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Phytoremediation by Duckweed in Municipal wastewater Treatment

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Abstract: Water pollution and the growing share of wastewater released into water bodies increased stress on water environment.. Proper environmental-friendly and sustainable techniques are important for treatment of these water resources. Phytoremediation in water deals with the application of aquatic plants for the remediation of pollutants in water resources or aquatic ecosystems. In this work, efforts were made to remove pollution load of municipal wastewater by using free-floating macrophytes like *Spirodela polyrhiza* and *Lemna minor*. Both were reported as very successful duckweeds for the phytoremediation and their low cost, long storage capacity, minimal chemical and biological sludge volume. Also, their ease of transport, ability to grow under different climatic conditions and fast reproduction rates are additional advantages. The interesting results obtained were percentage reduction in COD (82.2% and 68.8%), BOD (84.3% and 63.8%), phosphate (63.8% and 59.02%,), ammonia nitrogen (75.4% and 55.9%) and nitrate (79.1% and 55.4%*)* for *Lemna minor and Spirodela polyrizha* respectively. The maximum growth of duckweeds was during the experimental period in the pH range 7-8. The study showed that this treatment can be successfully carried out on a large scale by using *Lemna minor* as the phytoremediation agent for about 17days as an effective, low-cost tertiary treatment for heavy loaded municipal wastewater. Also, it could be given as a stand-alone treatment with preliminary facilities, in small villages.

Index Terms – Phytoremediation, duckweeds, *Spirodela polyrhiza*, *Lemna minor*, Biological treatment, Nutrient removal

I. INTRODUCTION

Globally, the water crisis is one of the most serious problems faced by the world today. According to the report of the World Health Organization of 2014, it is estimated that by 2025 more than half of the world's population will be facing water scarcity. The deterioration in the quality of available water due to the pollution and degradation of the environment results in this scarcity of water. The National Institution for Transforming India (NITI Ayog) of Government of India has released report 'Composite Water Management Index' in June 2018 which listed Delhi and other 21 cities in India that would run out of groundwater by 2020. The increasing strain on the surface water is due to untreated or partially treated wastewater entering the main streams.

Phytoremediation is an emerging technology that is bioremediation with the application of plants for the remediation of the polluted environment. Phytoremediation is such a method where the ability of plants to survive in polluted water and to facilitate pollutants removal from the environment is exploited [14]. It is also an aesthetically pleasing technique that is cost-effective, conserves environment in-situ [8]. The duckweeds are invasive floating aquatic macrophytes with ecological and economic implications wherever the colony of the plant exists. Duckweeds are small, simply constructed aquatic plants or macrophytes that float on the surface of quiet bodies of water [15]. The duckweed vegetative body, or frond, is a thallus-like structure of only a few cells in thickness that represents a fusion of leaves and stems and thus the extreme reduction of an entire vascular plant. The duckweeds constitute the family Lemnaceae that consists of 37 species distributed among 5 genera [1][15].

The giant duckweed i.e., *Spirodela polyrrhiza* characterized by their widespread availability, and potential to grow at wide temperature range is the most commonly accepted wetland plant for the phytoremediation. On the other hand, *L.minor* has been reported as a very successful floating macrophyte for the phytoremediation of organic pollutants.

Duckweed has the capability of purify wastewater in collaboration with both aerobic and anoxic bacteria. The duckweed mat, which covers the water surface, results in two zones. In aerobic zone, organic materials are oxidized by aerobic bacteria using atmospheric oxygen transferred by duckweed roots. Nitrification and denitrification takes place in anoxic zones, where organic nitrogen is decomposed by anoxic bacteria into ammonium and ortho-phosphate, which immediate products used as nutrients by the duckweeds [12].

II. MATERIALS AND METHODS

2.1 Sample and material collection

The municipal wastewater was collected from the primary settling tank of Elamkulam Sewage Treatment Plant, which works under the Kerala Water Authority, near Kadavanthara in Kochi Corporation. The phytoremediation agents, *Spirodela polyrhiza* and *Lemna minor* were collected from a garden center in Thrissur.

2.2 Species identification

The collected duckweeds were confirmed by species identification from the book of 'Flowering plants of Kerala: a checklist', Kerala forest research institute, Peechi. *Lemna minor* and *Spirodela polyrizha* were from the family of Lemnaceae. Individual plants of Lemna minor consist of single, flat oval leaf, not more than ¼ inch long that floats on the surface of still-moving ponds or lakes. The inflorescence, consists of two microscopic staminate flowers and one yiny pistillate flower in a pouch-like sag, mostly unseen. They grow quickly and proguce offshoots rapidly. The description of *Spirodela polyrizha* was the fronds solitary or cohering in groups of 2 or 5, symmetric or asymmetric, orbicular-ovate, base obtuse, apex obtuse or rounded, upper side- flat, smooth and green, lower side- flat, red to purple. Daughter fronts budding from near the point of root intersection in a slit in the parent frond. Flowers surrounded by a small open spathe in lateral dilt- pouch with one pistillte flower and 2-3 staminate flower each consisting of a single stamen.

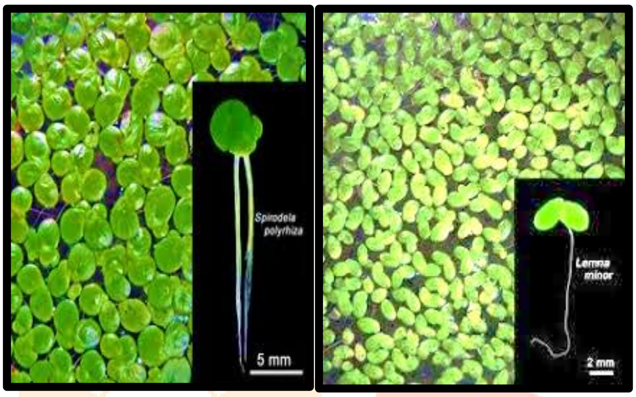

Figure 1 *Spirodela polyrhiza* (great duckweed) and *Lemna minor* (common duckweed)[6]

2.3 Experimental study

Following the identification, the duckweeds were washed with deionized water and made ready for use. Experiments were carried out at ambient temperature around 30°C. The system consist of a trays of 1440 cm^2 surface area and an effective volume of 5 litres. The key operational parameters investigate for their influence on COD, BOD, nitrate, ammonia nitrogen and phosphate removal efficiencies were hydraulic retention time, pH and biomass dosage. The samples were taken out at 7^{th}, 16^{th} and 25^{th} day and filtered through Whatman filter No.1 for the chemical analysis to determine optimum results [8].

III. RESULTS AND DISCUSSIONS

3.1 Characteristics of sample

The municipal wastewater sample was mainly analysed for pH, total dissolved solids (TDS), BOD, COD, nitrate, ammonia nitrogen and phosphate. Various analytical methods have been used for the analysis based on APHA standards. The nitrate, ammonia nitrogen and phosphate were analysed using UV- Visible spectrophotometer. The physicochemical characteristics of the sample were listed in the table. Then the synthetic sample was prepared based on the characteristics obtained from original wastewater. The components used were found by trial and error method and found as peptone, beef extract, urea, potassium dihydrogen orthophosphate, potassium nitrate, ammonium chloride, D Glucose and cow dung.

Table 1 Characterization of wastewater

Parameter	Unit	Value
pH	-	7.04
TDS	mg/L	746
COD	mg/L	927.5
BOD	mg/L	317.98
Nitrate	mg/L	36
Phosphate	mg/L	14.4
Ammonia nitrogen	mg/L	16

3.2 Process optimization

Optimizing is the process which is done to obtain maximum benefit from the system. Thus the main objective of the optimization was to determine the optimum values of the key parameters pH, hydraulic retention time and biomass dosage for maximum removal of nitrate, ammonia nitrogen, phosphate, BOD and COD. The process was done with the help of one of the most relevant multivariate techniques by using RSM. Response Surface Methodology (RSM), is a collection of mathematical and statistical techniques used for developing, improving and optimizing processes. It is employed for multiple regression analysis to solve multivariate equations simultaneously. RSM is an efficient statistical tool for optimizing multifaceted processes. RSM minimizes the number of experimental trials required to evaluate several parameters and their interactions. The experiments were established based on a Box- Behnken design[10]. Here, the total number of experimental runs need to be performed was obtained as 15 for both the species. The experiments were conducted in ambient temperature.

The optimization plot shows the effect of each factor (columns) on the responses or composite desirability (rows). The numbers displayed at the top of a column shows the optimized values of factors (in red). The horizontal blue lines and numbers represent the responses for the optimized conditions. The figures show the optimization plots for % removal Lemna minor and Spirodela polyrizha respectively.

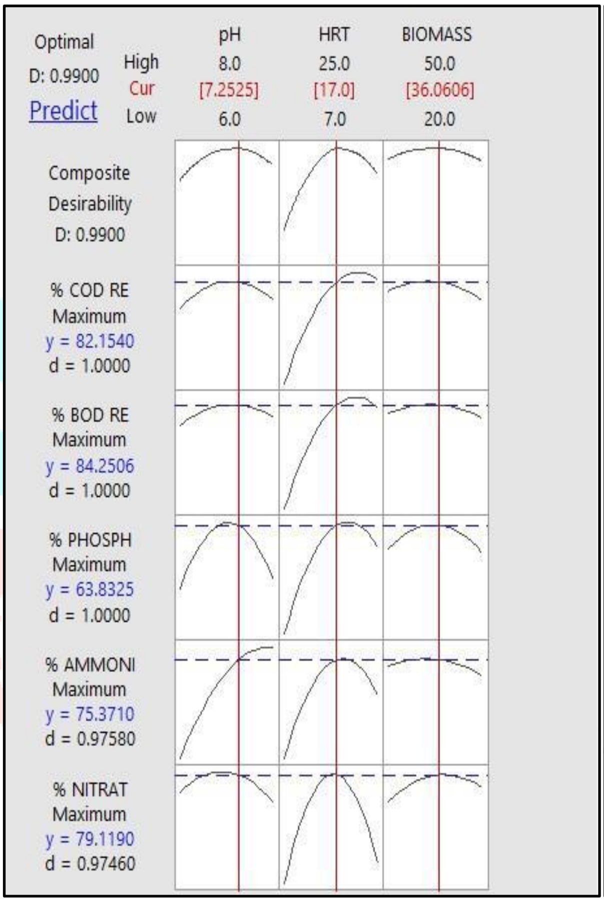

Figure 2 Optimization plot of *Lemna minor*

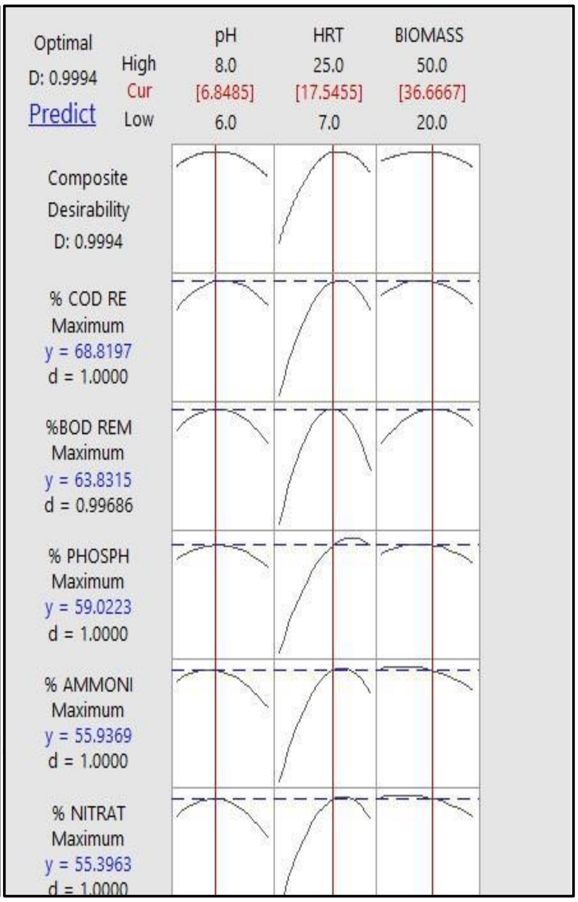

Figure 3 Optimization plot of *Spirodela polyrizha*

The optimized values of the variables from 15 trails obtained from RSM were summarized in the table 2.

Table 2 The optimized values of process variables

Variables	Optimum value	
	Lemna minor	*Spirodela polyrizha*
pH	7.25	6.85
HRT(days)	17	17.5
Biomass Dosage(g/5L)	36.06	36.67

3.3 Effect of *Lemna minor* in phytoremediation

The result of characterization before and after phytoremediation of municipal wastewater at optimum conditions were tabulated. The removal efficiencies of nitrate, ammonia nitrogen, phosphate, BOD and COD were 79%, 75%, 63%, 84% and 82% respectively. The final values for most of the parameters were not in the limit, but a small pretreatment could be given for the municipal wastewater so that the performance of the unit will be more satisfactory.

Table 3 Performance of *Lemna minor*

Parameter	Initial value (mg/L)	Final value (mg/L)	Removal Efficiency (%)	Effluent Standards* (mg/L)
Nitrate	65.26	13.62	79.12	45
Ammonia nitrogen	20.73	5.10	75.37	5
Phosphate	16.53	5.97	63.83	4
BOD	303.45	47.79	84.25	30
COD	880	157.08	82.15	50

*CPCB enforced effluent discharge standards for STP

3.4 Effect of *Spirodela polyrizha* in phytoremediation

The removal efficiencies of nitrate, ammonia nitrogen, phosphate, BOD and COD were obtained as 55%, 56%, 59%, 64% and 69% respectively which is tabulated. Here also the parameters were not within the limit.

Table 4 Performance of *Spirodela polyrizha*

Parameter	Initial value (mg/L)	Final value (mg/L)	Removal Efficiency (%)	Effluent Standards* (mg/L)
Nitrate	65.26	29.11	55.39	45
Ammonia nitrogen	20.73	9.13	55.94	5
Phosphate	16.53	6.77	59.02	2
BOD	303.45	109.75	63.83	30
COD	880	274.38	68.82	50

*CPCB enforced effluent discharge standards for STP

The result shows that, only nitrate met the EPA discharge limit but rest of the parameters shows good removal efficiencies with *L. minor.* So, clearly it can be inferred that, the use of *L. minor* for municipal wastewater treatment is a better option in rural areas or small villages.

IV. CONCLUSIONS

Wastewater treatment using macrophytes have good popularity due to its efficient mechanism in bioremediation and is an eco-friendly approach. Duckweeds were considered as a promising macrophyte as they show rapid growth and proven history of remediating ponds and many wastewaters. In the present study, phytoremediation of municipal wastewater was carried out using two different duckweeds, *Lemna minor* and *Spirodela polyrizha.* Since both were from same family, the factors affecting the remediation process were set same and the effect of such factors like pH, retention time and biomass dosage of the duckweed were assessed in both cases and were optimized using RSM in MINITAB. The results conveyed that all the factors affect the process performance significantly for both the duckweeds. In the case of *L. minor* and *Sp. polyrizha*, the model showed high R2 values conveying a good accuracy for both the models.

Thus, this intensive study on both the species showed a considerable reduction in COD, BOD, phosphate, ammonia nitrogen and nitrate. The mechanism behind the removal of BOD and COD was phytodegradation and the nutrient uptake using roots and fronds of the duckweed by phytoextraction, nitrification and denitrification would be the reason behind the nitrate and ammonia nitrogen reduction. The phosphate removal mechanisms may be absorption and phytoextraction. While observing the percentage removal efficiencies of each parameter, it was very clear that, *L. minor* shows more dominant effect than *Sp. polyrizha* in municipal wastewater treatment in the optimized condition. The percentage removal obtained using *L. minor* were 82.2%, 84.3%, 63.8%, 75.4% and 79.1% for COD, BOD, phosphate, ammonia nitrogen and nitrate respectively whereas by using *Sp. polyrizha* the obtained percentage removal were 68.82%, 63.83%, 59.02%, 55.94% and 55.39% respectively.

The result shows that, only nitrate met the EPA discharge limit. So, we cannot fully depend on phytoremediation when the pollutant concentration is high. Hence, *L. minor* could be used after primary and secondary treatment when the pollutant concentration is high and could give a maximum output in 17 days in an optimum pH of 7.3. Also, the local availability of the L. minor makes it a low-cost solution to water treatment problems. Because of the high percentage removal, it could be suggested as a suitable solution for raw wastewater as a stand-alone treatment with preliminary facilities, for grit settling, in small villages.

V. ACKNOWLEDGMENT

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