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Research review paper

Recent advances in extracellular biopolymer flocculants

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ABSTRACT

Extracellular biopolymer flocculants (EBFs) are flocculating substances, consisting of polysaccharides, proteins, and lipids, which are secreted in the culture broth by many microorganisms. Some of EBFs have attracted much attention as biodegradable and nontoxic substitutes for conventional chemical flocculants. This paper reviews the recent development of EBFs. Aspects discussed include an introduction to conventional chemical flocculants and EBFs, isolation of novel bioflocculant-producing microorganisms, culture conditions, chemical structure and molecular weight of EBFs, the physico-chemical factors affecting flocculating activity, fermentation process design and recent and emerging application fields of EBFs.

Contents

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Introduction

Flocculating agents are widely used in different industrial fields such as tap water and wastewater treatment, dredging, textile, mining, cosmetology, pharmacology, food and fermentation industries and downstream processes (Buthelezi et al., 2012; C. Wan et al., 2013; Deng et al., 2005; Li et al., 2009a, 2013; Salehizadeh and Shojaosadati, 2001; Ugbenyen and Okoh, 2014; Vijayalakshmi and Raichur, 2003; Zhang et al., 2013). They are generally classified into three groups; i) inorganic flocculants, such as aluminium sulfate, poly-aluminium chloride, ferric chloride and ferrous sulfate; ii) organic synthetic flocculants, such as polyacrylamide derivatives and polyethyleneimine; and iii) natural flocculants or bioflocculants, such as chitosan, sodium alginate, gelatin and EBFs (Salehizadeh and Shojaosadati, 2001; Shih et al., 2001).

Although the first two groups are most commonly used due to their effective flocculating performance and low cost, their use has caused many serious environmental and health problems. For example, acrylamide monomer is not only a strong carcinogen and neurotoxic to humans but also non-biodegradable in the environment (Ruden, 2004). There is evidence that the aluminium salts may induce Alzheimer's disease (Campbell, 2002).

Due to the above concerns, EBFs produced by microorganisms have been attracting more attention in utilization. Some of EBFs are nontoxic and benign to the environment (Gao et al., 2009; Salehizadeh and Shojaosadati, 2001; Zhuang et al., 2012).

The price of raw materials and yield of flocculants play important roles for bioflocculants' practical application. So far, with the aim of commercialization, a considerable effort has gone into reducing the production cost of EBFs through developing better strains and using cheap substrates (Fujita et al., 2001; Huang et al., 2005; J.N. Wang et al., 2013; More et al., 2014; Nwodo et al., 2014; Pei et al., 2013; Peng et al., 2014; Sam et al., 2011; Sun et al., 2012; Wang et al., 2006; Wong et al., 2012; Yang et al., 2007, 2013; Zhang et al., 2007, 2013; Zhao et al., 2012).

This manuscript describes the recent trends in the development of EBFs. Aspects reviewed include isolation of novel bioflocculant-producing microorganisms, culture conditions, chemical structure and molecular weight of EBFs, the physico-chemical factors affecting on flocculating activity, fermentation process design and EBFs applications.

Isolation of novel bioflocculant-producing microorganisms

Bioflocculant producers are generally selected based on their sticky mucoid colony morphology, Congo red staining to identify the presence of slimy extracellular polysaccharides (EPS) (Neu, 2000), and capsule staining using crystal violet and $CuSO_4$ solution (Cain et al., 2009). Use of chelating agents such as ethylene di-amine tetra acetic acid (EDTA) and many hydrolytic enzymes facilitates the release of the potential EBFs and Ca^{2+}-binding bioflocculants from the floc network into the supernatant (Vossoughi et al., 2001). The flocculating efficiency of the strains was measured using suspended solids' (SS) removal capability, decolorization test and chemical oxygen demand (COD) reduction of solution (Gong et al., 2008; Salehizadeh and Shojaosadati, 2001; Wang et al., 2014; Zulkeflee et al., 2012).

Activated sludge, soil and sediments, river and deep sea water samples are the best sources for isolating EBF-producing microorganisms (Bala Subramanian et al., 2010; Salehizadeh and Shojaosadati, 2001). Bioflocculation is a dynamic process which usually occurs in activated sludge during an aerobic process. Therefore, activated sludge can be supposed as a well-known mixed culture source for bioflocculant-producing microorganisms. The excess biological sludge contains the aggregate of microorganisms which mainly secrete flocculating agents such as polysaccharides, proteins, glycoproteins, cellulose derivatives and so on (Bala Subramanian et al., 2010; Guang-Hui et al., 2009; Lin et al., 2010, 2012; Liu et al., 2009; More et al., 2012a, 2014; Sun et al., 2012; Van Loosdercht and Heijnen, 2002).

Proteus mirabilis, *Saccharomycete*, *Achromobacter* sp., *Rhodococcus erythropolis* and *Solibacillus silvestris*, with the ability to flocculate kaolin suspension, were isolated from activated sludge or wastewater samples (Batta et al., 2013; C. Wan et al., 2013; Cheng et al., 2004; Gao et al., 2006; Peng et al., 2014; Xia et al., 2008). *Chryseobacterium daeguense* was isolated from backwashing sludge (Liu et al., 2009, 2010). *Vagococcus* sp. was screened from wastewater samples collected from the Little Moon River in Beijin (Gao et al., 2006). The *Brachybacterium* sp. belonged to Actinobacteria from a freshwater environment in South Africa (Nwodo et al., 2013).

Halomonas sp. (He et al., 2010), *Halobacillus* (Cosa et al., 2013), *Pseudoalteromonas* sp. (Li et al., 2008), and *Gyrodinium impudicum* (Yim et al., 2007) were isolated from deep sea sediments or muds. *Klebsiella mobilis* (Wang et al., 2007), *Corynebacterium glutamicum* (He et al., 2002, 2004a), *Serratia ficaria* (Gong et al., 2008), and *Bacillus licheniformis* (Li et al., 2009a) have been isolated from soil samples. Many researchers have been isolating microorganisms which secrete the extracellular biopolymer flocculant belong to the *Bacillus* family (Adebayo-Tayo and Adebami, 2014; Deng et al., 2003; Kumar et al., 2004; Li et al., 2009a,b; Salehizadeh and Shojaosadati, 2002; Sathiyanarayanan et al., 2013; Shih et al., 2001; Suh et al., 2002; Zaki et al., 2013). Table 1 lists some bioflocculant-producing isolates since the last decade.

Culture conditions

The major key factors affecting the flocculating activity of bioflocculants in culture broth are carbon and nitrogen sources, C/N ratio, initial pH of medium, culture temperature, culture time, inoculum size, metal ions, ionic strength, aeration rate and shaking speed (Radchenkova et al., 2014; Salehizadeh and Shojaosadati, 2001). Therefore, these factors need to be optimized to enhance yield, productivity and the flocculating efficiency of a culture.

Effect of carbon and nitrogen sources and C/N ratio on bioflocculant production

So far, a considerable effort has gone into producing bioflocculants from simple carbon sources such as glucose, sucrose, lactose, fructose, and maltose (Cosa et al., 2011; Gong et al., 2008; He et al., 2004a, 2009; Ji et al., 2010; Kim et al., 2011; Li et al., 2010; Lian et al., 2008; Liu and Cheng, 2010; Liu et al., 2010; Mabinya et al., 2011; Wu et al., 2010; Xia et al., 2008; Yang et al., 2009; Zhang et al., 2009, 2010); alcohols such as ethanol (Wang et al., 2007; Zhang et al., 2008); or organic acids such as acetic and propionic acids (Elkady et al., 2011; Fujita et al., 2000, 2001).

Carbon and nitrogen sources play an important role on the bioflocculant production. The optimal carbon and nitrogen sources and C/N ratio lead to the maximum flocculating activity with the shortest incubation time (Xiong et al., 2010).

Table 2 indicates the effect of carbon and nitrogen sources and the importance of C/N ratio and other parameters on bioflocculant production using various microorganisms. For example, sucrose, glucose and starch were favorable for producing bioflocculant by *Aspergillus flavus*.