Food and agriculture waste as an organic and inorganic nutrient source for the cultivation of *Desmodesmus armatus*

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

by

Anushka Kashyap

(Roll No: 151841)

Tanvi Taneja

(Roll No: 151822)

Under the Guidance of

Dr. Garlapati Vijay Kumar

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS

HP-173234, INDIA

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **"Food and agriculture waste as an organic and inorganic nutrient source for the cultivation of *Desmodesmus armatus*"**, submitted by Anushka Kashyap (151841) and Tanvi Taneja (151822) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of his original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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Date:

<u>DECLARATION</u>

We hereby declare that the work reported in the B. Tech. thesis entitled **“Food and agriculture waste as an organic and inorganic nutrient source for the cultivation of *Desmodesmus armatus*”** submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of our work carried out under the supervision of Dr. Garlapati Vijay Kumar, Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. We have not submitted this work elsewhere for any other degree or diploma.

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(Tanvi Taneja, 151822) **(Anushka Kashyap, 151841)**

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LIST OF SYMBOLS AND ACRONYMS

°C	Degree Celsius
µg/ml	Microgram per milliliter
mg/ml	Milligram per milliliter
mm	Millimeter
rpm	Revolutions per minute
µl	micro-liter
BBM	Bold Basal's Media
nm	Nanometre
OD	Optical Density

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ABSTRACT

The present examination explores the imminent of substituting organic (food waste) and inorganic (agriculture) medium as a supplement for the development of *Desmodesmus armatus.* Different percentages of agriculture waste and food waste were blends were supplanted in the inorganic medium to look at the algal development and biochemical structures. The utilization of organic medium was found to be better than the inorganic medium as higher biomass fixation algal growth was found in food waste and better lipid and protein content contrasted and micro algae development in completely inorganic medium. These outcomes displayed the capability of joining the inorganic medium with food waste medium as a powerful method to lessen the development cost of micro algae and to build the biochemical substance in the developed micro algae.

Keywords: *Desmodesmus armatus*; *Agri-compost; Food waste; Growth media*; *Protein; Carbohydrate; Lipid*

CHAPTER 1

INTRODUCTION

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More than one billion tons of nourishment squanders (food waste) are created and these nourishment squanders either require treatments or are arranged in landfills. The change of nourishment squander to esteem included items by natural methods, for example, treating the soil is an ecological agreeable technique for compelling natural waste administration (Cerda et al., 2018). The finished result of treating the soil is a balanced out natural issue to be rich in nutrients and precious metals which contains fundamentals supplements to be utilized as the natural manure (Zhouetal., 2016). Contrasted and inorganic fertilizer, the generation of natural manure can accomplish a spared discharges of 4– 81 kg CO_2 for every ton of food waste and agriculture waste and 4– 67 kg CO2 for each ton of garden squander (Boldrin et al., 2009).

The nitrogen and phosphorus content in fertilizer delivered by nourishment waste can be utilized by agriculture for sustenance creation. In addition, food waste contains high content of natural segments, for example, starches, proteins, lipids and natural acids which makes it a sensible feed stock for developing microorganisms (Zeng et al., 2018). The reusing of the supplements from sustenance waste could conceivably supplant by the non-sustainable feed stock in different microorganisms' development, which will prompt by the mitigation of the worldwide vitality emergency. And it will add to better supplement use and a decrease in contamination level that is somehow or brought by another inorganic treatment. Micro algae is developed and connected broadly because of its promising nature as a biomass feed stock. Micro algae has high development rates, and very less challenges by the earthly harvests and a worldwide distribution.

The generation of biofuel from micro algae was observed to be a lot more noteworthy than nourishment of the crops and non-sustenance crops feed stock, moreover to the shorter development time requirement for micro algae and (Chia et al., 2017). Aside from this, their undiscovered property to deliver numerous profitable items notwithstanding bio-fuels has excited the interest of different ventures (Chew et al., 2017; Yen et al., 2013). The extending interest in the business improvement of items with utilitarian mixes keep the center around micro algae metabolites which can be utilized in sustenance, medicines ,food items (Cuellar- Bermudez et al., 2015). Micro algae can deliver a wide scope of functional fixings which can incorporate proteins, polysaccharides, regular pigments, nutrients, basic Amino acids. The utilitarian protein bio molecules from micro algae are particularly of enthusiasm of their well being advancing impact natural properties like cancer prevention agents, anticancer, anticoagulant, anti tumor, anti hypertensive what's more, safe modulator exercises (Samarakoon and Jeon et al., 2012).

The production and scaling up of micro algae creation for bio molecules extraction requires a substantial amount of freshwater and surprising expense supplements. This makes the need to locate a practical minimal effort elective medium which can give comparative supplements expected to micro algae development (Chia et al., 2018). Waste waters are being utilized to develop micro algae since their development can purge the waste water before releasing them into water bodies (Wang et al., 2016). By the way , the use of waste water represents a couple of vulnerabilities for example, the likelihood of pollution, nearness of perilous chemicals and changing supplement substance which is going to influence the development of micro algae. Another important wellspring of supplement medium is the utilization of food waste for micro-algae development. Sustenance squanders hydrosylates which have effectively been utilized for the development of micro algal and demonstrated great development of micro algae (Pleissner et al., 2013). A coupled arrangement of dim aging and micro algal cultivation is likewise done utilizing substrates of nourishment squander and gave a supportable course of proficient vitality recuperation (Ren et al., 2018). The significant contamination of sustenance squander is mostly due to ammonia and phosphates, and the centralization of the toxins present which can be decreased and can be used effectively to develop micro algae, realizing a minimization of the pollution level in water (Cerda et al., 2018; Zhang et al., 2018). The combination of sustenance squander manure medium and inorganic medium could possibly substitute the real supplements, for example, nitrate and phosphate required for micro algae development. This will prompt and lead to maintainable modern micro algal development mechanism for resulting bio-refinery. The point of this examination was to explore the capability of utilizing sustenance squander fertilizer as a supplement hot-spot towards the development of *Chlorella vulgaris*. For the achievability in supplanting in organic manure with organic manure for development were investigated by utilizing different convergences of nourishment squander manure blend and to decide the algal biomass gathering from every blend. The ideal focus blend was distinguished the biochemical organizations in micro algae, for example, lipid, protein and sugars substance were removed from the micro algae biomass to assess the profitability of the biomass for fuel, nourishment and feed creations.

Micro algal biomass is known for its nutritious arrangement consisting of lipids, sugars, and proteins. Micro algal lipids may contain high measures of x-3 poly UN-immersed unsaturated fats (PUFA), e.g., (DHA, C22:6) and (ALA, C18:3), with "extraordinary significance to the prevention of abrupt passing from ventricular fibrillation" and basic for mental health of newborn children (Connor, 2000). are typically found at high concentration in marine biomass e.g., shellfish (Pleissner et al., 2012), yet micro algae have been expert acted like an elective source.

Also, micro algal biomass rich in carbohydrates and proteins is needed as feed in aquaculture. Besides, soaked and monounsaturated unsaturated fats present in micro algal lipids are considered for bio diesel production. The potential outcomes of utilizing algal biomass as sustenance and feed, PUFA as sustenance added substances, and changing over lipids into bio fuels make micro algae intriguing for sustenance and feed ventures, and as a sustainable power source. So as to deliver bio fuels from micro algal biomass two noteworthy monetary disadvantages must be survived. Development of hetero tropic algal strains relies upon cost-concentrated carbon sources making development forms unfavorable. For instance, lipids from hetero-tropic micro algae are considered to incompletely supplant fossil oils in bio diesel creation yet from a financial perspective this idea is confined by high costs for supplements and 80% of the general costs account alone for glucose. Moreover, costs for nitrogen, phosphorous, nutrients, and follow metals should be taken in thought. In any case, the financial disadvantage of lipid extraction can be defeat by improving the yield of separated lipids as appeared *Chlorella vulgaris* utilizing distinctive extraction techniques.

Aim of this study was to use the food and agricultural waste medium can be used as a nutrient medium for the cultivation of micro algae .The transformation of waste into value added products through biological means such as composting in an environment-friendly method for effective organic waste management. The nitrogen and phosphorus content in the compost produced from food and agricultural wastes can be used in agriculture for food production.Growth kinetics study of *Desmodesmus armatus* using food agricultural compost wastes as nutrient sources Vs Standard Media. To check out the algal biomass concentration grown in waste composts Vs Standard Media .To analyzes the biochemical con. Of lipid, protein and carbohydrates contents in micro algae cultivated in waste compost

CHAPTER 2

REVIEW OF LITERATURE

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Microalgae are found in numerous marine spineless creatures, including corals, mollusks, and wipes. These photosynthetic symbionts give their hosts supplements. On account of corals, the micro-algae, usually called zooxanthellae, give over half of the carbon and vitality necessities of their hosts by delivering sugars by means of photosynthesis and after that exchanging the sugar to the coral. Hence, the beneficial interaction of corals and zooxanthellae makes conceivable thickly populated coral reefs in oligotropic (supplement poor) marine situations.

Micro algae have a critical potential, contrasted with different biomass feed stocks, to enhance current transportation petroleum products. A few animal varieties, for example, Chlorella can collect as much as 60% of capacity mixes (lipids or polysaccharides) under supplement confinement. There are a few focal points offered by microbial over higher plants as a wellspring of bio-diesel or bio-ethanol, for instance:

- the yield of capacity mixes per unit of region for micro-algae can extraordinarily surpass that of customary harvests
- Micro-algae can be developed in zones unsatisfactory for ordinary farming.
- They can be refined in controlled aquaculture.
- Micro-algae develop either in seawater or in saline water which is unusable for typical horticulture.

- Micro-algae permit the recuperation of P and N from waste water, and the usage of waste CO_2 (for example from pipe gases)

- Then again, the development of micro algae is a more costly procedure than that of customary yields since it requires vitality for culture blending, collecting and biomass handling. The base current expense of micro algal biomass generation is around 5 US$ per kg, however regardless it surpasses by one to two requests of greatness the rate required for monetary bio-fuel creation. The cost decrease depends on appropriate and novel strain choice, ease photo-bio reactors, and lower vitality inputs.

- The present innovation is somewhat improbable to deliver a positive vitality and carbon balance for micro algal bio-fuels as an elective vitality source. Be that as it may, finding extra an incentive in biomass deposits may extraordinarily improve the practicality of bio-fuel innovation. Supplement supplies through waste water treatment, joined with the utilization of remaining biomass for methane creation, could possibly bolster the probability of delivering bio fuels that could rival customary energizes. Endeavors to deliver bio fuels from micro algae are deserving of study, however one must know that this innovation may just turn out to be financially practical inside 10– 15 years.

Micro algal societies have been utilized in high-rate oxidation lakes, went for the misuse of biomass for option bio-fuel feed-stock. Micro algae-remediation frameworks ingest supplements and produce oxygen by photosynthesis which upgrades the development of microscopic organisms utilized in debasing natural issue.

2.1 Microalgae cultivation

The first micro-algae development arrangements depended on soil water separates and, from that point forward, a great deal of work has been done through the plan of new media and the streamlining of recently utilized media, to support the capability of micro-algae development. A portion of the factors that are typically controlled are by and large supplement focus; saltiness; centralization of significant ionic segments; and nitrogen and carbon sources. The choice of the development medium will depend essentially on the micro-algae of intrigue (e.g., in the event that it is a salt water or freshwater species) and the development reason, in particular

- Culture high or low maintenance.
- getting most extreme biomass productivity
- Ideal metabolite creation (e.g., through nitrogen hardship conditions) and
- physiological investigations.

The tropic course to be favored will likewise widely impact the medium piece, uniquely the carbon source. All these diverse alternatives require distinctive development media or, in any event, unique definitions of the formulas, yet in practically all media macro-nutrients are provided in the request of g/L and the micro nutrients in mg/L. The choice on the immaculateness evaluation of the synthetic compounds utilized in the medium arrangement will be impacted by the development reason and cost. In the event that the micro-algae culture will be utilized for physiologic examinations, the most astounding virtue accessible ought to be utilized to evade medium tainting with follow metals that are regularly present in certain synthetics. Then again, the use of these profoundly unadulterated synthetic concoctions isn't doable in micro-algae mass development, where virtue is frequently not an issue but rather the cost is. The greater part of the formulas utilized in micro-algae large scale manufacturing is not detailed by the organizations to keep up their upper hand. Be that as it may, it is realized that the greater part of these organizations develop micro-algae under auto tropic conditions utilizing business manures weakened in regular waters (groundwater or seawater). The use of waste-waters is additionally expanding as an alternative to get the required supplements for micro algae development (media endured a few alterations amid the years, being the reason for practically the majority of the at present utilized micro-algae development media.

2.2 Cultivation of microalgae in wastewater

Waste water is a general term used to speak to the water with low quality that contains more measures of toxins and microorganisms. In the event that waste water is released into the adjacent water bodies, it can cause genuine natural and well being issues to individuals. Waste water treatment is a vital measure to lessen the toxin and other contaminants present in waste water. The initial phase in waste water treatment technique is essential treatment which evacuates the solids, oil, and oil from waste water. Auxiliary treatment or natural treatment is the second step, which abuses microorganisms to dispose of the synthetic substances present in waste water. Last advance is the tertiary treatment; which disposes of the microorganisms from waste water previously releasing into the river.

Waste water contains substantial measure of materials like poisons, organisms, and so on. Preparing is the primary advance to evacuate these undesirable materials from waste water. The sort of handling thus relies upon the attributes of waste water. For supplement expulsion from the waste water, single micro-algae strain or on the other hand consortium of micro algae alongside micro-algae development advancing microscopic organisms are utilized. A few investigations were performed to examine the ability of micro-algae consortium alongside cooperative microorganisms for supplement expulsion limit from waste water. One such investigation was the examination of cyan o bacterium, green micro-algae and two sorts of indigenous micro-algae consortium alongside advantageous microbes for the supplement expulsion ability from weakened (1:4 and 1:8) piggery waste-water.

The aftereffect of this examination demonstrated that the uni-algal societies like *Euglena viridis* (*E. viridis*), *Chlorella sorokiniana* (*C. sorokiniana*) had the capacity to develop in the two sorts of weakened waste water, while *Scendesmus obliquus* (*S. obliquus*) and consortium 2 had the capacity to develop in multiple times (1:8) weakened waste water, though consortium1 and *Spirulina platensis* demonstrated no development. If there should be an occurrence of phosphorus and nitrogen expulsion limit, *E. viridis* and *C. sorokiniana* indicated more nitrogen expulsion in both the weakening; on the other hand, *C. sorokiniana*, *S. obliquus* and *E.viridis* appeared phosphorus (20 - 65%) evacuation in multiple times weakened waste water

2.3 Cultivation of microalgae in dairy farm wastewater without sterilization

The creation of bio diesel has gotten a lot of attention worldwide because of the worldwide deficiencies of non-renewable energy sources, particularly oil and flammable gas. In this way, it is fundamental to develop feasible and clean vitality hot-spots for supplanting fossil fills. Micro algae, the third era bio diesel feed stock, is a promising possibility for bio diesel generation as a result of its higher unit territory oil yield, higher biomass creation, quicker development and photosynthetic proficiency examination with conventional oil seed crops (Miao et al. 2006; Wang et al. 2010b;Widjaja et al. 2009; Wu et al. 2012). In China, waste water is a tremendous wellspring of contamination with a total yearly release of 40 billion tons. These effluents ought to not be dumped straightforwardly into waterways, lakes or the ocean before treatment to lessen contaminants to earth safe dimensions. Waste water without treatment contains a lot of in-natural substances, for example, nitrogen and phosphates, which can prompt the eutrophication of the water bodies and ecosystem harm (Christenson et al., 2011; Martinez et al., 2000). The negative impacts of such supplement over-burdening incorporate quick engendering of microscopic fish, diminishing of broke up oxygen, deterioration of water, demise of fish and other living things, and toxin generation. In this manner, various investigations have focused on nitrogen and phosphorus expulsion from waste water.

While concoction and physical based advances are accessible to expel those supplements, they require surprising expenses and vitality, complex activity, and synthetic compounds (Christenson et al., 2011; Ruiz et al., 2012). In addition, compound treatment could prompt auxiliary pollution of the ooze result (Hoffmann, 1998). Examination with physical and substance treatment technology, micro algae based treatment of waste water can evacuate supplements and metals in a more affordable, progressively productive and safe way (Christenson et al., 2011; Hoffman, 1998). Cultivating micro algae in waste water has been considered as an option approach for waste water treatment for the greater part a century (Li et al., 2011; Min et al., 2011), and micro algae have been considered as a device for removing this vitality from waste water for bio fuel generation. Treatment waste water with micro algae couldn't just expel supplements, for example, natural carbon, ammonia, nitrate, phosphates and Chemical Oxygen Demand (COD), yet in addition could acquire micro algae biomass which can be utilized as a potential hot spot for proteins, sugars, pigments, lipids, hydrocarbons, compost, soil conditioners, and feed for fish or animals, and give a progression of various kinds of sustainable bio fuels, for example, methane, biodiesel, and photograph naturally delivered bio hydrogen (Chisti, 2007; Christenson et al., 2011; Converti et al., 2006; Hodaifa et al., 2008; Mallick, 2002; Wang et al., 2010a). Developing micro algae in waste water can give a few focal points including: the minimal effort of the activity and vitality necessities, expelling substantial metals what's more, xenobiotic substances, the likelihood of reusing assimilated nitrogen and phosphorus into green growth biomass as manure, what's more, the well being in light of the normal biological systems (Aslan et al., 2006; Converti et al., 2006; Sydney et al., 2011). More importantly, there were no microscopic organisms and pathogens found on circulated air through excrement when green growths are developed (Kumar et al., 2010).

2.4 The recycling of food waste to use as nutrients for microalgae

The food waste is defined as the waste, degraded or get lost during the production at the consumer level .it is a promising source of nutrients, proteins, vitamins, minerals, carbohydrates. When the hydrolysis is done after the fermentation process it becomes a promising source of nutrients .the hydrolysis of protein ,carbohydrates ,phosphates is done by using enzymes which can be fungal enzymes .and these enzymes help to reduce the overall amount of food waste which needs to disposed. The utilization of food waste can be used as a nutrient source for fermentation process which also contributes to new waste treatment strategies. It also offers opportunities for the recycling of organic waste matters by the production of algal biomass

It can be produced by using different methods

- handling of different microbes
- bakery and waste food items & Fermentation
- quantification of nitrate and phosphorus

- by using the submerged hydrolysis of food waste
- by growing in food waste and defined media

2.5 Algal culture media

In their regular living spaces green growth get every one of the supplements, minerals and nutrients they require from the water in which they live. To develop them in the lab in any case, you should furnish them with these basic assets for example you should make up a few development media. Green growth media alludes to the arrangement or culture in which green growth develop. Every one of the media has a few segments in like manner: wellsprings of nitrogen (in the form of nitrate, nitrite), phosphorus, nutrients and follow metals. Anyway the particular kind of these supplements, their concentrations and proportions change between the media**.**

The micro algal culture can be characterized during the time that a counterfeit situation in which the green growth develop. In principle, micro algal cultural state ought to look like the alga's regular habitat beyond what many would consider possible; as a general rule numerous critical contrasts exist, a large portion of which are purposely forced.

A micro algae culture has 3 particular segments:

- Micro algal culture media hold in a reasonable vessel.
- the micro algae biomass developing in the media
- The trade of carbon dioxide between micro algal culture and media is done through air

The components require for the micro algae for the development and growth are sunlight+ carbon dioxide +H_20+ supplements + follow components. By methods for photosynthesis the alga will most likely orchestrate all the biochemical mixes fundamental for development. Just a minority of alga appear, in any case, to be completely auto tropic; many are unfit to orchestrate certain biochemical exacerbates (certain nutrients, for instance) and will require these to be available in the medium. This condition is known as auto-trophic.

2.2.1 Physical properties

2.2.1.1 Temperature

The temperature at which the different algal societies grow are arctic creatures grow at (<10°C); mild (10-25°C); tropical (>20°C). The temperature is maintained at a rate of 17-20°C is regularly utilized. Temperature regulated hatcheries more often than not utilize steady temperature (exchanges to various temperatures ought to be led in ventures of 2°C/week), albeit a few models license temperature cycling. In calm districts surrounding temperature is commonly worthy for refined purposes.

2.2.1.2 Light

Light should be typically adequate to maintain the societies. Societies ought to never be presented to coordinate daylight (which may cause photo pigment harm), and ought to in this way be set alongside a north-bound window (in the northern side of the equator). Counterfeit lighting by bright light bulbs is regularly utilized to keep the culture maintained so that societies can grow rapidly .The force of light should in between 0.2-half of full sunshine (= 1660 µE/s/m2), with 5-10% (c. 80-160µE/s/m2) frequently utilized. The quality of (range) relies upon sort of knob utilized. The quality and power of light can be controlled by the channels. Numerous micro algal species don't develop well under consistent brightening, and henceforth a light/dim (LD) cycle is utilized (most extreme 16:8 LD, typically 14:10 or 12:12).

2.2.1.3 Mixing

The Blending of micro algae societies might be vital in specific situations. It is noticed sometimes in the sea cells only sometimes experience choppiness and thus blending come out to be delicate. The accompanying techniques might be utilized: rising with air (may or may not harm cells); tiny fish wheel or roller table (around 1 rpm); so the manual twirling is done.

Many societies do good without blending, especially when they aren't excessively thought, yet when conceivable delicate twirling is done which is done by manual methods

Objectives

- Growth kinetics study of *Desmodesmusarmatus* using food agricultural compost wastes as nutrient sources Vs Standard Media.
- To check out the algal biomass concentration grown in waste composts Vs Standard Media.
- To analyze the biochemical con. Of lipid, protein and carbohydrates contents in micro algae cultivated in waste compost.

CHAPTER 3

MATERIALS AND METHODS

3.1 *Desmodesmus armatus* cultivation

The Micro algal strain of *Desmodesmus armatus* sub cultured in Bold Basal Medium under photosynthetic light of 1500 lux intensity in period of dark: light (12h: 12h). The culture was grown at 25^0C at shaking conditions of 100 rpm. And the OD is taken at 680 nm after every 3^{rd} day to check the micro algal biomass concentration in the media. The *Desmodesmus armatus* of 15^{th} day cultivation was taken as inoculum for further experimentation

.3.2 Composition of BBM Media (Bold's Basal medium)

Table 3.1. Components of Bold Basal Medium

Components	**Stock solution (g/l)**	**Components**	**Stock solution (g/l)**
$NaNO_3$	25	Trace elements	
$CaCl_2.2H_20$	2.50	$ZNSO_4$. 7 H_2O	0.307µM
$MgSO_4$. $2H_20$	7.50	$MnCl_2$.4 H_2O	7.28 µM
NaCl	2.50	MO. O_3	4.93 µM
K_2HPO_4	7.50	$CuSO_4$.5 H_2O	6.29 µM
KH_2PO_4	17.50	$CO(NO_3)_2$.6 H_2O	1.68 µM
EDTA	50		
KOH	31		
FeSO4.7 H_20	4.98		
H_2SO_4	1 ml		
H_3BO_3	11.42		

Preparation of Culture Solution add chemicals and stock solutions as indicated above to 1000 ml of distilled water. And after that prepared working solution of the media from stock solution. The final pH should be 6.6.

3.3 Sample collection

- **Food waste compost:**

  Prepared by using leftover food waste obtained from mess and cafeteria composted with cow dung and soil kept for 15 days.

- **Agricultural compost:**

  The compost is collected from farm waste like sugarcane trash, paddy straw, weeds, and other plants.

3.4 Preparation of compost mixture

- The compost mixtures were prepared by immersing 25g of compost in 500 ml of water into an individual flask.
- The flask was placed in a shaker incubator and rotated at 150 rpm at room temperature for 24 hrs.
- The compost mixture was then filtered through whatmann filter paper to remove large particles.
- And mixture was autoclave to remove other contaminants and media used for algal cultivation (Chew et al. 2018).

Fig. 3.1 Agriculture and food compost media for microalgal cultivation after filtration

3.5 Composition percentage of Food compost and Agriculture compost media for micro algae cultivation

The *Desmodesmus armatus* cultivated in different concentration of the food and agriculture compost with standard media at 1500 lux intensity photosynthetic light in period of dark: light (12h: 12h). The culture was grown at 25^0C at shaking conditions of 100 rpm. The optical density was checked for biomass growth at 680 nm for 30 days in different concentration of food and agriculture compost. The best growing concentration of compost mixture with standard (BBM) media were selected for further analysis of the components.

Table 3.2. % of different concentration of organic (Food and Agriculture compost) and inorganic medium (BBM)

Organic Media (%) (Food compost and Agriculture Compost	Inorganic Media (%) (BBM media)
25%	75%
50%	50%
75%	25%
100% Control	-
	100% Control

3.6 Determination of micro algae cell concentration

The microalgal biomass concentration of *Desmodesmus armatus* in foremost compost mixture of food and agriculture was determined by measuring OD at 680 nm using UV-VIS spectroscopy for 30 days.

The Dry cell weight of micro algae was obtained by placing the 1 ml of micro algal culture in pre weighted eppendorff, and then micro algal culture subjected to centrifugation at 5000 rpm for 15 min. The clear supernatant was removed and the pellet was kept for drying in hot air oven. After drying the pellet again take the weight of eppendorf. The dry cell weight of microalgae was calculated in mg/ml.

3.7 Micro algal lipid Extraction

The biomass of the micro algal culture to extract the lipid content from it by using the method of Bligh and Dyer et al., 1959) with some modifications. The micro algal biomass was first centrifuged at 5000 rpm for 5 minutes and then the biomass was kept for drying so that the biomass settles down .After that the biomass was mixed with 1 ml of chloroform, 0.8 ml of distilled water and 2 ml of methanol. The sonication was done for 5 min. by using an ultrasonic water bath. After that again 2 ml of chloroform and distilled water was added and the mixture was sonicated again for 5 minutes. After the sonication is done the mixture was vortexes for 30 seconds and centrifuged at 3000 rpm for 5 minutes.

There will be a formation of two layers and the bottom layer is the chloroform layer. The bottom layer was transferred to pre weighed vial and was kept in a hot air oven and then the vials were transferred into a desiccator at room temperature and were allowed to cool before being weighed. The weight % of the lipids was determined.

3.8 Protein extraction

The protein was extracted from the micro algae biomass, the micro algae was subjected to cell disruption using sonication (Phong et al., 2018). The micro algae were centrifuged at 5000 rpm for 5 minutes and the pellet was kept for drying. 1ml of algal biomass was mixed with 9 ml of water and 9 ml of KOH. Then the mixture was vortexes were done for 1 minute and then the ultasonication is done at the 25 pulse rate for 10 minutes. After the ultrasonication is done the mixture was centrifuged at 7000 rpm for 5 minutes. The pellet was discarded and the supernatant was collected for protein analysis. The protein which was extracted from the micro algae was tested by using the Bradford method OD was taken at 595 nm. BSA standard was also made to establish the correlation between optical density (595nm) and protein concentration.

3.9 Estimation of carbohydrate contents

The carbohydrate content from micro algae biomass was extracted by using a method by (pleissner et al., 2013) with some modifications. The algal biomass was subjected to centrifuge at 5000 rpm for 5 minutes and the supernatant was discarded and the pellet was kept for drying. The dried algal biomass was mixed with 0.5 ml of H_2S04 and it was kept for 30 minutes reaction time. Transfer the mixture to falcon tubes and add 4.5 ml of distilled water. The mixture was kept in a water bath at 90^{o}C for 90 minutes .followed by centrifugation at 5000 rpm 10 minutes. The clear supernatant which was achieved was used for carbohydrate quantification by using the Phenol- sulphuric method (Dubois et al., 1951).

The known amount of supernatant was added to 5 ml of H_2S04 was heated at 95^{o}C for 10 minutes. And then phenol was added followed by vortexing for 5 minutes. After that OD was taken at 490 nm using UV-VIS Spectroscopy. The starch samples of known concentrations was used to establish the standard correlation between absorbance and carbohydrate content

CHAPTER 4

RESULTS AND DISCUSSION

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4.1 Microscopic examination of *Desmodesmus armatus*

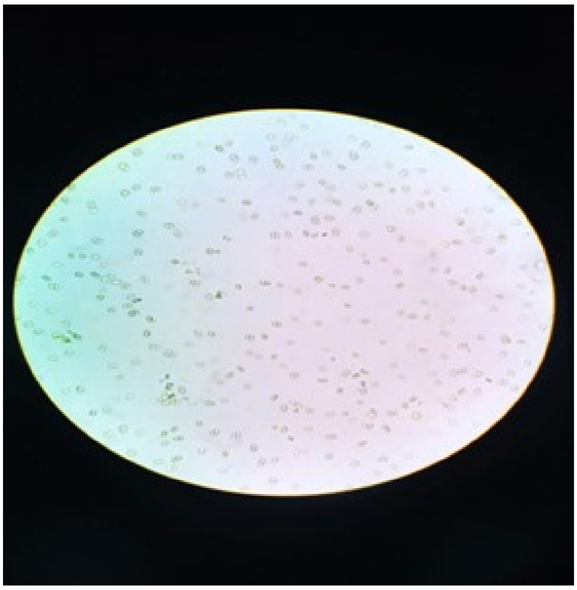

Fig.4.1 The cells were observed under UV-Visible microscope

4.2 Biomass growth of *Desmodesmus armatus* in food compost

The microalgae *Desmodesmus armatus* were grown at the different concentration of food compost with standard medium (BBM medium) as the source of nutrient. The *Desmodesmus armatus* growth studied in the dilutions of food compost such as 75:25, 50:50, 25:75 and 100 as control of food compost and BBM media. The food waste generally contains various amounts of micronutrients which enhance the growth of microalgae. The composting of food degrades the organic matter which acts as source of micro algal growth (Zhang et al., 2018).

Microalgae growth induced by the addition of extra nutrients through compost mixture. There is symbolic increase in the biomass of microalgae at appropriate dilution. The microalgae biomass concentration of different composition as shown in Fig.4.2.There is increase in biomass at the 6th day of cultivation in food waste (75) and standard medium (25). Whereas, on the other hand the control BBM did not show that much biomass concentration at the beginning of microalgal cultivation but as it entered the log phase on day 12th it showed significant increase in biomass concentration .

For different compositions of food and standard BBM media which were in the ratio of 50 BBM: 50 FW and 75BBM:25FW. Both the concentration showed quite similar trend till the end of growth phase. The pure food compost solution contains large number of organic matter. For the first 15 days media containing food waste did not show any increase in biomass after entering the log phase biomass concentration enhanced.

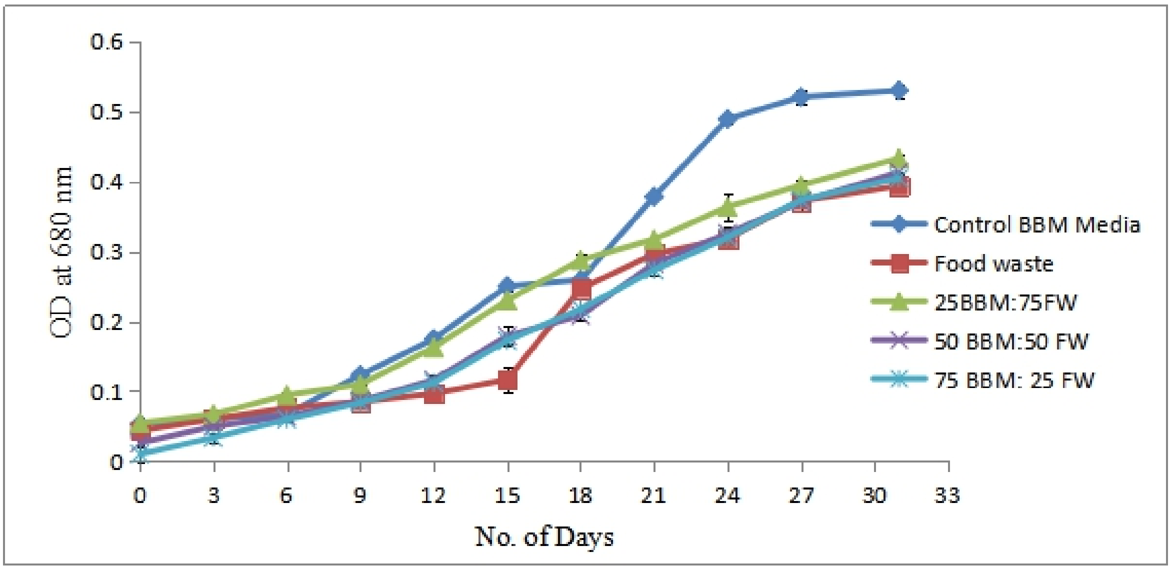

Fig.4.2 . Cultivation of *Desmodesmus armatus* in different concentration of organic (food) and inorganic medium (BBM media).

The combinations of organic (food compost) and inorganic media shows the increase in overall biomass productivity. The food compost inclusion provides the micronutrients for micro algal growth rate and its bio products. The food compost addition up to 50% in the standard medium is beneficial for the micro algal growth (Chew et al., 2018).

4.3 Biomass growth of *Desmodesmus armatus* in agricultural compost

The agriculture compost from the rice and wheat straw field compost for cultivation medium. The organic media as natural fertilizer for growth of microalgae. The different compositions of agriculture compost with standard BBM medium were used for cultivation of microalgae *Desmodesmus armatus*. Biomass concentration in different compositions as shown in Fig.4.3. There is notable increase in media have the ratio of 25 BBM: 75AW from day 9th up till the end of log phase. The agriculture waste compost also started to show escalation from 9th day as well but towards the end of the 31st day the biomass concentration came out to be less than that from the agriculture compost.

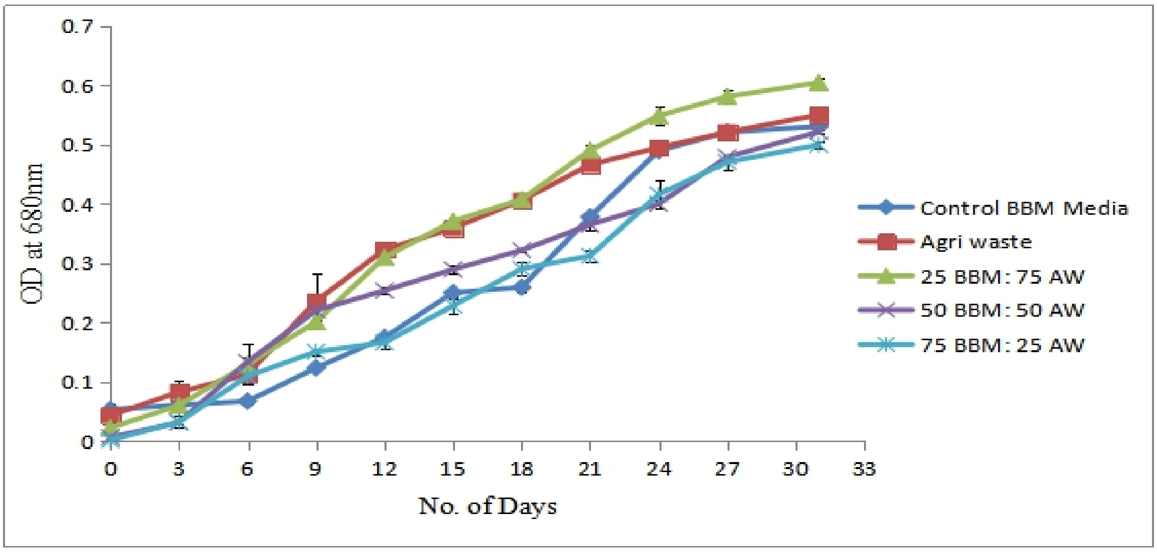

Fig. 4.3 Cultivation of *Desmodesmus armatus* in different concentration of organic (agricultural) and inorganic medium (BBM media).

The other different compositions used in the ratio of BBM media (control), 50 BBM: 50 AW, 75BBM:25 AW. For the media containing 25 AW started to show rise in biomass concentration from the start of 15th day. On the other hand media containing BBM (inorganic medium) and agriculture in equal parts started to rise from 9th day of cultivation. The different combination of organic and inorganic cultivation media analyze for its growth of microalgae and selected for further studies.

4.3 Analysis of Lipids, Carbohydrates and Proteins in food and agriculture compost media

4.3.1 *Desmodesmus armatus* biomass growth in food and agriculture compost

The microalgae *Desmodesmus armatus* were grown at different combinations of food as well as agriculture waste compost. The fig 4.4 showed the biomass concentration of *Desmodesmus armatus* in both medium the best growth was analyzed in combinations of 75% food waste compost and agriculture compost with 25% BBM media (inorganic) with respect to standard in comparison with standard media.

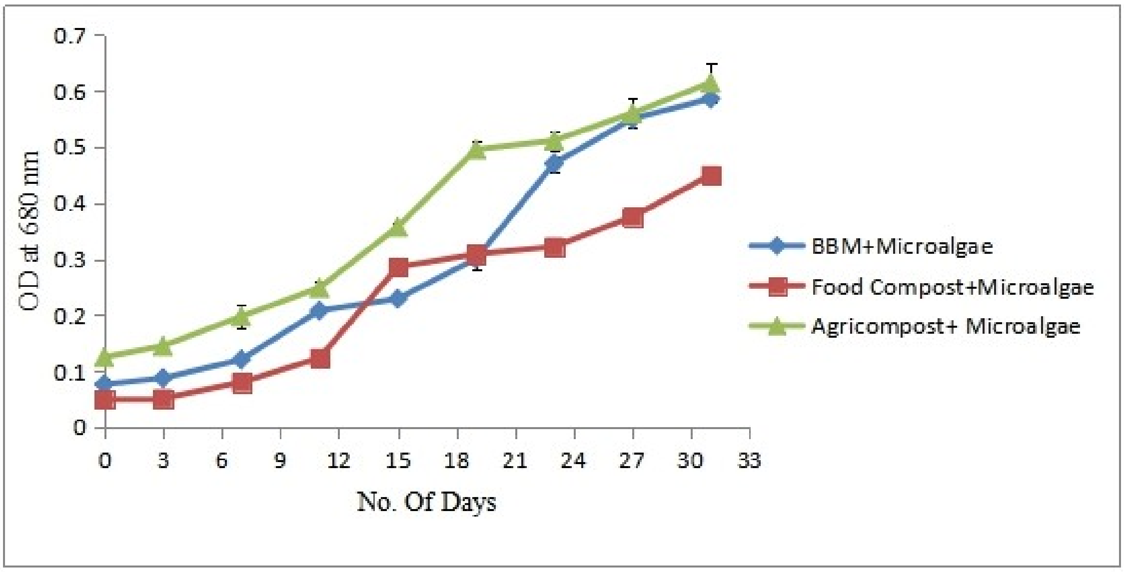

Fig. 4.4 Cultivation of *D. armatus* in the best combination of organic and inorganic medium in food and agricultural compost

The Fig.4.4 showed above depicts the growth of selected compositions (75:25) in food and agriculture waste with BBM medium respectively. As seen food waste compost did not showed much elevation till day 7 but at the start of the 15th day there was significant increase in biomass concentration. In case of BBM media, microalgae showed escalation in growth as it entered log phase on the day 15th and showed a significant increase after wards. The agriculture waste compost increased rapidly and showed tremendous growth in comparison to other two media it showed the finest growth among food compost and standard medium.

Dry cell weight was obtained at regular intervals of 3 days in accordance with optical density. Weights obtained were in mg/ml. For microalgae grown in standard media dry cell obtained at 0 day the initial biomass came out to be 0.292545mg /ml but towards the end the biomass concentration came out to be 1.45mg /ml. food waste compost showed least growth in comparison to agriculture waste compost and standard media in case of optical density as well as dry cell weight (Fig. 4.5).

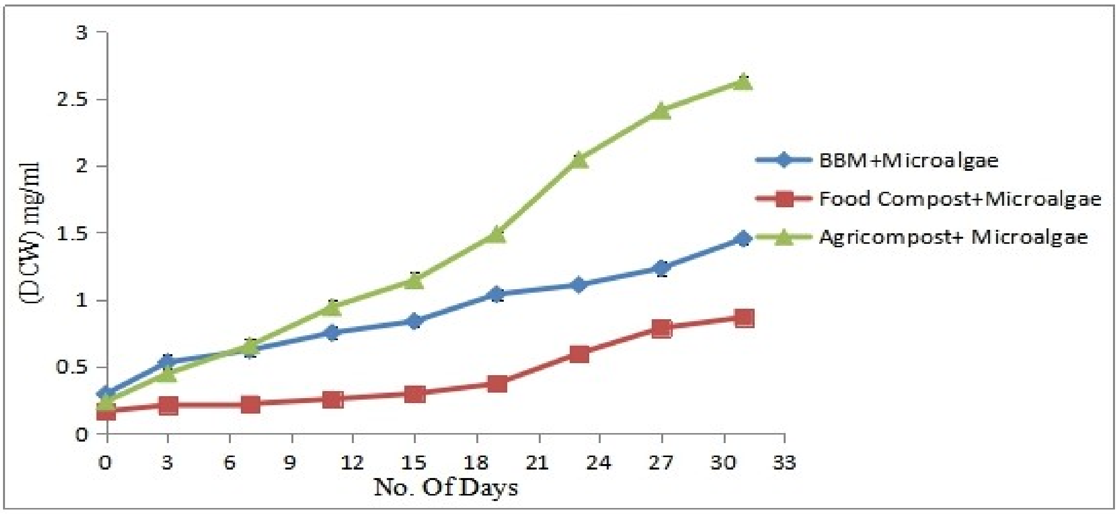

Fig. 4.5. Dry cell weight (mg/ml) of *D. armatus* in the best combination of organic and inorganic medium in food and agricultural compost

The agriculture compost biomass concentration up surged from the start. Initially the dry cell weight came out to be 0.235 mg/ml and at the end it came out to be 2.625 mg/ml which was best in contrast with food as well as standard media. The potential of growth of microalgae in agriculture and food compost were further analyses for its better biochemical composition

4.3.2 Estimation of lipids in food and Agricultural Compost

The total estimation of lipids in weight % was calculated on two different compost and standard media. Agriculture waste compost showed more lipid content up till last day among all three which is 9.925 wt% in contrast with food waste which showed lower lipid content from others. The lipid content in the agriculture compost has con. of organic and inorganic medium (75:25) showed enhancement of lipids content to 4.1%-9.925%. The lipid content is due to the presence of organic matter which enhances the lipids accumulation in the microalgae (Lin and Wu, 2015). Whereas standard media contained much more lipid content than food waste but a bit lower than agriculture waste which is 7.035 wt%. The lipid content in microalgae due to the presence of micronutrients which directly increased the biochemical components. The addition of both organic and inorganic compost mixture in the cultivation media increases the production of lipids. The lipids content in the different concentration of food waste compost is 219.7 mg/g and 215 mg/g (Chew et al., 2018).

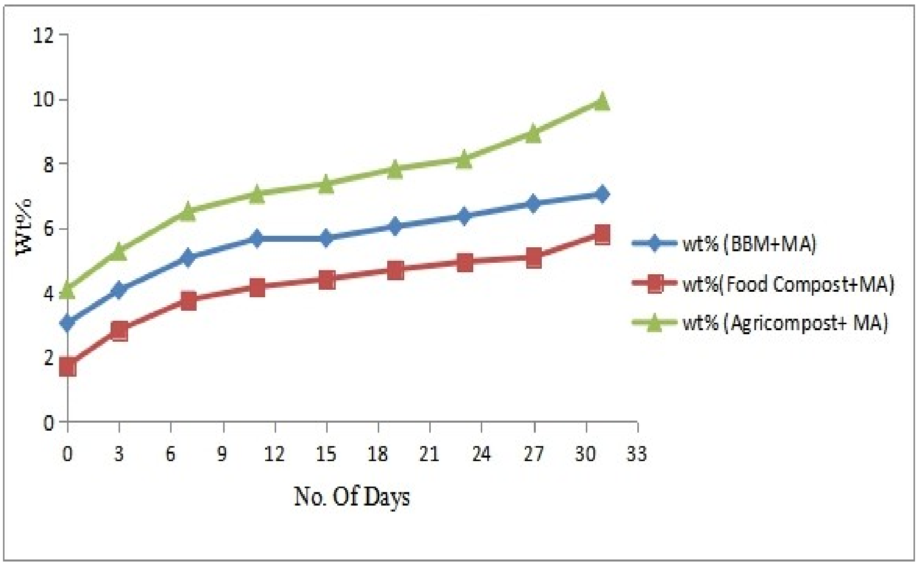

Fig. 4.6 Lipids contents (wt %) in the selective concentration of food and agriculture waste compost medium

The Fig. 4.6 showed the food compost had 1.73% of lipid content at start of growth of microalgae and it reaches 5.815% at end growth phase. The methods of microalgae growth conditions is same, but the compost medium color and its thickness varies. The light intensity is the main source of growth of microalgae. The medium thickness and color affects the light intensity penetration and ultimately affect the lipid content (Chew et al., 2018; Mandotra et al., 2016). The lipids which mainly used for biodiesel production the microalgal biomass form 37.6 kJg−1 of lipids (Wilhelm and Jakob, 2011).

4.3.3 Estimation of carbohydrates in food and agricultural compost

The microalgae has cellulose rich cell walls or plastids which accumulate starch as main carbohydrates source. The microalgae can accumulate 50% of starch and cellulose in their cell wall under proper cultivation conditions (Chen et al., 2013). The production of carbohydrates concentration is altered by providing different medium and their cultivation conditions. The carbohydrate content was estimated on standard media in comparison with food and agriculture waste compost. Among all three food waste compost showed the most upsurge till last day which is 8.75µg/ml. There was no significant increase in carbohydrate content until the 12^{th} day. Standard media and agricultural waste showed quite similar trend till the last and their carbohydrate content came out to be quite similar on last day of estimation which is 3.95µg/ml, 3.6µg/ml respectively which was very less than that of food waste (Fig.4.7).

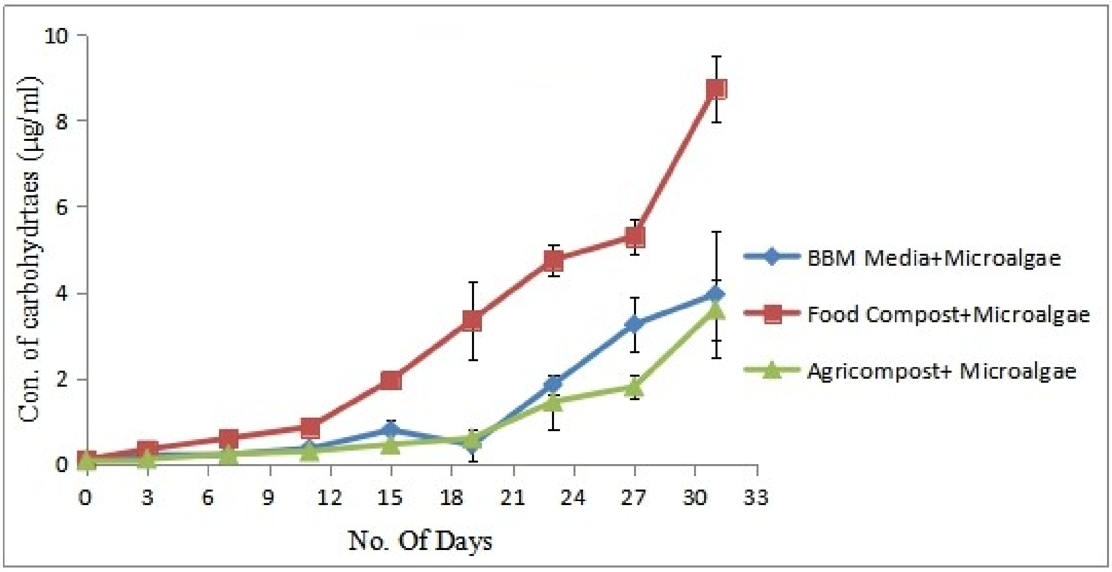

Fig. 4.7 Carbohydrates contents in (µg/ml) in the selective concentration of food and agriculture waste compost medium.

The carbohydrate content also depends on the micraolagal species to species. The microalgae has carbohydrate content such as Spirogyra have (33-64%) (Harun et al., 2010). *Chlorella vulgaris* (21%) (Pleissner et al., 2013). The food compost added with inorganic medium showed more carbohydrate content which coupled with carbon assimilation and carbohydrates accumulation in the microalgae (Zhan et al., 2014).

4.3.4 Estimation of protein in food and agriculture waste compost

The microalgae generally contain 40-50% of the protein content but its content varies from the cultivation conditions of microalgae to its species variability (Soto-Sierra et al., 2018). The protein evaluation was done on standard media (BBM), food waste compost and agriculture waste compost. No significant protein content was observed till 15th day in the agriculture waste compost but after 15th day it showed a notable rise it went from 0.1µg/ml on day 1 to 106µg/ml on the last day of estimation. The standard media had the highest protein concentration from the other two which is 112.5µg/ml. On the other hand food waste compost almost had same protein concentration to that BBM medium cultivating microalgae (Fig.4.8).

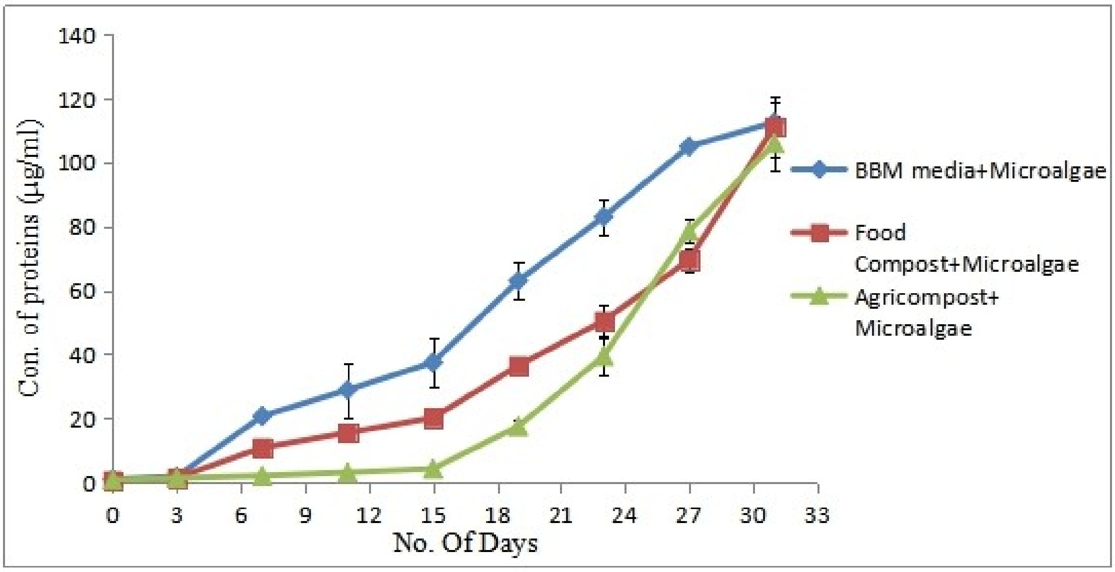

Fig. 4.8 protein contents in (µg/ml) in the selective concentration of food and agriculture waste compost medium.

The food waste generally contains more amount of protein 60-100 mg g^{-1} (Pleissner et al., 2014) due to which this waste become one of the major nutritive source for micro algal growth (lau et al., 2014).The selected concentration of both compost mixtures showed max. protein con.112.5 µg/m l, 111.25 µg/ml, 106 µg/ml in standard medium, food waste and agriculture compost respectively. The substitution of the organic and inorganic mixture produces almost same amount of the protein compared with the standard medium.

CHAPTER 5

CONCLUSION & FUTURE PROSPECTS

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5.1 Conclusion

- The reconciliation of natural medium with inorganic mechanism for the development of micro algae offers numerous ecological and monetary benefits.
- Substituting inorganic medium with 25% fertilizer blend yielded higher micro algae biomass just as more noteworthy lipid and protein content.
- The utilization of natural medium can spare micro algae development cost and increment the generation of biochemical mixes.
- Utilizing natural manure will likewise bring ecological points of interest as these manure sources are broadly accessible and can help in sustenance squander the board.
- Use of natural medium in micro algae development framework to supplant fossil-based manures ought to be actualized to make an increasingly maintainable future

5.2 Future prospects

The adopted agri- food waste media have to be assessed for the efficiency of

- ✓ Bioremediation of industrial effluents
- ✓ Microalgal lipid production
- ✓ PHA's production

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