998), co-digestion of algal biomass with cow dung was done. With co digestion of same quantity of Cow dung and Algae the C/N ratio was 18.1:1.

Table 4.4: Characteristics of algal biomass and fresh cow dung

| Parameters | Algal biomass | Cow dung |
|---|---|---|
| C (% ) | 57 | 36.1 |
| H (% ) | 6.8 | 5 |
| N (% ) | 7.1 | 1.7 |
| C/N ratio | 8.02 | 21.2 |

## 4.9 Anaerobic digestion of cultured microalgae and treated water

Table 4.5 summarizes the experimental conditions and the corresponding methane conversion yield in this study. The suitability of fresh microalgal biomass as substrate for the production of biogas was assessed in anaerobic fermentation batch tests over a period of 20 days. This increased biogas production in the first fifteen days of experiment was most likely due to low levels of complex sugars and lignin present in the microalgae composition that facilitates biodegradability Nigam *et al.* (2011).

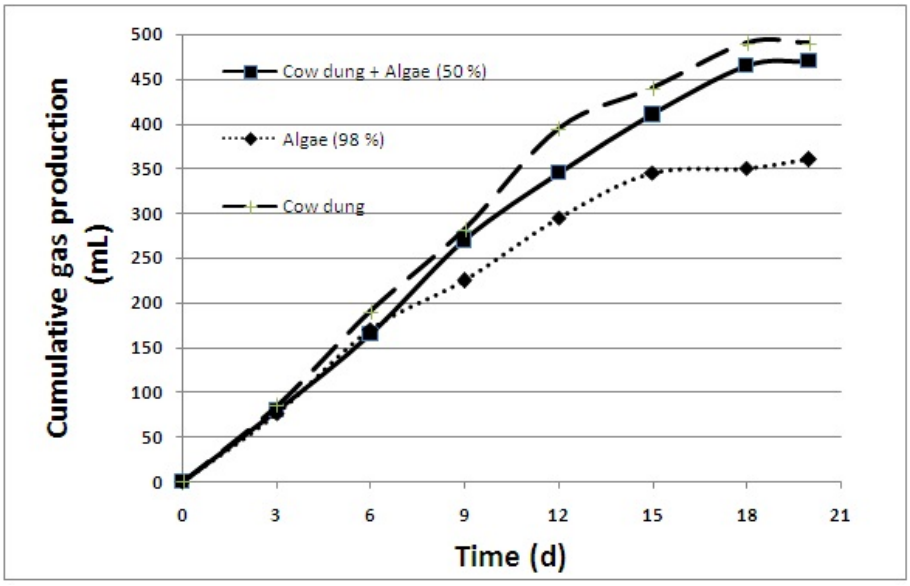


Figure 4.4: Daily biogas production profiles of algal biomass, cow dung and their mixture

It was clear that the set that contained algae alone can effectively digested compared to the cow dung alone as shown in Figure 4.4.

It was clear from the Table 4.5 that the algae alone can effectively be digested in comparison to the cow dung alone as shown in Figure 4.4.

Table 4.5: Comparison of mesophilic digestion of microalgae and sewage sludge

| Substrate | HRT (d) | Biogas yield (ml) | Methane (%) |
|---|---|---|---|
| Cow dung (50 %) + Algae (50 %) | 20 | 470 | 61 |
| Cow dung | 20 | 490 | 62 |
| Algae (98 %) | 20 | 360 | 56 |

## 4.10 The closed process involving codigestion and algae culturing has higher bioenergy generation potential

The present study revealed the suitability of closed algae culturing with treatment of AD waste water and biogas production. In order to keep the nutrient balance in anaerobic digestion, the released nitrogen could be recycled either for the growth of microalgae as nutrient, in co-digestion material (for cow dung and microalgae) or as fertilizer. Since one of the goals for the anaerobic digestion concept is to eliminate the need for chemical nitrogen fertilizer, the first strategy is chosen as shown in Table 4.5. By co-digesting cow dung-microalgae and treated water mixture, the nitrogen balance can be maintained. Mass balance for Nitrate Nitrogen and water for closed biogas cum biomass production is shown in Figure 4.5. By integrating 2 L of anaerobic digester and 10 L of photobioreactor, continuous production of 1720 ml of biogas was found. This closed loop was successfully operated in outdoor conditions. The mass balances were used to estimate the potential for biogas production and nutrient recovery from the anaerobic digestion of the algal biomass, in order to recycle the nutrients hydrolyzed back to the cultivation stage.

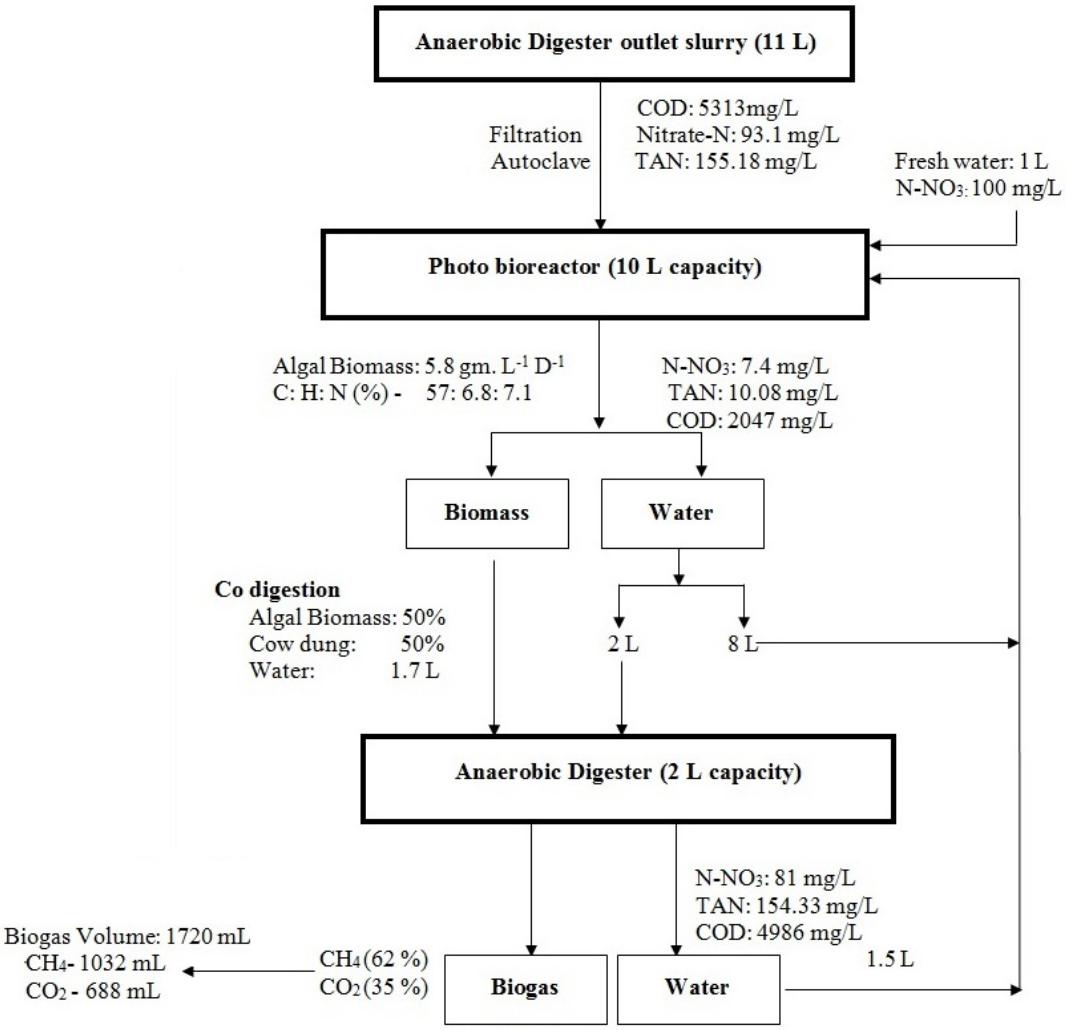


Figure 4.5: Mass Balance for closed biogas cum microalgae culturing system

## 4.11 Agent based modeling

### 4.11.1 Simulation based on biogas as a $CO_2$ source

In this study, agent based modeling of microalgae was done using $CO_2$ as a source of nutrient. The effect of $CO_2$ concentration, saturation constant for $CO_2$ utilization and reproduction ability was modelled in NetLogo software. Lipid production rate was also modelled in this simulation only. A comparison of how two different species behaved with respect to these variables was done by running the model with assigned selected values for these variables.

Twelve cases shows the effects of six variables (energy for reproduction, $CO_2$ utilization

and saturation constant for each species) on the growth or yield of micro algal species.

As per the factorial design model, which is shown in Table 4.6. Six variables with two level factorial design model explains the lipid production from the algae cells for the production of biofuels as a renewable source of energy.

Table 4.6: Factorial Design model for six variable

| Sr. No. | $RE_1$ | $RE_2$ | $CO_2$ 1 | $CO_2$ 2 | $K_1$ | $K_2$ | Yield(1) | Yield(2) |
|---|---|---|---|---|---|---|---|---|
| 1 | H | L | H | H | H | H | 2454 | 6008 |
| 2 | l | H | H | H | H | H | 6125 | 2336 |
| 3 | H | L | L | L | L | L | 162 | 3324 |
| 4 | L | H | L | L | L | L | 3364 | 148 |
| 5 | H | H | H | L | H | H | 6373 | 321 |
| 6 | H | H | L | H | H | H | 276 | 6424 |
| 7 | L | L | H | L | L | L | 2065 | 1460 |
| 8 | L | L | L | H | L | L | 1364 | 2146 |
| 9 | H | H | H | H | H | L | 5907 | 2 |
| 10 | H | H | H | H | L | H | 1 | 6001 |
| 11 | L | L | L | L | H | L | 8338 | 0 |
| 12 | L | L | L | L | L | H | 0 | 8318 |

The stages in analyzing the data in Table 4.6 using Nelson's method (1982) example are as:

Determine the difference between the average of the H as high and L as low responses for each variable.

Therefore the difference is

$$RE_1 = \sum(H) \text{ - } \sum(L)$$

The effect of an independent variable on the response is the difference between the average response at the high level and the average value at the low level.

Thus the effect of each variable is calculated as

$$RE_1 = \sum RE1 \text{ (H) } /6 \text{ - } \sum RE1 \text{ (L) } /6$$

As per the Table 4.7 it is found that Factors $K_1$ and $K_2$ i.e saturation constants show large effects which are very significant, whereas $CO_2$ utilization value shows a almost half

Table 4.7: Analysis of the yields shown in Table 4.6

| | Factor | | | | | |
|---|---|---|---|---|---|---|
| | $RE_1$ | $RE_2$ | $CO_2$ 1 | $CO_2$ 2 | $K_1$ | $K_2$ |
| $\sum H$ | 15173 | 15232 | 22925 | 22917 | 29473 | 29408 |
| $\sum L$ | 21256 | 21256 | 13504 | 13571 | 6956 | 7080 |
| Difference | -6083 | -6024 | 9421 | 9346 | 22517 | 22328 |
| Effect | -1013 | -1004 | 1570 | 1557 | 3752 | 3721 |

effect as compare to saturation constant value. The value for the reproduction energy is inversely proportional to the Yield of the cells as shown by the negative values of the effect. The requirement of high level of reproduction energy naturally generates the lower yield of cells.

Agent based simulation of mixed algal cultures (two and three species) using biogas as $CO_2$ source

A comparison of how three different species behaved with respect to these variables was done by running the model with assigned selected values for these variables. This modeling can help in choosing the microalgal species combination for stable production. The effect of two species and three species were studied. The effect of saturation constant for $CO_2$ utilization had large effect in the yield of species in both two and three species combination model.

To simulate algal growth simulation, a set of experiments were done. Eighteen cases with average as per Plackett Burman factorial design shows the effect of three variables on each species ( $CO_2$ utilization, energy require to double and saturation constant for $CO_2$ utilization for each species) on the growth of micro algal species.

Yield of cells using $CO_2$ as a source of nutrient in the these cases is summarized in Table 4.8.

The stages in analyzing the data of Table 4.9 were done using Nelson's method. The effect of an independent variable on the response is the difference between the average

Table 4.8: Factorial Design model of Three variable and two level for three species

| Sr. | Energy Utilization | | | $CO_2$ Utilization | | | K value | | | yield of cells | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $1^{st}$ | $2^{nd}$ | $3^{rd}$ |
| 1 | L | H | H | H | H | H | H | H | H | 1204 | 931 | 876 |
| 2 | H | L | H | H | H | H | H | H | H | 988 | 1296 | 902 |
| 3 | H | H | L | H | H | H | H | H | H | 945 | 802 | 1338 |
| 4 | H | H | H | L | H | H | H | H | H | 467 | 1196 | 1168 |
| 5 | H | H | H | H | L | H | H | H | H | 1310 | 564 | 1384 |
| 6 | H | H | H | H | H | L | H | H | H | 1267 | 1324 | 456 |
| 7 | H | H | H | H | H | H | L | H | H | 98 | 1023 | 1028 |
| 8 | H | H | H | H | H | H | H | L | H | 1307 | 72 | 1316 |
| 9 | H | H | H | H | H | H | H | H | L | 1154 | 1161 | 56 |
| 10 | H | L | L | L | L | L | L | L | L | 169 | 364 | 389 |
| 11 | L | H | L | L | L | L | L | L | L | 391 | 151 | 415 |
| 12 | L | L | H | L | L | L | L | L | L | 451 | 442 | 118 |
| 13 | L | L | L | H | L | L | L | L | L | 403 | 223 | 177 |
| 14 | L | L | L | L | H | L | L | L | L | 223 | 376 | 215 |
| 15 | L | L | L | L | L | H | L | L | L | 252 | 211 | 374 |
| 16 | L | L | L | L | L | L | H | L | L | 2377 | 98 | 109 |
| 17 | L | L | L | L | L | L | L | H | L | 105 | 2496 | 115 |
| 18 | L | L | L | L | L | L | L | L | H | 136 | 93 | 2279 |

response at the high level and the average value at the low level.

Thus the effect of each variable is calculated as

$$\frac{\sum H}{n_H} - \frac{\sum L}{n_L} \tag{4.1}$$

Table 4.9: Analysis of the yields which is shown in Table 4.8

| | Factor | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Effect of variables for three species factorial design model | | | | | | | | |
| | Energy Utilization | | | $CO_2$ Utilization | | | K value | | |
| | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $1^{st}$ | $2^{nd}$ | $3^{rd}$ | $1^{st}$ | $2^{nd}$ | $3^{rd}$ |
| $\sum H$ | 7705 | 7224 | 7304 | 8698 | 8181 | 8442 | 11041 | 10793 | 10747 |
| $\sum L$ | 5564 | 5599 | 5411 | 4571 | 4642 | 4273 | 2228 | 2030 | 1968 |
| Difference | 2141 | 1625 | 1893 | 4127 | 3539 | 4169 | 8813 | 8763 | 8779 |
| Effect | 237 | 180 | 315 | 458 | 393 | 463 | 979 | 973 | 975 |

Simulated data showed a linear relationship between lipid accumulation and algal growth. Effect of saturation constant for $CO_2$ utilization has a large effect and its effect in the yield of species also goes on increasing from two species combination to three species

combination.

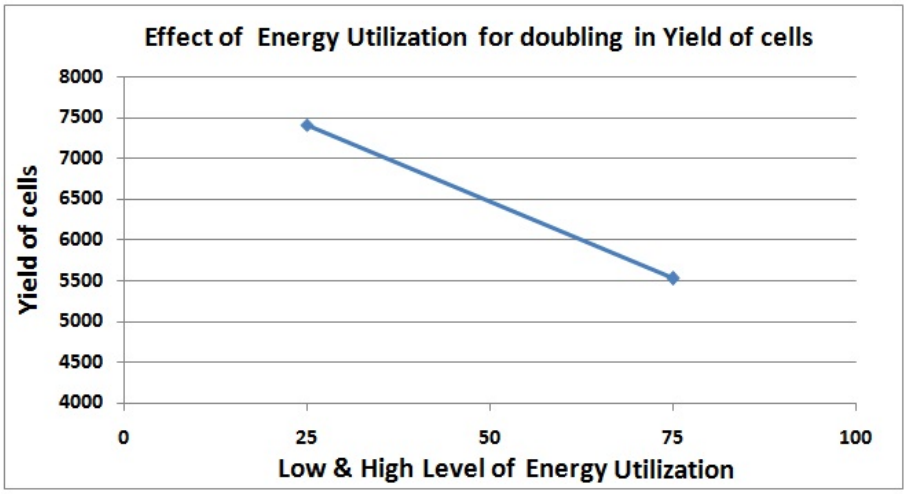


Figure 4.6: Effects of high and low level of Energy Utilization in the yield of species

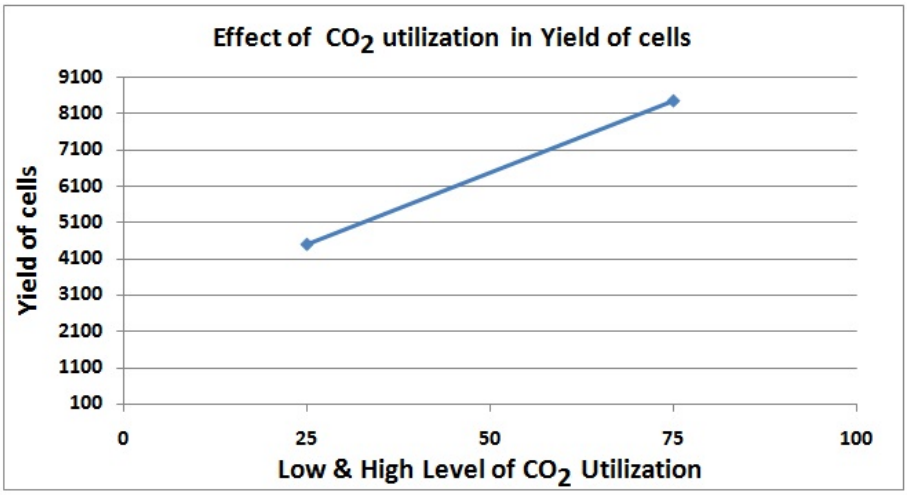


Figure 4.7: Effects of high and low level of $CO_2$ utilization in the yield of species

Based on the analysis, effect of the variable on the yield of the species in shown in the Figures 4.6, 4.7, 4.8 . The observed substrate consumption dependent biomass production suggests that $CO_2$ concentration most likely regulates the growth pattern of microalgae and was done by the model developed under this study.

Simulated data showed a linear relationship between lipid accumulation and algal growth. Effect of saturation constant for $CO_2$ utilization has a large effect and its effect in the yield of species also goes on increasing from two species combination to three species combination.

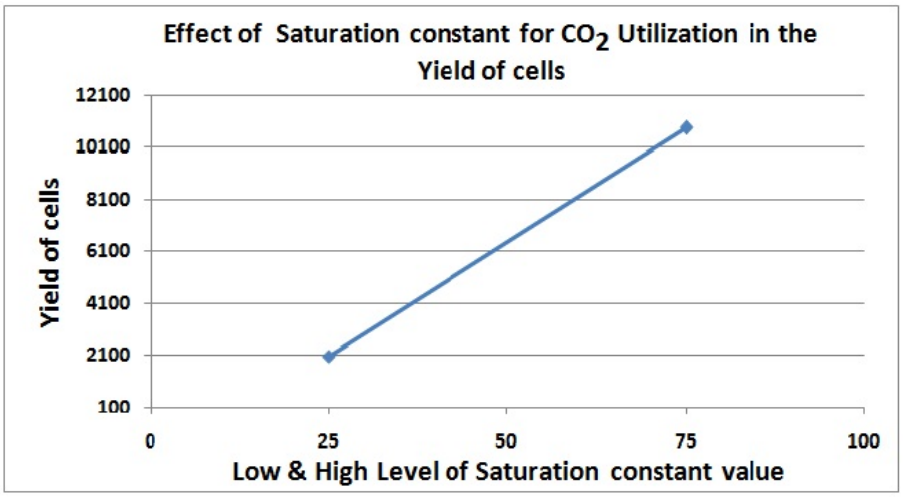


Figure 4.8: Effects of high and low level of saturation constant value of $CO_2$ utilization in the yield of species

It is found that saturation constant for $CO_2$ utilization had large effect which are very significant. This gives the optimized effects for the $CO_2$ utilization and saturation constant value. Furthermore, it was found that lipid production is microalgal growth associated. The model was applied to determine the optimum values of the parameters controlling and the choosing of species combination in the batch process. This information will be useful in the process scaling up and commercial biofuels production.

### 4.11.2 Simulation based on AD outlet slurry as a nitrogen source for microalgae

The same parameters (net specific growth rate $\mu_g$, nitrogen utilization value and saturation constant for nitrogen utilization) were used to run the model for a batch culture. Graph plotted through the Netlogo software satisfactory follows the Monod equation for microalgae cultivation and substrate utilization.

$$\frac{dx}{dt} = \mu_m \frac{s}{k_s + s} x_2 \tag{4.2}$$

$$\tau_d = \frac{ln2}{\mu_{max}} = \frac{0.6931}{\mu_{max}} \tag{4.3}$$

$$\mu_{max} = \frac{\ln x_2 - x_1}{t_2 - t_1} \tag{4.4}$$

Where $\tau_d$ is doubling time, $\mu_{max}$ is maximum specific growth rate, $K_s$ is saturation constant, $x_2$ and $x_1$ are the cell concentration and final time $t_2$ and initial time $t_1$. We can calculate doubling time based on the cell number or cell concentration and the net specific rate of replication. This model was run with a hydraulic retention time (HRT) of 20 days. The model predicted almost similar results for both the experimental study and simulation study as well.

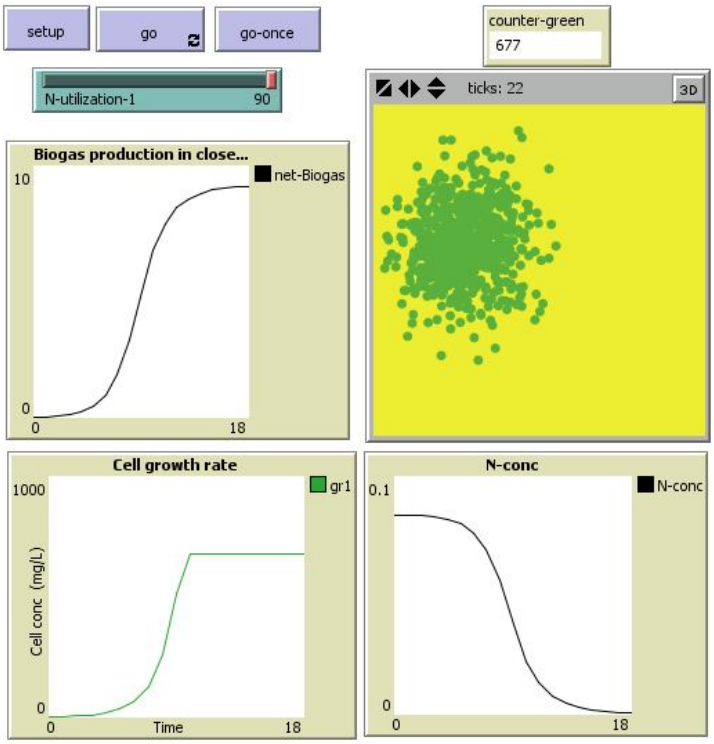


Figure 4.9: Modeled biomass concentration, substrate utilization and biogas production through netlogo

The NetLogo graph for Agent Based modelling or simulation is shown in Figure 4.9. It shows the nitrogen utilization and microalgae growth with biogas production. The growth simulation is based on the experimental values of specific growth and nitrogen utilization. The patch for modelling is shown in Figure 4.9. Green with round shape shows the microalgae growth and multiplication in nutrient rich medium. This model helps to predict the growth of microalgae species cultured based on the specific growth rate value and saturation constant value at different nitrogen utilization rates. Value of nitrogen utilization seems to be directly proportional to the biomass concentration and effect also goes on increasing with increasing the nitrogen utilization value. Biomass concentration predicted by the model followed the same pattern as it is measured in the photobioreactor. Overall, the proposed model has been able to simulate the pattern of biomass concentration change in a nutrient limited culture.

### 4.11.3 Model Prediction of Biomass Concentration

The change in biomass concentration (in terms of cell number) versus time is shown in Figure 4.10. Graph shows the three phases of growth kinetics: Lag phase, Exponential phase and Stationary phase. Simulation of microalgae based on nitrogen source satisfactorily followed Monod growth kinetics. As shown in Graph (Figure 4.10), Cell number goes on increasing with increase in nitrogen utilization percentage. Abrupt increase in cell concentration is due to the presence of nitrogen in the culture. Therefore, the algae growth is controlled by nitrogen source. The algae biomass density begins to constant after $12^{th}$ day because nitrogen and phosphorus concentration in the influent became very low. After stationary phase, if biomass is not harvested, a relatively sharper biomass decline occurs. This is due to the nutrient depletion in the batch culture.

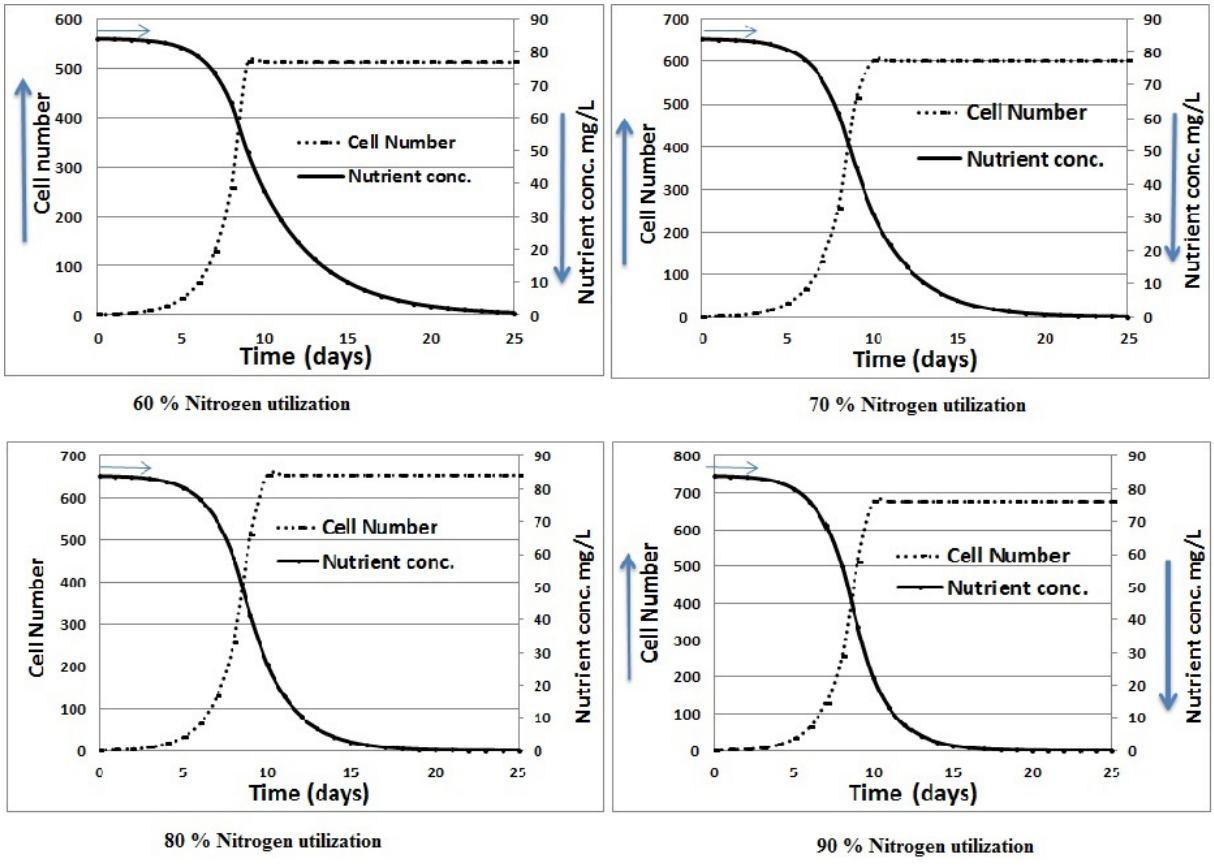


Figure 4.10: Effect of Nitrogen Utilization on microalgae growth

### 4.11.4 Model Prediction of Nitrogen Concentration

Figure 4.10 shows a model prediction for nitrogen concentration in the photobioreactor as a function of time. Initial concentration of nitrogen was 87 mg/L (based on experiments). Nitrogen is then consumed due to the algae uptake. After 4-7 days, nitrogen concentration starts to decrease. This is because the biomass concentration starts to increase after 4-5 days and nitrogen uptake by algae increases.

### 4.11.5 Comparison of Modeled Biomass Concentration with Measured Data

This section compares Nitrogen utilization or reduction with algae biomass density predicted by the model with the measured data in the month of February (as discussed in section 4.5). The modelled results was used for comparison, because we assumed nitrogen was the only source of nutrient, and the concentration of nitrogen in influent flow was better controlled during this time period. The modeled and experimental results are shown in Figure 4.11. The model simulations of nitrogen change follow the same pattern as th=e experimental results.

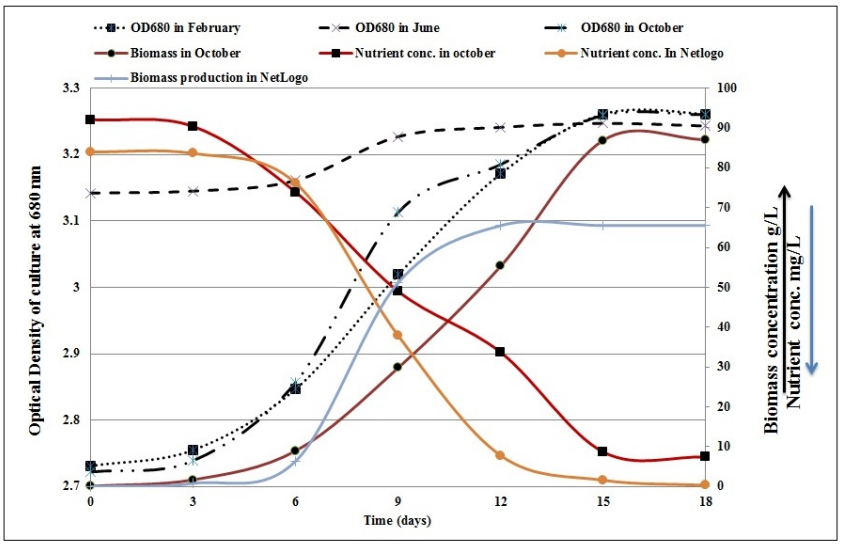


Figure 4.11: Comparison of experimental results with model simulations for nitrogen utilization

The discrepancy between model predictions and experimental data could be because of the three main reasons. As previously mentioned in model limitations section, some of the model's parameters (e.g., K and $\mu_{max}$ values) were not determined by kinetic experiments directly. Instead, those parameters were obtained from other studies of algae growth described in the literature. Secondly, some parameters such as the yield coefficient for nitrogen were derived experimentally and were assumed to remain constant during the experiment. However, yield coefficients vary with the change of the light intensity (Ogbonna and Tanaka (1996)). Lastly, the experiments were not conducted in a fully controlled environment and many other factors could impact the algae growth, which could be one reason for scattered experimental data. And some of those factors were not considered in the model developed, such as temperature and pH.

# CHAPTER 5

# Conclusion and Future recommendations

## 5.1 Overview

To conclude the thesis, the original objectives are reiterated and findings are presented. In Section 5.1 a brief overview of the work in experimental and agent based modeling is presented. A summary is provided in Section 5.2. Opportunities for future work are numerous, which is outlined in Section 5.3.

This thesis has investigated two novel approaches: an integrated closed algae-biofuel production process with nutrient as well as water recycling and the simulation of closed loop in Netlogo software using ABM technique. An alternative system for algal biofuel production was proposed. This system integrates algal biomass production, wastewater treatment and conversion of biomass to bio-methane. *Chlorella pyrenoidosa* were cultured successfully in anaerodic digester outlet slurry during outdoor conditions. This alternative approach provides an opportunity to leverage the nutrient content of wastewater for maximum biomass and biofuel production.

**Experimental Studies**

In this thesis, we investigated the potential of *Chlorella pyrenoidosa* strain for biogas production and waste water treatment. *Chlorella pyrenoidosa* could adapt well in biogas wastewater outlet slurry with small lag phases observed at the beginning. Algal growth was significantly enhanced in biogas wastewater outlet slurry because of the much higher levels of nitrogen concentration. The microalgae *Chlorella pyrenoidosa* has shown good growth in the biogas wastewater and its weight reached 3.71 gm, 1.40 gm in 750 ml medium and 87.1 gm/L in 10 L photobioreactor, after cultivation for fifteen (15) days. Although, the concentrations of nitrate nitrogen in the wastewater were extremely high,

the microalgae could still grow well in the wastewater. Thus biogas waste water is the suitable culture medium for the cultivation of microalgae.

Microalgae *Chlorella pyrenoidosa* utilized around 92.8%, 76 % and 93.6 % of the concentration of nitrate in the biogas waste water in the 15 days of inoculation period in month of Feburary, june and October. The waste water obtained after removal of algae could be reused for anaerobic digestion in a lab scale batch type anaerobic digester for the production of bio gas as a renewable source of energy. This concludes that the cultivation of *Chlorella pyrenoidosa* in biogas wastewater would be efficient, economic and saving water for anaerobic digestion as well as producing biogas.

By altering the mixture of the feedstock entering the anaerobic digester, an optimal nitrogen balance for the system was achieved. The model proved itself to be a powerful tool for understanding the symbioses and dynamics of the concept. Many symbiotic features were identified such as: nutrient removal from the digestate and nutrient supply for the algae cultivation; the need for external water source in different processes; and production of biogas.

**Agent Based Modeling**

A model was developed for predicting the algae growth in a batch culture of a photobioreactor and the biogas production. Two expressions were proposed based on the Monod model and weighted average of the Monod model. Unlike the Monod model which relates specific growth rate of algae to concentration of the limiting compound in a single limited culture, the proposed expressions account for the effect of four factors that control the growth rate in a multi-limited culture: inorganic carbon, nitrogen, doubling time and their utilization values. Biomass and substrate concentrations of culture media as a function of time were predicted by solving mass balances around the Photobioreactor and anaerobic digestion. Modeled results were compared with experimental data obtained by the algae growth and digestion in outdoor conditions, which was done for a batch culture.

The model predicted almost the same results using both (nitrogen and carbon) expressions. Biomass concentration predicted by the model followed the same pattern as

the measured biomass concentration in the photobioreactor. Overall, the proposed model has been able to simulate the pattern of biomass concentration change in a growth-limited culture.

However, there are discrepancies between model perditions and experimental data. This could be attributed to the fact that the environmental conditions varied during the experiment and some factors were not considered in the model developed. The parameters used in the model were measured directly, but either obtained from literature or derived theoretically. Model simulations for a continuous culture indicated that the HRT is very low for the growth of algae. Continuous production of biogas was also simulated. Simulated results based on both nitrogen and carbon limited follows same pattern with the experimental data.

## 5.2 Conclusion

In this work we investigated the potential of *Chlorella pyrenoidosa* for biogas production as well as waste water treatment. Our results indicate that microalgae *Chlorella pyrenoidosa* can be a good substrate for anaerobic fermentation, resulting in the production of biogas with relatively high methane content and in this respect have the potential to replace other feedstocks like maize which is generally used today. The biogas production potential is strongly dependent on the algal strain used. The adoption of low energy harvest and algal biomass cultivation has been shown to have a significant impact on the overall suitability of using algae for nutrient removal in wastewater treatt.

The major conclusion of this work was that microalgae *Chlorella pyrenoidosa* represents a viable alternative for wastewater nutrients removal systems, improving the energy balance of a integrated microalgae wastewater treatment when combined with low cost harvesting technologies in outdoor conditions.

**Specific conclusions were as follows:**

**1.** Compared to alternative conventional low-energy wastewater treatments such as aerated wetlands, the integrated microalgae with waste water treatment was demonstrated to be more economic and sustainable, reducing operational costs and carbon emissions.

**2.** The methane yields from both cow dung co-digested *Chlorella pyrenoidosa* and cow dung alone, are relatively same and they correspond well to those presented in the literature. They are almost equal to the methane yields of other cultivated crops and about half of the biowaste methane yields.

**3.** *Chlorella pyrenoidosa* could adapt well in biogas wastewater outlet slurry with small lag phases observed at beginning.

**4.** The cultivation cost of algal biomass (including harvesting and nutritional cost) was reduced by coupling the algal biomass production with wastewater treatment.

**5.** These benefits combined with the possibility of $CO_2$, waste water and nutrients recycling from the anaerobic effluents make anaerobic digestion the best technology for energy production from microalgae.

**6.** From the experimental studies, problems related to removal of toxic contaminants, biogas production and water availability was solved.

**7.** Continuous production of bio-methane was achieved by integrating the cultivation of *Chlorella pyrenoidosa* with biogas plants.

**8.** The cultivation of *Chlorella pyrenoidosa* in biogas wastewater would be efficient, economic and saving water for anaerobic digestion as well as for producing bio-methane.

**9.** This developed the sustainability of culturing low cost $3^rd$ generation feedstock for the continuous production of bio-methane in throughout the year.

**10.** Developed agent based model was applied to determine the optimum values of the microalgae culturing parameters, there controlling and the choosing of species combination in the batch process.

## 5.3 Future Recommendations

There is scope for expanding on this research in multiple areas. Few recommendations are as follows:

**1.** Furthermore, to test if inhibitory compounds are present in the anaerobic digester, it would be of great interest to perform anaerobic toxicity assays (ATAs) with the supernatants of reactors after a sufficient time of operation, as described by Tartakovsky (2013). As the risk of inhibition is higher when using greater OLRs, ATAs would be essential if the value of this parameter is increased in the future.

**2.** It is recommended that long term experiments with the integrated system combining the AWTS with the existing wastewater treatment plants are conducted to assess its performance limit. Moreover, Process scale-up is needed to integrate it with the dairy farming and cattle dung based biogas plants worldwide.

**3.** Role of inhibitors in growth medium is needed to simulate using Agent based model.

**4.** Pilot- and full-scale facilities for AD of microalgae biomass are still limited. A pilot-scale study will confirm the overall feasibility of the process. In addition, the behaviour of the algae biomass when pretreated and/or co-digested with wastewater sludge or other biomass, or when using a mix of different algae species needs to be addressed with further research.

**5.** Genome sequencing is a crucial next step for this research to identify the specific genes associated with the observed photosynthetic differences and possibly consider the transfer of those genes to other advantageous algae species.

**6.** Finally, Commercialization is needed for closed cycle.

# Publications

## Journal Publications

**1. Rohit Sharma**, Avanish K Tiwari. Sustainability of $3^{rd}$ generation feedstock for the continuous production of bio-methane under outdoor conditions. *Akshay Urja, April 2015*, , Volume 8, Issue 6, June 2015, Ministry of New and Renewable Energy, Govt, of India, New Delhi.

**2. Rohit Sharma**, Avanish K Tiwari, G. Sanjay Kumar, Bhawna Y. Lamba. Cost effective and economic method for cultivation of *Chlorella pyrenoidosa* for the simultaneous treatment of biogas digester wastewater and biogas production. *International Journal of Pharma Sciences and Research (IJPSR)*, Feb 2015; 6(2): 318-321.

**3. Rohit Sharma**, Avanish K Tiwari, G Sanjay Kumar, Bhawna Y Lamba, and Girdhar Joshi. Simulation and experimental Study of a closed model for algae based biogas Production and bioremediation of waste water. *Journal of Energy and Chemical Engineering* Nov. 2014; 2(4): 116-129.

**4. Rohit Sharma,** Avanish K Tiwari, G Sanjay Kumar and Bhawna Yadav Lamba, 2014. Development of integrated bioremediation and anaerobic digestion process using $3^{rd}$ Generation Feedstock. *International Journal of Advanced Research in Engineering and Technology (IJARET)* Oct 2014; 5(7):57-62.

**5. Rohit Sharma,** G Sanjay Kumar, Avanish K Tiwari. Novel modelling paradigm for the algal production of bio fuel. $5^{th}$ International Conference on Modeling Simulation and Applied Optimization (ICMSO'13), Tunisia, Africa. Published in IEEE explore digital library. July 2013, $978-1-4673-5812-5$. www.ieeexplore.ieee.org (International Conference Oral and Online publication).

**6. Rohit Sharma**, Avanish K Tiwari, G Sanjay Kumar, Vishaka Goel. Simulation of Algal Growth by $CO_2$ Removal from Wastewater. *International Journal of Pharma & Bio Sciences* Oct 2013; ISSN 0975-6299.

## Conference publications

**1. Rohit Sharma**, G Sanjay Kumar, Dr. Avanish K. Tiwari, *Modeling Biogas Production & $CO_2$ Removal by Algal Growth for Mixed Culture* presented in National Conference on Biolife: The Amalgamation of Multidisciplinary Life Sciences" (NCBL-2013), Lukhnow. 9-10th March, 2013 (Oral).

**2. Rohit Sharma**, G Sanjay Kumar, Dr. Avanish K Tiwari, *Cost effective & economic method for cultivation of Chlorella pyrenoidosa for the simultaneous treatment of biogas digester wastewater and biogas production* $3^{rd}$ National Conference on Recent Advances in Bioenergy Research' NIRE, Kapurthala, 22-24 Nov, 2013 (Oral).

**3. Rohit Sharma**, G Sanjay Kumar, Avanish K Tiwari *Cultivation of Chlorella pyrenoidosa for the simultaneous treatment of biogas digester wastewater and biogas production* CHEMCON 2013, ICT, Mumbai, 27-30 Dec, 2013 (Poster).

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# Appendix A

**NetLogo coding for Simulation based on AD slurry as a nutrient source for microalgae cultivation**

```
    turtles-own [ growth-rate ]
globals [ gr Biogas N-used-1 net-Biogas N-conc x i1 to-double f1 no-of-green r ]

    to setup
clear-all
setup-turtles
setup-patches
reset-ticks
end


    to setup-turtles
crt 1
ask turtle 0 [
set shape ”circle”
setcolor green
setxy random-xcor random-ycor
set growth-rate growth-rate-max
] end


    to-report N-concentration-patch
report N-conc
end


    to setup-patches
```

```
ask patches [
setpcolor yellow
set N-conc (92.3 )
]
end


    to move
if not any? turtles [ stop ]
ask turtles [
right random 360
forward 1
]
end


    to uptake-N
ask turtles [
ifcolor = green [
set N-used-1 (N-utilization-1 / 100 * N-conc)
set growth-rate ( ( growth-rate-max * N-used-1) * no-of-green / K1 + N-used-1 )
set N-conc ( (( N-conc * 4 * max-pxcor * max-pycor) - N-used-1)/ (4 * max-pxcor *
max-pycor))
]
] end


    to double ;; algae procedure
ask turtles
    ifcolor = green [
;; give birth to a new algae, but it uses growth rate
if growth-rate > growth-rate-max
```

```
] ] ] end

    to Biogas-production
ask turtles [
ifcolor = green [
set Biogas ( 2 / 1000 * growth-rate )
set net-Biogas net-Biogas + Biogas
] ] end

    to death
ask turtles [
if N-conc = 0 [
die]
] end

    to-report counter-green
report count turtles with [color = green]
end

    to initial-count
set i1 count turtles with [color = green]
end

    to final-count
set f1 count turtles with [color = green]
end
```

```
    to do-plots-count
;; set-current-plot ”Yield of Cells”
;;set-current-plot-pen ”green”
;;plot f1

    set-current-plot ”Biogas Production”
set-current-plot-pen ”Biogas”
plot net-Biogas

    set-current-plot ”Biomass Vs. Substrate conc”
set-current-plot-pen ”gr1”
plot f1 / 19
set-current-plot ”N-conc”
set-current-plot-pen ”N-conc”
plot N-conc
end

    to go
if ticks = 25
    stop

if count turtles = 0
    stop

initial-count
move
uptake-N
double
death
```

```
Biogas-production
final-count
;;do-plots-count
tick
end


    to go-once
if count turtles = 0
    stop


if ticks = 25
    stop


initial-count
move
uptake-N
double
death
Biogas-production
final-count
do-plots-count
tick
end
```

# Appendix B

**Netlogo coding for simulation based on biogas as $CO_2$ source**

```
    turtles-own [ energy ]
globals [ gr1 gr2 lpd-1 lpd-2 CO2-used-1 CO2-used-2 lpd-net-1 lpd-net-2 CO2-conc x i1
i2 f1 f2 no-of-pink no-of-blue r ]


    to setup
clear-all
setup-turtles
setup-patches
reset-ticks
end


    to setup-turtles
crt 2
ask turtle 0 [
set shape ”circle”
set color pink
setxy random-xcor random-ycor
set energy to-reproduce-1
]
ask turtle 1 [
set color blue
setxy random-xcor random-ycor
set shape ”circle”
set energy to-reproduce-2
```

```
]
end

    to-report CO2-concentration-patch
report CO2-conc
end

    to setup-patches
ask patches [
set pcolor yellow
set CO2-conc (0.6875 / ( 4 * max-pxcor * max-pycor ))
]
end

    to move
ask turtles [
right random 360
forward 1
]
end

    to uptake-CO2
ask turtles [
if color = pink [
set CO2-used-1 ( CO2-utilization-1 / 100 * CO2-conc)
set energy energy + ( 1210 * CO2-used-1 )
set CO2-conc ( (( CO2-conc * 4 * max-pxcor * max-pycor) - CO2-used-1)/ (4 * max-pxcor
* max-pycor))
```

```
]
if color = blue [
set CO2-used-2 ( CO2-utilization-2 / 100 * CO2-conc)
set energy energy + (1210 * CO2-used-2 )
set CO2-conc ((( CO2-conc * 4 * max-pxcor * max-pycor) - CO2-used-2)/ (4 * max-pxcor
* max-pycor))
] ]
ask patches[
set pcolor pcolor + 0.05 ]
end


    to reproduce
ask turtles [
if color = pink [
set x ( count turtles-here)
ifelse x <= K1 [
if energy >= to-reproduce-1 [
;; p = no of pink
set no-of-pink ( count turtles-here with [color = pink] )
;; b = no of blue
set no-of-blue ( count turtles-here with [color = blue] )
;; r = rand(0 -> 1)
random-seed (ticks)
;; if r < p / (p + b)
set r ( random 100 )
if (r / 100) < ( no-of-pink / (no-of-pink + no-of-blue) )[
set energy energy - to-reproduce-1
hatch 1 [
set energy to-reproduce-1
```

```
] ] ] ]
    die
]
if color = blue [
set x (count turtles-here)
ifelse x <= K2 [
if energy >= to-reproduce-2 [
;; no of pink
set no-of-pink ( count turtles-here with [color = pink])
;; no of blue
set no-of-blue ( count turtles-here with [color = blue])
;; r = random(0 -> 1)
random-seed (ticks)
;; if r < p / (p + b)
set r ( random 100 )
if (r / 100 ) < ( no-of-blue / (no-of-pink + no-of-blue) ) [
set energy energy - to-reproduce-2
hatch 1 [
set energy to-reproduce-2
] ]] ]
    die

]]
end


    to lipid-production
ask turtles [
if color = pink [
set lpd-1 ( 1 / 1.23 * CO2-used-1 )
set lpd-net-1 lpd-net-1 + lpd-1
```

```
]
if color = blue [
set lpd-2 ( 1 / 1.23 * CO2-used-2)
set lpd-net-2 lpd-net-2 + lpd-2
] ]
end


    to death
ask turtles [
if energy = 0 [
die]
]
end


    to-report counter-pink
report count turtles with [color = pink]
end


    to-report counter-blue
report count turtles with [color = blue]
end


    to initial-count
set i1 count turtles with [color = pink]
set i2 count turtles with [color = blue]
end


    to final-count
```

```
set f1 count turtles with [color = pink]
set f2 count turtles with [color = blue]
end


    to do-plots-count
set-current-plot ”Algae”
set-current-plot-pen ”pink”
plot f1
set-current-plot-pen ”blue”
plot f2
set-current-plot ”lipid”
set-current-plot-pen ”lpd-1”
plot lpd-net-1
set-current-plot-pen ”lpd-2”
plot lpd-net-2
set-current-plot ”CO2”
set-current-plot-pen ”CO2-conc”
plot CO2-conc
end


    to go
if ticks = 36
    stop


if count turtles = 0
    stop


initial-count
move
```

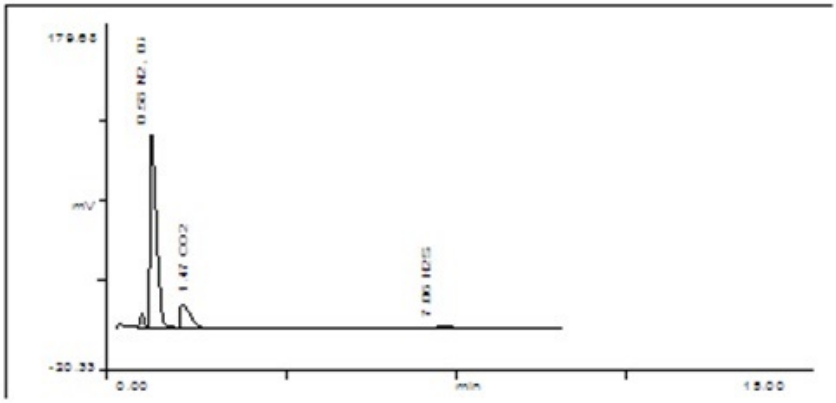
```
uptake-CO2
reproduce
death
lipid-production
final-count
;;do-plots-count
tick
end


    to go-once
if count turtles = 0
    stop


if ticks = 50
    stop


initial-count
move
uptake-CO2
reproduce
death
lipid-production
final-count
;;do-plots-count
tick
end
```

# Appendix C

REPORT

Sample Name: 26feb2012bgjd
Data File: D:\backupgc\26feb2012bgjd.DAT
Method File: BIO GAS STD NORM.MET
Detector: TCD. System: GC
Date: 30 Mar 2014 Time: 6:48:38
Run: ch1: 0
Type of Analysis : Normalisation On Area
Report printed on : 15/5/2014 at : 21:13:53

| Pk.Wdth | Peak Thrsh. | Area Rej. | Ht.Rej. | Time Scale |
|---|---|---|---|---|
| 8 | 30 | 5 | 4 | 20.0 |




| No. | R.T. | Area | Resp Fact | Con Amt | Pk Ty | Comp Name | CI |
|---|---|---|---|---|---|---|---|
| 1 | 0.58 | 499116 | 3.706740e-06 | 1.4946 | BV | N2 | |
| 2 | 0.80 | 12457610 | 6.193760e-06 | 62.3326 | VV | CH4 | |
| 3 | 1.47 | 2404036 | 1.758260e-05 | 34.1468 | VB | CO2 | |
| 4 | 7.06 | 699665 | 3.584340e-06 | 2.0259 | BB | H2S | |
| | | 16060427 | | 100.0000 | | | |

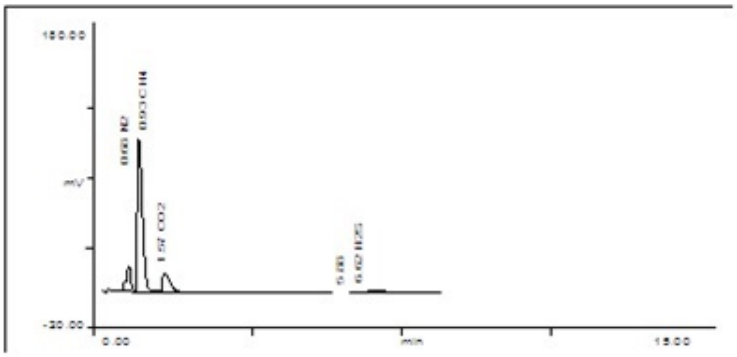
Page No. :1

Figure 5.1: Chromatograph usinng microalgae 98 % as a substrate

REPORT

Sample Name: BIO GAS STD NORM
Data File: D:\backupgc\BIO GAS STD NORM.DAT
Method File: BIO GAS STD NORM.MET
Detector: TCD. System: GC
Date: 30 Mar 2014 Time: 17:55:26
Run: ch1: 0
Type of Analysis : Normalization On Area
Report printed on : 15/5/2014 at : 22:13:57

| Pk.Wdth | Peak Thrsh. | Area Rej. | Ht.Rej. | Time Scale |
|---|---|---|---|---|
| 8 | 30 | 5 | 4 | 20.0 |




| No. | R.T. | Area | Resp Fact | Con Amt | Pk Ty | Comp Name | CI |
|---|---|---|---|---|---|---|---|
| 1 | 0.68 | 1599787 | 3.706740e-06 | 5.9300 | BV | N2 | |
| 2 | 0.93 | 9901897 | 6.193760e-06 | 61.3300 | VV | CH4 | |
| 3 | 1.57 | 1784720 | 1.758260e-05 | 31.3800 | VB | CO2 | |
| 4 | 6.62 | 379428 | 3.584340e-06 | 1.3600 | PB | H2S | |
| | | 13665832 | | 100.0000 | | | |

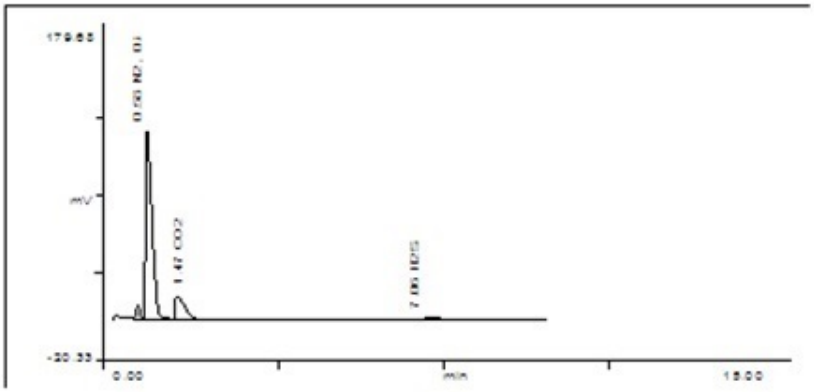
Page No. :1

Figure 5.2: Chromatograph using Cowdung as a substrate

REPORT

Sample Name: 26feb2012bgjd
Data File: D:\backupgc\26feb2012bgjd.DAT
Method File: BIO GAS STD NORM.MET
Detector: TCD. System: GC
Date: 30 Mar 2014 Time: 6:48:38
Run: ch1: 0
Type of Analysis : Normalisation On Area
Report printed on : 15/5/2014 at : 21:13:53

| Pk.Wdth | Peak Thrsh. | Area Rej. | Ht.Rej. | Time Scale |
|---|---|---|---|---|
| 8 | 30 | 5 | 4 | 20.0 |

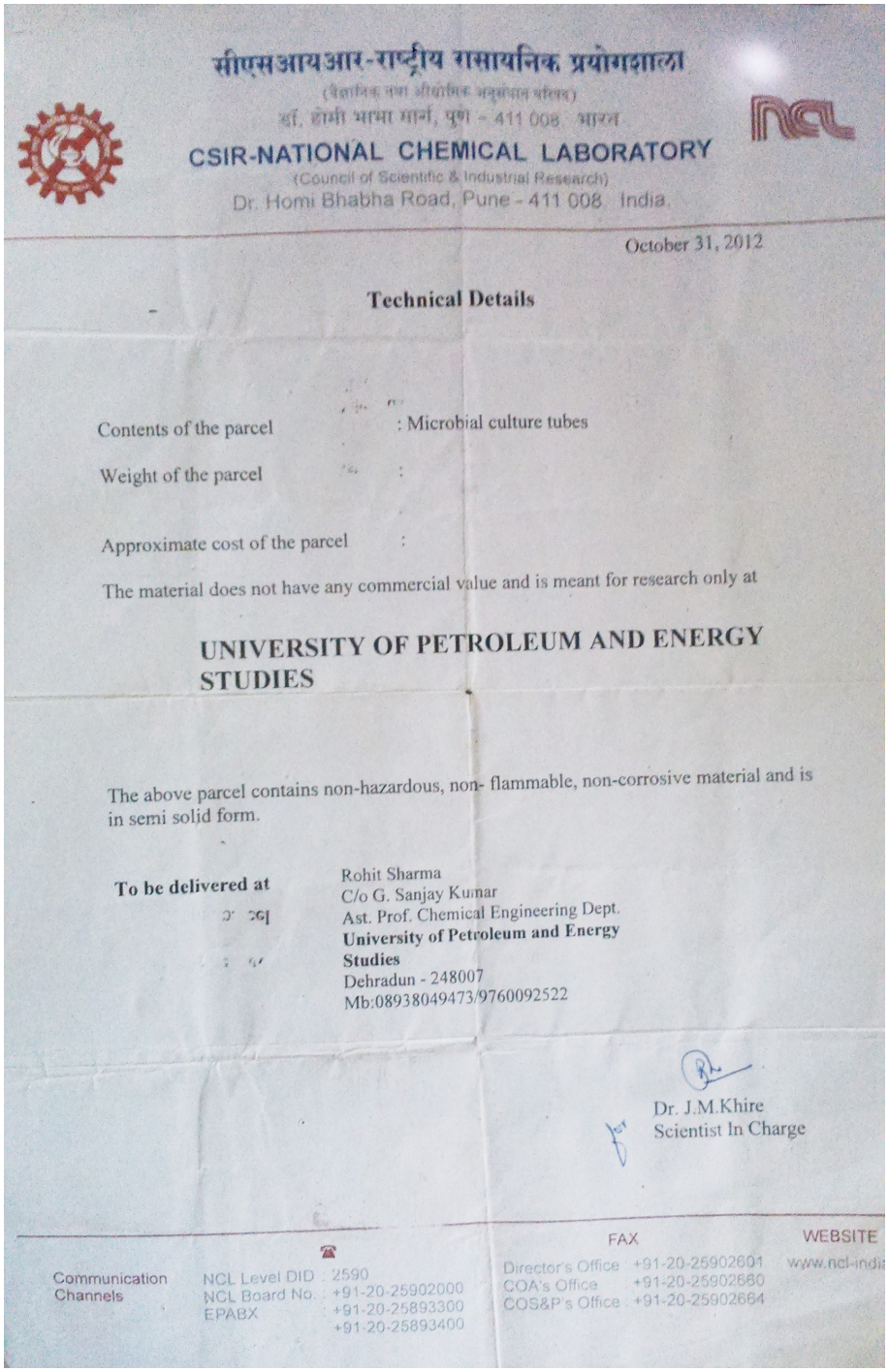



| No. | R.T. | Area | Resp Fact | Con Amt | Pk Ty | Comp Name | CI |
|---|---|---|---|---|---|---|---|
| 1 | 0.58 | 499116 | 3.706740e-06 | 1.4946 | BV | N2 | |
| 2 | 0.80 | 12457610 | 6.193760e-06 | 62.3326 | VV | CH4 | |
| 3 | 1.47 | 2404036 | 1.758260e-05 | 34.1468 | VB | CO2 | |
| 4 | 7.06 | 699665 | 3.584340e-06 | 2.0259 | BB | H2S | |
| | | 16060427 | | 100.0000 | | | |

Page No. :1

Figure 5.3: Chromatograph using codigestion of Cow dung & Algae 50 % as a substrate

# Appendix D



सीएसआयआर-राष्ट्रीय रासायनिक प्रयोगशाला
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CSIR-NATIONAL CHEMICAL LABORATORY
(Council of Scientific & Industrial Research)
Dr. Homi Bhabha Road, Pune - 411 008 India.

October 31, 2012

**Technical Details**

Contents of the parcel : Microbial culture tubes

Weight of the parcel :

Approximate cost of the parcel :

The material does not have any commercial value and is meant for research only at

**UNIVERSITY OF PETROLEUM AND ENERGY STUDIES**

The above parcel contains non-hazardous, non- flammable, non-corrosive material and is in semi solid form.

**To be delivered at**

Rohit Sharma
C/o G. Sanjay Kumar
Ast. Prof. Chemical Engineering Dept.
**University of Petroleum and Energy Studies**
Dehradun - 248007
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