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A Comparison Between Intracellular Compartmentation and Detoxification of *Pseudomonas Putida* and Extracellular Precipitation and Crystallization of *Alcaligenes Faecalis* in Cadmium Concentrations.

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ABSTRACT

Cadmium has a high toxic potential, with it being implicated in cases of poisoning through food. Scarce quantities of Cadmium are suspected of causing adverse renal arterial changes in human kidneys. Concentrations of 200 micrograms have been found toxic to certain fish, yet it may be that it could also be a dietary essential. The concentration of cadmium in the U.S. for drinking water is reported to be between 0.4 and 60 micrograms with a mean of 8.2 micrograms. It may enter drinking water as a result of industrial discharges or the deterioration of galvanized pipe. Wastewater treatment plants have recently had to refine their efforts to try to diffuse toxic levels of Cadmium that have been introduced into the environment by refining plants. One way they have gone about this is to introduce bacteria that compartmentalizes, and precipitates Cadmium ions. Two example of such bacteria are *Pseudomonas putida* and *Alcaligenes faecalis*, which were chosen to be worked with as a way of breaking down the Cadmium into a non-toxic by product.

Keywords: *cadmium, Pseudomonas Putida, Alcaligenes Faecalis*

INTRODUCTION

Cadmium is a naturally occurring element in the earth's crust. The soft, blue-white, metal or grayish white powder that is insoluble in water and reacts readily with dilute nitric acid. It occurs naturally in ores, and is obtained as a by-product from the extraction, separation, and recovery of those metals that are in the refinery. Cadmium exists in +2 Valence State, and does not form stable alkyl compounds or other organometallic compounds of known toxicological significance. It is most often encountered in combination with other elements such as oxygen, chlorine, and sulfur. Most cadmium in the country is obtained as a by-product from the smelting of zinc, lead, or copper ore. Cadmium has a number of industrial applications, but is used mostly in metal plating, pigments, batteries, and plastics. The primary source of cadmium exposure, for most people is through food. Although the largest source of cadmium released is due to the burning of fossil fuels (such as coal or oil) or by the incineration of municipal waste materials. Kidney damage has been reported from high levels of cadmium through inhalation or diet. Increased risk to lung cancer and emphysema has been reported from overexposure to cadmium. Other organs and tissues that have is reported to be affected include the liver, testes, the immune system, the nervous system, and the blood. So the government to ensure that there will be no repercussions that could come from it has regulated the prevention of cadmium exposure. The purpose of this experiment is to expose two different types of bacteria that are used in wastewater treatment of cadmium to see whether one is better suited for the absorbance of cadmium. I suspect that the extracellular precipitation and crystallization of *A. faecalis* will be able to retain more cadmium due to the way that the cadmium is present in the samples, by an aqueous solution.

MATERIALS AND METHODS

Cadmium ions under suitable conditions react with dithizone to form a pink to red color that can be extracted with chloroform. The extracts of the chloroform can be measured photometrically and the cadmium concentrations are obtained from a calibration curve that is prepared from the standard cadmium solution treated in the same manner as the sample. Under the conditions of this method, concentrations of metal ions usually found in water do not interfere.

Equipment and solutions needed:
Spectrophotometer, for use at 518 mμ with a minimum light path of 1cm. Separatory funnels, 125-150 ml. All glassware including sample bottles should be cleaned with 1 + 1 HCL and then rinsed thoroughly with tap water and then distilled water. Stock cadmium solution: weigh 100 mg pure cadmium metal and dissolve in a solution composed of 20 ml distilled water plus 5 ml conc. HCl. Use heat to assist dissolution of the metal. Transfer the solution to a 1 liter volumetric flask and dilute to the mark with distilled water: 1.00 ml = 100 μg Cd. Store in a polyethylene container. Standard cadmium stock solution: pipette 10 ml stock cadmium solution into a 1 liter volumetric flask, add 10 ml conc. HCl, and dilute to the mark with distilled water, 1.00 ml = 1.00 μg Cd. Sodium potassium tartrate solution: Dissolve 250 g $NaKC_4H_4O_6$-$4H_2o$ in distilled water and make up to 1 liter. Sodium hydroxide-potassium cyanide solutions: Solution (I) Dissolve 400 g NaOH and 10 g KCN in distilled water and make up to 1 liter. Solution (II) Dissolve 400 g NaOH and 0.5 g KCN in distilled water and make up to 1 liter. Hydroxylamine

hydrochloride solution: Dissolve 20 g NH_2OH-HCl in distilled water and make up to 1 ml. Stock dithizone solution: Dissolve 100 mg diphenylthiocarbazone in 1 liter chloroform. Keep in a brown bottle in the refrigerator until required and use while still cold. Tartaric acid solution: Dissolve 20 g $H_2C_4H_4O_6$ in distilled water and make up to 1 liter. Keep the solution in the refrigerator, as it must be cold when used. Standard dithizone solution: Dilute 100-ml stock dithizone solution to 1liter with $CHCL_3$. Keep in a brown bottle in the refrigerator and allow to warm to room temperature before using. Thymol blue indicator solution: Dissolve 0.4-g thymolsulfonephthalein sodium salt in 100 ml distilled water.

The standards are pipette into a series of separatory funnels at 0(blank), 2.00, 4.00, 6.00, 8.00, 10.00 μg Cd. Then distilled water is added to a final volume of 25ml.

Color development, extraction, and measurement
Add reagents in following order, mixing after each addition: 1ml sodium potassium tartrate solution, 5ml NaOH-KCN solution (I), 1ml hydroxylamine hydrochloride solution, and 15ml stock dithizone solution. Stopper the funnels and shake for 1 minute, relieving the pressure in the funnels through the stopper rather than the stopcock. Drain the $CHCl_3$ layer into a second funnel containing 25 ml of cold tartaric acid solution. Add 10 ml $CHCl_3$ to the first funnel; shake for 1 min. and drain into the second funnel again. Do not permit the aqueous layer to enter the second funnel in these operations. As the time of contact of the $CHCl_3$ with the strong alkali must be kept to a minimum, perform the two extractions without delay after addition of the dithizone (cadmium dithizonate decomposes on prolonged contact with strong alkali saturated with $CHCl_3$).

Shake the second funnel for 2 min. and discard the $CHCl_3$ layer. Add5 ml $CHCl_3$, shake, and discard the $CHCl_3$ layer, making as close a separation as possible. In the following order, add 0.25-ml hydroxylamine hydrochloride solution and 15.0 ml standard dithizone solution. Add 5 ml NaOH-KCN solution (II), and immediately shake for 1 min. Insert a pledget of cotton in the stem of the funnel and filter the $CHCl_3$ layer into a dry photometer tube. Read the absorbency at 518 mμ against the blank.

Treatment of samples: Pipette the appropriate volume of the sample containing 1-10 μg CD into a separatory funnel and make up to 25 ml with distilled water. In the case of potable water containing 10 μg/1 CD or less, add 0.5 ml concentrated HCL to 200 ml sample and evaporate to 20 ml. Add a few drops of tyhmol blue indicator to solution and then 6N NaOH solution until the indicator just turns yellow at a pH of approximately 2.8. Make up to 25 ml with distilled water. Adjust in a similar fashion the pH of a sample which has been processed by acid digestion. Unless the calibration curve is being prepared at the same time, prepare a blank and a standard containing 6.00 μg Cd in a final volume of 25 ml and run it in conjunction with the unknown. Proceed as in above to find the concentration of the Cd.

Calculation:
The following equation is used to calculate the CD concentration from the absorbency readings:

$$\text{Mg/1 Cd} = \frac{A2 \times C}{A1 \times S}$$

In which A1 = absorbency of the standard taken, A2 = absorbency of unknown water sample, C = micrograms Cd in standard taken, and S = milligrams of unknown water sample used.

The strands were split into two separate types one containing just the nutrient broth and the strand and the other consisting of the nutrient broth, the strand, and a cadmium solution. Then they were incubated for thirty days, after which all the cultures were centrifuged and then separated. The bacteria strands were then frozen to stop the progression of incubation any further than was already done. From there the cultures were then set up to be run through the dithizone method and ran through a spectrophotometer at a wavelength of 518 mμ with at least a 1cm light path.

RESULTS

The effects of the cadmium on the *P. Putida* strain was better tolerated and compartmentalized into the bacteria than there was for the *A. Faecalis* strain. The *Putida* strain averaged a lower amount of cadmium absorbency that was detectable by the dithzone method of detection for cadmium. I tried to compare the weight of the bacteria to that of the concentrations of cadmium, but the bacteria was too small to be calculated as a weight that could have been used. The results that I received were all over the place, there really was no consistency that was present in either of the two strains of bacteria used, both had a wide variance between the samples. Also both were not even on the scale so I doubled all concentrations to make up for the lack concentration of the cadmium solution.

DISCUSSION

There were several problems that I encountered in this experiment. One problem that occurred is with the standards that were taken. All standards were run up to five separate times and each time whenever reaching the concentrations of six micrograms or higher the readings went off the scale. The higher concentrations were up to two times larger than the blank. This might have been due to the fact that at such a high concentration that the sample was too highly saturated, the fact that there was too much cadmium dithizonate to be handled by the chloroform to separate out of the funnels. Also another problem was that of the samples, when running a dithizone method for cadmium the cadmium when present is to show up from a pink to

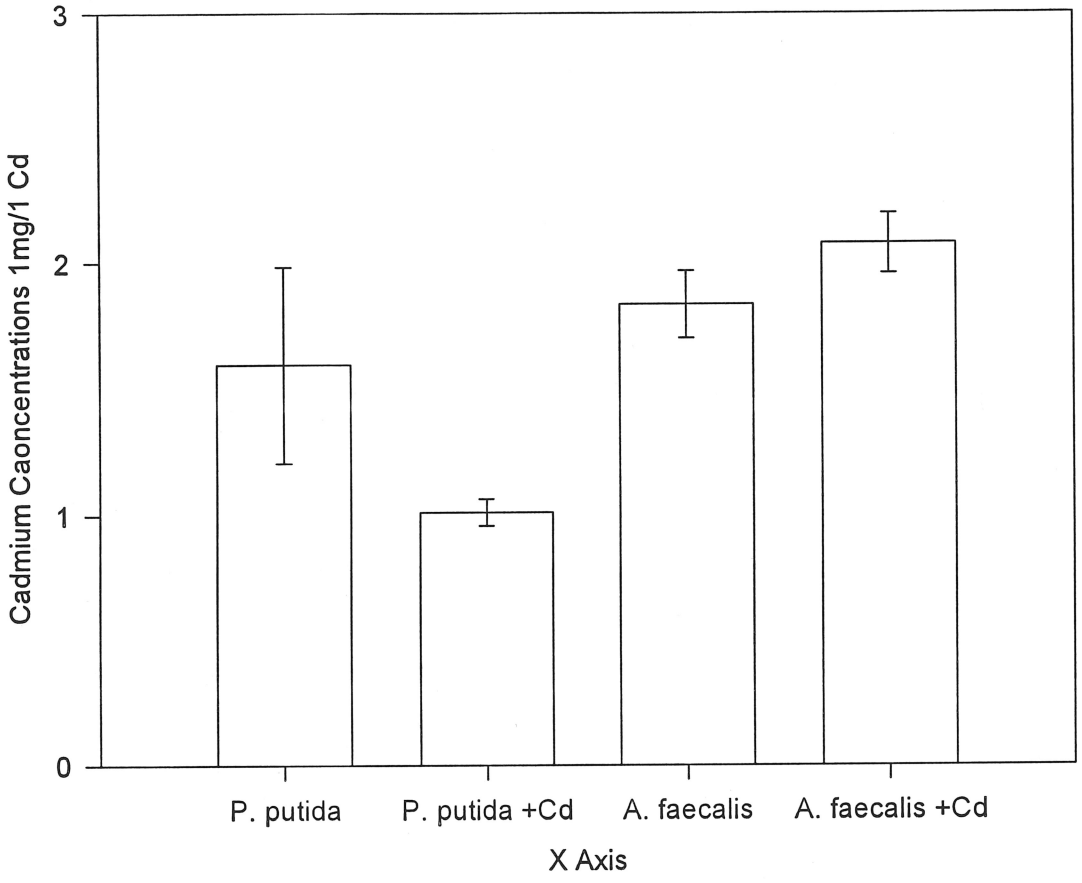

Figure 1. Random sample concentrations (1ml=1μg/1 Cd) of the P. putida and the P. putida with cadmium strains, also the A. faecalis and the A. faecalis with cadmium strain.

light red color, which is extracted and separated with chloroform. In the samples however the color of the dithizone was not pink but rather a light shade of orange, it looked more like a glass of Tang. This may be due to the low volume of the sample taken; there was only five ml. of each sample to be put through the dithizone method of detection.

The *A. Faecalis* was not as useful as I thought it would be. The fact that the bacteria were exposed to an aqueous solution of cadmium might not be the way that both bacteria would be used in treatment means. It seemed that the *P. Putida* had a lower rate of concentration of cadmium than *A. Faecalis* leads me to believe that intracellular compartmentalization and crystallization is better suited to be used as a treatment for the presence of cadmium due to the decrease of cadmium that was detected from the samples.

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