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Bioflocculants' production from a cellulase-free xylanase-producing *Pseudomonas boreopolis* G22 by degrading biomass and its application in cost-effective harvest of microalgae

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ABSTRACT

The major problem for industrial application of bioflocculants is its high production cost. Here, a novel bacterium *Pseudomonas boreopolis* G22, which can secret a cellulase-free xylanase and simultaneously produce bioflocculants (MBF-G22) through directly converting untreated biomass, was isolated. The bioflocculants' production of G22 was closely related to its xylanase activity, hydrolysis ability of biomass and the hemicellulose loss caused by G22. The optimal fermentation conditions with the highest bioflocculants' yield (3.75 mg g^{-1} dry biomass) were obtained at the fermentation time of 96 h, incubation temperature of 30 °C, inoculum concentration of 1.0% and biomass concentration of 1.0% in an initial pH value of 7.0. MBF-G22 mainly consisted of polysaccharides (63.3%) with a molecular weight of 3.982×10^6 Da and showed the highest flocculating efficiency of 97.1% at a dosage of 3.5 mg L^{-1}. In addition, MBF-G22 showed high flocculating efficiency of microalgae (95.7%) at a dosage of 80 mg L^{-1}.

1. Introduction

Flocculants are widely used to settle down suspended solids in various solutions by neutralizing surface charges on solids and binding particles together by physical or chemical forces (Chai et al., 2014; Haver and Nayar, 2017). In general, flocculants are divided into chemical flocculants (organic inorganic flocculants) and bioflocculants according to their raw material differences. Chemical flocculants have been successfully applied in various industrial fields such as dye wastewater removal, municipal sewage treatment, drinking water purification, and downstream treatment in fermentation and food industry, due to their low production costs and high flocculating efficiency (Gupta and Ako, 2005; Yue et al., 2008). However, most of these chemical flocculants, such as aluminum sulfate and polyacrylamide derivatives, are toxic to humans and the environment, and are difficult to degrade, which severely hampers their application in industrial processes (Buczek et al., 2017; Ummalyma et al., 2016). To overcome these shortcomings, an increasing number of researchers have focused on bioflocculants because they are easily degraded without producing secondary pollution, and harmless to humans, animals and our environment (Deng et al., 2003).

Bioflocculants produced by microorganisms have been widely reported and applied to wastewater treatment, heavy metal removal and sludge dewatering (Guo et al., 2015; Kurane and Nohata, 2006). Recently, bioflocculants were also reported to successfully harvest various microalgae (Liu et al., 2017; Ndikubwimana et al., 2016). However, the high production cost of bioflocculants still hinders their commercial application. It has been proposed that production costs can be reduced by either screening high production strains or seeking low-cost substrates as culture medium (Chen et al., 2016; Liu et al., 2017). A number of novel bioflocculant-producing bacteria have been isolated from various wastewaters, activated sludges and soil samples, and have been used to produce high yields of bioflocculants by optimizing their fermentation conditions (Liu et al., 2015b; Peng et al., 2014). However, for commercial-scale cultivation of bacteria, large amounts of water and nutrients are required. To solve this puzzle, various wastewaters like

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phenol-containing wastewater (Chen et al., 2016), livestock wastewater (Peng et al., 2014), palm oil mill effluent (Chaisorn et al., 2016) etc., have been successfully used for bacterial growth and bioflocculants' production. Although most of these wastewaters contain enough nutrition for bacterial growth, their nutrients are imbalanced (i.e., extremely high/low nitrogen and phosphorus concentration), which is likely to restrict the growth of bacteria. In addition, bioflocculants' production using wastewater is still fairly limited in industry because these wastewaters usually require preliminary removal of the impurities, toxins and microorganisms before being used for bacterial growth, which may increase the production cost (Adebami and Adebayo-Tayo, 2013; Ramsden et al., 2010).

Lignocellulosic biomasses like wood biomass, some agricultural wastes and wild grasses are thought to be the most abundantly sustainable raw materials on Earth which can be used as carbon source for producing biofuels and chemicals (Salas et al., 2010). Recently, it has been reported that some bacteria can produce bioflocculants through directly utilizing the raw material of biomass. A lignocellulose-degrading strain *Cellulosimicrobium cellulans* L804 isolated from corn farmland soil has the ability to produce bioflocculants through degrading lignocellulosic biomass directly (Liu et al., 2015b). Liu et al. (2017) reported that an alkaliphilic strain *Bacillus agaradhaerens* C9 produced up to 12.94 g L^{-1} bioflocculants when 20 g L^{-1} of untreated rice bran was used as carbon source in optimal fermentation conditions by secreting xylanase and cellulase. In addition, biomass is nontoxic and contains other abundant nutrients, such as nitrogen and various essential metals, which may be beneficial for bioflocculants' production by biomass-degrading bacteria in theory. In this study, we firstly reported a novel cellulase-free xylanase-producing bacterium G22 with the potential ability to directly convert various biomasses to bioflocculants. The biodegradability of G22 was determined and bioflocculants' production of G22 was evaluated by optimizing its fermentation conditions. Subsequently, the flocculating properties and applications in microalgal harvesting of these bioflocculants were also measured in this study.

2. Materials and methods

2.1. Materials

The microalgal strain *Scenedesmus abundans* (*S. abundans*) was donated by Dr. Lu of Algaen Corporation (Winston Salem, USA). The algal cells were grown in aerated BG-11 medium in an environmentally controlled growth room with a constant temperature at 25 °C (± 2 °C) and a photoperiod of 16 h:8 h light:dark cycle. Light intensity was provided by two cool fluorescence tube-bulbs on the top of the reactors (5 cm distance) with average light intensity of 200 μmol photons $m^{-2} s^{-1}$. After 2 weeks of cultivation, the algae culture was used for the harvest experiment. Carboxymethyl cellulose (CMC), beechwood xylan, 3,5-dinitrosalicylic acid (DNS) and kaolin clay were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.2. Isolation and identification of bioflocculant-producing bacteria

A bacterium that simultaneously degrades biomass and produces bioflocculants, named G22, was isolated from paper mill sludge (Thunder Bay, Ontario, Canada). Briefly, about 1.0 g samples were suspended in 10 ml of sterile distilled water and boiled for 5 min; the mixture was shaken at 200 rpm and 37 °C for 30 min. Then 1 ml of above culture was inoculated in mineral salt medium (0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.05% $MgSO_4$, 0.05% yeast and 0.3% peptone) containing 0.5% wood dust biomass at 37 °C with agitation at 200 rpm for 48 h. The bioflocculant-producing strains settled at the bottom of the flask together with biomass due to their attachment to the biomass surface. Then the supernatant was removed carefully and the solid residues were resuspended in 0.9% sodium chloride solution by vortex for 15 s. The isolate was spread via the standard serial dilution plate method using the mineral salt medium containing 0.5% wood dust biomass and 1.5% agar. After 2 d of incubation at 37 °C, all isolates were purified and cultured in 50 ml of the mineral salt medium containing 0.5% wood dust biomass. The samples were collected to determine their CMCase and xylanase activities and flocculating efficiencies each day. Finally, a cellulase-free xylanase-producing bacterium showed the highest flocculating activity was selected for further study. To identify the species of this strain, its genome was extracted with a Bacteria DNA kit (Bio Basic, Markham, Ontario, Canada). The internal transcribed spacer (ITS) region was amplified using the universal primers HAD-1 (5′-GACTCCTACGGGAGGCAG CAT-3′) and E1115R (5′-AGGGTTGCGCTCGTTGCGGG-3′) and purified for sequencing according to our previous study (Guo et al., 2017c).

2.3. Determination of flocculating efficiency

The flocculating efficiency was measured by using kaolin clay as substrate according to the method of Kurane et al. (2008) with some minor modifications. Briefly, 200 μl of diluted sample and 1.0 ml of 10% (w/v) $CaCl_2$ were added into 40 ml of 0.5% (w/v) kaolin clay suspension in a 50 ml glass-beaker, and the mixture was left to stand for 1 min after shaking at 100 rpm for 2 min at room temperature. The absorbance of the upper phase was measured at 550 nm using a microplate spectrophotometer (Epoch, Bio Tek Instruments, Inc., Vermont, USA). The flocculating efficiency was calculated as follows: Flocculanting efficiency (%) = (A − B)/A × 100 × D, where A and B are the optical densities of the control and the samples at 550 nm, respectively, and D is the dilution time of the supernatant of the fermentation broth.

2.4. Evaluation of biomass hydrolysis ability and enzyme activity assay

The biomass hydrolysis ability of this strain was determined by using various lignocellulosic biomasses as carbon source according to the description of Guo et al. (2017a). Briefly, 5 μl of overnight-grown culture were dropped on the center of agar plates containing above mineral salt medium, 1.5% agar and 0.5% agave, corn stover, *Miscanthus*, pine powder, wheat bran, wood dust, CMC or xylan. After cultivation at 37 °C for 48 h, the plates were stained with Gram's iodine solution for 3–5 min. Then the hydrolysis abilities of different biomasses were calculated by measuring the diameters of the halo region and bacterial colony according to our previous published paper (Guo et al., 2017a).

The CMCase and xylanase activities were obtained by measuring the contents of reducing sugars released from CMC and xylan, respectively, according to the description of Guo et al. (2017c).

2.5. Determination of hemicellulose and cellulose

The contents of hemicellulose and cellulose were determined according to our previous descriptions (Guo et al., 2017c). Briefly, 0.1 g various dry biomasses were washed by 1.5 ml of hot water, absolute ethanol and acetone, respectively, at 65 °C for 30 min each. Then the residue was air-dried in a chemical hood for 2 days and hydrolyzed by 0.5 ml of 72% sulfuric acid at 30 °C. After 1 h, the hydrolysate was diluted to 3% sulfuric acid by the addition of deionized water and autoclaved at 121 °C for 1 h. Finally, the supernatant was collected by centrifugation at 12,000*g* for 3 min and used to measure total pentose and hexose contents. The contents of hemicellulose and cellulose were calculated according to the contents of pentose and hexose, respectively.

The total hexose and pentose contents were determined according to the method of Xu et al. (2012), with some minor modifications. For determination of total hexoses, 80 μl of diluted sample was added to

160 µl of 0.2% (w/v) anthrone in concentrated H_2SO_4, mixed well and incubated in boiling water for 5 min. After cooling down, 200 µl of the mixture was removed to the 96-well microtiter plate and the absorbance was determined at 620 nm using a microplate spectrophotometer (Epoch, Bio Tek Instruments, Inc., Vermont, USA). For determination of total pentoses, 75 µl of diluted sample and 10 µl of 6% orcinol (0.6 g orcinol dissolved in 10 ml ethanol) were mixed with 150 µl of 0.1% (w/v) ferric chloride in concentrated HCl, mixed well and incubated in the boiling water for 20 min. After cooling down, 200 µl of the mixture was transferred to the 96-well microtiter plate and the absorbance was determined at 660 nm using a microplate spectrophotometer (Epoch, Bio Tek Instruments, Inc., Vermont, USA). A range of D-glucose (0–100 µg ml^{-1}) and D-xylose (0–40 µg ml^{-1}) concentrations were used to plot the standard curves for hexoses and pentoses, respectively.

2.6. Optimization of bioflocculants' production

To screen the optimal biomass for bioflocculants' production using G22 strain, the strain was cultivated in the mineral salt medium containing 1.0% (w/v) untreated agave, corn stover, *Miscanthus*, pine powder, wheat bran and wood dust at 37 °C with agitation at 200 rpm, and the flocculating efficiency and xylanase activity were monitored every day for 6 days. The substrates of xylan and CMC were used as positive and negative controls, respectively. To investigate bioflocculants' production under different pH and temperature conditions, flocculating efficiency was determined after culturing the strain at different initial pH values (5–10) and various temperatures (25–40 °C). The effects of the initial inoculum concentration on bioflocculants' production were evaluated by inoculating the strain at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/v) concentrations. To determine the effects of biomass concentration on bioflocculants' production, six biomass concentrations (0.25, 0.5, 1.0, 2.0, 3.0 and 4.0%, w/v) were used for bioflocculants' production and the 1.0% xylan (w/v) was selected as the control.

2.7. Extraction and characteristics of the bioflocculants

After 4 d of fermentation in the optimal conditions, the culture of G22 was centrifuged at 5000*g* for 30 min at 4 °C to settle the solids. The supernatant was mixed with two volumes of pre-cooled absolute ethanol to precipitate the bioflocculants. The resultant precipitate was collected by centrifugation at 5000*g* for 10 min, washed three times with 75% ethanol and finally lyophilized to obtain extracted MBF-G22. The dry weight was recorded to calculate the bioflocculants' yield.

To analyze the characteristics of MBF-G22, the total sugars were determined using the anthrone-sulfuric acid method described by Leyva et al. (2008); the total protein was obtained by using the Bradford Protein Assay Kit (Bio Basic Canada Inc., Markhan, ON, CA) according to our previous description (Guo et al., 2017b); the uronic acid content was measured by cabazoic-sulfuric acid method developed by Barker and Young (1966); while the nucleic acid content was directly quantified by the spectrophotometer. The molecular weight, charge density and Zeta potential of MBF-G22 were measured according to the method of Wang et al. (2016). The viscosity of MBF-G22 was recorded using an OB-C218 Ubbelohde Viscometer (Cannon instrument company, USA) at room temperature according to the manufacturer's instructions. Fourier transform infrared spectroscopy (FTIR) analysis was analyzed by a Bruker Tensor 37 FTIR Spectrophotometer (Bruker Optics, Inc., Billerica, MA) according to the description of Guo et al. (2017c).

2.8. Effects of MBF-G22 dosage, pH and temperature on flocculating efficiency

To evaluate the flocculating properties of MBF-G22, the effects of MBF-G22 dosage, pH and temperature on the flocculating efficiency of kaolin clay suspension were determined. The MBF-G22 dosage was ranged from 0.5 to 5.5 mg L^{-1}; the optimal pH value for MBF-G22 was determined in the region of 2–12; while the optimal temperature for MBF-G22 was assayed over the temperature range of 4–80 °C.

2.9. Evaluation of MBF-G22 on the harvest of microalgae

After two weeks of culture, the biomass yield of the green microalgal strain *S. abundans* was about 2.5 g L^{-1}. The flocculating efficiency of *S. abundans* was evaluated by using different dosage of MBF-G22. Briefly, 10–120 mg L^{-1} of MBF-G22 was mixed with 40 ml of algal culture containing 1 ml of 10% $CaCl_2$ solution. The mixture was stirred at 100 rpm for 2 min, and settled for 10 min at room temperature. Then the absorbance of the upper phase was measured at 680 nm using a microplate spectrophotometer (Epoch, Bio Tek Instruments, Inc., Vermont, USA). The flocculating efficiency of the microalgae was calculated as the follow: Flocculating efficiency (%) = (A − B)/A × 100, where A and B are the optical densities of the control and the samples at 680 nm, respectively.

2.10. Statistical analysis

Correlation coefficients were calculated by performing spearman rank correlation analysis. All the experiments were performed in quadruplicates, and the results were shown as mean ± SD. Statistical analysis was carried out by one-way analysis of variance using SPSS (SPSS Inc., USA, version 13.0).

3. Results and discussion

3.1. Identification and biomass hydrolysis ability of G22 strain

In total 11 different bacterial strains were isolated from paper mill sludge and 4 strains showed bioflocculants' production ability when wood dust biomass was used as sole carbon source. The strain of G22, which showed the highest flocculating efficiency under 0.5% kaolin clay suspensions, was selected as bioflocculant-producing strain for next experiments. According to the lignocellulolytic enzyme ability assay, the G22 strain simultaneously produced a cellulase-free xylanase. It is a Gram-negative and aerobic bacterium, and the colonies are yellow, mucoid and rounded. The 16S rRNA gene sequence of G22 showed 99.9% similarity with that of *Pseudomonas boreopolis*. Therefore, the isolated strain G22 was finally identified as *Pseudomonas boreopolis* according to the morphological and phylogenetic characteristics and the16S rRNA sequence of this strain. The bioflocculants produced by this strain were named as MBF-G22. The sequence was submitted to NCBI and the accession number was given as MF449425. Several *Pseudomonas* strains have been reported to produce bioflocculants and applied in various fields. For examples, bioflocculants' production from *Pseudomonas veronii* L918 using hydrolysate of peanut hull was used to treat the ash-flushing wastewater (Liu et al., 2016). *Pseudomonas aeruginosa* ATCC-10,145 was identified as a bioflocculant-producing bacterium and the bioflocculants were applied in the removal of heavy metals (Eman, 2012). It has not been reported that *Pseudomonas boreopolis* can be used as a bioflocculant-producing strain and its application in microalgae harvest. In this study, *Pseudomonas boreopolis* G22 was found to produce a low cost bioflocculant by directly hydrolyzing untreated biomass.

To evaluate its hydrolysis ability of biomass, strain G22 was first cultured on the agar plate containing 0.5% untreated various biomass at 37 °C for 2 d. Then the plates were stained by using 5 ml of Gram's iodine solution for 3–5 min. Gram's iodine, which will form a bluish-black region with polysaccharide compounds but not with their hydrolysates, has been regarded as an easy and fast method for screening lignocellulolytic enzyme-producing bacteria (Guo et al., 2017c; Kasana et al., 2008). In this study, G22 produced a large halo region in all biomass-containing agar plate, indicating that G22 strain can effectively degrade these untreated biomasses (Fig. 1). In addition, the

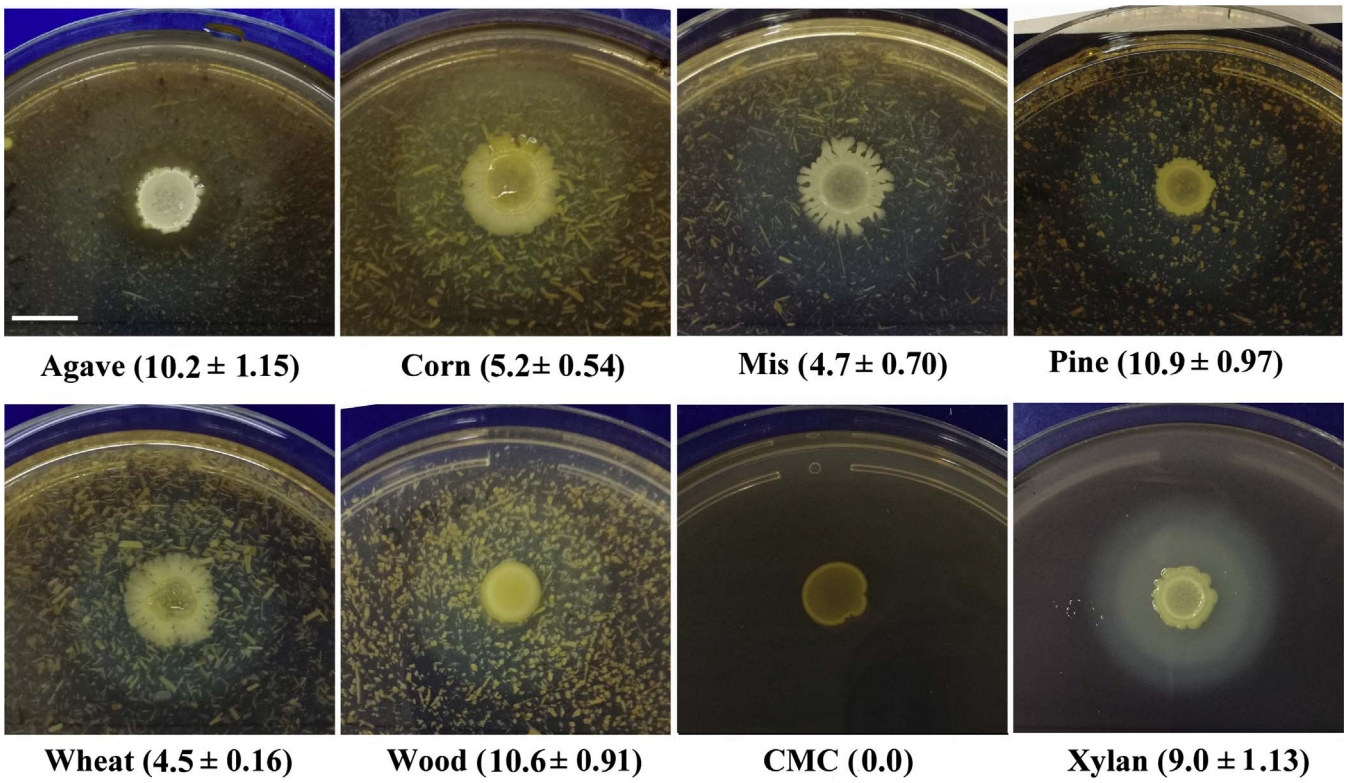

Fig. 1. Evaluating the hydrolysis ability of *Pseudomonas boreopolis* G22 using different biomass, CMC or xylan as carbon source by Gram's iodine staining. The numbers in brackets indicate the hydrolysis ability of G22 to different biomass. Values represent mean ± SDs (n = 3). Bar = 1 cm.

hydrolysis activities of this strain to different biomasses were evaluated by calculating square of the halo diameter: colony diameter ratio according to the method of Guo et al. (2017a). The hydrolysis ability values of grass lignocelluloses (agave, corn stover, *Miscanthus* and wheat bran) ranged from 4.5 to 10.2; while the hydrolysis ability values of wood dust and pine powder were 10.6 and 10.9, respectively (Fig. 1). Strain G22 produced a clear halo region with a hydrolysis ability value of 9.0 when xylan was used as the substrate, while it cannot form a halo region when CMC was used as sole carbon source, indicating that G22 secreted a cellulase-free xylanase (Fig. 1). This was consistent with that only xylanase activity can be detected rather than CMCase activity in the fermentation broths of G22 when untreated biomass was used as sole carbon source (date not shown). The higher hydrolysis ability of wood biomass than grass biomass may be due to the hemicellulose including more xylan in hardwood than that in grass lignocelluloses (Mazumder et al., 2012; Shrestha et al., 2015), and thus wood biomass was hydrolyzed sooner by the cellulase-free xylanase-producing bacterium.

3.2. Screening of the best biomass substrate for MBF-G22 production

Lignocellulose biomasses, which mainly consist of cellulose, hemicellulose and lignin, have been regarded as an alternative to produce biofuels, and value-added chemicals and bio-products (Jr et al., 2017). In this study, six kinds of lignocellulosic biomasses (agave, corn stover, *Miscanthus*, pine powder, wheat bran, and wood dust) and two commercial chemical compounds (CMC and xylan) were used as the sole carbon source to produce MBF-G22 in *Pseudomonas boreopolis* G22. The results showed that all of these lignocellulosic biomasses and xylan can significantly induce MBF-G22 production in G22 strain after 2 d of incubation (Fig. 2a). The maximum flocculating efficiencies in the presence of agave and *Miscanthus* were 91.2 and 81.8%, respectively, after 3 d of incubation. The highest flocculating efficiencies were 88.2, 98.5, 92.5, 98.8 and 91.3%, respectively, when corn stover, pine powder, wheat bran, wood dust and xylan was used as carbon source after 4 d of fermentation (Fig. 2a). However, the peak flocculating efficiency was only 15.0% in the presence of CMC after 4 d of culture, and the flocculating efficiency disappeared by day 6 (Fig. 2a), further indicating that MBF-G22 was mainly produced from the hydrolysates of xylan by G22. Moreover, for all of these substrates the highest xylanase activity was detected after 1–2 d of culture, which occurred before the maximum flocculating efficiency (Fig. 2b), suggesting that the production of MBF-G22 depended on the available sugars released from biomass due to the secretion of xylanase.

The cell wall compositions of these biomasses were different before and after incubation with G22 (Table 1). After 6 d of incubation, the hemicellulose contents of all tested biomasses were decreased by G22 strain, especially for agave, *Miscanthus*, pine powder and wood dust, of which hemicellulose contents were significantly reduced by 31.0, 7.4, 22.1 and 20.4%, respectively, compared to that of their controls. Moreover, G22 treatment extremely enhanced the cellulose contents of corn stover, *Miscanthus*, wheat bran and wood dust, with the increase of 12.0, 11.3, 19.2 and 12.7%, compared to that of their controls (Table 1). The results showed that G22 strain is a main xylanase producer, which more efficiently degraded the hemicellulose of biomass, especially of the wood biomass. To further conform that MBF-G22 was produced by directly converting the biomass, an in-depth statistical analysis was carried out to understand the correlations between flocculating efficiency and the degradation of biomass (Fig. 3a–c). The results showed that the flocculating efficiency was positively related to hydrolysis ability and hemicellulose loss with the correlation coefficient (R^2) values of 0.6686 and 0.5348, respectively (Fig. 3a and c); while a negative linear correlation was observed between flocculating efficiency and xylanase activity with R^2 value of 2.883 (Fig. 3b). Although the R^2 values of the correlation equations are low, all of R^2 reached markedly significant levels at $p < 0.01$. Therefore, the production of xylanase at the early stage resulted in the hydrolysis of hemicellulose and the hydrolysates were directly converted into MBF-G22 by

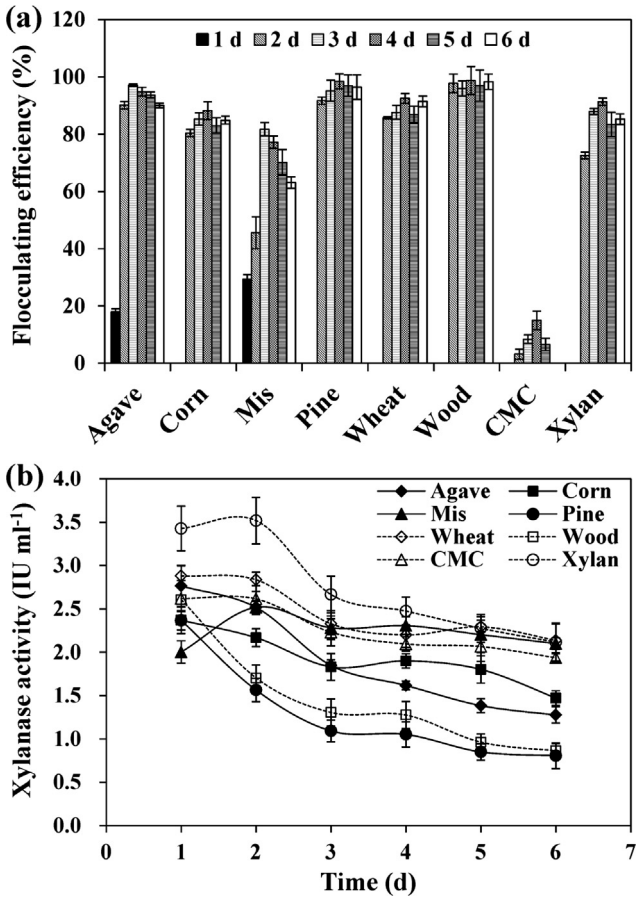

Fig. 2. Flocculating efficiency (a) and xylanase production (b) by *Pseudomonas boreopolis* G22 using 1.0% untreated agave, corn stover, *Miscanthus*, wheat bran, wood dust or pine biomass as carbon source. CMC and xylan as the control, values represent mean ± SDs (n = 4).

Pseudomonas boreopolis G22. It has been reported that some biomass-degrading bacteria have the ability to directly convert the biomass into bioflocculants by secreting various lignocellulosic enzymes. *Cellulosimicrobium cellulans* L804 produced bioflocculants from 2.0% corn stover with the maximum flocculating efficiency of 90.9% (Liu et al., 2015b), and *Bacillus agaradhaerens* C9 showed the ability to synthesize bioflocculants by using 2.0% rice bran as the sole carbon source with the highest flocculating efficiency of 87.2% (Liu et al., 2017), which was significantly lower than the flocculating efficiency induced by 1.0% wood dust in G22. Therefore, the wood dust was selected as the best biomass for MBF-G22 production due to its high flocculating efficiency, significant decrease in hemicellulose as well as relatively easy acquisition as raw material.

3.3. Optimization of fermentation conditions for MBF-G22 production

The fermentation conditions play an important role in enzyme secretion and bioflocculants' production due to their effects on electrification state, redox potential, enzyme reaction, and nutritional absorption and assimilation of microbial cells (Liu et al., 2016; Zhao et al., 2014). In this study, the effects of initial pH, incubation temperature, inoculum concentration and biomass concentration were measured to optimize MBF-G22 production in G22 strain when wood dust was used as the sole carbon source. The results showed that the flocculating efficiencies were not significantly different among different pH values although the xylanase activities were enhanced with the increase of pH (Fig. 4a). The similar flocculating efficiencies at different initial pH values may mainly depend on the final formation of alkali pH (above 8.5) by G22 after 1 d of incubation whatever the initial pH value is. This reflected how the bioflocculants were easily synthesized in the alkali environments by some bacteria. The maximum bioflocculation efficiency was found at pH 8.2 in *Cellulosimicrobium cellulans* L804 (Liu et al., 2015b), pH 9.0 in *Oceanobacillus polygoni* HG6 (Li et al., 2017), and pH 10.2 in *Bacillus agaradhaerens* C9 (Liu et al., 2015a). The optimal temperature and inoculum concentration for MBF-G22 production in G22 was 30 °C and 1.0%, respectively, which also induced the highest xylanase production (Fig. 4b and c). The high lignocellulosic enzymes ability helped to produce more available sugars, which can be used as the substrate for bioflocculants' production (Liu et al., 2017; Liu et al., 2015b). In addition, the flocculating efficiency increased with biomass concentration up to 1.0%, and then decreased with additional increase of wood dust concentration (Fig. 4d). The different biomass concentrations may change the carbon/nitrogen ratio of the fermentation broth, which played a crucial role in cell growth and bioflocculants' production (Chaisorn et al., 2016; Liu et al., 2016). High ratio of carbon/nitrogen can improve bacterial cell growth but will inhibit its flocculating efficiency; while low carbon/nitrogen ratio can increase flocculating efficiency by maintaining a stable pH of the fermentation broth (Liu et al., 2015a; Xia et al., 2008). In addition, high solid-liquid ratio of the culture may limit the access of oxygen into medium and thus refining cell growth, enzyme and bioflocculants' production of the microorganisms (Wang et al., 2011; Xia et al., 2008).

3.4. Characteristics of MBF-G22

The chemical compositions of purified MBF-G22 mainly consisted of 63.3% polysaccharides, 10.4% protein and 12.6% nucleic acid (Table 2), which are similar with that carbohydrate and protein are the main functional components in most of bacterial bioflocculants (Aljuboori et al., 2014; Liu et al., 2017). According to the assay of gel permeation chromatography, the average molecular weight of MBF-G22 was about 3.982×10^6 Da (Table 2), which was higher than that of reported bioflocculants with the molecular weight ranged from 1.8×10^4 to 3.22×10^6 Da (He et al., 2011; Nwodo et al., 2014). A larger bioflocculant molecular weight results in a larger floc size in the process of flocculation due to its large chain length and greater number

Table 1
Change of hemicellulose and cellulose contents after incubation with *Pseudomonas boreopolis* G22 for 6 d. Values represent mean ± SDs (n = 4). Different asterisks indicate a significant difference at $^*p < 0.05$; $^{**}p < 0.01$.

Biomass	Before incubation		After incubation	
	Hemicellulose (%)	Cellulose (%)	Hemicellulose (%)	Cellulose (%)
Agave	18.7 ± 2.02	39.1 ± 1.47	12.9 ± 0.83**	38.3 ± 6.81
Corn stover	34.8 ± 2.30	41.9 ± 2.99	33.0 ± 2.01	47.6 ± 1.30*
Miscanthus	29.6 ± 1.32	49.4 ± 6.09	27.4 ± 1.40*	55.0 ± 3.01*
Pine powder	12.2 ± 1.23	56.5 ± 4.07	9.5 ± 0.84*	53.6 ± 3.64
Wheat bran	29.1 ± 1.56	40.7 ± 3.42	27.4 ± 2.66	50.4 ± 3.47**
Wood dust	16.1 ± 3.19	43.8 ± 4.79	12.7 ± 1.36*	50.2 ± 4.69*

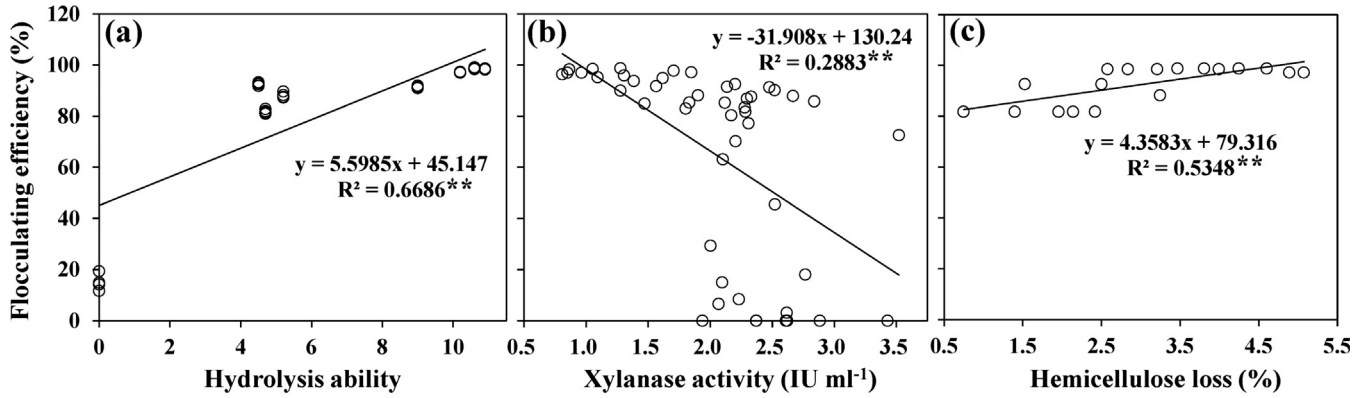

Fig. 3. Correlation analysis between flocculating efficiency and hydrolysis ability, xylanase activity or hemicellulose loss. (a) Between flocculating efficiency and hydrolysis ability (n = 24); (b) between flocculating efficiency and xylanase activity (n = 48); (c) between flocculating efficiency and hemicellulose loss (n = 18).

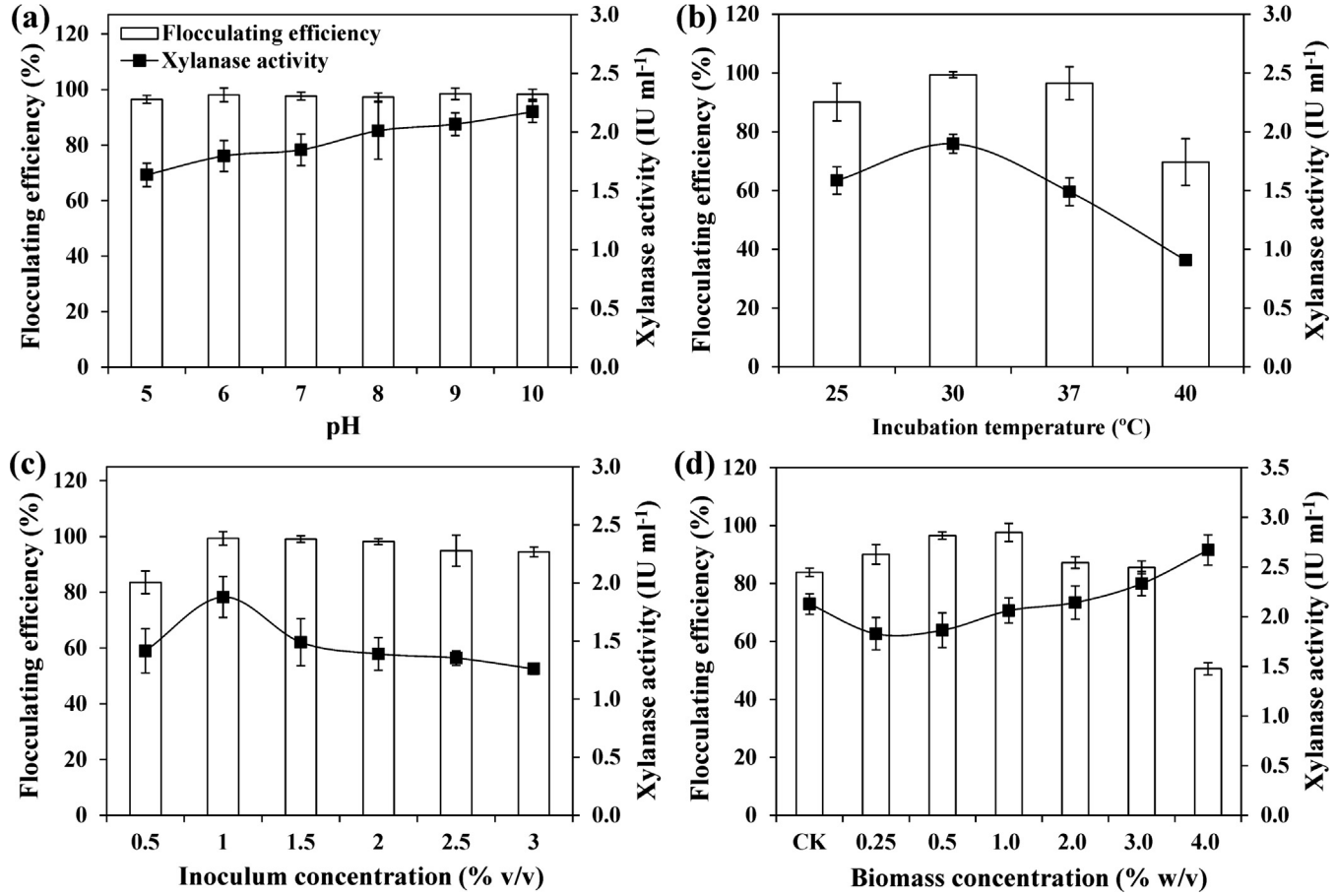

Fig. 4. Effects of pH (a), Temperature (b), inoculum concentration (c) and biomass concentration (d) on flocculating efficiency and xylanase activity. Values represent mean ± SDs (n = 4).

of function groups (Suh et al., 1997). In the optimal fermentation conditions, the maximum purified bioflocculants' yield was 375 mg per g biomass (Table 2), which was 57.6% higher that of MBF-L804 produced by *Cellulosimicrobium cellulans* L804 when 20 g L^{-1} corn stover was used as carbon source (Liu et al., 2015b). The high bioflocculants' yield of G22 may main due to its high secretion of xylanase (2.3–3.5 IU ml^{-1}) (Fig. 2 b), which was about 3-fold higher than the xylanase-secreted by *Cellulosimicrobium cellulans* L804 (Liu et al., 2015b). The charge density and zeta potential of MBF-G22 were −1.30 meq per g dry bioflocculant and −48.89 mV, respectively (Table 2), which was similar with that most bioflocculants produced by bacteria were negatively charged (Wang et al., 2011; Wu and Ye, 2007). Moreover, the viscosity of the MBF-G22 is 1.343 cPs when the bioflocculants' concentration is 1.0 g L^{-1} (Table 2). The FTIR spectrum of MBF-G22 was recorded in the 4000–600 cm^{-1} region. The results showed that a number of clear absorption peaks were found at 3255, 2899, 1632, 1445, 1352, 1234, 1001 and 887 cm^{-1} wavenumber. The broad O—H stretching absorption peak was observed at 3255 cm^{-1} and a small C—H stretching vibration band at 2899 cm^{-1}. The peaks at 1632 and 1445 cm^{-1} were the typical characteristic of C═O stretchings in the carboxylate, respectively. The signals at 1352 and 1234 cm^{-1} indicated symmetric CH bending and S═O stretching and the peaks ranging from 1200 to 800 cm^{-1} meant the presence of sugar derivatives. The FTIR result was similar with the findings of most bacterial

Table 2
The compositions and characteristics of the bioflocculants produced from 1.0% (w/v) untreated wood dust by *Pseudomonas boreopolis* G22. Values represent mean ± SDs (n = 4).

Compositions	
Polysaccharide	63.3 ± 3.51%
Protein	10.4 ± 1.67%
Nucleic acid	12.6 ± 2.90%
Characteristics	
Molecular weight	3.982×10^6 Da
Maximum yield	375 ± 15.9 mg g^{-1} dry biomass
Charge density	−1.30 ± 0.013 meq g^{-1} bioflocculants
Zeta potential	−48.89 ± 1.14 mV
Viscosity	1.343 ± 0.003 cPs (1.0 g L^{-1})

bioflocculants (Deng et al., 2003; Liu et al., 2015b; Yim et al., 2007).

3.5. Effects of MBF-G22 dosage, pH and temperature on flocculating efficiency

For better understanding the flocculating properties of MBF-G22, the effects of bioflocculants' dosage, pH, and temperature on the flocculating efficiency were determined when the kaolin clay was used as substrate. As shown in Fig. 5a, over 80% flocculating efficiency was found when the MBF-G22 dosage was as low as 1.0 mg L^{-1}, and the maximum flocculating efficiency with the value of 97.1% was obtained at the dosage of 3.5 mg L^{-1}, which was significantly lower than that of other bacterial bioflocculants. In total of 12 mg L^{-1} purified bioflocculants from *Rhizobium radiobacter* F2 and *Cellulosimicrobium cellulans* L804 were needed to obtain the highest flocculating efficiency (Liu et al., 2015b; Wang et al., 2011). The high flocculating efficiency of MBF-G22 at low dosage may be due to its big molecular weight and high viscosity (Haver and Nayar, 2017), and its low flocculating efficiency at a dosage of 0.5 mg L^{-1} was the result of inadequate bridging between particles (Zulkeflee et al., 2012). The pH values ranging from 4 to 11 were suitable to obtain over 93% flocculating efficiency of kaolin clay suspension for MBF-G22 (Fig. 5b). In addition, MBF-G22 was stable at all tested temperatures, which can achieve 92.1–97.6% flocculating efficiency in the region of 30–80 °C (Fig. 5c). The high temperature tolerance of MBF-G22 may mainly depend on its high polysaccharide component, which has been reported more stable than protein and other compounds comprising bioflocculants (Li et al., 2010; Sekelwa et al., 2013). Its excellent flocculating efficiency and stability in a broad range of pH and temperature make the MBF-G22 indicate great potential for industrial applications without worrying about the pH and temperature of the solutions (Makapela et al., 2016).

3.6. Utilization of MBF-G22 for microalgae harvest

Bioflocculation has been regarded as a potential technology to reduce the cost of microalgae harvest. It has been reported that the bioflocculants produced by *Desmodesmus* sp. F51 (Ndikubwimana et al., 2014), *Cellulosimicrobium cellulans* L804 (Liu et al., 2015b), *Bacillus agaradhaerens* C9 (Liu et al., 2015a), and *Cobetia marina* L03 (Lei et al., 2015) can be used to harvest various microalgae. In this study, the flocculation of *S. abundans* was evaluated by adding the extracted MBF-G22. The results showed the flocculating efficiency of *S. abundans* significantly increased initially and then decreased with increasing dosage of MBF-G22. Over 90% flocculating efficiency was obtained at the MBF-G22 dosage of 60–90 mg L^{-1} with the highest flocculating efficiency of 95.7% at the dosage of 80 mg L^{-1}(Fig. 6). Compared to kaolin clay, the flocculation of microalgae needed more bioflocculants due to its smaller particles and existence of more negative charges, which requires more negatively charged bioflocculants to bridge with metal cations (Wang et al., 2011). Large doses of bioflocculants usually resulted in the enhancement of repulsion between particles because of the excessive

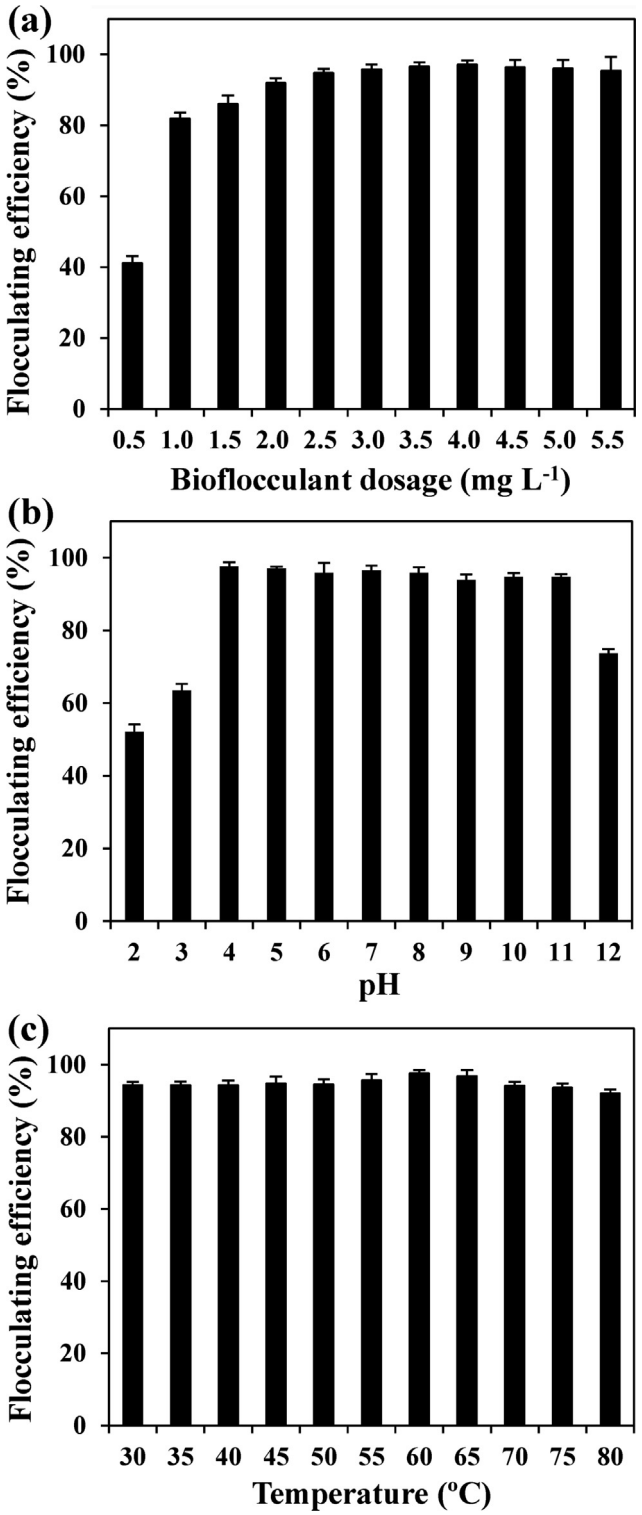

Fig. 5. Effects of MBF-G22 dosage, pH and temperature on flocculating efficiency. (a) Dosage; (b) pH; (c) Temperature. Values represent mean ± SDs (n = 4).

introduction of negatively charged bioflocculants (Abd-El-Haleem et al., 2008). In addition, the viscosity generated at high bioflocculants' concentrations can also inhibit the sedimentation of floccules (Wang et al., 2011).

4. Conclusions

Lignocellulosic biomass, especially wood biomass, was a suitable

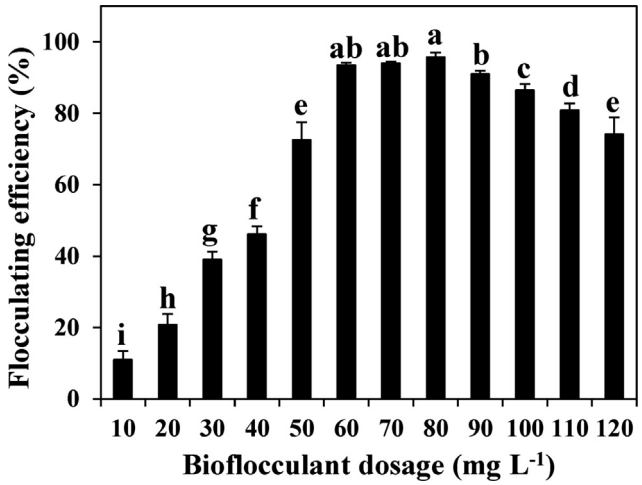

Fig. 6. Effects of MBF-G22 dosage on the flocculanting efficiencies of microalgae, *S. abundans*. Values represent mean ± SDs (n = 4). Different letters indicate significant differences at $p < 0.05$.

substrate for MBF-G22 production by G22 strain. The maximum yield of MBF-G22 (3.75 mg g^{-1} dry biomass) was achieved after 96 h of incubation at the optimal conditions as follows: 1.0% untreated wood dust, 1.0% inoculum concentration, initial pH 7.0 and a temperature of 30 °C. MBF-G22 included 63.3% polysaccharides with an average molecular weight of 3.982 × 10^6 Da. MBF-G22 showed 97.1% flocculating efficiency at a low dosage (3.5 mg L^{-1}) and was stable at wide ranges of pH and temperature. Moreover, MBF-G22 was also considered as a potential alternative for harvesting 95.7% microalgae.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2018.01.082.

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