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

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ABSTRACT

Urban wastewater is composed of various organic and inorganic substances which are capable of destroying the ecology of receiving water bodies. Therefore, for the treatment purpose, various efficient conventional processes have been applied so far to remove the easily settleable matter to oxidize the oxidizing compounds. However, this secondary effluent contains excessive inorganic nitrogen and phosphorus content whose biological treatments are rarely found. For this reason, microalgae, which has biodiesel potential, is used in this study for the removal of inorganic nitrogen and phosphorus from the secondary effluent. In this study, algal strains have been collected from locally available natural algal blooms and cultured in BG-11 medium. *Spirullina sp.*, the blue-green algae has been found to be dominant over the other species within the natural bloom. Ten different dosage (0.2-2.5 g/L) of *Spirullina sp.* have been applied to the synthetic wastewater and 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. Note that a 3-day hydraulic retention time has been kept in this experiment for all algal dosages. Linear forms of Langmuir and Freundlich isotherms have been used for biosorption modelling, 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: *Wastewater treatment, Urban wastewater, Microalgae, Biosorption.*

1. INTRODUCTION

Water is a fundamental necessity for life. Every metabolism inside a body depend 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 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, Park, & Li, 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, Al-Homaidan, & Ibraheem, 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, Smith, Brunner, & Farrell, 1976). More often these pollutants initiate into the environment without proper treatment (Mouchet, 1986).These anthropogenic activities affect the aquatic ecosystem severely (Mennaa, Arbib, & Perales, 2015). Eutrophication is a common phenomenon causes due to an excess amount of nitrogen and phosphorus. It means a dense population of algae on the water surface (Hutchinson, 1973). 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 acquatic organisms (Henze, Harremoes, la Cour Jansen, & Arvin, 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 imbalance 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 excess amount of Nitrate may cause gastric cancer, impose threats to newborn child and pregnant ladies, and changes the composition of hemoglobin which is responsible for methaemoglobinemia or blue baby syndrome in infant (Ghafari, Hasan, & Aroua, 2008; Mayo & 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 have carcinogenic, teratogenic and mutagenic properties (Abel, 2014). Ammonia and Phosphorus don not have any direct effect on human or animals. The necessity of removing them from municipal wastewater is protecting the food chain (Klaassen & Amdur, 2013).

Therefore, the necessity of wastewater recycling and energy recovery have arisen for potable water, energy, and food. (de la Noue & de Pauw, 1988). 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 a higher removal of biodegradable material by using bacteria in primary and secondary treatments of effluent. This apparently clean secondary treated effluent contains a large number of inorganic compounds, like nitrogen and phosphorus. he disposal of 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 (Oswald, 1988). For removing nitrates, ammonia and phosphate from wastewater completely, a tertiary treatment process costs four times more than primary treatment process (de la Noue & de Pauw, 1988). Lastly, the conventional treatment processes lead to incomplete utilization of natural resources (Guterstam & Todd, 1990; Phang, 1990).

On the other hand, the capacity of microalgae for inorganic nutrient uptake is very high (Blier, Laliberte, & De la Noüe, 1995; Talbot & De la Noüe, 1993). Therefore, as a potential alternative, wastewater treatment by using microalgae has been proposed as a tertiary treatment process (Olguín, 2003; Sturm & Lamer, 2011). Biological removal of nutrients by means of microalgae offers several advantages over tertiary chemical and physicochemical treatments (De la Noüe & Basseres, 1989; Proulx & De la Noue, 1988). Algal treatment is favorable because of 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 (Cai et al., 2013; de la Noue & de Pauw, 1988; Martínez, Sánchez, Jiménez, El Yousfi, & Muñoz, 2000; Tam & Wong, 1995). It is cost effective and environment-friendly method (Pittman, Dean, & Osundeko, 2011; Tang, Vincent, Proulx, Lessard, & De La Noüe, 1997). Microalgae also produce oxygen during photosynthesis process and also have a disinfection effect due to elevated pH (Mara & Pearson, 1986; Martínez et al., 2000). The main challenges for this technology lie in the wastewater composition, microalgae species and the final utilization of biomass yield (Jais, Mohamed, Al-Gheethi, & Hashim, 2017). The harvested microalgae can be used for the production of biofuel, fertilizers, various high-value products like Pharmaceuticals and genetically engineered products (Javanmardian & Palsson, 1991; Mallick, 2002; Mennaa et al., 2015). These include antibacterial, antiviral, antitumor/ anticancer, antihistamine and many other biologically valuable products (Haroun, Sharaf, & Ibrahee, 1995; Ibraheem, 1995). Blue-green algae are a prominent form of microalgae within the natural bloom in freshwater (Cai et al., 2013; Kotak et al., 1993; Smith, 1998). They are also potential for the removal of inorganic nutrients from wastewater (Sawayama, Rao, & Hall, 1998). It is also well established that blue-green algae are capable of reducing nitrate, ammonia and phosphate (Garbisu, Hall, & Serra, 1993; Romero, Coronil, Lara, & Guerrero, 1987).

Nutrients from wastewater generally uptake by algae for their cellular growth. For the growth of organisms, nitrogen is considered as 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 & 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, Jimenez, & El Yousfi, 1999).

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, Tam, & Wong, 1997). The study shows 86% and 70% removal of inorganic nitrogen and phosphorus respectively by *Chlorella vulgaris* too (Lau, Tam, & Wong, 1996). For *Natural bloom*, the efficiency is more than 87% and 80% for removing nitrogen and phosphorus respectively (Mennaa et al., 2015). About 97.8% of phosphorus is removed from domestic sewage by algae (Colak & Kaya, 1988). *Spirulina maxima* show the removal of 87% nitrogen and 60% phosphorus at HRT of 4 days (Kosaric, Nguyen, & Bergougnou, 1974). Promising nutrient removal by *Spirulina plantensis* also confirmed (Lodi, Binaghi, Solisio, Converti, & Del Borghi, 2003).

1.1. Knowledge Gap

The theory of wastewater treatment with microalgae has established around 50 years ago. Since then several studies have conducted by using laboratory cultured single microalgae species. A single microalgae strain collected from any laboratory having a controlled atmosphere needs high maintenance. The removal efficiency also decreases due to seasonal temperature variation. However, a complete study using algae only from the local source does not conduct yet. To fill in this gap, the wastewater has been treated in this study with locally available microalgae.

2. METHODOLOGY

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. 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, Kunisawa, Mandel, & Cohen-Bazire, 1971). The pH of the BG-11 culture medium maintained 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 air pump. A 16:8 hr light:dark cycle was maintaind throughout the culture condition. pH had measured once a day and maintained as 7.1.

2.3 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:

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

where, OD_0 represents the optical density at initial day and OD_t represents optical density measured at day t (L. 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 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.

2.4 Synthetic Wastewater Composition

Synthetic wastewater is a chemically derived wastewater. The chemical content of the synthetic wastewater used in this study had chosen to compare the nutrient content of wastewater within Sylhet city and from the literature review. Seven random points had chosen for the collection of wastewater samples. The selection of collecting points was based on: upstream and downstream of canals, location of residential areas and reconnaissance survey. The measured nutrient content of Sylhet city wastewater was compared with the values obtained from the literature review (Table 1). The highest values of nitrate, ammonia and phosphate were selected for 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 were 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 sysnthetic 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, Luong, Lee, Oh, & Lee, 2011), [b](Zhou et al., 2012)

3. 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, magnetic stirrer at 350 rpm was used (Figure 1(b)).

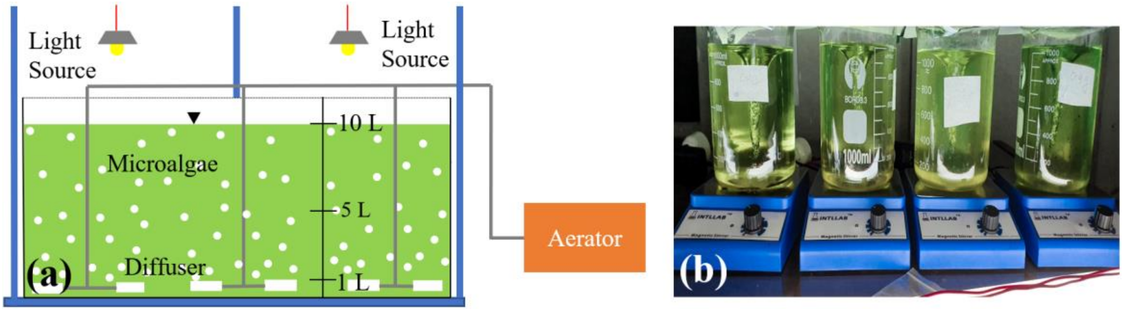

Figure 1: Panel (a) represents the schematic diagram of mass culture of microalgae using 60 cm × 20 cm tank and Panel (b) represents the experimental setup of synthetic wastewater treatment

4. Biosorption Modelling

Nutrient removal by microalgae is a biosorption process. Biosorption is a physicochemical and metabolically-independent process. The significance of biosorption processes in the environment and conventional biotreatment processes is irrefutable. The aim of biosorption modeling is experimental data analyses, understanding process mechanisms, prediction of operational condition changes and optimizing processes (Fomina & 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 & Yun, 2008). For simple single component models, Langmuir and Freundlich's versions are used widely (Gadd, 2009; Pagnanelli, Esposito, & Vegliò, 2002). In the Langmuir isotherm model, monolayer adsorbate adsorption is occurred at or before a relative pressure of unity is reached (Liu, Luo, Ding, & Luo, 2019). No further sorption can take place when the layer is filled. This causes the surface to be saturated where the maximum adsorption is achieved (Boparai, Joseph, & O'Carroll, 2011). The Freundlich equilibrium isotherm is an empirical exponential equation. It assumes that due to the increase of Adsorbate concentration, the concentration of Adsorbate on the adsorbent surface also increases (Hamdaoui & Naffrechoux, 2007; Liu et al., 2019). Freundlich isotherm describes the surface heterogeneity. The exponential distribution of active sites and their energies are also expressed by Freundlich equilibrium isotherm model (Ayawei, Ekubo, Wankasi, & Dikio, 2015; C. Wang, Hu, Chen, & Wu, 2005). For biosorption modelling, Langmuir (Langmuir, 1916) and Freundlich (Freundlich, 1906) isotherm models were used in this study.

5. RESULT AND DISCUSSION

Growth rate and nutrient removal by *Spirulina sp.*

The microalgae species had been identified from microscopic photograph after two months of culture. The dominant algae found in the culture was *Spirulina sp.* The growth rate of microalgae was measured in terms of OD_{680}. From the growth rate curve, the behavior of microalgae in cultural medium can be stated (Figure 2(a)). No lag phase was observed throughout the curve which indicated the well adaptation of microalgae in cultural medium. An exponential growth phase had observed after 8 days of culture. The exponential growth phase was remain for 1 day, and thereafter a comparatively stationary growth phase had achieved.

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 in an aim of reducing maximum amount of nutrient enrichments within 3 days of HRT. Figure 2(b) shows that the maximum reduction in nitrate, ammonia and phosphate was about 66%, 30% and 54% respectively when the algal dose was 2.5 g/L.

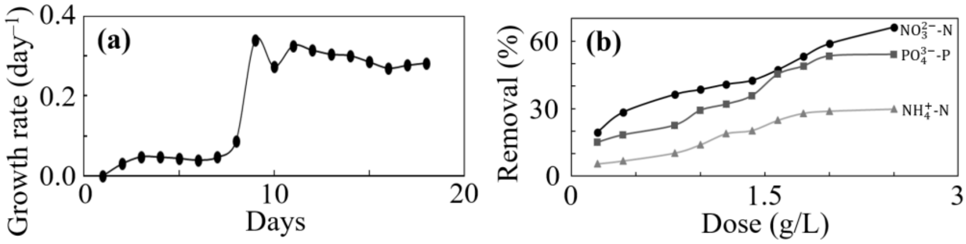

Figure 2: Growth Rate of *Spirulina sp.* and its efficiency to remove nutrients from wastewater

Calibration results

Langmuir isotherm model expresses the monolayer adsorption on adsorbent. The essential characteristics 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 represents the data are well fitted in the model (see Figure 3(a-b)).

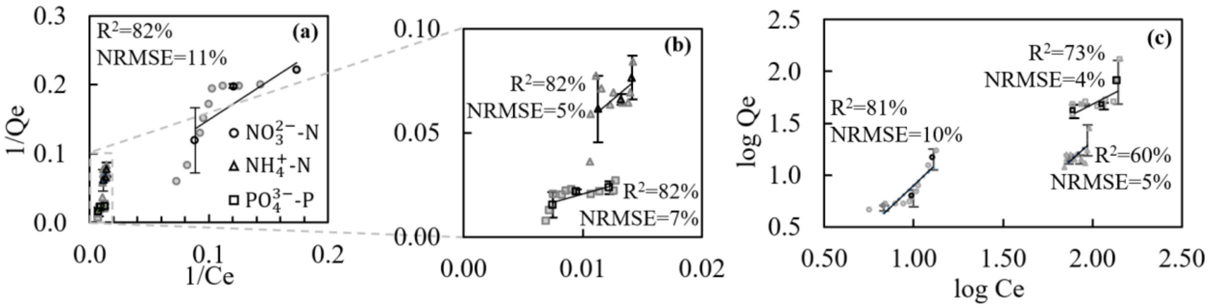

Figure 3: Graphical representation of Langmuir and Freundlich Isotherm Models. Panel (a) and (b) represents Langmuir model, where Panel (a) includes all three nutrients and Panel (b) represents the magnified section of Panel (a). Panel (c) represents Freundlich model.

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 3(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 modelled 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 monolayer. Again, the value of 1/n within the range of 1-10 expresses the favorability 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

6. CONCLUSION

Municipal wastewater possess a huge amount of nutrient load. Untreated nutrient content pollute the receiving water bodies and causes eutrophication which is harmful for human, animals and aquatic ecosystem. To solve the situation, this study has been conducted to find out the efficiency of microalgae in case of nutrient removal. The compatibility of natural bloom culture in BG-11 media also showed here. Among the species of natural bloom, Spirulina sp. has become dominant. Though Spirullina sp. is a Prokaryotic cell, it is able to do the photosynthesis process just like the Eukaryotic microalgae. However, the debate of cyanobacteria being Prokaryotic or Eukaryotic is still exiting.

In this study, Spirullina sp., a blue-green algae is used for treatment purpose. This blue-green algae has shown a promising removal efficiency of nitrate, ammonia and phosphate. However, around 30%, 70% and 40% of nitrate, ammonia and phosphate respectively could not be removed by Spirullina sp. These results has been obtained for 3-day HRT and 2.5 g/L algal dose. The feasibility of this biosorption treatment has confirmed by the Langmuir and Freundlich isotherm models. In both cases, the separation factor and the adsorption intensity have been found to below unity. Therefore, not only the favorable adsorption of nitrate, ammonia and phosphate has established but also well goodness of fit of both models have established by blue-green algae Spirulina sp.

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