

Algal Bioassay Studies in Waste Water

*Dr. A. Sridhar Kumar¹, Dr. P. Manikya Reddy², Dr. P.L.K.M.Rao³,

¹ Department of environmental Science, Osmania University, Hyderabad- Telangana State-India

² Professor, Department of Botany, Osmania University-Hyderabad

³ Consultant, CDT Laboratory, Hyderabad

Correspondence E-mail. meetsreedhar1@gmail.com

Abstract: In order to assessing whether algae can reduce the pollution concentration of the effluents by absorbing the nutrients, it is found that effluents can effectively be treated by employing algal organisms such as *Oscillatoria* and *Stigeoclonium* species and these organisms are frequently found in the polluted waters and they were recorded as pollution tolerant forms. In the laboratory procedures out of the several media tested Modified CHU No. 10 medium was found to be quite suitable for both the test organisms. It was found that up to 87% and 85% of phosphate uptake was achieved by *Oscillatoria* and *Stigeoclonium* respectively with 13% and 16% increase of D.O. in the effluents by the tenth day. In case of organic matter *Oscillatoria* removed 73% and *Stigeoclonium* 70% up to tenth day.

Keywords: *Oscillatoria*, *Stigeoclonium*, Culture medium, Industrial pollution and Nutrients

I. Introduction

In recent years the importance of biological waste treatment system has drawn the attention of scientist and has helped in developing relatively efficient low cost waste treatment systems. Because of highly discharging of untreated and partially treated industrial waste and municipal waste in to the river adversely affects the aquatic life and degrades the water quality. Though municipal waste mainly composed of sewage, on biodegradation and enriches the water with nutrients like nitrates, phosphates and their continued addition gradually leads to eutrophication problems. Industrial wastes on the other hand are highly toxic and generally contain algal and animal toxicants and algae growth stimulants [1 & 2]. They also contain heavy metals [3]. There higher concentrations are always toxic to algae [4 & 5] and may result in cellular abnormalities. Many studies demonstrated the success of using algal cultures to remove nutrients from wastewater rich in nitrogenous and phosphorus compounds [6]. Their analysis of long term data revealed that the Chlorophyta was dominant both in variety and quantity followed by Cyanophyta, Bascillariophyta and Euglenophyta. Palmer [7] listed the algae in the order of their tolerance to organic pollutants as reported by 165 authors. The list was compiled for 60 genera and 80 species. The most tolerant eight genera were found to be *Euglena*, *Oscillatoria*, *Chlamidomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*.

Addition of industrial effluents in a water body induce change in its algal population dynamics, species composition, morphology and physiology all of which may have a significant effect on the algal community structure [8] disturbing the primary food chain, natural purification system and ultimately the ecosystem [9].

Recently it is realized that, micro algae have much significant in fresh water ecology. The algal components of the biota produce a profound influence on the water quality. Hedge [10] recorded the good growth of algae intake water under eutrophication.

II. Materials And Methods

The study area is a effluent channel of Hussainsagar lake, the channels carrying industrial effluent and a domestic effluents which finally find their way in to the Hussainagar lake. The sample collected away from the joining both the channels (Fig 1) during the year 2014.

Though physico-chemical and biological analysis of water body have been used [11 & 12]. Conventionally to study water quality, recently the algal bio assay methods have been found advantageous for pollution evaluation [13]. Use of algae in bio assay method in laboratory has been interesting as explained by [14]. The use of algae for example, *Selenastrum capricornutum* [15] and *Scenedesmus obliquus* [16] in toxicity testing proved to be advantageous. Tiwary [17] utilized *Chlorella vulgaris* and *Scenedesmus bijuga* to remove nutrient from water body.

However, algal bioassay tests are relatively sensitive tools for evaluating water quality. This method is having its practical importance in distinguishing the occurrence and availability of the nutrients to the test organisms in bioassay because of its more sensitive nature and for relatively simple and inexpensive short-term test results [18].

Out of the three different bio assay techniques such as 1. Batch bioassay 2. The primary productivity incubation bioassay and 3. The continuous culture, the first method utilizing a chemostat was proved to be reliable by [19]. Algal bio assay have been variously employed by many workers [20, 21 & 22] for assaying the toxic effects, nutrient potentiality, effluent treatment and nutrient limitation studies.

Considering the above information, the algal batch bioassay was conducted taking two indigenous algal species belonging to two different groups. *Oscillatoria tenuis* and *Stigeoclonium tenue*.

In the present study, algal bioassay technique was used for assessing

1. Whether the effluents was toxic to algae reducing algicidal or algistatic effects
2. If toxic, to what extent, it should be diluted to support the algal growth?
3. Whether algae can reduce the pollution potential of the effluent by gleaned nutrients, if so to what extent.

2.1. Toxicity Test:

Sample collection: 4 to 5 uniform pebbles (2" x 2") coated with brown or green scum were collected from the habitat in a wide mouthed bottle containing 250 ml of water with clean forceps. The pebbles were scrapped carefully with scalpel and brush. The samples were preserved with 4% formaldehyde solution and the final volume of sample was reduced to 50 ml by sedimentation. This concentrated material was used for frequency measurement and species identification.

Laboratory Cultures: *Oscillatoria tenuis* from Cyanophyceae and *Stigeoclonium tenue* from Chlorophyceae are selected for the experimental work. They were isolated from the polluted waters of the effluent channel. These two organisms are frequently found in the polluted waters and they were recorded as pollution tolerant forms.

Isolation and Purification: Algal samples from the unialgal forms were diluted and inoculated on sold agar plates (1% agar in CHU 10 medium). From the filaments appearing on the plates after a week or so, some clean and uncontaminated filaments of *Oscillatoria* and *Stigeoclonium* are picked up and inoculated into culture flasks containing basal medium. The processes were repeated till unialgal cultures were obtained.

2.2. Culture Medium:

Out of the several media tested Modified CHU No. 10 medium was found to be quite suitable for both the test organisms.

1. 6 stock solutions were prepared by dissolving the chemical listed in the amounts indicated (in grams) each 100 ml double distilled water.

S.No	Component	Stock Solution Concentration (g/100 ml)
1	CaCl ₂ ·2H ₂ O	3.67
2	MgSO ₄ ·7H ₂ O	3.69
3	NaH CO ₃	12.6
4	K ₂ HPO ₄	0.87
5	NaNO ₃	8.5
6	Na ₂ SiO ₃ ·9H ₂ O	2.84

2. Iron solution was prepared by dissolving 3.35g. citric acid (C₆H₈O₇·H₂O) in 100 ml of distilled water and to this 3.35g. ferric citrate (FeC₆H₅O₃·5H₂O) was added and autoclaved to dissolve and kept in a refrigerator.

3. Trace element solution was prepared by dissolving the chemicals in quantities indicated in 1 liter of distilled water.

S.No	Component	Stock Solution Concentration (milligrams/1000 ml)
1	CuSO ₄ ·5H ₂ O	19.6
2	ZnSO ₄ ·7H ₂ O	44.0
3	CaCl ₂ ·6H ₂ O	20.0
4	MnCl ₂ ·6H ₂ O	36.0
5	Na ₂ MoO ₄ ·2H ₂ O	12.6
6	H ₃ BO ₃	61.84

To prepare the definite solution 1ml of each of the stock solutions were added aseptically in step 1 to a liter of sterile double distilled water. Then added aseptically 1 ml of the trace elements solution in step 3.

2.3. Culture Methods and Experimental Procedure and Environmental Requirements.

The cultures were maintained in 250 ml conical flask containing the 100 ml of the medium and stopped with cotton plugs. After inoculation, the cultures were kept in a culture room at about 22 to 24°C temperature

and exposed to continuous fluorescent light (2000 LUX). The cultures were shaken twice a day to avoid clumping of cells and sticking to the walls of culture flasks.

For both the test organisms' one loopful of filaments equivalent to 1 mg dry weight of inoculums from satisfactorily grown (2-3 weeks old) and thoroughly shaken culture was pipetted out and inoculated in to the experimental flasks. For each week a set of 3 conical flasks were maintained apart from control flasks for the estimation of growth and various physico-chemical parameters under investigation at regular intervals for a period of 15 days. The growth of cultures was estimated by measuring the density of algal cultures photo metrically at 660 nm in a spectrophotometer against a reference blank of basal culture medium.

2.4. Experimental procedure:

Industrial effluents from station 1 at balanagar were collected as per standard procedure and brought to the laboratory for experimental purpose. They were filtered through whattman No 44 filter paper to remove suspended solids and then through millipore filter to remove bacteria and other micro organisms. The filtrate was used to study the toxic effect, if any, on the algae and the nutrient up take of the algae.

Different dilutions of the industrial effluents were prepared with distilled water separately per both the test organisms. Culture flasks containing 50 ml of different diluted solutions were inoculated with the test organisms. Pure culture medium was taken as control. For both the test organisms loopful of filaments equivalent to 1mg dry weight was inoculated in to experimental flasks. Culture flasks were then incubated and the growth of cultures was estimated by measuring the density of the algal cultures photo metrically.

For both the test organisms the growth was estimated once in every five days and up to the 15th day after inoculation. The good growth of algae was noticed in 33% and 25% dilution of the industrial effluents, whereas 100% and 50% dilution of the effluents not supported the growth of algae. This indicates that the industrial effluents exhibit toxicity on the algal growth.

III. Results And Discussions

The Physico-chemical analysis of industrial effluents prior to experiment indicate that it consists of higher amounts of chlorides, organic matter, besides heavy doses of heavy metals particularly iron followed by manganese and zinc. These highly toxic metal effects the growth of algae [23 & 24] observed that high oxidizable organic content and low dissolved oxygen content of the effluent can cause growth inhibition of algae. Whereas in 33 % and 25% dilutions of industrial effluents supported the good growth of the algae and the growth continued up to 15th day. Perhaps, the dilution might have reduced the lethal effect of the effluents and at the same time nutrients present in the industrial effluents favored growth. Thus it is apparent that no growth in pure effluent was not due to nutrient deficiency but due to the presence of toxicants. The good growth of algae could also be explained from the biological property of extracellular substances liberated by the algae. It is well known that algae liberate extracellular substances in to the medium and these may form complexes with certain inorganic and organic ions present in the medium Fogg [25 & 26] found that extracellular materials may have the effect of reducing the toxicity in the medium.

The data on growth, pH, Phosphates, Chlorides, Dissolved Oxygen and organic matter on the initial day and thereafter at 5 days intervals or incorporated in tables 1 and 2.

Table-1. Nutrient removal from industrial effluents by *Oscillatoria tenuis*

Factors	Zero	5 days	10 days	15 days
pH	6.1	6.8	7.6	7.1
Cl	1870.2	718	437.9	452
D.O.	3.6	4.1	3.8	2.8
O.M.	6.16	1.62	1.9	2.43
PO ₄	4	0.5	0.2	0.5
O.D. at 660 nm	0.06	0.27	0.56	0.32
% removal				
Cl		62	77	75
O.M.		73	69	60
PO ₄		87	95	87
% increase of D.O.		13	5	-23

Table-2. Nutrient removal from industrial effluents by *Stigeoclonium tenue*

Factors	Zero	5 days	10 days	15 days
pH	6.1	6.6	8.2	7.4
Cl	1870.2	1308.3	392.4	182.1
D.O.	3.6	4.2	3.8	2.5
O.M.	6.16	1.8	1.73	2.36
PO ₄	4	0.6	0.4	0.9
O.D. at 660 nm	0.04	0.28	0.62	0.58
% removal				

Cl		10	79	90
O.M.		71	72	61
PO ₄		85	90	77
% increase of D.O.		16	6	-30

Oscillatoria tenuis

It is clear from the data that uptake of phosphate in *Oscillatoria* was high from the initial day to 10th day after that there is slight decrease in phosphate uptake from 10th the 15th day in both 33% and 25% dilutions.

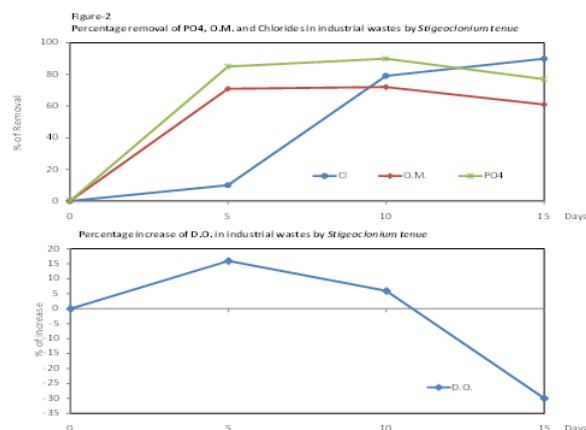
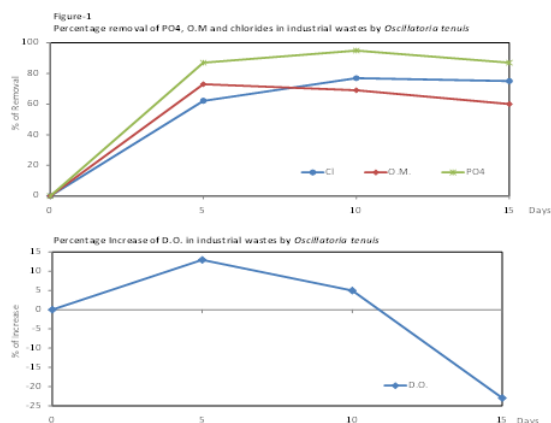
Uptake of chlorides was high from initial day to tenth day after which there is a slight decrease was observed in the uptake of chlorides both 33% and 25% dilutions.

Similarly the D.O. of the medium gradually increased from initial day to fifth day and after wards it showed a gradual decreasing trend reaching its minimum concentration on fifteenth day. In correspondence with D.O. the organic matter also exhibits a similar trend in gradual decrease in concentration up to fifth day there after it shows a relative increase in its concentration (Fig 1).

Stigeoclonium tenue

In case of *Stigeoclonium tenue* phosphate uptake was high from the initial day to tenth day. After that there is slight decrease in phosphate uptake from tenth day to fifteenth day in both 33% and 25% dilutions.

Uptake of chlorides was shown a gradually increase from fifth day to fifteenth day in 33% dilution whereas in 25% dilution there is slight decrease in its uptake from tenth day to fifteenth day. Similarly the D.O. of the medium gradually increased from initial day to fifth day and after which it showed a gradual decreasing trend reaching its minimum concentration on fifteenth day in both 33% and 25% dilution. In correspondence with dissolved oxygen the organic matter also exhibits similar trends gradual decrease in concentration up to fifth day. Thereafter it shows a relative increase in its concentration (Fig 2).



From the results it is evident that nutrient uptake was quite high in the beginning when the algae was exponential stage i.e., nearly up to tenth day. From tenth day to fifteenth day the medium showed slight decreasing nutrient uptake in corresponds to its growth. The medium became more alkaline with ageing of culture. Thus enhanced phosphate removal could be apparently due to high pH level and possible precipitation of phosphorus compounds with ageing of culture. The increase in the phosphate concentration after tenth day onwards may be due to gradual reaching of cells to the senescence stage which causes intracellular excretion of phosphate in to the medium.

The above experiment clearly indicates that the *Oscillatoria* removed 87% and *Stigeoclonium* 85% phosphate

by the fifth day and 95% and 90% respectively by the tenth day and 87% and 77% by the fifteenth day. In case of chlorides the *Oscillatoria* removed 61% and *Stigeoclonium* 30% by the fifth day and 76% and 79% respectively by the tenth day and 75% and 90% by the fifteenth day. In case of *Oscillatoria* the D.O. reaches its maximum increase of 13% on fifth day while in *Stigeoclonium* D.O. reaches of maximum of 16% on fifth day. From fifth day to fifteenth day there is gradual decrease of D.O. in both the test organisms. In case of organic matter *Oscillatoria* removed 73% and *Stigeoclonium* 70% by the fifth day and 69% and 72% respectively by the tenth day and 60% and 61% by the fifteenth day.

The near total recovery of nutrients and relatively high algal yields [27] point to the economic advantages and environmental acceptability of high rate pond mass culture systems for tertiary treatment of effluents.

Algal bioassay methods have been found advantageous for pollution evaluation. Use of algae in bioassay methods in laboratory has been interesting as explained by [28 & 29] proved that use of algae in toxicity testing was advantageous. However, algal bioassay tests are relatively sensitive tools for evaluating water quality. Tam [30 & 31] employed some organisms for algal bioassay tests.

In the present study both *Oscillatoria* and *Stigeoclonium tenue* were employed in the bioassay test. It is evident from the experiment that these two organisms survived well in 33% and 25% dilutions and they exhibited good growth. From the results it is evident that nutrient uptake was quite high in the beginning when the algae was in exponential stage up to the tenth day. From tenth day onwards the medium showed decrease in nutrient uptake in corresponds to its growth. This may be due to aging of culture as well as exudation of intra cellular substance in to the medium. The experiment clearly indicates that the *Oscillatoria* removed 87% *Stigeoclonium* 85% phosphate. In case of chlorides the *Oscillatoria* removed 61% and *Stigeoclonium* 30% by the fifth day and 76% and 79% respectively by the tenth day and 75% and 90% by the fifteenth day. In case of *Oscillatoria* the D.O. reaches its maximum increase of 13% on fifth day while in *Stigeoclonium* D.O. reaches of maximum of 16% on fifth day from fifth day to fifteenth day there is gradual decrease of D.O. in both the test organisms. In case of organic matter *Oscillatoria* removed 73% and *Stigeoclonium* 70%.

IV. Conclusions

In the present study, assimilation of nutrients by the test organisms seemed to be the only cause of nutrient removal from the medium. Removal of good amount of nutrients from the experimental solution by the test algae points to the fact that these algae are quite capable of treating the effluents by gleaning nutrients and pollutants and thereby reducing the pollution potential of the effluents.

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