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REMOVAL OF EXCESSIVE NITROGEN AND PHOSPHORUS FROM URBAN WASTEWATER USING LOCAL MICROALGAL BLOOM

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ABSTRACT

The organic content from urban wastewater is treated with various conventional processes efficiently. However, for biological treatment of secondary effluent containing excessive inorganic nitrogen and phosphorus, microalgae can be used. In this study, algal strains have been collected from locally available natural blooms and cultured in a BG-11 medium. Spirulina sp., the blue-green algae, dominant over the other species within the natural bloom, is applied in ten different dosages (0.2-2.5 g/L) to the synthetic wastewater with a 3-day hydraulic retention time. The removal efficiency of nitrate, ammonia, and phosphate have been observed to be about 60%, 30%, and 54% respectively. The highest removal efficiency has been found at 2.5 g/L of microalgae dose. Linear forms of Langmuir and Freundlich isotherms have been used for biosorption modeling, and both isotherms fit well with R^2>60% and NRMSE<11% in all cases. Additionally, the separation factor and the adsorption intensity represent the favorability of the biosorption process.

Keywords:*Biosorption; Microalgae; Urban wastewater; Wastewater treatment.*

1. INTRODUCTION

Water is a fundamental necessity for life. Every metabolism inside a body depends on water. However, only 2.66% of the total water resource is freshwater. For this reason, the conservation of freshwater sources has become a priority throughout the world. The qualitative and quantitative threats to water resources have been imposed due to man-made pollution. The urban municipal wastewater generally originated from domestic and community uses. An urban city having a population of 5,00,000 and water consumption of 0.2 $td^{-1}capita^{-1}$, produces 85,000 td^{-1} of wastewater approximately(Cai *et al.* 2013).The inorganic component of municipal wastewater contributes greatly to the accumulation of nitrogen and phosphorus in receiving water bodies. The source of nitrogen and phosphorus in municipal wastewater is various household activities (Abdel-Raouf *et al.* 2012).About 30-50% of the phosphorus originated from human wastes such as feces, urine, and waste food. The remaining phosphorus content (about 50-70%) comes from Detergents which are used for laundering of clothes (Barth *et al.* 1976).Initiating these pollutants into the environment without proper treatment has a significant negative impact on the aquatic ecosystem (Mennaa *et al.* 2015).Eutrophication is a common phenomenon causes due to an excess amount of nitrogen and phosphorus. Due to eutrophication, the growth of algae and higher forms of plants are accelerated. As a result of eutrophication, oxygen depletion and toxic effect occurs in the receiving water body along with several adverse ecological impacts and decreased lifespan of aquatic organisms (Henze *et al.* 2001). The water quality degrades gradually (Commission 2002).The area of surface water polluted with algal bloom is not suitable for uses such as drinking, irrigation, industry, recreation, or fishing (Carpenter *et al.* 1998).The toxic effect may enter into the food chain and causes various kind of diseases to human.

Aside from the imbalance in the aquatic ecosystem, humans and animals who depend on the water of receiving water bodies are facing a greater threat to their health. Exposure to an excess amount of Nitrate may cause gastric cancer, impose threats to the newborn child and pregnant ladies, and changes the composition of hemoglobin which is responsible for methemoglobinemia or blue baby syndrome in an infant (Ghafari *et al.* 2008, Mayo and Hanai 2014). Due to blue baby syndrome, respiratory problems, digestive problems like diarrhea, vomiting, and in extreme cases even death in young children may occur. Nitrate also has carcinogenic, teratogenic, and mutagenic properties (Abel 2014). Ammonia and Phosphorus donot have any direct effect on humans or animals. The necessity of removing them from municipal wastewater is protecting the food chain (Klaassen and Amdur 2013).

However, it is very difficult to find a solution for the treatment and safe discharge of wastewater. Since the solution involves integrated processes, the technical, economic, and financial issues must be considered. Activate sludge process is a very popular conventional method for wastewater treatment. This process shows ahigher removal of biodegradable material by using bacteria in primary and secondary treatments of effluent. This apparently clean

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secondary treated effluent contains a large number of inorganic compounds, like nitrogen and phosphorus. The disposal of a large volume of sludge is also troublesome and may lead to secondary pollution (Olguín 2012). The total cost of wastewater treatment increases for each additional step. For removing nitrates, ammonia, and phosphate from wastewater completely, a tertiary treatment process costs four times more than the primary treatment process. Lastly, the conventional treatment processes lead to incomplete utilization of natural resources (Molinos-Senante *et al.* 2010, Abdel-Raouf *et al.* 2012).

On the other hand, the capacity of microalgae for inorganic nutrient uptake is very high (Talbot and De la Noüe 1993, Blier *et al.* 1995). Numerous studies have shown that treating the wastewater with microalgae can decrease the nutrient concentrations up to 95% (Arbib *et al.* 2014, Gao *et al.* 2016, Lavrinovičs and Juhna 2017, Wang *et al.* 2017). Therefore, as a potential alternative, wastewater treatment by using microalgae has been proposed as a tertiary treatment process (Olguín 2003, Sturm and Lamer 2011). Biological removal of nutrients using microalgae offers several advantages over tertiary chemical and physicochemical treatments (Proulx and De la Noue 1988, De la Noüe and Basseres 1989). Algal treatment is favorable because of the photosynthesis characteristics of algae. During photosynthesis, solar energy converts into useful biomasses by combining nutrients such as nitrogen and phosphorus and carbon-di-oxide from the environment (Martínez *et al.* 2000, Cai *et al.* 2013). It is a cost-effective and environment-friendly method(Pittman *et al.* 2011). Microalgae also produce oxygen during the photosynthesis process and also have a disinfection effect due to elevated pH (Martínez *et al.* 2000). The main challenge for this technology lies in the wastewater composition, microalgae species, and the final utilization of biomass yield (Jais *et al.* 2017). The harvested microalgae can be used for the production of biofuel, fertilizers, various high-value products like Pharmaceuticals and genetically engineered products (Mallick 2002, Mennaa *et al.* 2015). These include antibacterial, antiviral, antitumor/ anticancer, antihistamine, and many other biologically valuable products (Borowitzka 2013). Blue-green algae are a prominent form of microalgae within the natural bloom in freshwater (Cai *et al.* 2013). They are also potential for the removal of inorganic nutrients from wastewater. It is also well established that blue-green algae are capable of reducing nitrate, ammonia and phosphate (Abdel-Raouf *et al.* 2012).

Nutrients from wastewater are generally uptake by algae for their cellular growth. For the growth of organisms, nitrogen is considered a critical nutrient. Within a cell of any organism, organic nitrogen is found in the form of biological substances. They are- peptides, proteins, enzymes, chlorophylls, energy transfer molecules (ADP, ATP), and genetic materials (RNA, DNA) (Barsanti and Gualtieri 2014). Microalgae convert inorganic nitrogen into organic nitrogen by the assimilation process. Eukaryotic algae perform the assimilation process by using nitrate, nitrite, and ammonium which are the forms of inorganic nitrogen (Cai *et al.* 2013). Inorganic phosphorus is also very important for microalgae growth and metabolism. It is found in nucleic acids, lipids, proteins, and the intermediates of carbohydrate metabolism. Phosphorus also helps to generation of ATP from adenosine diphosphate (ADP), accompanied by a form of energy input (Martinez *et al.* 1999, Wu *et al.* 2012, Beuckels *et al.* 2015, Whitton *et al.* 2015).

The efficiency of microalgae for removing nitrogen and phosphorus content from municipal wastewater has been shown in many studies. *Chlorella vulgaris* has an efficiency of removing 86% of inorganic nitrogen and 78% of inorganic phosphorus (Lau *et al.* 1997). About 97.8% of phosphorus is removed from domestic sewage by algae (Colak and Kaya 1988, Abdel-Raouf *et al.* 2012). Another study shows that *Chlorella vulgaris* can remove ammonia and nitrogen completely from the wastewater. Whereas, the removal rate of phosphate is about 78% (Aslan and Kapdan 2006). The strain of *Chlorella sorokiniana* has shown high efficiency of removing 31-62.2 % nitrate, 30.6-39.5 % phosphate,54.1-95.1 % ammonium (Saidu *et al.* 2017). About 30-100% of nitrate, ammonia, and phosphate can be removed by immobilized *Scenedesmus* sp. isolated from municipal wastewater in 21 days (Zhang *et al.* 2008).For *Natural bloom,* the efficiency is more than 87% and 80% for removing nitrogen and phosphorus respectively (Mennaa *et al.* 2015). *Spirulina maxima* show the removal of 87% nitrogen and 60% phosphorus at HRT of 4 days (Kosaric *et al.* 1974). Promising nutrient removal by *Spirulina plantensis* was also confirmed (Lodi *et al.* 2003).

The theory of wastewater treatment with microalgae had established around 50 years ago. In most cases, to ensure better wastewater treatment potential, the microalgal strains are chosen considering several criteria. The choice of microalgae depends on Wastewater's properties, the required magnitude of treatment efficiency, the cost, and energy requirement of biomass harvesting, and the application of the harvested biomass (Al-Jabri *et al.* 2021). These microalgae strains from any laboratory having a controlled atmosphere need high maintenance throughout the operation. Various environmental elements also have a significant impact on them. To minimize these effects, using microalgae from natural blooms in local fish ponds is a potential alternative.

2. METHODOLOGY

2.1 Natural-Bloom Culture

Microalgae sample for this study was collected from the natural bloom in pond by the side of university avenue, Shahjalal University of Science and Technology, Sylhet. The collected sample was primarily stored in filtered pond water in a 0.5 L plastic bottle.Numerous studies showed that the cell size of microalgae is ranged from 1.75 µm to 8 µm (Reynolds and Walsby 1975, M *et al.* 2013, Taghipour Heidari *et al.* 2018). As a result, in this study, Grade 2 qualitative filter paper with a pore size of 8 µm was used. Naturally grown microalgae may arise sporadically as water blooms in ponds. Generally, a large number of these natural blooms are consist of unicellular blue-green algae (Geitler 1932). For this reason, the BG-11 cultural medium has been selected to ensure the growth of microalgae. Medium BG-11 is neutral after sterilization. It supports the growth of the algal population in the air (Stanier *et al.* 1971). The pH of the BG-11 culture medium was maintained at 7.1 by using 1M NaOH. After adjusting the pH, the solution was autoclaved at 121°C for 15 min. The medium was cooled before adding the collected microalgae sample. The collected microalgae were separated from pond water by filtration. Then the filtrate was added to a sterilized BG-11 medium in three 1L beakers. Continuous air supply had maintained by using an air pump. A 16:8 hr light:dark cycle was maintained throughout the culture condition. pH had measured once a day and maintained as 7.1.

2.2 Growth Rate Measurement

A 10 mL sample from each batch has been collected daily at mid-day. Spectrophotometric analyses of the collected samples are done by using a UV spectrophotometer. Optical density has been measured from the absorbance of 680 nm wavelength. The optical density indicates the density of microalgae. Using the optical density (OD_{680}) the growth rate of microalgae is calculated by using the following exponential formula:

$$\text{Growth rate, GR (per day)} = \frac{\ln OD_t - \ln OD_0}{t}, \ldots\ldots\ldots \quad (1)$$

where OD_0 represents the optical density at the initial day and OD_t represents optical density measured at day t (Wang *et al.* 2010). After attaining a satisfactory growth rate after 18 days, the mass culture of microalgae has been started. For mass culture, the microalgae were moved from a 1L beaker to a large tank. The dimension of the tank is 60 cm × 20 cm. A total 10L BG-11 medium is used for mass culture.

2.3 Synthetic Wastewater Composition

Synthetic wastewater is chemically derived wastewater. For determining the chemical composition of the synthetic wastewater, wastewater samples have collected from seven random points. They were selected based on upstream and downstream of canals, residential areas, and reconnaissance surveys. However, the measured nutrient levels of Sylhet municipal wastewater as shown in Table 1 are significantly lower than the standard value. According to the environment conservation rules, 1997 the standards of nitrate, ammonia, and phosphate for inland water surface of Bangladesh are 10mg/L, 50 mg/L, and 35 mg/L respectively(Rules 1997). As a result, the acquired values had compared to those obtained from the literature review (Table 1). The highest values of nitrate, ammonia, and phosphate were selected for the chemical content of synthetic wastewater. For the chemical composition of synthetic wastewater, the highest nutrient content was selected from Table 1. The concentration of the nutrients in synthetic wastewater was set as 17mg/L, 100 mg/L, and 212 mg/L respectively.

Table 1: Nutrient Content of Wastewater

Nutrient	Samples from Sylhet City [mg/L]							Concentration in synthetic wastewater [mg/L]
	1	2	3	4	5	6	7	
Nitrate	3.4	2.8	4.3	1.2	1.1	0.4	0.5	17[a]
Ammonia	21.0	4.5	9.6	22.0	1.4	20.1	16.3	100[b]
Phosphate	5.3	3.5	4.5	5.7	0.9	5.4	1.3	212[b]

[a]Cho *et al.* (2011), [b](Zhou *et al.* 2012)

2.4 Experimental Setup

The experiments with synthetic wastewater were done on a laboratory scale. For each dose, three 1L solutions of synthetic wastewater were made. The hydraulic retention time (HRT) for all doses remained constant and it was 3 days. As a light source, two 23W fluorescent lights were used (Figure 1(a)). The fluorescent lights had provided 3600 lumens light intensity continuously for the photosynthesis process. To provide turbulence within the wastewater sample, a magnetic stirrer at 350 rpm was used (Figure 1(b)).

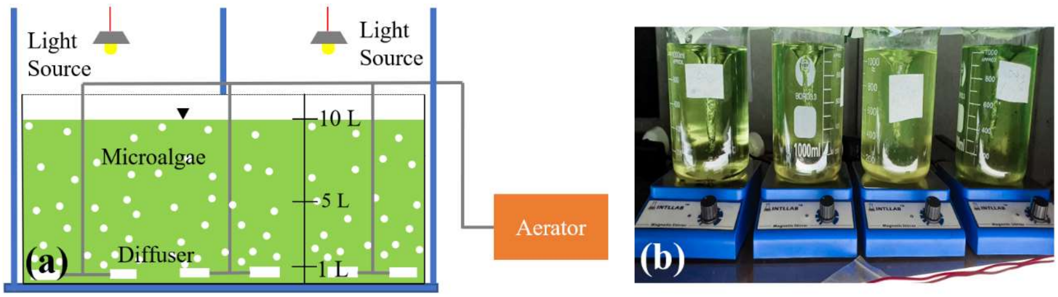

Figure 1: Panel (a) represents the schematic diagram of mass culture of microalgae using 60 cm × 20 cm tank and Panel (b) represents the experimental setup of synthetic wastewater treatment.

2.5 Biosorption Modelling

Nutrient removal by microalgae is a biosorption process. Biosorption is a physicochemical and metabolically independent process. The aim of biosorption modeling is experimental data analyses, understanding process mechanisms, prediction of operational condition changes, and optimizing processes (Fomina and Gadd 2014). For comparison among different types of biosorbents and their capacities of pollutant uptake, the biosorption process can be expressed as an equilibrium isotherm curve (Vijayaraghavan and Yun 2008). For simple single-component models, Langmuir and Freundlich's versions are used widely (Pagnanelli *et al.* 2002, Gadd 2009) Biosorption modeling helps to develop a design model for the removal of nutrients from wastewater more effectively and accurately. For biosorption modelling, Langmuir (Langmuir 1916) and Freundlich (Freundlich 1906) isotherm models were used in this study.

The initial and final concentrations of nutrients are measured using HACH DR 6000 UV- spectrophotometer. All HACH products were used according to the manufacturer's instructions. Percentage removal for each compound was calculated using equation 1 (Moris *et al.* 2018).

$$Q_e = \frac{(C_o - C_e)}{C_o} \times 100 \quad \text{.................. (2)}$$

The characteristic of the Langmuir isotherm is represented by the separation factor RL. RL is a dimensionless constant. R_L is a dimensionless constant.

$$R_L = \frac{1}{1 + K_L C_o} \quad \text{.................. (1)}$$

The value of R_L>1 represents the adsorption to be unfavorable. For R_L=1, the adsorption is linear. For 0< R_L<1, the adsorption is favorable. Lastly, for R_L =0, the adsorption is irreversible (Hamdaoui and Naffrechoux 2007).

The linear form of the Freundlich Isotherm Model (Hill 1946, Hamdaoui and Naffrechoux 2007, Boparai *et al.* 2011, Liu *et al.* 2019) for log Q_e vs. log C_e plot is:

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \quad \text{................. (2)}$$

Where, K_F represents the adsorption capacity $[\frac{L}{mg}]$ and $\frac{1}{n}$ represents the Adsorption intensity.

The strength of the used absorbent material is represented by the function $\frac{1}{n}$. The value $\frac{1}{n} > 1$ indicates favorable biosorption and $\frac{1}{n} < 1$ indicates poor condition (Aziz *et al.* 2004, Liu *et al.* 2019).

3. RESULT AND DISCUSSION

3.1 Growth rate and nutrient removal by *Spirulina sp.*

The growth rate of microalgae was measured in terms of OD_{680}. From the growth rate curve, the behavior of microalgae in the culture medium can be stated (Figure 3(a)). No lag phase was observed throughout the curve which indicated the good adaptation of microalgae in the culture medium. An exponential growth phase had observed after 8 days of culture. The exponential growth phase remained for 1 day, and thereafter a comparatively stationary growth phase had achieved.

After reaching the constant growth rate, the collected microalgae had cultured in the glass tank of Figure 1(a) for mass production. After two months of culture, the colonies were observed under a microscope and photographed with a lens of 40X magnification for taxonomic identification. The dominant algae found in the culture was *Spirulina sp.*(Figure 2). The *Spirulina sp.* were identified taxonomically according to Ciferri and Tomasseli (Ciferri 1983, Tomaselli 1997, Rout 2013).

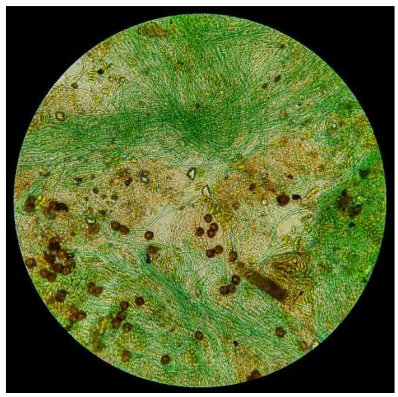

Figure 2: Microscopic Photograph of *Spirulina* sp. (Magnification 40X)

Removal of nitrate, ammonia and phosphate from municipal wastewater is a vital issue for saving the natural water source from pollution. This study was conducted to reducethe maximum amount of nutrient enrichments within 3 days of HRT. A total of ten different algal doses i.e. 0.2, 0.4, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and 2.5 g/L were added. Algae were added as a suspension with a ratio of 1:9.5 dry weight (DW) to fresh weight (FW). Figure 3(b) shows that the maximum reduction in nitrate, ammonia, and phosphate was about 66%, 30%, and 54% respectively for 2.5 g/L algal dose. As from Figure 3(a), it can be seen that the algal growth in BG-11 medium for the first 5-6 days is very slow and according to another study, algal growth in wastewater is negligible before 5-6 days (Kothari *et al.* 2012). As a result, the algal growth rate was not investigated during the nutrient removal from wastewater.

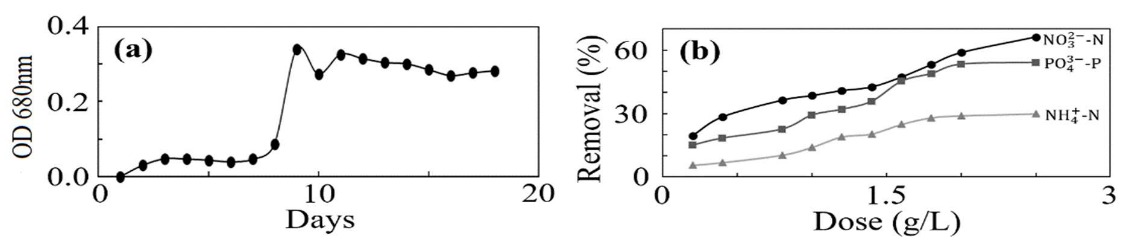

Figure 3: Growth Rate of *Spirulina sp.* and its efficiency to remove nutrients from wastewater.

3.2 Calibration results

Langmuir isotherm model expresses the monolayer adsorption on the adsorbent. The essential characteristic of the model is expressed by the dimensionless constant R_L. For all three nutrients, the values of R_L are within the range $0<R_L<1$ (Table 2). Therefore, the biosorption of the nutrients can be considered as favorable and the values of R^2 and NRMSE also represent the data are well fitted in the model (see Figure 4(a-b)).

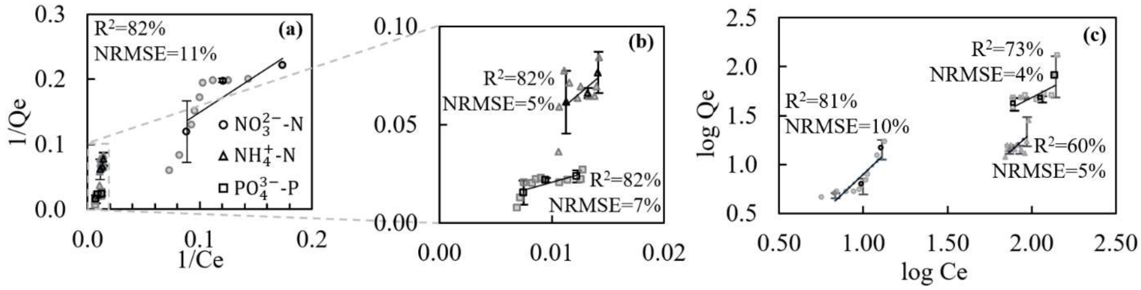

Figure 4: Graphical representation of Langmuir and Freundlich Isotherm Models. Panel (a) and (b) represents Langmuir model, where Panel (a) includes all three nutrients and Panel (b) represents the magnified section of Panel (a). Panel (c) represents Freundlich model.

Freundlich isotherm model supports the surface heterogeneity. The parameters K_F and 1/n from this model express the adsorption capacity (L/mg) and adsorption intensity respectively. Figure 4(c) shows the values of R^2 and NRMSE are around 60-80% and 3-10% respectively which represents the sound goodness of fit of the modeled data. The values of 1/n for all nutrients are seen to be greater than unity (Table 2), which symbolizes the increment of absorption coefficient with the increment of solution concentration. As a result, hydrophobic surface characteristics increase after the monolayer. Again, the value of 1/n within the range of 1-10 expresses the favourability of the biosorption for all components.

Table 2: Calibrated values of both Langmuir and Freundlich model parameters.

Parameters	$1/Q_m$	K_L	R_L	$logK_F$	1/n
Nitrate	0.036	0.033	0.64	-0.71	1.61
Ammonia	0.006	0.001	0.89	-1.73	1.53
Phosphate	0.004	0.003	0.79	-0.24	1

2. CONCLUSION

In this study, the BG-11 culture medium is used for the mass production of the collected natural bloom. After obtaining the dominant species, the microalgae doses of 0.2 g/L, 0.4 g/L, 0.6 g/L, 0.8 g/L, 1.0 g/L, 1.2 g/L, 1.4 g/L, 1.6 g/L, 1.8 g/L, 2.0 g/L, and 2.5 g/L are added from mass culture into 1L synthetic wastewater. The hydraulic retention time for all algal doses is maintained as 3 days.

The microalgae *Spirulina platensis* reduced ammonia nutrients by 82% and phosphate by 65.2% within the time period of 48hrs having initial concentrations of 10 mg/L and 5 mg/L respectively (Sofiyah and Suryawan 2021). Higher Yields can be obtained by another study with a residence time of 10 days, ammonia removal by Spirulina platensis was 97.8% and phosphate has 64.5% reduction (Kun *et al.* 2010). In another study, the Spirulina platensis was cultured in a 75% wastewater medium for 20 days. the percent reduction in the level of nitrate, phosphate, and ammonium in the medium was 99%, 99%, and 99%, respectively (Can *et al.* 2015). In this study,the maximum removal of nitrate, ammonia, and phosphate by *Spirulina sp.* are 66%, 30%, and 54% respectively which is much lower than the other studies. These results have been obtained for 3-day HRT. The maximum nutrient removal has been found at a 2.5 g/L algal dose.

The feasibility of this biosorption treatment with *Spirulina sp.*was also confirmed by the Langmuir and Freundlich isotherm models. In both cases, the separation factor and the adsorption intensity are below unity. Therefore, not only has the favorable adsorption of nitrate, ammonia, and phosphate has established but also well goodness of fit of both models have been established by blue-green algae *Spirulina sp.*

However, after treating the wastewater with 2.5 g/L algal dose for 3 days, the remaining amount of nitrate, ammonia, and phosphate in the synthetic wastewater are 6 mg/L, 70 mg/L, and 97.5 mg/L respectively. The amount of remaining nutrients is more than the standard value determined by the environment conservation rules, 1997 for both ammonia and phosphate. Therefore, a higher algal dose is required to obtain the desired removal rate in 3 daysof HRT.

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