Natural Cellulosic Adsorbent for Recovery of Nitrate from Aquaculture Effluent

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Abstract. Nitrogen based nutrients are among the main components in aquaculture effluent to cause eutrophication condition, as it may cause algal bloom and depletion in oxygen once its concentration reaches the threshold. Nitrogen based nutrients are released into water in a form of nitrate after going through nitrification and denitrification. Cellulose based adsorbent are increasingly gaining attention due to its adsorption capability which comes in useful for recovery of nitrogen based nutrients. This study aims to evaluate the efficiency of powder cellulose as a potential natural cellulosic adsorbent for the recovery of nitrate ions in water under a constant pH 6.6. The uptake of nitrate by natural cellulosic adsorbent was investigated with variation of parameters such as initial concentration of 5.0 mg L\(^{-1}\) and 30.0 mg L\(^{-1}\), adsorbent dosage of 5.0 mg and 40.0 mg, temperature of 25.0 °C and 45.0 °C, and contact time of 10 minutes and 60 minutes. The experiment was conducted based on the experiment design by Design Expert Version 11 software in order to observe the recovery percentage of powder cellulose in different condition. The best recovery percentage was achieved at the condition of 5.0 mg L\(^{-1}\), 5.0 mg, 25.0 °C and 10 minutes with 96.50 % of recovery. This shows that the electrostatic force of attraction between powdered cellulose and nitrate anion nutrients have the potential to occur without heat and vacant pores around powdered cellulose was filled by nitrate anions in a limited amount of time. The model for the experiment which is most suited in this experiment is a natural log with a significant R\(^2\) value of 0.9982. This indicates that the adsorption of nitrate anion nutrients using natural cellulose adsorbent is indeed feasible. Therefore, there are potential of using cellulose extracted from biomasses in Malaysia, which will expand its product-value.

1. Introduction
Aquaculture is one of the important industries in keeping up with the market demanding of seafood supplies, as the world’s population kept increasing year by year. According to Global Aquaculture Alliance, the current global aquaculture production rate is at 46.4 million metric tonnes (MT) and it is expected to grow to 93.6 million metric tonnes by 2030 [1]. However, there are still improvement needed in the aquaculture effluent treatment due to the dangerous concentration level of nitrate anions in the effluent content. Nitrate anions present in the aquaculture effluent are caused by the suspended particles (excess feed) and feces in the aquaculture water [2]. Once the concentration of nitrate anion goes beyond its allowable threshold, it turns the surrounding water condition into toxic and it is one of the anions causing eutrophication condition [3]–[10]. Hence, creating a massive loss in farmed fishes as such happened at Nibong Tebal, Penang [11]. According to the National Water Quality Standards for
Malaysia, the allowable concentration of nitrate anion is capped at 7.0 mg L$^{-1}$ [12]. Therefore, any level above the threshold is considered toxic towards farmed fishes as well as dangerous for human consumption which may lead to baby blue syndrome.

The recovery of nitrate anion nutrients from aquaculture effluent has been in the interest in research field for quite some time. Nitrate anion nutrients can be removed via biologically and chemically [13]. These methods include hydroponics [14], electrodialysis [15], ion exchange, reverse osmosis, and adsorption [13], [16], [17] methods. However amongst these various techniques, adsorption or ion exchange is considerably the most simple, efficient, relatively low cost, and renewable method to remove nitrate anion nutrients [13].

The adsorbent dosage used in the study of nitrate anion nutrients are commonly consist of active carbon or zeolite, which soon became an issue in the costing of the material purchasing itself [18]. Hence, it was then replaced with biomass lignocellulose material which was readily available in the respective countries. The treatment of lignocellulosic material needs to undergo high temperature to become hydrochar, which makes the cellulose even more desirable as it does not need high temperature to treat. Unfortunately, many has overlooked that the nitrate anion nutrients can be reuse as fertilizer as it is one of the important components for plants.

Thus, in this paper, we focus on recovering nitrate anion nutrients from aquaculture effluent using powdered cellulose under a fixed pH to determine the other factor in improving the adsorption of nitrate anion nutrients. The adsorption trend is then studied deeply under Design Experiment software Version 11.

2. Experimental Section

2.1. Materials
All the chemicals used were of analytical grade. Powdered cellulose was purchased from Sigma-Aldrich (USA), sodium nitrate from R&M chemicals was used as a source of nitrate anion. Stock solution of 1000 mg L$^{-1}$ was prepared by diluting 6.0711g of NaNO$_3$ in 1L of deionized water. Experimental solutions were prepared by diluting the stock solution to the desired concentration of 5 and 30 mg L$^{-1}$.

Solution used to adjust the pH of solution to pH 6.6 were sodium hydroxide from Merck (Germany) and hydrochloric acid from J. Kollins Chemicals.

2.2. Batch adsorption studies
Fifty mL of sample solution was prepared according to the desirable concentration and adjusted the pH to pH 6.6 using 0.1M of sodium hydroxide or 0.1M of hydrochloric acid. Each experimental set was prepared according to Table 1, where is initial concentration of sample solution was adjusted accordingly, adsorbent dosage (powdered cellulose) was weighed, temperature of sample solution and contact time of stirring was taken into consideration as well. After each experimental set was completed, the solution was taken to centrifugation and filtered to remove excess suspended cellulose in the solution. The filtrate was then measured to 3.5 mL and placed into a quartz cuvette to measure the concentration under UV-vis spectroscopic (LAMBDA 365 by Perkin Elmer) at 210 nm. The recovery percentage was then taken to be calculated according to the equation below.

\[
Recovery \ Percentage \ (%) = \frac{C_i - C_f}{C_i} \times 100
\]

Where $C_i$ is the initial concentration of solution (mg L$^{-1}$) and $C_f$ is the final concentration of solution (mg L$^{-1}$).
### Table 1. Experimental set arranged by Design Expert Software Version 11.

| Experimental Set | Initial Concentration (mg/L) | Adsorbent Dosage (mg) | Temperature (°C) | Contact Time (minutes) |
|------------------|-----------------------------|-----------------------|------------------|-----------------------|
| 1                | 5.0                         | 40.0                  | 25.0             | 60.0                  |
| 2                | 5.0                         | 5.0                   | 25.0             | 10.0                  |
| 3                | 30.0                        | 40.0                  | 25.0             | 10.0                  |
| 4                | 30.0                        | 5.0                   | 45.0             | 10.0                  |
| 5                | 5.0                         | 5.0                   | 45.0             | 60.0                  |
| 6                | 30.0                        | 5.0                   | 25.0             | 60.0                  |
| 7                | 5.0                         | 40.0                  | 45.0             | 10.0                  |
| 8                | 30.0                        | 40.0                  | 45.0             | 60.0                  |

3. Results & Discussion

3.1. Adsorption of Nitrate anion nutrients study

UV-Vis spectrophotometry was used to detect the concentration of nitrate anion nutrient after the adsorption experiment, at a wavelength of 210 nm. The results obtained is shown plotted in a histogram as shown in Figure 3.1.

![Figure 1](image_url)

**Figure 1.** Removal efficiency and adsorption capacity of each experimental set.

Figure 1 shows a graph obtained from the adsorption experiment under different sets of experiment arrangement. As seen in Figure 3.1, the experimental set 1 achieved the lowest recovery percentage of nitrate anion nutrients, 53.80%. The adsorbent dosage required to adsorb 5.0 mg L⁻¹ of nitrate anion nutrients are above threshold, which may result to internal attraction between the adsorbents vacant sites to occur rather than attracting the nitrate anion nutrients. Experimental set 2 and 3 were, by far, the highest in recovery percentage (96.50% and 93.7%, respectively) due to the balance ratio of initial concentration and adsorbent dosage. The balance of both parameters affected the efficiency of recovery percentage which induced the electrostatic force of attraction between the nitrate anion nutrients with the active site on the surface of the powdered cellulose. The effect of room temperature helps eliminate the possibility of breakage of electrostatic bond between the nitrate anion nutrient and the adsorbent. Experimental set 4 to 6 experiences a similar range of recovery percentage (85.10% to 85.60%), due to
the same initial concentration and adsorbent dosage. However, experimental set 6 result a slightly higher recovery percentage as compared to experimental set 4 may be due to the lower temperature in experimental set 6. Higher temperature promotes breakage of bond which affects the electrostatic force of attraction between the nitrate anion nutrients with the active sites of the powdered cellulose, as seen occurring in experimental set 4. Experimental set 5, on the other hand, had a lower recovery range of 80.50 % even with a balance ratio of initial concentration with adsorbent dosage could be under the influence of higher temperature promotes bond breakage as well. Experimental set 7 and 8 obtained similar recovery percentage, with 88.30 % and 88.70 % respectively. As the adsorbent dosage is higher than required in experimental set 7, the recovery percentage is affected making it slightly lower than experimental set 8. Hence, the electrostatic force may have reaction between the powdered cellulose active sites, rather than nitrate anion nutrient with the powdered cellulose active sites. As seen through the experiment, the longer the contact time between the nitrate anion nutrients with the powdered cellulose adsorbent, the lower the recovery percentage is especially with higher amount of adsorbent dosage in a lower initial concentration experimental set. Hence, the adsorption reaction is sufficient under 10 minutes of contact time.

3.2. Design Expert Data Analysis

Two level-half factorial was used to conduct the experimental based on the arrangement set by the Design Expert Version 11, as seen in Table 4.1. There were 8 sets of experiment with different parameter condition. Once the adsorption capacity (mg g⁻¹) of each experimental set is calculated, the data are keyed into the Design Expert Version 11 to observe the response graphs. Table 2 shows that the data obtained from two level-half factorial agrees on a natural log model, with a standard deviation of 0.1136 and the coefficient variation (C.V) of 8.89, indicating the natural log model is reasonably reproducible as it is lesser than 10%. While the coefficient determination (R²) is 0.9985, which provides a satisfactory adjustment of the natural log model towards the experimental data.

| Table 2. Data obtained from Design Expert Version 11. |
|---------------------------------|-----------------|------|--------|------------------|
| Model                          | Standard Deviation | C.V (%) | R-squared | Remarks         |
| Natural Log                    | 0.1136           | 8.89  | 0.9985 | Suggested       |

Table 3 is the tabulated results obtained from ANOVA, and it is shown that the Fisher F-value of the model is 262.04. This large F-value represents the variation of responses that can be analyzed by the regression equation. Besides, the p-value lesser than 0.05, represents the significance of the model and the associated terms. Hence, supporting that this model with the other parameter to be significant.

| Table 3. Analysis of variance (ANOVA) and statistical parameters of model |
|---------------------------------|-----------------|------|--------|------------------|
| Source                          | Sum of Squares | Mean Square | F-value | p-value | Remarks         |
| Model                           | 16.91           | 3.38 | 262.04 | 0.0038 | Significant     |
| A-Initial Concentration         | 7.34            | 7.34 | 568.42 | 0.0018 | Significant     |
| B-Adsorbent Dosage              | 9.41            | 9.41 | 729.12 | 0.0014 | Significant     |
| C-Temperature                   | 0.0081          | 0.0081 | 0.2098 | 0.6781 |                |
| D-Contact Time                  | 0.0656          | 0.0656 | 5.08   | 0.1529 |                |
| AB                              | 0.0484          | 0.0484 | 3.75   | 0.1924 |                |
| AD                              | 0.0492          | 0.0492 | 3.81   | 0.1900 |                |
| Residual                        | 0.0258          | 0.0129 |        |        |                |
A natural log equation was created to express the relationship between the input variable and the experimental results. Hence, the generated equation is as follow:

\[
\ln(\text{Adsorbance capacity}) = 1.28 + 0.9577 \cdot A - 1.08 \cdot B - 0.0906 \cdot D + 0.0778 \cdot AB + 0.0785 \cdot AD
\]  

(4.1)

3.3. Model Adequacy

Each model had to be verified in order to provide an adequate approximation to the actual values. Therefore, a predicted value versus experimental values is an important plot to estimate the model’s precision, and display the correlation of the predicted and experimental data.

![Figure 2. A comparison of predicted values versus actual experimental values.](image)

Based on Figure 2, the experimental data is seen plotted closely towards the linear line. This indicates that the actual experimental data obtained is in agreement with the predicted values. Hence, supporting the model adequacy. Besides, Figure 3 shows a 3D response surface plot, where the initial concentration and adsorbent dosage played a bigger impact on the experimental outcome as compared to the other two parameters, temperature and contact time. This plot can be supported based on the p-value shown in Table 3.

![Figure 3. Response surface plot (3D) of the effect of initial concentration and adsorbent dosage on adsorbance of nitrate anion nutrients.](image)
Overall, the results obtained from the actual experimental data and the predicted data generated by Design Expert Version 11 are sufficiently in agreement of one another. Hence, this supports the model design of natural log and the natural powdered cellulose’s ability to act as an adsorbent for the recovery of nitrate anion nutrients.

4. Conclusion
In conclusion, the usage of powdered cellulose as an adsorbent to recover nitrate anion nutrient is adequately doable. However, it is highly dependent on the ratio of initial concentration and adsorbent dosage. The powder cellulose could further be improved by functionalizing it, in order to obtain a higher recovery percentage with a proper agreement between all four parameters, which is initial concentration, adsorbent dosage, temperature, and contact time.

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