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Optimized production of a novel bioflocculant M-C11 by Klebsiella sp. and its application in sludge dewatering

Jiewei Liu¹, Junwei Ma¹,⁎, Yanzhong Liu¹, Ya Yang¹, Dongbei Yue², Hongtao Wang²

¹. State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Beijing Normal University, Beijing 100875, China. E-mail: liujiw123@gmail.com
². School of Environment, Tsinghua University, Beijing 100084, China

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Abstract

The optimized production of a novel bioflocculant M-C11 produced by Klebsiella sp. and its application in sludge dewatering were investigated. The optimal medium carbon source, nitrogen source, metal ion, initial pH and culture temperature for the bioflocculant production were glucose, NaNO₃, MgSO₄, and pH 7.0 and 25°C, respectively. A compositional analysis indicated that the purified M-C11 consisted of 91.2% sugar, 4.6% protein and 3.9% nucleic acids (m/m). A Fourier transform infrared spectrum confirmed the presence of carboxyl, hydroxyl, methoxyl and amino groups. The microbial flocculant exhibited excellent pH and thermal stability in a kaolin suspension over a pH range of 4.0 to 8.0 and a temperature range of 20 to 60°C. The optimum bioflocculating activity was observed as 92.37% for 2.56 mL M-C11 and 0.37 g/L CaCl₂ dosages using response surface methodology. The sludge resistance in filtration (SRF) decreased from 11.6 × 10¹² to 4.7 × 10¹² m/kg, which indicated that the sludge dewaterability was remarkably enhanced by the bioflocculant conditioning. The sludge dewatering performance conditioned by M-C11 was more efficient than that of inorganic flocculating reagents, such as aluminum sulfate and polymeric aluminum chloride. The bioflocculant has advantages over traditional sludge conditioners due to its lower cost, benign biodegradability and negligible secondary pollution. In addition, the bioflocculant was favorably adapted to the specific sludge pH and salinity.

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Introduction

Sludge dewatering is of great significance in sludge processing because it reduces the sludge volume and, as a result, decreases the cost of the transportation and disposal (Yuan et al., 2011b). Chemical flocculation (Lo et al., 2001), thermal treatments (Guan et al., 2012), ultrasound conditioning (Feng et al., 2009), electrolysis (Yuan et al., 2010) and Fenton oxidation (Tony et al., 2008, 2009) have been investigated as means of enhancing sludge dewaterability. Inorganic and organic synthetic flocculants are extensively employed in sludge dewatering because of their high coagulating effectiveness, despite the fact that they posed consequent environmental threats and risks. As chemical flocculating substances, polyacrylamides (PAM) were confirmed to be harmful and carcinogenic (Dearfield et al., 1988), resulting in human health problems, including Alzheimer’s disease, which is correlated with the application of aluminum salts (Bondy, 2010).

Bioflocculants are organic macromolecular substances produced during microorganism growth that can efficiently coagulate and aggregate suspended colloids. Attention has been focused on the production and component analysis of novel bioflocculants with high flocculation efficiencies (Li et al., 2009; Liu et al., 2010; Zheng et al., 2008). Various strains have been reported to be bioflocculant-producing microorganisms (BPMs),
of which Bacillus sp. has been the most widely reported (Lian et al., 2008; Yuan et al., 2011c; Zheng et al., 2008). Previously, bioflocculants were reported to remove metal ions (Salehizadeh and Shojaosadati, 2003), defecate trona suspension (Lu et al., 2005), and treat dye solutions (Deng et al., 2003) and low-temperature drinking water (Li et al., 2009). Nevertheless, relevant research studies on improved sludge dewaterability by bioflocculant conditioning have rarely been reported. Compared with conventional flocculating agents, bioflocculants have attracted considerable scientific and biotechnological attention because of their lower costs, benign biodegradability, and negligible secondary pollution (Liu et al., 2010). Salinity has been correlated with lipid content and cell growth, which was believed to have a significant effect on the sludge dewaterability (Bartley et al., 2013; Chu et al., 1997; Lo et al., 2001). In particular, the salinity levels of the wastewater and the activated sludge were relatively high. The reported bioflocculants for wastewater treatment were primarily screened and isolated from soils, wastewaters, rivers, and foods (Gao et al., 2009). Bioflocculants from other environmental media may not efficiently adapt to the sludge salinity, which may inhibit the sludge conditioning effect.

The cation dosage was proven to neutralize the negative charges in the sludge particles, and to decrease the absolute value of the zeta potential during the flocculating process (Zhang et al., 2010b). The bioflocculant concentration also exhibited a remarkable influence on the flocculating activity (Zheng et al., 2008). To obtain the optimum flocculating activity under the combined effects of the pH value, the bioflocculant dosage and the cation dosage, response surface methodology (RSM) was used to estimate the interaction between the multiple factors. Compared with conventional and single-factor methods, which are time-consuming because they require a large number of experiments to determine the optimal content of each factor one at a time, the response surface methodology can distinguish the interaction between individual variables (He et al., 2009). In this study, a novel bioflocculant named M-C11, produced by Klebsiella sp., was isolated from the activated sludge. The optimal cultivation and flocculation conditions were determined using response surface methodology. In addition, the bioflocculant was finally employed in sludge dewatering such that it was adjusted in the specific pH, salinity, and biological conditions.

1. Materials and methods

1.1. Isolation and culture composition

Bioflocculant-producing bacteria were isolated from the activated sludge of a secondary sedimentation tank in a wastewater treatment plant (WWTP) located in Jiangsu Province, China. To obtain bioflocculant-producing microorganisms (BPMs), repeated screening and purification experiments were conducted with a culture-medium composition as follows (per liter): 0.5 g KCI; 2.0 g NaNO3; 0.01 g FeSO4; 1.0 g K2HPO4; 0.5 g MgSO4; and 30.0 g sucrose. All medium solutions were prepared in triplicate for a mean calculation, and the standard deviations were included in the figure plotting. SigmaPlot software (v10.0) was used to create the figures.

1.2. Optimization of bioflocculant production

The effects of the carbon source, nitrogen source, metal ions, initial pH and culture temperature on the M-C11 production were investigated to identify the optimal cultivation conditions. Sucrose (30.0 g/L) was compared with glucose, starch, compound carbon (glucose: starch = 1:1, m/m) and beef extract to determine the effect of the carbon source on the bioflocculant production; NaNO3 (2.0 g/L) was compared with NH4Cl, yeast, and urea to determine the effect of the nitrogen source on the bioflocculant production; MgSO4 (0.5 g/L) was compared with NaCl, CaCl2, FeSO4, CuSO4 and Al2(SO4)3 to determine the effect of the metal ions on the bioflocculant production; the initial pH was adjusted to 4.0–10.0 by NaOH (0.1 mol/L) and HCl (0.1 mol/L), and the culture temperature was adjusted to 20 to 40°C to determine the effect of the initial pH and the culture temperature, respectively, on the bioflocculant production. All experiments were conducted in triplicate for a mean calculation, and the standard deviations were included in the figure plotting. SigmaPlot software (v10.0) was used to create the figures.

1.3. Measurement of flocculating activity

Kaolin clay was chosen as the suspended solid to calculate the flocculating activity. First, 93 mL kaolin suspension (4.0 g/L), 5.0 mL CaCl2 (1%, m/v) and 2.0 mL liquid bioflocculant were mixed and stirred at 400 r/min for 1 min and then stirred at 150 r/min for 5 min. After settling for 5 min in the cylinder, the supernatant absorbance was measured by a spectrophotometer at 550 nm (Shimadzu UV-2550, Japan). The flocculation supernatant was replaced with a culture medium at the same concentration in the control experiment. The flocculating activity (FA, %) was calculated according to Eq. (1):

$$FA = \frac{A_0 - A}{A_0} \times 100\%$$

where, $A_0$ and $A$ were the absorbance variables at 550 nm of the control and the sample supernatant, respectively.

1.4. Bioflocculant purification

According to Salehizadeh and Shojaosadati (2003), to obtain the purified bioflocculant, the fermentation broth was centrifuged to remove the cells by centrifugal separation (5000 r/min, 30 min). Two volumes of cold ethanol were then added to the supernatant and left overnight at 4°C. The precipitate was re-dissolved in distilled water, followed by the addition of 2% cetylpyridinium chloride solution (CPC) with stirring. After 2 hr, the resulting precipitate was collected by centrifugation at 5000 r/min for 30 min and re-dissolved in NaCl (0.5 mol/L). Three volumes of cold ethanol was added to obtain the precipitate, which was then washed two times with ethanol, and then the purified bioflocculant was vacuum-dried.

1.5. Physical and chemical analysis of M-C11

The total sugar content of the M-C11 bioflocculant was measured according to the phenol-sulfuric acid method using glucose as the standard (Chaplin and Kennedy, 1994). The total protein content was measured by the Bradford method with bovine

1.6. Physical and chemical analysis of M-C11
serum albumin as the standard (Brady, 1976). The DNA content was measured by the diphenylamine colorimetric method using calf thymus DNA sodium salt as the standard (Aduv and Lee, 2008). Ultraviolet scanning of the purified biofloculant was conducted over a wavelength range of 200–400 nm (Shimadzu UV-2550, Japan). Fourier transform infrared (FT-IR) spectroscopy of purified M-C11 sample was obtained on a KBr disk over a wavenumber range of 400–4000 cm⁻¹ to determine the functional groups (Thermo Nicolet IS-5, USA).

1.6. Flocculation characteristics of M-C11

1.6.1. pH and thermal stability of M-C11

To investigate the pH stability of M-C11, the pH was adjusted in the range of 3.0–9.0; to investigate the thermal stability of M-C11, a biofloculant and kaolin suspension mixture was kept for 30 min in a water bath with various temperatures from 0 to 100°C.

1.6.2. Response surface methodology and experimental design

The response surface methodology is an empirical statistical modeling method that performs multiple-regression analysis of quantitative data to solve multivariable equations simultaneously (Rao et al., 2000). A preliminary experiment to determine the narrower ranges of two significant variables, the M-C11 and CaCl₂ dosages on the flocculating properties, was conducted. As a result, the ranges were chosen as 1.0 to 2.5 mL for M-C11 and 0.1% to 1.0% for CaCl₂ (1.0%, m/V). To obtain the optimum biofloculant activity, a 2-factor-5-level central composite design (CCD) was employed (Table 1).

The quadratic polynomial model for predicting the optimal conditions can be expressed according the following Eq. (2):

\[ Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j \]  

(2)

where, \( Y \) (%) represents the predicted response (floculating activity); \( X_i \) and \( X_j \) (\( i = 1, 2 \) and \( j = 1, 2 \)) are the variables; \( \beta_0 \) is the interception coefficient; \( \beta_i \) is the linear coefficient; \( \beta_{ij} \) is the quadratic coefficient; and \( \beta_{ij} \) is the interaction coefficient.

The Design Expert software package (version 7.0, Stat-Ease, Inc., US) was applied for the experimental design. Analysis of variance was used for the data analysis to obtain the interaction between the process variables and the responses.

1.7. Activated sludge dewatering experiment

The sludge dewaterability was characterized by the specific resistance in filtration (SRF) and the filtrate curve by a Buchner funnel test (Lo et al., 2001). In the sludge conditioning experiment, aluminum sulfate, polymeric aluminum chloride (PAC) and cationic polyacrylamides (CPAM) were selected as the representative conventional sludge conditioners and compared with the novel biofloculant M-C11 to enhance dewaterability. Dissolved conditioners (0.1%, m/V) were added into a 50-mL beaker with 25 mL sludge samples and stirred at 400 r/min for 1 min and then at 150 r/min for 5 min. The conditioned sludge was poured into the funnel fitted with a fiberglass filter paper at vacuum of 0.05 MPa and the filtrate volumes collected at various times were recorded. SRF is calculated by the following Eq. (3):

\[ SRF = \frac{2bP}{A} \]  

(3)

where, \( P \) (N/m²) is the pressure of filtration; \( A \) (m²) is the filtration area; \( \mu \) (N · sec/m²) is the filtrate viscosity; \( C \) (kg/m³) is the weight of the solids per unit volume of filtrate.

\[ 1/C = C_i/(100-C_i)-C_f/(100-C_f) \]  

(4)

where, \( C_i \) (%) is the initial moisture content; \( C_f \) (%) is the final cake moisture; \( b \) is the slope determined from the \( t/V-V \) plot; \( V \) (m³) is the volume of the filtrate; and \( t \) (sec) is the filtration time.

2. Results and discussion

2.1. Isolation and identification of the biofloculant-producing strain

A novel biofloculant-producing bacterium with a high and stable flocculating activity, named C11, was isolated from the activated sludge. A creamy color was observed in both the fermentation broth and the strain colony cultivated on the agar medium. The strain was proven to be positive in the fungal catalase reaction. A rod-shaped strain (Fig. 1) was observed, and the diameter of the cells was 1.0–1.5 μm, measured by scanning electron microscopy (FEI Quanta 200, the Netherlands). C11 was identified as Klebsiella pneumoniae with the GenBank sequence accession number CP000647. The similarity between C11 and Klebsiella sp. was as high as 99%.

2.2. Biofloculant production

2.2.1. Effects of the carbon source, nitrogen source and metal ions on M-C11 production

Most microbial flocculants consist of polysaccharides, protein and nucleic acids. The medium carbon and nitrogen source provide the necessary nutrients for biofloculant production and further influence the flocculating activity. The carbon

<table>
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<th>Table 1</th>
<th>Range and levels of the natural and corresponding coded variables for the response surface methodology (RSM).</th>
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<tr>
<td>Variable</td>
<td>Symbols</td>
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<tr>
<td>M-C11 (mL)</td>
<td>( X_1 )</td>
</tr>
<tr>
<td>CaCl₂ dosage (g/L)</td>
<td>( X_2 )</td>
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</table>

\( \zeta_1 = (X_1 - 2.5)/1.5 \), \( \zeta_2 = (X_2 - 0.3)/0.2 \); where, \( \zeta_1 \) and \( \zeta_2 \) are coded values, with the ranges from -1.414 to 1.414, according to the central composite design (CCD); \( X_1 \) and \( X_2 \) are natural values.
source contributes to cell growth and metabolite synthesis during the microorganism cultivation. Among the carbon sources studied, glucose was the most favorable for the M-C11 flocculating activity (in a kaolin suspension), whereas the flocculation rates by sucrose, compound carbon, beef extract and starch were lower (Table 2). A flocculating activity as high as 86.7% was obtained with glucose as the carbon source. The flocculating activity was higher using glucose as the carbon source because the monosaccharide was easily degraded compared with the polysaccharides, such as sucrose and starch, indicating that the flocculating activity of Klebsiella sp. was correlated with the carbon source degradability. In contrast to the results with M-C11, the most favorable carbon for the bioflocculant produced by Bacillus licheniformis X14 was starch (Li et al., 2009); the optimum carbon source varies with bioflocculant-producing strains. Gluconic acids were produced in the fermentation broth during the starch degradation process. An increasing gluconic acid concentration resulted in a decrease in the pH value and a rise in the extracellular osmotic pressure (Zheng et al., 2008), which explains the lower activity when cultured in compound carbon media. As shown in Table 2, the nitrogen sources were sequenced by the M-C11 flocculating activity as follows: NaNO₃ > yeast > urea > NH₄Cl > blank. Considering the incomplete absorption of the organic nitrogen sources (yeast and urea) and the preference for NO₃⁻ instead of NH₄⁺ for bioflocculant production, NaNO₃ was selected as the optimal nitrogen source, for which the flocculating activity is 82.6%.

Cationic metal ions can neutralize the negative charges of both the polysaccharide and suspended particles and increase the adsorption of the polysaccharide onto the suspended particles (Elkady et al., 2011). The effect of the metal ions on the M-C11 production was also investigated; most of the ions can promote the flocculant production to varying degrees, except for the negative effect of Cu²⁺. The result showed that MgSO₄ was the most favorable cation for M-C11 production. The optimal metal ion was consistent with the conclusion of Liu et al. (2010). Compared with monovalent and trivalent cations, divalent cations enhanced the kaolin clay floc formation more efficiently, resulting in a higher flocculating activity, which is confirmed in this research. The effects of the metal cations on bioflocculation were also influenced by the ionic valency and strength (Salehizadeh et al., 2000).

2.2.2. Effects of the initial pH and the culture temperature on M-C11 production

The effects of the pH variation within the range of 4.0–10.0 on M-C11 production are shown in Table 2. In the initial pH range of 6.0–10.0, M-C11 maintained a high flocculating activity, and the optimum initial pH was 7.0. The medium initial pH determines the surface electric charge and the redox potential. As a result, the nutrient absorption and the enzymatic reaction were inhibited. Under acidic conditions, the flocculating activity was low because the excess H⁺ concentration changed the zymoprotein dissociation and electric charge. Higher or lower temperature could exert negative effects on the reaction rate and the most favorable culture temperature for M-C11 production was 25°C. The bioflocculant was produced under the enzymatic reaction by zymoprotein catalysis, which was related to the culture temperature. A higher temperature may influence the molecular chain length and decrease the bioflocculant-synthesis enzyme activity (Salehizadeh and Shojaosadati, 2003).

2.3. Characterization of the purified bioflocculant M-C11

Bioflocculants were mainly composed of neutral sugar, uronic acid, protein and nucleic acid (Li et al., 2009; Liu et al., 2010; Zhang et al., 2010b). Approximately 1.68 g purified M-C11 was recovered per liter culture broth by the cold ethanol method. Tyrosine and tryptophan in the protein derivatives present strong absorption at short wavelengths of approximately

![Fig. 1 - Scanning electron microscopy (SEM) image of strain C11.](image-url)
280 nm (Yuan et al., 2011c; Zheng et al., 2008). The ultraviolet scanning spectrum of M-C11 (Fig. 2a) displayed a weak peak at 280 nm, confirming the presence of protein in the bioflocculant. The strong absorption peak at 250–260 nm was proven to be characteristic of nucleic acids. The purified M-C11 consisted of 91.2% sugar, 4.6% protein and 3.9% nucleic acids \((m/m)\), indicating that M-C11 was an exopolysaccharide bioflocculant. The compositional analysis indicated that the bioflocculant M-C11 consisted of polysaccharide, protein and nucleic acids.

As illustrated in Fig. 2b, the FT-IR spectrum of the purified bioflocculant M-C11 displayed an intense broad stretching peak at 3430 cm\(^{-1}\) characteristic of the hydroxyl and amino groups, and a weak stretching band at 2930 cm\(^{-1}\) as an indication of aliphatic C–H (Elkady et al., 2011). The absorption in the 1625 cm\(^{-1}\) region of the spectra can be assigned to the COO\(^{-}\) and C–C groups (Tremblay et al., 2011). The peak at 1383 cm\(^{-1}\) may be attributed to the C–O symmetrical and asymmetrical stretching of a carboxylate group in the bioflocculant (Xiong et al., 2010). The strong absorption at 1057 cm\(^{-1}\) indicated the C–O stretching vibration and the presence of methoxyl groups, which is known to be characteristic for all sugar derivatives. The FT-IR spectrum confirmed the presence of the carboxyl, hydroxyl, methoxyl and amino groups, which was in accordance with the results of most bioflocculants produced by different microorganisms (Liu et al., 2010; Xia et al., 2008).

2.4. Flocculating properties of the bioflocculant M-C11

2.4.1. pH and thermal stability of M-C11

The flocculating properties of the purified bioflocculant were influenced by the system conditions such as the kaolin suspension pH and temperature. A flocculating activity over 80.0% was achieved in the wide acid and neutral pH range of 4.0–8.0, as illustrated in Fig. 3. The optimal activity of 91.7% was observed at pH 7.0. The favorable pH range varies for the bioflocculants produced by different strains. For example, the optimal pH range was 5.0 to 9.0 for Bacillus mojavensis 32A (Elkady et al., 2011), and the flocculating activity was higher than 80.0% in the range of 7.0–9.0 for Rothia sp. ZHT4-13 (Gao et al., 2009). As the concentration of hydroxide ions (OH\(^{-}\)) in the kaolin suspension increased, the charge density on the clay particles was simultaneously increased, which in turn inhibited the neutralization effect of calcium chloride. After being heated in a water bath for 30 min, the relationship between the clay-suspension temperature and the flocculating activity was investigated. M-C11 was thermally stable over a temperature range of 20–60°C. Additionally, the polysaccharide-backbone composition of M-C11 was assumed to explain the excellent thermal stability of the bioflocculant produced by Klebsiella sp.

2.4.2. Effects of the M-C11 and CaCl\(_2\) dosages on the flocculating activity

The addition of the bioflocculant resulted in remarkable precipitation for the kaolin suspension (4.0 g/L), and the optimal M-C11 dosage was 2.0 mL (Fig. 4). In the single-factor experiment, an insufficient bioflocculant dosage could not efficiently promote the formation of the bridging structure. However,
excess negatively charged M-C11 increased the repulsion of the kaolin particles and, coupled with the competitive adsorption, inhibited small flocs from growing into larger ones. The relationship between the M-C11 dosage and the flocculating activity agreed with the results described by earlier researchers (He et al., 2010).

The effect of the CaCl2 dosage on the flocculating activity was investigated by adding 0–0.5 g/L CaCl2 and 2.0 mL M-C11 into the kaolin clay suspension. As showed in Table 3, the flocculating activity increased from 78.4% to 92.2% at CaCl2 dose ranging between 0 and 0.5 g/L, and then reached the optimized activity at 0.3 g/L. During the flocculating process, the main role of CaCl2 was to neutralize the surface negative charges and to promote stable-floc formation. On the other hand, an excessive concentration of CaCl2 stabilized the suspended particles and organic materials again in their original states, as a result of the superfluous positive charges.

2.4.3. Flocculating activity optimization using the response surface methodology

To obtain the optimum flocculation conditions of M-C11, central composite design (CCD) and response surface methodology (RSM) were applied in the experimental design based on the single-factor experiment. The following second-order fitting polynomial Eq. (5) was then obtained after data fitting and the observed and predicted flocculating activity (%) are shown in Table 4:

\[ Y = 61.94 + 10.01X_1 + 94.59X_2 - 6.81X_1X_2 - 1.46X_1^2 - 103.96X_2^2 \]  

where, \( Y \) (%) is the flocculating activity; and \( X_1 \) and \( X_2 \) are the M-C11 dosage and CaCl2 dosage, respectively.

Good agreement of the data between the observed and predicted response values was achieved with a regression coefficient \( R^2 \) value of 0.9824. The analysis of variance results illustrated that the fitted quadratic polynomial model was statistically valid given an F-test with a low-probability value (\( p_{\text{Model}} < 0.0001 \)). The results showed that the lack-of-fit value of 3.3 is not significant relative to the pure error.

The three-dimensional response surface plot is shown in Fig. 5. The CaCl2 dosage (\( p < 0.0001 \)) had a greater influence on the flocculating activity than the M-C11 dosage (\( p = 0.0131 \)). According to the quadratic polynomial model, the maximum flocculating activity of 92.30% was obtained under the following conditions: \( X_1 = 2.56 \) and \( X_2 = 0.37 \). The observed flocculating activity by the optimized condition was 92.37%, which is in close agreement with the model prediction. Thus, it is reasonable to believe that the polynomial model is reliable for describing the effects of the M-C11 and CaCl2 dosages on the flocculating activity.

2.5. Application of M-C11 in activated sludge dewatering

Several applications in the sludge treatment of bioflocculants have been reported from distinct viewpoints (Subramanian et
After sludge flocculation with the bioflocculant produced by Rothia sp., the filamentous fungi that caused the sludge bulking disappeared, and the protozoa increased (Gao et al., 2009). The screening, cultivation and optimization for flocculating activity of M-C11 were investigated using the kaolin-clay suspension. The novel bioflocculant produced by Klebsiella sp. was then used for improving the waste activated sludge dewaterability.

Fig. 6a presents a comparison of the sludge dewatering results using the bioflocculant M-C11 and other conventional chemical flocculants, such as Al2(SO4)3, PAC and CPAM. The addition of sludge conditioners resulted in a promoted dewatering performance to a varying extent. The sludge resistance in filtration decreased from 11.6 × 10¹² to 4.7 × 10¹² m/kg by M-C11 conditioning with a dewatering efficiency of 59.9%. The filtrate volumes at specific times were recorded with 3.0 mL flocculant dosages (Fig. 6b). Compared with the raw sludge and the performance of inorganic flocculants, the sludge dewatering performance was considerably enhanced by M-C11. In the application of the novel bioflocculant in waste sludge dewatering, the bioflocculant M-C11 enhanced the sludge dewaterability more efficiently than the inorganic flocculants. The positive charges made the inorganic coagulants neutralize the suspended flocs with negative charges and reduce the repulsion between the sludge particles, after which the colloidal particles agglomerated and formed into bigger flocs (Qi et al., 2011). According to the bridging mechanism, bioflocculants and organic polymers could further promote the dewatering ability by adsorption onto the molecular chain structure.

With a high pH and thermal stability, the sludge dewatering performance by M-C11 conditioning was similar to that of biopolymer cationic polyacrylamides but avoid the environmental problems and human-health risks caused by CPAM (He et al., 2009; Yuan et al., 2011a). In addition, M-C11 favorably accommodates the specific pH and high salinity levels in waste sludge. The bioflocculant could serve as an efficient method to enhance sludge dewatering performance, and a promising substitute for conventional flocculants because of its excellent stability and negligible environmental impact.

3. Conclusions

A novel bioflocculant M-C11, produced by Klebsiella sp., was obtained from activated sludge. The optimal carbon, nitrogen, metal ion, initial pH and temperature for the bioflocculant production were glucose, NaNO3, MgSO4, pH 7.0 and 25°C, respectively. The FT-IR spectrum indicated that the bioflocculant contained carboxyl, hydroxyl, methoxyl and amino groups. The bioflocculant showed excellent stability over wide pH and thermal ranges. Insufficient or excessive M-C11 and CaCl2 dosages could inhibit the flocculating activity as a result of competitive adsorption. The optimized bioflocculating activity was observed as 92.37% for 2.56 mL M-C11 and 0.37 g/L CaCl2 dosages using response surface methodology. The sludge dewaterability was obviously enhanced by the bioflocculant conditioning, which was more efficient than the inorganic flocculants. The novel bioflocculant M-C11 can favorably accommodate the specific pH and high salinity levels in waste sludge.

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