Overcoming Microalgae Harvesting Barrier by Activated Algae Granules

Olga Tiron1, Costel Bumbac1, Elena Manea1, Mihai Stefanescu1 & Mihai Nita Lazar2

The economic factor of the microalgae harvesting step acts as a barrier to scaling up microalgae-based technology designed for wastewater treatment. In view of that, this study presents an alternative microalgae-bacteria system, which is proposed for eliminating the economic obstacle. Instead of the microalgae-bacteria (activated algae) flocs, the study aimed to develop activated algae granules comprising the microalgae Chlorella sp. as a target species. The presence of the filamentous microalgae (Phormidium sp.) was necessary for the occurrence of the granulation processes. A progressive decrease in frequency of the free Chlorella sp. cells was achieved once with the development of the activated algae granules as a result of the target microalgae being captured in the dense and tangled network of filaments. The mature activated algae granules ranged between 600 and 2,000 µm, and were characterized by a compact structure and significant settling ability (21.6 ± 0.9 m/h). In relation to the main aim of this study, a microalgae recovery efficiency of higher than 99% was achieved only by fast sedimentation of the granules; this performance highlighted the viability of the granular activated algae system for sustaining a microalgae harvesting procedure with neither cost nor energy inputs.

Integrating microalgae biomass production with the wastewater treatment step is a promising approach for the implementation of a sustainable wastewater treatment technology that considers economical, ecological, and social impacts. This statement is based on the fact that microalgae-bacteria processes could sustain a greenhouse gas mitigation strategy1 and the elimination of the aeration costs with simultaneous production of a valuable residual biomass that could be used for the production of bioenergy and high-value chemicals2, 3 (Fig. 1). Moreover, the production of microalgae biomass during wastewater treatment is considered as a condition for sustaining the economic viability of algae-based biofuel production4, microalgae biomass being classified as a third generation renewable resource for bioenergy production5. Microalgae-bacteria flocs developed during wastewater treatment are called ‘activated algae’ flocs, the term being used for the first time several decades ago during experimental research performed by McGriff and McKinney6.

However, wastewater treatment technology based on activated algae processes faces several drawbacks. Microalgae harvesting is one of the major constraints in microalgae biotechnology development at an industrial scale7, 8 due to the substantial costs and energy involved in this step. Frequently applied harvesting techniques can account for between 20 and 30% of total microalgae cultivation costs8. Moreover, the economic impact of the microalgae harvesting step compromises the development of a microalgae-based biofuel production industry8, with up to 50% of the total costs for biofuel production being attributed to the harvesting step9. This economic inefficiency is caused by the low settling velocity of the microalgae (lower than 0.0036 m/h) and their density, which is similar to that of water10, with the microalgae species commonly used in wastewater treatment processes and the bioenergy production sector usually having a cell size of less than 30 µm11.

No universal method was delineated for the microalgae harvesting step11, and several physical, chemical, and biological techniques have been proposed to date. Processes such as centrifugation, chemical coagulation-flocculation, filtration, gravity sedimentation, and flotation represent frequently applied harvesting techniques12. Centrifugation is known as the most time-efficient method, and also as the most energy-intensive when it is applied as a primary step for microalgae harvesting; an energy consumption of higher than 3,000 kWh/t microalgae has been reported12. Compared with centrifugation, chemical flocculation requires less energy13. However, the method presents several important drawbacks, such as end-product contamination with metal salts, high costs for flocculant acquisition (especially at an industrial scale), as well as the sensitivity of the procedure

1Department of Environmental Technologies and Technological Transfer, National Research and Development Institute for Industrial Ecology - ECOIND, 71–73 Drumul Podu Dambovitei, 060652, Bucharest, Romania.
2Department of Pollution Control, National Research and Development Institute for Industrial Ecology - ECOIND, 71–73 Drumul Podu Dambovitei, 060652, Bucharest, Romania. Correspondence and requests for materials should be addressed to O.T. (email: olga.tiron@incdecoind.ro)
for the following factors: pH, the morphology of the microalgal cells, the stage of the microalgal life cycle, and flocculant type. Gravity sedimentation is applied for activated sludge recovery, being the most cost-suitable harvesting method. However, in the case of the activated algae process, it is best applied to algae species larger than 70 µm, otherwise it becomes a very time-consuming method (several days), which compromises cell integrity. In comparison with sedimentation, the flotation technique is considered to be more effective.

Bio-flocculation represents an environmentally friendly harvesting technique comprising microalgae cells aggregation with different microorganisms, such as bacteria, autoflocculating microalgae and filamentous fungi. Therefore, use of microalgae species in wastewater treatment processes leads to an increase in microalgae recovery efficiency by developing the microalgae-bacteria flocs. For instance, the microalgae-bacteria flocs used by de Godos et al. for wastewater treatment exhibited a settling rate of 0.28–0.42 m/h.

Taking into consideration the particularities of the discussed harvesting techniques, as well as the emerging studies in the microalgal biotechnology field, it seems that none of the proposed harvesting techniques are economically and/or ecologically viable at an industrial scale; therefore, the development of a cost and energy efficient, effective, and environmentally friendly harvesting technique continues to be one of the most discussed strategies in microalgae-based technology.

This study focused on developing and describing an alternatively activated algae system, comprising granular entities, proposed as a solution for sustaining a cost and energy efficient microalgae harvesting step. The assessment of the activated algae granules' viability for microalgae biomass recovery was conducted by presenting the main granules features, such as morphology, biological structure and settleability, and by analyzing the microalgae recovery efficiency that could be obtained after granules sedimentation.

Results and Discussion

Evolution of the activated algae flocs size vs. granules. Chlorella sp. represents one of the smallest taxa from the microalgae category. In the hereby conducted experiments, the size of the Chlorella sp. cells ranged between 1.3 and 7.6 µm, with mean cell size being 3.51 ± 0.18 µm.

The activated algae system used as an inoculum in the granulation process was represented by irregular, mainly open flocs, with a high frequency of the filamentous microalgae and target microalgae (Fig. 2a). A high abundance of filaments and free Chlorella sp. cells was also recorded in the liquor. The volume distribution of the activated algae flocs size is illustrated in Fig. 3a. The distribution curve presented two sections: section I, which represented the volume distribution of the free target microalgae cells size, and section II, which represented the volume distribution of the activated algae flocs size. According to the recorded results, only a small percentage (1.26 ± 0.06%) of the total volume was attributed to the flocs under 25 µm, with the highest proportion (78.8 ± 1.21%) being represented by large flocs (>250 µm), the remaining percentage of 19.9 ± 1.12% comprising medium-sized flocs (25–250 µm). The mean size of the activated algae flocs was 598.24 ± 22.71 µm.
With the increase in number of the culturing batches during the granulation process, a decrease in distribution of the free target microalgae was noticed. As a result, after 59 culturing batches, the volume of the *Chlorella* sp. cells outside flocs was 74.6 ± 2.3% lower compared to the first batch. In spite of this sharply decreasing tendency, the presence – with a high frequency – of free target cells in liquor was still noticed. Compared with the first batch, after 59 batches, the volume of the small and medium-sized activated algae flocs decreased by about 52.3 ± 1.6 and 36.5 ± 0.63%, respectively. As a result, the volume of the large-size flocs increased by 22.2 ± 1.16% and the mean size of the activated algae flocs increased to 730.62 ± 11.28 µm. The microscopy investigations underlined an evolution of the flocs from an irregular shape to a round shape. Floc structure remained open, being governed by the filaments’ development (Fig. 2b).

After 122 batches, the round shape of the activated algae aggregates was clearly defined (Fig. 2c) and complete removal of particles smaller than 10 µm was obtained (Fig. 3a). These results showed that target microalgae cells were efficiently entrapped into activated algae aggregates whose mean size increased to 968.3 ± 19.7 µm. Moreover, no presence of the small flocs was detected, the activated algae system being mainly represented by large flocs (96.6 ± 0.26%), with the volume of medium-sized flocs being 3.41 ± 0.25%. At this stage, development of compact flocs was emphasized.

At the applied operational conditions, the granulation of the activated algae flocs was considered accomplished after 153 culturing batches. At this stage, granules size was higher than 300 µm, with almost 65.9 ± 2.3% of them ranging between 720 and 1,450 µm. The mean size of the granules was 1,034.8 ± 1.9 µm. Developed activated algae granules were characterized by a compact structure (Fig. 2d). During this stage, a high frequency of rotifer population was also noticed.

Further culturing of the activated algae granules contributed to the increase of the granules’ mean size to 1,176.1 ± 5.4 (batch 197) and 1,343.7 ± 1.8 µm (batch 237; Fig. 3b). Moreover, light microscopy images showed an increase of granule compactness level, property also underlined by the dark-green colour of the granules (Fig. 4c) compared to the light green observed during the granulation process (Fig. 4a,b). The dry weight of the granules, reached after 197 batches, was found to be 75.6 ± 17.6 µg/granule; at the end of the experimental part of the study, granule weight increased to 155.7 ± 20.9 µg/granule.

It is worth pointing out that after 237 batches, the volume of the granules with a diameter between 720 and 1,450 µm increased to 90.4 ± 1.13%. Moreover, during the entire culturing period (assigned to the granulation process).
The volume of the granules larger than 1,450 µm was kept lower than 11%, with less than 2% reaching 1,900 µm. Taking into consideration the results stated above, it could be concluded that the optimum size of the granules ranged between 700 and 1,500 µm. An increase in granule size to above 1,500 µm could act as a limiting factor for granule development, possibly due to the decrease of nutrients and light diffusion caused by the highly compact structure. However, the adaptation processes could strongly influence the evolution of the volume distribution curve of the granule size. Once with granules formation, due to light and nutrients diffusion gradients, different growth conditions (aerobic, anoxic, anaerobic) could be achieved causing the organization of aggregates into multilayer structures, each layer providing conditions for the development of the specific microorganisms populations. As a result, the development of granules above 2,000 µm could be expected, with further research being needed in this case.

Properties of the activated algae granules. Light microscopy images of the crushed activated algae granules showed the presence of bacteria – target microalgae Chlorella sp., and filamentous microalgal Phormidium sp. – populations inside granules (Fig. 5a). According to the SEM images, it was concluded that the granular structure was developed through both non-flocculative and bioflocculative processes. The non-flocculative process comprised the microalgal filaments interwoven in a tangled, dense, globular network.
(Fig. 6a,b), the set-up stirring conditions being one of the key factors for the occurrence of this process. As a result, during the activated algae granules' development, the free target microalgae cells were captured in a dense network of filaments, with the filamentous microalgae acting as an efficient 'biological filter.' The performance achieved in this case was emphasized not only by the volume distribution curve of the activated algae aggregates size (as represented in Fig. 3a), but also by the light microscopy investigations. For instance, after granule crushing, the microscopy images revealed the release of a rich population of the target microalgae cells from the granular structure (Fig. 5b,c).

The bioflocculation process consisted of the aggregation of microalgae and bacteria biomass by released biopolymers. The occurrence of this process inside granules is shown in Fig. 6c. It was observed that the filaments acted as a biological support for both the microalgae and bacteria cells, promoting the development of these species inside granules. Therefore, besides the 'biological filter' role, the filamentous microalgae had a similar role to that of the filamentous bacteria present in conventional activated sludge flocs, where these species represent flocs'
Moreover, the filamentous microalgae *Phormidium* sp. have the ability to self-aggregate\textsuperscript{22} and present a gelatinous matrix\textsuperscript{23}, properties that could also influence the efficiency of the target microalgae entrapment and maintenance inside the granular structure. In addition to the target microalgae, the presence of *Nitzschia palea* diatoms was also noticed inside the granules (Fig. 6d). Thus, the developed activated algae granules could be considered for harvesting larger-size species than *Chlorella* sp., according to the microscopy investigations, with the presented diatoms having a cell size greater than 4 µm wide and 20 µm long.

Developing the activated algae flocs and activated algae granules in real dairy wastewater promoted the development of a viable biomass, with potential application in wastewater treatment processes. The viability of the developed activated algae granules in wastewater treatment processes have been proven in our previous research\textsuperscript{24} where the symbiotic relationship developed between bacteria and microalgae inside the granular structure contributed to: (1) almost complete removal of organic matter, using only the oxygen provided through photosynthesis processes; (2) the complete removal of ammonium, with the performance promoted through cell assimilation and nitrification processes; and (3) the occurrence of the denitrification processes sustained by the variation of oxygen saturation during the conducted treatment batches and possibly by the dense granular structure. In addition to the role of the *Phormidium* sp. taxa in sustaining the granular structure, the cyanobacterial populations were also involved in wastewater treatment processes along with the target microalgae cells, with both populations representing oxygen-producing microorganisms. The co-existence between filamentous cyanobacteria *Phormidium* sp. and other microalgae could have been achieved due to the fact that the *Phormidium* genus is characterized by a high tolerance for microalgae diversity, as shown by Pouliot et al.\textsuperscript{25}. Thus, *Phormidium* sp. could be considered for sustaining in each granule the development of a stable trophic network. Moreover, these properties could play an important role in the analysis of biotechnology viability beyond laboratory scale, where the microalgae community could undergo severe taxonomic changes.

However, more research is still required for decreasing the duration of granulation process (from suspended biomass to granules). Possible industrial scale start-up of a wastewater treatment plant based on activated algae granules could be performed either from suspended biomass followed by granulation, either by inoculating with previously developed granules (in pilot scale or in another wastewater treatment plant).

Based on the SEM investigations, it could be assumed that a ‘porous’ granular structure could promote a more efficient diffusion of the organic matter, nutrients and oxygen inside granules compared to granular activated sludge. This property could have a considerable influence on granules’ speciation and on the efficiency of their use in wastewater treatment processes, with further investigations being required in this case.

**Granules settleability.** The settling rate of the target microalgae cells *Chlorella* sp. was found to be lower than $0.54 \times 10^{-2}$ m/h. In comparison with the target microalgae, the settling rate of the filamentous cyanobacteria *Phormidium* sp. could reach 12 m/h\textsuperscript{26}, mostly due to their self-aggregation ability, morphology and larger size.

![Figure 6. SEM images of the activated algae granule. (a) Activated algae granule with emphasis on (b) granule structure, and (c) inside populations: target microalgae *Chlorella* sp. (I), bacterial biomass (II), filamentous microalgae (III), and (d) *Nitzschia palea* diatoms.](image-url)
the settling phase and (c) in the first seconds of remaining suspended due to their small cell size, and mature activated algae granules: (d) after 2 min of settling.

Figure 7. The influence of activated algae granules development on biomass settling velocity. (a) Settling rate of the activated algae biomass recorded during the granulation procedure in respect to the investigated batch number. (b) Sedimentation of the activated algae flocs (after 1 h), the free target microalgae cells (Chlorella sp.) remaining suspended due to their small cell size, and mature activated algae granules: (c) in the first seconds of the settling phase and (d) after 2 min of settling.

Microalgae recovery efficiency. Due to the small cell size, harvesting Chlorella sp. biomass is an expensive procedure. Even the centrifugation method is insufficient for harvesting these microalgae species36, with application of the combined methods being necessary to increase recovery efficiency. However, the use of two or more harvesting techniques rarely contributes to a microalgae recovery efficiency higher than 95%.

In this study, the residual chlorophyll a concentration found in effluents, after activated algae granules settling, ranged between 2.65 ± 0.41 and 54.13 ± 6.9 µg/L, with the mean result being 21.8 ± 2.5 µg/L. As a result, the microalgae recovery efficiency varied between 99.24 ± 0.08 and 99.93 ± 0.014%, with a mean value of 99.63 ± 0.029%. The results obtained suggest that the granulation of the activated algae flocs using filamentous microalgae (Phormidium sp.) represents a viable solution for almost complete recovery of the microalgae biomass (and implicitly of the target Chlorella sp. cells) using gravity sedimentation as a single harvesting technique. The development of the dense activated algae granules could be considered a promising alternative solution, not only for saving both costs and energy in the harvesting step, but also in the microalgae dewatering step, which is another barrier for the development of microalgae-based technology36, with further research being needed to sustain this statement.

As reported above, the activated algae biocenosis included rotifer populations. The presence of these species in liquor could influence microalgae recovery performance by capturing the remaining free target microalgae cells, as rotifers are well known for being grazers of these species3. In practice, the optimum harvesting method is chosen based on several factors, such as microalgae species, cell density and culturing conditions7,8. Using activated algae granules, the previously mentioned conditions could be overlooked. Moreover, taking into consideration the presented granules characteristics, it could be stated that the granulation procedure could be applied in the case of other Chlorella-like microalgae with a similar cell size characterized by poor settleability.

Almost complete microalgae recovery was also reported by Zhou et al.32. This particularity could be a major barrier for the integration of algae-fungal pellets into conventional wastewater treatment processes, with the efficiency of the activated sludge process decreasing at pH values lower than 6.5. Moreover, the optimal activity of several important bacteria (such as nitrifiers) occurred between 7.2 and 8.0 pH values33. Compared with fungi-algae pellets, the activated algae granules were developed at common pH values registered during conventional wastewater treatment processes (pH 6.5–8.5). Moreover, our previous investigations showed a high tolerance of granule structure at higher variations of pH (5–8.5)24. Such properties sustain the feasible character of the activated algae granules for...
further application in wastewater treatment processes. Further investigations must be taken into consideration for describing the response of the granular activated algae system at industrial scale conditions.

Considering potential industrial scale application for wastewater treatment, the harvesting process of activated algae granules can be performed during the settling phase (considering SBR operation) or in the secondary settler (for continuous flow conditions).

**Methods**

**Materials.** The granulation process was performed using conventional activated algae flocs as inoculum. Taking into consideration that this approach was followed in order to use the proposed method to develop activated algae granules for wastewater treatment, it was considered appropriate to use activated algae flocs, which comprise native conventional microalgae and bacteria species adapted to wastewater treatment conditions. As a result, microalgae and bacteria taxa were obtained by sampling a biofilm developed on the inner wall of a laboratory-scale sequencing batch reactor used for dairy wastewater treatment. In this study, conventional microalgae were considered to be any microalgae species smaller than 30 µm, which could be used in wastewater treatment processes, except filamentous microalgae.

The hereby discussed method of activated algae flocs granulation is a biological method that relies on the use of filamentous microalgae. Therefore, besides conventional microalgae and bacteria species, it was necessary to include filamentous microalgae in the biomass. The microscopic investigations performed on sampled biofilm showed the presence of all the above-mentioned populations (conventional microalgae, filamentous microalgae, and bacteria). Thus, in this study, it was not necessary to use an external source of filamentous microalgae. According to the microscopic investigations, the filamentous microalgae were represented by cyanobacteria populations *Phormidium* sp. The prevalent genus from the *Chlorophyte* category was *Chlorella* sp., single-cell species, which are frequently used in wastewater treatment processes, as well as for biomass production34. In view of the above findings, *Chlorella* sp. taxa were chosen as target microalgae.

**Experimental set-up.** The experimental part of the study comprised four major steps, which are illustrated schematically in Fig. 8.

*Step 1.* The sampled biomass was enriched in mixed raw dairy wastewater – *Chlorella* broth media using Erlenmeyer flasks (250 mL), as described in our previous research35. This step was conducted to increase the biomass of the target microalgae while simultaneously maintaining the native bacterial populations. The biomass cultivation was carried out for about 3 months.

**Figure 8.** Schematic illustration of the experimental steps. Step 1 – cultivation of the microalgae – bacteria inoculum; step 2 – development of the conventional microalgae-bacteria system; step 3 – implementation of the activated algae granulation procedure; step 4 – validation of the obtained alternative activated algae system in downstream process (microalgae harvesting).
Step 2. The biomass obtained from the first step was used for the development of the conventional activated algae flocs. The process was carried out in a 4 L stirred tank photobioreactor (PBR) Bplus (BIOSTAT®, Sartorius, Germany). The development of the activated algae flocs was carried out in sequential batch operation mode (SBR), with each culturing batch consisting of the following consecutively applied phases: (I) PBR feeding with 2 L of pretreated, unsterilized dairy wastewater supplemented with NH4Cl (45 mg/L), MgSO4· 7H2O (100 mg/L), K2HPO4 (15 mg/L), and CaCl2· 2H2O (30 mg/L), to increase the concentration of NH4+, Mg2+, PO43−, and Ca2+, respectively. As a result, the physicochemical parameters of the influent varied at the following intervals: pH (6.5–7.3), O2 (1–3%), chemical oxygen demand (COD; 127.4–195.6 mg O2/L), NH4+ (15.3–21.8 mg/L), NO3− (<0.1 mg/L), NO2− (7.3–14.2 mg/L). The set-up operational conditions were as applied during activated algae granulation, except that the hydraulic retention time was maintained at 23 h 35 min, the liquor was stirred at a frequency of 120 rpm, and the time allocated for the granules’ sedimentation was 5 min. The PBR feeding and effluent withdrawing phases lasted 10 min each. In these conditions, 83 batches were conducted. During this step, the research mainly focused on the following issues: the determination of the microalgae recovery efficiency after the activated algae granules’ sedimentation (during the settling phase), the investigation of the dynamics of the volume distribution curve of the granules’ size, the microscopy examination of the mature activated algae granules, and the monitoring of the granules’ settleability and weight.

Microalgae recovery efficiency. The microalgae recovery efficiency was determined based on chlorophyll a concentration44, according to the following equation:

\[ R = \frac{[\text{Chla}_I - \text{Chla}_F]}{\text{Chla}_I} \times 100\%, \quad (1) \]

where R represents the microalgae recovery efficiency recorded after the activated algae granules’ sedimentation in the settling phase (5 min) (%), ChlaI is the chlorophyll a concentration of the granular activated algae biomass (mg/L), and ChlaF represents the residual chlorophyll a concentration from effluent withdrawn after granules settling (mg/L).

Activated algae granule weight. The dry weight of the mature activated algae granule was calculated according to the method described in the previous study39 and by applying the following formula:

\[ DW = \frac{[(F_{90} - F_9) \times 10^6]}{N}, \quad (2) \]

where DW represents the dry weight of a granule (µg), F90 is the weight of the glass-microfibre filter (pore size 0.45 µm) with activated algae granules measured after drying at 90°C for 24 h (g), F9 is the weight of the glass microfibre filter (g), and N represents the number of activated algae granules on the filter. The analysis was performed in triplicate.

Particle size distribution and microscopy investigations. The volume distribution of the target microalgae cells, activated algae flocs and activated algae granules size was measured using analyzer Mastersizer 2000 (Malvern Instruments, Malvern, UK), based on the laser diffraction method, the refractive index being set at 1.06044. The biological samples were examined using the Optech B1 light microscope (Germany) and the scanning electron microscope (SEM) Quanta 250 FEG (FEI, Netherlands).

Biomass settling velocity. 250 mL of homogenous sample was transferred into a graduated cylinder (250 mL, 0.23 m height). The activated algae aggregates were allowed to settle and the biomass position in the cylinder was recorded during the settling time. The settling velocity of the activated algae aggregates was calculated as follows:
where $S$ represents the settling velocity of the activated algae aggregates (m/h) and $P$ is the height (m) crossed by biomass between initial time ($T_0$) and final time ($T_f$) (h).

**Analytical methods.** The pH and oxygen saturation values were determined by EasyFerm Plus K8 (200) electrode (Hamilton, Bonaduz, Switzerland) and OxyFerm FDA (225) sensor (Hamilton, Bonaduz, Switzerland), respectively. COD was analysed according to SR ISO 6060 standard based on the potassium dichromate method. The SR ISO 14911 standard was used for measurement of NH$_4$ concentration, while the SR EN ISO 10304/1 standard was applied in the case of NO$_3$-, NO$_2$-, and PO$_4^{3-}$, analyses performed using the ion chromatography system ICS-3000 ( Dionex, Sunnyvale, CA, USA). The chlorophyll $a$ concentration was determined according to ISO standard SR 10260 using UV-Vis spectrophotometer (Model DR/5000 TM, Hach Lange, Düsseldorf, Germany). The analyses was performed by using ethanol ($\geq 96\%$) purchased from Chemical Company (Iasi, Romania).

All analyses were carried out on duplicate samples, the results being expressed as the means and standard errors.

**References**

1. Wilson, M. H. *et al.* CO$_2$ recycling using microalgae for the production of fuels. *Appl. Petrochem. Res.* 4, 41–53 (2014).
2. Savvidou, M. & Kolisis, F. Studies on the role of different culture conditions on the growth and biochemical composition of marine microalgae *Nannochloropsis* sp. *New Biotechnol.* 31, Supplment, 599 (2014).
3. Mata, M. T., Martins, A. A. & Caetano, S. N. Microalgae for biodiesel production and other applications: a review. *Renew. Sust. Energy. Rev.* 14, 217–232 (2010).
4. Christenson, L. & Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv.* 29, 686–702 (2011).
5. Brennan, L. & Owende, P. Biofuels from microalgae - a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energy. Rev.* 14, 557–577 (2010).
6. McGriff, C. E. & McKinney, E. R. The removal of nutrients and organics by activated algae. *Water Res.* 6, 1155–1164 (1972).
7. Gonzalez-Fernandez, C. & Ballesteros, M. Microalgae autoflocculation: an alternative to high-energy consuming harvesting methods. *J. Appl. Phycol.* 25, 991–999 (2013).
8. Pragya, N., Pandey, K. K. & Sahoo, P. K. A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renew. Sust. Energy. Rev.* 24, 159–171 (2013).
9. Molina Grima, E., Belarbi, F.-H., Fernandez, F. G. A., Medina, A. R. & Chisti, Y. Recovery of microalgal biomass and metabolites: process option and economics. *Biotechnol. Adv.* 20, 491–515 (2003).
10. Gerde, A., Yao, L., Lio, J.-Y., Wen, Z. & Wang, T. Microalgae flocculation: impact of flocculant type, algae species and cell concentration. *Algol Res.* 3, 30–35 (2014).
11. Muradov, N. *et al.* Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. *Biotechnol. Biofuels* 8, 24 (2015).
12. Granados, M. R., Acien, F. G., Gomez, C., Fernandez-Sevilla, J. M. & Grima Molina, E. Evaluation of flocculants for the recovery of *Phormidium* sp. Microalgae harvesting and subsequent biodiesel production. *Bioresour. Technol.* 118, 102–110 (2012).
13. Wang, H., Gao, L., Chen, L., Guo, F. & Liu, T. Integration process of biodiesel production from filamentous oelaginous microalgae *Trichonema minus*. *Biotechnol. Biofuels* 6, 39–44 (2013).
14. Rashid, N., Ur Rahman, M. S., Sadiq, M., Mahmood, T. & Han, J.-I. Current status, issues and developments in microalgae derived biodiesel production. *Renew. Sust. Energy. Rev.* 40, 760–778 (2014).
15. Chen, C.-Y., Yeh, K.-L., Ayisah, R., Lee, D.-I. & Chang, J.-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresour. Technol.* 102, 71–81 (2011).
16. Schenk, M. P. *et al.* Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenerg. Res.* 1, 20–43 (2008).
17. Tran, D.-T. *et al.*. Microalgae harvesting and subsequent biodiesel conversion. *Bioresour. Technol.* 140, 179–186 (2015).
18. Chatsungnoen, T. & Chisti, Y. Harvesting microalgae by flocculation-sedimentation. *Algal Res.* 13, 271–283 (2016).
19. Laamanen, C. A., Ross, G. M. & Scott, J. A. Floation harvesting of microalgae. *Renew. Sust. Energy. Rev.* 58, 75–86 (2016).
20. de Godois, I. *et al.* Assessing carbon and nitrogen removal in a novel anoxic-aerobic cyanobacterial-bacterial photobioreactor configuration with enhanced biomass settleability. *Water Res.* 61, 77–85 (2014).
21. Jenkins, D., Richard, G. M. & Daigler, T. G. *Manual On The Causes And Control Of Activated Sludge Bulking, Foaming, And Other Solids Separation Problems*. Third ed., Lewis Publishers, London (2004).
22. Talbot, P. & de la Noüe, J. Tertiary treatment of wastewater with *Phormidium bolnieri* (Schmidle) under various light and temperature conditions. *Water Res.* 27, 153–159 (1993).
23. Bellinger, E. G. & Sige, D. C. *Freshwater Algae: Identification And Use As Bioindicators*. John Wiley & Sons Ltd, Chichester (2010).
24. Tiron, O., Bumbac, C., Patroescu, I. V., Badescu, V. R. & Postolache, C. Granular activated algae for wastewater treatment. *Water Sci. Technol.* 71(6), 832–839 (2015).
25. Pouliot, Y., Buelna, G., Racine, C. & de la Noüe, J. Culture of cyanobacteria for tertiary wastewater treatment and biomass production. *Biol. Waste* 29, 81–91 (1989).
26. Bui, T. T. & Han, M. Removal of *Phormidium* sp. by positively charged bubble flotation. *Miner. Eng.* 72, 108–114 (2015).
27. Adar, S. S., Lee, D.-J., Show, K.-Y. & Tay, J.-H. *Aerobic granular sludge: recent advances*. *Biotechnol. Adv.* 26, 411–423 (2008).
28. Zheng, Y.-M., Yu, H.-Q. & Sheng, G.-P. Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor. *Process Biochem.* 40, 645–650 (2005).
29. Rusten, B. & Sahu, K. A. Microalgae growth nutrient recovery from sludge liquor and production of renewable bioenergy. *Water Sci. Technol.* 64, 1195–1201 (2011).
30. Guldhe, A., Misra, R., Singh, P., Rawat, I. & Bux, F. An innovative electrochemical process to alleviate the challenges for harvesting of small size microalga by using non-sacrificial carbon electrodes. *Algal Res.* 19, 292–298 (2016).
31. Zhou, W. *et al.* Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microbial cells. *Sep. Purif. Technol.* 107, 158–165 (2013).
32. Xie, S., Sun, S., Dai, S. Y. & Yuan, J. S. Efficient coagulation of microalgae in cultures with filamentous fungi. *Algal Res.* 2, 28–33 (2013).
33. Gerardi, M. H. *Nitritification And Denitrification In The Activated Sludge Process*. John Wiley & Sons Inc., New York (2002).
34. Abdel-Raouf, N., Al-Homaidan, A. A. & Ibraheem, I. B. M. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* 19, 257–275 (2012).
35. Gerardi, M. H. *Nitrification And Denitrification In The Activated Sludge Process*. John Wiley & Sons Inc., New York (2002).
36. Abdel-Raouf, N., Al-Homaidan, A. A. & Ibraheem, I. B. M. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* 19, 257–275 (2012).
35. Tricolici, O., Bumbac, C., Patroescu, V. & Postolache, C. Dairy wastewater treatment using activated sludge – microalgae system at different light intensities. *Water Sci. Technol.* **69**, 1598–1605 (2014).

36. Aas, E. Refractive index of phytoplankton derived from its metabolite composition. *J. Plankton Res.* **18**, 2223–2249 (1996).

37. SR ISO 6060. *Water Quality - Determination Of The Chemical Oxygen Demand*. Romanian Standards Association, Bucharest (1996).

38. SR EN ISO 14911. *Water Quality - Determination of Dissolved Li⁺, Na⁺, NH₄⁺, K⁺, Mn²⁺, Ca²⁺, Mg²⁺, Sr²⁺ and Ba²⁺ Using Ion Chromatography. Method For Water And Wastewater*. Romanian Standards Association, Bucharest (2003).

39. SR EN ISO 10304/1. *Water Quality - Determination Of Dissolved Anions By Liquid Chromatography Of Ions (Part I). Determination Of Bromide, Chloride, Fluoride, Nitrate, Nitrite, Phosphate And Sulfate*. Romanian Standards Association, Bucharest (2009).

40. SR ISO 10260. *Water Quality - Measurement Of Biochemical Parameters. Spectrometric Determination Of The Chlorophyll a Concentration*. Romanian Standards Association, Bucharest (1996).

41. Tricolici, O., Bumbac, C. & Postolache, C. Microalgae-bacteria system for biological wastewater treatment. *J. Environ. Prot. Ecol.* **15**, 268–276 (2014).

Acknowledgements
The study was funded under the Nucleu Program (PN 16-25 03 03) by the Romanian Ministry of National Education – State Authority for Scientific Research, Technological Development and Innovation.

Author Contributions
O.T. and C.B. conceived the project, designed the experiments and performed the experiments; O.T. created the figures and interpreted microscopy images; M.S. conducted particles size distribution analysis and analysed the data; O.T. and C.B. wrote the manuscript with comments from M.N.L. and E.M.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017