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Evaluation of a Causative Species of Harmful Algal Blooming, *Prorocentrum triestinum*, as a Sustainable Source of Biosorption on Cadmium

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Abstract: Biosorption is an effective method for removing heavy metal ions from wastewater. In the current study, the biosorption capacity of a microalgae *Prorocentrum triestinum* strain *AD1* was investigated for cadmium removal. The efficient biomass concentration was found to be 5 g/L. Based on the Langmuir adsorption model, the maximum adsorption capacity (q_{max}) value of cadmium removal was found to be 0.0196 mmol/g. The investigation results of the *AD1* biosorption kinetics showed that the effective contact time on biosorption was 3 h, and the adsorption kinetics fitted well with the pseudo-second-order model. The optimum pH of biosorption was found to be 5. On the other hand, HCl could act as an efficient desorbent for cadmium recovery from AD1, with an optimum concentration of 0.01 M. These results suggest that the biomass of *P. triestinum* has great potential for the removal of cadmium from wastewater as an efficient biosorbent.

Keywords: biosorption; dinoflagellate; harmful algal blooms; *Prorocentrum triestinum*

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1. Introduction

Heavy metal contamination in aquatic systems is nowadays one of the most serious environmental pollution problems. Heavy metal pollutants are produced and released into the environment by industrial activities. In Hong Kong, the typhoon shelters in Victoria Harbour often show poor water quality, and the sediments are contaminated by heavy metals because of the emission of effluents containing cadmium from industries such as the textile, printed circuit board and electroplating industries [1]. Biosorption is an environmental biotechnology that uses biomaterials, such as bacteria, yeast, fungi and microalgae, instead of conventional chemicals, to remove heavy metals from aqueous solutions efficiently [2,3].

The industrial uses of cadmium (Cd) include electroplating, silver-cadmium batteries and stabilizers for plastics. Wastewater from electroplating, plastic industries and battery manufacturing plants are major sources of cadmium in marine environments [4]. Cadmium and other heavy metals, e.g., mercury, chromium, lead, arsenic and thallium, are highly toxic to humans and have the greatest potential hazard to the environment [4]. In recent years, biosorption has been widely studied for the removal of heavy metals from aqueous solutions [3,5]. The mechanisms of biosorption involve simultaneous surface sorption, partitioning processes and chemical reactions. Biosorption and bioaccumulation are two different processes. Biosorption is a process whereby sorbates are passively adsorbed onto dead biomass and is based on the 'affinity' between sorbates and the biosorbent, while bioaccumulation is an active process whereby live biomass absorbs contaminants into their cells [6]. Biosorption is a reversible process that occurs at a faster rate and produces a higher recovery of pollutants. Therefore, biosorption is preferable to bioaccumulation for the bioremoval of heavy metals. It has been found that various biomaterials, including

bacteria, yeast, fungi and microalgae, can be used to reduce the heavy metal concentrations in aqueous solutions [2].

Since metal-bearing wastewater is usually of a large volume, the use of abundant and inexpensive biomaterials as biosorbents in the removal process shall effectively reduce the cost of wastewater treatment. The performance of various marine and freshwater microalgal species in removing heavy metals has been elucidated in recent years [7,8]. Most of the studies focus on green microalgae; however, dinoflagellates, as a group of ecologically important phytoplankton, have scarcely been explored [8]. In this study, an armored dinoflagellate *Prorocentrum triestinum* strain *AD1* was selected to evaluate its effectiveness in the removal of Cd(II) from waters. The potential application of *AD1* in Cd(II)-containing wastewater treatment was also explored.

2. Materials and Methods

2.1. Microalgal Cultivation

A monoculture of the *Prorocentrum triestinum* strain *AD1* was obtained from the Environmental Laboratory of Hong Kong Metropolitan University, and the culture was maintained as previously described [9,10]. f/2-Si media in synthetic seawater were used for strain cultivation [11]. It was prepared by adding 90.5 g of sea salt (Instant Ocean® Sea Salt, Instant Ocean, Blacksburg, VA, USA) into 2.5 L of double-distilled water to reach a salinity of 25 parts per 1000 (salinity was routinely checked using a refractometer) and stored at 4 °C until use. The synthetic seawater was autoclaved before subculture. The f/2 medium [11] required nutrients were added to the autoclaved synthetic seawater aseptically. All chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA) unless otherwise stated. *P. triestinum* strain *AD1* was cultivated in 8 L NALGENE® polycarbonate carboy and was transferred to a new medium weekly at a ratio of 1:10 *v/v*. The algal cells were cultivated in a Conviron growth chamber at 25 °C and under a 12/12 h light/dark cycle. The light intensity was 120 μE/m^2/s.

2.2. Preparation of Biosorbent

The microalgal cells were centrifuged in a centrifuge (Eppendorf® 5804, Eppendorf, Hamburg, Germany) with 4000 rpm for 5 min. After discarding the supernatant, the concentrated biomass was dried in the oven at 70 °C to obtain the dry biomass and finally weighed.

2.3. Preparation of Cadmium Solution

For the determination of heavy metal concentrations, a flame atomic absorption spectrometer (AAS, HITACHI Z-5000, Berkshire, UK) was used. The cadmium (Cd) 100 ppm solution was prepared with 10 mL of 1000 ppm stock solutions marked up with deionized water.

2.4. Biosorption Equilibrium Experiments

Samples were prepared with the *AD1* algal biomass with a heavy metal solution, where the sample and blanks were prepared with the *AD1* biomass with an f/2-Si medium, *AD1* biomass with deionized water and a blank heavy metal solution. These sets of solutions were treated at 250 rpm at 27 °C by a shaking incubator (GALLENKAMP®, Cambridge, UK). After 24 h or other time intervals, the supernatant was extracted from the samples with a pipette for AAS determination. Algal biomass was spun down by a centrifuge at 5000 rpm for 5 min. The supernatant of each sample was transferred to a clean centrifuge tube and followed by AAS determination. The following equation was used to calculate the maximum adsorption capacity (q_{max}) [5,12]:

$$\frac{C}{q} = \frac{C}{q_{max}} + \frac{1}{k \cdot q_{max}}$$

q_{max}: the maximum adsorption capacity (mmol/g); k: the Langmuir adsorption constant (m^3/mg); C: the equilibrium concentration (mg/m^3).

2.5. Cadmium Removal Kinetics

The pseudo-first-order model and pseudo-second-order model [13] were applied to study the cadmium removal kinetics. The kinetic equation of the pseudo-first-order model is shown as below [14]:

$$\ln(q_e - q) = \ln q_e - k_1 t$$

q_e: equilibrium metal concentration in the solid phase; q: metal concentration in the solid phase at time t.

The kinetic equation of the pseudo-second-order model is shown below [13,14]:

$$\frac{t}{q} = \frac{t}{q_e} + \frac{1}{k_1 q_e^2}$$

q_e: equilibrium metal concentration in solid phase; q: metal concentration in solid phase at time t.

2.6. Desorption Experiments

Volumes of 20 mL of desorbents were added into 50 mL conical flasks and mixed well with 5 g/L metal-loaded *AD1* algal biomass. These samples were treated at 250 rpm at 27 °C by a shaking incubator (GALLENKAMP®, Cambridge, UK). After 1 h, samples were spun down by the centrifuge at 5000 rpm for 5 min, and the supernatant of each sample was then transferred to another clean centrifuge tube and followed by AAS determination.

2.7. Desorption Studies of Cd

For desorbing heavy metal ions from metal-loaded *P. triestinum* biomass, various types of desorbents for the screening experiment were investigated: 0.1 M NaOH, 0.1 M HNO_3, 0.1 M HCl and 0.1 M EDTA. The desorption efficiency (% of metal recovery) was determined as follows:

$$\% \text{ of Metal Recovery} = \frac{[Cd]_{des}}{[Cd]_{ads}} \times 100\% \quad (1)$$

where $[Cd]_{des}$ is the amount of cadmium desorbed (ppm), and $[Cd]_{ads}$ is the amount of cadmium adsorbed (ppm).

2.8. Statistical Analysis

All data were from three independent replicates (n = 3) at the 5% significance level and are presented as the mean ± standard deviation.

3. Results and Discussion

3.1. Influence of Biomass Dosage on Cadmium Biosorption

The tendency of the percentage of Cd removal increased following the higher algal biomass concentration and eventually tended toward 100% metal removal (Figure 1). The heavy metal ion removal rate of *AD1* biomass increased from 68.9% of 0.5 g/L to 92.7% of 5 g/L, and the removal efficiency increased to 97.2% when algal biomass reached 25 g/L. Therefore, the optimum algal biomass dose was found to be 5 g/L, which was selected for the rest of the experimental studies. On the other hand, the removal percentage of the 7.5 g/L biomass dosage was less than the 5 g/L dosages by approximately 1.3%. However, the expected result was that the removal percentage of the 7.5 g/L biomass concentration should be higher than that of 5 g/L. The actual result might have been due to the various *AD1* biomass conditions, such as the cell density of the biomass and the integrity of each cell in the biomass, illustrating that the broken cell in the biomass may affect the adsorption ability. In summary, it can be considered that the Cd removal depended on the

concentration of microalgal biomass used in the adsorption environment. The efficiency of removal performance increases with the number of adsorption sites [15]. The design and planning of the following testing concentrations were based on these results.

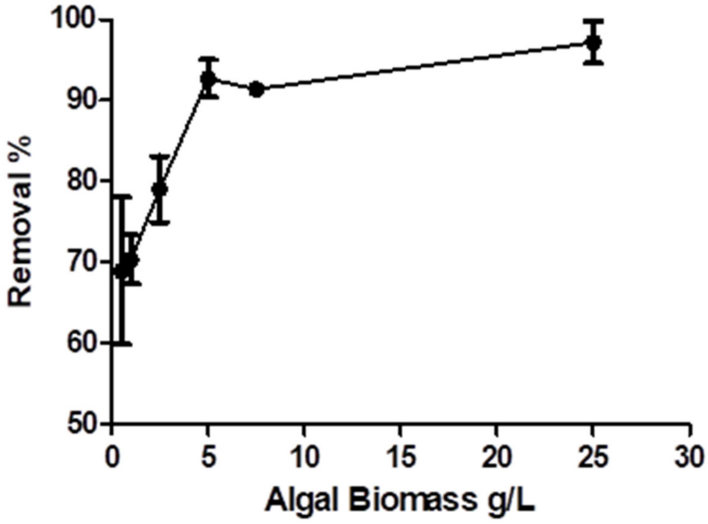

Figure 1. Influence of biomass dosage on Cd removal (initial concentration: 4 mg/L; pH: 4.0; temperature: 27 °C; contact time: 24 h).

3.2. Sorption Isotherms

The equilibrium isotherm model can be utilized to explain and predict the experimental behavior. In this study, the Langmuir isotherm of the single-sorbate isotherms was used. This isotherm was obtained by plotting the sorption capacity q (mmol/g) versus the equilibrium metal concentration (mg/L) (Figure 2). Thus, the maximum adsorption capacity (q_{max}) can be calculated. The q is expressed as the number of binding sites occupied by the sorbate at the concentration (C). In these relationships, q_{max} was calculated, which indicated the maximum sorbate uptake under the given conditions. The q_{max} showed the maximum adsorption as mmol/g based on the Langmuir adsorption model. It correlated with the covering or adsorption of molecules on a solid surface to the concentration of the medium above a solid surface at a fixed temperature. It is also assumed that all sorption sites are uniform and that there is a fixed number of adsorption sites at equilibrium.

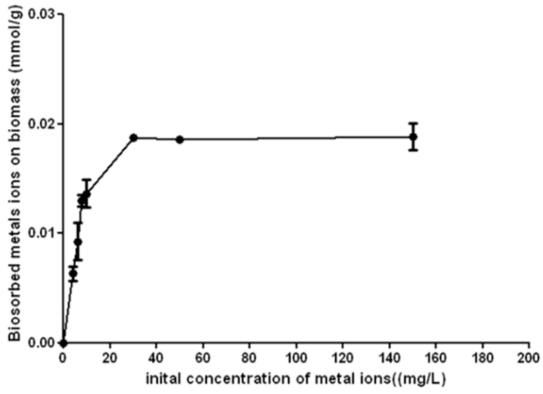

Figure 2. Adsorption isotherm of cadmium on microalgal biomass (pH: 4.0; temperature: 27 °C; biomass dosage: 5 g/L).

The Langmuir isotherm was obtained by plotting the sorption capacity q value (mmol/g) versus the equilibrium metal concentration (mg/L). The ratio of the metal concentration over the q value (C/q) was plotted versus the concentration (C) to fit the experimental results into a straight line. Based on the linear plot of the Langmuir equation,

q_{max} and k can be obtained according to the formulation introduced in Refs [5,12]. Table 1 summarizes the preliminary findings of the adsorption isotherm. The fit of experimental data to a Langmuir model was assessed by the regression coefficient R^2. The closer the value to 1, the better the sorption isotherm fit into this model. In this study, the sorption isotherm was well suited for the Langmuir isotherm, since the R^2 value was found to be 0.9979 (Table 1), illustrating that the Langmuir model would be applied to this system.

Table 1. Summary on the adsorption isotherm.

Metal Ions	Calculated Langmuir Constant		
Cadmium	q_{max} (mmol/g) 0.0196	k (m^3/mg) 4.5260	R^2 0.9979

Based on the experimental findings, the adsorption ability on the cadmium of *P. triestinum* strain *AD1* can be compared with other microalgal biosorbents (Table 2). The q_{max} value of *P. triestinum is* higher than *Spirulina* sp., which is a blue green algal species, indicating that the adsorption ability of *P. triestinum* is higher than that of *Spirulina* sp. However, another blue green alga *Spirulina platensis* and two green algae *Codium fragile* and *Chlorella* sp. show a higher Cd adsorption ability than *AD1* (Table 2). The adsorption capacity of AD1 is not the highest compared to other algae. However, culturing other microalgal species incurs costs, with respect to land, containers and media, while high amounts of AD1 can be obtained 'for free' from 'natural' harmful algal bloom events (assuming it is relatively less expensive to collect this red tide species from the sea). In addition, salvaging the red tide algae out of the water can also prevent secondary disasters, such as hypoxia in seawater, caused by the decomposition of red tide algae by marine bacteria after the red tide outbreak. All these favorable factors can be incentives for further study of dinoflagellate algae as adsorbents.

Table 2. Maximum adsorption capacity of *AD1* and other biosorbents.

Biosorbent	Category	Maximum Adsorption Capacity q_{max} (mmol/g)	References
Procentrium triestinum (*AD1*)	Dinoflagellate	0.018	This study
Spirulina sp.	Blue green algae	0.012	[16]
Spirulina platensis	Blue green algae	0.072	[17]
Codium fragile	Green algae	0.083	[18]
Chlorella sp.	Green algae	0.193	[19]

3.3. pH Dependence of Biosorption

The pH value is one of the most crucial parameters to determine the effect of biosorption. As the biosorption describes the ion exchange from the binding places with heavy metals, the variation in the pH value may influence the uptake capacity. There are a variety of functional groups on the cell surface, for example, the carboxylic group, which means pH dependence of the biosorbent is largely related to their surface functional groups [20–22].

The result of the adsorption capacity of the biosorbent at pH 2 was found to be 0.080 mg/g, which was significantly increasing with the increase in pH until pH 5 (0.740 mg/g) (Figure 3). The increasing biosorption ability was mainly due to the decrease in hydrogen ions (H^+), which compete for the binding sites with heavy metal ions (Cd).

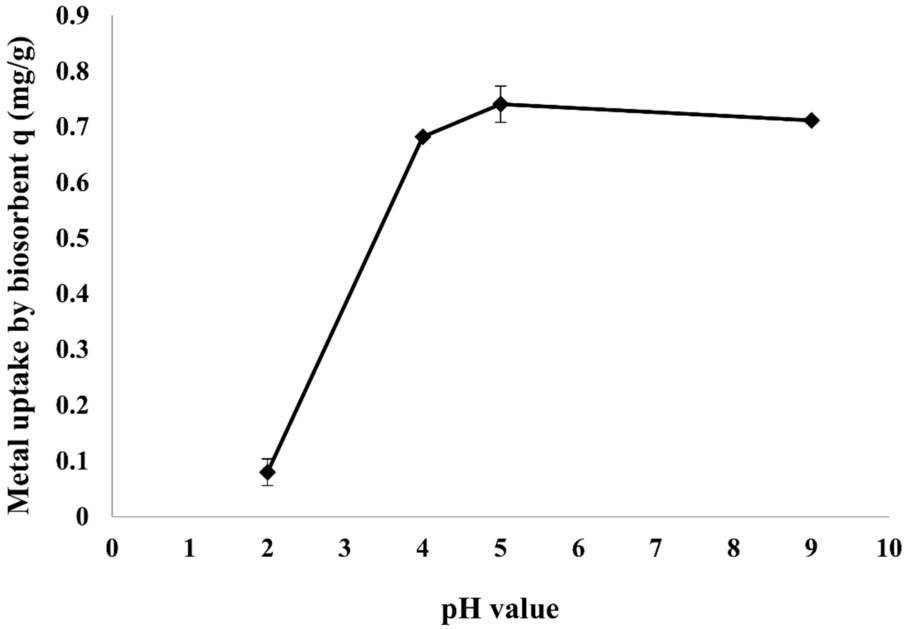

Figure 3. Effect of pH on the biosorption of Cd (pH: 4.0; temperature: 27 °C; biomass dosage: 5 g/L; time: 3 h).

At pH 2, the increasing concentration of H^+ ions led to a decrease in binding sites in the biosorbent for cation sorption. Therefore, the Cd biosorption ability was decreased. There is a competition between Cd^{2+} ions and H^+ ions for the binding sites on the cell wall. At the same time, most of the carboxylic groups may not be dissociated at a lower pH, so they may not bind with heavy metal ions in the medium [21].

As the pH increased (pH 5), more ligands were available for heavy metals, and the adsorption capacity of the biosorbent was then increased. The following equation shows the relationship between hydrogen ions, cadmium ions and ligands.

$$Cd^{2+} + 2HL \quad \leftrightarrow \quad [CdL_2] + 2H^+ \tag{2}$$

L: ligands on the surface of the *AD1* cell wall.

The cadmium biosorption ability of the biosorbent increased from pH 2.0 to 5.0 and then declined with a further increase in pH (pH 9). The adsorption capacity of the biosorbent at pH 9 was found to be 0.711 mg/g (Figure 3). The biosorption ability of the biosorbent decreased due to the formation of the insoluble compound $Cd(OH)_2$. From this investigation, the greatest biosorption capacity was obtained at pH 5. Therefore, the optimum pH value for the biosorption of cadmium was found to be pH 5.

The metal solution chemistry and the functional groups on cell walls of the biosorbent were affected by the pH of the medium [21,22]. These functional groups can justify the pH dependency on the metal ions uptake by the biosorbent by association and dissociation. These functional groups on a cell wall in a different pH range determine the extent of biosorption. Different types of microalgae have different functional groups for heavy metal biosorption. The differences between functional groups lead to a variation in the optimum pH of different species, as shown in Table 3.

Table 3. Comparison on optimum pH of cadmium sorption among different algal species.

Metal Ion	Optimum pH	Algal Species	Reference
Cadmium (Cd)	5	*Procentrium triestinum*	This study
Cadmium (Cd)	4–6	*Gracilaria Fisheri*	[13]
Cadmium (Cd)	6	*Scenedesmus obliquus*	[23]
Cadmium (Cd)	6	*Didymogenes palatina XR*	[24]
Cadmium (Cd)	7	*Spirulina* sp.	[20]

3.4. Kinetic studies of Cadmium Removal

Kinetic studies of metal biosorption focus on the heavy metal biosorption equilibrium between the residence time and the reactor dimensions [13]. Most of the studies have found that the uptake kinetics profiles are an initial rapid decrease in metal followed by a subsequent slower and insignificant reduction. In order to distinguish which factor is dependent, the pseudo-first-order model and pseudo-second-order model are applied [13]. The pseudo-first-order model is a kinetic equation based on the adsorption capacities of the biosorbent. The parameters q_e and k_1 can be determined by using plots $\ln(q_e - q)$ against t. The calculated q_e values are then compared with the experimental results. If there is a large difference between these two values, the reaction cannot be regarded as a pseudo-first-order model, and even high regression coefficients can be obtained. The pseudo-first-order model is utilized in many biosorption kinetic studies, but it may apply only to the initial period of the biosorption process.

The pseudo-second-order model is a kinetic equation dependent on the sorption rate at available sites. The q_e and k_2 parameters can be obtained by using plots t/q against t. It was noted that, in batch reactors, the pseudo-second-order model is more suitable than the pseudo-first-order model for representing the kinetic profiles of the biosorption processes [25]. Most of the regression coefficients were higher than 0.98, and the calculated q_e values were well fitted with the experimental results [25].

The Cd(II) uptake kinetics for the algal biomass was investigated, as shown in Figure 4. Two phases can be observed on the kinetic profile. The first phase was non-linear and was characterized by a rapid uptake in the first 3 h. The Cd(II) concentration in the metal solution was quantitatively reduced. The equilibrium phase was almost reached at about 5 h; the amount of metal uptake did not change significantly subsequently. Therefore, the maximum adsorption took place within the first 3 h. A linear plot of ln $(q_e - q_t)$ versus t shows the slight applicability of first-order kinetics, and the plot of t/q_t versus t gives a straight line. This indicates that second-order kinetics is applicable. Table 4 lists the results of rate constant studies with the pseudo-first-order and pseudo-second-order models. In the pseudo-second-order adsorption model, the value of the correlation coefficient R^2 is much higher (0.999), and the calculated adsorption capacities (q_e) fitted very well with experimental ones. On the other hand, the value of R^2 and the calculated adsorption capacities (q_e) for the pseudo-first-order are not so satisfactory (0.528). Therefore, it can be concluded that pseudo-second-order adsorption model would be more appropriate to describe the Cd adsorption kinetics over microalgal biomass.

A comparison between the results from this experiment and others found in the literature is shown in Table 5. The effective contact time on Cd of *P. triestinum* is shorter than *Mucor rouxii*, which is a fungi species, indicating that the adsorption rate of *P. triestinum* is faster than *Mucor rouxii*. However, the effective contact time on Cd of *P. triestinum* is longer than *Gracilaria fisheri* (a red seaweed). Therefore, *G. fisheri* has the fastest adsorption rate in this comparison and has the best adsorption efficiency.

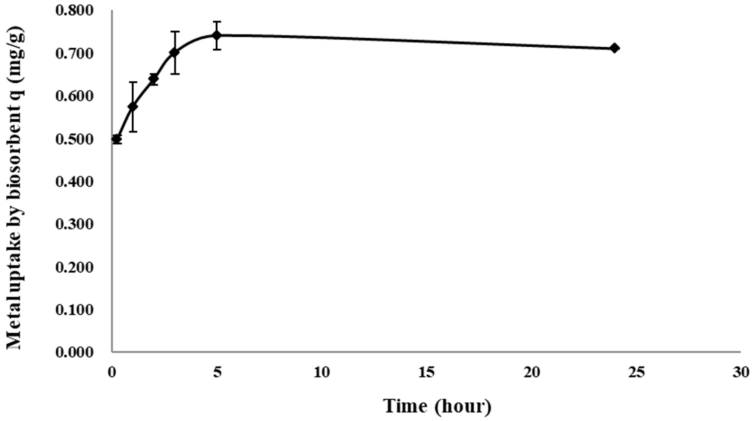

Figure 4. Effect of contact time on biosorption of Cd (biomass dosage: 5 g/L; Cd initial conc.: 4 ppm; temperature: 27 °C; pH: 5.0).

Table 4. Comparison between adsorption rate constants (k), the estimated q_e and the coefficient of correlation (R^2) associated with the pseudo-first-order and pseudo-second-order adsorption.

Experimental q_e (mg/g)	Pseudo-First-Order Model			Pseudo-Second-Order Model		
	k_1 (/min)	$q_{e,\,cal}$ (mg/g)	R^2	k_2 (g/mg/min)	$q_{e,\,cal}$ (mg/g)	R^2
0.7407	0.0011	0.129	0.528	0.224	0.714	0.999

Table 5. Comparison of effective contact time with other biosorbents on Cd.

Type of Biosorbent	Category	Effective Contact Time (h)	Reference
Prorocentrum triestinum	Dinoflagellate	3	This study
Gracilaria fisheri	Red alga	1	[13]
Mucor rouxii	Fungi	6	[26]

3.5. Desorption Studies of Cd Adsorbed on Biomass

A suitable desorbent shall be non-damaging to biomass, environmentally friendly and cost effective [27]. A screening experiment is one of the methods for identifying a suitable desorbent for the desorption process. The percentage of Cd recovery represents the desorption efficiency of each desorbent. A higher percentage of Cd recovery means that such desorbent has a high efficiency of desorption and is effective for Cd recovery.

As shown in Table 6, 0.1 M HCl has the highest yield on Cd recovery, which is a 90.7% total cadmium content desorbed from *AD1* algal biomass by HCl. Both 0.1 M HNO_3 and 0.1 M EDTA solutions also have high yields on Cd recovery—87.0% and 80.7% total cadmium content desorbed, respectively. However, 0.1 M NaOH only has 22.8% total cadmium content desorbed. Predictably, deionized water has a very low yield on Cd recovery (0.7%), which is probably not involved in the desorption process. The result illustrates that NaOH and deionized water have low efficiencies for replacing or eluting Cd^{2+} ions from *AD1* algal biomass. This may show that replacing hydroxyl ion (OH^-) with algal biomass was not the dominant process of the desorption process.

Table 6. Metal recovery of different desorbents.

Desorbent	HCl (0.1 M)	HNO_3 (0.1 M)	EDTA (0.1 M)	NaOH (0.1 M)	Deionized Water
Recovery	90.7%	87.0%	80.7%	22.8%	0.7%

Based on the experimental result, 0.1 M HCl has the highest yield on Cd recovery. Therefore, HCl was found to be an effective desorbent for Cd recovery. The desorption

ability of hydrochloric acid (HCl) was then investigated. The performance of three concentrations of HCl—0.01 M, 0.10 M and 1.00 M—was determined and is shown in Figure 5. The Cd adsorption capacities of the biosorbent were similar in percentage (79.05%, 80.63% and 80.86%) in the previous biosorption experiment. The Cd removal abilities of 0.01 M, 0.10 M and 1.00 M HCl were found to be 88.06%, 91.44% and 92.12%, showing an increasing trend with increasing HCl concentration. It was found that the highest removal ability was 92.12% using 1.00 M HCl. A higher concentration of HCl leads to a better desorption ability, since a higher concentration of HCl can provide a higher ability to bind with cadmium ions that are bound with the biomass.

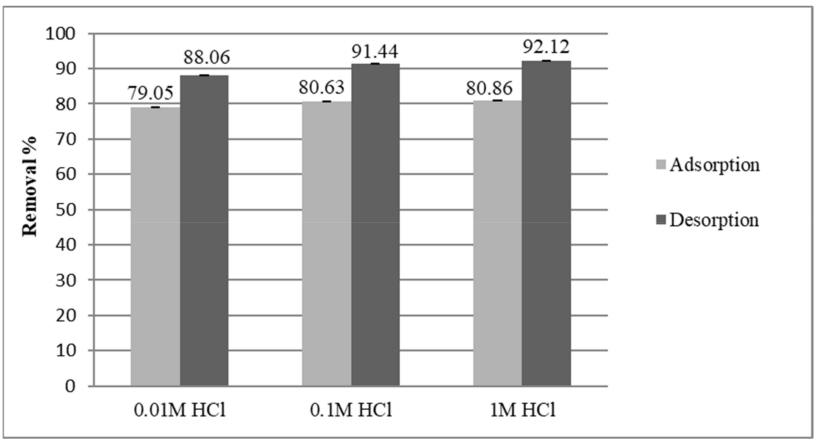

Figure 5. Adsorption and desorption of Cd with three concentrations of HCl—0.01 M, 0.1 M and 1 M.

4. Conclusions

In the current stage, the *P. triestinum* strain *AD1* was found to be handled and cultivated easily in the laboratory environment. The results of sorption experiments illustrated that Cd could be removed by *AD1*. The q_{max} value was found to be 0.018 mmol/g, and the effective contact time of biosorption was found to be 3 h. Based on the result of the kinetic study, the adsorption kinetics fit well with the pseudo-second-order model. Based on the result of the pH study, the optimum pH was found to be 5. HCl was an effective desorbent for cadmium recovery, which was found to be 90.7%, indicating that cadmium can be recovered. For the desorption experiment, the optimum concentration of HCl was found to be 0.01 M. Although *P. triestinum* is not the most efficient adsorbent species, large amounts of this dinoflagellate species can be salvaged 'for free' from 'natural' HAB events, greatly reducing the cost of land, containers and media for culturing other species. In addition, harvesting the blooming algae can also prevent secondary disasters, such as seawater hypoxia. Therefore, with all these favorable factors, the biomass of *P. triestinum* has great potential as an efficient biosorbent for cadmium removal from wastewater.

Author Contributions: Conceptualization, S.J.-L.X., K.-C.W. and F.W.-F.L.; methodology, S.J.-L.X., K.-C.W. and F.W.-F.L.; software, S.J.-L.X., K.-C.W. and F.W.-F.L.; validation, S.J.-L.X. and F.W.-F.L.; formal analysis, W.L., S.J.-L.X. and F.W.-F.L.; investigation, S.J.-L.X., K.-C.W. and F.W.-F.L.; resources, F.W.-F.L.; data curation, S.J.-L.X. and K.-C.W.; writing—original draft preparation, S.J.-L.X. and K.-C.W.; writing—review and editing, W.L., S.J.-L.X. and F.W.-F.L.; visualization, S.J.-L.X., K.-C.W. and F.W.-F.L.; supervision, S.J.-L.X. and F.W.-F.L.; project administration, F.W.-F.L.; funding acquisition, F.W.-F.L. All authors have read and agreed to the published version of the manuscript.

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