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Critical review of EPS production, synthesis and composition for sludge flocculation

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ABSTRACT

Extracellular polymeric substances (EPS) produced by microorganisms represent biological macromolecules with unfathomable potentials and they are required to be explored further for their potential application as a bioflocculant in various wastewater sludge treatment. Although several studies already exist on biosynthetic pathways of different classical biopolymers like alginate and xanthan, no dedicated studies are available for EPS in sludge. This review highlights the EPS composition, functionality, and biodegradability for its potential use as a carbon source for production of other metabolites. Furthermore, the effect of various extraction methods (physical and chemical) on compositional, structural, physical and functional properties of microbial EPS has been addressed. The vital knowledge of the effect of extraction method on various important attributes of EPS can help to choose the suitable extraction method depending upon the intended use of EPS. The possible use of different molecular biological techniques for enhanced production of desired EPS was summarized.

Contents

Abbreviations: C/N, carbon to nitrogen molar ratio; CER, cation exchange resin; EDTA, *Ethylene di amine tetra acetate* group; EPS, exopolysaccharide or extra cellular polymeric substances; FTIR, *Fourier Transform Infrared* spectroscopy technique; GDP, Guanosine di phosphate; GT, Glucosyltransferase; MBR, membrane bioreactor; SEC, size exclusion chromatography; SS, suspended solids; UDP, uridine diphosphate; VSS, volatile suspended solids; WWTP, waste water treatment plant or wastewater treatment process.

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Introduction

In general, sludge settling is improved by the addition of synthetic polymers, but they are known to be expensive and may further pollute the environment (Deng et al., 2003). To minimize the use of synthetic flocculants in sludge settling applications, a novel alternative approach will be to use eco-friendly bio coagulants/bioflocculants. The role of extracellular polymeric substances (EPSs) produced by sludge microorganisms during the wastewater treatment process have been extensively studied (Hay et al., 2010; More et al., 2014; Subramanian et al., 2010). Recently, a demand of biopolymers for various industrial, biotechnological and environmental applications like flocculation, settling, dewatering of sludge, dyes and metal removal from wastewater has rekindled the interest in EPS production (Nontembiso et al., 2011; Zhang et al., 2012).

The main characteristic of EPS is to enhance aggregation of bacterial cells and suspended solids (SS). Adhesion and cohesion occur between EPS and the biomass along with suspended solids by complex interactions such as London forces, electrostatics interactions and hydrogen bonding, which leads to the formation of flocs. These EPS properties make them suitable for many applications such as sludge flocculation, settling, dewatering, metal binding and removal of toxic organic compounds (Chien et al., 2013; Jia et al., 2011; Nouha et al. 2016; Solís et al., 2012).

Microbial EPS biosynthesis promotes the attachment of the cells to a solid support. It helps in the establishment and continuation of microbial colonies to a mature biofilm structure and protects from environmental stress. Rehm (2010) published a review on critical EPS biosynthesis and metabolic pathways. EPS biosynthesis pathway depends on the type of EPS being produced *i.e.*, homopolysaccharides or heteropolysaccharides. Three major steps involved in EPS synthesis are (i) assimilation of a carbon substrate, (ii) intracellular synthesis of the polysaccharides and (iii) EPS exudation out of the cell (Vandamme et al., 2002). However, these EPS production steps depend on multiple factors like the microbial species (genes involved in EPS synthesis), media composition (carbon and nitrogen source, C/N ratio), and operating conditions (pH, temperature, dissolved oxygen).

Many EPS extraction methods have been used to extract EPS produced by pure microbial cultures (laboratory conditions) and mixed culture (activated sludge) (Nguyen et al., 2016; Nouha et al., 2016a, 2016b). Chemical, physical and combination of both methods were used for EPS extraction (Comte et al., 2006a; Nguyen et al., 2016; Nouha et al., 2016a, 2016b). The efficiency of EPS extraction by different methods have been compared (Comte et al., 2006a; Liu and Fang, 2002) based on the quantity and the composition of extracted EPS. EPS is mainly composed of carbohydrates and proteins. Carbohydrate was mainly observed in EPS produced from pure cultures, whereas proteins were found in higher quantities in the sludge-EPS of many wastewater treatment plants (Liu and Fang, 2002). However, the EPS chemical structure (functional group), molecular weight (MW) and its effect on bioflocculant activity were greatly limited by extraction methods, which were never reviewed.

Scientific findings on general metabolism required for EPS precursor biosynthesis and different metabolic engineering strategies for EPS overproduction in some bacterial strains are reported in this review. Secondly, the significant recent developments concerning the impact of extraction methods on EPS composition, chemical structure and molecular weight was critically reviewed and discussed in the ambit of sludge flocculation.

1. Composition of EPS

The chemical structure of polymeric substances secreted by the microbial cells depends on the different environmental conditions they grew, which are highly diversified. The most investigated components of EPS are polysaccharides and proteins (More et al., 2012; Nouha et al. 2016; Subramanian et al., 2010). The presence of humic substances and nucleic acids as part of EPS extracted from sludge were also reported

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