Chemical Engineering Journal 303 (2016) 627–635

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Probing the contribution of extracellular polymeric substance fractions to activated-sludge bioflocculation using particle image velocimetry in combination with extended DLVO analysis

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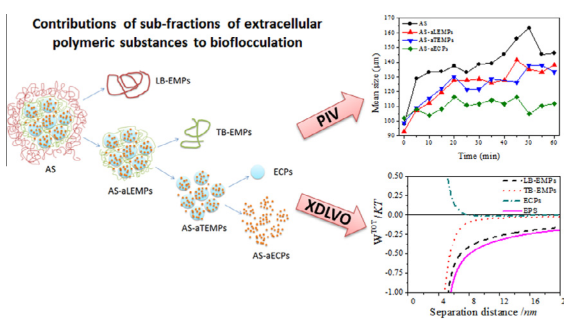
HIGHLIGHTS

- PIV was employed to determine the activated sludge flocculation ability.
- XDLVO theory was applied to describe the sludge aggregation properties.
- Contributions of different EPS sub-fractions to sludge flocculation was quantified.
- EMPs played an important positive role in sludge flocculation.
- ECPs may play different roles in the flocculation of sludge cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 6 June 2016
Accepted 8 June 2016
Available online 8 June 2016

Keywords:
Activated sludge
DLVO theory
Extracellular polymeric substances (EPS)
Flocculation
Particle image velocimetry (PIV)

ABSTRACT

Extracellular polymeric substances (EPS) play a crucial role in the flocculation of activated sludge during biological wastewater treatment. However, as EPS may be classified into sub-fractions, the exact roles of different EPS fractions in sludge flocculation remain to be further defined. This study investigated the contribution of EPS fractions to the sludge flocculation based on a refined EPS classification considering the function and composition of the EPS sub-fractions, including loosely-bound extra-microcolony polymers (LB-EMPs), tightly-bound extra-microcolony polymers (TB-EMPs) and extra-cellular polymers (ECPs). The flocculation ability of the sludge before and after the extraction of the EPS fractions was determined using the particle image velocimetry (PIV) technique. Combined with the flocculation test and sludge characterization, the effects of the EPS fractions on the interaction energy and attachment of microbial cells were evaluated by extended DLVO analysis. The PIV results indicate that sludge flocculation ability decreases with the extraction of EPS. The interaction-energy curves obtained from the sludge before and after the stepped EPS extraction suggest that different EPS fractions contribute to sludge flocculation to different degrees. The interaction energy of LB-EMPs and TB-EMPs is always negative, indicating their important and positive roles in sludge flocculation. In contrast, the contribution of ECPs to sludge flocculation is not always positive, depending on the separation distance between the sludge cells. The quantitative findings will help develop more effective approaches to enhance sludge flocculation by regulating the EPS contents.

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http://dx.doi.org/10.1016/j.cej.2016.06.042
1385-8947/

Nomenclature

A	projected area of floc (m^2)
R	radius of sludge cell (m)
H	separation distance (m)
A_{BLB}	effective Hamaker constant (J)
T	temperature (K)
k_B	Boltzmann constant (J/K)
I	ionic strength (mol/L)
N_A	Avogadro constant (mol^{-1})
l_0	minimum equilibrium distance (m)
ε	dielectricity constant (F/m)
e	elementary charge of electron (C)
γ	surface tension (J/m^2)
ψ	Stern potential (V)
κ	inverse of Debye length (m^{-1})
λ	correlation length (m)

Superscripts

LW	Lifshitz-van der Waals
EL	electrical double layer
AB	Lewis acid base
$+$	electron-acceptor parameter
$-$	electron-donor parameter

Subscripts

B	sludge
L	liquid medium
i	surface

Abbreviations

AS	activated sludge
EPS	extracellular polymeric substance
FA	flocculation ability
LB	loosely bound
TB	tightly bound
EMPs	extra-microcolony polymers
ECPs	extra-cellular polymers
AS-aLEMPs	sludge sample after LB-EMPs extraction
AS-aTEMPs	sludge sample after TB-EMPs extraction
AS-aECPs	sludge sample after ECPs extraction
RH	relative hydrophobicity

1. Introduction

In biological wastewater treatment systems, a good flocculation ability of activated sludge (AS) is essential to the quality of the effluent. AS usually has a loose structure, and its microbial cells are glued together into flocs by a matrix of extracellular polymeric substances (EPS) which are crucial to AS flocculation [1–4]. EPS are generally depicted using a two-layer model: the inner layer consists of tightly bound EPS (TB-EPS), which has a definite shape and are bound stably to microbial cells; the outer layer comprises loosely bound EPS (LB-EPS), which is a dispersible slime layer without an obvious edge [5]. To investigate the influence of different EPS sub-fractions on AS flocculation, various methods have been developed to extract LB-EPS and TB-EPS from sludge, leading to inconsistent classification and varying results [6,7].

However, a new EPS classification scheme based on function and composition was recently proposed. The refined EPS classification brings about an approach to distinguish EPS at the floc level (extra-microcolony polymers, EMPs) and at the microcolony level (extra-cellular polymers, ECPs) [8]. Wang et al. (2014) clarified that traditional EPS extraction methods can efficiently disaggregate sludge flocs for EMPs extraction, but are inefficient in the disaggregation of microcolonies for ECPs harvesting. Therefore, most previous researchers claiming to investigate EPS and their roles in sludge flocculation have referred only to EMPs [6,7]. In addition, the contribution of ECPs to sludge flocculation has not been confirmed, due to a lack of appropriate extraction methods. Wang et al. (2014) showed that a combination of ultrasonication and high-speed centrifugation is an effective means of separating ECPs from AS flocs. Accordingly, combined with the conventional two-layer model, EPS may be classified into fractions of LB-EMPs, TB-EMPs and ECPs. With the new EPS classification and extraction method, the contributions made by these EPS sub-fractions to AS flocculation can be better quantified.

AS flocculation properties are conventionally evaluated in terms of parameter Flocculation Ability (FA) by measuring the absorbance of the sludge supernatant at 650 nm after sonication and stirring [9]. This method, however, is inaccurate because the absorbance of the wastewater supernatant fluctuates greatly. Moreover, measuring the supernatant does not directly reveal the properties of sludge flocs. Particle image velocimetry (PIV) is an advanced and powerful flow visualization and particle tracking technique [10]. As a non-intrusive optical setup, PIV can be used to capture the images of flocs *in-situ* without the risk of breaking their fragile structures. With these particular advantages, the PIV technology has been applied to investigate the floc formation of kaolin [11] and to characterize the nature organic matter flocculation after chemical flocculation [12]. A combination of PIV and image analysis offers a sophisticated method to determine the flocculation ability of sludge by tracking the change of floc sizes during the flocculation process, replacing the conventional FA measurement.

The flocculation properties of AS are primarily governed by the interaction between microbial cells, which can be described by the extended DLVO (XDLVO) theory [7,13,14]. According to this theory, the van der Waals forces, polar interaction, electrical double layer interaction and Brownian-motion are taken into account in calculating the total energy of adhesion. The XDLVO theory has been applied as both qualitative and quantitative models to explain microbial adhesion in biomass systems, including pure cultures and activated sludge [2,15].

In this study, to identify the effects of EPS sub-fractions on sludge flocculation, the sludge samples at different EPS extraction stages, including raw sludge, sludge after LB-EMPs extraction, sludge after TB-EMPs extraction and sludge after ECPs extraction, were resuspended for the flocculation tests by PIV tracking. Combined with the flocculation tests and sludge characterization, the effects of the EPS sub-fractions on the attachment of sludge cells and the interaction energy were evaluated by XDLVO analysis. Comparing the experimental results with theoretical calculations, the crucial roles of EPS in sludge flocculation was clarified based on a comprehensive EPS classification. The improved understanding of EPS sub-fractions and their functions will help develop more effective approaches to enhance sludge flocculation by manipulating the EPS contents in AS operation.

2. Materials and methods

2.1. EPS extraction and analysis

The AS samples were collected from the Stanley Sewage Treatment Works in Hong Kong. The process of extracting the EPS sub-fractions is depicted in Fig. 1. LB-EMPs and TB-EMPs were