TREATMENT OF PHARMACEUTICAL INDUSTRY EFFLUENT USING IMMOBILIZED OSCILLATORIA SPECIES

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ABSTRACT

The present study showed that the potential degradation of pharmaceutical industrial effluents by environmental species of *Oscillatoria.* *Oscillatoria* species collected from coastal regions of Rameswaram, south east coast of India. Biodegradation and biosorption capacity of some potential Oscillatoria species: *Oscillatoria subsalsa, Oscillatoriaflos-aquae, Oscillatoria salina* and *Oscillatoria amphigranulata.* The occurrence of *Oscillatoria* species in the pharmaceutical industry effluent was due to favorable contents of organic matter, rich calcium and nutrients such as nitrates and phosphates with less dissolved oxygen. Removal efficiencies of the different organic, inorganic metal and non-metallic elements were evaluated and compared.

KEYWORDS: Cyanobacteria, *Oscillatoria*, Pharmaceutical Industry Effluent, Immobilization, Rameswaram.

INTRODUCTION

Microorganisms are the vital important in biological treatment systems. Mahaswari Devi and Gopal[1] stated that the role of heterotrophic bacteria in aerobic and anaerobic digestion of wastewaters has been exploited for the production of fuel. Microorganisms excrete the

extracellular polysaccharides during the stationary phase of growth which helps in bringing down the pollution load in biological treatment systems.[2]

The aerobic biological processes used for treatments of various effluents. Subramanian[3] and Subramanian and Shanmugasundaram[4] reported that the biological treatment systems using bacteria have been well studied and controlled photosynthesis involving algae-bacterial symbiosis is an inexpensive process and is used in the reclamation of wastewater like sewage.

In 1984, Elnabarawy and Welter[5] stated that algae serve as excellent indicators of pollution as they respond typically to many toxicants. The use of algae in waste treatment is beneficial in oxygenation, mineralization and also for serving as food for aquatic species.

A marine filamentous cyanobacterium, *Oscillatoria salina* BDU 10142 was introduced in the effluent treatment. It brought down calcium, chloride by 30% and 12% respectively. The cyanobacterium biomass was increased 80 times more than that inoculated. Manoharan and Subramanian[6] reported early that the effluent containing phosphate, chlorides, calcium and magnesium was treated with marine cyanobacterial strains namely *Oscillatoria wellei* BDU 130511 and *Oscillatoria formosa* BDU 40261 were able to grow and metabolize magnesium, phosphate and nitrate completely and to certain extent calcium.

MATERIALS AND METHODS
Source of Organisms: Marine cyanobacteria were collected in polythene bags from Rameswaram costal region, south east coast of India. They were transported to laboratory for identification and purification.

Purification: Cyanobacteria were purified with standard plate methods like streak, spread and pour plate techniques. The purified cyanobacteria species are namely *Oscillatoria subsalsa, Oscillatoria flos-aquae, Oscillatoria salina* and *Oscillatoria amphigranulata.*

Culture conditions: Cultures were maintained in 250 mL Erlenmeyer flasks containing 100 mL of sterile ASN III medium and incubated under white cool fluorescent light 13.7 μ Einsteins m^{-2}. s^{-1}at room temperature of 27 +2°C with 14 ± 10 hr L/D cycle.

Source of Pharmaceutical effluents: The effluent was collected from Hosur, Tamil Nadu at that time the effluents temperature was 21°C and the physical characteristic were examined. The raw effluent sample was taken, sterilized and filtered through Whatman No.1 paper and

then cooled. The effluent pH was adjusted to 7.5, which was stored in brown bottles at room temperature. This effluent was ready for treatment.

Inoculum preparation: The purified and selected marine forms of cyanobacteria were inoculated into polyurethane foam pieces (1cm x 1cm) and allowed one day to grow with small amount of ASN III medium containing Petridis.

Experimental setups: The living cultures were introduced into polyurethane form with ASN III medium. The cyanobacteria loaded polyurethane forms were packed into column (60cm x 10mm dia). Initially ASN III medium was run into column for standardizing the liquid flow per minute and then sterilized pharmaceutical industrial effluent was run through the cyanobacteria loaded column in an identical speed and standardized quantity of out let collection of effluent which was considered as treated one. The effluent with no organisms served as control.

Analysis of Treated Effluent with standard procedure: The analyzing parameters are such as 1. Physico - Chemical Measurements; Colour, Physical appearances (i) Flat formation and (ii) Foam), Odour, Temperature, pH, Suspended solids, Dissolved solids and Settable solids, 2. Measurement of Organic Pollution; Bio – chemical Oxygen Demand (BOD)[7] Chemical oxygen Demand (COD)[7] and Ammonia (Nitrogen), 3. Inorganic Constituents – Metals (Calcium and Magnesium)[8] and 4. Inorganic Constituents – Non Metallic (Chloride)[9] Nitrate, Nitrite, Sulfate and Total Phosphate.[7]

RESULTS AND DISCUSSION

Pharmaceutical Industrial effluent was taken for this current study, it has dark brownish colour, which was intolerable one. The colour, foam formation, flat formations and odour were totally disappeared in the treated effluents with the four marine cyanobacteria such as *Oscillatoria subsalsa, Oscillatoria flos-aquae, Oscillatoria salina* and *Oscillatoria amphigranulata.* [Table-1].

Temperature: The untreated raw pharmaceutical effluent consisted $20^{\circ}C$ temperature which was increased to $25^{\circ}C$ in *Oscillatoria subsalsa* and *Oscillatoria salina* and 26°C in *Oscillatoria flos-aquae* and *Oscillatoria amphigranulata* species of immobilized cyanobacteria which were inoculated into the pharmaceutical industrial effluent [Table-1].

Table 1: Reduction of Physicochemical Parameters of Pharmaceutical Industry Effluent with Marine Immobilized Cyanobacteria [untreated effluent as a control and treated effluent reduction is expressed in percentage].

Oscillatoria species	Color (Whitish Grey)	Flat Formation (Thin Film)	Foam (Present)	Vile Odor	Temp. (20°C)	pH (5.5)
O. subsalsa	-	-	-	-	25°C	6.5
O. flos-aquae	-	-	-	-	26°C	6.1
O. salina	-	-	-	-	25°C	6.2
O. amphigranulata	-	-	-	-	26°C	6.3

(-) indicates not detected.

pH: The raw pharmaceutical effluent has with 5.5 pH after the treatment, the pH was decreased 6.5in *O. subsalsa*, 6.1 in *O. flosaquae*, 6.2 in *O. salina*, 6.3 and in *O. amphigranulata* (Table-1). This results of pH in the pharmaceutical Industrial effluent was similar to previous study with the cyanobacterium *Phormidium valderianum* BDU 30501.[10]

Suspended Solids: The maximum reduction of the suspended solids in pharmaceutical industry effluent was 87.69% in *O.flos-aquae* and the minimum of 80%, in *O. subsalsa* (Table-2).

Dissolved Solids: The living cell of cyanobacterial species showed the result with the dissolved solids the maximum reduction of 84.76% with *O.amphigranulata*in the phamacetical industry effluent (Table-2).

Total Solids: Of the algal cell with the pharmaceutical effluent that an extreme reduction of 90%, 83.52%, 87.64% and 88.82% with cyanobacterial species *O.subsalsa*, *O.flosaquae*, *O.salina*, *O. amphigranulata* respectively (Table-2).

The above results showed in suspended solids, dissolved solids and total solids that the selected cyanobacteria have the unique photosynthetic organisms bring about oxygenation and mineralization which is related to the earlier report of Elnabarawy and Welter.[5]

Cyanobacteria excretion of exopolysaccharides in the stationary phase which were used as detoxifiers and biofloculants.

Table 2: Reduction of Solid Waste in Pharmaceutical Industry Effluent with Marine Immobilized Cyanobacteria [untreated effluent as a control and treated effluent reduction is expressed in percentage].

Oscillatoria species	Suspended Solid in %	Dissolved Solids in %	Total Solids in %
O. subsalsa	80	80.95	90
O. flos-aquae	87.69	83.8	83.52
O. salina	83.07	81.9	87.64
O. amphigranulata	84.61	84.76	88.82

Measurement of Organic Pollutants

Biological Oxygen Demand (BOD): The reduction of BOD in pharmaceutical industrial effluent was minimum93.90% and the maximum of 96.50% with an immobilized cell of *O. salina* and *O. amphigranulata* species respectively (Table-3).

Chemical Oxygen Demand (COD): The maximum of 89.77% COD reduction in raw pharmaceutical industrial effluent by *O. flosaquae* and the minimum of 87.50% with *O. salina*, the intermediate percent at 88.30% and 87.36% with *O. subsalsa* and *O. amphigranulata* respectively (Table-3).

Ammonia: Pharmaceutical industrial effluent marine cyanobacterial species showed about 85.36% of minimum of reduction with *O. flosaquae*, 95.93% of maximum reduction of ammonia with *O. amphigranulata* (Table -3). This study was supported to previous study of Govindan (1981) and Mitsui *et al.,* (1985) in BOD and COD with algal cultures and bacterium *Rhodopseudomonas* in pharmaceutical industrials effluents.

Table 3: Reduction of Organic Pollution in Pharmaceutical Industry Effluent with Marine Immobilized Cyanobacteria [untreated effluent as a control and treated effluent reduction is expressed in percentage].

Oscillatoria species	BOD in %	COD in %	Ammonia in %
O. subsalsa	95.52	88.30	92.68
O. flos-aquae	94.70	89.77	85.36
O. salina	93.90	87.50	91.86
O. amphigranulata	96.50	87.36	95.93

Inorganic Constituents – Metals

Calcium: The element calcium reduction in this raw pharmaceutical industrial effluent was an extreme 88.33% with *O. salina*, 81.66% of calcium reduction with the two marine cyanobacteria species such as *O. subsalsa* and *O. amphigranulata* and the remaining one

species of *O. flosaquae* was showed 78.33% calcium reduction in the raw pharmaceutical industrial effluent (Table – 4).

Magnesium: The selected four marine cyanobacterial species such as *O. subsalsa*, *O. flosaquae*, *O. salina*, *O. amphigranulata* were extremely reduced the magnesium elements from the raw pharmaceutical industrial effluent about 74.54%, 83.63%, 80% and 89.09% respectively (Table – 4).

Table 4: Reduction of Inorganic – Metals in Pharmaceutical Industry Effluent with Marine Immobilized Cyanobacteria [untreated effluent as a control and treated effluent reduction is expressed in percentage].

Oscillatoria species	Calcium in %	Magnesium in %
O. subsalsa	81.66	74.54
O. flos-aquae	78.33	83.63
O. salina	88.33	80.00
O. amphigranulata	81.66	89.09

Inorganic constituents Non – metals
Chloride: In the pharmaceutical industrial effluent degradation, there are about more than 96% reduction with *O. flosaquae* and *O. amphigranulata* about 97% biodegradation of chloride in the effluent by *O. salina*, *O. subsalsa* (Table -5).

Nitrate: Of the pharmaceutical industrial effluent with the biological treatment of cyanobacteria species. The minimum of 87.57% and the maximum of 92.09% with *O. salina*, and *O. subsalsa* respectively. The remaining two species of marine cyanobacteria such as *O. flosaquae*, *O. amphigranulata* showed 88.7% and 89.83% respectively (Table -5).

Nitrite: The industrial pharmaceutical effluent basically consist 81.1mg/liter of nitrite. The amount of nitrite extremely reduced to 85.20, 88.90, 90.13 and 91.36% with the selected marine cyanobacterial four species a Genus *Oscillatoria*, such as *O. amphigranulata, O. subsalsa*, *O. salina* and *O.flosaquae* respectively (Table -5). Fogg[11] reported that the variety of nitrogenous compounds such as nitrate and nitrite at low concentration serve as nitrogen source for the growth of algae, when nitrogen is taken up in an oxidized form as nitrate or nitrite; it gets reduced to ammonia by the action of nitrate and nitrite reductase, before being incorporated into organic molecules.

Total Phosphorus: All the four selected species of marine cyanobacterial were used in the industrial effluent treatment that showed 94.83%, 93.42%, 92.95% and 90.14% of total phosphorus removed from the effluent with *O. subsalsa*, *O. amphigranulata*, *O. flosaquae* and *O. salina* respectively (Table -5). Tailing [12] stated that the phosphorous is one of the major nutrients required for the normal growth of cyanobacteria. It plays a key role in most cellular processes, particularly those involved in energy transfer and nucleic acid synthesis.

Sulphate: In the pharmaceutical industrial effluent the inorganic elements. Sulphate was maximally reduced from 83.5%, 84%, 85.5% and 89% with *O. subsalsa*, *O. amphigranulata*, *O. flosaquae* and *O.salina* respectively (Table -5). Sulphur is also an essential element and most of the algae obtained sulphur from inorganic sulphate. Uptake of sulphate from the pharmaceutical Industrial effluent by living cells were fed with sterilized is clearly evident. The possible reason that could be attributed for reduction observed with dead cells could be sulphate getting precipitated as calcium sulphate. Subramaniyan and Manoharan[13] reported that immobilized cyanobacteria were removed more than 75% of colour from effluent with nitrites, phosphates and ammonia and increase in Dissolved Oxygen (DO) content and reduction of Biological Oxygen demand (BOD), Chemical Oxygen demand (COD) up to 95% than that of free cells.

Borowitzka & Borowitzka[14] reported that microalgae are being implicated in food, cosmetic, aquaculture and pharmaceutical industries and also supported to the biotechnology, but Jyothi[15] prospered the cell immobilization techniques to solve the problem of using small size cells in the application of biotechnological processes.

Table 5: Reduction of Inorganic – Non- Metals in Pharmaceutical Industry Effluent with Marine Immobilized Cyanobacteria [untreated effluent as a control and treated effluent reduction is expressed in percentage].

Oscillatoria species	Chloride in %	Nitrate in %	Nitrite in %	Total Phosphorous in %	Sulphate in %
O. subsalsa	97.12	92.09	88.90	94.83	83.50
O. flos-aquae	96.57	88.70	91.36	92.95	85.50
O. salina	97.67	87.57	90.13	90.14	89.00
O. amphigranulata	96.16	89.83	85.20	93.42	84.00

CONCLUSION

The above results clearly indicate that the pharmaceutical Industrial effluent could be effectively treated by the immobilized living cells of *Oscillatoria subsalsa, Oscillatoria flos-aquae, Oscillatoria salina* and *Oscillatoria amphigranulata* which were efficient in bringing down the total solids, dissolved solids, suspended solids, sulphate, nitrate, nitrite, phosphorous levels to a considerable one.

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