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Production of a High Valued Biofertilizer From Ammonical Waste Water Using A Novel And Synergistic Phycoremediation Method.

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Abstract:

Expansion of industrial areas increased the industrial sewerage discharge which leads to the environmental health and water pollution issues. Wastewater treatment is having much importance in recent years with the intension of reusing it. Presently with upcoming technologies in developing countries for treating wastewater is not sufficient and cost effective solution. A novel advanced method of algal culture offers an effective solution for wastewater treatment coupled with the production of potentially valuable biomass and utile water which can be used for different purposes. This invention uses cheap and efficient, ecologically safe hybrid photobioreactor for the simultaneous detoxification of effluent and advantage of this result with no pollution of hazardous substances and lesser sludge production. The present study demonstrates that the algae growth merged on effluent without sterilization in presence of sunlight and the percent elimination of nitrate (99.55%), phosphate (90.83%), B.O.D (78.32%), C.O.D (80.45%) with 95.41% increasing efficiency of D.O were evaluated. Algal biomass with high nutrients is used as a biofertilizers which provides an effective and non-polluting approach in improving the productivity of crop by both nitrogen fixation and photosynthesis. This paper is briefly underlining the environment friendly way to utilize effluent and usage of algal biofertilzer which will be applicable on laboratory and large scale treatment for ecology betterment.

Keywords: Industrial Sewerage, algae culture, photobioreactor, detoxification, biofertilizer.

1. Introduction:

Fertilizer manufacturing industries on process operations discharge sewerage containing ammonical nitrogen, nitrate, phosphate, BOD, COD and total dissolved solids. Conventional technology has been widely applied in treatment of sewerage. The process removes most of the organic contaminants, nitrogen (N) and phosphorus (P) in the wastewater. However, the secondary effluent still contains high concentration level of N and P, which leads to eutrophication, ecosystem damage and cause various health problems in humans. The negative effects of such nutrients overloading of receiver aquatic systems include undesirable pH, low dissolved oxygen concentrations and effects marine aquatic life [1] [2].

The presence of these impurities in water affects to the water resource on the earth and cause high level of water environment damage. In other hand, due to the high demand on the fresh or clean water for daily use and due to water shortage, new technologies of treating the wastewater has to be introduced and worked out for the society to provide good quality of treated water that can be used for daily activities and to have recycle in industry process itself [3]. Compared to physical and chemical treatment processes, biopurification of effluent is of the major importance to the environment, as this technology may be in process to develop an efficient, low cost and environment friendly process which may lead for removal of excess wastes efficiently at minimal cost and the feasible cheapest technology for the treatment of sewerage is phycoremediation using photobioreactor [1] [4].

The use of macro algae or microalgae for effective removal or biotransformation of Pollutants, including nutrients and xenobiotics from wastewater and CO2 from waste air called phycoremediation [5]. Wastewater treatment in Waste Stabilization Ponds (WSPs) is "green treatment" achieved by the mutualistic growth of microalgae and heterotrophic bacteria and shallow mixed open high rate stabilized photobioreactor unit provides efficient wastewater treatment than conventional oxidation ponds [4] [5].

Algae are sustainable sources of food, feed and essential for their growth are light, CO_2 and inorganic nutrients like nitrogen and phosphorus. Algae has an important role in self- purification of organic matter due to this apply the algal system as secondary rather than tertiary treatment [4]. They generally comsumes nitrate, phosphorus, BOD, COD, total dissolved solids which are available in varying amounts (concentration) in the fertilizer industrial effluent which having growth of 20-30 times faster than food crops within a short period [3]. Algae support aerobic bacterial oxidation of organic matter producing oxygen through photosynthesis while release carbon dioxide in aerobic oxidation used for algal biomass [5]. The oxygen and pH variation induced by algal photosynthesis help to reduce coliform and other pathogenic bacteria in the effluent [4].

The algal biomass could be used as an ideal feedstock for the production of biofertilizer [1]. A continuous increasing demand of food crops and decrease in the productivity due to continuous use of chemical fertilizer has not only resulted in decline of crop yield, loss of fertility and degradation of soil, but which has also led us one step back in achieving sustainable agriculture. With the growing realization that chemical based agriculture is unsuitable and is slowly leading to ecological imbalance, the latter part of the last century witnessed the emergence of the concept of “organic agriculture” advocating minimum use of chemical fertilizers and increasing dependence on biological inputs like biofertilizers, compost, farmyard manure and green manure [6].

Biofertilizers are the natural fertilizer that contains bacterial, fangal or algal strains and enhance the productivity of soil by fixing atmospheric nitrogen or by solubilizing soil phosphate or by stimulating plant growth for synthesis of growth promoting substances by increasing the availability of primary nutrients and improve the health of the soil in eco-friendly manner [7]. Among the array of biofertilizers developed algal biomass constitutes the most important inputs for nutrients management in plants. Culturing algae using inorganic media is relatively expensive because of the need to provide full complement of nutrients [8] [9]. Low cost alternative such as nutrient rich effluent as a natural medium has been evaluated as a more cost effective method of algal biomass cultivation [6]. Algae are simple in operation and easy in adaptability for Indian farmers. India being one of the largest producer and consumer of pulses requires abundant amount of pulse production to fulfill the demands of growing populations which can also be achieved by using this cost effective fertilizers [10].

The benefits of treated effluent reuse derived mainly from savings in freshwater supply and a reduction in generation of waste. Cooling systems are major water consumers in many industries and therefore using treated effluent for this purpose may bring considerable saving in freshwater consumption. Concrete industry is consuming annually 1 billion tons of mixing water in the world and large quantity of freshwater are used for curing of concrete and hence recycle of treated wastewater to make fresh concrete. The main key point of the present research has been made to explore the possibility of cultivation of mixed culture algae from domestic sewerage source using nutrient fulfill effluent as a growth medium, from which treated effluent as a growth medium, from which treated effluent as a growth medium, from which treated effluent can be recycled and utilized in industry to overcome the water shortage problem and production of biofertilizers as a byproduct which is nature friendly.

2. Methodology:

2.1. Algal Sampling

Sample of merge algae was collected from domestic sewage treatment tank.

Figure 1: Algal source from Domestic Sewage Treatment Tank

2.2. Effluent Collection

The effluent used in this study was collected after secondary treatment deammonification process containing nitrate, phosphate, BOD, COD, total dissolved solids and small amount of other elements such as calcium, magnesium, chloride, iron etc.

Figure 2: Deammonified Effluent collection

2.3. Physico-chemical analysis of effluent

The analyses carried out for effluent are pH, nitrate, phosphate, BOD, COD, total suspended solids, turbidity, total hardness, calcium hardness, magnesium hardness, M-alkalinity, Conductivity, iron, Free Ammonia and TAN respectively.

2.4. Phycoremediation Technology

Algae cultivation was initiated in bioreactor from 1000 ml of beaker containing 500 ml of effluent. The experiment was conducted without sterilization of effluent in presence of sunlight with open air. In order to compare growth of algae in effluent and synthetic medium, an artificial medium was synthesized in the following way: 12.5 g of sodium nitrate ($NaNO_3$) and potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water.

Figure 3: Photobioreactor with Nitrate Effluent

Figure 4: Photobioreactor with Synthetic Media

2.5. Determination of algal growth

Growth of algae was determined by using spectrophotometer.

***2.5.1. Optical density*:**

For the comparison of the growth of algae in synthetic medium and effluent, equal quantity of algal culture was inoculated in measured quantity of both the medium (1 liter) under same climatic condition. Optical density of samples, taken for consecutive 7 days from both media, was measured by spectrophotometer at 680 nm of wavelength. The growth rate (GR) was determined by fitting the optical density (OD_{680}) in the following formula:

$$GR = (\ln OD_t - \ln OD_0)/t$$

Where OD_0 is the optical density at the initial day, OD_t is the optical density measured on day t. Each recorded OD_t was corrected by taking away that of the corresponding blank sample [3].

2.6. Biomass Harvesting

Biomass was harvested by using filtration method with whatmann filter paper No.1.The filtrate was used for different processes. After harvesting, the biomass was dried in shadow open air. Then, the dried algae were ground to fine powder by using mortar and pestle.

The algae were applied as biofertilizer for the soil treatment at the rate of 1 grams powdered algae/kg soil, seven days before planting and twice water daily.

2.7. Nutrient analysis of algal biomass

2.7.1. Organic Carbon

The organic carbon content in the dried biomass was checked by using the complete combustion of the biomass

2.7.2. Nitrate and Phosphate

Nutrients from the algal biomass were extracted using various solvents such as 1:1 HCl, ethanol and acetone. For the extraction process, 1 gram of biomass is dissolved in 100 ml of each solvents and heat for 5 minutes on hot plate. Then the solution was filtered through whatman filter paper no.1. The nitrate and phosphate content of biomass was determined using standard methods after heating with the 1:100 dilutions for biofertilizer and combusted biofertilizer.

3. Results and Discussion:

3.1. Physicochemical characteristics of water

Different water samples were collected from effluent treatment plant for the feasibility study of various parameters as shown in **Table.1**.

Table.1: Comparison of pollution load before and after treatment in Nitrate effluent with raw water using merged Algae.

Parameters	Raw water	Nitrate wastewater	Phycoremediated water	% change	
				%Increase	%Decrease
Physical					
Color	Transparent	Light Green	Dark Green	-	-
Odour	No odour	Muddy	Manure	-	-
pH	7.2	6	8.63	30.47	-
TSS	15	584	52	-	91.09
Chemical					
Free ammonia (ppm)	0.1	22	N.T	-	-
TAN (ppm)	0	0.074	N.T	-	-
Nitrate(ppm)	1	3122	14	-	99.55
M-Alkalinity (ppm)	18	20	120	83.33	-
Turbidity (NTU)	0.8	60	18	-	70
Conductivity (µs or ms)	93 µs	3.56 ms	4.8 ms	25.83	-
Total Hardness (ppm)	30	305	196	-	35.73
Ca-Hardness (ppm)	17	186	152	-	18.27
Mg-Hardness (ppm)	16	124	75	-	39.51
Dissolved Oxygen (ppm)	5	0.335	7.3	95.41	-
B.O.D (ppm)	4	286	62	-	78.32
C.O.D (ppm)	8	655	128	-	80.45
Phosphate (ppm)	0	15.884	1.4561	-	90.83
Iron (ppm)	0.08	1.183	0.561	-	52.57

Phycoremediation of Nitrate waste water increased pH and Dissolved Oxygen by 30.47% and 95.41% while retarded Nitrate, Phosphate, B.O.D, C.O.D and TSS respectively.

Comparision of parameters for Nitrate and Phycoremediated water was done for further experimental study as shown in **Fig.5**

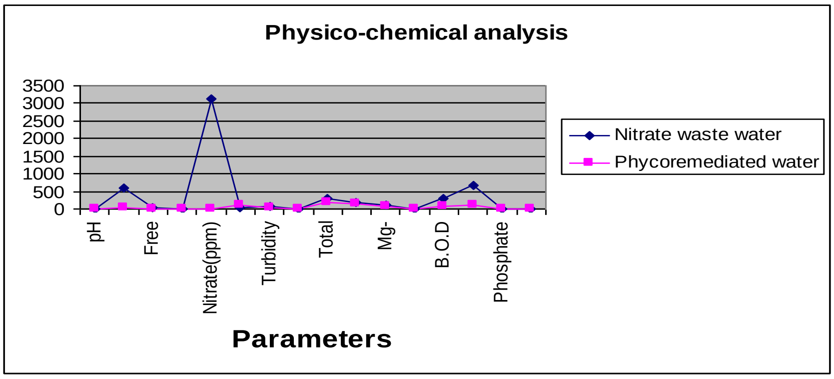

Figure 5: Comparative study of Nitrate and Phycoremediated water.

3.2. Algal Growth curve for effluent and synthetic media:

Chlorophyll content reached up to the maximum value of absorbance i.e. 0.328 nm in Nitrate effluent while 0.281 nm was recorded for synthetic media after 7 days incubation period under sunlight (**Table.2**). Growth rate and curve of Algae in terms of absorbance in both nutrient media is as shown in **Table.3** and **Fig.6**.

Table.2: Absorbance of Algae cultivated in Nitrate effluent and Synthetic media.

Days	Chlorophyll content (Absorbance)	
	Nitrate Effluent	Synthetic media
1	0.144	0.008
2	0.185	0.013
3	0.197	0.015
4	0.254	0.134
5	0.289	0.171
6	0.316	0.231
7	0.328	0.281

Table.3: Algal Growth Rate determination

Days	Growth Rate (GR)	
	Nitrate Effluent	Synthetic media
1	0	0
2	0.2505	0.4855
3	0.0628	0.1431
4	0.2541	2.1897
5	0.1290	0.2438
6	0.0893	0.3007
7	0.0372	0.1959

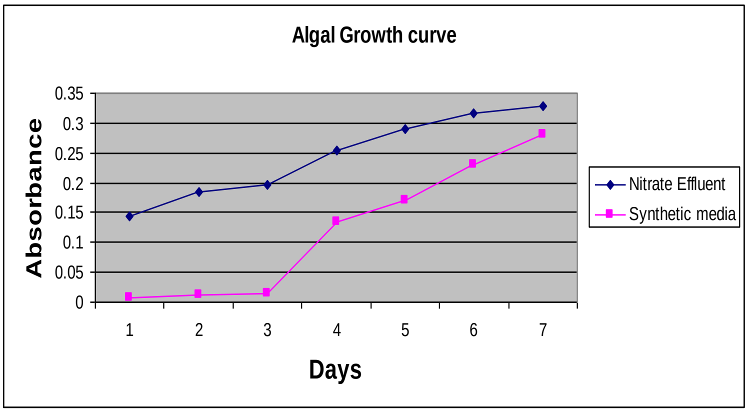

Figure 6: Growth Rate Curve of Algae in Nutrient Effluent and Synthetic media.

The growth curve revealed that Nitrate waste water acted as a stimulatory agent for algal growth, this may be due to the presence of available nitrogenous compounds, organic matter and phosphates.

3.3. Efficiency of algae for absorption of pollutants in nitrate waste water.

As the day's increases, the removal efficiency of various pollutants also increases.

3.3.1. Nitrate and Phosphate Removal.

Data in **Table.4** showed that the maximum removal efficiency of Nitrate Nitrogen and Phosphate was achieved by 99.55% and 90.83% at the end of incubation period (30 days). Significant reduction in Nitrate and Phosphate indicates that algae consumed Nitrogen in the form of Nitrate and also Phosphate as its food source.

Table.4: Percentage Removal of Nitrate and Phosphate in Wastewater after 30 days of treatment.

Days	Nitrate (ppm)		Phosphate (ppm)	
	Concentration (ppm)	% Removal	Concentration (ppm)	% Removal
1	3122	0	15.884	0
2	2455	21.36	15.6219	1.65
3	2001	35.90	14.0091	11.80
4	1631	47.75	14	11.86
5	1128	63.86	13.921	12.35
6	988	68.35	13.6215	14.24
7	933	70.11	12.1253	23.66
8	858	72.51	12.0067	24.41
9	743	76.20	11.0056	30.71
10	717	77.03	10.9821	30.86
11	662	78.79	10.2252	35.62
12	522	83.27	10.1073	36.36
13	389	87.54	9.8976	37.68
14	292	90.64	8.6966	45.24
15	231	92.60	8.1005	49.00
16	175	94.39	7.885	50.35
17	118	96.22	7.7749	51.05
18	85	97.27	7.1231	55.15
19	72	97.69	6.0023	62.21
20	61	98.04	6.0011	62.21
21	47	98.49	5.9919	62.27
22	33	98.94	5.7912	63.54
23	30	99.03	4.4523	71.96
24	28	99.10	4.0021	74.80
25	25	99.19	3.929	75.26
26	21	99.32	3.634	77.12
27	18	99.42	3.2222	79.71
28	17	99.45	2.959	81.37
29	15	99.51	2.5671	83.83
30	14	99.55	1.4561	90.83

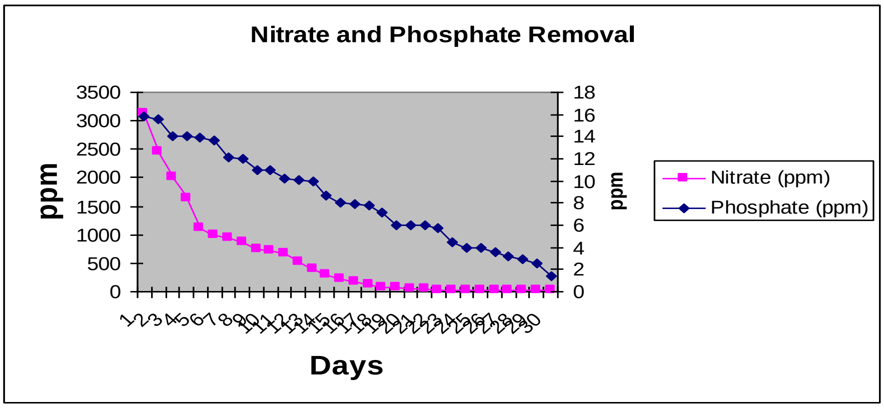

Figure 7: Nitrate and Phosphate removal efficiency.

3.3.2. Reduction of B.O.D and C.O.D with increase in D.O.

Initial D.O level was 0.335 ppm and it increased to 2.75 ppm. B.O.D and C.O.D was recorded 286 ppm and 655 ppm respectively before treatment, after treatment it was reduced by 78.32% and 80.45% respectively was found to be 62 ppm and 128 ppm (**Table.5**).

Table.5: Removal of B.O.D and C.O.D with changes in D.O

Days	B.O.D Concentration (ppm)	B.O.D % Removal	C.O.D Concentration (ppm)	C.O.D % Removal	D.O Concentration (ppm)	D.O % Increase
2	286	0	655	0	0.335	0
4	261	8.74	571	12.82	0.85	60.58
6	244	14.68	508	22.44	1.27	73.62
8	230	19.58	485	25.95	2.9	88.44
10	225	21.32	411	37.25	3.03	88.94
12	215	24.82	363	44.58	3.76	91.09
14	198	30.76	301	54.04	4.55	92.63
16	185	35.31	258	60.61	4.98	93.27
18	171	40.20	221	66.25	5.22	93.58
20	153	46.50	202	69.16	5.67	94.09
22	130	54.54	188	71.29	6.04	94.45
24	107	62.58	172	73.74	6.34	94.71
26	83	70.97	158	75.87	6.88	95.13
28	70	75.52	139	78.77	7.09	95.27
30	62	78.32	128	80.45	7.3	95.41

As per the **Fig.8**, Algae photosynthesis produces a very high oxygen concentration in the water which increases the biodegradation of organic matter and release of CO_2 is consumed by algae for their growth as a carbon source.

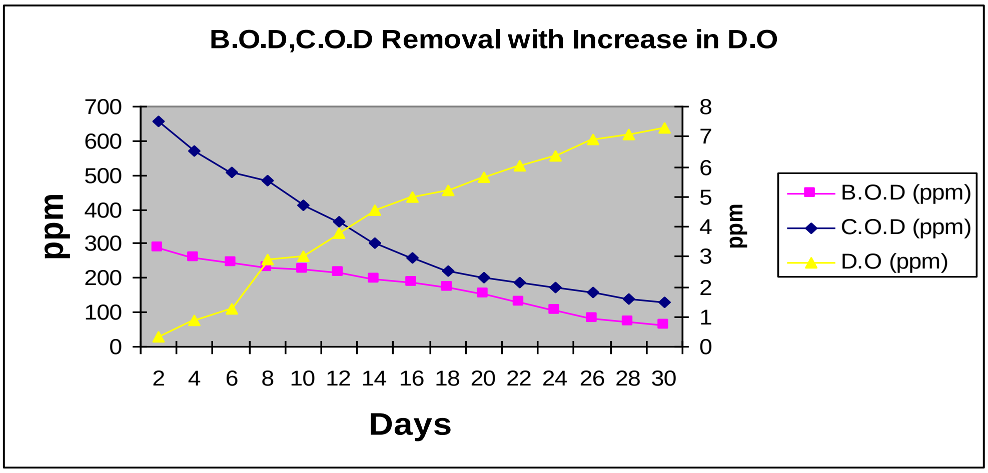

Figure 8: B.O.D, C.O.D Reduction with D.O Increment

3.3.3. Relationship between Turbidity and TSS.

Turbidity is closely related to TSS and is most often used to estimate the TSS (ppm). High concentration of suspended solids can lower water quality by absorbing light. Turbidity in water is caused by suspended matter such as clay, slit, organic matter and other microscopic organisms that interfere with the passages of light through the water.

Table.6: Comparative relation of Turbidity and TSS.

Days	Turbidity (NTU)	TSS (ppm)
1	60	584
2	51	408
3	42.1	363
4	35	240
5	27.1	153
6	22.3	91
7	18	52

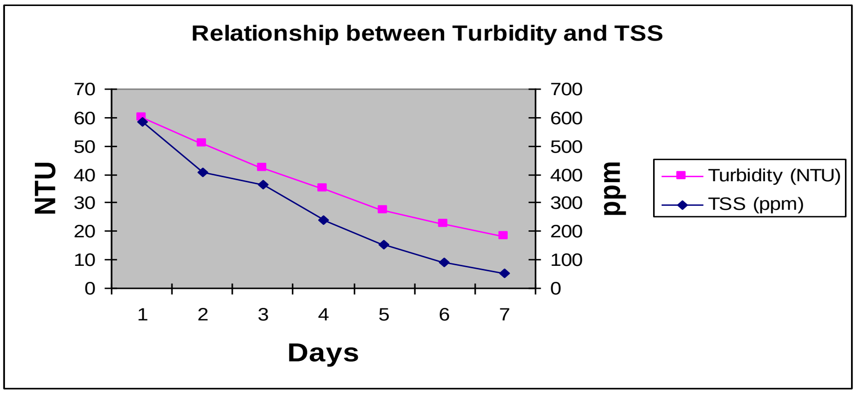

Figure 9: Relationship between Turbidity and TSS

3.3.4. Variations in pH and M-Alkalinity.

pH range for Nitrate containing water is 6.5-7.5 (Acidic) and algal biomass increases due to Nitrate consumption which leads to basic condition i.e. pH up to 8.63 and in turn M-Alkalinity increases as shown in **Fig.10**

Table.7: Change in pH during 7 days of treatment

Days	pH	M-Alkalinity
1	6.75	20
2	6.98	37
3	7.2	49
4	7.5	64
5	8.01	85
6	8.22	93
7	8.63	120

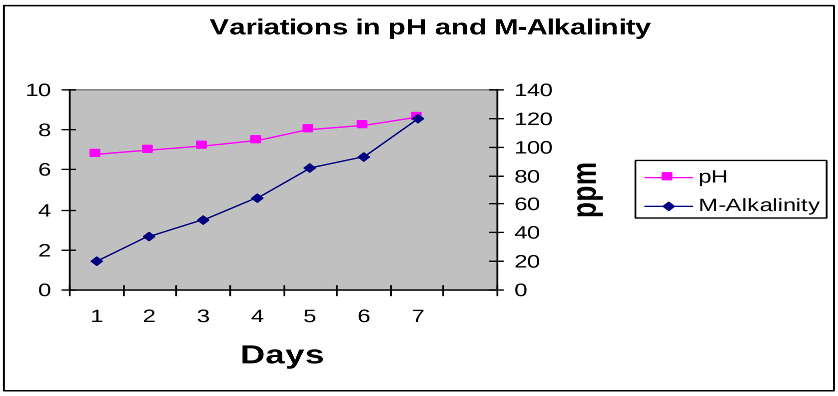

Figure 10: Variations in pH and M-Alkalinity

3.4 Biomass Processing:
Algal biomass was dried under shadow condition after harvesting and were packed into polythene bags for further nutrient analysis test.

Figure 11: Algal Biomass grown on Nitrate Wastewater

Wet Biomass **Dried Biomass**

Figure 12: Processing of Biomass

3.5. Nutrient Analysis:

3.5.1. Organic Carbon:

Carbon content= weight before combustion – weight after combustion

= (1-0.774) grams

=0.226-gram C-content/ 1 gram of Biofertilizer

Thus, 22.6 grams of organic carbon was found in 100 grams of Biofertlizer.

3.5.2. Nitrate and Phosphate:

Presently algae biomass is rich in nutrients, as the algae consumed nutrients such as Nitrate, Phosphate from deammonified effluent. Extraction of these nutrients was possible using different solvents for the confirmation of N and P contents required for plant growth. The high amount of N and P was extracted by using 1:1 HCl i.e. 2270000 ppm and 2989 ppm respectively.

Table.8: Nutrient Analysis Test

Biofertilizer Sample	**Nitrate content (ppm)**	**Phosphate content (ppm)**
Biofertilizer dissolved in 1:1 HCl (Set 1)	2270000	2984
Biofertilizer dissolved in 1:1 HCl (Set 2)	524000	10000

Percentage calculation:

Set 1

Nitrate = 227%, Phosphate = 0.2984 %, Organic Matter (Carbon)=22.6 %

Set 2

Nitrate = 52.4%, Phosphate = 1%, Organic Matter (Carbon) =46.6%

3.6. Effect of the physicochemical parameters on cooling tower and on concrete:

3.6.1. Impacts of effluent on cooling tower:

The pH of the water used for the cooling tower should be neutral. If it is alkaline then carbonate and bicarbonate are formed, it becomes hard water. It solubility decreases and gets deposited. In case of the acidic effluent the sulphate is formed and it causes corrosion. Temperature of the effluent should be normal. High concentration of total dissolved solids (T-hard, Mg-hard, Ca-hard, conductivity, PO_4^{2-}) increases the conductivity. Inorganic salts dissolved in water may increase the possibility of the corrosion. If nitrate content is more in water, then the acidity of water increases and pH decreases which results into the corrosion. Higher rate of BOD in water causes biological fouling that is the biofilm formation which leads to MIC (Microbiologically Influenced Corrosion). Deposition of organic matter occurs due to COD and causes fouling and corrosion. Higher PO_4^{2-} concentration in water helps the algal growth and may damage wood and tower.

3.6.2. Impacts of effluent on concrete:

BOD, COD and total suspended solids basically affects hydration process of the concrete. The effect of BOD on concrete compressive strength and tensile strength is during hydration process. It basically effects the chemical reaction of the main cement constituents such as Di-calcium Silicate (C_2S), Tri-calcium Silicate (C_3S), Tri-calcium Aluminate (C_3A), Tetra-calcium Alumino-ferrite (C_4AF). Also this leads to the formation of pores in concrete matrix which forms the weaker bond between aggregate and cement paste, thus decreasing the durability and resistance of the concrete. Aslo the COD and TSS decreases the strength of the cement.

3.6.3. Utilization of Treated water for Cooling Tower and Concrete Mixing:

Treated waste water overcomes all the above limitations of different parameters and contents which basically meet the raw water standards. (**Table 1**)

4. Conclusion:

The present study can open an opportunity to fully utilize the advanced waste water based algal biofertilizer production technology as a dual purpose of removing pollutants and producing biofertilizer at an economically viable feedstock. The estimated 22.6 grams of organic carbon may influence nutrient holding capacity, nutrient turnover and improves soil stability by binding of microorganisms and therefore, facilitates healthier plant life preventing water pollution as well as eliminating the need of synthetic fertilizers. The analyzed amount of nitrate and phosphate can be better nutrients supplements for the plant growth in proportional N: P ratio (3:2). On the other hand, this Phycoremediation technology has proved its importance in Industrial Wastewater Treatment as per the Maharashtra Pollution Control Board (MPCB) limits which can be reutilized in industry to overcome water shortage.

5. Discussion:

In upcoming future, Wastewater purification through algae will prove itself, because this treatment costs less for the removal of pollutants and toxicity purification from waste water that prevents the pollution as compared to different chemical waste water purification. The water purified by using phycoremediation and the algal biofertilizer can viably used by farmers to solve the problems faced by the farmers nowadays. The algal purified water can be used as their raw material for manufacturing in industries such as concrete, fermentation. Combining algae for waste water remediation with biofuel production, methane, ethane forming industries, electricity generation as a renewable energy source that can also be one of the additional benefits of the wastewater treatment and feed for animal and fish which may be economically feasible processes. The waste water treatment plants are an invaluable source of algae as a feedstock which may be in use for various purposes.

The phycoremediated water can also be utilized in various purposes and the cultivated algal biomass can be supplied as a suplementary of Biofertilizer for the crops as this will be cost effective. Being cheap and cost effectiveness biofertiizer can easily be adopted by farmers as well as common people because it requires inexpensive technique for production process and therefore may be preferable. Considering all this ability of algae to purify polluted water, It is worthwhile to emphasize that algal technology in waste water treatment system are expected to be even more common to use in near future.

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