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Bioflocculation formation of microalgae-bacteria in enhancing microalgae harvesting and nutrient removal from wastewater effluent

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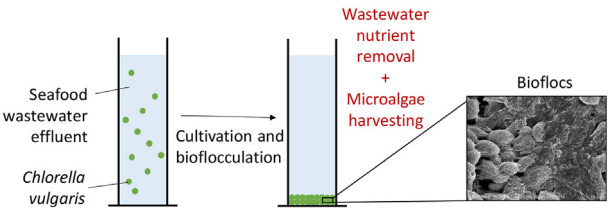
GRAPHICAL ABSTRACT



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ABSTRACT

Microalgal bacterial flocs can be a promising approach for microalgae harvesting and wastewater treatment. The present study provides an insight on the bioflocs formation to enhance harvesting of *Chlorella vulgaris* and the removal of nutrients from seafood wastewater effluent. The results showed that the untreated seafood wastewater was the optimal culture medium for the cultivation and bioflocculation of *C. vulgaris*, with the flocculating activity of 92.0 ± 6.0%, total suspended solids removal of 93.0 ± 5.5%, and nutrient removal of 88.0 ± 2.2%. The bioflocs collected under this optimal condition contained dry matter of 107.2 ± 5.6 $g{\cdot}L^{-1}$ and chlorophyll content of 25.5 ± 0.2 $mg{\cdot}L^{-1}$. The results were promising when compared to those obtained from the auto-flocculation process that induced by the addition of calcium chloride and pH adjustment. Additionally, bacteria present in the wastewater aided to promote the formation of bioflocculation process.

1. Introduction

Microalgae have appeared as promising renewable raw materials to provide a wide variety of compounds with commercial interest, such as lipids, proteins, pigments and carbohydrates (Becker, 2007; Borowitzka, 2013; Lee et al., 2017). However, large-scale production of microalgae is limited by the high-energy inputs required for the harvesting of microalgae (Ummalyma et al., 2017; Vandamme et al., 2013). Microalgae harvesting requires an intensive effort to separate a small amount of biomass from a large volume of culture broth, either from open pond reactor or photobioreactors. Besides, the small size of microalgae cells (from 2 to 20 μm) has contributed to their high colloidal stability in liquid suspension, and thus making the harvesting by simple sedimentation process is not feasible. The cost of microalgae harvesting can easily achieve 20–30% of the total cost of microalgae production and, in some circumstances, might reach 60% of the total

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cost when post-production is needed (Greenwell et al., 2009; Grima et al., 2003; Van Den Hende et al., 2011). Therefore, many studies have devoted to develop a cost-effective and economical strategy for microalgae harvesting (Grima et al., 2003; Milledge and Heaven, 2013; Quijano et al., 2017).

Various approaches for solid-liquid separation have been investigated, including adherence techniques using coagulation, flocculation and flotation, and the force applications such as centrifugation and filtration (Barros et al., 2015; Laamanen et al., 2016; Singh and Patidar, 2018; Wan et al., 2015). Amongst these methods, adherence approach is promising. However, chemical coagulation method using metal coagulants like alum and iron chloride might consume large amounts of coagulants and flocculants which lead to high operation cost and metal contamination of the harvested biomass. In view of these constraints, bioflocculation is an alternative technological approach. Bioflocculation is a flocculation process of microalgae cells assisted with microorganisms (Lee et al., 2013). During bioflocculation process, the aggregation of bacteria and microalgae cells creates large flocs and settle down by gravity, without the use of any metal and chemical flocculants, or the alteration of medium's pH (Vandamme et al., 2013). Beside of allowing speedy harvesting of microalgae, the microalgal bacterial flocs (MaB-flocs) tend to adsorb suspended compounds in surrounding medium to form co-bioflocculate and thus enhance the removal of nitrogen and phosphorous (Alcántara et al., 2015; Ummalyma et al., 2017). Therefore, the MaB-flocs technique was incorporated into conventional aerobic activated sludge technologies for wastewater treatment to enhance nutrient removal and effluent recovery (Tang et al., 2018; Van Den Hende et al., 2016; Van Den Hende et al., 2011; Yan et al., 2019). This technology was reviewed for its fundamentals and applications recently (Abinandan et al., 2018; Posadas et al., 2017). Moreover, several studies reported that the technique can be applied for the wastewater treatment coupled with the flue gas treatment or biogas upgrading simultaneously (Posadas et al., 2016; Tang et al., 2018; Van Den Hende et al., 2016; Van Den Hende et al., 2011). Despite that it is generally accepted that bacteria aids in inducing flocculation, there is still lack of the investigation on the contribution of the bacteria to the overall wastewater treatment performance (Posadas et al., 2017). Additionally, the key factor for the formation of the MaB-flocs is not clear yet. It is therefore important to provide more insights of the technique to optimize its efficiency. Moreover, the bioflocculation formation without adjusting of bioflocculants plays an important role in this investigation.

In this study, the seafood wastewater effluent (SWE) obtained from the cleaning units of a seafood production factory was used as culture medium for microalgae production. *Chlorella vulgaris* was selected as a strain of microalgae in this study owing to its fast growth rate and lipid productivity compared to others strains (Fu et al., 2012; Kang et al., 2014). The study started with the investigation of the effect of initial concentration of *C. vulgaris* on the MaB-flocs formation. After obtaining the optimal initial microalgae concentration, different culture media, as presented in Table 1, were prepared for the cultivation of microalgae, in order to examine the key factors for the formation of bioflocculation. Aiming to compare the bioflocculation process with the autoflocculation induced by pH modulation and presence of metal ions, Sueoka broth was prepared with added calcium chloride and adjusted pH, and their results were addressed. Besides, *Escherichia coli* was added as bacteria model in several media, in order to verify its effect. The results were evaluated based on the flocculating activity, total suspended solids (TSS) removal, dry matter and chlorophyll content of the flocs collected, and the wastewater nutrient removal. Furthermore, all the flocs collected from different culture media were treated and observed using compound microscope and scanning electron microscope (SEM).

Table 1
Different culture media prepared for the investigation of flocculation process.

Medium	M1	M2	M3	M4	M5	M6	M7	M8
Description	Sterilised SWE	Sterilized SWE + *E. coli*	Treated SWE with low Ca^{2+} and Mg^{2+} content	SWE	Sueoka broth + *E. coli*	Sueoka broth	Microalgae grown in M6 were harvested by centrifugation	Sueoka broth + $CaCl_2$ and pH at 10.0 ± 0.5
Bacteria content (CFU/mL)	[a]	4.0×10^5	720.0×10^5 for aerobic bacteria, 2.1×10^5 for *Coliforms*	240.0×10^6 for aerobic bacteria 5.0×10^5 *Coliforms*	4.0×10^5	[a]	[a]	[a]
Divalent metal concentration (mg/L)	107.7 Ca^{2+} + 14.1 Mg^{2+}	107.7 Ca^{2+} + 14.1 Mg^{2+}	56.2 Ca^{2+} + 2.1 Mg^{2+}	107.7 Ca^{2+} + 14.1 Mg^{2+}	6.8 Ca^{2+} + 13.8 Mg^{2+}	6.8 Ca^{2+} + 13.8 Mg^{2+}	6.8 Ca^{2+} + 13.8 Mg^{2+}	126.8 Ca^{2+} [b] + 13.8 Mg^{2+}

[a] No bacteria present in the medium.
[b] Total calcium content was derived from calcium concentration in Sueoka broth and calcium chloride added in.

2. Material and methods

2.1. Microalgal strain and wastewater

The microalgal strain used in this study was *C. vulgaris* SAG 211-19 and the cultivation process was performed using the protocols described elsewhere (Nguyen et al., 2014). The SWE was collected from the cleaning units of fish and shrimp in a seafood production factory in Vietnam. Before using for all the experiments throughout this study, the SWE was filtered to remove the suspended grease layer. The quality parameters of the SWE were measured and listed as follow ($mg{\cdot}L^{-1}$): NH_4^+, 277.5; PO_4^{3-}, 39.3; CO_3^{2-}, 405.0; Ca^{2+}, 107.7; Mg^{2+}, 14.1; Na^+, 186.5; chemical oxygen demand (COD), 362.0; biochemical oxygen demand (BOD), 215.5; and TSS, 468.5.

2.2. Cultivation and harvesting of microalgae in different media and conditions

First, the effect of initial concentration of *C. vulgaris* on the bioflocs formation was investigated. *C. vulgaris* inoculum that cultivated in Sueoka medium was obtained at $1000.0 \pm 2.0\ mg{\cdot}L^{-1}$, and was mixed with 1 L of the SWE to make up the culture medium at different initial concentration of *C. vulgaris* starting at $10.0\ mg{\cdot}L^{-1}$ with a concentration interval of $5.0\ mg{\cdot}L^{-1}$. The cultivation process was performed in a continuous aeration mode for 14 days. The microalgae productivity ratio, namely the ratio of the COD concentration in the SWE (C_{COD}, $mg{\cdot}L^{-1}$) and initial microalgae concentration ($C_{microalgae}$, $mg{\cdot}L^{-1}$), was defined in Eq. (1).

$$Microalgae\ \ productivity\ \ ratio = C_{COD}/C_{microalgae} \quad (1)$$

After obtaining the optimal microalgae productivity ratio, the experimental studies were carried out to investigate the formation of bioflocs, specifically the self-settlement of microalgae cells by bacteria aggregation on the microalgae cell surface, and the removal of nutrients from the SWE. Several different cultivation media were prepared at a pH of 8.2 ± 0.5, unless otherwise stated, as presented in Table 1. The sterilization process of the SWE was run in an autoclave at 121 °C for 30 min. While for the preparation of M3, the SWE was treated using a Pyrex Squibb separatory funnel containing cation exchange resins (Indion 220Na) to reduce the content of Mg^{2+} and Ca^{2+}. The cation exchange process was conducted using 1 kg resin to treat 1L of the SWE. After the SWE passed through the resin bed, the filtered suspension was collected for the analysis. The quality parameters of the treated SWE were as follow ($mg{\cdot}L^{-1}$): NH_4^+, 168.5; PO_4^{3-}, 8.5; CO_3^{2-}, 315.2; Ca^{2+}, 56.2; Mg^{2+}, 2.1; Na^+, 144.6; COD, 217.8; BOD, 165.5; and TSS, 206.7. For the preparation of M7, the microalgae grown in M6 were harvested using centrifugation step. Whereas for the preparation of M8, Sueoka broth was prepared and $333.0\ mg{\cdot}L^{-1}$ of $CaCl_2$ was added and pH was adjusted to 10.0 ± 0.5 (Nguyen et al., 2014).

The microalgae cultivation process was performed under a light intensity of $150\ \mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and at 27 ± 2 °C until all the microalgae cells have been reduced to content. The flocs settled down in the bottom of flask were collected for the determination of the dry matter and chlorophyll contents. Besides, the culture medium was measured for the COD contents.

2.3. Flocculation activity

The supernatant of culture broth before and after the cultivation process was counted for *C. vulgaris* cells on a Malassez counting chamber using a microscope (ProWay China, PW-BK 5000). The flocculation activity was determined using Eq. (2), by measuring the optical density of the sample at 680 nm (OD_{680}) (Kim et al., 2011; Lee et al., 2013; Oh et al., 2001).

$$Flocculation\ \ activity\,(\%) = (1 - OD_{680,s}/OD_{680,i}) \times 100 \quad (2)$$

where $OD_{680,s}$ is the optical density of the sample at the sample collection time and $OD_{680,i}$ is the optical density of the sample before the flocculation process.

2.4. Dry matter and chlorophyll content

The flocs formed at the bottom of culture broth was collected for the measurement of their dry matter and chlorophyll contents. Prior to passing through the vacuum filtration device for dewatering the flocs, the supernatant was withdrawn to allow efficient separation of flocs from the culture broth. The flocs were then settled down in a glass graduated cylinder of 100 mL for 2 h to allow a complete separation of a thick stable flocs layer. The flocs were dewatered in a linen filter bag (150–200 μm pore size). Then, the samples were dried at 105 °C for 24 h and cooled in a desiccator for 10 min, before weighed using a reusable filter holder.

Besides, the chlorophyll content of microalgae can be used as an indicative parameter for the growth performance of microalgae in different culture media. In this study, the chlorophyll that contained in microalgae cells was extracted using methanol. 0.5 mL of culture broth was centrifuged using a centrifuge (Eppendorf, Minispin) at 13400 rpm for 5 min for the separation of biomass and supernatant. Then, the supernatant was discarded and 1.5 mL of methanol was added to extract the chlorophyll from microalgae cells. The biomass suspension was isolated from the light for 1 h and incubated at 44 °C in an oven. After that, the suspension was centrifuged at 13400 rpm for 5 min to remove the cell debris. The absorbance of the supernatant was measured using a UV–vis spectrophotometer (Lambda 2S, Perkin Elmer). The absorbance peaks of the supernatant at wavelengths of 652 nm (chlorophyll *b*), 665 nm (chlorophyll *a*) and 750 nm (turbidity of suspension) were obtained, and were used to determine the chlorophyll content ($mg{\cdot}L^{-1}$), using Eq. (3) (Ritchie, 2006).

$$Chlorophyll = [-8.0962(OD_{652} - OD_{750}) + 16.5169(OD_{665} - OD_{750})]\frac{V_2}{V_1 \cdot l} \quad (3)$$

where V_1 and V_2 are volume of sample suspension and methanol used, and l is the optical path, which is 1 cm. For high concentration of chlorophyll that is more than $10\ mg{\cdot}L^{-1}$, the protocols can be simplified by adding 0.25 mL of sample suspension (V_1) to 1.25 mL of methanol (V_2) before the incubation process. The rest of the procedures followed the same as described.

2.5. Seafood wastewater quality parameters

The measurement of quality parameters of the SWE, including TSS, BOD, COD, NH_4^+, Ca^{2+}, Mg^{2+}, NO_3^- and PO_4^{3-}, was performed by referring to the American Public Health Association (APHA) standard methods (APHA/AWWA/WEF, 2012). The measurements were carried out before and after the flocculation process to determine the efficiency of the nutrient removal by the formation of flocs. The efficiency of nutrient removal (E_r), particularly the reduction of the COD in wastewater effluent, was expressed in Eq. (4).

$$E_r(\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (4)$$

where C_i and C_f are the initial and final concentrations of COD ($mg{\cdot}L^{-1}$) in wastewater effluent.

2.6. Compound microscope and scanning electron microscope

The flocs collected after the centrifugation operation at 3000 rpm for 1 min were resuspended in distilled water and observed using a compound microscope to verify the presence of extracellular polymeric substances.

Besides, the flocs collected was immersed in 2.5% glutaraldehyde in

0.1 M cacodylate buffer at pH 7.2 for overnight. After that, the specimen was washed 3 times using 0.1 M cacodylate buffer and followed by a post-fixing treatment using 1% osmium tetroxide in 0.1 M cacodylate buffer for 20 min. Then, the specimen was washed 3 times using 0.1 M cacodylate buffer and dehydrated in 50, 70, 90, and 100% ethanol. After the dehydration process, the sample was transferred to the chamber of a critical point drying apparatus for drying. The sample was mounted onto a metal stub and sputter coated with gold by an ion-coater, before being examined using a SEM (Hitachi, FE-SEM S4800).

3. Results and discussion

3.1. Initial concentration of microalgae

In the study, the SWE served to supply nutrients for the growth of microalgae, and the nutrient utilization allow wastewater treatment (Cai et al., 2013). In a typical condition, microalgae consume wastewater mineral nutrients and CO_2 to produce biomass and release the O_2 required by bacteria. Whereas, bacteria ingest O_2 and release CO_2 during their action to degrade COD to mineral components (Muñoz and Guieysse, 2006).

The *C. vulgaris* SAG 211-19 was cultivated in the SWE for 14 days with different initial microalgae concentration from 10.0 to 50.0 $mg{\cdot}L^{-1}$. The flocculating activity was evaluated through the observation of flocs formation at the bottom of flask and the measurement of TSS. TSS is one of the most essential parameters for water quality since both organic and inorganic particles that are larger than 2 μm, including bacteria and algae, can contribute to the concentration of TSS (Ayana et al., 2015). Table 2 shows the results of the culture media with different initial microalgae concentration. Significant built up of flocs volume was observed at initial microalgae concentration of 20.0 $mg{\cdot}L^{-1}$, namely at microalgae productivity ratio of 18, with the TSS removal performance of 90.0 ± 5.5%. At the culture media with the microalgae productivity ratio less than 18, the SWE medium was dense without clear formation of flocs layer due to slow settlement of biomass and low microalgae density. The cell might not sufficient to grow in low-dense culture medium and thus prevent the aggregation of microalgae to form bioflocs (Grima et al., 2003). Therefore, the initial concentration of *C. vulgaris* added into the culture medium of 20.0 $mg{\cdot}L^{-1}$ was chosen for further studies.

3.2. Flocculation process in different media

The adhesion of bacteria and microalgae to form MaB-flocs is closely related to the secretion of the extracellular polymeric substances by bacteria (Salehizadeh et al., 2000). Many studies reported the close relationship between extracellular polymeric substances and TSS to form bioflocs (Dertli et al., 2015; Jimenez et al., 2007). The researchers reported that the amount of extracellular polymeric substances released by bacteria was closely related to the available space in bioflocs. The flocculating activity increases with the increase of the extracellular polymeric substances content. The suggestion was supported by the results presented in Table 3 with different types of culture media prepared. The decrease of TSS content in supernatant of culture medium altered the performance of TSS removal and flocs concentration in M4 that consisted of various aerobic bacteria that are capable to produce extracellular polymeric substances layer.

Besides, wastewater nutrient removal efficiency is important in a wastewater treatment protocol. The effects of bacteria on flocs formation and simultaneously the nutrient removal performance are one of the main focus of this work. Therefore, it is essential to evaluate the growth of microalgae in the SWE. The results showed that the growth of *C. vulgaris* in M1, M2, M3 and M4 was similar by the utilization of nutrients from the SWE to microalgae cells. This nutrient conversion can be evaluated by analyzing nutrient removal efficiency and chlorophyll content of flocs (Ji et al., 2013; Lee et al., 2013). Among the culture media composed of the SWE, M4 showed the highest nutrient removal efficiency of 88.0 ± 2.2% and relatively chlorophyll concentration of 25.5 ± 0.2 $mg{\cdot}L^{-1}$. Therefore, it can be concluded that the formation of flocs has positive impact in both wastewater treatment and microalgal biomass production. Moreover, the chlorophyll content of flocs obtainned from the untreated SWE (M4) is relatively comparable to those obtained from Sueoka medium (M8), indicating the successful bioconversion of wastewater nutrients and CO_2 into microalgae biomass.

Compared to the TSS removal performance, flocculating activity was determined in relative values by comparing the optical density of the culture medium before and after the flocculation process. It is worth noting that the untreated SWE in M4 promoted a valuable flocculating activity, which indicating that *C. vulgaris* was capable to settle down by gravity in the culture broth without any harvesting technique applied. M4 consisted of excess nutrients that were sufficient to support the growth of both microalgae and bacteria to enhance the formation of MaB-flocs. Compared to M4, M1 with the sterilized SWE offered significant low flocculating activity. This might be due to the lack of bacteria, and insufficient metal cations content as well as pH value in performing flocs formation. Additionally, it was observed that the flocculating activity of at least 78.0 ± 6.4% was achieved for those culture media with the presence of bacteria (M2, M3 and M4). Nonetheless, it was found that the highest flocculating activity achieved at 99.0 ± 5.5 for M8 containing Sueoka broth with the addition of Ca^{2+} and adjusted pH value to 10.0 ± 0.5. Both high pH and sufficient magnesium concentration (> 0.15 mM) are essential to induce the autoflocculation process (Vandamme et al., 2012a). This autoflocculation process occurs due to the change of surface properties of microalgae cells when there are changes in nitrogen, pH, dissolved oxygen and presence of calcium and magnesium ions in the culture media (Ummalyma et al., 2017; Vandamme et al., 2013). The results reported were consistent with those reported by other researchers specifically about the impact of bacteria, divalent cation and pH in inducing flocculation formation (Han et al., 2016; Pacheco-Vega et al., 2018; Quijano et al., 2017; Van Den Hende et al., 2016; Vandamme et al., 2013).

The results showed that the microalgae harvested were able to grow well when they were co-cultivated together with bacteria as a bioflocculant. This technique might reduce the production cost of microalgae and at the same time improve wastewater treatment strategies without using chemical substances and the coercion in increment of pH value. The use of wastewater like the SWE in this study allows the cultivation and harvesting of microalgae, and simultaneously the removal of nutrients which eases the further wastewater treatment steps (Alcántara et al., 2015; Cuellar-Bermudez et al., 2017; Ummalyma et al., 2017; Wang et al., 2017).

Table 2
The effects of different initial microalgae concentrations on the occurrence of bioflocculation and the results of TSS removal.

Microalgae concentration ($mg{\cdot}L^{-1}$)	10.0 ± 1.3	15.0 ± 1.1	20.0 ± 1.2	25.0 ± 1.1	35.0 ± 1.0	40.0 ± 1.1	45.0 ± 1.1	50.0 ± 1.2
Microalgae productivity ratio	36	24	18	14.5	12	10	9	8
Flocculation occurrence	No clear formation	No clear formation	Yes	Yes	Yes	Yes	Yes	Yes
TSS removal (%)	20.1 ± 7.0	20.6 ± 10.5	90.0 ± 5.5	94.5 ± 4.2	95.0 ± 6.0	95.0 ± 7.2	94.2 ± 4.5	94.0 ± 6.8

Table 3
The results of different culture media prepared in terms of flocculating activity, TSS removal, chlorophyll and dry matter contents of flocs, and wastewater nutrient removal.

Medium	M1	M2	M3	M4	M5	M6	M7	M8
Flocculation activity (%)	8.7 ± 2.5	78.0 ± 6.4	85.0 ± 2.2	92.0 ± 6.0	75.0 ± 2.5	– [a]	– [a]	99.0 ± 5.5
TSS removal (%)	14.3 ± 3.5	80.0 ± 3.5	81.0 ± 7.0	93.0 ± 5.5	78.0 ± 8.2	– [a]	– [a]	90.0 ± 4.0
Flocs chlorophyll ($mg{\cdot}L^{-1}$)	3.5 ± 0.3	22.8 ± 2.4	29.0 ± 1.6	25.5 ± 0.2	22.4 ± 2.1	– [a]	– [a]	26.1 ± 0.8
Flocs dry matter ($g{\cdot}L^{-1}$)	8.3 ± 4.72	81.2 ± 3.5	82.2 ± 2.6	107.2 ± 5.6	91.1 ± 3.4	– [a]	– [a]	47.5 ± 7.8
Nutrient removal (%)	78.4 ± 2.3	82.3 ± 5.2	78.1 ± 2.5	88.0 ± 2.2	– [b]	– [b]	– [b]	– [b]

[a] No formation of flocculation in the respective culture medium.
[b] Sueoko broth was used instead of the SWE for M5 to M8. Therefore, no measurement was needed for wastewater nutrient removal performance.

3.3. Flocculation formation

To further understand the key factors for the formation of bioflocs, the results of different culture media presented in Table 3 were discussed. The flocculating activities in M3 and M4 were relatively high at 85.0 ± 2.2 and 92.0 ± 6.0%, respectively. The results were consistent with the removal of TSS calculated. Despite the absence of flocculation process in M6, M5 containing Sueoka broth with added *E. coli* demonstrated flocculation activity of 75.0 ± 2.5%. Likewise, M2 containing sterilized SWE with added *E. coli* promoted flocculation activity of 78.0 ± 6.4% when compared to M1 consisting of only sterilized SWE with the flocculation activity of only 8.7 ± 2.5 acquired. All these results suggested that the bacteria play a key role in the formation of flocculation. When compared to M5, M3 that consisted of diverse aerobic bacteria can lead to a more efficient adhesion of cells although the concentration of primary divalent cations present in the culture medium was not sufficient to promote the aggregations of microalgae cells. The adhesion of bacteria on microalgae cell surface created a large biofilm to enhance the attachment of microalgae cells around this layer until the aggregation size is sufficient for auto-settlement by gravity.

The use of chemical flocculants for microalgae harvesting might not appreciated owing to their high pH dependence, high cost, and large accumulation of many chemical compounds on microalgae cells (Farid et al., 2013; Wan et al., 2015). It must be noted that the presence of high pH in culture medium is a major factor for the creation of auto-flocculation (Bhola et al., 2011; Nguyen et al., 2014; Vandamme et al., 2012b; Vandamme et al., 2013). The same conclusion was also drawn in this study. There was no formation of flocs in M8 until the medium's pH was increased to 10.0 ± 0.5. At the pH of 10.0 ± 0.5, the flocs formed instantly and increased up to 99.0 ± 5.5%. Besides, the presence of high concentration Ca^{2+} and Mg^{2+} content in culture medium has aided in improving the formation of flocs when comparing the results obtained for M3 and M4. Nevertheless, there was no flocculation occurred in M8 containing added Ca^{2+} of 120.0 $mg{\cdot}L^{-1}$ at pH of 8.2 ± 0.5, although the concentration of Ca^{2+} in M8 is almost equivalent to those in M4, namely at 107.7 $mg{\cdot}L^{-1}$.

3.4. Microscopic images of the harvested biomass

The flocs formation mechanism was well formulated by the location of bacteria (Nguyen et al., 2014; Vandamme et al., 2013). The results of SEM images of the harvested microalgae from different media were presented in electronic supporting information. The converging of aerobic bacteria around *C. vulgaris* cells in M4 was remarkable compared to M5 containing *E. coli* that the adsorption of *E. coli* and microalgae was relatively sporadic. The higher flocculation activity of M4 than M5 might be due to the significant interactions of various bacteria on microalgae cell surface forming conditional films. The findings were in good agreements with several works published concerning the increment of bioflocs content in culture medium containing various bacteria (Pacheco-Vega et al., 2018; Quijano et al., 2017). The results suggested that the bacteria play important role in the adhesion of suspended microalgae in order to perform bioflocculation process.

Bacteria attachment on microalgae cells is the critical step in bioflocs formation. The attachment was performed by the extracellular polymeric substances excreted by bacteria to derive a conditional film (Bos et al., 1999). The layers of extracellular polymeric substances were found in those samples collected from M2, M3, M4 and M5 that contain bacteria in their medium, as illustrated in electronic supporting information. Whereas, the microscopic image of harvested samples from culture media such as M1, M7 and M8 demonstrated the absence of extracellular polymeric substance. The microalgae cells have the tendency to become a large bioflocs when slimy layers of extracellular polymeric substances were formed. Moreover, the aggregation of microbial population can be observed from the harvested biomass obtained from M3 and M4. For M3 consisting of *E. coli*, the adhesion of *E. coli* with planktonic microorganism might be difficult. The interference with other cells has led to a weak absorbance of these specific molecules. Compared to M3, the accumulation of diverse aerobic bacteria in M4 was developed fast, which might be attributed to the specific interactions between localized molecular groups. The cohesion occurred between bacteria species has accelerated the produce of extracellular polymeric substances that were beneficial for the attachment of planktonic microorganism. When the microorganisms have adhered to each other, they grew progressively to create an accumulation of large number of bacteria on microalgae cell surface.

In order to compare the difference of adherence of microalgae supporting by divalent cations and bacteria, the SEM images of the harvested biomass obtained from M1, M7 and M8 were observed. There was a discrete connection of microalgae cells in M1 and M8, while bacteria in other mediums, suggesting that the bacteria size was much smaller than microalgae. Moreover, the extracellular polymeric substances appeared as a messy stacking in every microalgae cells when observed under compound microscope. It was therefore concluded that the role of bacteria on aggregation of microalgae cells to form the bioflocs is indispensable because of their small size and the capacity to secrete extracellular polymeric substances. The proposed bioflocculation technique in this work is viable for microalgae harvesting and wastewater treatment in a single step. This method is associated with several advantages, such as simplicity, low cost, low energy consumption and environmentally friendly.

4. Conclusions

Bioflocculation using bacteria in wastewater effluent is an alternative approach for harvesting the microalgae biomass from the huge volume of culture broth. The study demonstrated the use of seafood wastewater in cultivation and harvesting of *C. vulgaris*. The direct use of untreated SWE as culture medium for *C. vulgaris* allowed the flocculation activity of 92.0 ± 6.0%, TTS removal of 93.0 ± 5.5%, and nutrient removal of 88.0 ± 2.2%. The MaB-flocs collected under this optimal condition contained dry matter of 107.2 ± 5.6 $g{\cdot}L^{-1}$ and chlorophyll content of 25.5 ± 0.2 $mg{\cdot}L^{-1}$.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2018.09.146.

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