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CADMIUM ACCUMULATION BY MICROBIAL CONSORTIUM KNOWN IN EGYPT AS "SAMAKAT EI-SHEFFA"

Refae, R.I.

Department of Microbiology, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

The ability of natural microbial consortium, known in Egypt as "Samakat El-Sheffa", to accumulate cadmium ions from aqueous solutions was examined and compared with that obtained by individual pure culture of *Zymomonas mobilis* or *Pseudomonas putida.* In continuous system using packed bed column reactor, the natural microbial consortium "Samakat El-Sheffa" effictively adsorbed Cd from solution. An uptake of 46 % (w/w) was observed after 30 min (initial concentration 50 ppm Cd^{2+}) and 45 % (w/w) after 250 min (initial concentration 245 ppm Cd^{2+}). By replacing the saturated microbial consortium in the column, at the end of the experiment, with a fresh consortium it was possible to achieve Cd adsorption efficiencies of 81 % (w/w) after 4 dayes. In batch system, however, no saturation was observed and Cd uptack was complete within only 210 min. The optimum pH for Cd accumulation was 7.0. Three pure microbial strains were isolated from the natural microbial consortium and identified as *Pseudomonas spp., Saccharomyces spp. and Candida spp*. Cadmium accumulation efficencies by the isolated strain of *Pseudomonas spp.* was higher (99.8 % w/w after 30 min) than those of both mixed population (74.2 % w/w after 90 min) or natural consortium (93.4 % w/w after 180 min). Compared to the other bacterial strains, microbial consortium has the most favourable feature for cadmium removal; high adsorption ability, good mechanical properties and an excellent applicability in a column system. The feasibility of using such microbial consortium on larger scale process was discussed.

INTRODUCTION

Microorganisms have long been known to accumulate metal ions from the environment and this ability offers an attractive option for the recovery of useful, or the removal of environmentally harmfull heavy metals from solution. In recent years interest has been focused on producing microorganisms as suitable biosorbents for metals. Several reports exist of cadmium accumulation by microorganisms (e.g. Kuhn and Pfister 1990; Ron et al. 1992; Wang et al. 1997 and Pazirandeh et al. 1998).

The natural microbial consortium (Samakat El-Sheffa) is known in Egypt for the public since a long time. Its unusual characteristic is the secretion of a massive amount of viscous extracellular material that bends the cells together to form a large aggregate (a jelly-like structure; ca. 8 cm diameter and 4 mm thicknis) within 3-5 days when is grown in a sucrose-containing solution. This exopolymer consists of multilayers with a very reproductive nature. It is believed that this jelly-like structure is a polysaccharide. Many bacteria produce large amounts of extracellular polymers that form capsules or loose aggregates around cells. These are often polysaccharides which act as polyelyctrolytes and adsorb metal ions such as Co^{2+}, Cu^{2+}, Cd^{2+}, Uo_2^{2+}, and Fe^{3+} (Parsons and Dugan 1971; Lester *et al*. 1984; Norberg and Person 1984 and Kuhan and Pfister 1990). Cadmium is of interest because it has been implicated as a mutagen,

carcinogen and teratogen (Degraeve 1981). This heavy metal is naturally present in the earth's crust at < 0.15 μg ml^{-1}, but continuous to increase in the environment (Scow *et al*. 1982; Delos 1985; Smith et al. 1987). The purpose of the present study was to determine the extent to which this microbial consortium accumulate cadmium from solutions compared with known bacterial strains (e.g. *Zymomonas mobilis* and *Pseudomonas putida*).

MATERIALS AND METHODS

1. Microorganisms:

Pseudomonas putida was obtained from Institute of Microbiology, University of Bayreuth, Germany. *Zymomonas mobilis* was obtained from American Type Culture Collection, Rockville, USA. The natural microbial consortium traditionally known as "Samakat El-Sheffa" was used. Three microbial strains were isolated from this cosortium and identified to the genus level. They included *Pseudomonas spp.*, *Saccharomyces spp.* and *Candida spp*.

2. Culture media:

Pseudomonas putida, Pseudomonas spp. or *Saccharomyces spp.* were grown on a rotary shaker (110 rpm) at 25°C in mineral medium (Meyer and Schlegel 1983) which contained: 9 g/l $Na_2HPO_4.12H_2O$, 1.5 g/l KH_2PO_4, 1.5 g/l NH_4Cl, 0.2 g/l $MgSO_4.7H_2O$, 20 mg/l $CaCl_2.2H_2O$, 1.2 mg/l Ferric ammonium citrate, 1 ml/l trace element solution and suplemented with 10g/l glucose as a carbon and energy source. Trace element solution contained (mg/l): $ZnSO_4.7H_2O$, 100; $MnCl_2.4H_2O$, 30; H_3BO_3, 300; $CoCl_2.6H_2O$, 200; $CuCl_2.2H_2O$, 10; $NiCl_2.6H_2O$, 20; $Na_2MoO_4.2H_2O$, 30; Na_2SeO_3, 20. For mixed culture experiments, *Pseudomonas spp., Saccharomyces spp.* and *Candida spp.* were mixed in equal volume and the mixed culture was grown as described above. *Zymomonas mobilis* was grown at 30°C for 48 hrs without shaking in basic medium described by Rogers et al. (1982) which contained (g/l): Peptone, 2.0; yeast extract, 5.0; $MgSO_4.7H_2O$, 1.0; KH_2PO_4, 2.0; sucrose, 50; $(NH_4)_2SO_4$, 2.0.

3. Cadmium accumulation experiments:

3.1. Using natural microbial consortium (Samakat El-Sheffa):

3.1.1. Batch experiments:

A 5-day old microbial consortium of a jelly-like structure (Samakat El-Sheffa) of ca. 8 cm diameter and 4 mm thickness was washed with deionized water and incubated at 25°C with agitation at 60 rpm for 4 hrs in flat fermentaion flasks (20 cm diameter) containing 200 ml phosphate buffer (pH 7.0 ; 50 ml of 0.1 M KH_2PO_4 + 29.1 ml of 0.1 M NaOH, diluted to 100 ml with deionized water) suplemented with cadmium chloride (final concentration of 76 ppm Cd^{2+}). Periodical samples (1.5 ml) were transferred to Eppendorf tubes and centrifuged at 10,000 x g for 3 min. Supernatents were analyzed for Cd concentrations using atomic absorption spectrophotometry.

3.1.2. Continuous experiments:

The jelly-like structure similar to the one used in the batch experiments (3.1.1) was packed into double walled glass column of 4 cm diameter and 16 cm height. Cadmium solution (initial concentration of 50 or 245 ppm Cd^{2+} as cadmium chloride dissolved in phosphate buffer) was fed at the bottom of the column, and from the top effluent was recieved and recirculated back to the column through an external unit using a peristaltic pump at a flow rate of 30 ml/h. Periodical samples were taken and centrifuged (as in 3.1.1). Supernatants were analyzed for Cd concentrations using atomic absorption spectrophotometry.

3.2. Using individual strains:

Bacteria grown in the media without cadmium were harvested by centrifugation (7,000 x g at 25 °C for 15 min), washed with deionized water, resuspended, and washed again. The pellets were resuspended in phosphate buffer (as described above) to give a final cell density of 4 mg . ml^{-1} fresh weight. Aliquots (100 ml) were transferred to 500 ml Erlenmeyer flasks, equilibrated at 30 °C for 30 min and cadmium (1 ml, as cadmium chloride) from stock solutions rapidly added to give a final concentration of 50 or 105 ppm Cd^{2+} . The cell suspentions were incubated at 25°C with agitation at 200 rpm for 3 h. Periodical samples (1.5 ml) of bacterial suspensions were transferred to Eppendorf tubes and centrifuged at 10,000 x g for 3 min. Supernatants were analyzed for Cd concentrations using atomic absorption spectrophotometry. The Cd^{2+} biosorbed was determined by subtracting the Cd concentration of the supernatant from the initial Cd concentration of the contacting test solution.

3.3. Effect of pH on Cd adsorption:

Effect of pH was studied using KH phthalate buffer (pH 3.0; 50 ml 0.1 M KH phthalate + 22.3 ml 0.1 M HCl diluted to 100 ml), phosphate buffer (pH 6.0; 50 ml 0.1 M KH_2PO_4 + 5.6 ml 0.1 M NaOH diluted to 100 ml) and phosphate buffer (pH 7.0; 50 ml 0.1 M KH_2PO_4 + 29.1 ml 0.1 M NaOH diluted to 100 ml).

RESULTS AND DISCUSSION

1. Removal of cadmium by natural microbial consortium (Samakat El-Sheffa):

1.1. Cadmium removal using a growing microbial consortium (Samakat El-Sheffa):

A set of experiments were conducted in order to investigate the removal efficiency of cadmium by Samakat El-Sheffa during various stages of its growth. In each experiment, a portion of 20 x 20 x 4 mm of the natural microbial consortium (Samakat El-Sheffa) was allowed to grow in a sucrose-containing solution to form its typical jelly-like structure. Cadmium was supplied at concentration of 61 ppm Cd^{2+}.The capacity of natural microbial consortium to sorb cadmium was a function of slime production (Figure 1). It

increased as slime production increased. The highest Cd accumulation efficiency could be achieved by the 5-day old microbial consortium (ca. 8 cm diameter) which adsorped 65.4 % of cadmium within only 1 hr of incubation (Figure 1). Cadmium adsorption for 3 cm diameter structure (lower surface area) was 20 % less efficient. The 1- 2-day old microbial consortium differed from 5-day structure in secretion of lower amounts of exopolymers which had 10 % lower efficiency (55.3 % after 1 hr). In general, the removal efficiency could be ranked as follows: 5-day old structure (8 cm diameter) > 1-2-day old structure > 5-day old structure (3 cm diameter) > slime-free consortium. Rudd et al. (1983) showed that maximum polymer production occured in stationary phase cultures of *Zoogloea ramigera*, and these were most efficient at metal removal. These results clearly indicate that the secreted slime contributed to the binding of cadmium. Abiotic uptack was not a significant contributer to the removal; the soluble Cd level decreased by less than 5% in cell/slime free control.

Both living and dead microbial cells are capable of uptake and accumulation and so are products produced by or derived from microbial cells such as excreted metabolites, polysaccharides, and cell wall constituents (Kelly et al., 1979 and Brierley et al., 1985, 1986). Experiments on Cu and Co by cell walls of *Cunninghamella blackesleeana* over a range of metal concentrations up to 0.4 mg . mg^{-1} wall showed that the binding of such metals increased as the concentration increased (Venkateswerlu and Stotzky 1989).

1.1.1. Removal of higher concentration of cadmium in batch system:

A 5-day old microbial consortium of a jelly-like structure of ca. 8 cm diameter and 4 mm thickness which has a fresh weight of ca. 10 g and a dry weight of ca. 1.5 g was used in this experiment. A solution of 77 ppm Cd^{2+} was employed. The natural microbial consortium effictively adsorbed cadmium from solution (Figure 2). The sorption of Cd was rapid. More than 50 % of the cadmium ions were tacken up from solution within 90 min and cadmium uptack was complete within 210 min (Figure 2). No saturation was observed. As shown in Figure 2, the rate of cadmium adsorbtion was ca. 51.1 mg Cd^{2+}/g microbial consortium on a dry weight basis (ca. 5.1 % of the biomass's dry weight) in less than 4 hrs, indicating that natural microbial consortium has a high adsorption ability. This binding capacity is obviously high, when compared with those reported by other investigators (0.16%-0.98% and 3.9-8.9%, Doyle et al. 1975; 1%, Tynecka et al. 1975; 0-2%, Macaskie and Dean 1982; 0.22%, Gadd 1988). However, extracellular polysaccharides produced by *Zoogloea ramigera* adsorped up to 1 g Cd/ g dry wt (100 %) (Norberg and Person 1984). Studies on *E. coli* expressing a metal binding motif showed a metal accumulation of 1.1 nmol $Cd^{2+}mg^{-1}$ wet weight cells in 1 h (Pazirandeh et al. 1998).

1.2. Removal of cadmium in continuous process:

The results in batch system (1.1.1) showed that the natural microbial consortium (Samakat El-Sheffa) had the ability to adsorp Cd ions from solution. For this reason the cadmium removal in a continuous system was investigated in order to study the feasibility of using such microbial consortium

on larger scale process. Two different cadmium concentrations (50 and 245 ppm Cd^{2+}) were studied. The binding efficiency of cadmium was dependent on its concentration and decreased as the concentration increased (Table 1). The highest cadmium removal efficiency achieved was 50.4 % after 90 min (low Cd concentration) and 44.7 % after 250 min (high Cd concentration). Prolonged exposure of the natural microbial consortium revealed only low Cd accumulation with low Cd concentration but did not result in further accumulation with high Cd concentration (Table 1). By replacing the saturated microbial consortium in the column at day 2 with a fresh consortium it was possible to achieve Cd adsorption efficiencies of 81 % after 4 days (Table 1).

Compared to batch removal of Cd (36.9 % after 30 min), the continuious removal efficiency reached 46.2 % after the same time (Figure 2 and Table 1). However, prolonged exposure to Cd revealed high Cd uptack only in batch system (100 % after 210 min) (Figure 2 and Table 1). These results suggest that the natural microbial consortium in batch system may posses a greater surface area - to - volume ratio than those in continious system and may therfore exhibit a greater potential for cadmium uptack. One important factor in metal sorption is the available surface area. Some investigators studied the Cd uptack by immobilized cells in flow systems, among them the experiments on *Citrobacter Sp*. carried out by Macaskie and Dean (1984). In this experiments 65 % of Cd was removed from solution (up to 13.5 % of the organism's dry weight).

The results presented so far indicate that natural microbial consortium (Samakat El-Sheffa) is potentially useful for removing cadmium from contaminated solutions. It has the added advatage that it possesses good mechanical properties and an excellent applicability in a column system. Compared to the immobilized cells, jelly-like structure of the natural microbial consortium may have a bitter capability of re-use and has no clogging properties in continious flow systems. For a biological metal removal system to work on an industrial scale, the organism used has to be readily contained and the set-up has to be reusable. Consequently, studies are now in progress to determine whether it is possible to desorb (separate) the bound cadmium from biomass in order to reuse the jelly-like structure.

1.2.1 Effect of pH on Cd adsorption in the continuous process:

In acedic pH-range (pH 3.0) no Cd adsorption was observed. At pH 6.0 the Cd could be removed from solution, but with low efficiency (Table 2). The highest Cd removal uptack (50.4 % after 90 min) could be achieved in neutral pH-range (pH 7.0; Table 2). These results suggest that natural microbial consortium (Samakat El-Sheffa) probably can not be recommended for acedic industrial effluents since high H^+ ion concentrations can compete with Cd binding sites. However, this microbial consortium can be very effective in neutral environments. In thier experiments on *Zoogloea ramigera*, Norberg and Person (1984) found that optimum pH value for maximal sorption of cadmium was 6.5. Other experiments on extracellular polymer of *Klebsiella aerogenes* showed that uptake of Cu, Cd, Co, Mn and Ni was reduced at low pH and little metal was bound at pH 4.5 as compared with pH 6.8 (Rudd *et al*., 1983).

Table (1): Effect of cadmium concentration on its continuous removal from solution by natural microbial consortium (Samakat El- Sheffa)*.

	Percentage of Cd removal**	
Time	50 ppm Cd^{2+}	245 ppm Cd^{2+}
12 min	25.8	13.5
30 min	46.2	15.9
90 min	50.4	30.3
170 min	57.2	35.3
250 min	60.9	44.7
2d	ND^{+}	44.7
2d***	ND	54.3
4d	ND	81.0

*** Experiments were performed as described in Material and Methods (1.2).**
****Determined as amount of Cd (mg) removed, divided by the amount of initial Cd (mg).**
*****Fresh microbial consortium. At the end of the experiment (after 2 days) the saturated natural microbial consortium was replaced with a fresh one.**
$^{+}$ND, Not determined.

Table (2): Effect of pH on Cd removal from solution by natural microbial consortium (Samakat El-Sheffa) in continuous process*.

	Percentage of Cd removal ** at pH		
	3.0	**6.0**	**7.0**
Time (min)			
12	0	4.0	25.8
30	0	5.6	46.2
90	0	22.0	50.4

***Experiment was performed as described in Material and Methods (3.1.2 and 3.3) [the concentration of Cd^{2+} was 50 ppm].**
****Determined as in table 1.**

2. Removal of cadmium by individual strains:

2.1. Removal of cadmium by isolated strains:

Three bacterial strains were isolated from the natural microbial consortium. One strain is a Gram negative non-pigmented short rod and classified as *Pseudomonas spp* according to Bergey's maual (1994). Visual observation (growth on solid and in liquid media) confirmed its ability to secrete a considerable amount of extracellular material. The other two genera were found to belonge to the yeast genus of *Saccharomyces* and *Candida* (classified according to Lodder 1970). The highest removal efficiency of cadmium (99.8 % within 30 min) was observed by the *Pseudomonas spp*. isolate (Figure 3). This may be due to the secreted extracellular material which appears to bind cadmium effictively. Other isolated strains had a poor removal efficiency (not exceeded 13.2 % within 90 min). However, when they are mixed with *Pseudomonas spp*. isolate, the uptack efficiency of the mixture reached 74.2 % within 90 min (Figure 3). This clearly indicate that the bacterium *Pseudomonas spp*. contributed significantly to Cd adsorption.

Although the natural microbial consortium (Samakat El-Sheffa) possessed a high Cd removal efficiency, this efficiency did not reach the levels achieved with cells of *Pseudomonas spp*. (Figure 2 and Figure 3).

These observations suggest that *Pseudomonas spp*. isolate represents a major effictive microbial component of the natural microbial consortium regarding the removal of cadmium from solution.

2.2. Removal of cadmium by pure strains of *Z. mobilis* and *P.putida:*

Z. mobilis is a gram negative facultative anaerobic bacterium which grow in liquid media abundantly with a flocculent deposit. The sorption of cadmium ions to flocs of the organism was rapid and complete within 1 hr (Figure 4). Cells of *P. putida* exhibited a similar potential for cadmium uptack (Figure 4). However, the cadmium removal efficiency by *P. putida* (74.9 % after 30 min) was slightly higher than those observed for *Z. mobilis* (67.5 % after the same time) [Table 3]. Compared to the natural microbial consortium (100 % removal efficiencies after 210 min), both *Z. mobilis* and *P. putida* removed all Cd ions from solution after only 60 min (Figure 2 and Figure 4).

Although cells of either *Z. mobilis* or *P. putida* showed a high efficiency of Cd removal (67.5 % and 74.9 % after 30 min, respectively), isolated strain of *Pseudomonas spp*. exhibited a greater efficiency of Cd uptack. Almost complete Cd uptake was observed over the same period (Table 3). The binding of Cd by cells of individual strains described in the present study is comparable to those with *P. aerogenosa* described by Wang et al. (1997). The cadmium binding studies described by those investigators, however, were performed under different conditions.The ability of cells of *Pseudomonas spp.* to effectively remove Cd from solutions (Figure 3 and Table 3) demonstrates superiority of these cells to be used at metal-contaminated sites.

Table (3): Removal efficiency of cadmium from solution by natural microbial consortium (Samakat El-Sheffa) compared with other strains.

Percentage of Cd removal*						
Time (min)	**natural consortium[1]**	***Saccharomyces spp.*[2]**	***Pseudomonas spp.*[3]**	**mixed population[4]**	***Z. mobilis*[5]**	***P. putida*[6]**
1	19.3	4.0	96.0	21.8	60.8	69.8
30	36.9	5.0	99.8	34.2	67.5	74.9
60	46.2	10.4	100	40	100	100
90	52.2	13.2	-	74.2	-	-

***Determined as in table 1.**

[1] Natural microbial consortium (Samakat El-Sheffa). Experiment was performed as in Figure 2.

[2,3] Isolated strains. Experiments were performed as in Figure 3.

[4] Mixed population of isolated strains of *Pseudomonas spp., Candida spp*. and *Saccharomyces spp*. Experiment was performed as in Figure 3.

[5,6]Experiments were performed as in Figure 4.

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ازالة الكادميوم من الاوساط السائله بواسطة خليط ميكروبى طبيعى يعرف فى مصر باسم "سمكة الشفاء"

رفاعى ابراهيم رفاعى
قسم الميكروبيولوجيا الزراعيه-كلية الزراعه-جامعة القاهره-الجيزه

تم دراسة قدرة خليط من الميكروبات الطبيعيه المعروف فى مصر باسم "سمكة الشفاء" على ازالة الكادميوم من المحاليل المائيه وتم مقارنته بسلالات بكتيريه نقيه مثل سلالة *Zymomonas mobilis* التى تمتاز بتكوين حبيبات عند نموها فى البيئات السائله و سلالة *Pseudomonas putida* . فى تجارب باستخدام النظام المستمر (continuous system) باستخدام عمود زجاجى معبأ (packed bed reactor) كان للخليط الميكروبى الطبيعى (سمكة الشفاء) كفاءه عاليه على ازالة الكادميوم من المحلول بلغت حوالى ٤٦ % بعد ٣٠ دقيقه (تركيز ٥٠ ميلليجرام/لتر) و ٤٥% بعد ٢٥٠ دقيقه (تركيز ٢٤٥ ميلليجرام/لتر). أدى احلال خليط الميكروبات فى العمود بخليط جديد فى نهاية التجربه الى زيادة كفاءة ازالة الكادميوم من المحلول بمقدار ٨١% بعد ٤ أيام. ومع ذلك فانه فى نظام المرحله الواحده (batch system) لم يلاحظ تشبع للمخلوط الميكروبى بالكادميوم حيث كانت ازالة الكادميوم من المحلول كامله فى خلال ٢١٠ دقيقه فقط. وجد أن أفضل درجة pH لازالة الكادميوم كانت فى النطاق المتعادل (pH 7.0).

ومن جهه أخرى، فقد أمكن عزل ثلاثة أنواع ميكروبيه نقيه أثناء المراحل المختلفه لنمو وتكاثر ميكروبات سمكة الشفاء وتم تصنيفها الى *Pseudomonas spp.* و *Saccharomyces spp.* و *Candida spp.* وتم مقارنة كفائتها فى ازالة عنصر الكادميوم (مفرده أو فى مخلوط) بكل من الخليط الطبيعى للميكروبات (سمكة الشفاء) و سلالات نقيه تتبع *Z. mobilis ,P. putida* وقد وجد أن أعلى كفاءه لازالة الكادميوم من المحلول (٩٩,٨ % بعد ٣٠ دقيقه) أمكن الحصول عليها بواسطة سلالة *Pseudomonas spp.* المعزوله اذا ما قورنت بمخلوط السلالات الثلاثه المعزوله (٢ ,٧٤% بعد ٣٠ دقيقه) والخليط الطبيعى (سمكة الشفاء) [٩٣,٤ % بعد ١٨٠ دقيقه] . كما تفوقت سلاله *Pseudomonas spp.* المعزوله على غيرها من *P. putida* التى بلغت ٩, ٧٤ % و *Z. mobilis* التى بلغت ٥, ٦٧% وذلك بعد نفس المده (٣٠ دقيقه). يتميز الخليط الميكروبى (سمكة الشفاء) بسهولة استخدامه بطريقه مستمره حيث يسهل تعبأته فى الأعمده وذلك على عكس الخلايا الميكروبيه العاديه التى يتطلب تحميلها على دعامات مما قد يزيد من تكلفة عملية المعالجه.