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# USE OF ADSORPTION ISOTHERMS: TO PREDICT THE CR (VI) DETOXIFICATION MECHANISMS BY *PS. AERUGINOSA 4442.*

**Rohini Kulkarni and Gupta S. G. Rohini Kulkarni (Pandhare)[1], Gupta S.G.[2]**

[1]Incharge Principal , Govt. College of Arts and Science, KileArk Aurangabad, (M.S.), India
[2]Director, Govt. Institute of Science , Nipatniranjan, Aurangabad, (M.S.), India

## ABSTRACT

Microorganisms can remove heavy metal ions from aqueous solution by various mechanisms which may or may not be releated to metabolic process of microorganisms. For the appilication of these mechanisms on commercial metal removal from effluent adsorption equilibrium isotherms can be appilied which will predict the capacity of the biomass to detoxify metals from the effluent. This study will help in designing the environmrntal clean up projects on large scale appilication. In this paper Cr (VI) detoxification by growth independent sorption, metabolism dependent accumulation and plasmid mediated reduction by *Ps. aeruginosa* 4442 is studied and the data thus obtained is analysed by equilibrium modeling.

**KEY WORDS:** Cr (VI), Detoxification mechanisms, *Ps. aeruginosa 4442*

## INTRODUCTION

The microbial community has capacity to alter the fate of metals entering into the aquatic or soil environment. They have various mechanisms to detoxify metal ions. It may be just sorption, growth dependent accumulation, reduction or transformation. Metals with a specific gravity greater than 5nhave been termed as heavy metals (Lapedes, 1974). Many heavy metals are carcinogenic (WHO1993). Chromium (VI) is toxic to biological systems due to its strong oxidizing potential that that can damage cells (Kotas and Stasicka, 2000). Some microorganisms in presence or absence ofoxygen can reduce the toxic hexavalent chromium to its trivalent form (Polti *et.al,* 2007) A variety of chromate resistant bacterial isolates has been reported and their resistnce mechanism is encoded by either plasmid or chromosomal genes(Cervantes and Campos-Garcia, 2007). Bacterial reduction of chromate is observed for both aerobic (Bopp and Ehrlich,1988) and anaerobic reduction system (Gvozdyak *et.al.* 1986).

Several active groups of microbial cell constituents like acetamido group of chitin, structural polysaccherides of fungal amine, sulphahydril and carboxyl group in proteins, phosphodiester teichoic acid, phosphates participates in biosorption (Thakur, 2006). Microbial metal accumulation often comprises of two stages (Trevors *et al.,* 1989) An initial rapid and passive process involves physical adsorption or ion exchange at cell surface, occasionally a subsequent phase that is slower involves active metabolism dependent transport into bacterial cell.

The adsorption models are useful means for describing the degree of biosorption as a function of equilibrium metal ion concentration at constant pH and temperature conditions. The models are mathematical representation of biosorption equilibrium and by providing metal uptake capacities for different mechanisms applied under the study, serve as a means of comparing different mechanisms or different groups of biosorbents in terms of their metal removal capacity. The two isotherms, which are widely used, are Langmuir (1918) and Freundlich isotherms (1926). Generally these isotherms show how adsorption takes place and may serve as design parameters in the treatment of heavy metals from waste waters. The data thus obtained is useful in giving a quantitative description of the process; it further helps in optimizing the operating condition for large scale operating systems at various conditions.

The process also helps in minimizing the number of experimental runs and to pin point the results of spot-check experiments. It helps in designing of reactor and optimization of operational conditions. Various commercial waste water system are based on the bacterial mechanism of metal detoxification include Homestake, (1999), Metex anaerobic sludge reactor (Morper, 1985).

## MATERIALS AND METHODS

- *Pseudomonas aeruginosa 4442*
- Sterile nutrient broth tubes containing different concentrations of Cr (VI) from 20-300ppm

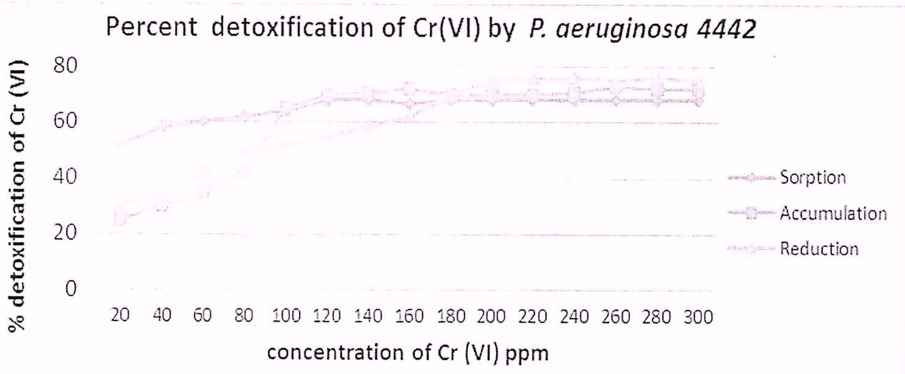
To study the detoxification capacity Cr (VI) by *P. aeruginosa* 4442 by sorption, accumulation and reduction, nutrient broth tubes with pH 7.0 containing various concentration of Cr (VI) from 20-300ppm were taken. The tubes were inoculated with 1% (w/v) *P. aeruginosa 4442.*For the sorption studies, tubes were incubated on a rotary shaker at $30^0$ C for 2hrs at 100rpm. For the accumulation (metabolism dependent) studies tubes were incubated at same temperature and condition for 48hrs and for bioreduction based detoxification studies screw cap tubes filled with broth up-to the neck were used. Tubes were also incubated at the similar conditions for 48hrs. After proper incubation the contents of the tubes were centrifuged and the % residual Cr (VI) concentration was calculated using AAS. The data thus obtained was processed for the different adsorption isotherms.

Adsorption isotherms were applied to the biosorption experiments carried out using growth dependent and independent % sorption of Cr(VI) 20-300 ppm with pH 7.0 at $30^0$C on a rotary shaker at 100rpm with 1 % inoculum concentration for varying period of time. In case of growth independent experiments after every 30 minutes results were taken and in case of growth dependent results were noted after each 24 hrs. The data thus obtained was applied to different adsorption isotherms like Langmuir and Freundlich and the graphs obtained were as follows.

## RESULTS AND DISCUSSION

In the Figure (1) percent detoxification of Cr (VI) at various concentrations by the three mechanisms sorption, accumulation and reduction by *P. aeruginosa* 4442 is represented. It was observed that all the three mechanisms showed near about the same % detoxification after 150 ppm of Cr (VI) concentration. At the initial low Cr (VI) concentration sorption % was more. Passive non-living cells is a good option because it can overcome the need for nutritional requirements, sensitivity to extreme pH and other environmental conditions as well as other toxic effects of effluents and at the same time it gives equal or in some cases more % detoxifications. At the same time production of such non-growing biomass and immobilization is also expensive. Generally effluent does not contain single metal at a time, it also contains various co-ions and other organic matter that can also be used for growth by the micro-organisms thus sounds to be a good option and thus comparison of all the three processes as a hybrid can be applied.

**Figure1. Percent Cr (VI) detoxification by 3 mechanism**




When the obtained data was applied to Langmuir and Freundlich (Fig.2. and 3) model the straight line obtained in Langmuir suggest that the data fits into Langmuir isotherm. From the adsorption studies for Cr (VI) detoxification by *P. aeruginosa* 4442 by sorption, accumulation and reduction experiments when applied to Langmuir and Freundlich isotherms to give straight line. It suggested that Langmuir isotherm is more suitable

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**Fig. 2. Langmuir plot for 3 detoxification mechanisms**

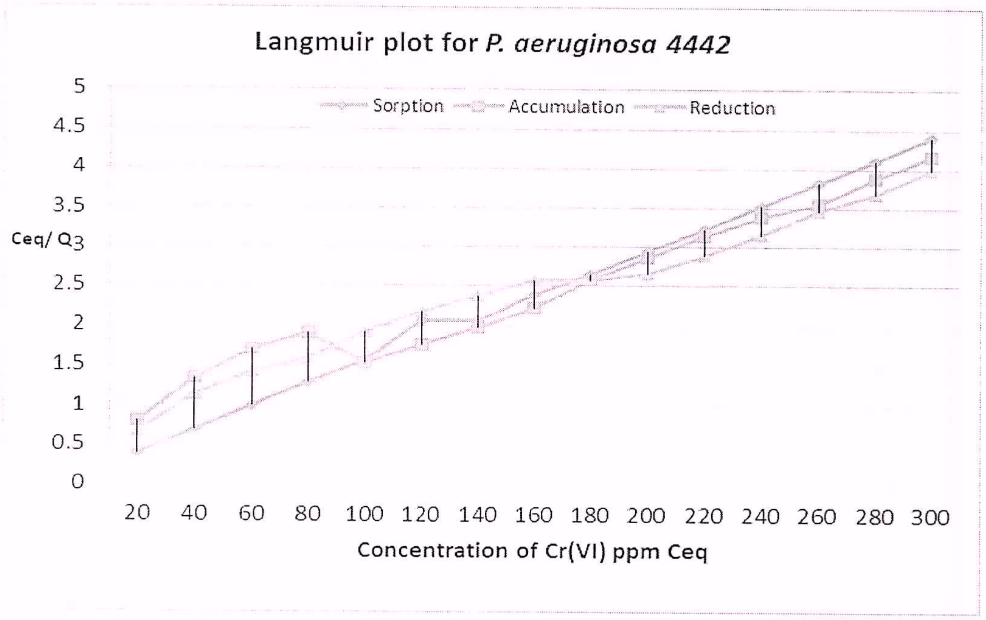


**Fig. 3 Freundlich isotherm for 3 detoxification mechanisms**

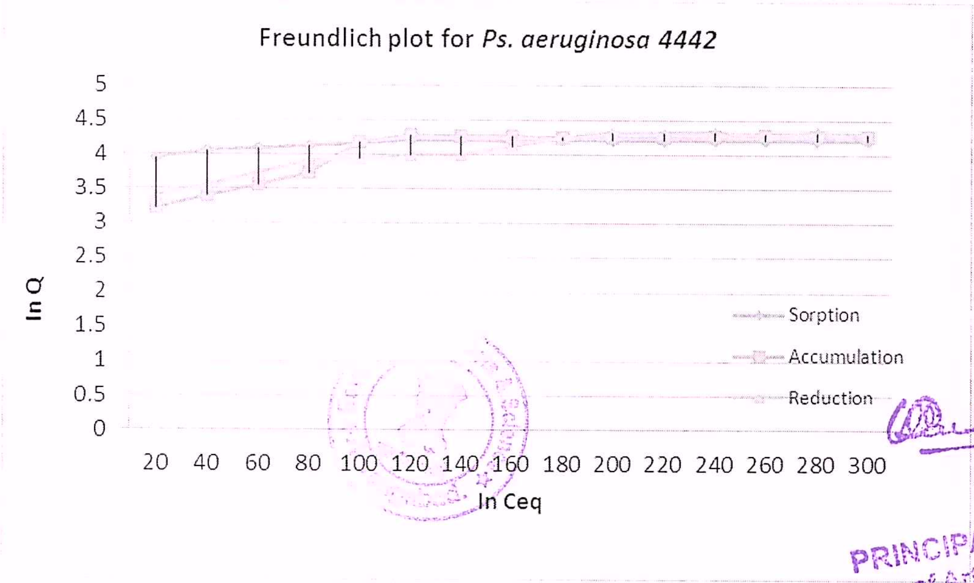




  

**Table 1: Comparison of adsorption isotherm parameters for Cr (VI) detoxification by *P. aeruginosa* 4442**

**Langmuir isotherm**

| **Detoxification mechanisms** | **Slope** | **Intercept** | **$R^2$** |
|---|---|---|---|
| Sorption | 0.0141433 | 0.1462781 | 0.9973 |
| Accumulation | 0.01100486 | 0.69363619 | 0.9576 |
| Reduction | 0.01056013 | 0.73241333 | 0.9778 |

**Table 2 Freundlich isotherm**

| **Detoxification mechanisms** | **Slope** | **Intercept** | **$R^2$** |
|---|---|---|---|
| Sorption | 0.09762 | 3.697449 | 0.8985 |
| Accumulation | 0.441631 | 1.905509 | 0.8597 |
| Reduction | 0.381308 | 2.20682 | 0.9690 |

**Table 3: $Q_{max}$ for *P. aeruginosa 4442* by different detoxification mechanisms**

| **Detoxification mechanisms** | **$Q_{max}$ for Cr (VI) 100ppm solution** |
|---|---|
| Sorption | 109.21mg/gm |
| Accumulation | 123.35mg/gm |
| Reduction | 101mg/gm |

The coefficient of determination ($R^2$) for Langmuir models was greater than 0.95. For sorption 0.99, for accumulation it was 0.95 and for reduction it was 0.97 all were close to 1 (Table 1 and 2).This indicates that this model adequately describe the experimental data of the detoxification of Cr (VI) compared to Freundlich. Langmuir data was used for the calculation of the maximum theoretical capacity by the test organism (Table 3) it was found to be 109 ppm/gm by growth independent sorption mechanism, 123.35 mg/gm by growth dependent accumulation and 101mg/gm by reduction. Our results are similar to that of Vasanthi (2004) who observed 149.25 percent mg/gm of chromium adsorption by *Pseudomonas sp*.S6 and 149.25 percent mg/gm by *Bacillus sp* S5.

So far, the most exhaustive studies and review on the occurrence of heavy metals in various soils and aquatic biotypes as well as the metal tolerance by bacteria has been presented by various authors. The selected bacterial strain *P. aeruginosa 4442* was found to be capable of tolerating differing metal tolerance of the different metals under study. The detoxification mechanism observed in the test organism was found to be metabolism independent sorption, metabolism dependent accumulation. The isolated *Pseudomonas aeruginosa 4442* strain was capable of Cr (VI) accumulation under aerobic as well as anaerobic conditions. While comparing the mechanisms of metal detoxification by the *P. aeruginosa 4442* aerobic growth dependent was found to be the most suitable that showed 123.35mg/gm of Cr (VI) detoxification, followed by growth independent sorption (109.21mg/gm) and then anaerobic reduction (101mg/gm). Chaturvedi *et. al.,* (2007) observed that the strain MN1 of *Bacillus circulans* was capable of Cr (VI) reduction under aerobic as well as anaerobic condition, and anaerobic conditions were found to be more suitable and a maximum 68% Cr (VI) reduction could be achieved in 28h. The studies so far carried out will be helpful in deciding the strategies that can be used to clean up the metal containing waste. If there are common seawares for the entire industrial zone, then brewing, Pharma industrial effluent containing various organic and inorganic constituents will support the growth of organisms that can be applied for clean-up procedure furnishing nutritional requirement of the organism under study. Veglio and Beolchini(1997) pointed out that investigations on the performance of free cells for metal uptake provide fundamental information on the equilibrium of biosorption process which is useful for application.

  

The study gives the clues towards multidimensional strategies which can be adapted for environmental clean-up. The isolated *P. aeruginosa 4442* culture may be suitable for Cr (VI) removal from industrial wastes. Optimized growth conditions would help in efficient growth of strains. The presence of Cr (VI) resistance gene on the plasmids makes possible the genetic manipulation of the strains for the enhanced efficiency. Mutagenesis can be achieved in the strains under consideration for better adaptation of the strains. The strain isolated show a potential towards the development of potential industrial strains.

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PRINCIPAL
Govt. College of Arts & Science
Aurangabad