MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Alginate microbeads for the treatment of the outlet of a remediated municipal wastewater

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Received:29/7/2021
Accepted: 20/8/2021

Abstract: As a result of dwindling water supplies, the wastewater disposal costs, and tougher discharge regulations that have reduced allowable contaminant levels in waste streams, highly efficient and eco-friendly wastewater treatment approaches is becoming highly significant. Alginate beads found play a key role in wastewater remediation. In this study, the removal efficiency of alginate microbeads (7.5 – 12.5µm diameter) for nitrite, nitrate, ammonia and phosphate from the outlet of a phytoremediated municipal wastewater was studies for 72 h at the treatment periods of ½, 1.0, 2.0, 4.0, 24, 48 and 72 h. Satisfying removal results was obtained as follow: 100% nitrite removal (after 24 h), 100% nitrate removal (after 48 h), 72.53% ammonia (after 72 h), 39.44 % for phosphorous after 48 h and declined to 32.90 % after 72 h. Also, a remarkable decline in concentrations of COD and BOD was detected indicating the improvement of the quality of the treated wastewater by alginate microbeads.

The use of alginate microbeads for wastewater treatment is less expensive and ecofriendly bioremediation technique, that performing tertiary treatment with a remarkable removal efficiency of nitrate, nitrite, ammonia and phosphate.

keywords: **:** Alginate microbeads, *Chlorella minutissima*, municipal wastewater, ammonia, nitrate, BOD and COD

1.Introduction

Water is a key material for sustaining life on the surface of the earth. The planet faces a wide range of toxins and contaminates from different construction activities due to the global population boom. Some of these contaminants were generated from industrial plants, chemical products, domestic activities and other community water uses [1]. The gradual increase of water demand and the limitation of water supply, the feasible solution to meet water requirements in Egypt is through the reuse of different agricultural, municipal and industrial wastes. The Egyptian irrigation system is considered a closed system, where different water losses return to the drainage system, as well as a mixed system. This led to an increase in pollution in the drainage water, and it makes its reuse a serious problem. Providing sewage service and water treatment were among the main priorities of the government in recent decades.

However, due to the economic challenges, the service did not catch up with rapid population growth. Therefore, it is important to find alternative solutions to mitigate the pollution problem that are economically and technically feasible. Many processes as ozonation, filtration by the membrane, precipitation, coagulation, reverse osmosis, and ion exchange have been applied for the elimination of pollutants from contaminated water [2]. However, these technologies have many disadvantages, since they often imply high costs because demand much energy and can carry on the formation of free radicals and sometimes more toxic than the initial pollutant [3]. Widen importance has been accorded on the bioremediation as it is a cost-effective, ecofriendly and safe wastewater treatment biotechnology that uses a biological material [4].

culture or immobilized cells in the form of NaCS-PDMDAAC capsules [5], alginate microbeads [6,7] and chitosan beads [8]. Sodium alginate is a highly promising biopolymer obtained from brown seaweeds and is applied well in drug delivery as it is biologically safe [9]. It has many applications as being thickening agent, gel forming and colloidal stabilizing agent in the food and beverage industries, it is also used as a binder in tablet formulation [10]. The hypothesis of this work was that micro-alginate beads may sustain economic removal potential and make it easier in handling and processing. The aim of the present study to evaluate the removal potential of alginate microbeads for nitrate, nitrite, ammonia and phosphate from municipal wastewater.

2.Materials and methods

1-Study area, sampling program and analysis

The municipal wastewater samples were collected from Samaha sewage treatment station which located in the west north of Mansoura city. It is a type of phytoremediation treatment by *Cyperus papyrus*. The output of the treatment station is 2,000 m^3/day. On the day of sampling, a clean polyethylene bottles with 20 L volume capacity were used for wastewater collection from each sampling station. Some physicochemical parameters includes water temperature (T), pH, total dissolved salts (TDS) and dissolved oxygen (DO) were measured in the field using portable multi-parameter. The collected wastewater samples were shipped to the laboratory of Mansoura University for further chemical analysis. Sampling procedure, handling and processing followed strictly [11]. Upon arrival to the laboratory, the wastewater samples were thoroughly mixed and two liters were filtered through GF/C glass fiber filters for determination of nitrite (NO_2), nitrate (NO_3), ammonia (NH_4), soluble reactive phosphorus (SRP) and the heavy metals (Pb, Fe, Cd, Cu). Other part of the unfiltrated wastewaters were used for the determination of total suspended solids (TSS), Chemical oxygen demand (COD) and biological oxygen

Microalgae have been used in wastewaters treatment in the form of suspended free-cells demand (BOD_5). All the analyses were done according to [12].

2-Preparation of alginate microbeads.

1.1-Preparation and characterization of alginate microbeads

A sodium alginate solution (2% w/v) was used for the preparation of microbeads using a sprayer with some modifications on Abdel-Hamid (1996)[7]. The microbeads were received into 0.3 M $CaCl_2$ solution being stirred on a magnetic stir with 250 rpm. The stirring was continued for 30 min to allow complete strengthening of the microbeads, then washed several times with distilled water, transferred to flasks containing distilled water and stored in dark at 4^{o}C until use. The microbeads size was measured using Scanning Electron Microscope (SEM).

1-Wastewater treatment experiment

A three glass tanks of 10 L capacity were used for the wastewater treatment, in which seven-liter raw wastewater was added. The alginate microbeads were added with a volumetric ratio of 4:1 v/v wastewater: microbeads. The bioremoval efficiency of nitrite, nitrate, ammonia and soluble reactive phosphorus were determined after ½, 1.0, 2.0, 4.0, 24, 48 and 72 h. all the analyses methods followed [12].

Bioremoval efficiency = $(C_i-C_f)*100/C_i$

4-Chemical analysis

1.1-Nitrate-N

It was determined using the method quoted by Taras, 1950[13]

Reagent:

1-Phenol disulphonic acid

2-Potassium hydroxide solution (approx. 12 N)

3-Potassium nitrate stock solution (1.0 ml = 100 µg nitrogen)

4-Standard nitrate solution. (1.0 ml = 10 µg nitrogen)

5-Standard silver sulphate solution (1.0 ml = 1.0 mg Cl)

Table 1: Physical and chemical analysis of Samaha raw municipal wastewater

<table>
<tr><th colspan="2">Parameters</th><th>Unit</th><th>Samaha sewage raw wastewater</th></tr>
<tr><td colspan="2">Temperature</td><td>^{o}C</td><td>19</td></tr>
<tr><td colspan="2">pH</td><td>Unit</td><td>8.47</td></tr>
<tr><td colspan="2">Alkalinity, Total as CaCO3</td><td rowspan="14">**mg l^{-1}**</td><td>75.2 ± 1.724</td></tr>
<tr><td colspan="2">Chloride (Cl^-)</td><td>54.91 ± 0.276</td></tr>
<tr><td colspan="2">Total Dissolved Solids (TDS)</td><td>227 ± 44</td></tr>
<tr><td colspan="2">Total settleable solids (TSS)</td><td>5.8 ± 0.085</td></tr>
<tr><td colspan="2">Dissolved oxygen (DO)</td><td>2.0 ± 0.085</td></tr>
<tr><td colspan="2">Biological oxygen demand (BOD)</td><td>69.4 ± 1.131</td></tr>
<tr><td colspan="2">Chemical oxygen demand (COD)</td><td>117.7 ± 1.131</td></tr>
<tr><td colspan="2">Nitrate</td><td>0.212 ± 0.025</td></tr>
<tr><td colspan="2">Nitrite</td><td>0.03 ± 0.006</td></tr>
<tr><td colspan="2">Ammonia</td><td>36 ± 0.849</td></tr>
<tr><td colspan="2">Soluble reactive phosphorus (SRP)</td><td>4.723 ± 0.045</td></tr>
<tr><td rowspan="3">Heavy metals</td><td>Fe</td><td>0.643</td></tr>
<tr><td>Cu</td><td>0.285</td></tr>
<tr><td>Cd & Pd</td><td>nd</td></tr>
</table>

nd = not detected

Procedures:

1- After the determination of the chloride content, a volume of 50 ml of the water sample was treated with a volume of the standard Ag_2SO_4 solution equivalent to its chloride content.

2- The $AgCl_2$ precipitate was coagulated by heat whenever necessary and then removed by filtration, using Whitman No.1 filter paper.

3- The clarified sample was neutralized by dil. HCl to ~ pH 7, transferred to a crucible and evaporated till dryness over a water bath.

4- The residue was rubbed with 2 ml phenol disulphonic reagent and then 10 ml of distilled water were added. Stirring was applied to ensure dissolution of all solids.

5- KOH (12 N) was added until maximum yellow color was developed. The solution was filtered and transferred to a 50 ml volumetric flask, diluted with distilled water to the mark.

1-The absorbance of the developed color was measured at 410 nm against a reagent blank.

Calculations:

The concentration of nitrate-N in the examined water samples were calculated from the standard curve.

1.1-Nitrite-N

It was determined using the method described by Barnes and Folkard, 1951 and Dewis and Freitas, 1970[14,15].

Reagents:

1-Sulphanilic acid -1- naphthylamine

2-Sodium nitrite stock solution (0.1 ml = 50 μg nitrogen)

3-Standard sodium nitrite solution. (1.0 ml = 1.0 μg nitrogen)

Procedure:

1- Add 5 ml of the Sulphanilic acid -1- naphthol reagent to 20 ml of the water sample and then checked for any pink color that may be developed.

2 - The absorbance of the developed color was measured at 520 nm against a reagent blank after 20 min.

Calculations:

The concentration of nitrite-N in water samples were calculated from the standard curve.

1.1-Ammonia-N

It was determined by phenate method according to standard methods for the examination of water and wastewater [12].

Reagents:

1- Phenol solution

2-Sodium nitroprusside, 0.5% w/v

3-Alkaline citrate

4-Sodium hypochlorite, commercial solution, about 5%

5-Oxidizing solution

Procedure:

1. To a 25-ml sample in a 50-ml Erlenmeyer flask, add, with thorough mixing after each addition, 1 ml phenol solution, 1 ml sodium nitroprusside solution, and 2.5 ml oxidizing solution.

2. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature (22 to 27°C) in subdued light for at least 1 h. Color is stable for 24 h.

3. Measure absorbance at 640 nm.

Calculations:

The concentration of ammonia-N in the water samples were calculated from the standard curve.

1.1-Soluble reactive phosphorus (SRP)

SRP was determined by the direct stannous chloride method described in standard methods for the examination of water and wastewater [12].

Reagent:

1-Ammonium molybdate solution

2-Sulfuric acid (50 %)

3-Hydrochloric acid (10%)

4-Ammonium molybdate reagent

5-Stannous chloride reagent

6-Potassium phosphate stock solution (1.0 ml = 50 μg p)

7-Standard Potassium phosphate solution (1.0 ml = 2 μg p)

Procedure:

1- In a reaction flask, 1 ml of ammonium molybdate reagent and 0.5 ml of stannous chloride reagent were added to 100 ml of the water sample with continuous stirring.

2- The absorbance of the developed color was measured at 690 nm against a reagent blank after 7 min. from stannous chloride solution addition.

The concentration of SRP of water samples were calculated from the standard curve.

1.1-Heavy metals (Pb, Fe, Cd, Cu)

All analysis were carried out in Atomic adsorption unit, Chemistry Department, Faculty of science, Mansoura University.

1-Statistical analysis

All the experiments were run in triplicate. The collected data were statistically analyzed by Tukey test to compare means using Statistical Package (Statistix 8.1).

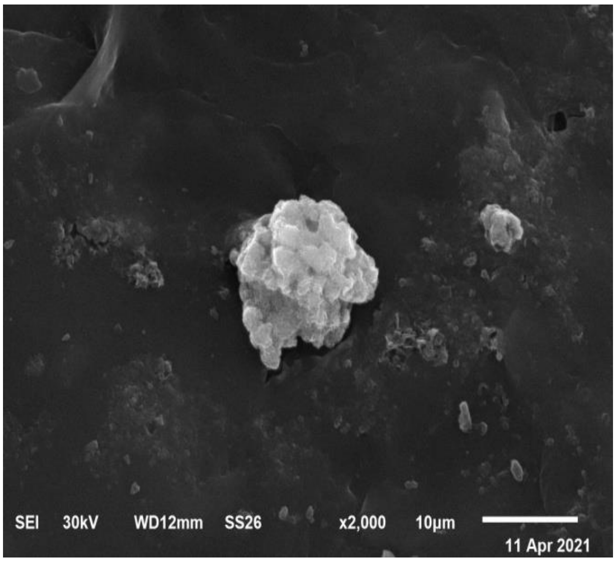
3. Results and Discussion

1-Physical and chemical properties of wastewater samples.

As the municipal wastewater was collected during winter season, the wastewaters temperature ranged from 19 to 21^{o}C. The wastewater pH was 8.47 and a total alkalinity of 75.2 mg l^{-1} was recorded (Table 1). While, the chlorides, total dissolved solids (TDS) and total settleable solids (TSS) record a values of 54.91 mg l^{-1}, 227 mg l^{-1} and 5.8 mg l^{-1}, respectively. Also, the dissolved oxygen, BOD and COD values were 2.0 mg l^{-1}, 69.4 mg l^{-1} and 117.7 mg l^{-1}. The concentrations of the total inorganic nitrogen (nitrate, nitrite and ammonia) and soluble reactive phosphorus (SRP) were 0.212 mg l^{-1} (nitrate), 0.03 mg l^{-1} (nitrite), 36 mg l^{-1} (ammonia) and 4.723 mg l^{-1} (SRP) (Table 1). The iron and cupper maintain low concentrations of 0.643 and 0.285 mg l^{-1}, meanwhile, cadmium and lead were undetectable.

1-Characterization of alginate microbeads

Scanning electron microscopy (SEM) has been a primary tool for characterizing the surface morphology and fundamental physical properties of the adsorbent surface. It is useful for determining the particle shape, porosity and appropriate size distribution of the adsorbent. In this study alginate microspheres are semi spherical with cavities, alginate sample dried at room temperature that may cause the semi spherical shape due to dehydration of microspheres. The diameter of alginate microspheres ranges from 7.5 µm to 12.5 µm



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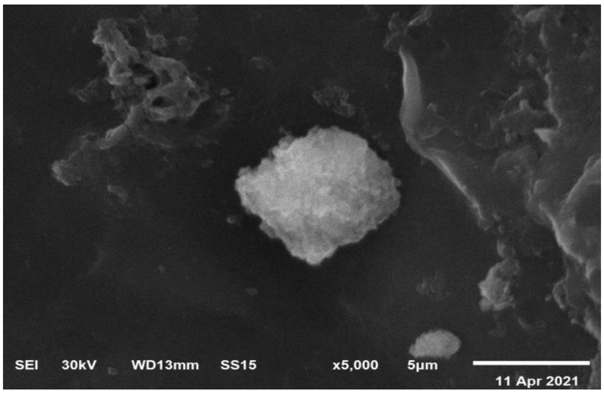

Figure 1: Scanning electron microscopy (SEM) of calcium alginate microbeads

1-Bioremoval efficiency of alginate microbeads

It is clear that the bioremoval efficiency depends on the treatment time and the elements concentration. For example and as seen from Figure 2 and Table 3, the removal percent of nitrate after half hour was 14.82% and increased by time, where after four hours reached to 47.77%, and after twenty four hours the removal percent was 90.56%. A similar removal trends were observed for nitrite, ammonia and soluble reactive phosphorus. However the SRP concentration starts to increase in the wastewater, changing the removal percent from 39.44% (after 48 h) to 32.9% (after 72 h) (Figure 2 and Table 3). These results highlight the impact of time, the initial concentration and the target element on bioremediation efficiency. As seen from table 4, a remarkable changes in water quality of the treated wastewater were detected.

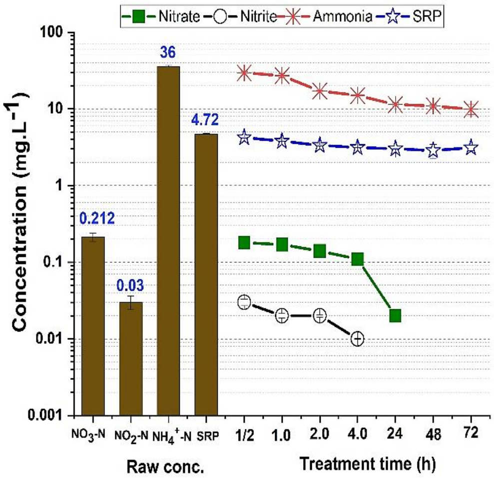

Figure 2: Time-dependent changes in nitrate, nitrite, ammonia and SRP concentrations in municipal wastewater tertiary treated with alginate microbeads. Bars represent SE

Table 2: Changes in removal percent (%) of nitrate, nitrite, ammonia and SRP concentrations in municipal wastewater tertiary treated with alginate microbeads.

Treatment time (h)	Removal percent (%)			
	Nitrate	Nitrite	Ammonia	SRP
½	14.82 ±2.83	0	18.07 ±2.98	10.17 ±1.55
1.0	19.28 ±5.49	37.96 ±2.53	24.49 ±2.40	19.49 ±0.14
2.0	34.88 ±9.58	36.72 ±6.56	52.51 ±1.37	29.24 ±0.69
4.0	47.77 ±1.57	66.56 ±5.52	58.32 ±0.78	33.47 ±0.22
24	90.56 ±8.08	100	68.34 ±1.72	36.02 ±1.33
48	100		69.58 ±1.22	39.44 ±6.42
72			72.53 ±4.03	32.90 ±18.63

Table 3: chemical analysis of Samaha treated municipal wastewater (after 72h treatment times).

Parameters	Concentration , $mg\ l^{-1}$	
	Raw wastewater	Treated wastewater
BOD	69.4 ± 1.131	39.8 ± 2.52
COD	117.7 ± 1.131	78.6 ± 4.23
Nitrate-N	0.212 ± 0.025	0
Nitrite-N	0.03 ± 0.006	0
Ammonia-N	36 ± 0.849	9.89 ± 1.54
SRP	4.723 ± 0.045	3.17 ± 0.88

Discussion

Pollutants and contaminates from various developmental activities are a serious problems facing our world. Naturally occurring microorganisms and other aspects of the natural environment are used to treat wastewater, which a phenomena known as bioremediation [16]. On the contrary, biosorption is a physicochemical process for the removal of materials (compounds, metal ions, etc.) using inactive, non-living materials of biological origin. The biosorption mechanisms includes absorption, adsorption, ion exchange, surface complexation and precipitation [17]. In this study, calcium alginate microbeads was used as a green sorbent for the treatment of wastewater, where it is known that the small molecules with upper limit of ∼140 kDa (e.g. molecules of nitrogen and phosphorus compounds) are easily diffused into the beads [18,19].

According to El-tayieb et *al*. (2016)[20], the removal efficiencies were 78.6% for ammonia nitrogen and 75.5% for nitrate after 2 hours, while in this study removal efficiencies were 100 % for nitrate after 48 hours, nitrite 100 % after 24 hours and ammonia 72.53 % after 72 hours (Table 3). However, the phosphate removal with alginate microbeads after 48 h was 39.44% and declined to 32.90% after 72 hours (Table 3), it is still higher than the values obtained by Zamani *et al.*, (2012)[21] in similar alginate based treatments (25%). The low phosphate removal efficiencies is most likely owing to phosphate precipitation, where phosphates have a strong affinity for calcium ions, they sequester them from the alginate matrix and precipitate as calcium phosphate [8,19,21,23]. Also, the decline in phosphate removal after 72 hours to 32.90% may be due to the degradation of the surface of alginate microbeads with longer retention time in wastewater by the soil microorganisms occur in wastewater [24]. It is also clear that the BOD and COD levels reduced significantly by treatment with alginate microbeads after 72 h (Table 3). Indication the decrease of the substances that consuming dissolved oxygen in the treatment [25].

Conclusion

This study explains the feasibility of bioremediation with alginate microbeads, that performing tertiary treatment of wastewater with a remarkable removal efficiency of nitrate, nitrite, ammonia and phosphate. Alginate microbeads give less expensive and ecofriendly bioremediation technique for wastewater treatment.

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