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RETREATMENT OF TOXIC INDUSTRIAL EFFLUENT BY MICROALGAE CULTIVATION FOR POTENTIAL BIOFUEL PRODUCTION

***Lakshmi Praba S., Subashini R., Dhivya K. and Nandhini Devi S.**

Assistant Professor, Department of Biotechnology, P.S.R Engineering College, Sivakasi. -626140.

***Corresponding Author: Lakshmi Praba S.**

Assistant Professor, Department of Biotechnology, P.S.R Engineering College, Sivakasi. -626140.

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ABSTRACT

Industrial effluent having Organic & Inorganic chemicals which were released into the environment as a result of domestic, agriculture and industrial water activities lead to environmental pollution, The process have been used to treat the industrial effluents to eliminate the easily settled organic chemicals present in effluent and which is reused for beneficial purpose. This effluent is however loaded with some inorganic chemicals like N_2 & P and causes eutrophication, longer term problems because of the heavy metals that are released. Microalgae are the third generation source provides a valuable way for treatment of effluents, because they provide a tertiary bio treatment along with the production of biofuel. Microalgae have the ability to utilize inorganic N_2 & p as a nutrient for their growth. And also, capacity to use heavy metals as well as some toxic chemicals. In this present study, the industrial effluents were treated by *Chlorella sp., Scenedesmus sp., Desmococcus sp.,* coupled with biofuel production. The species (*Chlorella sp., Scenedesmus sp.,*) were collected from Phycospectrum Environmental Research Centre and the *Desmococcus sp.,* was isolated from nearby drainage areas and the effluent was collected from various dye industry and match industry. The collected species were subjected to screening for the effective utilization of nutrients from effluent. And also the growth was observed in the addition of Macronutrients, Micronutrients and effluent. The bio treated effluent was undergone for physico-chemical properties. From this study we concludes that the *Chlorella sp., and Desmococcus sp.*, shows the maximum growth and highlighted on effective utilization of organic & inorganic substances and should be used for biofuel production.

KEYWORDS: Effluents, Inorganic n2&p, Microalgae, Macro &Micronutrients, Biofuel production.

INTRODUCTION

Now days, due to overpopulation the earth atmosphere get increased by the emission of excessive greenhouse gases and carbon derivatives. Due to the pollution created by the industrial activities the living beings get affected. Also the waste water from the various industries is directly released into the environment with or without treatment. The effluents which are not treated within the industry causes various diseases to the human like respiratory problem by in healing the polluted air and by drinking the water the digestive system also get disturbed. Due to the over emission of carbon di oxide, the Ozone layer also get depleted which leads to the UV rays directly to enter into the earth. Most of the diseases are commonly due to the contamination of drinking water. It also diminishes the nutrients present in the soil which can be used for the cultivation. The pollution control board insist the industry should have the Waste water treatment plant based on the volume of effluent get released into the environment. So there is a need for the efficient system to treat the effluent in a less expensive and should give the beneficial activities towards the environment. Various generations are available for the production of biofuel. They are first generation, second generation and third generation biofuel. In every industry there will be the emission of waste water which may contain more toxic compounds. This waste water when released into the cultivable land causes land pollution which may affect the growth of commercial and cash crops.

2. MATERIALS AND METHODS

2.1. Sample collection: *Desmococcus species* were obtained from drainage areas confirmed by photographic view and the *chlorella species & Scenedesmus species* were purchased from Phycospectrum Environment Research Centre, Chennai.

2.2. Chemicals and medium: Calcium chloride, Sodium chloride, Sodium bi carbonate, Sodium nitrate, Dipotassium hydrogen phosphate, Potassium sulphate, Magnesium sulphate hepa hydrate, Ferrous sulphate, Calcium acetate are the chemicals used for the preparation of medium. The prepared medium was CFTRI Medium, BBM Medium, Industrial effluent (Match industry effluent, coloring dye effluent Dye industry effluent 1, 2).

2.3. Pre-inoculum Preparation: Microalgae were collected from the drainage areas and cultivated in 250mL Erlenmeyer

flasks containing 100mL of CFTRI medium and Micronutrients: (10ml each /940 ml)NaNO3 (10g/400ml), CaCl2.2H2O (1g/400ml), MgSO4.7H2O(3g/400ml), K2HPO4 (3g/400ml), KH2PO4(7g/400ml), NaCl (1g/400ml), EDTA (1ml/lit): EDTA (50g/lit, KOH(31g/lit) Iron (1ml/lit): FeSO4.7H2O (4.98g/lit, H2SO4(1.0ml/lit), Macronutrients(per liter): 4.98 ZnSO4.7H2O , 1.44g MnCl2.4H2O, 0.71g MoO3, 1.57g CuSO4.5H2O, 0.49g Co(NO3)2.6H2O. The composition of the CFTRI medium was as follows (perliter):4.5g NaHCO3, 0.5g K2HPO4, 1.5g NaNO3, 1.0g K2SO4, 1.0 NaCl, 0.2g MgSO4.7H2O, 0.04g CaCl2, and 0.01g FeSO4.The flasks were subjected to direct sunlight and optimum temperature until they reached sufficient growth.

2.4. Effluent treatment with microalgae: The effluents was treated with *Chlorella sp* and *Scenedesmus sp* in a boiling tube with varying volume of effluent and CFTRI medium was taken also act as control. The growth was measured at regular time interval using Hemocytometer. The specific growth rate was calculated as per the cell count.

2.5. Observation of growth by addition of nutrients: The various micronutrients such as organic, Inorganic and SAP (fertilizer) to the effluents in the concentration of 0.5g/lit in a 250ml of Erlenmeyer flasks and treated with the *Chlorella sp. The* cell count was taken till 15th day and the specific growth rate was calculated.

2.6. Screening of medium by micro algae: The different medium includes nutrients (carbon, nitrate, phosphate) for the effective utilization of micro algal species. It was done by varying the volume of BBM medium which was made up to 10ml with the effluent (food coloring dye) and 1ml of inoculum was added to each boiling tube for which the initial cell count was made on the day of inoculation till 10th day. The specific growth rate was calculated.

2.7. Comparative study with existing work: The Three species of micro algae were cultivated in the optimized effluent (ie..Match industry effluent) in a boiling tube by varying volume of CFTRI Medium and made up to 10ml with effluent. The growth was measured in a periodic interval of time and the specific growth rate was calculated and the cell count was done with Hemocytometer.

2.8. Specific growth rate: The specific growth rate was calculated for the day on which the cell count was made by using the formula, **$\mu = \ln \mu 2 - \ln \mu 1 / 2t$.**

2.9. Biomass recovery: The biomass was recovered on 15th day and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected in a separate vessel for analysis physico-chemical properties and used for biochemical test. The recovered biomass was allowed to dry in a direct sunlight for 20 minutes. The dry cell of microalgae was weighed.

2.10. Analytical methods: **Physico-chemical parameter analysis:** The different physico- chemical parameter of a medium was estimated using TWAD manual protocol. The parameter such as Appearance, odor, Turbidity NT units, total dissolved solids, Electrical conductivity, Color, PH, Alkalinity, Total hardness, calcium, Magnesium, free ammonia, Nitrate, Chloride, Fluoride, Sulphate, Phosphate, Tidy`s test 4hrs., COD , BOD were monitored.

3. RESULTS AND DISCUSSION

3.1. Preparation of seed inoculum: *Desmococcus species, Chlorella species and Scenedesmus species* was cultivated in Erlenmeyer flask containing 250mL of CFTRI Medium. The cultures were maintained at a temperature of 16ºC. The Light and Dark cycles was maintained at 8hrs. The culture was used as a seed inoculum for further studies.

3.2. Cultivation of micro algal species in different effluents for the effective utilization of chemicals

The three different species were cultivated in 250mL Erlenmeyer flasks containing 100mL of different effluent. The cultures were maintained at a temperature range of 16ºC, Dark and light cycle period. The cultures were placed in light intensity of 2000 lux in light period.

3.3. Growth curve analysis

The 1mL of seed inoculum was inoculated into different effluents and the cell growth was continuously monitored at 4th and 8th day. Among these two species the chlorella sp., shows the maximum growth due to effective utilization of inorganic nitrate and phosphate in food coloring dye as shown in the figures.

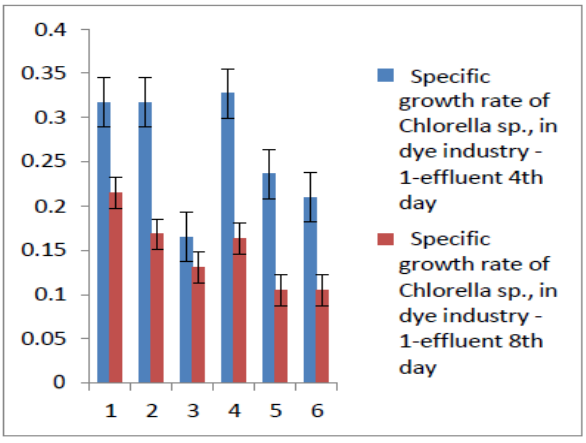

Fig. 1: Specific growth rate of *Chlorella sp.*, in *sp.*,dye industry-1- effluent.

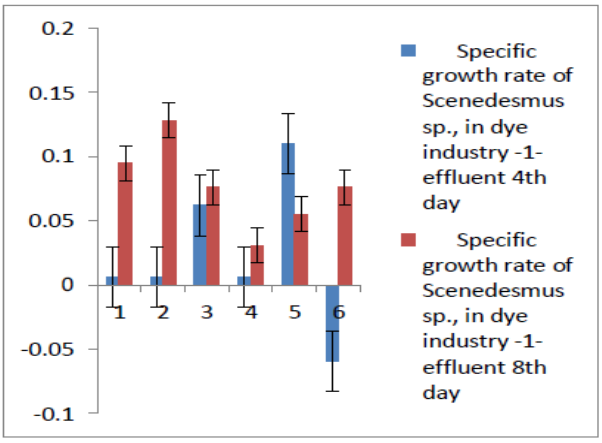

Fig. 2: Specific growth rate of *Scenedesmus* in dye industry-2-effluent.

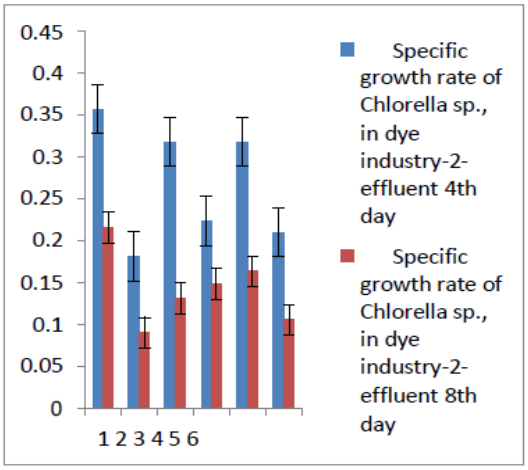

Fig. 3: Specific growth rate of *Chlorella sp.*, in *sp.*, in dye industry-2-effluent.

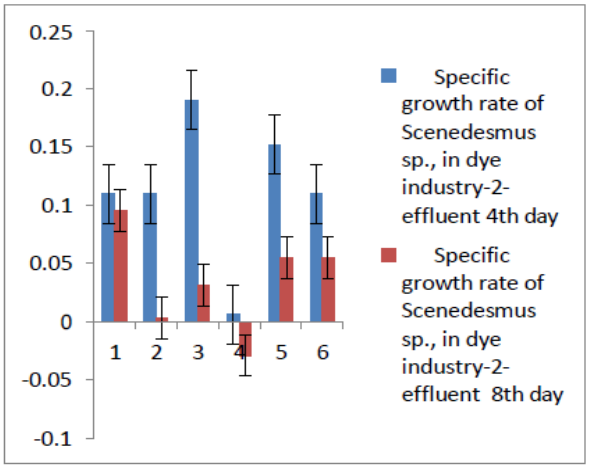

Fig 4: Specific growth rate of *Scenedesmus sp.*,dye industry-2-effluent.

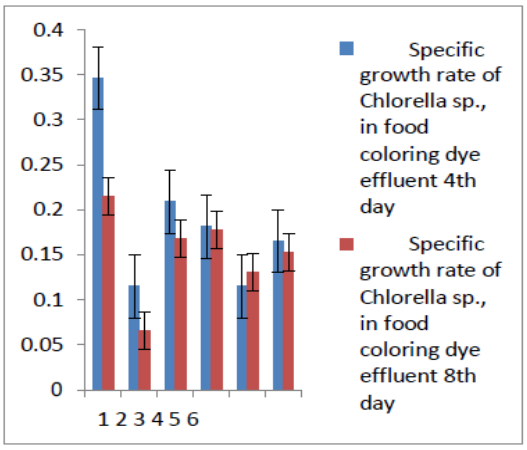

Fig. 5: Specific growth rate of *Chlorella sp.*, in food coloring dye effluent.

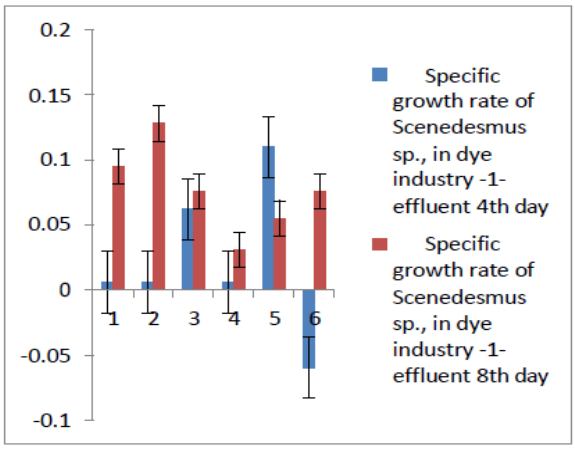

Fig. 6: Specific growth rate of *Scenedesmus sp.*, in food coloring dye effluent.

3.4. Growth curve analysis by the addition of micronutrients

250ml Erlenmeyer flask containing 100ml of effluents and 10ml of seed inoculum was added with organic , inorganic and SAP(fertilizer) in the concentration of 0.5g/lit in every flask. The Chlorella sp., shows a maximum specific growth rate of 0.224(day)-1, 0.276(day)-1, 0.276(day)-1 in dye industry-1- effluent, food coloring dye effluent, dye industry-2-effluent by the addition of inorganic, SAP, organic micronutrients to the different effluents as shown in figures.

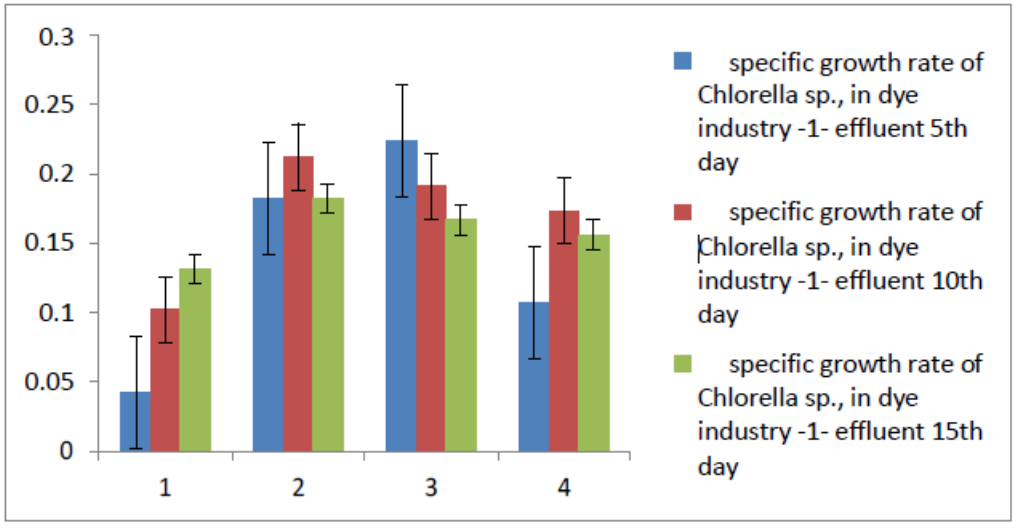

Fig. 7: Specific growth rate of *Chlorella sp.*, in dye industry-1-effluent.

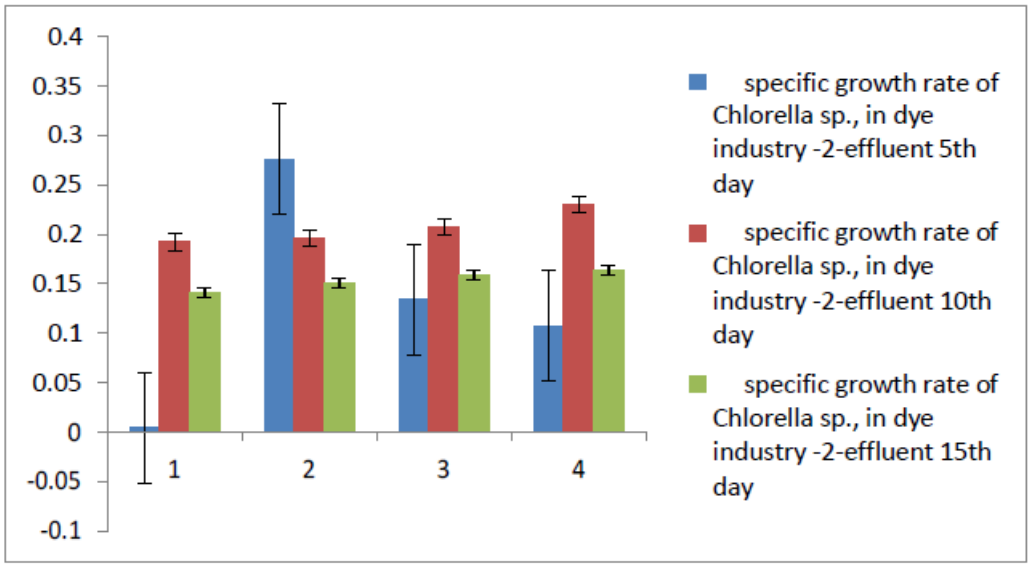

Fig. 8: Specific growth rate of *Chlorella sp*., in dye industry-2-effluent.

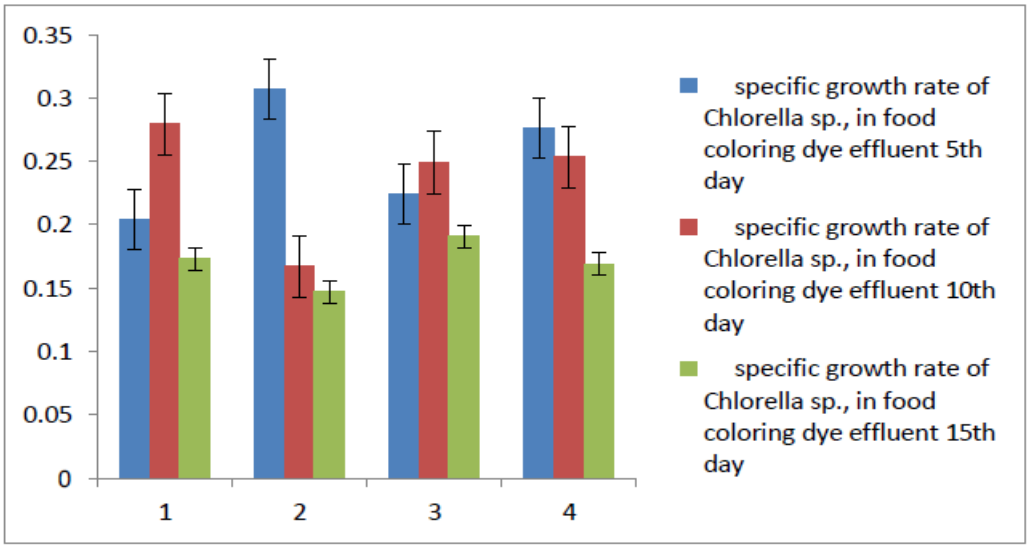

Fig. 9: Specific growth rate of *Chlorella sp*., in food coloring dye effluent.

3.5 Screening of media by microalgae: 1ml of seed inoculum of three microalgae species was added with varying volume of effluent (2ml, 4ml, 6ml, 8ml, and 10ml) was made up to 10 ml with BBM Medium. The Chlorella sp., & Scenedesmus sp., shows the maximum growth on 5th day and the Desmococcus sp., shows the maximum growth on 9th day in food coloring dye effluent containing urea, phosphate and nitrate. The growth of microalgae depends on the macronutrients like carbon, Nitrogen and Phosphorus (Larsdotter et al, 2006). The graph was plotted between the specific growth rate (day)-1 and the varying volume of effluent as shown in table.

Table 1: Specific growth rate of Chlorella sp., on food coloring dye effluent in Nitrate, Urea, Phosphate on 5th day.

S. No.	Effluent rate in ml	Specific growth rate of *Chlorella sp.*, on food coloring dye effluent		
		Nitrate on 5th day	Urea on 5th day	Phosphate on 5th day
1	Control(BBM)	**0.11**	**0.230**	**0.21**
2	2	**0.16**	**0.150**	**0.25**
3	4	**0.16**	**0.200**	**0.22**
4	6	**0.21**	**0.200**	**0.15**
5	8	**0.11**	**0.120**	**0.16**
6	10	**0.25**	**0.270**	**0.15**

Table 2: Specific growth rate of Scenedesmus sp., on food coloring dye effluent in Nitrate, Urea, Phosphate on 5th day.

S. No.	Effluent rate in ml	Specific growth rate of Scenedesmus sp., on food coloring dye effluent		
		Nitrate on 5th day	Urea on 5th day	Phosphate on 5th day
1	Control(BBM)	0.088	0.21	0.24
2	2	0.039	0.1	0.21
3	4	0.073	0.16	0.13
4	6	0.1	0.073	0.2
5	8	0.16	0.18	0.1
6	10	0.17	0.24	0.26

Table 3: Specific growth rate of Desmococcus sp., on food coloring dye effluent in Nitrate, Urea, Phosphate on 9th day.

S. No	Effluent rate in ml	Specific growth rate of Desmococcus sp., on food coloring dye effluent		
		Nitrate on 9th day	Urea on 9th day	Phosphate on 9th day
1	Control(BBM)	0.44	0.32	0.38
2	2	0.43	0.46	0.48
3	4	0.46	0.43	0.47
4	6	0.45	0.41	0.47
5	8	0.43	0.31	0.45
6	10	0.37	0.45	0.32

3.6. Comparative study with the existing work: In previous work, the bio treated effluent was optimized as match industry effluent for the cultivation of *Desmococcus species.* It was compared with the growth of *Chlorella sp.,* and *Desmococcus sp.,* 1ml of seed inoculum of *Chlorella sp.,* was added with varyin6g volume of match industry effluent and it was made up to 10ml of CFTRI medium in a boiling tube. The specific growth rate was measured on a regular time interval (3rd, 5th, 7th, 9thday). Maximum growth of Chlorella sp., and Desmococcus sp., was compared and tabulated. The graph was plotted between the specific growth rate (day) -1 and varying effluent rate (ml).

Table 4: Specific growth rate of microalgal species in match industry effluent.

Effluent rate in ml	Specific growth rate of microalgal species in match industry effluent					
	Initial count of *Chlorella species*	Count of Chlorella species on 5th day	Initial count of Scenedesmus species	Count of Scenedesmus species on 7th day	Initial count of Desmococcus species	Count of Desmococcus species on 9th day
Control (CFTRI)	17	0.239	14	0.127	2	0.611
2	17	0.316	14	0.111	2	0.444
4	17	0.208	14	0.188	2	0.369
6	17	0.232	14	0.093	2	0.423
8	17	0.232	14	0.17	2	0.515
10	17	0.239	14	0.127	2	0.512

CONCLUSION

Three micro algal species (*Chlorella sp., Scenedesmus sp., Desmococcus sp.,*) was used as an agent for bioremediation of industrial wastewater. The bio treated effluent contains the biomass of *Desmococcus sp.,* 0.45g/lit. Level of Inorganic Nitrate and Phosphate reduced in the bio treated effluent was Nitrate-96.96% and phosphate-42.49%**.** The Optimized match industry effluent from existing work has compared with food coloring dye effluent to cultivate different microalgae species to obtain maximum growth.

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