Isolation and Characterization of Green Microalgae for Carbon Sequestration, Waste Water Treatment and Bio-fuel Production

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Abstract

Green microalgae Chlorella vulgaris species was collected from Bhavani Lake at Erode District, Tamil Nadu State, India. The Fourier Transform Infrared spectroscopy and Scanning Electron Microscopic studies were utilized to examine morphology of Chlorella Vulgaris. This Chlorella vulgaris is attempted its efficiency in removing Nitrate and Phosphate from waste water medium. These experimental studies reveal that, Chlorella Vulgaris is having full-fledged efficiency in removing Nitrates and Phosphates from waste water medium. This microalgae specimen 'Chlorella Vulgaris' was attained 100% removal efficiency of Nitrates and Phosphates at a time intravel of 6 days period.

Keywords*: Chorella Vulgaris, UV-Visible, FTIR, SEM, Nitrate removal, Phosphate removal*

1. Introduction

Algae are the major health indicator of oceans in which 71% of the earth surface is covered by these species. Algae are the original source of fossil carbon found in the crude oil and in natural gas (Andersen, [1]). Microalgae, which cover almost 75% algae species, contribute approximately 40% of the oxygen in the atmosphere.

The simple and common green alage genus chlorella are placed below the order Chlorococcales and family Chlorellacese (Hoek, *et al.*, [12]). Reproduction is actual and achieved by production non-motile autospores .Species of this genus are widespread in fresh water and in the sea, air and soil. The discovery of pure culture, fast-growing micro-organisms can be used as the ideal experimental materials for research in photosynthesis, Nitrate, Phosphate reduction physiology and in biochemistry. Chlorella vulgaris have been studied and employed in various applications namely in agriculture, biotechnology. Chlorella vulgaris are used as protein – rich food and also employed in sewage oxidation process (Kessler, [13]).

Microalgae *Chlorella* sp. are known to used in the treatment of wastewater, production of biodiesel, production of electricity using microbial fuel cells, animal food supplements and providing valuable extracts for chemical products (Becker, [2], Chisti [6]).

The traditional taxonomic characteristics of Chlorella vulgaris indicated that morphological biochemical properties are used in its identification, the cell size and shape are variable and largely depends upon varying age, nutrition and environmental factors. The traditional identification of micro algae is achieved by microscopic studies; these identification results are questionable to certain degree. Most methods used in measuring algal nutritional and physiological changes are limited to detecting whole community responses because of the relatively large quantity of material needed to analysis. In the past few years, spectroscopic

technique has developed to become a very powerful and flexible tool for the differentiation and identification of microorganisms.

FTIR Spectroscopy has been widely used to provide the information on range of vibrationally active functional groups (including O-H, N-H, C=O, =C-H, -CH2, -CH3, C-O-C, and >P=O) in biological specimens (Stuart, [20]). Although the technique has been largely used with isolated macromolecules and molecular complexes such as nucleic acid (Liquier, Taillandier, [15]), Proteins (Stuart, [20]), Lipids (Lewis, [14]), Polysaccharides (Brandenburg and Seydel, [5]), studies carried out on whole organism. The FTIR spectroscopy has successfully been established as a tool reailably, quikly and easily identifying microalgae (Bastert, [4]).

Immobilization of microalgae in polysaccharide gels is an experimental way to use these microorganisms for wastewater treatment (Chevalier, [7]). The major difficulty is collecting enormous populations of cells developed during the treatment will hamper the regular microalgae treatments (De la Noue, [9]).

The tertiary treatment of waste waters are achieved by C. vulgaris (Valderrama, *et al.*, [22]), this is not been demonstrated that the observed growth promotion may be due to improve capabilities of microalgae to remove nutrients from natural wastewater. The microbial carrier chosen in this study were alginate beads.

2. Materials and Methods

During the studies on carbon sequestration technique and waste water treatment by utilizing the micro green algae the Chlorella Vulgaris was collected from Bhavani Lake. This micro algae transferred to a bath containing preliminarily treated waste Water (Bath water) from KCT hostel. The quality parameter of this water is tabled in Table 1. The variation of the quality of bath water due to carbon sequestration after 2, 4, 6, 8, 10&12 days of interval are estimated and tabulated. The Spectroscopic technique was selected as a testing tool and validated with dry mass measurements in this research. The percentage transmission, concentration, optical density. After substantial growth was determined during the period, this growth a portion of the algae biomass was separated. Then the biomass was filtered and dried to find out its weight and it is preserved for isolation.

2.1 Light Microscopy and Scanning Electron Microscopy

The algal cells were observed under light microscope for their morphological features and other cellular details, the cells were further studied using scanning electron microscope (SEM) method.

The sample was screened by SEM (Scanning Electron Microscopy) for their absolute morphological studies. The basic steps for SEM sample preparations are fixing it with buffered aldehyde, post fixing it in Osmium tetraoxide, dehydrating it in ethanol, drying it with air dryer, mounting it on a specimen stub, coating it with Carbon and examining under the HRSEM (Quanta FEG 200). The topography of green micro algae is analyzed (Figure 1 and 2).

2.2 Fourier transforms infrared spectroscopy

The IR spectrum of dried algal biomass was recorded on Nicolet IR spectrometer at room temperature. The dried algal powder was blended with potassium bromide (KBr) powder, and pressed into tablets before measurement. A region of 4000–400 cm^{-1} was used for scanning.

2.3 Analysis of chlorophyll a

The algae was mixed with methanol and distilled water (1:1) and heated to 60℃ 30mins and kept at the in water bath. Then it is after cooled at room temperature it is centrifuged it for 10mins, at 5000 rpm. This extract was analyzed by UV-Visible spectrophotometer (Evolution 201).

3. Results and Discussion

Sample obtained from Bhavani Lake were cultured wastewater and allowed to grow by exposing to direct sunlight. Characteristics and Morphological feature of the isolate have demonstrated its close similarity with genus chlorella vulgaris. The individual cells of the colonies were in the range of 10µm.Cells are green colour, unicellular, spherical in shape its shows the Figure1 and 2.

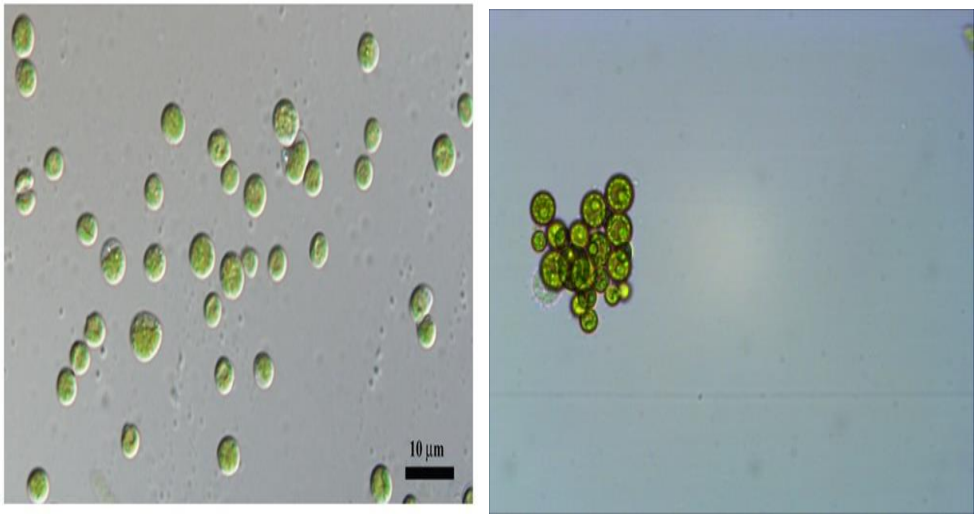
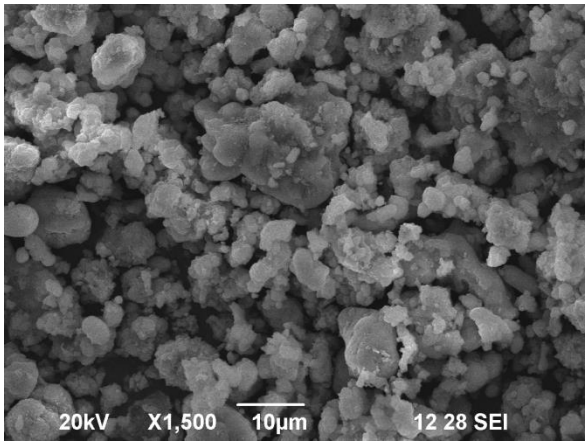


Figure 1. Light Microscope Image of chlorella vulgaris



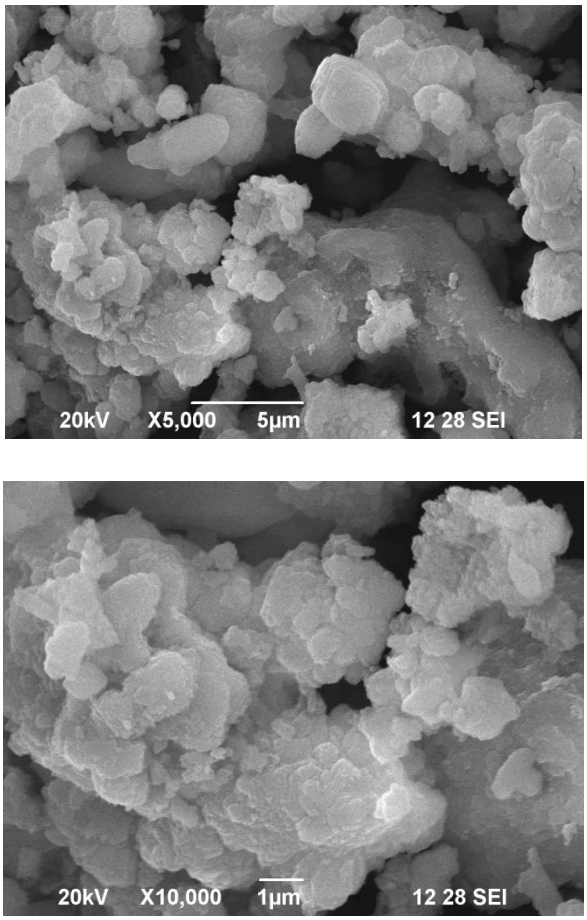


Figure 2. Scanning Electron Microscope images of chlorella vulgaris

FTIR spectra (Figure 3) in relation to specific groups (Table 1). Each peak assigned a functional group. The molecular assignments of bands are based on published data phytoplankton, bacteria and other biological materials. In this study chlorella vulgaris protein spectra characterized by strong peaks 1656 cm^{-1} (amide I) and 1536 cm^{-1} (amide II). These bands were due primarily to C=O stretching vibration and a combination of N-H and C-H Stretching vibrations in amide complexes. Lipid and carbohydrates were characterized by strong vibrations the C-H 2925cm^{-1},C-O-C of polysaccharides at 1079cm^{-1},1047cm^{-1}respectively (Brandenburg, Seydel, [5]).while carbohydrates are the strongest absorbers between 1200 and 1000 cm^{-1}.Several other classes of compounds, such as nucleic acids have functional groups with absorption bands in the same region of the spectrum. The strongest peaks 1536 and 1422 shows that bending modes of methyl groups of protein(Dean, [8]).The peak 1243 shows carboxylic acid present in the algae (Benning, *et al.*,[3]).In this study ,the close correlation between the peaks and the existence of with band 2 suggested that lipid content very high and also carbohydrate, nucleic acid also present in chlorella vulgaris.

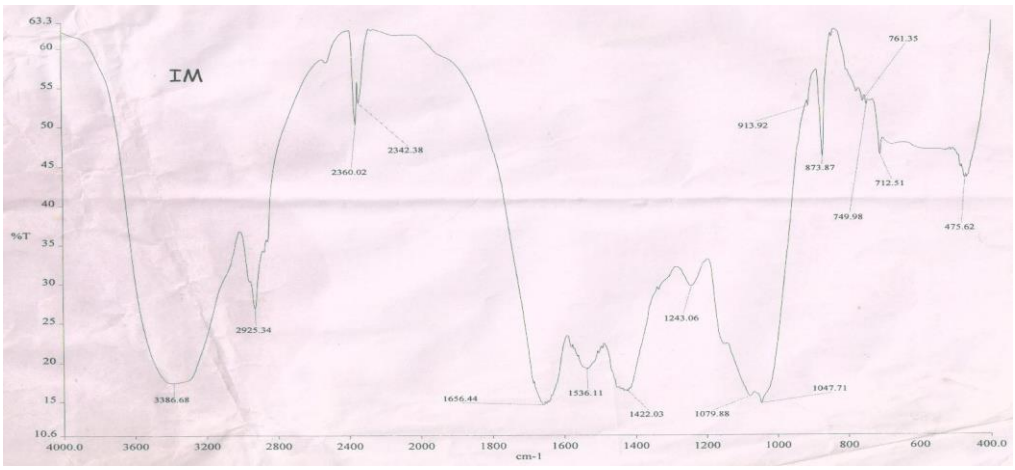


Figure 3. FTIR spectra for chlorella vulgaris

Table 1. Tentative assignment of bands found in FTIR spectra of chlorella vulgaris

Band	Main peak cm^{-1}	Typical band	Wave number range cm^{-1}
1	3386	Water V(O-H) stretching Protein V(N-H) stretching	3029-3639
2	2925	Lipid –carbohydrate mainly V_{as} (CH_2) and V_s(CH_2) stretching	2809-3012
3	1656	Protein amide I band mainly V(C=O) stretching	1583-1709
4	1536	Protein amide II band mainly σ(N-H)bending V(C-N) stretching	1481-1585
5	1422	Protein σ_{as} (CH_2) and σ_s(CH_3) bending of methyl lipid $_{as}$ (CH_2) bending of methyl	1425-1477
6	1243	Nucleic acid (other phosphate containing compounds) V_{as}> P=0 stretching of phosphodiesters	1191-1356
7	1079	Carbohydrate V (-O-C) of polysaccharides. Nucleic acid (other phosphate containing compounds) V_{as}> P=0 stretching of phosphodiesters	1072-1099
8	1047	Carbohydrate V(C-O-C) of polysaccharides	980-1072

The growth of the biomass was continuously monitored by conducting analysis using spectrophotometer. This includes % transmission; concentration and optical density are determined and used as growth parameters. Simultaneously weight of biomass was recorded.

Table 2 shows the spectroscopic analysis of the biomass and dry weight measurements. The Evolution201 spectrophotometer is used for spectrophotometer analysis of a solution of

any concentration. The output is available on the digital display in the forms of optical density (Absorbance), percentage transmission (%T) and concentration (C). The instrument operates at wavelength of 340 nm to 960nm. Figure 4 shows the percentage transmission as function of growth period and Figure 5 shows the optical density of algae biomass as function of growth period obtained from the Spectroscopic Analysis of Biomass for Kinetic Study. Figure 6, variation in algae density as a function of time exhibits a linear growth kinetics

Table 2. Spectroscopic Analysis of Algae Biomass for Kinetic Study and Dry Weight Measurements

Days	% Transmission	Concentration	Optical Density	Dry Weight Measurements(gm)
0	96	15	0.0	0.9
2	95	21	0.025	1.7
4	82	80	0.08	2.7
6	67	86	0.121	3.5
8	57	98	0.134	4.4
10	45	109	0.154	5.6
12	38	117	0.165	6.4

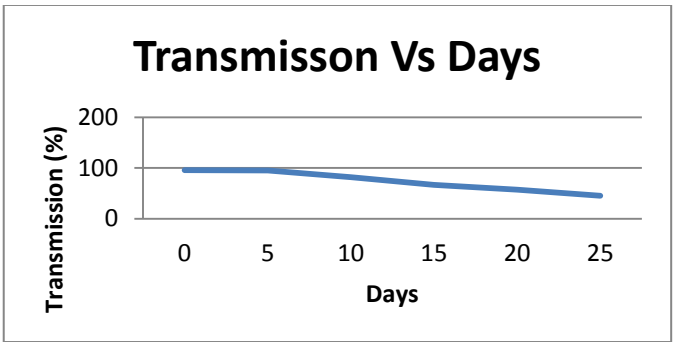


Figure 4. Transmission Vs Days

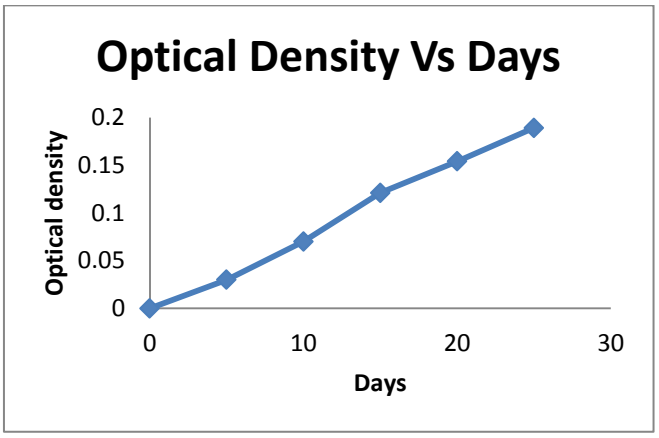


Figure 5. Optical Density Vs Days

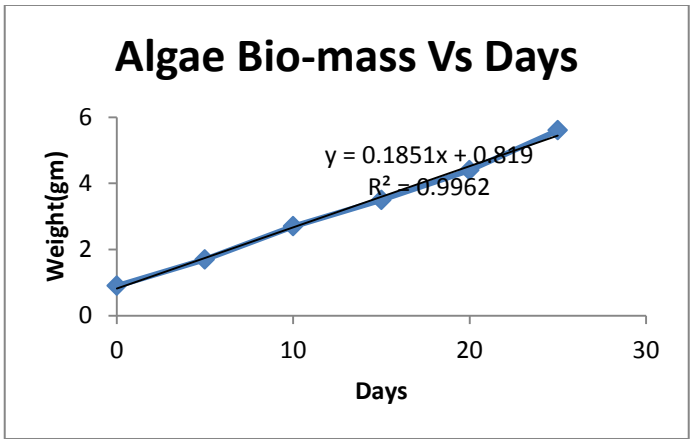


Figure 6. Algae Bio-mass Vs Days

The pretreated bath waste water from Kumaraguru college of Technology hostel is used as a medium to grow the biomass assay. As per the linear growth kinetics studies, the waste water medium supports the growth of the biomass algae. The quality parameters of the waste water are determined and the values are tabled in Table 3, It reveals that the quality of waste water is considerable improved after the growth of algae. The p^H, conductivity, Total hardness, Calcium hardness, Magnesium hardness, Total dissolve solids, Chlorid, Sulphate, Phosphate, Nitrate, Iron ,Total Alkalinity, Carbonate Alkalinity, Bicarbonate Alkalinity, Hydroxide Alkalinity, Fluoride are determined. It reveals that, CO_2 sequestration studies. The decrease in TDS, Total hardness, Chloride, Sulphate, Phosphate, Nitrate, Iron etc, indicates the uptake of nutrients by algae for its growth. It is evident that, waste water qualities are improved. This indicates that, micro algae can be utilized to treat bath waste waters from hostels. The linear growth of algae is the effective tool to examine sequestration of carbon form the atmosphere.

This waste water is completely screened to remove bio-mass assay and it is subjected to pass through activated carbon columns to obtain odour less, transperant clear water.

Table 3. Water Quality Parameters

S.No	parameter	Sample water	After 2 days	After 4 days	After 6 days	After 8 days	After 10 days	After 12 days
1	PH	8.5	8.4	8.7	8.4	8.0	7.9	7.8
2	Conductivity	3.38	3.03	3.53	3.67	4.21	5.35	6.64
3	TDS	2.31	2.06	2.45	2.54	2.94	3.67	4.42
4	Phosphate	0.069	0.005	0.003	Nil	nil	Nil	Nil
5	Nitrate	0.857	0.801	0.387	nil	Nil	Nil	Nil
6	Iron	Nil	Nil	Nil	Nil	Nil	Nil	Nil
7	Fluoride	Nil	Nil	Nil	Nil	Nil	Nil	Nil
8	Chloride	280	223	280	309	436	684	649
9	Total hardness	1423	1150	1600	1350	2150	2970	3660

10	Calcium hardness	620	300	210	310	300	250	390
11	Magnesium hardness	803	850	1390	1040	1850	2720	3270
12	Sulphate	385	142	280	633	1059	1896	2422
13	Total alkalinity	705	600	310	270	420	235	330
14	Phenothalin alkalinity	67	75	35	20	15	10	Nil
15	Methyl orange alkalinity	638	525	275	250	405	225	330

In microalgae, the ratio of chlorophyll a to biomass dry weight (Geider and Osborne, [11]). The concentration of chlorophyll varies with the cell concentration. Variation of chlorophyll concentration follows the same pattern as the growth of cells (Young, [22]). Hence, the highest concentration of chlorophyll will be obtained at the highest cell concentration or at the end of the exponential phase of growth. In this study, chlorophyll content were observed 17 mg/L.

The main commercial processes for removing phosphorus from wastewater effluents are chemical precipitation with iron, alum, or lime (Donnert, [10]) achieving over 95% removal, and to a lesser extent biological treatment (Stratful, [19]). Practical biological methods of removal are far less efficient, ranging between 20% and 30% of P with various microorganisms, while up to 90% removal with some bacterial species has been recorded in laboratory tests (Nagadomi, *et al.*, [17]). In this study shows the 100% removal of nitrate and phosphate from wastewater are evident from the Table 3.

4. Conclusion

In this study Bio-mass growth rate, Isolation and Characterization of microalgae by utilizing hostel waste water medium reveals that.

- Lipid-producing microalgae species were isolated, using microscopic analysis, the culture identified as Chlorella Vulgaris.
- Algal species FTIR spectroscopy determination shows that high amount of protein, carbohydrate, nucleic acid were present in the Chlorella Vulgaris.
- The Phosphate and Nitrate were removed completed within a very short period.
- Economics of producing microalgal biodiesel is competitive with petrodiesel.

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References

[1] R. A. Andersen, "Algal Cultural Techniques", Elsevier academic press, London, **(2005)**.
[2] W. Becker, "Microalgae in human and animal nutrition", In: A. Richmond, Ed., Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Publishing, **(2004)**, pp. 312-346**.**
[3] L. G. Benning, V. R. Phoenix, N. Yee and M. J. Tobin, "molecular characterization of cynaobacterial silification using synchrotron infrared micro-spectroscopy", Geocimica et Cosmochimica acta., vol. 68, **(2004)**, pp. 729-741**.**
[4] Bastert, H. C. Korting, P. Traenkle and A. F. Schmalreck, "Identification of dermatophytes BY Fourier Transform Infrared Spectroscopy", Mycoses ISSN 09333.42:525-528, **(1999).**
[5] K. Brandenburg and U. Seydel, "Fourier Transform Infrared Spectroscopy of cell surface polysaccharides", In H. H. Manstsch and D. Chapman, Infrared Spectroscopy of biomolicules, Wiley: chichester, **(1996)**, pp. 203-278**.**
[6] M. Y. Chisti, "Biodiesel from microalgae", Biotechnology Advances, **(2007)**, pp. 294-306.
[7] P. Chevalier and J. De la Noue, "Wastewater nutrient removal with microalgae immobilized in carrageenan", Enzyme Microbiol Technol., vol. 7, **(1985)**, pp. 621–624.
[8] A. P. Dean, M. C. Martin and D. C. Sigee, "resolution of co dominant phytoplankton species in atrophic lake using synchrotron –based Fourier Transform Infrared Spectroscopy", Phycologia, vol. 46, no. 2, **(2007)**, pp. 151-159.
[9] J. De la Noue and N. De Pauw, "The potential of microalgal biotechnology: a review of production and uses of microalgae", Biotechnol Adv., vol. 6, **(1988)**, pp. 725–70.
[10] D. Donnert and M. Salecker, "Elimination of phosphorus from municipal and industrial waste water", Water Sci Techol., vol. 40, **(1999)**, pp. 195–202**.**
[11] R. J. Geider and B. Osborne, "AAlgal Photosynthesis - The measurement of algal gas exchange", New York: Chapman and Hall, **(1992).**
[12] C. Hoek and D. Mann, "Algae an introduction to physiology", Cambridge university press, **(1995)**.
[13] E. Kessler, "A general view on the contribution of chemo-taxonomy to the systematic green algae", Systematic Association.,Acdamic pres London, vol. 17, **(1984)**, pp. 391-407.
[14] R. N. Lewis and R. N. McElhancy, "Fourier Transform Infrared Spectroscopy in the study of hydrated lipids and lipid bilayer membranes", in H. H. Manstsch and D. Chapman, Infrared Spectroscopy of biomolicules, Wiley: chichester, **(1996)**, pp. 159-202**.**
[15] J. Liquir and E. Tailander, "Infrared Spectroscopy of nucleic acids", in H. H. Manstsch and D. Chapman, Infrared Spectroscopy of biomolicules, Wiley: chichester, **(1996)**, pp. 131-158**.**
[16] Mass culture of Botryococcus braunii under open cultivation system for bio-diesel production, Aban Informatics Pvt. Ltd., Chennai, **(2009).**
[17] H. Nagadomi, T. Kitamura, M. Watanabe and K. Sasaki, "Simultaneous removal of chemical oxygen demand (COD), phosphate, nitrate and H2S in the synthetic sewage wastewater using porous ceramic immobilized photosynthetic bacteria", Biotechnol Lett., vol. 22, **(2000)**, pp. 1369–74**.**
[18] Optimization of conditions for mass culture of Botryococcus braunii under open race way ponds, Aban Informatics Pvt. Ltd., Chennai, **(2009).**
[19] I. Stratful, S. Brett, M. B. Scrimshaw and J. N. Lester, "Biological phosphorus removal, its role in phosphorus recycling", Environ Technol., vol. 20, **(1999)**, pp. 681–95.
[20] B. Stuart, "Biological applications of Infrared Spectroscopy", willey: chichester, **(1997)**, pp. 25-180.
[21] O. Tokusoglu and M. K. Unal, "Biomass nutrient profiles of three microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana", Journal of food science, **(2003)**, pp. 1144-1148**.**
[22] L. T. Valderrama, C. M. Del Campo, C. M. Rodriguez, L. E. de- Bashan and Y. Bashan, "Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula", Water Res., vol. 36, **(2002)**, pp. 4185–92.
[23] A. J. Young, A. J. Tsavalos and M. Harker, "Autotrophic growth and carotenoid production of Haematococcus pluvalis in a 30 liter air-lift photobioreactor", Journal of fermentation and bioengineering, **(1996)**, pp. 113-118**.**

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