Separation and Purification Technology 201 (2018) 51–59

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Flocculation efficiency of the *Sinorhizobium meliloti* 1021 exopolysaccharide relative to mineral oxide suspensions – A preliminary study for wastewater treatment

Katarzyna Szewczuk-Karpisz[a,*], Małgorzata Wiśniewska[b]

[a] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland
[b] Department of Radiochemistry and Colloid Chemistry, Faculty of Chemistry, Maria Curie-Sklodowska University, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland

ARTICLE INFO

Keywords:
Adsorption
Suspension stability
Exopolysaccharide
Wastewater treatment

ABSTRACT

The main aim of the study was to examine the *Sinorhizobium meliloti*1021 exopolysaccharide (EPS) flocculation efficiency relative to mineral oxide suspensions and verify if EPS can be used in the wastewater treatment procedure. The paper compares the EPS adsorption influence on the stability of various mineral oxide suspensions (chromium(III) oxide, silica, zirconia). Moreover, it indicates the solid whose aqueous suspension is the most destabilized in the EPS presence. The selected adsorbents have different surface properties. They are characterized by different pH_{pzc} (zero surface charge) and pH_{iep} (isoelectric) points. The highest adsorption amount was observed on the chromium(III) oxide surface. It resulted in the greatest changes in the particle zeta potential and surface charge density. The exopolysaccharide addition affects the stability of all mineral oxide suspensions. The most significant changes were observed for chromium(III) oxide and zirconia at pH 9. Under these conditions there is a bridging flocculation in the system, so the *S. meliloti* exopolysaccharide can be regarded as a potential flocculant related to these solids. In turn, the silica suspension stability changes insignificantly in the EPS presence.

1. Introduction

Exopolysaccharides (EPS) are natural polymers synthesized by soil bacteria belonging to the *Rhizobiaceae* family. They are produced in large quantities to the environment and play a key role in the commencement of the microorganism-legume symbiosis [1]. *Sinorhizobium meliloti* 1021 is one of the bacteria producing exopolysaccharide. It can synthesize two EPS types, i.e. EPS I and EPS II. The first is a succinyloglycan composed of octasaccharide subunits containing seven glucose molecules and one galactose molecule linked by the β-1,3-; β-1,4- and β-1,6-glycosidic bonds [2]. This polysaccharide chain is modified by various substituents, e.g. succinyl, pyrunyl and acetyl [3]. EPS II is a galactoglucan composed of disaccharide units containing acetylated glucose and galactose substituted with the pyruvic acid residue joined by the β-1,3- and α-1,3-glycosidic bonds. It is only synthesized in the phosphorus-poor environment as well as after mutations in bacteria regulatory genes [4,5]. Both types of exopolysaccharide are produced in two fractions: high molecular weight (HMW) and low molecular weight (LMW). The HMW fractions include the EPS macromolecules made up of several hundred / several thousand units [6]. On the other hand, the LMW fraction is the monomer, dimer and trimer for EPS I or oligomers of 15–20 units for EPS II [7].

Exopolysaccharides are non-toxic and biodegradable [8]. What is more, their synthesis is simpler and faster compared to plant or alga polysaccharide production and one microorganism is able to produce much more substance than the individual plant [9]. Owing to these features, exopolysaccharides have wide application possibilities, among others, in biomedicine, food and pharmaceutical industries as well as in cosmetics [10–12]. In the food industry, they are mainly synthesized by lactic bacteria. They act as stabilizers, emulsifiers or viscosifiers [13,14]. For example, the dextran produced by *Leuconostoc mesenteroides* is a gelling and thickening agent, which can also prevent sugar crystallization and give adequate viscosity and moisture to products [14]. EPS is also a source of prebiotics and immunomodulators [13]. Some of them are also used in the pharmaceutical industry. They have antitumour properties [15] and can reduce cholesterol level [14].

However, the EPS usage as a flocculant in the wastewater treatment is the most promising. It accelerates the colloidal impurity aggregation and, as a result, the large flocs falling to the tank bottom are formed. Microbial flocculants are competitive with the synthetic ones because they do not cause secondary pollution and thus are safe for the environment [16]. As demonstrated in the literature, the EPS

* Corresponding author.
E-mail address: k.szewczuk-karpisz@ipan.lublin.pl (K. Szewczuk-Karpisz).

https://doi.org/10.1016/j.seppur.2018.02.028
Received 30 October 2017; Received in revised form 22 January 2018; Accepted 13 February 2018

macromolecules neutralize the particle charge and form the polymer bridges between them. For example, the GY03 biopolymer synthesized by *Bacillus mucilaginosus*, composed essentially of polysaccharides and a small amount of proteins and nucleic acids, improves the efficiency of wastewater purification (municipal, pharmaceutical and brewery effluents) based on the polymer bridge formation [17]. The microbial flocculation efficiency strongly depends on the solution pH value. For example, the biopolymer of *Bacillus subtilis* PY-90 is the most effective at pH 3–5 [18].

Due to the problem of water lack in many world regions, accurate water and wastewater treatment is a very current issue. New substances and equipment that improve the process effectiveness are constantly being tested [19–21].

In this paper, the flocculation efficiency of *S. meliloti* exopolysaccharide related to mineral oxide suspensions was examined. What is more, the solid of which aqueous suspension is the most destabilized after the EPS addition was indicated. The stabilization/destabilization mechanism of the mineral oxide suspensions in the biopolymer presence was explained based on the numerous studies: stability, electrokinetic (zeta potential, surface charge density), particle aggregate size and adsorption measurements. It should be emphasized that the described experiments constitute a significant issue of practical application. The may be helpful in the development of innovative wastewater treatment procedure using microbial flocculant. The mineral oxides selected for the study, i.e. chromium(III) oxide, zirconia and silica, are widely used in the industry and their presence in the effluents is inevitable. Chromium(III) oxide is a green dye used in construction and paintings [22,23]. Zirconia is an important material in implantology and jewelry making [24]. In turn, silica is used as a humectant and raw material in glass and ceramic industry [25]. The removal of these solids is extremely important because they make the aqueous solutions turbid and chromium(III) oxide also colours them green. In the solutions contaminated with colloidal mineral oxides, the light penetration into the reservoir is limited and consequently the productivity of photosynthesis and other life processes is reduced [26].

However, it must be noted that the presented study is preliminary. Practical application of the exopolisaccharide requires further experiments including, inter alia, the effect of other pollutants present in wastewater on the biopolymer properties. The *S. meliloti* EPS flocculating ability relative to Cr_2O_3, SiO_2 and ZrO_2 have not been compared in the literature yet. So this paper can be considered as novel.

2. Experimental

2.1. Materials

Exopolysacchaide (EPS) of bacteria *Sinorhizobium meliloti* 1021, used as an adsorbate, was isolated according to the literature report [27]. In this way the EPSI HMW (High Molecular Weight) fraction was obtained. Its molecular mass is in the range of 10^3–10^4 kDa [6]. The EPSI subunit structure is presented in Fig. 1 [28].

The pK_a value of expolysaccharide was 3.8, i.e. at pH 3.8 the concentrations of dissociated (COO^-) and undissociated carboxylic groups (COOH) in the EPS macromolecules are identical. The EPS dissociation degree (α) as a pH function was also calculated. At pH 3 it is equal to 0.14, at pH 4.6–0.86, at pH 6, 7.6 and 9–0.99 [27]. The higher the dissociation degree was, the more expanded structure of EPS macromolecules was observed. The dissociation of practically all carboxylic groups at pH 6 and higher is equivalent to the largest biopolymer sizes [29].

Chromium(III) oxide (Cr_2O_3, *Sigma Aldrich*), silicon(IV) oxide (silica, SiO_2, *POCh*) and zirconium(IV) oxide (zirconia, ZrO_2, *Sigma Aldrich*) were used as adsorbents in the experiments. All of them were finely crystalline and have a mesoporous structure. Adsorbents had a different specific surface area: Cr_2O_3 – 7.12 m^2/g, ZrO_2 – 21.7 m^2/g, SiO_2 – 145 m^2/g. In addition, they differed in their mean particle size, i.e. Cr_2O_3 – 265 nm, SiO_2 – 225 nm and ZrO_2 – 100 nm. The average pore diameter and the specific surface area were calculated using the BET method whereas the average particle size was measured by a zetameter (*Zetasizer 3000, Malvern Instruments*).

2.2. Methods

0.01 M NaCl was used as a supporting electrolyte. All measurements were performed at 25 °C.

2.2.1. Potentiometric titration

In the supporting electrolyte solution, the mineral oxide surface charge is formed by the interactions of the surface hydroxyl groups (−OH) with the solution components: electrolyte as well as hydrogen ions. H^+ ions affect the surface charge density (σ_0) according to the following acid-base reactions:

$$\equiv SOH^0 + H^+ \leftrightarrow \equiv SOH_2{}^+ \quad (1)$$

$$\equiv SOH^0 \leftrightarrow \equiv SO^- + H^+ \quad (2)$$

where:

S – the metal or semi-metal.

On the other hand, the supporting electrolyte ions form complexes with −OH groups, which is shown by the reactions:

$$\equiv SOH_2{}^+Cl^- \leftrightarrow \equiv SOH^0 + H^+ + Cl^- \quad (3)$$

$$\equiv SOH^0 + Na^+ \leftrightarrow \equiv SO^-Na^+ + H^+ \quad (4)$$

Potentiometric titration allows the determination of solid surface charge density. This parameter is established based on the equation [30]:

$$\sigma_0 = \frac{\Delta V \cdot c \cdot F}{m \cdot S_w} \quad (5)$$

where: ΔV – the difference in the base volume added to a suspension

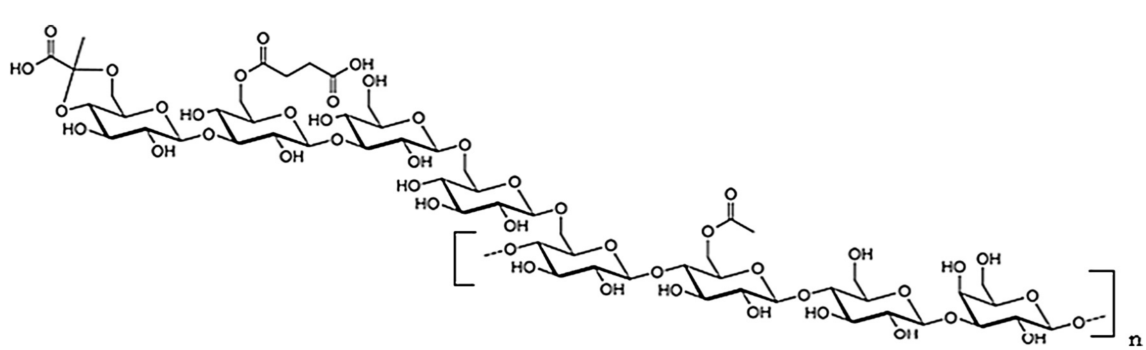

Fig. 1. EPSI subunit structure [28].