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STRIKING SYNERGISM BETWEEN WATER QUALITY RESTORATION WITH ALGAL SINGLE CELL PROTEIN (SCP) PRODUCTION

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ABSTRACT

Phycoremediation is a process that uses microalgae for water remediation. The limitations to the slow growth rate and low biomass of plants in case of phytoremediation can be effectively managed by Phycoremediation. The present study efficiently couples remediation of nitrate, phosphate levels in a fresh water source with single cell protein production. The *Spirulina-Chlorella* consortia was tested for its remediation efficacy in the form of a floating wetland for a maximum period of 10 days. The results were promising with a reduction of 34.36 ± 1.19 % of nitrates, 83.39 ± 0.66 % of phosphates and 97.79 ± 0.79% in bacterial load (cfu/mL). The consortia thus reared was found to have 79 ± 0.2 % protein, 19.8 ± 0.34 % lipids and 9 ± 0.42 % carbohydrates making it a prospective feed supplement. This pilot scale set up thus seems to be a promising solution for onsite remediation of agricultural run-offs that are rich in nitrates and phosphates.

KEYWORDS: *Phycoremediation, Chlorella-Spirulina, Nitrate, Phosphates, Feed supplement*

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INTRODUCTION

Agricultural practices of random fertilizer as well as pesticide applications are major sources of nitrates and phosphates in aquifers[1]. Conventional methods for water treatment that are in practice for contaminant removal and water purification prove to be either costly or non-ecofriendly[2]. Phytoremediation however, is an economical as well as eco-friendly approach which employs plants for removing environmental pollutants thus detoxifying the contaminated water and restoring its quality[3].Like plants, algae are also one of the prime ecofriendly remediating agents and have been employed widely in a process termed as phycoremediation[3]. Microalgae capable of growing mixotropically have been efficiently used for remediation of water containing organic and inorganic nutrients like nitrogen, phosphorus, heavy metals, pesticides, hydrocarbons and other pollutants[4]. Unlike plants, the higher growth productivity of microalgae due to their enhanced photosynthetic yield provides a large amount of biomass (Single Cell Protein) in a short period of time[5]. Also, a longer remediation time as well as expenses incurred for harvesting the plant for biomass management presents another lacuna in phytoremediation[6]. Microalgal bioremediation thus not just offers an eco-friendly but also an economical approach to removal of inorganic nutrients while producing potentially valuable biomass which can be directly sold as feed without any processing expenses. In addition to the removal or biotransformation of nutrients, algae functions to release free oxygen during photosynthesis which enhances the aerobic degradation of organically enriched water aided by aerobic micro-organisms[7-8].The conventional process of phycoremediation however has limitations in terms of the downstream processing involved during harvesting of the uniformly dispersed algal mass used either as single cells or immobilized beads after remediation. The present study was therefore designed in order to overcome this challenge by the use of algal biofilms in the form of floating wetland. As reported earlier, algal production systems can be coupled with the treatment of biodegradable nutrients from the wastewater for economical production of single cell protein[9]. Algae while removing the inorganic nutrients from the water (nitrate, phosphates) incorporate them thereby enriching its biomass with high value added products like proteins and lipids[10]. The biomass generated after remediation can be subsequently processed to produce energy or raw material for feed thus renewing interest in microalgal wastewater bioremediation[11]. A faster growth rate together with an easier mixotrophic cultivation of *Chlorella* strains coupled with the ability of the cyanobacterium *Spirulina* to thrive on alkaline environments makes them good candidates for remediation purposes[12-13]. Also the microalgal feed market is currently dominated by *Chlorella* and *Spirulina* due to their high protein content. They are either directly sold as fish food or are used to produce valuable compounds like proteins, lipids and pigments[14]. The present study was thus designed to evaluate the efficacy of *Chlorella-Spirulina* consortia as remediators to restore the water quality in terms of nitrate, phosphate, dissolved oxygen (DO), biological oxygen demand (BOD), bacterial load (cfu/mL) alongwith nutritional composition.The biomass at the end of remediation was analyzed for its carbohydrate, protein and lipid content.

MATERIALS AND METHODS

Consortia culturing

Chlorella emersonii KJ725233 as well as *Spirulina* species isolated from a freshwater source from the western regions of Maharashtra were used[15]. This *Chlorella – Spirulina* consortia was grown in BG-11 medium for a period of 20 days under 12 hours : 12 hours light : dark conditions and with 12 hours of aeration.

Culture and set up

The floating wetland was fabricated using a nylon sieve of 0.5-0.8 mm pore size and diameter 20 cm. After 20 d of incubation, the consortia grown in the form of a film was placed on the mesh that was subsequently kept in contact with the water to be treated. The experimental set up was maintained and monitored for period of 10 days for its nitrates, phosphates, DO, BOD, pH as well as bacterial counts. Appropriate control without the algal film was maintained for the set up.

Determination of the physico-chemical and biological parameters

The change in the parameters was determined by appropriate spectrophotometric methods. Nitrates were detected by the Ultraviolet spectrophotometric screening method[16], Phosphates by Stannous Chloride method[16], BOD and DO by the Azide modification method[16]. pH was monitored (Hanna instrument) pH meter. The decrease in bacterial load (cfu/mL) was determined by Heterotrophic plate count method before and after 10 d of treatment.

Determination of the nutritional composition

Proteins were extracted in 1 N NaOH and quantified by the Bradford's method[17]. Carbohydrates were extracted in 2.5 M HCl and determined by the anthrone sulfuric method[18-19]. Lipid content was determined gravimetrically by extraction in 2:1 Chloroform: methanol as reported[20].

STATISTICAL ANALYSIS

All the tests were performed in triplicates. Results are expressed as mean ± SD. p value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Phycoremediation by means of floating wetland is a novel technique which exploits the natural ability of the alga to take up, accumulate and degrade the contaminants present in their environment thus assisting the clean up of polluted water[21]. Similar to the matrix-immobilized culture remediation technology, substrate-attached floating algal biofilm offers same culture density and lower land as well as water requirements however, a floating wetland approach reduces the time

as well as cost associated with immobilization processes. In comparison to suspended cultures, a floating wetland system integrates production as well as harvesting operations thus potentially leading to cost effective downstream processes[22].

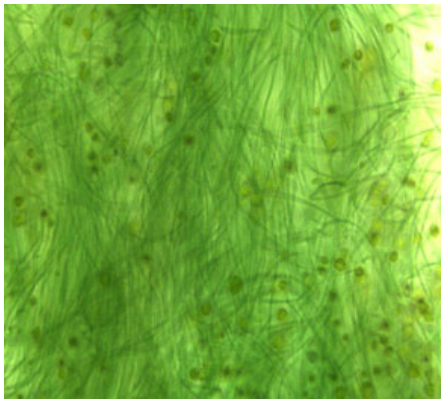


Figure 1
***Spirulina-Chlorella* consortia (40x)**

Figure 2
Consortia biofilm

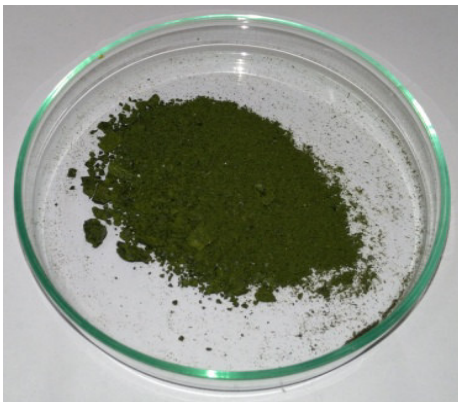


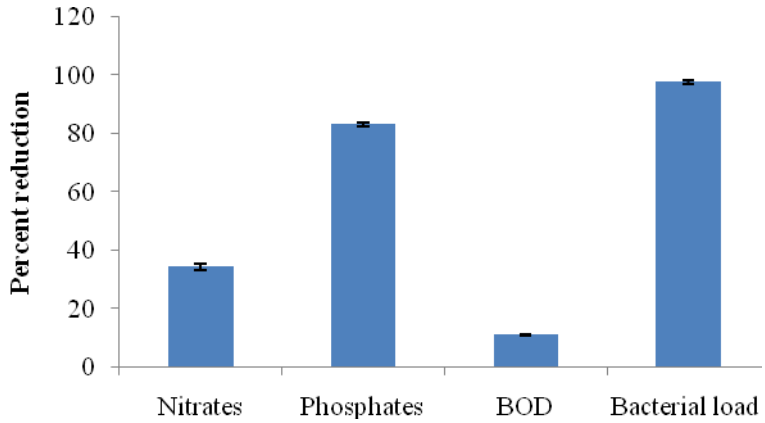
Figure 3
Dried biomass

The present study thus evaluated the efficiency of a *Chlorella-Spirulina* consortia (Figure 1, Figure 2) in restoring the water quality of the contaminated drinking water source. The results (Figure 4, Table 1, Table 2) indicated a 34.36±1.19 % and 83.39±0.66 % decrease in the nitrates and phosphates respectively after 10 d of phycoremediation. Since BOD of the water is the amount of oxygen used by the non-photosynthesizing bacteria to degrade the organic compounds, a high BOD and a low DO is indicative of contamination of the water. As seen in Fig 5 there was a 97.79±0.79% decrease in the bacterial load (cfu/mL) corresponding to a 11.11±0.14% decrease in biological oxygen demand (BOD) and a 37.5±0.38 % increase in the dissolved oxygen content.

Table 1
Percent Reduction in the parameters of the treated water over a period of 10 d.

Days	Nitrates	Phosphates	BOD	Bacterial load
0	0	0	0	0
3	20.76±0.41	68.63±0.44	47.05±0.34	-
5	24.84±0.60	71.58±0.61	26.31±0.43	-
7	31.15±0.49	81.91±0.43	15.81±0.49	-
10	34.36±1.19	83.39 ± 0.66	11.11 ± 0.14	97.79 ± 0.79

Results are expressed as mean ± SD.
P value for reduction in Nitrates, Phosphates, BOD as well as the bacterial load was found to be less than 0.05 as compared to that of the control.



The present results demonstrated a correlation of 0.9334 and 0.7544 between a decrease in BOD to a decrease in the bacterial count and increase in DO respectively. Thus, a decrease in BOD with a corresponding increase in DO indicates improved water quality.

Figure 4
Percent reduction in the parameters after 10 d of phycoremediation

Table 2
Change in DO (percent) and pH (units) of the water over 10 d during phycoremediation

Days	DO	pH
0	0	7.8±0.2
3	25.19±0.43	7.6±0.1
5	30.52±0.56	7.4±0.2
7	32.68±0.66	7.4±0.2
10	37.5±0.38	7.0±0.1

Results are expressed as mean ± SD.
p value (DO) < 0.04 as compared to control.

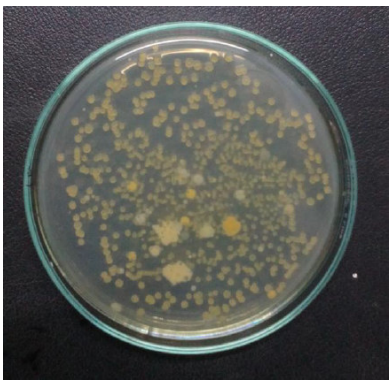


Figure 5a
0 day bacterial load (cfu/mL)

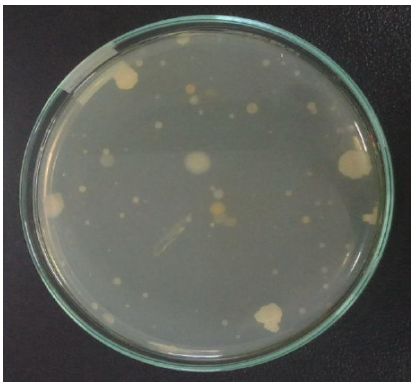


Figure 5b
10th day bacterial load (cfu/mL)

Mansor *et al*[23] have earlier reported the use of *Spirulina platensis* for the treatment of leachate which resulted in a 27%, 53%, 86% decrease in COD, BOD and TSS respectively with a 10% increase in the pH from 7.72 – 8.5. Use of *Chlorella vulgaris* for the remediation of sewage water showed 85.6% and 44.7% reduction in the phosphates and nitrates after 18 d of treatment[24]. *Chlorella vulgaris* and *Spirulina platensis* have showed a 79.04% and 84.21% decrease in nitrate respectively whereas a 57.75% and 41.81% decrease in phosphate respectively from municipal wastewater sludge[25]. Though a lower nitrate reduction was observed in the present study as compared to that reported in the literature for *Chlorella* and *Spirulina* alone but 83.39% reduction in phosphate concentration is analogous to the earlier reports. Reports suggest microalgal – bacterium consortia of *Chlorella vulgaris* and *Bacillus licheniformis* have shown 78% reduction in nitrate after 6 d[26]. However, the 34.36% and 83.39% reduction in nitrate and phosphates achieved in the present study is higher as compared to that reported for *C.vulgaris* and *A.brasilense* by De Bashan *et al*[27]. A 47.05% reduction in BOD was observed (Table 1) after 3 d which is equivalent to that observed for the *C.vulgaris* and *S.dimorphus* consortia as reported by Sakthivel and Elumalai[28].The dried biomass (Figure 3) was further harvested and analyzed for its nutritional composition in terms of proteins, lipids and carbohydrates (Figure 6). However, though the nitrate absorption was less as compared to that of phosphates the algal biomass showed a higher protein as well as a higher lipid: carbohydrate ratio as compared to the analyzed commercial post larval feed.

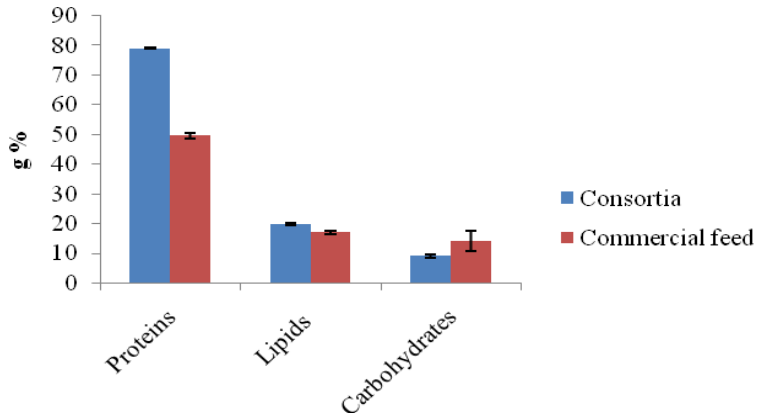


Figure 6
Comparison of nutritional composition of consortia and commercial feed.

Algal feeds are known to induce positive physiological changes viz., improved liver function, stress responses, health status in fish. Commercially available microalgal feeds are typically characterized with a high protein and a low carbohydrate content. A high carbohydrate content is associated with a lower protein fraction. Also the presence of complex polysaccharides in feed are known to affect the protein digestibility of the fish fed and hence carbohydrates are not sought after as a large percentage of microalgal feed supplements[29-30]. The low carbohydrate and a high protein content of the consortia makes it a potential feed supplement.

CONCLUSION

The technical feasibility of the novel floating wetland mediated phycoremediation makes it a viable solution to the water treatment.The removal of nitrates-phosphates together with the generation of high protein, valuable biomass is an added advantage of the technique. This

protein rich biomass can be further transformed into food or feed additives. The approach thus coupled, to the strains used, proves to be a true "Waste to Wealth" transformer.

CONFLICT OF INTEREST

Conflict of interest declared none.

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