



**DEVELOPMENT OF COST EFFECTIVE AND ECONOMIC METHOD FOR
CULTIVATION OF *CHLORELLA SINGULARIS* USING WASTE CARBON AND
NITROGEN SOURCE**

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ABSTRACT

Single cell organism based oil from oleaginous microorganisms has involved the attention because of its significance in the production of biodiesel as well as reducing pollution and waste. This possibility of algal biomass for utilizing waste carbon and nitrogen content was tested in this study. In this work, *Chlorella singularis* was cultivated in medium containing waste carbon, nitrogen as a nutrient source and BBM medium as control. The growth kinetics of the algae on these mediums was observed. The microalgae, *Chlorella singularis* can utilize the nitrogen content present in biogas digester wastewater as a substrate for its growth. Different low cost and easily available carbon and nitrogen based feedstock such as Potato peel, and Pea peel was used in this study. The growth of microalgae was found to follow the Monod growth model satisfactorily. Under the optimum photobioreactor condition, a maximum biomass of 3.75 gm/l in waste carbon and nitrogen medium and 1.5 gm/l in BBM was obtained in fifteen days of batch process. Based on Monod growth kinetics, net specific growth rate and doubling time of *Chlorella singularis* was found to be 0.054 day⁻¹ and 12.83 hr⁻¹.

KEYWORDS: Orange peel, Pea peel, *Chlorella singularis*, Monod kinetics, closed photo-bioreactor cultivation.

INTRODUCTION

Today the world is facing problems with a variety of pollutants and contaminants from various developmental activities. Microalgae have vast industrial and economic potential as valuable sources for pharmaceuticals, health foods, carotenoids, dyes, new chemicals, bio fuels, and others.^[1] Bioremediation of wastewater by microalgae can provide the microalgae feedstock for their biomass energy, as well as reduce the material cost of the bio fuel.^[2]

The algal cells were able to consume high concentrations of nitrate ion and, therefore, can possibly contribute to purification of industrial and domestic wastewater.^[3] The coupled process of algae cultivation and succeeding biogas production is a better option compared to algal biodiesel production.^[4]

A possible solution for overcoming the high cost of production is to integrate algae cultivation with an existing biogas plant, where algae can be cultivated using the discharges of CO₂ and digestate as nutrient input, and then the attained biomass can be converted directly to biogas or bio methane by the existing infrastructures.^[4]

Energy and GHGs balances of algal bio methane production were assessed in the perspective of life cycle, and comparison with ley crop was conducted.^[4] The growth of green algae *Chlorella sp.* on wastewaters sampled from four different points of the treatment process flow of a local municipal wastewater treatment plant (MWTP) was done. They investigate how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the wastewaters.^[5]

The domestic wastewater samples were collected from sewage wastewater treatment plant Bopodi from Pune city was used to study the role of microalgae in wastewater treatment. *Chlorella sp.* shows the best removal capacity of nitrate and phosphate reduction.^[6] Microalgae have been used for the bioremediation of textile dyes in wastewater from industrial textile processes.^[7]

Microalgae such as *Chlorella* and *Scenedesmus* have shown tolerance and bioremediation capabilities to certain heavy metals.^[7] Both nitrogen and phosphorus are the major sources of eutrophication, therefore, high

concentrations of nitrogen or phosphorus can cause algal blooms and other hazardous environmental problems.^[1]

Nitrate in wastewater is generally produced as an intermediate of nitrogen metabolism by microorganisms, beginning with ammonification of proteins or other nitrogen-containing compounds, followed by nitrification of ammonia into nitrite, and later, oxidation of nitrite into nitrate. Based on the understanding nitrate accumulation becomes a concern in water quality management.^[8] Algae can capture carbon dioxide in the flue gas from coal red power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into bio-fuel. *Chlorella*, *Scenedesmus* and *Spirulina* are the most widely used algae for nutrient removal.^[9]

2. MATERIAL AND METHODOLOGY

2.1 Sample Preparation

Potato peel and pea peel was used as the source of carbon and nitrogen in this work. These low cost substrates were sun dried until they were crispy to touch and were grounded into fine powder. The fine powdered carbon source and nitrogen source were used as raw materials were then sieved with 0.2 mm in diameter sieve and kept in desiccators until required for use.

2.2. Media Preparation

100 mg of potato and pea peels were dissolved in 1 liter of water. This media containing waste carbon and nitrogen source were then autoclaved at 121°C for 20 minutes for the removal of foreign contaminants. BBM culture media was used as control to compare the growth kinetics of *Chlorella singularis*.

2.3 Algal Strains Culture

Chlorella singularis were purchased from the National Collection of Industrial Microorganisms (NCIM) for this work. It was cultivated in the fog medium at room

temperature which is about $25 \pm 3^\circ\text{C}$ each day before the microalgae was inoculated to the other media.

2.4 Incubation

0.1 gm of microalgae strain was aseptically transferred into the sterilized 1 litre flask of media containing waste carbon and nitrogen source. The flask was incubated under optimized conditions of the photobioreactor till the stationary phase of monod kinetics started.

2.5 Data Collection and Analysis

In order to study the growth of *Chlorella singularis* in the waste carbon and nitrogen source, absorbance of sample was checked at 680 nm.^[10] Microalgae growth process can be explained by the 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.

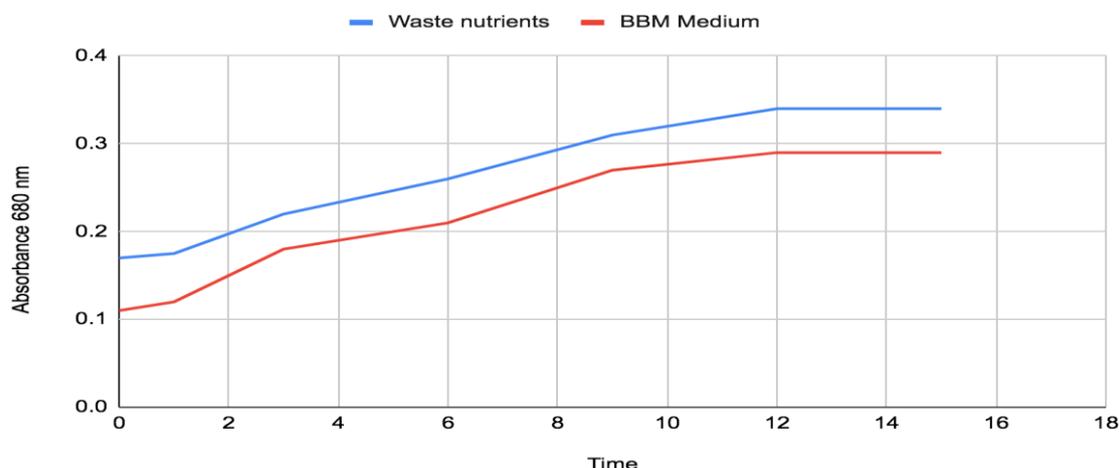
$$\mu_g = \frac{1}{x} \frac{dx}{dt}$$

μ_g is the net specific growth rate of the microalgae; x is the conc. of microalgae in the medium; t is the time of incubation. Doubling time of *Chlorella singularis* is calculated as.

$$\mu_g = \frac{1}{x} \frac{dx}{dt}$$

RESULTS AND DISCUSSION

The growth curve plotted for *Chlorella singularis* involving media containing waste carbon and nitrogen revealed better growth as compared to the growth of the *Chlorella singularis* in the BBM culture medium. The different stages of growth kinetics of *Chlorella singularis* media containing waste biomass and BBM culture medium is shown in Figure 1.



0.1gm of *Chlorella singularis* was inoculated separately into the BBM culture medium and also in the waste

carbon and nitrogen source. After the culture samples were centrifuged, the algal biomass were extracted and

the final weights of each were noted. By comparing the initial and final weights, the growth rates and doubling time of each algae species was determined. The final weight of *Chlorella singularis* was found to be 3.75 gm in media containing waste carbon and nitrogen source; Whereas, 1.5 gm of *Chlorella singularis* found in BBM culture medium. The net specific growth rate of *Chlorella singularis* in media containing waste carbon and nitrogen source was found to be 0.054 day^{-1} with a doubling time of 12.83 hr^{-1} .

CONCLUSION

The growth and cultivation of the microalga, *Chlorella singularis* in the medium containing waste carbon and nitrogen as the source of nutrients was studied, compared and analyzed. The results from this study demonstrated the feasibility of cultivating *Chlorella singularis* in any waste biomass containing carbon and nitrogen source. *Chlorella singularis* could adapt well in waste biomass with small lag phases observed. The microalgae *Chlorella singularis* had good growth in the waste biomass containing carbon and nitrogen as the source of nutrients and its wet weight reached 3.75 gm in 1000 ml after cultivation for fifteen^[15] days as compared to 1.5 gm of *Chlorella singularis* found in BBM culture medium. The net specific growth rate of *Chlorella singularis* in media containing waste carbon and nitrogen source was found to be 0.054 day^{-1} with a doubling time of 12.83 hr^{-1} . This concludes that the cultivation of *Chlorella singularis* in waste biomass would be efficient, economic and saving water as well as producing more yield as compared to BBM nutrient media.

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REFERENCES

1. Choul-gyun Lee. Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources, 2002; 12: 979-985.
2. Lu Yinghua Tang Xuemin Lu Bin Li Yuanyue Lu Zhiqiang Lin Yaojiang Zheng Jiang, Li Zhongbao and Zhou Jixin. Cultivation of the microalga, *Chlorella pyrenoidosa*, in biogas wastewater. African Journal of Biotechnology, 2011; 10:13115-13120.
3. Laila Al-balushi, Nitin Rout, Sahar Talebi, Ahmed Al Darmaki, and Maryam Al-qasmi. Removal of Nitrate from Wastewater Using *Trentepohlia Aurea* Microalgae. Engineering, 2012; I: 8-10.
4. Xiaoqiang Wang, Eva Nordlander, Eva Thorin, and Jinyue Yan. Microalgal biomethane production integrated with an existing biogas plant: A case study in Sweden, 2012.
5. Liang Wang & Min Min & Yecong Li & Paul Chen & Yifeng Chen & Yuhuan Liu & Yingkuan Wang & Roger Ruan. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Appl Biochem Biotechnol, 2009; 7: 10-13.
6. Ayodhya D Kshirsagar. Bioremediation of waste water by using microalgae: An experimental study. IJLBPR, 2013; 2: 339-346.
7. Asif Rahman, Joshua T Ellis, and Charles D Miller. Bioremediation of Domestic Wastewater and Production of Bioproducts from Microalgae Using Waste Stabilization Ponds. J Bioremed Biodeg, 2012; 3(6): 6194-99.
8. Jalan Sultan, Ahmad Shah, Bandar Indera Mahkota, Kuantan Pahang, Jalan Gombak, and Kuala Lumpur. Removal of nitrate and phosphate from municipal waste water sludge by *Chlorella vulgaris*, *Spirulina plantensis*. Biotechnology, 2011; 12(4): 125-132.
9. Seema Dwivedi. Bioremediation of heavy metal by algae: Current and future perspective biotechnology. Journal of advanced laboratory research in biology, 2012; III: 229-233.
10. Dwivedi, S.: Bioremediation of heavy metal by algae: Current and future perspective biotechnology. Journal of advanced laboratory research in biology, 2012; 3: 229-233.