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Response Surface Methodology Design and Optimization of Inorganic Phosphate Removal from Simulated Wastewater Effluent Utilizing *Caulerpa lentillifera* Algal Powder

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The excessive amount of phosphate is a critical issue in several countries since this leads to eutrophication that may result in algal bloom. Biosorption processes are currently gaining recognition to remove wastewater contaminants since the sorbent source can either be alive or dead, which, for the latter can be beneficial in terms of end-of-life utilization as well as cost. Response surface methodology (RSM) design was used to evaluate the effect of pH, contact time, initial phosphate concentration, and biosorbent dosage in the percent removal of inorganic phosphate from simulated wastewater with an alga (*Caulerpa lentillifera*) as the biological raw material. This method of experimental design concentrated on using a specific window for each parameter and obtaining the most optimal combination of these parameters by comparing their predicted and actual responses to better understand their interactive effects. The raw seaweed was subjected to drying and size reduction for it to become powder form. This algal powder used was characterized by Fourier Transform Infrared Spectroscopy to determine the functional groups present. The highest percent removal obtained in the experiment proper was 42.29 % on average. The data from the experiment proper was assessed by making use of a statistical analysis software, JMP® (SAS institute), and showcased about 45.46 % as the predicted optimum percent phosphate removal. Running the test based on the best parameter combination and comparing the actual percentage removal resulting from the validation provided only a 3.89 % difference to the predicted value of the software. Analysis of variance (ANOVA) was applied to the RSM results, and the predicted R^2 value, as well as that of the adjusted R^2 was found to have good interaction with each other with the difference between the values much less than 0.2.

1. Introduction

Water scarcity is a pressing global concern that poses significant challenges to sustainable development and the preservation of human health (Tarras and Benjelloun, 2017) and the environment. Contributing to this issue is the continual increase of untreated wastewater from agriculture, industries, and households, as well as urbanization, specifically in developing countries. As population grows and industrial activities continue to multiply, the demand for clean water has increased dramatically (van Vliet et al., 2021), while the availability of freshwater resources has become increasingly limited. Effective management strategies must be implemented to ensure the provision of safe and sustainable water supplies due to the lack of water resources that are easily utilized for an array of uses.

Phosphates, derived primarily from human activities such as agricultural runoff, industrial discharges (Smil, 2000), and domestic sewage (Omwene and Kobya, 2017), have become a widespread pollutant in freshwater systems (Richardson et al., 2021), posing significant environmental and health risks (Jiao et al., 2021). Extreme levels of phosphate concentrations can lead to eutrophication, a process that triggers harmful algal blooms and depletes dissolved oxygen, resulting in the degradation of aquatic ecosystems. To address these intertwined challenges of water scarcity and phosphate contamination, wastewater treatment is necessary in water resources management. Wastewater treatment processes not only offer the potential to recover and reuse water resources but also provide effective means to remove pollutants, including phosphates, from wastewater before it is discharged into the environment.

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Arumugam et al. (2018) stated that seaweed is largely explored and used for the treatment of wastewater as an adsorbent to replace the functional activated carbon. Generally, green, brown, or red seaweed can be used in many chemical experiments as an adsorbent and is widely used for wastewater treatment. According to a study conducted by Mithra et al. (2012), phosphate adsorption using brown algae is not simply explained using ion-exchange mechanisms. Phosphate groups undergo a substitution process, which is an exchange in existing hydroxide (OH-) groups bonded on the surface of the seaweed. Birungi and Chirwa (2018), utilized biological material, specifically algal sorbent, to remove toxic metals from wastewater and emphasized the fact that this method is emergent and environmentally friendly. This highlights the idea that algal sorbents may be explored in either heavy metal or inorganic contaminant removal. According to a study done by Rathod et al. (2014), in strongly acidic conditions (pH < 2), the neutral species (H_3PO_4) is dominant while $H_2PO_4^-$and $H_2PO_4^{2-}$ are the main species in weakly acidic to basic conditions (pH 2-9). The same study utilized *Kappaphycus alvarezii* algae and was able to remove phosphate with a maximum efficiency of 40% at pH 6, 80 min of contact time and 25 °C. With this, a pH value less than or equal to 6 is explored in this research. Several studies investigated long contact time, which is essentially not recommended when looking at costs; hence, investigating lower time values was looked into in this study. With the same concern on expenses, a lowered range of values from Rathod (2014) for the adsorbent dosage was used. Novel to this study is the use of *Caulerpa lentillifera* specific to inorganic phosphate removal.

In the Philippines, the Department of Environment and Natural Resources (DENR) has set Water Quality Guidelines (WES) and General Effluent Standards (GES). With the most recent update, referring to DENR Administrative Order (DAO) 2021-19, the Total Phosphate concentration that is allowable in either freshwater or marine water falls between 0.025 to 0.4 mg/L depending on the water body classification as set by the DENR. According to Sivaraos et al. (2014), the utilization of Design of Experiments (DOE) has been proven to be effective by several studies in terms of making One-Factor-At-A-Time (OFAT) type experiments less expensive and more accurate and precise due to statistical approaches. Response Surface Methodology (RSM), a subset of DOE, is a viable approach in terms of biosorption since this will utilize a range of parameters and obtain the optimal value within those parameters based on actual responses. Central Composite Design (CCD) is a design that can be employed to work on a quadratic model. It is often used when the design requires sequential experimentation. Box-Behnken design (BBD) is a design that uses fewer design points than CCD which makes them less expensive to run under the same factors, but it does not include runs from a factorial experiment. Unlike CCD, BBD never include runs where all factors are at their extreme setting, such as all the low settings, which limits the optimization values of the design. For this study, RSM CCD was used since there are no research employing this method in analyzing inorganic phosphate removal.

2. Materials and Methods

2.1 Biosorbent Preparation

Caulerpa lentillifera was bought from Biñan, Laguna, Philippines. The pre-treatment procedure of the biosorbent was adapted from the study of Rathod et al. (2014) with modifications. The alga was washed thrice with running water and four times with distilled water. It was then sundried for about 2 weeks prior to grinding. The powder formed is sieved to a size of 0.50 mm and stored in an airtight glass container.

2.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed on the algal powder to determine the different functional groups present using Shimadzu UV-Visible Spectrophotometer UV-2600 from De La Salle University.

2.3 Simulated Wastewater Preparation

Potassium Dihydrogen Phosphate (KH_2PO_4) powder and distilled water were used to prepare the simulated wastewater. Three different KH_2PO_4 concentrations were prepared: 0.53, 0.73, and 0.93 mg/L. The concentrations were chosen with an initial basis on the inorganic phosphate concentration in Cabuyao River as reported for Quarter 1 of the year 2019 range (LLDA, 2019) with adjustments both at the lower and upper values for a wider analysis.

2.4 Biosorption Experiments

The methodology in biosorption experiments was adapted from Rathod et al. (2014) with several variations. All the experiments were conducted at room temperature. Each of the batch trials was set up primarily considering 2 levels (low and high) of these factors or parameters: pH, contact time, initial phosphate concentration, and biosorbent dosage, as seen in Table 1. Mid-points between the 2 levels were also added for better data analysis.

Table 1: CCD factors and levels controlled in the biosorption experiments.

Parameters	-1	1
Biosorbent dosage, g	0.300± 0.001	1.000± 0.001
Initial Phosphate concentration, mg/L	0.53	0.93
Contact time, min	20	120
pH	2	6

To statistically design the experiments and consider the interactive effects of the four independent parameters for optimizing the biosorption, a full factorial CCD was utilized. In general, the total number of runs required for CCD is ($2^k + 2k + N_0$), where k is the number of factors, 2^k are factorial points, 2k are axial points, and N_0 is the number of experiments carried out at the center (Verma et al., 2016). Considering 4 factors and 2 experiments in the center, it resulted to 26 runs and each run is subjected to 2 trials.

For each run, a 30-mL aliquot of simulated wastewater was placed in a 50-mL beaker. The biosorbent was added to the beaker, and pH was adjusted as needed using hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions. The beaker is then placed on a magnetic stirrer with the speed set at 195 rpm for a specified duration equivalent to the contact time per setting. The final phosphate concentrations were measured using a HANNA Phosphate colorimeter (HI774 Checker® HC).

3. Results and Discussion

3.1 FTIR Result

The IR spectra (%Transmittance vs. Wavelength) as illustrated in Figure 1 showcased that the *C. lentillifera* algal powder has a high presence of alcohol and carboxylic acid (R-COOH) groups, followed by Alkanes (C-H), Alkenes (C=C) and Halides (C-Cl) groups. This corresponds to the general idea that seaweed possesses a wide surface area of available electronegative sites, given the high level of present hydroxyl sites from the alcohol groups. The peaks 1,405 cm^{-1} and 1,640 cm^{-1} represent the absorption of amide band II and amide band I from proteins in line with the study of Ji et al (2020), with the former peak showing N–H bending coupled with C–N stretching and the latter exhibiting C=O stretching. As stated by Ruoff et al (2006), the absorption peaks in the regions ranging from 1,250 to 900 cm^{-1} in the IR spectrum are made by the C–O and C–C groups which are characteristic absorption peaks mainly of saccharides.

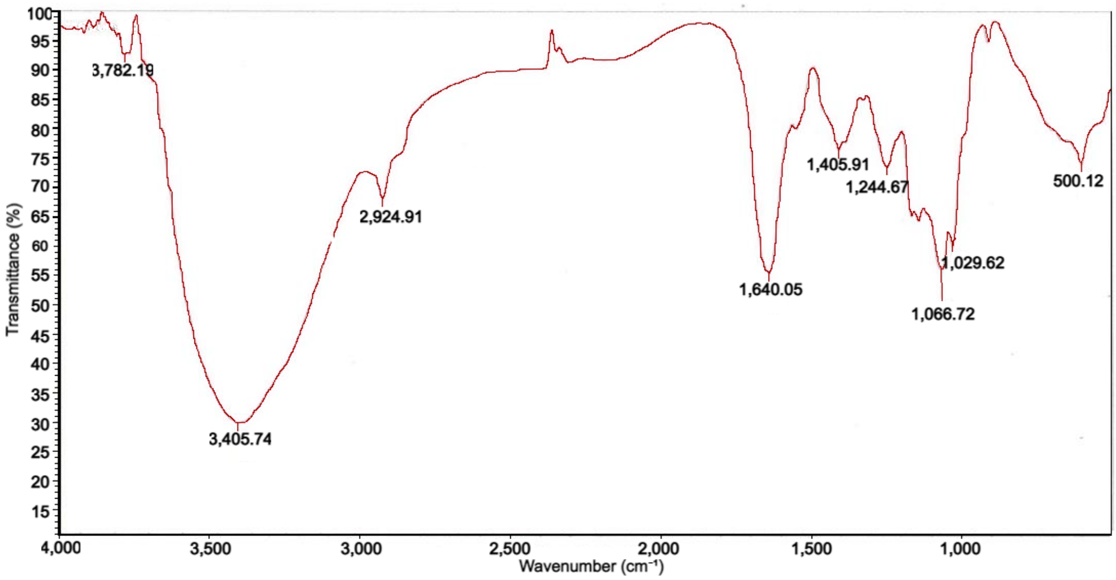

Figure 1: FTIR characterization of C. lentillifera algal powder

3.2 Biosorption Experiments Result

The percent phosphate (%P) removal was computed using Eq(1), where C_i and C_f are the initial and final phosphate concentrations of the solution in mg/L. The parameter combinations for each run, including the resulting %P removal, are summarized in Table 2. All gathered data were encoded in JMP® (SAS institute) and processed accordingly to determine the ideal parameter combination that would yield the optimum %P removal using the software's prediction profiler.

$$\%P\ removal = \frac{(c_i - c_f)}{c_i} \cdot 100 \tag{1}$$

The results in Table 2 show that the lowest removal is 2.35 %, with its duplicate run at 7.54 % at the parameter values of 1 g dosage, 20 min contact time, pH of 2, and initial phosphate concentration of 0.93 mg/L. This can be due to not having enough active binding sites within the said contact time at low pH or due to the abundance of hydroxyl ions that replaces active binding sites faster than phosphate binding to it. The highest %P removal is 56.24 % while, its duplicate only showed 34.12 %, hence, it was ruled out as an outlier value. The resulting second highest %P removal value is 41.95 %, with its duplicate at 42.62 % given the parameters of 0.3 g adsorbent dosage, 120 min contact time, pH 2, and an initial phosphate concentration of 0.93 mg/L. This setting was considered to have the highest %P removal. Comparing this with the run that got the lowest %P removal, the contact time is higher, although the adsorbent dosage is about 70 % lower. The reduction in phosphate ion removal at higher pH is due to higher amount of hydroxyl ions in the mixture that competes with the PO_4^{3-} ions for the biosorption sites. This is supported by a study of Vikrant et al. (2017) which stated that hydroxyl ions are utilized through the surface complexation reaction happening on the surface of the adsorbent where surface hydroxyl groups dissociate or are substituted with the phosphate groups. Higher pH value also causes the dissociation of functional groups (-COOH, -OH, etc) of the biosorbent, rendering the seaweed unable to carry more negative charges, which repel phosphate ions from the surfaces, thus reducing phosphate biosorption.

Table 2: Experimental Matrix and %P removal results

Setting	Adsorbent Dosage, g	Initial Phosphate Concentration, mg/L	Contact Time, min	pH	%P removal (Trial 1)	(Trial 2)
1	0.3	0.53	20	2	6.48	8.75
2	0.3	0.53	20	6	23.3	21.85
3	0.3	0.53	120	2	37.54	38.26
4	0.3	0.53	120	6	20.64	21.32
5	0.3	0.73	70	4	22.31	23.12
6	0.3	0.93	20	2	3.65	4.12
7	0.3	0.93	120	2	42.62	41.95
8	0.3	0.93	20	6	11.34	12.32
9	0.3	0.93	120	6	31.85	32.64
10	0.65	0.93	70	4	20.84	21.15
11	0.65	0.53	70	4	22.64	23.85
12	0.65	0.73	70	2	25.26	26.77
13	0.65	0.73	20	4	18.74	19.21
14	0.65	0.73	70	4	32.45	35.42
15	0.65	0.73	70	4	30.12	33.52
16	0.65	0.73	70	6	28.74	27.95
17	0.65	0.73	120	4	38.21	39.14
18	1	0.93	20	6	56.24	34.12
19	1	0.93	120	2	12.43	14.62
20	1	0.93	120	6	28.12	34.12
21	1	0.53	20	2	34.62	35.34
22	1	0.53	120	2	39.85	40.52
23	1	0.53	20	6	20.13	19.85
24	1	0.53	120	6	22.81	23.45
25	1	0.73	70	4	38.62	39.15
26	1	0.93	20	2	2.35	7.54

3.3 Regression Data

The coefficient of determination (R^2) of the model is 0.8804. The RSM Analysis of Variance (ANOVA) exhibited a suitable positive interaction between predicted and adjusted R^2 values, as seen in Table 3.

Table 3: Post-regression data of percent removal obtained from JMP®.

Std. Dev.	Mean	CV (%)	R^2	Adjusted R^2	Predicted R^2
6.13	25.38	24.14	0.8804	0.8770	0.8690

The predicted R^2 is 0.8690 while the adjusted R^2 is 0.8770, and according to StatEase® (2022), the predicted and adjusted R^2 values must be within 0.20 difference with each other for the values to be in realistic concurrence.

3.4 Modeled Equation

An empirical relation between %P removal and significant parameters can be calculated using Eq(2) which is a second-order polynomial function.

$$Y = 29.452 + 2.773\left(\frac{A-0.65}{0.35}\right) - 1.341\left(\frac{B-0.73}{0.2}\right) + 6.115\left(\frac{C-70}{50}\right) + 1.379\left(\frac{D-4}{2}\right) - 1.543\left(\frac{B-0.73}{0.2}\right)\left(\frac{A-0.65}{0.35}\right)$$
$$- 5.290\left(\frac{C-70}{50}\right)\left(\frac{A-0.65}{0.35}\right) + 1.019\left(\frac{C-70}{50}\right)\left(\frac{B-0.73}{0.2}\right) + 1.865\left(\frac{D-4}{2}\right)\left(\frac{A-0.65}{0.35}\right) + 5.609\left(\frac{D-4}{2}\right)\left(\frac{B-0.73}{0.2}\right) \quad (2)$$
$$- 4.661\left(\frac{D-4}{2}\right)\left(\frac{C-70}{50}\right) + 2.490\left(\frac{A-0.65}{0.35}\right)^2 - 6.569\left(\frac{B-0.73}{0.2}\right)^2 + 0.515\left(\frac{C-70}{50}\right)^2 - 1.507\left(\frac{D-4}{2}\right)^2$$

where Y = %P removal, A = adsorbent dosage, B = initial phosphate concentration, C = contact time, and D = pH. The synergistic effect of the components and interactions is indicated by the positive sign of the coefficients, whereas the antagonistic effect is denoted by the negative sign of the coefficients (Kanafin et al., 2022). In this case, an increase in adsorbent dosage, contact time, and pH had a positive effect on the %P removal.

3.5 ANOVA Results

According to Mohammed (2020), pairing the F-value with the p-value determines how significant the values are in the design. The design showed an F-value of 12.51 and a p-value of less than 0.0001 for the model, therefore making the design a good fit to the experiment performed. The ANOVA results are presented in , and it shows that parameters in Eq(2) of AB, AC, AD, BD, and CD have significant interactions, while interactions of BC have a less significant effect on the biosorption experiment. This less significant interaction result is aligned to the findings by Rathod et al. (2014) that mostly the dosage and pH are the parameters that affect the %P removal.

Table 4: ANOVA results from JMP®.

Source / Block	F-value	p-value	
Model	12.51	<0.0001	significant
AB	10.95	0.0041	
AC	28.13	<0.0001	significant
AD	16.17	0.0009	
BC	1.30	0.2704	
BD	16.88	0.0007	
CD	23.87	0.0001	

3.6 Validation of Optimum Parameter Setting

Using the JMP® prediction profiler to analyze and interpret the data, an ideal setting for the four parameters was found that would reportedly result in the optimal %P removal of 45.46 %. The parameter settings were 0.3 g of adsorbent dosage, initial phosphate concentration of 0.66 mg/L, contact time of 120 min with pH of 2. This parameter combination was validated using the same method of previously performed biosorption experiments. Actual %P removal results were computed as 43.57 % and 43.81 % for the initial and the duplicate run of the optimal parameter setting. The average of these is 43.69 % which has about 3.89 % difference when compared to the predicted %P removal of the software. This means that the %P removal using *C. lentillifera* algal powder did not fall too far off its predicted value at a percentage difference below 5 %.

4. Conclusions

In this study, phosphate removal was investigated using biosorption onto *C. lentillifera* algal powder. The FTIR results also showed the presence of hydroxyl groups confirming its ability to adsorb phosphate. The process was concentrated on the influence of operating variables such as adsorbent dosage, initial phosphate concentration, pH, and contact time using RSM CCD. Furthermore, the interaction between parameters was investigated, and the ANOVA results indicated that there are interactions between the majority of the parameters.

The adjusted and predicted R^2 were 0.8770 and 0.8690, respectively, showing that the actual and predicted data is a good fit to the model. And the optimum parameters predicted by JMP® established that a 45.46 %

predicted %P removal is observed to be nearly accurate, with the average validation result of 43.69 % having a percentage difference of only 3.89 %.

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