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EMPLOYING ADSORPTION ISOTHERMS TO EXPLAIN METAL-MICROBE INTERACTION: A LITERATURE REVIEW.

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ABSTRACT

Adsorption is a process occurring in Microbe –metal interactions and hence can be explained using isotherms. The different types of adsorption isotherms have been discussed along with their equations and assumptions. Several of these microbes can be used as low cost biotechnological tools for treatment of effluents containing heavy metals. This is mainly on account of their flexibility and their aptitude to adapt in different environments. Microbial processes of detoxification of chromate and other heavy metals can be through a number of processes occurring intracellularly and extracellularly. Biosorption can also be one of these methods and hence can be explained using these isotherms.

KEY WORDS: Adsorbents, adsorption, sorbate, sorption, Freundlich,, Langmuir, biosorption, reduction.

INTRODUCTION

Adsorption isotherms are used to study the process of adsorption through graphs. Several isotherm models such as single layer phenomenon given by Langmuir (1918), Freundlich (1926), and Scatchard (1949), Multilayer adsorption explained by the Brunaur-Emmett-Teller (BET) isotherm which also reduces to the Langmuir model when the limit of adsorption is a monolayer has been stated by Brunaur *et al.* (1938).Other isotherms include the Temkim isotherm, the Frumkin, the Gibbs, Redlich –Peterson, Lineweaver-Burk etc. Generally these isotherms show how adsorptions take place and may serve as design parameters in the treatment of heavy metals from waste waters. Adsorption has been treated as a first order pseudo first order and pseudo second order processes and the kinetics of adsorption controls the process efficiency. The electron donors implicated in a Cr (VI) reduction are NADH and NADPH (Bopp and Ehrlich,1988); they are active within a wide range of temperatures from 40 to 70^{0}C and pH 6 to 9 under laboratory conditions. Riboflavin derivates FAD and FMN have been found to be essential coenzymes for chromate-reducing flavoenzymes (Masayasu,1991).

LITRETURE REVIEW

Adsorption isotherms:

Adsorption isotherms are used to study the process of adsorption through graphs. It is the graph between the amounts of adsorbate (x) adsorbed on the surface of adsorbent (m) and pressure at constant temperature. In order to estimate practical or dynamic adsorption capacity, it is essential to have enough information on adsorption equilibrium. Since adsorption equilibrium is the most fundamental property, a number of studies have been conducted to determine: The amount of species adsorbed under a given set of conditions (concentration and temperature) or how selective adsorption takes place when two or more absorbable components co-exist. When an adsorbent is in contact with the surrounding fluid of a certain composition, adsorption takes place and after a sufficiently long time, the adsorbent and the surrounding fluid reach equilibrium. This means that the equilibrium distribution of metal ions between the sorbent and the solution is important in determining the maximum sorption capacity.

Adsorption isotherms have been classified into six characteristic types. Microporous adsorbents produce adsorption isotherms of Type I which has a convex shape and it is also associated with mono-molecular layer adsorption. Type II and III depict adsorption for multi-molecular layer formation while Types IV and V describe the adsorption process of multi-molecular layer formation and condensation in pores. Type VI represents surface phase transition of a mono-molecular layer on a homogenous surface (Fried *et. al.,* 1977). Type III has a concave shape whereas II, IV, V, VI are sigmoid shape showing a plateau that is as pressure or concentration increases, amount adsorbed increases slowly first, sharply and then flattens out.

Several isotherm models are available to describe this equilibrium sorption distribution. Single layer phenomenon was referred by Langmuir (1918), Freundlich (1926), and Scatchard (1949). Multilayer adsorption is referred to by the Brunaur-Emmett-Teller (BET) isotherm and reduces to the Langmuir model when the limit of adsorption is a monolayer (Brunaur *et. al.,* 1938).Other isotherms include the Temkim isotherm, the Frumkin, the Gibbs, Redlich – Peterson, Lineweaver-Burk etc. Generally these isotherms show how adsorptions take place and may serve as design parameters in the treatment of heavy metals from waste waters.

Langmuir isotherm

In 1916 Langmuir proposed this isotherm known as Langmuir adsorption isotherm. This equation is used to estimate the maximum adsorption capacity corresponding to complex monolayer coverage on the adsorbent surface. It represents the equilibrium distribution of metal ions between the solid and liquid phases. The following equation can be used to model the adsorption isotherm:-

$q_e = QbC_e/1+bC_e$ where,

q_e = amount of adsorbate adsorbed per unit weight of adsorbent,mg/g

C_e =equilibrium concentration of the adsorbate mg/l.

The constants Q and b are Langmuir constants. The values of Q and b are calculated from the intercept and the slope of the plot of Ce/q_e versus C_e.

This isotherm is based on following assumptions:-

- Metal ions are chemically adsorbed at a fixed number of well defined sites.
- Each site can hold only one ion
- All sites are energetically equivalent and
- There is no interaction between the ions.

When the initial metal concentration rises, adsorption increases while the binding sites are not saturated. It gives linear graph showing that adsorption follows Langmuir adsorption model.

Freundlich adsorption isotherm

The relation between the metal uptake capacity q_e (mg/g) of adsorbent and the residual metal concentration C_e (mg/l) at equilibrium is given by:-

$$\ln q_e = \ln k + 1/n \ln Ce$$

Where intercept in k is a measure of adsorbent capacity and the slope 1/n is the sorption intensity; k and n are calculated from the intercept and slope of the plot In q_e versus In C_e respectively. Freundlich model is basically empirical and was developed for heterogeneous surfaces. The model is useful means of data description (Sudha and Abraham, 2001).

Scatchard isotherm

Scatchard developed a model to describe the attraction of proteins for small molecules and ions, and also used to describe the biosorption equilibrium. The interaction of metal ion with the cell surface binding sites can be described.

Adsorption kinetics

The kinetics of adsorption controls the process efficiency. Adsorption has been treated as a first order pseudo first order and pseudo second order processes. In the present study, adsorption of copper (II) on activated fly ash has been described by first and pseudo second order models and also by a diffusion model.

Lagergren model

Lagergren proposed a pseudo first order kinetic model as

$$\text{Log}\,(q_e\text{-}q) = \log q_e - \frac{K_{ad}}{2.303}\,t$$

Where q= amount of copper (II) sorbed (mg/g) at time t (min), q_e= amount of copper adsorbed at equilibrium (mg/g) and K_{ad} = equilibrium rate constant of pseudo-first order adsorption (min^{-1}).

The plot of log $(q_e\text{-}q_t)$ versus t gives a straight line for the first order adsorption kinetics. First order rate constant K_{ad} is obtained from the slope of the straight line.

Pseudo second order model

Pseudo-second order rate equation is given by

$$\frac{1}{qt} = \frac{1}{K2qe2} + \frac{t}{qe}$$

Where K_2 = rate constant for the pseudo second order adsorption kinetics, g/mg min. the slope of the plot (t/q_t) verses t gives the value of q_e and form the intercept K_2 can be calculated.

Bacterial mechanisms of chromate resistance:

Chromium (VI) is toxic to biological systems due to its strong oxidizing potential that can damage cells (Kotas and Stasicka, 2000). However, some microorganisms in the presence or absence of oxygen can reduce the toxic form of Cr (VI) to its trivalent form (Polti *et. al.,* 2007). These microorganisms are known as chromium reducing bacteria (CRB). It has been demonstrated in a variety of bacterial species that chromate activity crosses biological membranes by means of the sulfate uptake pathway, which reflects the chemical analogy between these two oxyanions (Cervantes and Campos-Garcia 2007). Cr (III) crosses cell membranes with a low efficiency because it forms insoluble compounds (Cary 1992). Inside the cell, Cr (VI) is readily reduced to Cr (III) by the action of various enzymatic or non-enzymatic activities; the Cr (III) generated may then exert diverse toxic effects in the cytoplasm (Cervantes *et. al.*, 2001). A variety of chromate-resistant bacterial isolates has been reported, and the mechanisms of resistance to this ion may be encoded either by plasmids or by chromosomal genes (Cervantes and Campos-Garcia 2007). Usually, the genes located in plasmids encode membrane transporters, which directly mediate efflux of chromate ions from the cell's cytoplasm. Bacteria capable of chromate reduction are isolated by various scientist using different isolation conditions and carbon source. Some of them include *Achromobacter* spcultivated anaerobically on luria broth using glucose and lactate as C source (Zhou *et.al.*2008) *Alcaligenes eutrophus* cultivated aerobically using sodium gluconate(Nies and silver,1989) *Bacillus spp* which is aerobic use citric acid and D-glucose (Chirwa and Wang,1997).

Microbiology of chromium (VI) reduction:

Bacterial reduction of chromate is observed for both aerobic and (Bopp and Ehrlich, 1988) and anaerobic reduction systems (Gvozdyak *et.al.,* 1986). Three Cr (VI) reduction mechanisms have been described (Cervantes and Campos-Garcia 2007). In aerobic conditions, chromate reduction has been commonly associated with soluble chromate reductases that use NADH or NADPH as cofactors. Under anaerobiosis, some bacteria, like *Pseudomonos fluorescens* LB300 (Bopp and Ehrlich 1988), can use Cr (VI) as an electron acceptor in the electron transport chain. Reduction of Cr (VI) may also be carried out by biochemical reactions associated with compounds such as amino acids, nucleotides, sugars, vitamins, ascorbate is capable of reducing Cr (VI), and riboflavin derivates FAD and FMN are essential coenzymes for chromate-reducing flavoenzymes (Masayasu, 1991).

a) Aerobic cultures

Studies conducted using various species of microorganisms have shown that some species isolated from Cr (VI) contaminated environments are capable of reducing Cr (VI) to Cr (III). Most of these microorganisms show remarkable resistance to Cr (VI) toxicity through cellular level mutations and other community level survival mechanisms.

The Cr (VI) reduction activity under aerobic condition is generally associated with soluble proteins that use NADH as an electron donor either as a requirement for growth (Ishibashi *et.al.,* 1990) or for enhanced activity (Horitsu *et.al.,* 1987) as shown in following equation:

$$2\ CrO_4^{2-} + 13\ NADH \longrightarrow 2\ Cr^{3+} + 3\ NAD^+ + 8\ H_2O$$

Microorganisms may also reduce Cr (VI) under anaerobic conditions via the mediation of a soluble reductase, a membrane-bound reductase or both (Wang and Shen, 1995).

b) Anaerobic cultures

In sub-surface conditions at the contaminated site may be severely oxygen depleted due to lack aeration in laminar flow groundwater systems. Under anaerobic conditions microorganisms reduce Cr (VI) via the mediation of a soluble reductase, a membrane-bound reductase or both (Cheung *et.al.*, 2006).Some of the organisms do not require organic carbon sources as energy sources and electron donors. Some of these utilize CO_2 and HCO_3 as carbon sources as shown in the following equation:

$$HCO_3^- + CrO_4^{2-} + 9\ H^+ + 3\ NADH \longrightarrow Cr^{3+} + 3\ NAD^+ + \frac{3}{2}H_2 + 5\ H_2O + CO_2$$

The above reaction is an energy intensive reaction involving the consumption of NADH and ATP, where Cr (VI) reduced at the expense of cellular growth and maintenance.

Biological chromium (VI) reduction pathways

a) Intra-cellular processes

In intra-cellular processes, Cr (VI) has been found to be reduced in the cytosol using cytoplasmic soluble reductase enzymes. These enzymes play an intermediate role between associated biological electron donors. The electron donors implicated in a Cr (VI) reduction are NADH and NADPH; they are active within a wide range of temperatures from 40 to 70^0C and pH 6 to 9 under laboratory conditions.

b) Extra-cellular processes

Gadd (1988) established the extra-cellular Cr (VI) reduction pathways in sulfate reducing bacteria through a mass balance in which 90% of the reduced Cr was detected in the supernatant. Yewalkar *et. al.*, (2007) detected 10-19% of the total Cr radioactivity inside the cells, 1-2% was attached to the cell wall and the remaining radioactivity was situated in the supernatant. Thus confirms the extracellular Cr (VI) reduction theory. In this case, only living cells could reduce Cr (VI).

c) Membrane associated Cr (VI) reduction

It is suggested that the reductase may be exported to the medium and that Cr (VI) is reduced in the external environment. This theory was investigated by Wang and Shen in (1993) using mass balance studies of chromium inside cells and in the supernatant in which Cr (VI) reduction pathways in and outside the cells were investigated using the Gram-negative bacteria – *E. coli* ATCC 33456.

In the membrane- associated Cr. (VI) reduction, a constitutive enzyme mediates the transfer of electrons from intercellular electron donors such as NADH and NADPH to Cr (VI) as the terminal electron acceptor. In this model, electron transport to the reductase is mediated by the trans membrane proton pumps such as NADH- dehydrogenase and the cytrochromes a to c_3. This type of Cr (VI) reduction has been also observed in *P. maltophilia* O-2 and *Bacillus megaterium* TKW3 (Cheung and Gu, 2007).

Based on the critical analysis of various microbial biomasses and the isotherm studies a few potent metal sequestering biosorbent have been commercialized. A potent algal biosorbent AlgaSorb^{TM} was developed using a fresh water alga *Chlorella vulgaris* to treat wastewater containing toxic metal ions. It can efficiently remove metallic ions from dilute solutions, i.e. 1-100mg/l and reduces the concentration of metal(s) down to 1 mg/l or even below.

Other metal sorption agent AMT-BIOCLAIM^{TM} (MRA) has employed *Bacillus* biomass to manufacture granulated material for wastewater treatment and metal recovery. This can accumulate metal cations with efficient removal of more than 99% from dilute solutions. It is non-selective and metal(s) can be stripped using H_2SO_4, HCl, HNO_3 NaOH or complexing agents and the granules can be regenerated for repeated use. Bio-Fix biosorbent uses biomass from a variety of sources including *Cyanabacterium* (*Spirulina*), yeast, algae and plants (*Lemna* sp. and *Sphagnum*). The biomass is blended with xanthum and guar gums to give a consistent product and immobilized as beads using polysulfone. Zinc binding to this biosorbent is approximately 4-fold higher than the ion exchange resins. Evaluations of the Cd, Cu and Zn biosorption in two metal systems using an algal biosorbent is studied by De Carvalho (1995)

The important wastewater treatment systems with full-scale applications

The Homestake Mine at Lead, South Dakota, (USA) is one of the oldest and largest underground gold mines in the western hemisphere. The plant began operating in 1984. The plant for the revolutionary process- which uses microorganisms to achieve water quality standards prior to mine water discharge- was donated to the South Dakota school of mines (Homestake Mining Company, 1999).

Bacteria of the genus *Pseudomonas* predominate in the biofilms in the first stage of the bioreactor which is followed by autotrophic nitrifying bacteria. The wastewater first enters a mix tank, and then proceeds to the train of five rotating biological contractors. Excess biomass continuously sloughs off from the disks and sediments in a clarifier, flocculants

(ferric chloride or polymeric flocculants) may be added to improve settling. After passing an emergency clarifier the final effluent is sand filtered (Mudder and Whitlock, 1984, Whitlock, 1987).

The Mersafin sand filter (Europe) is the biologically active moving-bed sand filter has been developed by a European consortium of 10 research and industrial partners from 1996 to 1999 funded by the European Union (Project 1). The system was designed to promote various known microbial processes of metal immobilization in a robust biofilms reactor: physico-chemical biosorption, biologically mediated precipitation and reduction. The filter is inoculated with a mixed population of wastewater adapted, non-pathogenic bacteria providing the desired performance (Pernfuss *et al.*, 1999).

The Bicmer concept operated in Beljium (Bacteria immobilized composite membrane reactor) was developed by the Vlaamse installing voor technologic onderzoek (Vito) in the early 1990's. In the tubular membrane reactor, with respect to heavy metals, Bicmer systems have been tested in pilot scale (upto $18M^2$ of membrane surface 50-300 liter/h) to treat waters containing metals (Diels, 2001).
Agharkar Research Institute (ARI), Pune, India, developed a microbiological process for the treatment of chromate-containing wastewaters. Using a "two-stage selection" procedure a strain of *Psedomonas mendocina* MCB B-180 was isolated which was able to reduce 2 m*M* chromate (100 mg/liter hexavalent chromium) with an efficiency >99.9% in 24 h (Rajwade and Paknikar, 1997). The strain was resistant to chromium

CONCLUSION

It may be thus seen from the above discussions that cell mediated or cell independent processes are involved in metal binding and neutralization of toxicity of heavy metals such as Chromium. Since biosorption is a passive and therefore independent process it is also found to be rapid and reversible. Bacterial cells, fungal mycelia, yeast and algal biomass have all been used as biosorbents. Thus the metal –microbe interactions using these cells, mycelia or biomass can be suitably explained using one of the above mentioned adsorption isotherms.

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