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Review

Extracellular polymeric substances of bacteria and their potential environmental applications

T.T. More [a,*], J.S.S. Yadav [a], S. Yan [a], R.D. Tyagi [a], R.Y. Surampalli [b]

[a] Institut national de la recherche scientifique, Centre Eau, Terre & Environnement, Université du Québec, 490 de la Couronne, Québec, QC G1K 9A9, Canada
[b] U. S. Environmental Protection Agency, P.O. Box 17-2141, Kansas City, KS 66117, USA

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A B S T R A C T

Biopolymers are considered a potential alternative to conventional chemical polymers because of their ease of biodegradability, high efficiency, non-toxicity and non-secondary pollution. Recently, extracellular polymeric substances (EPS, biopolymers produced by the microorganisms) have been recognised by many researchers as a potential flocculent for their applications in various water, wastewater and sludge treatment processes. In this context, literature information on EPS is widely dispersed and is very scarce. Thus, this review marginalizes various studies conducted so far about EPS nature-production-recovery, properties, environmental applications and moreover, critically examines future research needs and advanced application prospective of the EPS. One of the most important aspect of chemical composition and structural details of different moieties of EPS in terms of carbohydrates, proteins, extracellular DNA, lipid and surfactants and humic substances are described. These chemical characteristics of EPS in relation to formation and properties of microbial aggregates as well as degradation of EPS in the matrix (biomass, flocs etc) are analyzed. *The important* engineering properties (based on structural characteristics) such as adsorption, biodegradability, hydrophilicity/hydrophobicity of EPS matrix are also discussed in details. Different aspects of EPS production process such as bacterial strain maintenance; inoculum and factors affecting EPS production were presented. The important factors affecting EPS production include growth phase, carbon and nitrogen sources and their ratio, role of other nutrients (phosphorus, micronutrients/trace elements, and vitamins), impact of pH, temperature, metals, aerobic versus anaerobic conditions and pure and mixed culture. The production of EPS in high concentration with high productivity is essential due to economic reasons. Therefore, the knowledge about all the aspects of EPS production (listed above) is highly essential to formulate a logical and scientific basis for the research and industrial activities. One of the very important issues in the production/application/biodegradation of EPS is how the EPS is extracted from the matrix or a culture broth. Moreover, EPS matrix available in different forms (crude, loosely bound, tightly bound, slime, capsular and purified) can be used as a bioflocculant material. Several chemical and physical methods for the extraction of EPS (crude form or purified form) from different sources have been analyzed and reported. There is ample information available in the literature about various EPS extraction methods. Flocculability, dewaterability and biosorption ability are the very attractive engineering properties of the EPS matrix. Recent information on important aspects of these properties qualitatively as well as quantitatively has been described. Recent information on the mechanism of flocculation mediated by EPS is presented. Potential role of EPS in sludge dewatering and biosorption phenomenon has been discussed in details. Different factors influencing the EPS ability to flocculate and dewaterability of different suspensions have been included. The factors considered for the discussion are cations, different forms of EPS, concentration of EPS, protein and carbohydrate content of EPS, molecular weight of EPS, pH of the suspension, temperature etc. These factors were selected for the study based upon their role in the flocculation and dewatering mechanism as well the most recent available literature findings on these factors. For example, only recently it has been demonstrated that there is an optimum EPS concentration for sludge flocculation/dewatering. High or low concentration of EPS can lead to destabilization of flocs. Role of EPS in environmental applications such as water treatment, wastewater flocculation and settling, colour removal from wastewater, sludge dewatering, metal removal and recovery, removal of toxic organic

* Corresponding author. Tel.: +1 418 654 2617; fax: +1 418 654 2600.
E-mail addresses: more.tanaji@gmail.com, tanaji.more@ete.inrs.ca (T.T More), tyagi@ete.inrs.ca (R.D Tyagi).

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compounds, landfill leachate treatment, soil remediation and reclamation has been presented based on the most recent available information. However, data available on environmental application of EPS are very limited. Investigations are required for exploring the potential of field applications of EPS. Finally, the limitations in the knowledge gap are outlined and the research needs as well as future perspectives are highlighted.

1. Introduction

Microbial flocculants (EPS, extracellular polymeric substances) are considered an eco-friendly, cost effective and sustainable alternatives to substitute the existing chemical flocculants. Microbial EPS are biopolymers (Wingender et al., 1999). EPS matrix is formed by different biochemicals secreted by microbes, release of cellular material/products by cell lysis or organic matter in the medium. EPS secretion is a general attribute of microorganisms in natural environments and occurs in prokaryotic as well as in eukaryotic microorganisms (Flemming and Wingender, 2001; Wingender et al., 1999). EPS consists of quite viscous biofilm matrix. In general, EPS in a biofilm varies from 50% to 90% of the total organic matter (Flemming and Wingender, 2001). EPS are defined as capsular (C-EPS), slime (S-EPS), loosely bound (LB-EPS) and tightly bound (TB-EPS) on the basis of the nature of their association with the cells or the method used to extract/separate EPS from the cells (Table 1). The organic compounds produced by activated sludge microorganisms can be grouped into three categories: the first category compounds are secreted by microorganisms due to their interaction with the environment, the second category includes the compounds produced as a result of substrate metabolism and the third involves bacterial growth and compounds released during the lysis and/or degradation of microorganisms or microbial components.

Basic functions of EPS are aggregations of bacterial cells, adherence to surfaces, formation of flocs and biofilms, cell–cell recognition (e.g., cell adhesion), structural elements of biofilms, protective barrier for cells, and water retention to minimise desiccation of the cell, sorption of exogenous organic compounds, and sorption of inorganic ions, enzymatic activities and interaction of polysaccharides with enzymes (Tian, 2008; Wingender et al., 1999). Carbohydrates, proteins, humic substances and nucleic acids are the main EPS components. Because of special EPS components, EPS matrix shows adsorption abilities, biodegradability and hydrophilicity/hydrophobicity. EPS have an important role in biofilm formation, mass transfer via biofilm, adsorption of different metals and organic/inorganic compounds by biofilm and most importantly it provides structural support to the biofilm (resistant to shear) (Czaczyk and Myszk, 2007; Flemming and Leis, 2003; Flemming et al., 2005; Neyens et al., 2004).

The occurrence or production of EPS in different systems (e.g., membrane bioreactors (MBR)) is unwanted and a serious issue. Fouling is a serious problem in membrane processes. The fouling problem makes MBR operation and control very difficult. This is mainly due to the prevalence of EPS in the system (Drews et al., 2006). EPS interactions with the membrane surface are not well established till date. There are numerous theories or considerations reported in the literature, which are often contradictory and very confusing. In general MBRs are operated at a constant-flux, however, due to fouling trans-membrane pressure increases. The increase in trans-membrane pressure occurs in three phases and fouling occurs because of pore clogging, floc adhesion and cake layer formation, changes of cake layer and/or osmotic pressure effect. As per the literature, in general, EPS concentrations and chemical characteristics are the two important factors that determine the extent and severity of the fouling condition (Drews et al., 2006; Lin et al., 2014). Based upon the present understanding, there are mainly three strategies to mitigate membrane fouling such as EPS production control, modifications of EPS characteristics and EPS removal from the system (Lin et al., 2014). Fouling mitigation strategies require extensive research on advanced analysis of different components of EPS and chemical and functional characterization of the EPS produced by different microorganisms to increase fundamental understanding of EPS's role in fouling mechanism. With the advancement of knowledge about EPS-fouling nexus, simplified and economical strategies for the fouling control are expected to come out in near future.

Recently, the use of bacterial EPS for environmental applications has been the focus of many researchers. On the other hand, the information on the environmental applications of EPS are scattered and there is no consolidated report so far, which highlights the various aspects of EPS and their correlation to the potential environmental applications. This review provides current state-of-the-art knowledge with regard to bacterial EPS and their environmental applications. Special emphasis has been laid on the EPS nature-production-recovery, interrelation among EPS characteristics and properties, and potential application of EPS in environmental pollution control. Throughout the review, the term EPS has been used synonymously to address microbial polymers, extracellular polysaccharides substances, bacterial polymers, biofilms matrix and/or bioflocculants.

Table 1
Types of EPS.

Classification basis	EPS	Remarks	References
Nature of EPS association with cells	Slime	Slimes are the polymers present in the supernatant after centrifugation of the biomass. Slimes are mostly in soluble form or unattached to the cells in the form of colloids.	(Barker and Stuckey, 1999; Comte et al., 2006a; Laspidou and Rittmann, 2002; Raszka et al., 2006; Tian, 2008; Wingender et al., 1999)
	Capsular	Capsular EPS are the permanent part of the cell membrane and are bound to pellets (bacterial cells).	
Physical–chemical states and composition of EPS	Soluble	Soluble EPS are the secretion from the cells in dissolved form in the surrounding environment. The main components are macromolecules, colloids and slimes.	(Barker and Stuckey, 1999; Comte et al., 2006a; Laspidou and Rittmann, 2002; Raszka et al., 2006; Tian, 2008; Wingender et al., 1999)
	Bound	The bound EPS attach to the cells. The components of bound EPS are sheaths, capsular polymers, condensed gel, loosely bound polymers and attached organic material.	