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Research article

Impact of extraction methods on bio-flocculants recovered from backwashed sludge of bio-filtration unit

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ABSTRACT

Effect of ten extraction methods on flocculation activity and chemical composition of bio-flocculants recovered from backwashed sludge of bio-filtration unit was studied. The results showed that the chemical method was better than physical method with respect to the extracted BFs weight and its flocculation activity. Cell lysis did not affect to the flocculation activity of BFs. Among ten extraction methods, EDTA (20 g/L) was the best one with extracted BFs dry weight of 6242 mg/L and flocculation activity of 83%. Optimization of EDTA concentration showed that 5 g EDTA/L (or 0.2 g EDTA/g SS) was suitable for recovery of BFs from backwashed sludge. The flocculation activity of BFs was 94% when using 2.4 mg of BFs/g of kaolin. The outcome of this study suggested that backwashed sludge of the bio-filtration unit was a potential source for exploiting bio-flocculants.

1. Introduction

The chemical flocculants are popularly used in wastewater treatment plant to increase the size of flocs in coagulation step prior to settling and increases dewaterability of waste sludge prior to dewatering process. However, using of chemical flocculants might have a negative impact on the environment and health (Kunle et al., 2016). Bio-flocculants (BFs) is an urgent need to replace chemical flocculants, as it is biodegradable and less harmful to the environment. Bio-flocculants (BFs) produced by bacteria have been studied and shown high ability in the flocculation of kaolin solution. Bio-flocculants also have been applied in treating of various types of pollutants present in wastewater such as humics in leachate (Zouboulis et al., 2004), color and dyes in textile wastewater (Gao et al., 2011), heavy metals (Guibaud et al., 1999), suspended solids and organic compounds (Gong et al., 2008). Many bacterial strains have been isolated to produce bio-flocculants (BFs) in both synthetic growth medium (Dermlim et al., 1999; Watanabe et al., 1998; Yang et al., 2012) and low-cost growth medium such as wastewater (Aguilera et al., 2008; Wang et al., 2007), wastewater sludge (Bezawada et al., 2013; More et al., 2012) and industrial waste and byproducts (Banik et al., 2007). However, production of bio-flocculants in industrial scale is still limited due to the high cost of growth medium, complex fermentation and bio-flocculants extraction process and maintenance of bacterial strains.

Wastewater sludge is an increasing environmental issue. Treatment and disposal of wastewater sludge represent approximately 50% of wastewater treatment cost. Produced sludge need to be reused or recycled to prevent the environmental problems and to improve the value of sludge. Wastewater treatment plants generate many million tons of bio-sludge per year around the world and contains varying concentration of polymeric substances (both extracellular and intracellular), which could be potentially used as bio-flocculants (Comte et al., 2006; Yu et al., 2009; Zhang et al., 2012). However, in bio-sludge or activated sludge, the polymeric substances are tightly attached with microbial cells and most of the functional groups are occupied by linking with cell membrane, therefore, they exhibit low flocculation activity (Yu et al., 2009). By chemical/physical means, polymeric substances can be detached from the cell, free the functional groups and thereafter can be collected as active bio-flocculants.

So far, many methods were proposed and applied to extract BFs from activated sludge and bacterial broth. However, these methods were used as an analytical tool to determine the composition of BFs in sludge or BFs produced by the isolated bacteria (Bezawada et al., 2013; Liu and Fang, 2002; More et al., 2012; Sun et al., 2012; Zhang et al., 2012) and to study the effect of extraction method on cell lysis (D'Abzac et al., 2010; Sun et al., 2012). The extraction methods have

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also been used to examine how BFs correlated to bio-flocs structure, sludge dewaterability and efficiency of the biological treatment unit such as bio-filtration, bio-membrane filtration (Duan et al., 2013; Jiao et al., 2010; Neyens et al., 2004; Zhang et al., 1999) or the presence of filamentous bacteria in activated sludge system (Al-Halbouni et al., 2008; Martins et al., 2004). There is no report on how the extraction methods affect the flocculation activity of the extracted BFs. Therefore, the aim of this study is to determine the effect of different bio-flocculants extraction methods on both quantities extracted and flocculation activity of the obtained BFs and thus proposes the most efficient method to recover BFs from backwashed wastewater sludge of bio-filtration unit.

2. Materials and methods

2.1. Sludge sample

Backwashed sludge was collected from the bio-filtration unit of CUQ (Communauté Urbain du Québec) wastewater treatment plant (Quebec, Canada). The sludge was washed twice with distilled water and then concentrated to 25*g* SS (suspended solids)/L by centrifugation. Concentrated sludge was stored at 4 °C for further studies.

The CUQ wastewater treatment plant is located in Quebec City in Canada. It treats domestic wastewater using bio-filtration technology with designed capacity of 231,000 m^3/day. Food per microorganism ration (F/M) was around 0.4. Concentration of main pollutants in the influent wastewater was presented in Table 1.

2.2. Extraction methods

The following ten different extraction methods were used to extract BFs from the sludge sample. The volume of the fresh sludge (25 g SS/L) used for extraction was 70 mL.

1) Centrifugation: Fresh sludge was centrifuged at 6000*g*, 4 °C for 15 min to release BFs to the supernatant.
2) Heating: Fresh sludge was heated in a water bath at 60 °C for 30 min.
3) Sonication: Fresh sludge sonication was performed at 40 W for 2 min using Ultrasonic Processor – Cole Parmer. The condition for extracting BFs by sonication was adopted from Comte et al. (2006).
4) EDTA: EDTA was used with concentration of 20 g EDTA/L of sludge with 25 g SS/L. After adding EDTA to fresh sludge, the sample was mixed well and incubated for 3 h at 4 °C (Liu and Fang, 2002).
5) Formaldehyde and NaOH (F/NaOH): 0.42 mL of formaldehyde (36.8% w/w or 368 g/L) was added to 70 mL of sludge (25 g SS/L) and incubated for 1 h at 4 °C. Thereafter, 3 mL NaOH 10 M was added and left for 3 h at 4 °C to complete the reaction (Liu and Fang, 2002).

Further, a combination of several methods was used to extract BFs from fresh sludge; each step was conducted under similar conditions as described previously. The following combinations were employed:

6) Formaldehyde followed by heating (F-Heat);
7) Formaldehyde followed by sonication and the NaOH treatment (F-Sonic-NaOH);
8) Formaldehyde followed by EDTA extraction (F-EDTA);
9) Formaldehyde followed by sonication and EDTA (F-Sonic-EDTA) and
10) Formaldehyde followed by sonication and heating (F-Sonic-Heat).

The use of formaldehyde in combined methods is to fix the cell and prevent cell lysis and sonication was used to reduce the floc size and therefore, increase the extraction rate and extraction efficiency. After completion of reaction, all samples were centrifuged at 6000*g* for 15 min to remove the pellet. Supernatant of each sample was considered crude BFs. To purify, the crude BFs was mixed with cold ethanol (98%) in a ratio of 1:2 (v/v) and precipitated at −20 °C overnight. The precipitated BFs was collected by centrifugation (6000*g*, 4 °C, 20 min) as a pellet. The pellet was dissolved in distilled water to initial volume and the resulting solution was considered purified BFs.

2.3. Chemical composition of BFs

For measuring dry weight, one volume of crude BFs was mixed in two volumes of cold ethanol (98%) and precipitated at −20 °C overnight. After precipitation, the sample was centrifuged at 6000*g* for 20 min. The pellet was dried at 50 °C until constant weight.

To determine the chemical composition, the purified BFs solution was used to determine the protein (PN), polysaccharide (PS) and nucleic acid concentration. Soluble protein was determined according to the method of Bradford (Bradford, 1976). Polysaccharide was analyzed by Phenol-Sulfuric acid method (DuBois et al., 1956) and nucleic acids were measured by Diphenylamine method (Burton, 1956). The results were presented as an average of the triplicate samples.

2.4. Flocculation activity test

Kaolin clay was used as a test material to measure the flocculation activity of BFs as kaolin has a negative surface charge (−32 mV), which is similar to the charge of particulate matter in wastewater and wastewater sludge (Bezawada et al., 2013). The flocculation activity of BFs was determined in Jar-test with 5 g/L of kaolin solution. Both crude and purified BFs were used for the flocculation activity test. Procedure of the flocculation activity test was as described briefly below.

$CaCl_2$ was added to the kaolin suspension to obtain a final concentration of 150 mg Ca^{2+}/L and pH was adjusted to 7.5 by NaOH 0.1 M. BFs solution was added into kaolin suspension and rapidly mixed at 100 rpm for an initial 5 min, then slowly mixed for

Table 1
Influent characteristics of CUQ wastewater treatment plant.

Parameter	Concentration (mg/L)
pH	6.9
Total solids (TS)	390
Total dissolved solids (TDS)	270
Suspended solids (SS)	120
Biochemical oxygen demand (BOD_5)	110
Chemical oxygen demand (COD)	250
Total organic carbon (TOC)	80
Total nitrogen (Ntot-N)	20
Kjeldahl nitrogen (TKN-N)	20
Organic nitrogen (Norg-N)	8
Ammonia nitrogen (NH4–N)	12
Nitrite (NO_2–N)	0
Nitrate (NO_3–N)	0
Total phosphorus (Ptot-P)	4
Inorganic phosphorus (PO4–P)	3
Chlorides	30
Alkalinity (as $CaCO_3$)	50
Sulfates	20