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Treatment of swine wastewater using chemically modified zeolite and bioflocculant from activated sludge

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HIGHLIGHTS

- Sludge was used as medium for bioflocculant production.
- Effect of sludge pretreatments on bioflocculant production was determined.
- A novel modified zeolite was prepared for ammonium adsorption.
- Composite of bioflocculant and zeolite was used for treatment of swine wastewater.
- Response surface methodology was applied to search optimal flocculation condition.

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ABSTRACT

Sterilization, alkaline-thermal and acid-thermal treatments were applied to activated sludge and the pretreated sludge was used as raw material for *Rhodococcus* R3 to produce polymeric substances. After 60 h of fermentation, bioflocculant of 2.7 and 4.2 g L^{-1} were produced in sterilized and alkaline-thermal treated sludge as compared to that of 0.9 g L^{-1} in acid-thermal treated sludge. Response surface methodology (RSM) was employed to optimize the treatment process of swine wastewater using the composite of bioflocculant and zeolite modified by calcining with MgO. The optimal flocculating conditions were bioflocculant of 24 mg L^{-1}, modified zeolite of 12 g L^{-1}, $CaCl_2$ of 16 mg L^{-1}, pH of 8.3 and contact time of 55 min, and the corresponding removal rates of COD, ammonium and turbidity were 87.9%, 86.9%, and 94.8%. The use of the composite by RSM provides a feasible way to improve the pollutant removal efficiencies and recycle high-level of ammonium from wastewater.

1. Introduction

Bioflocculant, secreted by microorganisms during their growth and cell-lysis, is a kind of environment-friendly material with the character of harmless and biodegradable (Salehizadeh and Shojaosadati, 2001). Although the use of bioflocculants has been considered as a potential solution to the toxicity to aquatic life and the environment pollution in recent years, flocculating activity and cultivation cost are still the major impediments to its application (Liu et al., 2010; Zhao et al., 2012). Activated sludge, produced from wastewater treatment plants when wastewater is treated through biological process, contains macromolecules compounds, such as polysaccharide, protein, cellulose and so on, which have been supposed to be a source of bioflocculants (Drouin et al., 2008; More et al., 2010). Wastewater, like swine wastewater, from livestock and poultry breeding, is one of the most abundant sources of nitrogen and phosphorus matters. Thus, strains that can effectively utilize the substrates in sludge and wastewater to produce bioflocculants are of academic and practical interests (Wang et al., 2007a; You et al., 2008). Furthermore, in the flocculating process, composite flocculant is considered as an effective way to reduce the cost, since it can reduce the dosage of bioflocculant and can improve the flocculating activity (Yang et al., 2009).

Over the past few years, biological systems have provided an effective solution for ammonium nitrogen removal, in which ammonium was transformed first to nitrite, then to nitrate, and finally to nitrogen gas (Thornton et al., 2007). However, since biological systems do not respond well to high shock loads of ammonium, unacceptable peaks over the discharging levels may frequently appear in the effluent ammonium concentrations (Huang et al., 2010; Saltale et al., 2007). In such solution,

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ammonium adsorption using zeolites as adsorbent are gaining on interests, due to its low cost and relative simplicity of application and operation (Lei et al., 2008; Wang et al., 2007a,b). Natural zeolite always needs to be treated before used in order to improve its adsorption capacity, and acid treatment, alkali treatment and hydrothermal treatment are the most commonly used methods. For example, the ammonium ion uptake values of NaOH-treated zeolite (7.5 g of the natural zeolite powder was placed in a Ni crucible and fused with 9 g of NaOH powder at 550 °C for 2 h), HCl-treated zeolite (natural zeolite was treated with 10% HCl at 25 °C over a period of 24 h) and hydrothermally-treated zeolite were 19.29 mg L^{-1}, 21.23 mg L^{-1} and 12.17 mg L^{-1}, respectively, higher than that of natural zeolite (10.49 mg L^{-1}) (Wang et al., 2007b; Watanabe et al., 2005; Zhao et al., 2004). Moreover, it is reported that the adsorption capacity of zeolites can be improved with the increasing relative content of alkaline metal cations which are of low valence and large ionic radius (Wang et al., 2007b). Thus, a novel zeolite modified by calcining with MgO was prepared in this study.

The zeolite modified by calcining with MgO was selected to be composited with the bioflocculant from pre-treated activate sludge. The response surface methodology (RSM), a statistical technique for building multivariable equation and evaluating their optimal values (Yang et al., 2009), was employed to search the optimum conditions of the flocculation process and to investigate the interactions of possible individual parameters including dosage of the bioflocculant, modified zeolite and $CaCl_2$, pH and contact time with a limited number of planned experiments. Swine wastewater was chosen as a representative suspended sample, the COD and ammonium removal rates were settled as the response variables, and the optimal conditions were their compromised result.

2. Methods

2.1. Zeolites

The natural zeolite with grain diameter of 30 μm used in this study was supplied by Jingyun Mining Processing Plant, Zhejiang province, China. Physical–chemical properties tests showed that the specific external area of the natural zeolite is 157.2–191.5 m^2 g^{-1}. The natural zeolite was firstly washed with distilled water to remove residual salinity and dirtiness such as ash and sand specimen, and then dried in an oven at 105 °C until a constant weight. Afterwards, the resulting zeolite was collected and calcined with MgO (4:1, W/W) at 400 °C for 4 h in a muffle, and the modified zeolite was finally obtained.

2.2. Swine wastewater quality

The swine wastewater in this study was taken from an anaerobic digestion pool of a swine wastewater treatment plant located in Fuhua pig farm, Hunan Province, China, with a treatment capacity of 160 m^3 h^{-1}. The concentrations of COD, ammonium and turbidity of this solution were 1000–1300 mg L^{-1}, 800–1200 mg L^{-1} and 150–200 NTU, respectively. The concentrations of K^+, Na^+, Ca^{2+}, and Mg^{2+} were 0.031, 0.026, 0.025, and 0.007 mg L^{-1}, respectively. The pH and VSS/TSS of the wastewater was 7.5–8.5 and of 78.4%, respectively.

2.3. Activated sludge sample

Activated sludge samples were obtained from a dewatering workshop at the Jinxia Wastewater Treatment Co., Ltd., Hunan province, China, which treated municipal domestic sewage mainly. The sludge suspension was prepared with swine wastewater at the wet sludge concentration of 100 g L^{-1}. Before the bioflocculant production, sludge solution samples were treated by sterilization (ST), alkaline-thermal (ALT) and acid-thermal (ACT) treatments, respectively. Sterilization was carried out by autoclaving (steam sterilization) at 121 °C for 30 min. In ALT treatment, first, pH of the sludge solution was raised to 10.0 by using 1.0 mol L^{-1} NaOH at room temperature (25 °C) and then autoclaved in the same procedure. In ACT treatment, first, pH was reduced to 2.0 using 1.0 mol L^{-1} HCl at room temperature (25 °C) and then autoclaved in the same procedure.

2.4. Assay of flocculating rate

The flocculating activity of the bioflocculant was described by measured the flocculating rate in jar tests based on the method reported by Kurane et al. (1986) with slight modifications, in which 4.0 g L^{-1} of kaolin clay (K2-500, Tianjin Hengxing Industrial Co., Ltd., China) was chosen as the suspended solid. After the pH value was adjusted to 7.5 using 1.0 mol L^{-1} NaOH or HCl, 15 mg of $CaCl_2$ and 2.0 mg of bioflocculant were added into the 100 mL of kaolin suspension in a 300 mL beaker. The mixture was vigorously stirred (180 rpm) for 1.0 min and slowly stirred (80 rpm) for 4.0 min, and then allowed to stand 10 min using a six-breaker jar tester (ZR4-6, SY.36-ZR4-6, Shenzhen Zhongrun Company, China). The optical density (OD) of the clarifying solution was measured with a spectrophotometer (Unic-7230, Shanghai Lianhua Company, China) at 550 nm. A control experiment was conducted in the same manner without adding bioflocculant. All the bioflocculant measurements were carried out in triplicates and the average values were presented (with standard error less than 5% of the mean). The flocculating rate was calculated by the following equation:

$$FR = \frac{(B - A)}{B} \times 100\% \quad (1)$$

where FR is the flocculating rate; A and B are the OD values of the sample and control.

2.5. Isolation and identification of functional strains

A total of 1.0 mL of activated sludge sample was serially diluted with distilled water (10^1–10^{10} folds), and subsequently, 1.0 mL of each dilution was spread onto agar plates. The composition of the agar plates was as follows (per liter): urea 5.0 g, yeast extracts 0.5 g, sucrose 20 g, K_2HPO_4 5.0 g, KH_2PO_4 2.0 g, $MgSO_4$ 2.0 g, NaCl 10 g, and agar 10 g. Then, the plates were sealed and inverted and incubated at 35 °C in an incubator. Visible colonies appeared after 48 h of cultivation. After 4–6 cycles of replanting onto the agar plates, a total of 34 morphologically different isolates were obtained, in which the 9 large and viscous colonies were chosen and individually inoculated on a reciprocal shaker (SHA-A, Shanghai Lianhua Industrial Co., Ltd., China) at 150 rpm and 35 °C for 24 h in 100 mL culture medium. The culture medium consisted of urea 5.0 g, yeast extract 0.5 g, sucrose 20 g, K_2HPO_4 5.0 g, KH_2PO_4 2.0 g, $MgSO_4$ 2.0 g, and NaCl 10 g dissolved in 1.0 L distilled water with the pH adjusted to 7.0.

The flocculating efficiency of the strains was measured by 4.0 g L^{-1} kaolin clay suspensions method and the strains which showed high flocculating efficiency were selected as bioflocculant-producing bacteria for further studies. All the 9 strains yielded flocculating activities above 70%, and the bioflocculant-producing bacterium with the highest flocculating rate of 92.3%, named R3, was selected for further tests. The strain R3 was stored at 4 °C and sub-cultured fortnightly, the medium for slant and subculture consisted of (per liter): peptone 10 g, yeast extracts 5.0 g, NaCl 10 g, agar 20 g, with initial pH of 7.0.

Cell forms and colony characteristics of the strain R3 on nutrient agar was observed with bio-microscope (CX31, Olympus, Japan), physiological and biochemical characteristics were identified according to Bergey's Manual of systematic bacteriology. The 16S rRNA gene fragment of the strain R3 was then amplified using individual bacterial colony PCR (Kim et al., 2006). PCR amplification of 16S rDNA was carried out using forward primer (5′-GAG AGT TTG ATC CTG GCT CAG-3′) and reverse primer (5′-CTA CGG CTA CCT TGT TAC GA-3′). The PCR amplification was run on a MyCycler thermal cycle (Bio-Rad, USA) using cycling conditions as follows: 94 °C for 4 min; followed by 30 cycles of 94 °C for 90 s, 55 °C for 60 s, 72 °C for 90 s; followed by 72 °C for 7 min, and end at 4 °C. The PCR product was sequenced and analyzed by Guangdong Institute of Microbiology (Guangzhou, China).

2.6. Bioflocculant production

The bacterial strain R3 was first inoculated in the sterilized sludge in 250 mL Erlenmeyer, and the sample was incubated on a reciprocal shaker at 150 rpm and 35 °C for 24 h. Subsequently, 2.0% V/V of the inoculum (prepared in sterilized sludge) was used to inoculate the different treated sludge samples, respectively. These inoculated sludge samples and the control sample (without inoculating) were incubated in the same procedure to produce crude bioflocculant. After 60 h of cultivation, the fermented broths with the flocculating components were obtained and it will be utilized directly in the flocculation.

2.7. Characteristics of the bioflocculant obtained from the lyophilized material

The total sugar content of the bioflocculant was determined by the phenol–sulfuric acid method using glucose as the standard solution (Chaplin and Kennedy, 1994). Protein content was measured by the Bradford method using bovine serum albumin as the standard solution (Bradford, 1976). Amino acid composition was estimated by amino acids auto analyzer (Hitachi L-8800, Hirayama Company, Japan). Elemental analysis was achieved using an elemental analyzer (PE 2400 II, Perkin Elmer Company, USA). The molecular weight of the bioflocculant was determined by gel permeation chromatography (GPC) using a Hitachi L-6200 system controller. The functional groups of the bioflocculant were determined using a Fourier transform infrared (FTIR) spectrophotometer (EQUINOX 55, Bruker Company, Germany).

2.8. Determination of the dose range

Preliminary experiments were conducted to discuss the effects of dosage of the modified zeolite and bioflocculant on the removal of ammonium and COD from swine wastewater. A Sample of 1.0 L wastewater was poured into a beaker and the pH was adjusted using 1.0 mol L^{-1} NaOH or HCl if necessary. The modified zeolite or bioflocculant was then added into the sample, and the mixture was vigorously stirred (300 rpm) for 1.0 min and slowly stirred (180 rpm) for 9.0 min, and then allowed to stand 10 min using a six-breaker jar tester (ZR4-6, SY.36-ZR4-6, Shenzhen Zhongrun Company, China). Residual COD, ammonium, and turbidity of the wastewater were determined according to the standard methods (EPA of China, 2002).

2.9. RSM experimental design

The central composite design (CCD), which is the standard RSM, was selected to investigate the interactions of parameters including the dosage of the bioflocculant (x_1), modified zeolite (x_2), pH (x_3), $CaCl_2$ (x_4), and contact time (x_5). The response variable (y) that represented COD or ammonium removal rate was fitted by a second-order model in the form of quadratic polynomial equation:

$$y = \beta_0 + \sum_{i=1}^{m}\beta_i x_i + \sum_{i<j}^{m}\beta_{ij} x_i x_j + \sum_{i=1}^{m}\beta_{ii} x_i^2 \quad (2)$$

where y is the response variable to be modeled, x_i and x_j are independent variables which determine y, β_0, β_i and β_{ii} are the offset term, the i linear coefficient and the quadratic coefficient, respectively. β_{ij} is the term that reflect the interaction between x_i and x_j. The actual design ran by the statistic software, Design-expert 7.1.3 (Stat-Ease Inc., USA), is presented in Table 1.

3. Results and discussion

3.1. Identification of bioflocculant-producing microorganism

A bioflocculant-producing bacterium with a flocculating rate exceeding 90%, named R3, was screened from the sludge collected from dewatering workshop where the activated sludge was mechanical dewatered. The colony of bacterial R3 is circular, incarnadine, and trim edge. It's gram-positive, obligate aerobic bacterium, non-endospore forming, and having no flagellum and immotile. Some of the physiological and biochemical characteristics of the bacterium were as follows: Glycolysis, methyl-red, nitrate reduction and catalase test were all positive, starch hydrolysis, oxidase and citrate test were all negative. The 16S rDNA gene sequences of strain R3 was registered in GenBank and the accession number is DQ166375, results showed that the similarity of the 16S rDNA sequences of the strain R3 and the *Rhodococcus erythropolis* CCTCC 10543 reached 99%. According to the 16S rDNA gene sequence and the physiological and biochemical characteristic, strain R3 could be identified as *Rhodococcus erythropolis*.

3.2. Effect of different sludge treatments on bioflocculant production

3.2.1. Bioflocculant production

According to section 2.6, after 60 h of cultivation, the fermented broths (10 mL) with the flocculating components were collected and were centrifuged at 3000 rpm for 60 min at 4 °C, the supernatant was collected and the bacterial cells were washed with distilled water and re-suspended in 10 mL phosphate solution (Subramanian et al., 2010). The flocculating rates of the broth, supernatant and cell suspension were assayed separately. While both the fermented broth and the supernatant had high flocculating rates (92.1% and 90.8%, respectively), the flocculating rate of the cell suspension was poor (5.3%). This information suggested that the bioflocculant produced by R3 was an extracellular product, and most of the flocculating activity was released into the culture medium. To determine the bioflocculant, the supernatant was precipitated with two volumes of absolute chilled acetone (containing 0.07% β-mercaptoethanol) by incubating the mixture at −20 °C for 4 h. The resulting precipitates were collected by centrifugation at 5000 rpm for 30 min and the crude bioflocculant were obtained (APHA, 2005). Concentrations of the bioflocculant harvested at

Table 1
Coded levels for five variables framed by the central composite design.

Factors	Codes	Codes levels		
		−1	0	1
Bioflocculant (mg L^{-1})	x_1	10	15	20
Modified zeolite (g L^{-1})	x_2	5.0	17.5	30
pH	x_3	5.0	8.5	12
$CaCl_2$ (mg L^{-1})	x_4	10	30	50
Contact time (min)	x_5	10	35	60

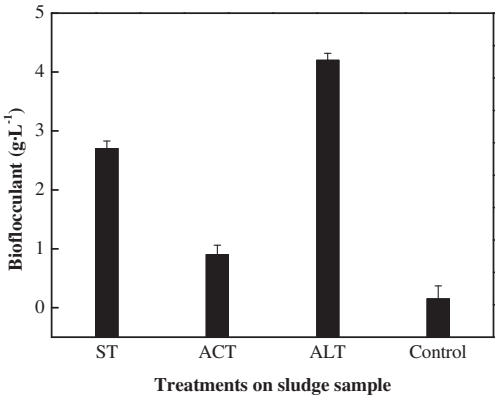

Fig. 1. Bioflocculants harvested from fermented broths of ALT, ST and ACT sludge.

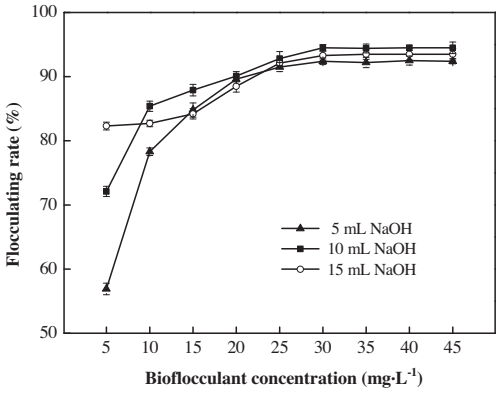

Fig. 2. Effect of sodium hydroxide (NaOH) dosage on flocculating rate.

60 h of cultivation from different samples of fermented broths were as shown in Fig. 1. High bioflocculant concentrations observed in the fermented broths were due to secretion by the strain R3 in three pre-treated sludge (4.2, 2.7 and 0.9 g L^{-1} of crude bioflocculant were harvested from fermented broths of ALT, ST and ACT sludge, respectively), while the control sample (without inoculating) had very low bioflocculant concentration of less than 0.15 g L^{-1} during 60 h of cultivation. Results clearly showed that the bioflocculant secreted by the strain R3 significantly varied with different sludge pre-treatments.

3.2.2. Effect of different sludge treatments

Although the sludge from the same sample was used for the bioflocculants production using the same bacterial strain, application of sludge produced by different treatments distinct bioflocculants yields. The variations in bioflocculants yields from fermented broths of ALT, ST and ACT sludge were attributed to the specific chemical/physical changes induced by different treatments in the treated sludge (More et al., 2012). In general, sludge treatments disintegrated the organic fractions and released soluble carbon into the sludge medium. Studies conducted by Verma et al. (2007) have confirmed the fact that the soluble carbon and nitrogen concentrations increased after sludge treatments (irrespective of treatment method), and this increase was more obvious in case of ALT sludge as compared to ST and ACT sludge. At alkaline conditions, sterilization of sludge increased soluble chemical oxygen demand and other low molecular weight soluble carbon compounds to an extent which was higher than acid treatments (Chen et al., 2007). Moreover, Capacity of HCl to induce cell lysis is lower than NaOH and hence, sludge solubilization significantly increased with alkaline treatment and to a lesser extent by acid treatment (Sun et al., 2012). Thus, the low concentration of bioflocculant from fermented broths of ACT sludge was observed due to the strong inhibition of microorganism growth and cell lysis at acidic condition. Overall, the carbon sources and nitrogenous organic materials available in the sludge medium changed with the type of treatment and therefore could change bioflocculants secretion pattern, and the specific carbon and nutrients content released in the ALT sludge medium was more favorable to bioflocculants secretion by R3 as compared to that of ST and ACT sludge.

Accordingly, NaOH solution was selected to disintegrate sludge to produce bioflocculant using R3. Results (Fig. 2) showed that more than 90% of flocculating rate for 4.0 g L^{-1} kaolin clay suspension could be reached when the flocculant concentration was adjusted to 20–40 mg L^{-1} at three NaOH dosages disintegrating. Fig. 2 also showed that 94.5% flocculating rate could be attained with 30 mg L^{-1} bioflocculant dosage when the preferable NaOH dosage was 10 mL for 100 mL sludge medium.

3.3. Time course assay of flocculating rate and cell quantity

The growth curve of R3 in cultivation medium (ALT sludge) was shown in Fig. 3. The cells were in logarithm growth phase during 6–54 h, with a rapid growth period occurring during 12–18 h. The cells entered stationary phase since 60 h, with a maximum biomass yield of 4.5 in OD_{600}. On 78 h and onward, the cells and flocculating rates were in death phase. Fig. 3 also showed that the cells produced bioflocculants along with their growth, and the crude bioflocculant yield was increased rapidly with cultivation time and peaked (4.2 g L^{-1}) at 60 h. Afterwards, the bioflocculant yield and flocculating rate were decreased, which may be due to cell autolysis and enzymatic activity decrease. Restated, the production of the bioflocculant was positively associated with cell growth.

3.4. Characteristics of the bioflocculant

3.4.1. Thermal stability

The thermal stability of the bioflocculant depends on its activity ingredients. It has been reported that the bioflocculants with protein or peptide backbone in the structure were generally sensitive to heat, while those made of sugars were thermostable (Salehizadeh and Shojaosadati, 2001), that is to say, if the main backbone of the bioflocculant is a polysaccharide, its flocculating activity varied a little with the changing temperature, for example, the flocculating activity of the bioflocculant SF-1 maintained above 90% after being heated at 80 °C for 30 min, and decreased by only 5% after being heated at 120 °C for 30 min (Gong et al., 2008). On the contrary, a protein bioflocculant has a poor thermal stability, for example, the flocculating activity of the bioflocculant DYU500

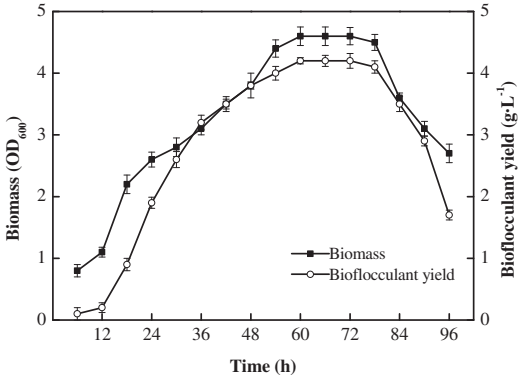

Fig. 3. Growth curve of strain R3.

Table 2
Thermal stability of the flocculation products of strain R3.

T (°C)	30	40	50	60	70	80	90	100	110	120	130
Flocculating rate (%)	96.2	92.2	91.8	90.6	62.5	37.7	25.2	20.9	15.7	9.4	3.6

decreased by about 50% after being heated at 80 °C for 30 min, and lost after being heated at 120 °C for 30 min (Wu and Ye, 2007). This can be explained by the denaturalization of proteins (destruction of the spatial structure of proteins, such as the S–S and hydrogen bonds) in the bioflocculant under hot conditions (Deng et al., 2005). In this study, as seen from Table 2, while the flocculating activity of the broth produced by bacterial R3 using ALT sludge medium can be maintained at 90.6% or more after being heated at a relatively low temperature (<60 °C) for 30 min, it was decreased by about 50% after being heated at 80 °C for 30 min and by about 80% after being heated at 120 °C for 30 min. The poor heat stability indicated that the main backbone of this bioflocculant was a protein rather than a polysaccharide.

3.4.2. Enzymatic stability

In order to further determine the activity ingredients of the bioflocculant, the effects of glucoamylase (glycogen phosphorylase), glycosidase and protease on the flocculating activity of the crude bioflocculant was discussed. In general, the molecular structure of the bioflocculant could be destroyed by the glucoamylase further reduced or loss its flocculating activity if the main backbone is a polysaccharide, the sugar chain structure of the bioflocculant with glycoprotein backbone could be destroyed by the glycosidase further reduced or loss its flocculating activity, the bioflocculants with protein or peptide backbone in the structure could be destroyed by the protease further reduced or loss its flocculating activity.

It is clearly showed that the flocculating activity of the bioflocculant retains its 99.6%, 99.8%, and 99.4% after digested by amylase, cellulase and glucoamylase, respectively, while these enzymes themselves have no flocculating activities, indicated that the main backbone of the main backbone of the bioflocculant is not a polysaccharide. The bioflocculant was further digested by glycosidase, results showed that the flocculating activity did not decrease or disappear, while the glycosidase itself has no flocculating activity, indicated that the activity ingredients of the bioflocculant contains no glycoprotein matters.

After catalytic hydrolysis by pepsin and trypsin, the flocculating activity of the bioflocculant decreased to 23.6% at 60 min and to 11.2% at 100 min, which further reduced to 0.2% by further extracting protein by acetocaustin, while the pepsin, trypsin and acetocaustin themselves have no flocculating activities. This information indicated that the activity ingredient of the bioflocculant was a protein rather than a polysaccharide or a glycoprotein. Thus, the bacterial strain R3 was able to generate a protein bioflocculant which lost the flocculating capability by enzymatic digestion.

3.4.3. Activity ingredients analysis of the bioflocculant

Chemical analysis of the crude bioflocculant revealed that the total protein and sugar content of bioflocculant were 84.6% and 15.2% (W/W), thus, the crude bioflocculant should be purified before determining its characteristics and chemical groups. Many studies utilized ethanol as extract ants to purify polysaccharide flocculants (Gao et al., 2009; Liu et al., 2010). In this study, the main backbone of the bioflocculant was a protein due to the examination of its thermal and enzymatic stability. Thus, the acetone-trichloroacetic acid solution, prepared by mixing acetone and trichloroacetic acid with a volume ratio of 9:1, was selected to purify the bioflocculant. The crude bioflocculant was completely dissolved in distilled water, and was poured into cold acetone-trichloroacetic acid solution (4 °C) containing 0.2% DL-Dithiothreitol (DDT) with a volume ratio of 1:2 at −20 °C for 24 h to precipitate the bioflocculant, the resulting precipitate collected by centrifuged at 5000 rpm for 30 min and washed by redissolving in distilled water. After three times repeat such trial, the precipitate was dried at room temperature in laminar hood for 6 h and its dry weight was measured and denoted as purified bioflocculant (APHA, 2005). Chemical analysis revealed that the total protein of the purified bioflocculant was 99.7% (W/W), including glutamic acid, alanine acid, aspartic acid with a mass proportion series of 13.3%, 18.5%, and 10.3% (W/W), respectively. There was almost no polysaccharide contained in the purified bioflocculant. Gel permeation chromatography indicated that the approximate molecular weight of the purified bioflocculant was 3.99×10^5 Da. The elemental analysis of the bioflocculant revealed that the mass proportions of C, H, O, N and S were 35.2%, 12.6%, 41.8%, 8.8%, and 1.6% (W/W).

3.4.4. Fourier Infrared Spectroscopy of the purified bioflocculant

FTIR spectrum analysis was utilized to detect functional groups existed in the bioflocculant molecules, in order to further characterize the above purified bioflocculant. The infrared spectrum of the bioflocculant extracted from activated sludge by NaOH disintegrating displayed a broad stretching peak in the range from 3400 to 3500 cm^{-1} which can be assigned to −OH and NH groups (Liu et al., 2010), and the peak at around 3430 cm^{-1} is an indication of −OH stretching from hydroxyl group (Yim et al., 2007). The peak around 1640 cm^{-1} is an indication of $-COO^-$ asymmetric stretching vibration (Lian et al., 2008). The peaks around 1620 cm^{-1} and 1082 cm^1 were characteristic of C–O groups, indicating the presence of carboxyl groups in the bioflocculant (Zheng et al., 2008). The peak at 1544 cm^{-1} is probably an indication of $-COO^-$ symmetric stretching vibration (Ahmad et al., 2013). In summary, the infrared spectrum of the purified bioflocculant shows the presence of hydroxyl, carbonyl and carboxyl groups, which are all preferable functional groups for the flocculation process in polyelectrolyte.

The bioflocculant participates in the flocculation mainly through available hydroxyl, carbonyl and carboxyl groups which induces very high binding capacity. On the one hand, the negative charge groups could react with the positively charged site of suspended particles in the wastewater, in this case, the particles can approach sufficiently close to each other so that attractive forces become effective. On the other hand, coagulant aid, like Ca^{2+}, draw closer to the negatively charged particles through columbic attraction and therefore Ca^{2+}-particles complexes were formed. Ca^{2+} reduced the thickness of the diffuse double layer of adjacent particles and hence, reducing the inter particle distance between particles. Chemical groups in the bioflocculants act like a bridging agent of two or more Ca^{2+}-particles complexes and reduce inter-particle distances through the ionic bonds mechanism, particles adsorbed onto one bioflocculant molecular chain, and they could be adsorbed simultaneously by other chains, leading to the formation of three-dimensional flocs, which were capable of rapid settling.

3.5. Characteristics and adsorption property of the modified zeolite

3.5.1. Characteristics of modified zeolite

Being analyzed by X-ray diffraction, the modified zeolite had the following chemical composition (in%): SiO_2 = 55.3, Al_2O_3 = 12.3, Na_2O = 2.2, CaO = 5.7, K_2O = 0.3, MgO = 9.1. The particle size of the modified zeolite was between 30 and 50 μm, and the diameter of the 50% of zeolite particle (D_{50}) was about 44 μm. The specific external area of the natural zeolite is 425.3–478.7 $m^2\ g^{-1}$, much larger than that of natural zeolite (157.2–191.5 $m^2\ g^{-1}$).

According to literature (Wahab et al., 2010), the formation of complexes between ammonium ions in wastewater solution and functional groups of the zeolite played an important role in ammonium adsorption. The infrared (IR) spectrum of the modified zeolite displayed a number of absorption peaks, indicating the complex nature of the specific functional groups. The peaks located in the range from 3400 to 3500 cm^{-1} indicate the presence of –OH group (Farinella et al., 2007). The peak at 1620 cm^{-1} is characteristic of a C–O group, while the $-COO^-$ stretching absorption bond is observed at 1500 cm^{-1} (Wahab et al., 2010). The peaks observed between 1375 and 1300 cm^{-1}, which reflect stretching vibrations of symmetrical or asymmetrical ionic carboxylic groups (Farinella et al., 2007). Ammonium ions could be adsorbed onto modified zeolite by complexation with functional groups such as carboxyl, hydroxyl, and carboxylic groups on both external surfaces and internal pores of modified zeolite.

3.5.2. Adsorption property of modified zeolite

The ammonium adsorption property of the zeolite modified by calcining with MgO (4:1, W/W) increased with an increasing in calcinations temperature, and the highest adsorption capacity of 24.7 mg g^{-1} was achieved at an optimum calcinations temperature of 400 °C, an increase by 96.1% compared to the zeolite not calcinated. However, the amount of ammonium adsorbed by modified zeolite (adsorption property) decreased when the calcinations temperature was above 400 °C, this may be ascribed to the destruction of the internal structure of the zeolite by calcining at high temperatures. According to the literatures (Wang et al., 2007a,b; Watanabe et al., 2005; Zhao et al., 2004), the ammonium ion uptake values of NaOH-treated, HCl-treated, and hydrothermally-treated zeolites were 19.29, 21.23 and 12.17 mg g^{-1}, lower than that of zeolite modified by calcining with MgO at 400 °C. Thus, the modified zeolite produced in this work possessed engineering application values.

To determine the functional groups involved in adsorption of ammonium by the modified zeolite, a comparison between the FTIR spectra before and after adsorption was done. The FTIR spectra confirmed changes in functional groups and surface properties of the modified zeolite, illustrated by the shift of some functional groups bands due to ammonium adsorption (Table 3). These shifts may be attributed to the changes in ammonium ions associated with carboxyl and hydroxyl groups, suggesting that these groups are predominant contributors in the complexation of ammonium ions. The changes in peaks observed on the spectrum between 3200 and 3550 cm^{-1}, may be attributed to complexation between ammonium ions and –OH groups. The changes in peaks observed between 1740 and 1710 cm^{-1} are indicative of stretching vibration of C–O bonds due to non-ionic carboxyl groups (–COOH, $-COOCH_3$) (Farinella et al., 2007). The changes in peaks observed between 1660 and 1500 cm^{-1}, caused by stretching vibration of the asymmetric and symmetric $-COO^-$ (Wahab et al., 2010). The changes in peaks observed between 1375 and 1300 cm^{-1}, which reflect stretching vibrations of symmetrical or asymmetrical ionic carboxylic groups (Farinella et al., 2007).

Table 3
FTIR spectral characteristics of the modified zeolite before and after ammonium adsorption.

σ (cm^{-1})	Wavelength range (cm^{-1})	Modified zeolites			Assignment
		Before adsorption	After adsorption	Differences	
3500–3400	3550–3200	3430	3223	207	–OH groups
1740–1600	1740–1720	1620	1740	–120	C–O bonds
	1720–1660	1620	1710	–90	C–O bonds
1660–1500	1600–1500	1500	1544	–44	Symmetric $-COO^-$
	1660–1500	1500	1640	–140	Asymmetric $-COO^-$
1375–1300	1350–1300	1345	1310	35	Ionic carboxylic groups

3.6. Determination of the dose range

Fig. 4a depicted the COD and ammonium removal rates at different dosage of the bioflocculant varying from 10 to 40 mg L^{-1}. And the adsorption using modified zeolite whose dosage was adjusted in the range of 5–60 g L^{-1} was presented in Fig. 4b.

In the bioflocculant flocculation process, it was observed that the removal efficiencies increased rapidly when the bioflocculant dosages were adjusted in the range of 5–20 mg L^{-1}, and the maximum COD removal efficiency (47.2%) and turbidity (72.9%) were achieved at the optimum bioflocculant dosage of 20 mg L^{-1}. While a rapid increase was observed when the bioflocculant dosage ranging from 5 to 20 mg L^{-1}, the increasing bioflocculant dosage above 20 mg L^{-1} had negligible effects on the increasing in removal efficiencies (Fig. 4a), this may be attributed to the formation of aggregates at higher solid/liquid ratios or to precipitation of particles. Thus, the maximum dosage of the bioflocculant in the RSM experiment was selected as 20 mg L^{-1}. Moreover, when the activated sludge itself (incubated in the same procedure) was used directly for swine wastewater treatment, the removal efficiencies of COD, ammonium, and turbidity were only 22–26%, 11–14%, and 35–42%, respectively. Fig. 4b showed the effects of modified zeolite dosage on ammonium adsorption. It can be observed that the amount of ammonium adsorbed onto modified zeolite increased with an increasing in the zeolite dosage, it rose from 50.0 mg to 495 mg with the increasing of zeolite dosage ranging from 5 to 30 g L^{-1}, and the removal efficiency of ammonium reached 48.6% when the zeolite dosage was 30 g L^{-1}. Increasing zeolite dosage above 30 g L^{-1} had negligible effects on the increasing in removal

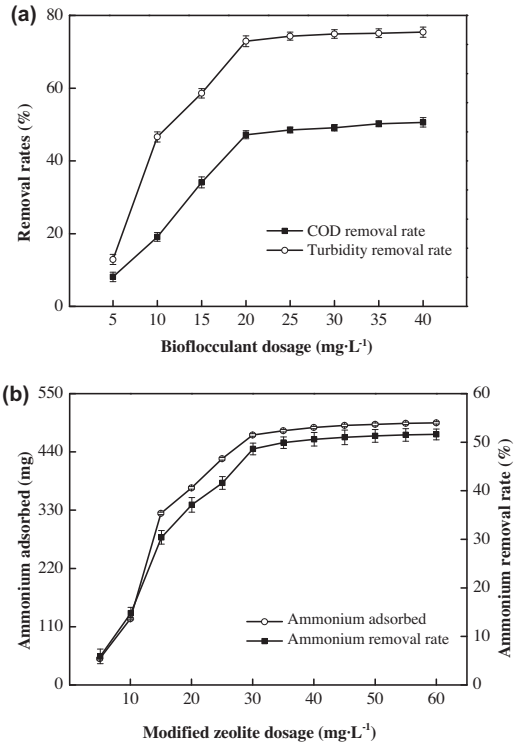

Fig. 4. Removal rates at different dosage of (a) bioflocculant and (b) modified zeolite.

efficiency of ammonium ions. Thus, the maximum dosage of the modified zeolite in the RSM experiment was selected as 30 g L^{-1}. Under this condition, the COD and turbidity removal efficiencies were poor, which were 12–16% and 24–33%, respectively.

3.7. Experimental results of RSM

In previous study, although the modified zeolite performed a good ammonium adsorption property, it demonstrated a poor ability for the COD and turbidity removal. On the contrary, the bioflocculant processed an industrial potential for COD and turbidity removal from wastewater. Thus, based on the removal of organic pollutants by bioflocculant and adsorption of high-level of ammonium by modified zeolite, this paper presents a study of the treatment of swine wastewater using the composite of bioflocculant and modified zeolite.

3.7.1. Data analysis of COD removal rate as the response variable

Following equation represents empirical relationship in the form of quadratic polynomial between the COD removal rate (y_1) and the other five factors (x_1–x_5).

$$\begin{aligned} y_1 = {} & 54.75 - 3.37x_1 - 1.96x_2 - 9.44x_3 - 0.64x_4 + 10.62x_5 \\ & + 6.75x_1x_2 + 0.37x_1x_3 + 0.25x_1x_4 + 0.05x_1x_5 \\ & - 12.75x_2x_3 + 4.57x_2x_4 + 3.40x_2x_5 - 16.92x_3x_4 \\ & + 1.05x_3x_5 + 0.35x_4x_5 + 2.96x_1^2 + 0.36x_2^2 - 2.62x_3^2 \\ & + 9.38x_4^2 + 5.68x_5^2 \end{aligned} \quad (3)$$

Statistical testing of this model was performed with the Fisher's statistical method for analysis of variance (ANOVA) (Mohana et al., 2008). The results of ANOVA for COD removal rate indicated that the second-order equation fitted well, because model value of $F_{statistic}$ (the ratio of mean square due to regression to mean square to real error) of 4.23 was greater than $F_{0.01}(20, 29)$ of 2.57, values of 'Prob > F' = 0.0486 less than 0.05, and the total correlation coefficient R reached 0.8843. Moreover, the value of the determination coefficient (R^2 = 0.7819) indicates that only 21.71% of the total variation could not be explained by the empirical model.

The significance testing for the coefficient of the equation whose variables are in terms of coded factors was listed in Table 4. As seen from Table 4, in the linear terms, pH was significant and unique, and it played a decisive role in the flocculating process. In acidic environment, the hydrogen ions concentration raised with the decrease in pH, which sharing functional groups of the bioflocculant such as −OH, −COOH groups, in this case, COD removal rate declined. While in the strong alkaline environment (pH > 10), negatively charge density raised with the increase of pH, and hence increased electrostatic repulsion of the negatively charged particles. As repulsion prevented particles from approaching the activity chain of the bioflocculant with the increased pH, there eventually results a depression of COD removal rate.

Among the higher order effects, the quadratic terms of bioflocculant dosage was significant. For the bioflocculant attributing to the absorption bridging action, its flocculating mechanism, could promote the flocculation by influence the size and density of flocs. Particles adsorbed to a bioflocculant molecular chain, and they could be adsorbed simultaneously to other chains, leading to the formation of three-dimensional flocs, which were capable of rapid settling. Besides this, the flocculation might be attributed to a decrease of electrostatic repulsion force between bioflocculant chains and particles by decreasing the negative charge on both the bioflocculant and particles surface by neutralizing in the presence of Ca^{2+}, eventually the negative charge on suspended particles might be reversed from negative to positive. Thus, the negative charge groups could react with the positively charged site of suspended particles, in this case, the particles can approach sufficiently close to each other so that attractive forces become effective.

The interaction terms with significant effect are shown in Fig. 5. Fig. 5a showed that, when the contact time, dosage of $CaCl_2$ and modified zeolite were kept at the central level, COD removal rate can get to the anticipant value at a small quantity of bioflocculant when pH is higher than 8.0 approximately, indicating that the activity of bioflocculant depends upon an alkaline environment. Fig. 5b also indicated the importance of a higher pH for flocculating process when the other three factors were kept at the central level.

3.7.2. Data analysis of ammonium removal rate as the response variable

Eq. (4) represents empirical relationship in the form of quadratic polynomial between the ammonium removal rate (y_2) and the other five factors (x_1–x_5).

$$\begin{aligned} y_2 = {} & 62.83 - 3.59x_1 + 1.26x_2 - 0.02x_3 + 0.37x_4 + 3.95x_5 \\ & + 0.70x_1x_2 + 11.57x_1x_3 + 2.50x_1x_4 - 12.63x_1x_5 \\ & - 2.40x_2x_3 + 6.25x_2x_4 + 4.02x_2x_5 - 3.95x_3x_4 - 7.15x_3x_5 \\ & + 1.77x_4x_5 - 8.88x_1^2 + 1.43x_2^2 - 0.76x_3^2 + 6.19x_4^2 \\ & - 4.11x_5^2 \end{aligned} \quad (4)$$

Table 4
Significance of quadratic model coefficient of COD removal rate.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > F
x_3	13.2100	1	3.38	0.0099
x_1x_3	−1.9340	1	6.76	0.0192
x_2x_3	−0.4857	1	6.76	0.0411
x_1^2	0.1501	1	4.58	0.0411

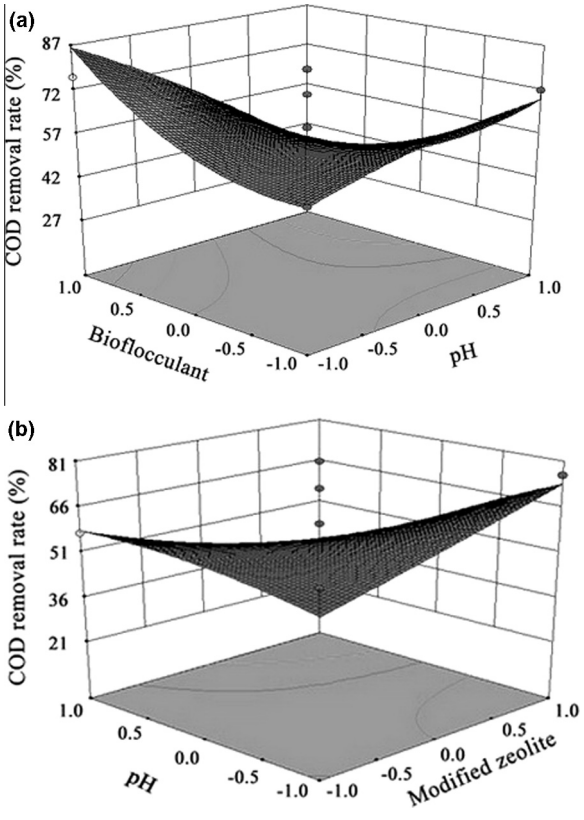

Fig. 5. Surface graphs of COD removal rate showing the effect of variables (a) Bioflocculant–pH and (b) Modified zeolite–pH.

Results of ANOVA for ammonium removal rate indicated that the second-order equation fitted well, because model value of $F_{statistic}$ of 4.23 was greater than $F_{0.01}$ (20, 29) of 2.57, values of 'Prob > F' = 0.0364 less than 0.05, and the total correlation coefficient R reached 0.9134. Moreover, the value of the determination coefficient (R^2 = 0.8343) indicated that only 16.57% of the total variation could not be explained by the empirical model.

The significance testing for the coefficient of Eq. (4) whose variables are in terms of coded factors was listed in Table 5. In the linear terms, pH and dosage of the modified zeolite were significant. Dosage of the modified zeolite was significant and unique for the reason that ammonium ions could be adsorbed onto the modified zeolite by pore adsorption, ion exchange, and complexation with some functional groups such as carboxyl and hydroxyl groups on both external surfaces and internal pores of modified zeolite. Among the higher order effects, the quadratic terms of bioflocculant dosage was significant.

The interaction terms with significant effects are shown in Fig. 6. Fig. 6a provides more evidence that the bioflocculant was relatively effective at weak alkaline conditions. Fig. 6b showed that ammonium removal rate is enhanced varying with the dosage of modified zeolite and bioflocculant, when the pH, contact time and dosage of $CaCl_2$ were kept at the central level. And it is predicted that, at a low level of modified zeolite, ammonium removal rate is enhanced as the bioflocculant and it can get to the peak ultimately. However this target obviously becomes more difficult when the concentration of modified zeolite was kept at a high level (Khosravi et al., 2012).

Table 5
Significance of quadratic model coefficient of ammonium removal rate.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > F
x_2	1.6532	1	2.35	0.0131
x_3	1.9998	1	2.35	0.0037
x_1x_2	−0.2020	1	4.71	0.0128
x_1x_3	1.3223	1	4.71	0.0212
x_1^2	−1.4213	1	3.19	0.0100

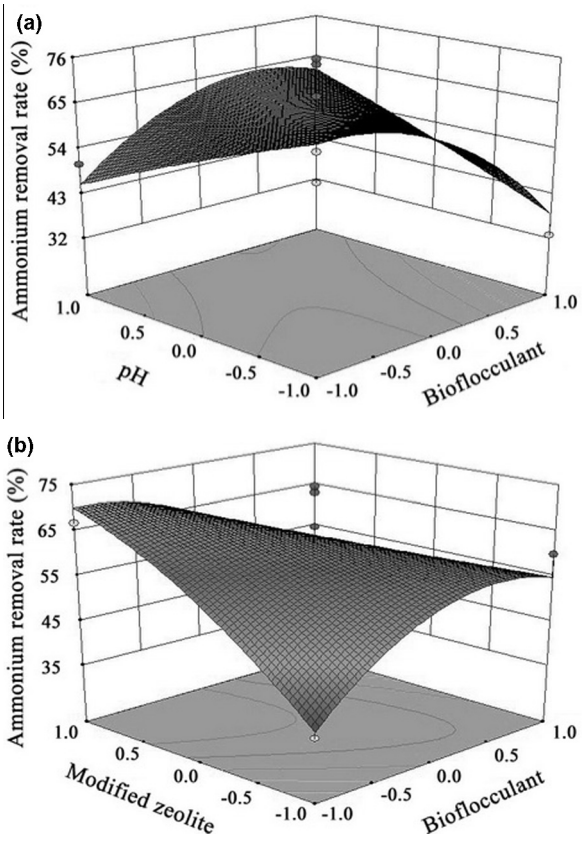

Fig. 6. Surface graphs of ammonium removal rate showing the effect of variables (a) Bioflocculant–pH and (b) Modified zeolite–Bioflocculant.

3.7.3. Optimal flocculent condition

According to the target values of the two responses, COD removal rate of 100% and ammonium removal rate of 100%, the optimal condition calculated from the regression equations were bioflocculant of 24 mg L^{-1}, modified zeolite of 12 g L^{-1}, $CaCl_2$ of 16 mg L^{-1}, pH of 8.3 and contact time of 55 min. Under this optimal condition, COD, ammonium and turbidity removal rates appeared as 87.9%, 86.9%, and 94.8%, respectively. The use of the composite by RSM was not only improves the pollutant removal and recycles the ammonium nitrogen, but also provides a feasible way to receive high removal efficiencies of pollutants from wastewater with high-levels of organic pollutes and ammonium.

4. Conclusions

A bioflocculant was produced by R3 using activated sludge pretreated by NaOH, and a modified zeolite was prepared by calcining with MgO for ammonium adsorption. RSM was employed to optimize the treatment process of swine wastewater using the composite of this bioflocculant and modified zeolite, and the optimal flocculating conditions for swine wastewater treatment were bioflocculant of 24 mg L^{-1}, modified zeolite of 12 g L^{-1}, $CaCl_2$ of 16 mg L^{-1}, pH of 8.3 and contact time of 55 min, and the corresponding removal rates of COD, ammonium and turbidity were 87.9%, 86.9%, and 94.8%, respectively.

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