A Seminar Paper on

Bacterial Extracellular Polymeric Substances: Characteristics and Bioremoval of Heavy Metals

Course Title: Seminar

Course Code: ENS 598

Term: Summer, 2020

Submitted To:

Course Instructors

Dr. A. K. M. Aminul Islam
Professor

Dr. Md. Mizanur Rahman
Professor

Dr. Dinesh Chandra Shaha
Associate Professor

Dr. Md. Sanaullah Biswas
Associate Professor

BSMRAU, Gazipur

Major Professor

Dr. Md. Manjurul Haque
Professor
Department of Environmental Science
BSMRAU, Gazipur

Submitted By:

Md. Mohiminul Haque Mithun

Reg. No. 15-05-3509

MS Student

Term: Summer, 2020

Department of Environmental Science

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY

GAZIPUR-1706

ABSTRACT

Extracellular polymeric substances (EPS) of microbial origin are a fancy mixture of biopolymers having polysaccharides, proteins, nucleic acids, uronic acids, humic substances, lipids, etc. Bacterial secretions, cell lysates and adsorption of organic constituents from the environment result in EPS formation in a wide variety of free-living bacteria as well as microbial aggregates like biofilms, bioflocs and biogranules. EPS could be loosely attached to the cell surface or bacteria may be embedded in EPS. Regulated by the organic and inorganic constituents of the microenvironment compositional variation exists amongst EPS extracted from pure bacterial cultures and heterogeneous microbial communities. EPS function mainly works as cell-to-cell aggregation, adhesion to substratum, formation of flocs, protection from dessication and resistance to harmful exogenous materials. Additionaly exopolymers fuction biosorbing agents by accumulating nutrients from the encircling environment and also play an important role in biosorption of heavy metals. EPS produced by *Bacillus* sp. reported for the removal of copper, lead and zinc from different solutions. Some other EPS produced bacterial strain like *Pseudomonas* sp. are also reported as good removal option that remove Copper, cadmium, lead etc. Many more bacterial strains that produce EPS are found effective in heavy metal removal. Moreover, in EPS enzymatic activity also assist detoxification of heavy metals by transformation and subsequent precipitation in the polymeric mass. Although using microbial exopolymer the core mechanism for metal binding and / or transformation remains identical. In future chemical processes will be avoided and bioremoval process will only be followed because of it’s eco-friendly, cost effective nature.

Keywords: Bacterial EPS, Function, Biofilm, Bioremoval etc.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The term Extracellular polymeric substance was first coined by Sutherland in 1972 to describe higher molecular weight carbohydrate produced by marine bacteria. The production of extracellular polymeric substances (EPS) involves a major investment of carbon and energy by microorganisms. Considering the tendency in nature to conserve instead of to waste, this expenditure of energy (in some cases quite 70%) is probably going to carry benefits to the producers of EPS, still as those organisms related to them. Bacteria are very efficient in converting nutrients into EPS; it's been calculated that one azotobacter cell can produce enough EPS to coat over 500 particles with a 0.4 μm diameter per day. the dimensions of one cell is often 1-2 μm by 0.5 μm, and sometimes much smaller, and thus this number is impressive (Underwood *et al.*, 1995). The importance of EPS has long been recognized and a range of functions are attributed to EPS as far because the benefits they supply to cells, that can live in heterogeneous communities or as single organism in binary association (Wingender *et al.,* 1999).

EPS composition analysis shows that every component has different role that works for surviving of cells in adverse environmental condition. Bacterial EPS has a main function as biofilm formation that Flemming *et al.,* 2007 said that "if metaphorically biofilm called as "city of microbes" then EPS represents the "House of biofilm cell". That component creates strong bonds and give support to form, to survive in adverse environment and hardiness that make able to adsorb metals. Though biofilm formation by EPS depends on gram negativity or positivity of bacteria. Positive strains found more effective to form biofilm in a very rapid way (Jayathilake *et al., 2017).*

The function of EPS is very critical to understand because of its components. EPS is a very complex mixture of polysaccharides, proteins, nucleic acids, humic substances, lipids etc (Pal and Paul, 2008). Bacterial secretions, detachment of cell surface materials, and organic constituent adsorption from the environment result in EPS formation in a wide variety of free-living bacteria as well as microbial aggregates like biofilms, bioflocs etc (Pal and Paul, 2008). According to different origins, EPS may be loosely attached to the cell surface or bacteria can be embedded in EPS. Many variation of components found in EPS extracted from pure bacterial cultures which are regulated by the organic and inorganic constituents of the microenvironment. Functionally, EPS works as cell-to-cell aggregation, adhesion to substratum, protection from dessication and resistance to harmful exogenous materials. By accumulating nutrients from surrounding environments EPS acts as biosorbing agent and play vital role in bioremoval of heavy metals. EPS forms complexes with metal cations resulting in metal immobilization within the exopolymeric matrix as EPS found as polyanionic in nature. Electrostatic interactions occurs between the metal and negatively charged components of biopolymers (Pal and Paul, 2008). EPS also detoxify the heavy metals by transformation and subsequent precipitation in the polymeric mass because of enzymatic activities. Although the important mechanism for metal binding and transformation using bacterial exopolymers remains identical, the existence and complexity of EPS from pure bacterial cultures, biofilms, biogranules and other systems differ significantly, which affects the EPS-metal interactions process. Day by day removal of heavy metals by microorganism is getting popular though there have so many chemical process that works better but have severe impact on environment. Some physico-chemical process like membrane filtration, electrodialysis, photoctalysis found very effective on heavy metal removal (Barakat, 2011). These chemical processes are very much costly and can hamper environmental balance. So that scientists want to focus on a biological process that can help to remove heavy metals from contamination and make environment healthier. In this phenomenon bacterial EPS have very important role to play

Keeping these above considerations in view, the present study has aimed with the following objectives:

Objectives:

1. To know about characteristics of bacterial extracelluar polymeric substances(EPS).

2. To get an overall idea about the function of bacterial EPS and role as bioremoval component of heavy metals.

Chapter II

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. Therefore, all the information was collected from secondary sources with a view to prepare this paper. Various relevant books and journals, which were available in internet, were used for the preparation of this paper. Good suggestions, valuable information and kind consideration from my honorable Major Professor, course instructors and other resources personnel were taken to enrich this paper. After collecting necessary information, it has compiled and arranged chronologically for better understanding and clarification.

CHAPTER III
REVIEW OF FINDINGS

3.1 Bacteria

Bacteria are single celled microorganism that that they constitute a large domain of prokaryotic microorganism. Bacteria have variety number of shapes ranging from spheres to rods and spirals. Normally few micrometers in length. Bacteria were one of the first life forms to appear on earth, and can be found most of earth's habitats like soil, water and also deep biosphere of the earth's crust. With plants and animals bacteria also live in symbiotic and parasitic relationship (Fredrickson *et al.*, 2004).

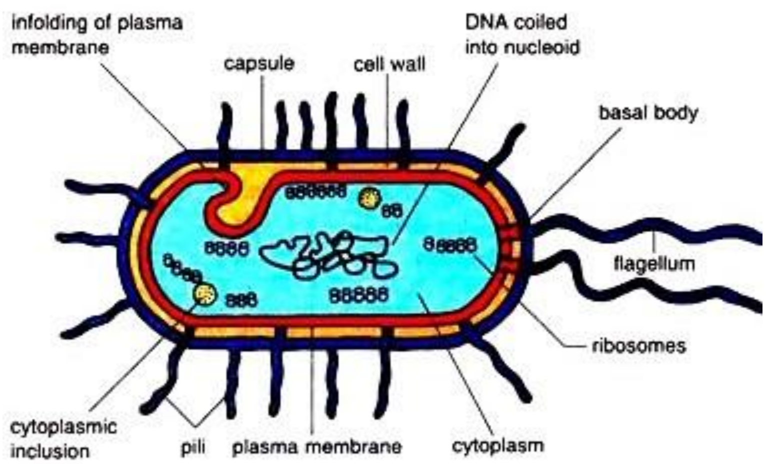

Figure 1. Sections of bacterial cells. (Source: https://biologydiscussion.com/bacterialcell)

3.2 Bacterial EPS (Extracellular polymeric substance)

The overwhelming majority of microorganisms live and grow in aggregated forms like biofilms and flocs ("planktonic biofilms"). This mode of existence is lumped within the somewhat inexact but generally accepted expression "biofilm"(Gupta and Diwan, 2017). The common feature of these phenomena is that the microorganisms are embedded by a matrix of extracellular polymeric substances (EPS). EPS was defined as "extracellular polymeric substances of biological origin that participate in the formation of microbial aggregates" (Geesey, 1982). The assembly of EPS may be a general property of microorganisms in natural environments and has been shown to occur both in prokaryotic (Bacteria, Archaea) and in eukaryotic (algae, fungi) microorganisms. EPS are mainly accountable for the structural and functional integrity of biofilms and are considered because the key components that determine the physicochemical and biological properties of biofilms. EPS form a gel-like, highly hydrated, three-dimensional, and sometimes charged biofilm matrix, within which the microorganisms are embedded and more or less immobilized. EPS create a microenvironment for sessile cells which is conditioned by the character of the EPS matrix (Wingender *et al.*, 1999).

3.3 Composition of bacterial EPS

EPS comprises polysaccharides, including cellulose nanofibers and sucrose-derived glucans and fructans, proteins, such as lectins, baplike proteins and proteinaceous appendages mainly curli fimbriae extracellular DNA, lipids, surfactants (e.g., rhamnolipids), and other biopolymers, including humic substances (Mosharaf *et al.*, 2018). Every component of EPS has some subunits and have different linkage types also that shown in table-1 below.

Table 1. General Composition of bacteria EPS; Humic substances also included

Main components of EPS	**Principal components (subunits, precursors**	**Main type of linkage between subunits**	**Structure of polymer backbone**
Polysaccharides	Monosaccharides, uronic acids and amino sugars	Glycosidic bonds	Linear, branched
Proteins (polypeptides)	Amino acid	Peptide bond	linear
Nucleic acids	nucleotides	phosphodiester bonds	linear
Phospholipids	fatty acids, glycerol, phosphate, ethanolamine, serine, choline and sugars	ester bonds	side-chains
Humic substances	phenolic compounds simple sugars amino acids	ether bonds, C-C bonds, peptide bonds	Cross-linked

(source: Wingender *et al.*, 1999)

3.3.1 Variation of EPS Content during aerobic sludge granulation

According to a study shows that in aerobic sludge granulation process protein and polysaccharide fluctuation occur that under the condition of high shear force, the metabolic pathway of microorganisms began to be regulated and the component of sludge EPS shifted. Along with decrease of sludge settling time, poor settling microorganism discharged and microbial community with good flocculation enrich which results in increase of protein and polysaccharide content (Zhu *et al.,* 2012). However, with the maturation of granular sludge, the PS(polysaccharide) content decreased probably due to the consumption of the microorganisms during the starvation phase (Wang *et al.*, 2005).

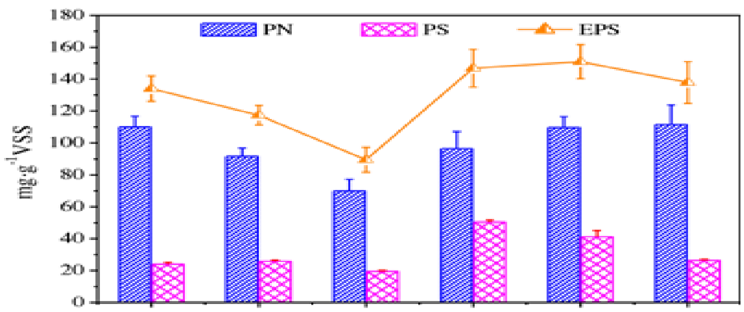

Figure 2. EPS content variation during aerobic sludge granulation. (source: Zhu *et al.*, 2012)

3.3.2 Bacterial EPS component by Fourier Transform Infrared Spectroscopy(FTIR) Test:

According to a study by FTIR test it was observed that all bacterial EPS were dominant with protein contents producing peaks at different amide region and also observed that EPS consisted with high content of polysaccharide. Also indicates the presence of small amount of lipids (Mosharaf *et al.,* 2018).

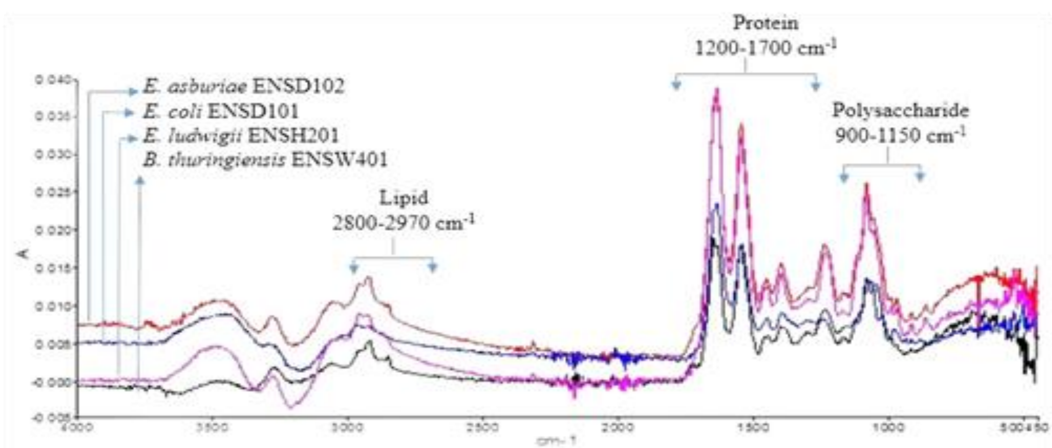

Figure 3. EPS component in different bacteria by FTIR test. (Source: Mosharaf *et al.*, 2018)

3.4 Function of bacterial EPS

Majority of gram-negative bacteria produce EPS that they invest 70% of their energy in its production. Many function have been proposed for bacterial EPS. They can be divided into groups (Weiner *et al.,* 1995). That shown in the table-2

Table 2. EPS function and cell survival

Function	**Survival advantages**
Physical/protective barrier	Protection from desiccation, predation and the immune system. Resistance to toxins, antibiotics and poisons
Cell-cell recognition and interaction	Plant symbiosis, formation of nodules and microcolonies, invertebrate larvae settlement
Response to environmental stress	Sequestering and import of charged ions, production of excess reducing power
Adhesion and biofilm formation	Immobilization onto nutrient-rich surfaces, dissociation from nutrient-depleted surfaces

(Source: Weiner *et al.,* 1995)

3.4.1 Function of EPS for biofilm formation

According to a study it was said that if metaphorically biofilm called as "city of microbes" then EPS represents the "house of biofilm cell" (Flemming *et al.,* 2007). The immediate conditions of life of biofilm cells living in this microenvironment by affecting porosity, density, water content, charge, sorption properties, hydrophobicity, and mechanical stability is determined by the EPS. EPS are biopolymers of microbial origin in which biofilm microorganisms are embedded (Flemming *et al.,* 2007). EPS functionality that effects of EPS component and role in biofilm shown below in table-3.

Table 3. Effects of EPS component on biofilm

Effects of EPS components	**Nature of EPS component**	**Role in Biofilm**
Constructive	*Neutral polysaccharides *Amyloids	*Structural component *Structural component
Sorptive	Charged or hydrophobic Polysaccharides	Ion exchange, sorption

Active	Extracellular enzymes	Polymer degradation
Surface active	*Amphiphilic *Membrane vesicles	*Interface interactions *Export from cell, sorption
Informative	*Lectins *Nucleic acids	*Specificity, recognition *Genetic information, structure
Redox Active	Bacterial refractory polymers	Electron donor or acceptor
Nutritive	Various polymers	Source of C, N, P

(Source: Flemming *et al.,* 2007)

3.4.2 Drought protection

Environment like in drought stress EPS can offer advantages to microorganism. It was observed that EPS have high water holding capacity produced by a *Pseudomonas* strain isolated from soil; this EPS can hold several times its weight in water (Costa *et al.*, 2018). By acting like a protective sponge EPS protect bacteria against desiccation and gives the bacteria time to make metabolic adjustment (Costa *et al.,* 2018).

3.4.3 Salt tolerance

Study revealed that microbial polymers are also responsible for tolerance of salt stress. The polymer prevents nutrient imbalance and osmotic stress which promote survival capacity of bacteria in highly salt stressed area (Costa *et al.,* 2018).

3.4.4 Protection against low/high temperature

In severe environmental condition like in winter season EPS have very important role that shields microorganisms. It was observed in samples that were collected from arctic sea ice (Krembs *et al.,* 2002). EPS can alter the microstructure and desalination of growing ice that improves microbes habitability and survivability (Krembs *et al.,* 2011). EPS also found as a protection factor for thermophilic bacteria by shielding microorganisms from very high temperature (Nicolaus *et al.,* 2000).

3.4.5 Protection from antimicrobials

EPS plays an important role for biofilm that decreased susceptibility to antimicrobials by surrounding microorganisms. Normally biofilm compounds are negatively charged and bind with positively charged compound, protecting the inner cells from contact (Costa *et al.,* 2018). Many studies have tested the inhibitory mechanisms of bacterial EPS against antimicrobial compounds, particularly for clinically important bacterial strains (Costa *et al.,* 2018).

3.5 Bacterial biofilm and EPS

3.5.1 Bacterial biofilm

Biofilm are multicellular, structured, surface-adherent, microbial communities that mainly consists of cells embedded in a self-produced extra cellular polymeric substances (EPS) (Mosharaf *et al.,* 2018). EPS plays an important role in bacterial biofilm matrix compared with their free living planktonic counterparts that EPS gives protection of the cells from adverse environmental condition like high concentration of toxic chemicals, change in pH, temperature, salt concentration and water content (Mosharaf *et al.,* 2018). Bacterial biofilm enclosed in a mixture of polymeric compounds, generally referred as extracellular polymeric substance(EPS) that over 90% of microorganisms on earth lives within these biopolymers (Vu *et al.*, 2009). With the attachment of a cell to a surface biofilm formation starts. Colony forms through division of the bacterium, and production of the biofilm matrix is initiated. Other bacteria can then be recruited as the biofilm expands owing to cell division and the further production of matrix components (**Figure 4a**) (Flemming and Wingender, 2010). The major matrix components-polysaccharides, proteins and DNA are distributed between the cells in a non-homogeneous pattern (**Figure 4b)** (Flemming and Wingender, 2010).

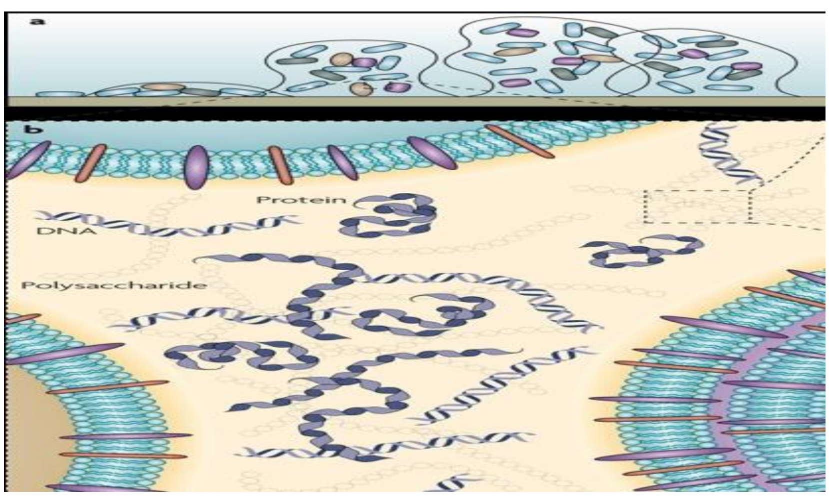

Figure 4. Biofilm formation on a surface (a), Major matrix components distributed between cells(b). (Source: Flemming and Wingender, 2010)

3.5.2 Some biofilm producing bacteria

Formation of biofilm by bacteria are very much different that many Gram positive and Gram negative bacteria produce biofilm. Type of bacteria produce biofilm differ with the difference of source.

Table 4. Different types of biofilm producing bacteria with source

Source	Bacterial strain	References
Dyeing industry	*Escherichia coli* *Enterobacter asburiae*	Mosharaf *et al.*, 2018
Household and different industrial wastewater	*Enterobacter ludwigii*	Mosharaf *et al.*, 2018
Garments industry	*Pseudomonas fluorescens* *Acinetobacter lwoffii* *Klebsiella pneumonia*	Mosharaf *et al.*, 2018
Washing plant industry	*Bacillus thuringiensis*	Mosharaf *et al.*, 2018
Tannery industry	*Escherichia coli*	Mosharaf *et al.*, 2018
Seeds, sprouts and food crops	*Pseudomonas putida*	Danhorn and Fuqua, 2007
Animals and plants	*Enterococcus faecalis*	Danhorn and Fuqua, 2007
Marine water	*Marinobacter sp.*	Bhaskar and Bhosle, 2006
Aqueous Solution	*Bacillus firmis*	Salehizadeh and Shojaosadati, 2003

3.6 Mechanism and application of bacterial EPS as heavy metal removal

The phrase heavy metal is defined as metals or metalloids with an atomic density greater than 5 g cm^{-1}. Though less amount of these heavy metals (Cu, Zn, ni etc.) are necessary for life in low concentration and also known as micro elements or trace elements. That these elements have an important role in metabolic processes. But higher concentration of these heavy metals and low concentration of non-essential heavy metals can damage environment very badly. That heavy metals cannot be destroyed and degraded in environment because of their persistency and stability in nature.

Many traditional practices are found to remove heavy metals but they are very much costly and have big threats for environments like bioaccumulation and biomagnification etc. These processes are also very much energy consuming.

Bioremoval is not cost effective but also it is very much ecofriendly and safe solution for heavy metal removal process. Because of having high surface-to-volume ratio among all forms of life bacteria have superior competence to absorb metals from solution (Beveridge, 1989). Bacterial EPS is associated with formation of biofilm. Biofilm EPS have an important role in bioremediation of heavy metals (Baker-Austin *et al.,* 2006). For survival of biofilm forming bacteria and for grow in metal contaminated environment EPS shows excellent protective role (Mohite *et al.,* 2017). With the increase of accumulation of metals the amounts of EPS production also becomes higher (Mohite *et al.,* 2017). EPS have strong binding capability and have large quantities of negatively charged functional group that binds positively charged heavy metals easily (Pal and Paul, 2008).

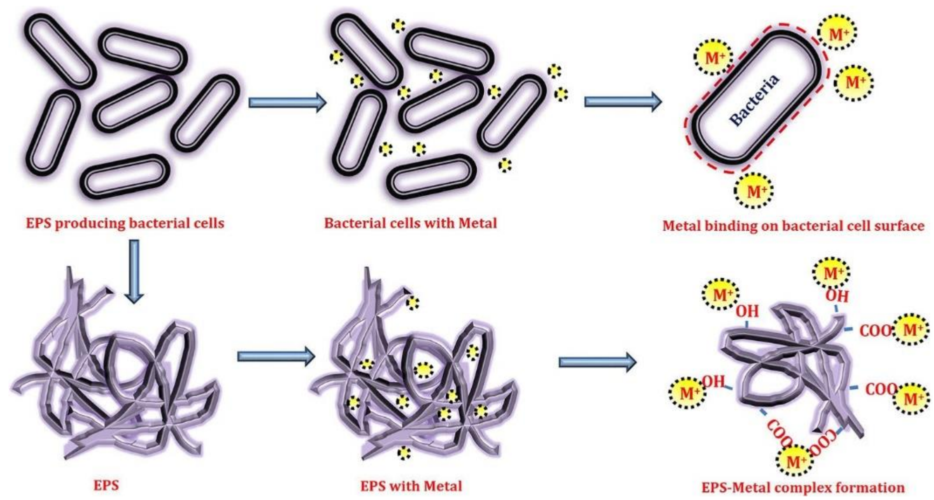

Figure 5: Schematic representation of the mechanism of Bacterial EPS interaction with heavy metals. (Source: Mohite *et al.,* 2017)

since the 1990s there is a remarkable increase in the number of publications (research papers and reviews) dealing with the use of EPS for heavy metal removal. It is accepted that naturally occurring biological methods are beneficial rather than the physicochemical techniques for remediation of lethal heavy metal ions. Many scientific work has been found that worked on application of Bacterial EPS and its ability to removing heavy metals. Some findings are added (Table 5) that shows application of Bacterial EPS and its result from different work.

Table 5. Application of Bacterial EPS and its results as heavy metal removal

Bacterial EPS-Producing microorganisms	**Metals (removed by particular organism)**	**Removal Efficiency**	**Reference**
Methylobacterium organophilum	Copper, Lead	21% Cu^{2+},18% Pb^{2+} removal	Kim *et al.*, 1996
Herminiimonas arsenicoxydans	Arsenic	Upto 5 mmol/L metal ion uptake	Marchal *et al.*, 2010
Pseudomonas sp	Copper	N.A	Lau *et al.*, 2005
Enterobacter cloaceae	Cadmium, Copper, Chromium	65% Cd^{2+}, 20% Cu^{2+}; 75% Cr^{6+} reduction from 100 ppm initial metal load	Gutierrez *et al.*, 2012; Iyer *et al.*, 2005
Shewenella oneidensis	Cadmium	80% Cd^{2+}	Ha *et al.*, 2010
Anabaena spiroides	Manganese	8.52 mg Mn^{2+} /g EPS	Freire-Nordi *et al.*, 2005
Gloeocapsa gelatinosa	Lead	82.22±4.82 mg Pb^{2+}/ g EPS	Raungsomboon *et al.*, 2006
Calothrix marchica	Lead	65 mg Pb^{2+} / g CPS	Ruangsomboon *et al.*, 2007
Gloeocapsa calcarea	Chromium	36mg Cr^{6+} /g EPS at 20ppm initial metal load	Sharma *et al.*, 2008
Nostoc punctiforme	Chromium	90.05mg Cr^{6+}/g EPS	Sharma *et al.*, 2008
Lyngbya putealis	Chromium	157 mg/g of EPS Cr^{6+} at 30 ppm initial load	Kiran and Kaushik, 2008
Pseudomonas putida	Cadmium	80% Cd^{2+} at 10 ppm initial	Kenney, 2010
Rhizobium tropici	Cadmium	80% Cd^{2+} at 10 ppm initial	Kenney, 2010
Bacillus firmus	Lead, Copper, Zinc	1103mg Pb^{2+} / g EPS (98.3%,) , 860 mg Cu^{2+} / g EPS (74.9%) , 722 mg Zn^{2+} /g EPS (61.8%)	Salehizadeh and Shojaosadati, 2003
Azotobacter chroococcum	Lead ,Mercury	40.48% Pb^{2+}(33.5 mg Pb^{2+}/g of EPS); 47.87% Hg^{2+} (38.9 mg of Hg/g EPS)	Rasulov *et al.*, 2013

Ensifer meliloti	Lead, Nickel, Zinc	89% Pb^{2+} , 85% Ni^{2+} , 66 % Zn^{2+} reduction from 50 ppm initial load	Lakzian, 2008
Paenibacillus jamilae	Lead, Cadmium, Nicle, Zinc, Coppur	200-300 mg Pb^{2+}/g EPS, 21 mg Cd^{2+}/g of EPS	Morillo *et al.*, 2006; Pérez *et al.*, 2008
Paenibacillus polymyxa	Copper	1602 mg Cu^{2+} / g EPS	Acosta *et al.*, 2005
Lactobacillus plantarum	Lead	276.44 mg Pb^{2+}/g EPS, at 1000 ppm initial metal load	Feng *et al.*, 2012
Bacillus cereus	Chromium	89.87% reduction from initial metal load of 50 ppm	Sultan *et al.*, 2012
Bacillus pumilus	Chromium	89.87% reduction from initial metal load of 50 ppm	Sultan *et al.*, 2012
Chryseomonas luteola	Cadmium, Cobalt, Copper, Nickel	82.5% reduction from initial metal load of 50 ppm	Sultan *et al.*, 2012

3.7 Drawbacks of using bacterial EPS as heavy metal removal

Biological process improvement potential is limited because bacteria have different environment demand and in all environment EPS not produce in exact way and also understanding of microorganism demand for growth is very difficult (Feng and Aldrich, 2004). For growth of bacterial EPS need a suitable pH, temperature, surface suitability etc. which is very difficult to maintain (Wingender *et al.*, 1999). The major problem is there have very limited process developed until now. Every known strains is working on a single or more about two heavy metal ions that will not actually work on contaminated site because there will be remain so many heavy metal ions contaminated (Feng and Aldrich, 2004).

CHAPTER IV

CONCLUSION

Bacterial EPS has been well known process that its already explored and appreciated as an environment friendly and safe process for bioremoval of heavy metals. It has rapid, efficient and sensitive response toward metal ion removal. If differentiated with chemical process bacterial EPS has proven as environment friendly and cost effective process. But EPS production is a very complex phenomenon that totally depends on environmental factors. Different Bacterial EPS produce in different environment. The reuse of EPS necessitates desorption after every use which may limit its use for not many times, as the efficiency may decrease on each use. Bacterial EPS is composed of protein, carbohydrate and various other components that indicates role of every component should be investigated to apply these EPS more appropriately. Many Works are ongoing. In future Bacterial EPS will be a common phenomenon in every sector for removing heavy metals.

References

Acosta, M. P., Valdman, E., Leite, S., Battaglini, F. & Ruzal, S. (2005). Biosorption of copper by Paenibacillus polymyxa cells and their exopolysaccharide. *World Journal of Microbiology and Biotechnology 21*, 1157-1163.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology, 14(4)*, 176–182.

Barakat, M. A. (2011). New trends in removing heavy metals from industrial wastewater. *Arabian journal of chemistry*, *4*(4), 361-377.

Beveridge, T. J. (1989). Role of cellular design in bacterial metal accumulation and mineralization. *Annual Reviews in Microbiology*, *43(1)*, 147–171.

Bhaskar, P. V., & Bhosle, N. B. (2006). Bacterial extracellular polymeric substance (EPS): a carrier of heavy metals in the marine food-chain. *Environment international, 32*(2), 191-198.

Costa, O. Y., Raaijmakers, J. M., & Kuramae, E. E. (2018). Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. *Frontiers in microbiology*, *9*, 1636.

Danhorn, T., & Fuqua, C. (2007). Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol.*, *61*, 401-422.

Feng, M., Chen, X., Li, C., Nurgul, R. & Dong, M. (2012). Isolation and Identification of an Exopolysaccharide-Producing Lactic Acid Bacterium Strain from Chinese Paocai and Biosorption of Pb (II) by Its Exopolysaccharide. *Journal of food science 77,* T111-T117.

Feng D and Aldrich C (2004) Adsorption of heavy metals by biomaterials derived from the marine algae *Ecklonia maxima Hydrometallurgy* 73:1-10

Flemming, H. C., Neu, T. R., & Wozniak, D. J. (2007). The EPS matrix: the "house of biofilm cells". *Journal of bacteriology*, *189*(22), 7945-7947.

Fredrickson, J. K., Zachara, J. M., Balkwill, D. L., Kennedy, D., Shu-mei, W. L., Kostandarithes, H. M., ... & Brockman, F. J. (2004). Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. *Appl. Environ. Microbiol.*, *70*(7), 4230-4241.

Freire-Nordi, C. S., Vieira, A. A. H. & Nascimento, O. R. (2005). The metal binding capacity of Anabaena spiroides extracellular polysaccharide: an EPR study. *Process Biochemistry 40*, 2215-2224.

Geesey GG (1982) Microbial exopolymers: ecological and economic considerations. ASM News 48:9-14.

Gupta, P., & Diwan, B. (2017). Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. *Biotechnology Reports, 13*, 58-71.

Gutierrez, T., Biller, D. V., Shimmield, T. & Green, D. H. (2012). Metal binding properties of the EPS produced by Halomonas sp. TG39 and its potential in enhancing trace element bioavailability to eukaryotic phytoplankton. *Biometals 25*, 1185-119.

Ha, J., Gélabert, A., Spormann, A. M. & Brown, G. E. (2010). Role of extracellular polymeric substances in metal ion complexation on Shewanella oneidensis: batch uptake, thermodynamic modeling, ATR-FTIR, and EXAFS study. Geochimica et Cosmochimica Acta 74, 1-15.

Jayathilake, P. G., Jana, S., Rushton, S., Swailes, D., Bridgens, B., Curtis, T., & Chen, J. (2017). Extracellular polymeric substance production and aggregated bacteria colonization influence the competition of microbes in biofilms. *Frontiers in microbiology*, *8*, 1865.

Kenney, J. P. (2010). Metal adsorption to bacterial cells and their products: University of Notre Dame.

Kim, S.-Y., Kim, J.-H., Kim, C.-J. & Oh, D.-K. (1996). Metal adsorption of the polysaccharide produced from Methylobacterium organophilum. *Biotechnology letters 18*, 1161-1164.

Kiran, B. & Kaushik, A. (2008). Chromium binding capacity of Lyngbya putealis exopolysaccharides. *Biochemical Engineering Journal 38*, 47-54.

Krembs, C., Eicken, H., and Deming, J. W. (2011). Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc. Natl. Acad. Sci. U.S.A. 108, 3653– 3658.

Krembs, C., Eicken, H., Junge, K., & Deming, J. W. (2002). High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(12), 2163-2181.

Lakzian, A. (2008). Adsorption Capability of Lead, Nickel and Zinc byExopolysaccharide and Dried Cell of Ensifer meliloti. *Asian Journal of Chemistry 20*, 6075-6080.

Lau, T., Wu, X., Chua, H., Qian, P. & Wong, P. (2005). Effect of exopolysaccharides on the adsorption of metal ions by Pseudomonas sp. CU-1. *Water Science & Technology 52*, 63-68.

Marchal, M., Briandet, R., Halter, D., Koechler, S., DuBow, M. S., Lett, M.-C. & Bertin, P. N. (2011). Subinhibitory arsenite concentrations lead to population dispersal in Thiomonas sp. PlOS one 6, e23181.

Mohite, B. V., Koli, S. H., Narkhede, C. P., Patil, S. N., & Patil, S. V. (2017). Prospective of microbial exopolysaccharide for heavy metal exclusion. *Applied biochemistry and biotechnology*, *183*(2), 582-600.

Morillo, J. A., Aguilera, M., Ramos-Cormenzana, A. & Monteoliva-Sánchez, M. (2006). Production of a metal-binding exopolysaccharide by Paenibacillus jamilae using two-phase olive-mill waste as fermentation substrate. *Current microbiology* 53, 189-193.

Mosharaf, M. K., Tanvir, M. Z. H., Haque, M. M., Haque, M. A., Khan, M. A. A., Molla, A. H., ... & Talukder, M. R. (2018). Metal-adapted bacteria isolated from wastewaters produce biofilms by expressing Proteinaceous Curli Fimbriae and cellulose nanofibers. *Frontiers in microbiology*, *9*, 1334.

Nicolaus, B., Panico, A., Manca, M. C., Lama, L., Gambacorta, A., Maugeri, T., et al. (2000). A thermophilic Bacillus isolated from an eolian shallow hydrothermal vent able to produce exopolysaccharides. *Syst. Appl. Microbiol. 23*, 426–432.

Pal, A., & Paul, A. K. (2008). Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. *Indian journal of microbiology*, *48*(1), 49.

Rasulov, B. A., Yili, A. & Aisa, H. A. (2013). Biosorption of Metal Ions by Exopolysaccharide Produced by Azotobacter chroococcum XU1. *Journal of Environmental Protection 4*, 989-993.

Raungsomboon, S., Chidthaisong, A., Bunnag, B., Inthorn, D. & Harvey, N. W. (2006). Production, composition and Pb2+ adsorption characteristics of capsular polysaccharides extracted from a cyanobacterium Gloeocapsa gelatinosa. *Water research 40,* 3759-3766.

Ruangsomboon, S., Chidthaisong, A., Bunnag, B., Inthorn, D. & Harvey, N. W. (2007). Lead (Pb2+) adsorption characteristics and sugar composition of capsular polysaccharides of cyanobacterium Calothrix marchica. *Songklanakarin J Sci Technol 29,* 529-541.

Salehizadeh, H. & Shojaosadati, S. (2003). Removal of metal ions from aqueous solution by polysaccharide produced from Bacillus firmus. *Water research 37,* 4231-4235.

Sharma, M., Kaushik, A., Bala, K. & Kamra, A. (2008). Sequestration of chromium by exopolysaccharides of Nostoc and Gloeocapsa from dilute aqueous solutions. *Journal of hazardous materials 157,* 315-318.

Sultan, S., Mubashar, K. & Faisal, M. (2012). Uptake of toxic Cr (VI) by biomass of exo-polysaccharides producing bacterial strains. *African Journal of Microbiology Research 6,* 3329-3336.

Underwood GJC, Paterson DM, Parkes RJ (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limnol Oceanogr 40*: 1243 – 1253

Vu, B., Chen, M., Crawford, R. J., & Ivanova, E. P. (2009). Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*, *14*(7), 2535-2554.

Wang, Z.W., Liu, Y., Tay, J.H., (2005). Distribution of EPS and cell surface hydrophobicity in aerobic granules. *Appl. Microbiol. Biotechnol. 69 (4)*, 469– 473.

Weiner, R., Langille, S., & Quintero, E. (1995). Structure, function and immunochemistry of bacterial exopolysaccharides. *Journal of industrial microbiology*, *15*(4), 339-346.

Wingender, J., Neu, T. R., & Flemming, H. C. (1999). What are bacterial extracellular polymeric substances? In *Microbial extracellular polymeric substances* (pp. 1-19). Springer, Berlin, Heidelberg.

Zhu, L., Qi, H. Y., Kong, Y., Yu, Y. W., & Xu, X. Y. (2012). Component analysis of extracellular polymeric substances (EPS) during aerobic sludge granulation using FTIR and 3D-EEM technologies. *Bioresource Technology*, *124,* 455-459.