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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF EXOPOLY SACCHARIDE PRODUCING BACTERIA FROM INDUSTRIAL WASTE WATER AS A BIOFLOCCULANT

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ABSTRACT

Exopolysaccharides (EPSs) are produced by micro-organisms that represent an inactive market. About 30 species of eukaryotic and prokaryotic microorganisms are known for their EPS production. EPSes are produced in response to biotic and abiotic stressors and/or adaptation to extreme conditions. Extracellular polymeric materials (EPS) in the soil are now gaining more attention due to the many challenges of the methods already overcome. In biofilms many microbes are present.These biofilms are represented by a large number of extracellular polymeric cells (EPS: mainly polysaccharides, glycoconjugates, and proteins) and embedded microbial cells. Exopolysaccharides (EPSs) play a broad role as biopolymers in the environment by replacing polymers that are synthetic, non-toxic, and produced by microorganisms. Exopolysaccharides (EPSs) are secreted by both eukaryotes (fungi, phytoplankton, and algae) and prokaryotes (archaeobacteria and eubacteria). EPS produced by bacteria encompasses a wide range of chemical properties and can be heteropolymeric or homopolymeric.

Keywords: Exopolysaccharide, Biofloculant, Heavy Metal Removal, Antimicrobial Activity.

I. INTRODUCTION

Bacterial exopolymeric substances (EPS) by cells are released in response to the physical pressure you encounter within the herbal environment. EPS is a structural component of the surface matrix of cells in which cells are inserted during biofilm development (Marvasi et al., 2010). In a sense, EPS can be defined as any long chain of polysaccharide or branched chains that are soluble in water, strong, solution, viscosity enhancement and/or gel form. Most microorganisms produce exopolysaccharides (EPS) and EPS of bacteria forming an important source of dissolved natural carbon in marine living resources. It is suggested that the bacterial EPS rich in uronic acid is evidence against the mineral extraction of bacteria and so you have a long time to live on a global ocean. To confirm this hypothesis, EPS-rich EPS-rich galacturonic acid was isolated from Alteromonas sp. JL2810.

Diesel oil is one of the most important crude oil products. It is a major source of pollution in the environment. By relying on a combination of diesel oil with fewer cars and generators, additional components are transported over long distances. So diesel oil can do it crash into oil tanks sports diesel oil, diesel cleaning tanks by traders, warships carrying diesel fuel and automotive equipment. (Nwaogu et al., 2008) Diesel oil spill on agricultural land reduces crop growth. The reasons for the decline in crop growth in diesel fuel oil from direct toxic effects on plants (Baker, 1982) and decline in germination (Udo and Fayemi, 1975) (UNwaogu et al, 2008)

Nigeria is the world's largest producer of crude oil and environmental pollution due to oil. The spills are slowly increases. In the Niger Delta Area alone, there were more than 550 reported cases of the crude oil spill in 1976, producing nearly 2.8 million barrels of crude oil in the environment (Korie-Siakpere, 1998; Odiete, 1999). Crude oil from various parts of the world will vary greatly in its physical and chemical properties. This diversity is very important in relation to the behavior of spilled oil in the tropics as well as the following cleaning techniques (Awabajo, 1981).

Soil is the foundation of sustainable economic and social development, and is one of the most valuable natural resources in all countries, especially in our country. With the continuous development of China's industrial level and urbanization, the call for oil products is on the rise (Tang, 2014). Now in China, a total of more than 400 oil and gas fields are distributed to 25 provinces and cities and private areas across the country. Most

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importantly the width of the oil fields is about 200000 km2, which covers an area of 320000 km2, which is almost 3% of the total area. Among them, the oil content in the soil of approximately 4.8 million hm2 may be more than the stable value (Liu et al, 2007). During the oil and gas exploration process, the development, collection, refining, storage and marketing, hazards, improper operation and maintenance of equipment and various causes can lead to the overflow and release of petroleum hydrocarbons. (Shi et al, 2013) The environmental impact is mainly due to the petroleum industry (Pala ne Freire, 2002). Petroleum-contaminated soils cause environmental pollution of groundwater (Wang and Fingas, 2008). Individual microorganisms are able to degrade very well the amount of crude oil depends on the presence of various chemical communities. EPS is the research topic of a wide range of scientific fields focused on their production process, the organisms that produce EPS, the Biosynthesis method involved in their compilation, their applications. EPS has a short production time and extraction is also easy (Freitas et al., 2017).

Habitat

- There are many small insects that produce EPSs such as isolated slime nearbyenvironment or as capsular attachments (Bajaj et al., 2007).
- EPSs that produce microorganisms contain a variety of algae, fungi and micro-organisms such as thermophilic, mesophilic, and halophilic. The popular small mesophilic organism contains Lactic Acid Bacteria (LAB). Other micro-microbial organisms are Escherichia spp., Bacillus spp., Pseudomonas spp., Streptococcus spp., Acetobacter spp., Aureobasidium spp., Escherichia spp. and Lactobacillus spp.Thermophilic archaebacteria are Sulfolobus, Thermococcus and Archaeoglobus fulgidus (Nicolaus, et al., 1993; Rinker & Kelly, 1996; Lapaglia and Hartzell, 1997).
- Many thermophilic microorganisms act as major producers of EPSs including *Geobacillusthermodenitrificans*, Bacillus *thermantarcticus* and Bacillus *licheniformis*.
- Methanococcus *jannaschii*, Thermotoga *maritima* and Geobacillus *tepidamans* V264 cocultures produce a large number of EPSs (Kambourova et al., 2009).
- Many halophilic Archaea include Haloferax, Halococcus, Natronococcus, Haloarcula and Halobacterium additionally produces exopolysaccharides.(Antón et al., 1988; Nicolaus et al., 1999; Paramonov, 1998)

Extraction, purification and detection methods

In below diagram extraction, purification and detection method is explained.

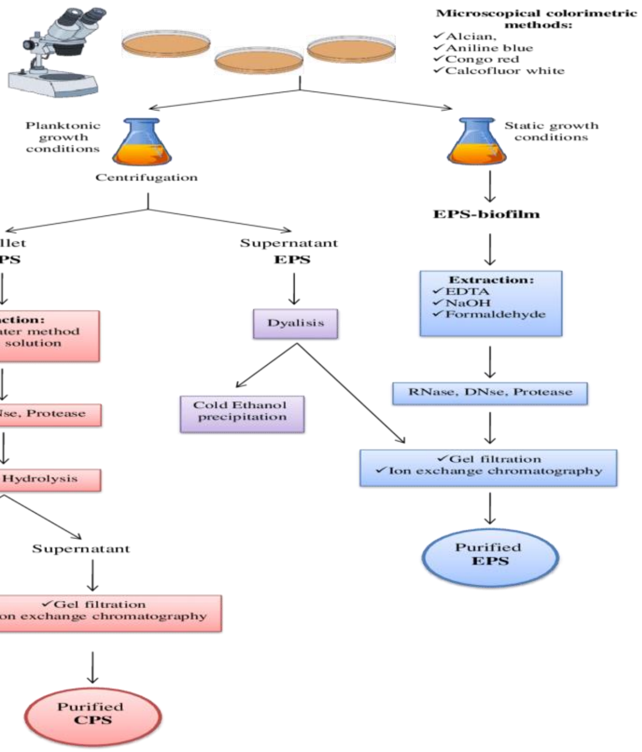

Figure 1: Extraction of EPS (Casillo et al., 2018)

Advantages

1. EPSs protect microorganisms as a well-known physical barrier. Their production is a direct response to selected environmental pressures such as osmotic pressure, temperature, pH, pressure and light intensity. In the case of acidophilic or thermophilic species with Archaea, a resource for EPSs to adapt to dynamic conditions.

2. EPS plays an important role in the microbial type developing a conducive environment waterretention in the case of water discharge and therefore kept hydrated microenvelo around biofilm and this is key to the survival of water scarcity places.

Major application of Microbial EPS:

A restricted quantity of monosaccharides include EPS, and its structural diversitydetermines its possible applications. (Colliec et al., 2001)

Table 1: Industrial application of EPS

Various Industries	Applications
Industrial	Gel formation,Soil condenser
Pharmaceutical	Immune modulation, Blood volume expanders
Biomedicinal	Antiviral, Antimicrobial, Pesticide
Medicine	Cholesterol-lowering ability, Controlled drug release, as a vaccine

EPSs are also mentioned for the prevention of tumor cell development, formation of white blood cells and in the treatment of rheumatoids arthritis. (Vanhooren and Vandamme 2000)

Table 2: Examples of microbial used macromolecules (adapted from(Delbarre-Ladrat et al., 2014))

EPS	**Source**	**Main applications**
Xanthan	*Xanthomonas campestris*	Food industry as a texturizing agent, petroleum industry, health care
Alginate	*Pseudomonas aeruginosa Azotobacter vinelandii*	food hydro colloid, wound care, drug encapsulating agent
Dextran	*Leuconostoc mesenteroides*	food industry, biomedical as plasma volume expander and biotechnological supports for separation
Cellulose	*Acetobacter xylinum*	food industry, biomedical as artificial temporary skin and biotechnological separations of hollow fiber and membranes
Hyaluronic acid	*Streptococcus equi Streptococcus zooepidemicus*	Human health cosmetics
Gellan	*Sphingomonas paucimobilis*	food industry and biotechnology (culture medium gelification)
Curdlan	*Sinorhizobium meliloti Agrobacterium radiobacter Alcaligenes faecalis*	*Sinorhizobium meliloti, Agrobacterium radiobacter,*
Succinoglycan	*Sinorhizobiummeliloti Alcaligenes faecalis*	food and pharmaceutical industries and oil recovery

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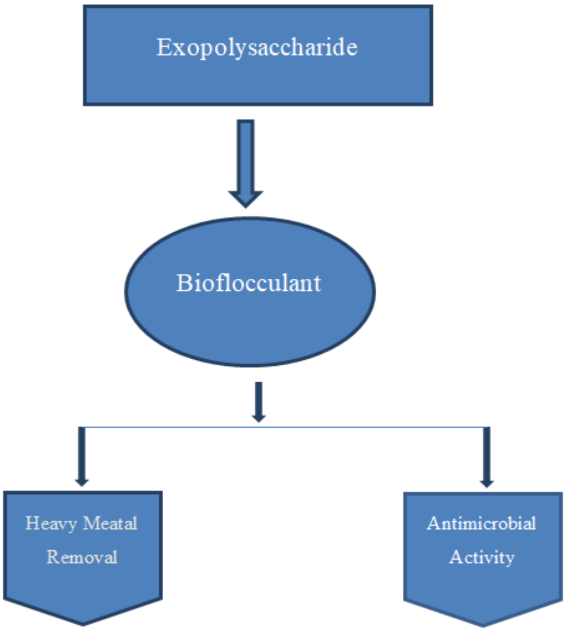
Levans	Levans Various	food industry (prebiotic)

Application



II. BIOFLOCCULANT

Naturally, microorganisms release other macromolecules which meet with the established firmness. Colloidal particles and impurities also allow for stability in the solution. Bioflocculant has two types depending on the case involved: cationic bioflocculant and cation bioflocculant, cation-based bioflocculant contains negative charge on its surface: allow well-charged macromolecule interactions. Example - Serratia ficaria, corynebacterium glutamicum, and Halmonas sp. independent cation biosurfactant contain positive charges that may reduce and disrupt particles charged poorly through advertising. For example- Bacillus mucilaginous, Klebsiella Pneumonia, and Aspergillus *flavus* (Aljuboori et al. 2015) have a soothing ability produced by various spp. for example,vagococcus sp. (Ggo et al., 2006), Pseudoaltermonas sp. (Liet al., 2008), Bacillus ficaria (Gong et al., 2008) subject to cations. Ca2 + is a highly charged link for flocculants and particles. (He et al., 2010). Flocculating agents are used in industrial activity such as purified drinking water, food process, and wastewater management. Bioflocculant has attracted industry and research, such as high flocculation efficiency,Friendly, and the production of industrial or agricultural waste.(Aljuboori et al., 2013, 2014; Bezawada et al., 2013) The affected bioflocculant may have the factors such as pH, temperature, iron, salt, mixing speed, etc. Bioflocculation is an active process due to the production of exopolymeric macromolecules caused by living cells. The process of flocculation was first reported with yeast by scientist Louis-Pasteur in 1876.

Bioflocculants are usually of three types. Unusual flocculants, Organic synthetic flocculants, Bioflocculants naturally. (Shin et al., 2001). The first two types are related to environmental and health problems and bioflocculants are perishable, eco-friendly, and environmentally friendly (Shin et al., 2001). Bioflocculant forms mainly biomacromolecules, a polysaccharide for the treatment of wastewater (Crini, 2005; Raza et al., 2011). A polysaccharide flocculant is used to remove pigment in the textile industry (Deng et al, 2005). Bioflocculant Biological Paenibacillus used to remove contaminants from contaminated water.

The Flocculation method is used to remove colloids attached to cells in the liquid phase. Bioflocculants produce secondary pollutants (Sun et al., 2015 and Zulkeflee et al., 2016). Bioflocculation is very important in cations. They form many multi-chain structures, extracts of sugar, glycoprotein, and polyols, etc. (Pathak et al., 2015) use organic waste to produce bioflocculant using microorganisms. (Deng et al., 2005; Zhang et al., 2007; Chen et al., 2017) Bioflocculant produced by microorganisms named *Pseudomonas aeruginosa* ZJU1, this organism removes the harmful algal bloom and Rhodococcus erythropolis used as sludge and wastewater and is used as an 8% dye removal solution (Peng ., 2014)

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Source

Some microorganisms and their sources

Table No.3

Microorganisms	Sources
Klebsiella pneumonia,K.mobiliss sp.	Sediments of a wastewater treatment plant, soil sample
Bacillus mucilaginosus	Soil samples, kaolin suspension, activated sludge
Bacillus sp.	
Rhodovulum sp.	Mud sample
Citrobacter sp.	Sewer pipes
Sorangium cellulosum sp.	Salt soil sample
Corynebacteriumglutamicum sp.	Soil samples
Nannocystis sp.	Salt soil sample(coast of the Huanghai sea)
Enterobacter aerogenes sp.	Soil sample
Cobetia sp.	Sediment samples of Algoa bay
Chryseomonas	Palm oil mills effluent
Luteola sp.	
Methylobacterium sp.	River Water
Enterobacter cloacae, E	
Agglomerans, E. cloaca	Activated sludge
Pseudomonas alcaligenes sp.	
Serratiaficaria sp.	Soil samples
Proteus mirabilis sp.	Activated sludge
Sarangiumcellulosun	Salt soil sample
Halomonas sp.	West Pacific Ocean (deep-sea)
Chryseobacterium daeguense	Activated sludge
Staphylococcus *cohnii*	Palm Oil Mill Effluent

Extraction metod of bioflocculant

In below diagram extraction, purification and detection method is explained

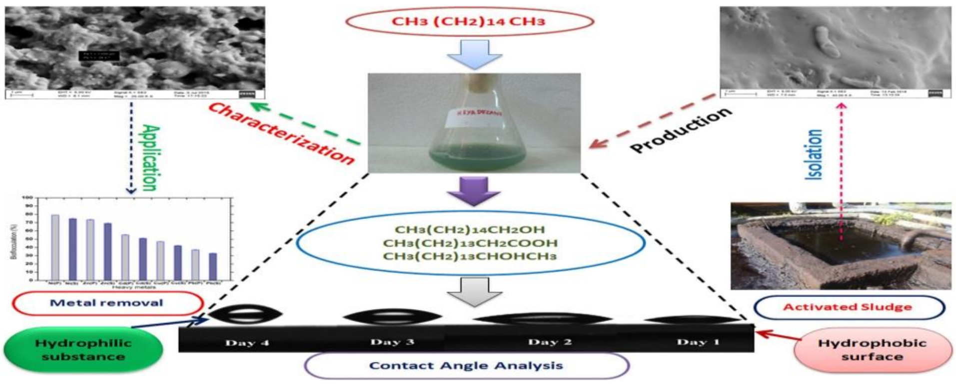

Fig.2: Extraction of bioflocculants

Applications

Table no. 4 – Application of Bioflocculant

Applications	Microorganisms	Remarks
Biomass Recovery and cell removal	Paenibacillus polymyxa Solibacillus silvestris Klebsiella pneumonia	Removed Scendesmus sp. Removed Nannochloropsis oceanica Removed Acanthamoeba cysts
Water and wastewater treatment	Oceanoacillus and Halobacillus Azotoacter indicus Cobetia sp. and Bacillus sp.	Treated brewery, dairy wastewater and river water Treated river water, wool, starch,and sugar industries wastewater Treated river water,dairy, and brewery wastewater
Decolorization	Rhodococcus erythropolis Serratia ficaria Chryseomonas luteola	Remove disperse dye solutions Decolourized pulp effluent Decolourized dye wastewater
Mining and other applications	Rhodopseudomas sphaeroides Bacillus subtilis Halomonas Maura	Focculated coal slurry Synthesis of ag nanoparticles(60nm) Synthesis of mauran/chitosan nanoparticles(30-200nm)

Bioflocculant as heavy metal removal :

Pollutants from the wild are a must before they can be used as agricultural fertilizers, due to environmental concerns. Local and international experts have conducted extensive studies on how to effectively remove heavy metals from sludge. Because the bioleaching method is recommended as a cost-effective and effective method of removing heavy metals from the mud, bioleaching is therefore used to remove heavy metals from the sludge. In recent years, microbial technology has made great strides in removing heavy metals from the wild that are widely used in the bioleaching process. The introduction of biological therapies has received a lot of attention in terms of ease of access, high efficiency, heavy metal regeneration, economic stress, low levels of mud, and the reuse of blood pressure. These components require special treatment methods while they must be environmentally friendly. Especially in animatebiological masses have a greater chance of saturating any nutrient utilization with these harmful substances as a heavy metal adsorbent could open a new window in water and wastewater care technique because it operates in many developing lands. Active sludge is another useful absorbent substance divided into different groups such as viruses, fungi, multicellular, and so on. The microorganisms present in the active sludge play an important role in the removal of heavy metals through different processes divided into two groups of organic reservoirs. Industrial activities including mining, metal, painting, fertilizer, and batter processing are major sources of heavy metals. Human activities have increased the levels behind liquids trace metals such as mercury, copper, lead, cadmium, nickel, and zinc into natural water. Many heavy metals contain toxic or non-toxic substances in fish and other organisms.Toxicity Cu (مر) d Cd (T) b Pb (to) fishing and aquatic environment can cause blastocolysis binding and growth. Low density of heavy metals in seawater, enrichment of heavy metals is essential before it can be discovered. In recent years, a numberof enrichment technologies have been proposed to focus on heavy metals in environmental samples, including solvent extraction, precipitation, marketing, and ion trade.

Biofluculant as Antimicrobial activity: The anti-bacterial molecules produced by marine bacteria can be divided into two main groups: secondary metabolites and antimicrobial protein. Second metabolites are found in chemicals that are not involved in the main metabolism but can increase the fitness of the manager. Antimicrobial proteins can be large molecules, such as glycoproteins, or low-weight peptides, such as bacteriocins. Coral reefs are one of the most useful marine organisms with a wide variety of micro-organisms associated with them and they are often an unused resource with a large group of unique biological outcome. These products contain many biotechnological systems. Hamamelis virginiana L (Hamamelidaceae) is a traditional medicinal plant with a long history of medicinal plants. Originally used by Native Americans in the treatment of burns and injuries, this plant is known for its high levels of tannins and other phenolic compounds, which can produce anti-bacterial activity. ZnO-NPS are among the most well-known nanomaterials that produce antibacterial and antifungal and silver properties. Zinc oxide is not toxic to human cells and is highly compatible. Antibacterial agents containing inorganic metal atoms or ions such as silver, copper and zinc are enhanced. Silicon ions are known to have higher antibacterial activity and lower toxicity compared to other iron ions. Zinc ions have some antimicrobial effect, good thermal stability and low cost and low toxicity, and rare earth ions have been used as antimicrobial agents in medicine for a long time due to their high safety and high antibacterial activity. However, the use of long-acting antibacterial agents containing zinc ions or earth ions that are rarely limited due to their poor antimicrobial activity.

III. CONCLUSION

This study aims to isolate,characterization and identification of microorganisms which are produce EPS. EPS are very important for microbial aggregation in biological wastewater treatment systems. The EPS was effective as a bioflocculant for removing heavy metals and show antimicrobial activity. EPS has become attractive in various industries such as pharmaceutical and cosmetic industry, food industry and wastewater treatment.

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