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Bioremediation of Heavy Metals

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BIOREMEDIATION OF HEAVY METALS

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ABSTRACT

Bioremediation of heavy metal is a method of removing metals from a polluted area using plants or biological organisms. Experiments were conducted with lambda-kappa carrageenan (LKC), a biopolymer, to determine if it can be used as a reagent in the bioremediation of copper. It was hypothesized that LKC could provide a highly effective solution for wastewater treatment. Through the experimental analysis herein, it was concluded that the biopolymer is a mediocre bioremediator of copper.

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1 INTRODUCTION

The amount of wastewater that is contaminated by heavy-metal is increasing each day because of the industrial growth throughout the world. This issue is encouraging a new scientific fever to find a better solution in quantity and cost. There are a number of bio materials that can be use to remove metal from wastewater, such molds, yeasts, bacteria, and seaweeds (Vieira and Volesky, 2000). This project investigated the use of a biopolymer extract from seaweed called carrageenan. There are three types of carrageenan: lambda, kappa, and iota found in natural seaweed. Recent research showed that lambda carrageenan, a similar biopolymer to the one used in this study, to be most effective at adsorbing copper (Cu), as compared with results for lead (Pb) and mercury (Hg) (Son et al., 2004). This study aimed to extend this research direction to include lambda-kappa carrageenan (LKC) in hopes of finding a more effective and economical way of removing Cu from wastewater. LKC is a non-gelling biopolymer extracted from seaweed, is fairly cheap and the raw material is easily found in nature. The project used an equilibrium dialysis apparatus and bathocuproine method to calculate how much Cu was bound by LKC.

2 BACKGROUND

2.1 Carrageenan

As mentioned in the introduction, Carrageenan is a group of polysaccharides that are extracted from seaweed, and lambda-kappa carrageenan is most likely to be extracted from *Chondrus crispus* known better as Irish moss that is shown in figure 1 (Fournet *et al*., 1993). Figure 2 shows the three primary structures of carrageenan: lambda, kappa, and iota. Carrageenans are sulfated polymers made of galactose units. The basic structure of carrageenan consists of repeating main chain of D-galactose residues linked alternately β - D – galactopyranose - (1 → 3) and α - D - galactopyranose - (1 → 4) (Cyber Colloids, LTD www.cybercolloids.net).

There are many uses of carrageenan. Typically, carrageenan can be found in daily food and drink, such the frosting of key lime pie, milk, and even shampoo because they can be used as gelling, emulsifying, and stabilizing agents (Tobacman, 2001). Carrageenan also has an outstanding research use because of its ability to block human papillomavirus and gonorrhea infections. It is also used in research for preventing the sexual transmission of HIV (Maguire et al., 1998).

Four basic processes are already known to produce carrageenan which are alcohol precipitation, KCL precipitation or gel press, Danisco process, and semi refined. Although the four processes have their own advantages and disadvantages, they all have similar procedures: wash seaweed, extract in hot alkali, filter, separation, and dry (Cyber Colloids, LTD www.cybercolloids.net).

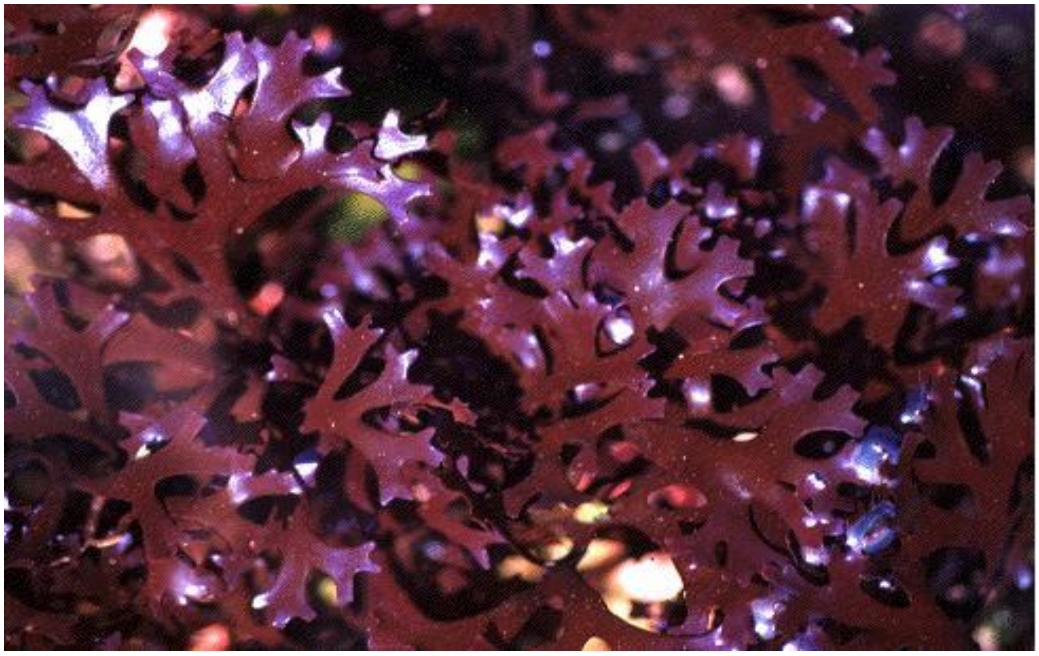
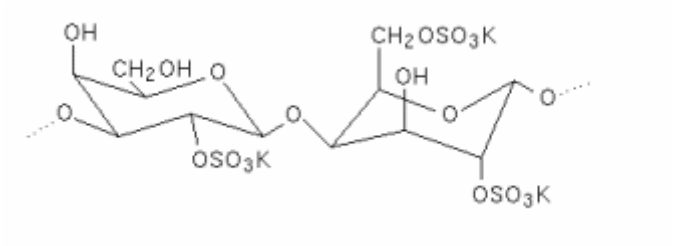
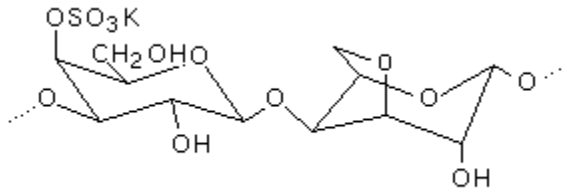


Figure 1: Chondrus crispus (Cyber Colloids, LTD www.cybercolloids.net)

a) Lambda Carrageenan



b) Kappa Carrageenan



c) Iota Carrageenan

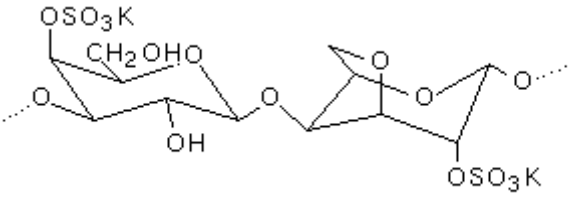


Figure 2: Primary structures (Cyber Colloids, LTD www.cybercolloids.net)

3 MATERIALS AND METHODS

A sample of powdered carrageenan (LKC) was obtained from Sigma. An ionic interaction was imparted between the LKC and Cu, with a cellulose-based membrane separating the two junctions. The LKC was treated to have a pH > 2 so that it would act as an anion with Cu^{2+} as the cation. The Cu was bound to the LKC polymer using an equilibrium dialysis apparatus and a rotatory incubator. This set up allowed for the creation of a solution with equal parts of free Cu ions on both sides of the membrane. The bathocuproine method was used to determine the quantity of Cu left unbound in solution. All of the glassware used was pre-treated with 1 + 1 (6M) HCl to remove any Cu ions and then rinsed with deionized water (dH_2O) to ensure the accuracy of the results.

Figure 3: Lambda-kappa carrageenan

3.1 Preparation of Dialysis Apparatus

An equilibrium dialysis apparatus (EDA) is a set of two plastic components separated by a cellulose-based membrane. Each component has 8 wells that are connected only in pairs with those of the other component across the membrane. Each well can hold 2.5 mL of solution. The wells of one component were filled with water containing LKC: this component is designated as the polymer-side (P). The wells of the other component were filled with water containing Cu: this component is designated as the metal-side (M). The cellulose-based membrane ensured that the solution in each component would not diffuse easily. Before the EDA was initially used, the apparatus was treated with dH2O to clean the wells. This was done to also test the condition of the membrane. If any dH2O diffused to the other side, the membrane would need to be replaced.

Figure 4: Equilibrium dialysis apparatus (EDA)

3.2 Equilibrium Dialysis

The water was removed from the wells using suction without poking the membrane; doing otherwise might have torn the membrane. 200 mg of LKC was dissolved in dH_2O and the pH of Cu stock 1000 ppm was adjusted to a value of 3 using hydrochloric acid (HCl). The wells of the EDA's P-side were filled with 2.5 mL of LKC. A mixture of {Cu + 0.2M KCl + dH_2O} was pipetted into the wells of the M side (see figure 5). Potassium chloride (KCl) had to be added to the solution because it helps prevent Cu from binding with the membrane.

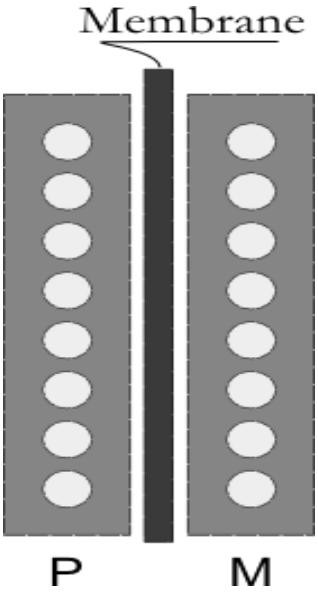


Figure 5: Schematic of the equilibrium dialysis apparatus

P: 2.5 mL polymer solution **M**: 2.5 mL of {Cu + 0.2 M KCl + dH_2O}

A control well was set up to determine the amount of Cu bound to the membrane. This was done with care so as not to add any LKC on the P side. Solutions of progressively greater Cu concentration were added in the remaining 7 wells of the M-side. dH_2O was added as needed to prepare a total of 2.5 mL in each well (see table 1).

Table 1: EDA Samples

Cu_o ppm	μL of 1000 ppm Cu	μL of 2M KCl	dH_2O [mL]
30Control	150 λ	500 λ	2.15 mL No polymer was added to the P side
30	150 λ	500 λ	2.15
40	200 λ	500 λ	2.10
50	250 λ	500 λ	2.05
60	350 λ	500 λ	2.00
80	400 λ	500 λ	1.90
100	500 λ	500 λ	1.80
120	600 λ	500 λ	1.70

The wells were covered with a plate of material that the components were comprised of. The apparatus was placed on a rotating incubator for 24 hours at 200 rpm to allow an equilibrium of free Cu to be established across the membrane (see figure 6), excluding the amount of Cu bound to the polymer. The initial concentration of free Cu is designated as Cu_o, and the equilibrium concentration as Cu_f.

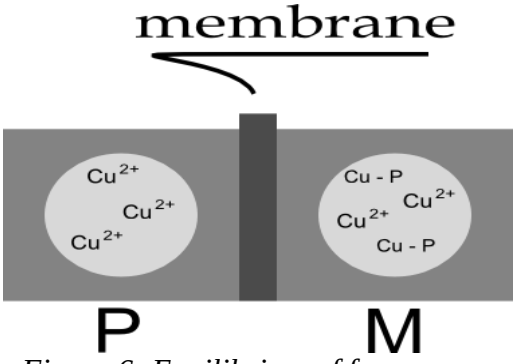


Figure 6: Equilibrium of free copper

3.3 Cu Assay

An assay process, called the bathocuproine method, was used to determine the amount of free Cu present in both sides of the EDA. The volume analyzed was 5 mL.

3.3.1 Preparation of Samples

To assay the Cu in the EDA, 5.0 mL samples were made by adding variable amounts of M-side solution to variable amounts of dH_2O, ensuring that the resultant solutions each contained 10 µg Cu. It was assumed that none of the Cu in the M-side solution was bound to either the membrane or polymer.

Table 2: Cu assay sample

Cu_o [C]	µL of M side solution needed for 10 µg of Cu	dH_2O needed [mL]
30	333	4.67
30	333	4.67
40	250	4.75
50	200	4.80
60	167	4.83
80	125	4.77
100	100	4.90
120	83	4.91

Certain samples were analyzed in identical groups to ascertain the accuracy of the calculations. Thirty Cu samples were prepared thusly: 4 identical controls; 3 identical groupings of 7 distinct unknowns; 3 identical calibration standards; and 2 identical zeroing blanks. The calibration standards were samples that contained 5.0 mL of dH_2O and 10 µL of 1000 ppm Cu stock, which contained 10 µg of Cu. The zeroing blanks were samples that contained 5.0 mL of dH_2O.

In preparation for the bathocuproine method, a 51 mL stock assay buffer for was made by mixing 16 mL of citric acid, 16 mL of hydroxylamine, 16 mL of bathocuproine disulfonate, and 3.2 mL of 1 + 1 HCl in a clean beaker. 1.6 mL of this buffer was added to each of the 30 sample tubes.

Citric acid functioned to sequester the solution because copper does not dissolve easily with water on its own. Hydroxylamine was the reducing agent, which reduced Cu^{2+} into a less stable form: Cu^{+} (Carter and Small, 2003). Bathocuproine disulfonate changed the solution's color to orange. The higher a sample's Cu concentration was, the darker its shade of orange would become. If a blank sample changed color, it could be deduced that the sample was contaminated with Cu. Each test tube was vortexed and incubated at room temperature for no less than 10 minutes but no longer than 60 minutes.

The next step was to read the samples on a spectrophotometer with a plastic cuvette at 484 nm. The spectrophotometer was first zeroed with one of the blank sample. Then, before reading other samples, the device needs to be left for about 10 minutes to make sure that the reading on blank sample stabilized. When the spectrophotometer was stable, all the samples were read at 484 nm using a same cuvette. This was done in samples ordered from the lowest concentration of Cu to the highest to avoid cross

contamination. By using a same cuvette, the reading inaccuracy could be avoided, because variances in cuvette properties between different devices are eliminated within the spectrophotometer. For each Cu triplicate concentration sample, the three readings were averaged, as well as the four of membrane binding controls.

At the end of the experiment, EDA was washed three times with a dH_2O, and filled with chloroform to prevent any bacterial growth on the membrane.

4 RESULTS

Results include the spectrophotometer readings for the 30 samples, the amount of Cu bound to the membrane and polymer.

Table 3: A484 of Cu Assay Samples

Tubes	First Reading	Second Reading	Third Reading	Average Reading
Standard	.327	.311	.306	.315
30 Control	.282	.282	.277	.280
30	.256	.262	.64	.261
40	.256	.258	.251	.255
50	.264	.262	.264	.263
60	.293	.297	.294	.295
80	.291	.291	.292	.291
100	.291	.293	.293	.292
120	.283	.285	.281	.293

Standard A_{484} = .315
µgx = (10µg Cu/.315) x Ax = 31.75 Ax

Table 4: Amount of Cu bound to the membrane and polymer

Concentration	µg in 1.0 mL	µg in 5.0 mL	µg of Cu bound to membrane and polymer
30 Control	26.7	133.5	**16.5 (only Cu_f)**
30	24.8	124.0	9.5
40	32.3	161.5	22.0
50	41.7	208.5	25.0
60	56.0	280.0	3.5
80	73.8	368.8	14.7
100	92.9	464.5	19.0
120	108.0	540.0	43.5

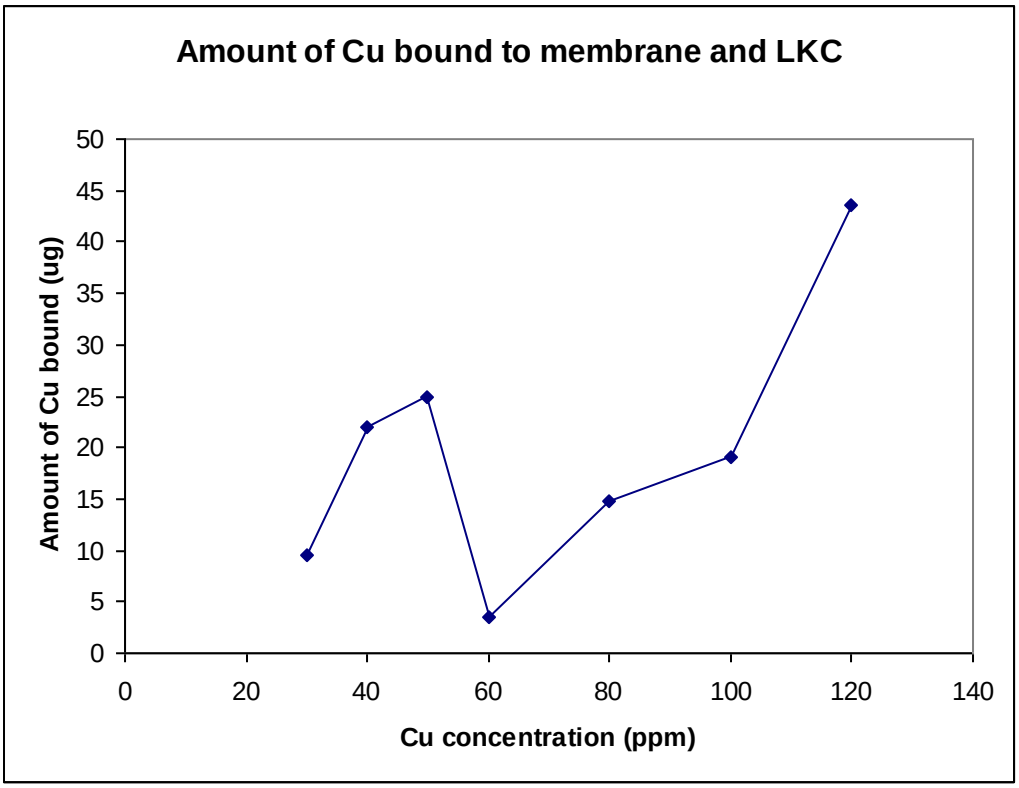


Figure 7: The amount of Cu bound to the membrane and polymer

5 Discussions

The ideal spectrometry reading for 484 nm when Cu presented is 10 µg supposed to be 0.3-0.31, and acceptable error is less than 1%. The actual result has more than 1% error, and the number did not increased progressively as it was expected to be, which made a solid conclusion difficult to raw for LKC. This may be a result of different things; the pH for the Cu stock not having the best properties to work with LKC, lack of precision in pipetting, spectrophotometer error, or the LKC cannot maintain its pH stability. But if a conclusion must be drawn solely from the actual result, the data shows that lambda-kappa carrageenan does bind copper, but not as good as was hope for. However, the conclusion is based on one set of experiments in the project, and further more experiments need to be carried out to deliver more extensive and significant results.

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