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Effects of settling time on the flocculation progress and treatment performance in the co-culture of microalgae-activated sludge photobioreactor

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Abstract:

The application of microalgae-based wastewater treatment systems using wastewater as a source of nutrients has been successfully developed in recent years and has brought about positive results in wastewater treatment and microalgal biomass recovery while producing valuable products. This study presents the application of a microalgae and activated sludge (AS) co-culture in a low agitation photobioreactor (aPBR), which could reduce energy usage. In addition, the results demonstrate the role of settling time on co-culture flocculation progress and wastewater treatment performance. The average Chemical Oxygen Demand (COD) removal was up to 76.1% of co-culture and was achieved using a PBR system with a low agitation speed of 80 rpm. Moreover, a high biomass growth was observed to be coupled with high nutrient removal. After 63 days of operation, heavy flocs with excellent settling ability were dominant in the photobioreactor. This would be a preliminary step for activated algae granulation in a co-culture system.

Keywords: **activated sludge, co-culture, flocculation, microalgae, wastewater treatment.**

Classification number: **5.3**

Introduction

Nowadays, biological processes are mainly applied to wastewater treatment plants, especially the activated sludge process (ASP). However, there are still many drawbacks of biological processes, for example, the need for pre-treatment, the high energy requirements for aeration and stirring, and low nutrient removal capacity [1, 2]. Moreover, the removal of phosphorus in wastewater, which is usually done by chemical and physical processes like flocculation, comes with high cost and low treatment efficiency. Due to these existing shortcomings, the application of microalgae-based wastewater treatment systems using wastewater as a source of nutrients has been successfully developed in recent years and brought about positive results to the treatment of nitrogen (N), phosphorus (P), and algal biomass recovery [3-5]. The advantage of using algae corresponds to the ability to remove nitrogen and phosphorus in wastewater through the assimilative process of biomasses, which has been studied as a post-treatment process [6], in urine [7], aquaculture wastewater [8], and livestock wastewater [9]. In addition, utilizing microalgae to convert carbon emissions into biomass is regarded as one of the most cost-effective methods of CO_2 reduction, and the obtained biomass of algae could become a high-profit product like biofuels, food sources, or nutraceuticals [10, 11]. However, the widespread adoption of microalgae-based technology is constrained due to high cost of operation and biomass harvesting. It is estimated that about 20-30% of operating costs during the cultivation of microalgae are from microalgal biomass harvesting through coagulation/flocculation, centrifugation, or flotation [12].

The symbiotic co-culture of microalgae and AS has been increasingly taken more advantage of due to the consistent ability of carbon dioxide to produce oxygen, which decreases the need for aeration, increases nutrient removal rates, and promotes high microalgae recovery [13-15]. On the other hand, a phenomenon called "bio-flocculation" appears when microalgae and other microorganisms are associated in a co-culture. Extracellular polymer compounds such as polysaccharides and proteins will be secreted into the medium to form flocs that cause bio-flocculation when co-cultivating microalgae and AS [16, 17]. Through the flocculation process, the size of the flocs increase by cell aggregation, which enhances the settling rate of the biomass. Bio-flocculation effectively eliminates the need for chemical flocculants, and thus represent an inexpensive, non-feasible, and toxic alternative for effective biomass harvesting. During flocculation, the sizes of the floc cells are increased by an aggregation of cells that can enhance the biomass settling rate. However, bio-flocculation is currently not widely applied in harvesting steps because of the high production cost of bio-flocculants and the challenge of controlling the process on an industrial scale. An ideal bio-flocculant should be inexpensive, nontoxic, and effective at low concentrations and it should preferably be derived from non-fossil fuel sources, thus being sustainable and renewable.

In this study, microalgae and activated sludge as co-cultures were experimented in a low aPBR to reduce the effort of energy usage. To promote an easy way to harvest the microalgae by gravity settling, two settling times of 3 h and 0.5 h were investigated in terms of wastewater treatment ability and bio-flocculation process of the co-culture system.

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Materials and methods

Microalgae-activated sludge co-culture

The co-culture of microalgae and activated sludge at the ratio 5:1 (%w:%w) was chosen for this experiment due to the higher nutrient removal efficiencies (5-40%) and biomass growth rate comparing to other inoculation ratios. The microalgae strain *Chlorella Vulgaris* was used in this study due to its well-known benefits (e.g., biofuels, medical application, etc.) [18]. The strain was taken from Aquaculture Research Institute 2, Ministry of Agriculture and Rural Development, Vietnam. The aerobic AS was taken from a membrane bioreactor (MBR) treating supermarket wastewater in Ho Chi Minh city. The initial biomass concentration of co-culture was 400 mg/l.

Synthetic wastewater

In this study, the co-culture was cultivated by synthetic wastewater with characteristics of 400±20 $mg.l^{-1}$ of COD. Total nitrogen (TN) was present in the form of ammonium at 40±1.4 $mg.l^{-1}$, and nitrate and nitrite nitrogen were not detected. The N/P mass ratio was about 10. In addition, 1 ml of trace elements was prepared following Bold's basal medium (BBM) [19, 20] per liter of feeding wastewater. The initial pH of synthetic wastewater was controlled by $NaHCO_3$ (10%) in the range 7-7.5 for the biological growth of microorganisms.

Experimental system set-up

The tubular PBR system consisted of a closed cylindrical acrylic column with a height of 41.75 cm and diameter of 17 cm. The water level was 30 cm with a total volume of 10 l and a working volume of 7 l. The PBR was operated at a low agitation speed of 80 rpm without aeration, which is called an PBR. The PBR system was installed in a wooden box with a thickness of 10 mm to prevent loss of light and temperature fluctuations (maintained around 27-32^{o}C). Light was provided by 3800-4000 lux LED lights (SMD 5050, China) with a light-dark cycle of 14:10 (h).

Operating conditions

The PBR system was operated with synthetic wastewater in a sequencing batch process of 24 h each cycle with a volume exchange ratio of 50%. Initially, one cycle consisted of 15 min influent addition, 20.5 h reaction phase (with agitation), and 3 h settling. The settling time was reduced based on the settling velocity at the steady state as follows: day 1-day 63 was 3 h, then day 63-day 90 was 0.5 h. The reaction phase correspondingly increased and a 15-min effluent withdrawal of the suspended biomass was done while the settled biomass was remained for use in the next batch. The effluent was withdrawn by an effluent valve and stored in an Erlenmeyer flask for water quality analysis within 1 d of sampling.

Analysis method

The samples of each reactor were collected daily. The influent was prepared weekly and analysed. For characterization of the microalgae and AS biomass, the mixed liquid suspended solids were collected via the middle valve in the reaction phase. Then, the COD, ammonia, NO_3-N, NO_2-N, total phosphorus (TP), and Mixed liquor suspended solids (MLSS) concentrations were analysed in accordance with standard methods [21].

In this research, microalgal biomass evaluation was conducted based on Chlorophyll-a (Chl-a) in the mixture of AS and microalgae [22]. Chl-a concentration was used to represent the growth of the algal biomass [23]. Chl-a in mixture of AS and microalgae was extracted by acetone solution [24] and a 20-ml well-mixed biomass sample was taken from the reactor to analyse sludge and algae characteristics. The biomass concentration and Chl-a concentration correlation was done through the following calibration curve of Chl-a and MLSS: y=7.7191x+759.04.

Results and discussion

As denoted in Fig. 1, after 11 day of operation, the total biomass concentration increased from 0.4 to 1.14 g/l for the co-culture system i.e., microalgae:AS inoculum ratio of 5:1 was strongly decreased to 1:2.33. It was observed during that time the flocculation process had yet not started (before day 63), the AS was dominant (microalgae fraction was below 10%), and the microalgae concentration decreased from 0.5 to around 0.105±0.041 g/l. The biomass fractions of microalgae and activated sludge also changed following the formation of bio-flocculants (Fig. 1). It is important to note that a major change of biomass fraction was observed between the initial and final stage of the experiment: the fraction of activated sludge remained stable at 84% in the first 30 day, then it decreased gradually to around 18% after day 63 when bio-flocculation occurred.

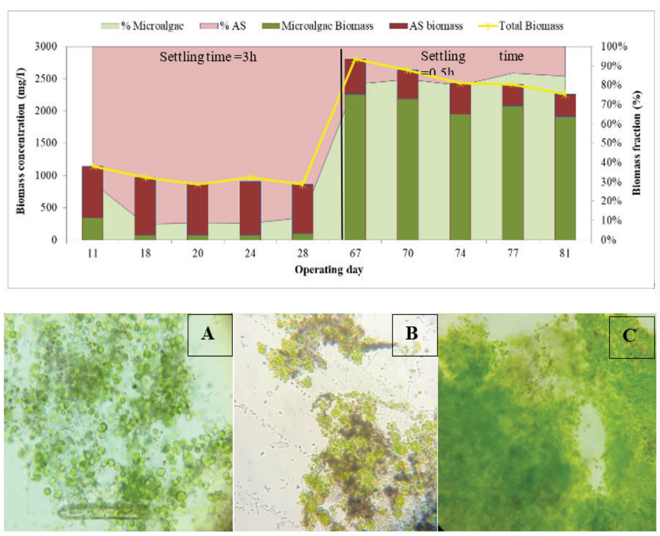

Fig. 1. Growth of microalgae and AS in the PBR at different settling times under low agitation speed (top) and microscopic observation on (A) day 14, (B) day 53, and (C) day 83 (x40) (bottom).

Another study [25] evaluated the growth of co-cultures in different microalgae where AS inoculum ratios were tested from 1:1 to 9:1, with a stirring speed of 100 rpm, and a low COD/N wastewater ratio of 2.5:1 and showed the opposite result. At the end of the experiment, the microalgae fraction inside all the co-culture systems were above 70% with the dominance of microalgae. The reason being that the composition of feeding wastewater for microalgae and AS comprised a high COD/N ratio (10:1). As it is known that microalgae exhibit shorter lifetimes than AS, there might have been a struggle to compete with AS for the nutrient uptake [26] and this high COD/N ratio could elevate the biomass growth rate of AS rather than that of microalgae due to sufficient COD in wastewater. Besides, under conditions of total biomass concentration over 1.0 g/l (before Day 63) with high AS density, a stirring speed of 80 rpm may not be sufficient to help microalgae access to light and overcome the dense cover of AS, thereby slowing their growth.

Biomass growth and flocculation

Despite growth being adversely affected, bio-flocculation was promoted and began being recognized clearly after day 63. In addition, reducing the settling time from 3 to 0.5 h enforces the bio-flocculation process by increasing the amount of small-sized biomass being washed out of the system. This will help classify the groups of microorganisms that tend to create high-density flocs, which are retained in the aPBR, and remove the low-density groups of microorganisms that tend to be suspended, which are released after discharge phase.

When appearing together in one co-culture, microorganisms can secrete extracellular polymer compounds, such as polysaccharides and proteins, to form bio-flocculants with microalgae. These results lead to a strong increase of microalgal biomass [27]. In the formed flocs, AS tends to act as a core to adhere the externally suspended algal cells (see Fig. 1B). This can be explained by the fact that microalgae are photoautotrophs, and the way they appear around the border of flocs could give them easier access to light outside the aPBR and overcome the covering phenomena by the AS. In addition, after reducing the settling time, the dense structure of microalgae-AS biomass (Fig. 1C) with higher settling rates in heavy flocs occupy less space inside the PBR but contain more microorganisms, which help to increase light penetration inside the co-culture and show positive results in enhancing microalgae growth.

With an agitation speed of 80 rpm, the microalgal growth significantly prevailed over AS growth. In fact, the bio-flocculant of algal-bacteria can accelerate the microalgal growth rates, enhance wastewater removal efficiency, boost the carbohydrates and lipid content in microalgae, promote microalgal flocculation processes, and facilitate microalgal cell wall disruption [28].

After reducing the settling time, aggregation of the biomass could already be observed, which also obviously influenced the settling velocity. Indeed, the co-culture flocculant obtained after day 63 exhibited a higher settling velocity of 3.56 m/h than only microalgae culture, which was less than 0.0036 m/h [29]. Therefore, after the flocculation process, the settling time was efficiently reduced from 3 to 0.5 h without any negative affect to the co-culture system. These results indicate that the effort to harvest microalgae could be reduced if the co-culture in PBR is operated under an appropriate operating condition by stimulating the flocculation process. However, bio-flocculation is a complex process that combines microalgae self-aggregation (i.e., filamentous microalgae), the flocculation of produced algae, and bio-flocculant-producing bacterial species (i.e., EPS) [30]. Agitation is responsible for creating cell-to-cell contact between microalgae and bacteria without cell stress or lysis over a period of time [31]. According to O. Tiron, et al. (2017) [29] and M. Xu, et al. (2015) [32], under certain hydrodynamic conditions, the formed flocs are strong and similar in size as the weaker ones break and re-flocculate until they are strong enough to resist the shear stress, i.e., turbulence, which can promote bio-flocculation by increasing the collision frequency while the shear stress can promote microbial cell aggregation.

Nitrogen removal

In terms of nitrogen pollutants, poor ammonium removal in co-cultures with low agitation speed was due to low hydraulic retention time and high ammonia concentration in feed water [33]. Fig. 2 has shown the ammonium removal performance during the whole experiment with initial concentrations of 40 mg/l for a 2 day hormone replacement therapy (HRT). After 1-2 days of co-cultivation, although the nitrifiers and microalgae did not present a good adaptation ability in the new environment in co-culture, heterotrophs in the activated sludge showed good ability in assimilated COD. During that time, when the ammonium removal only reached higher than 20%, nitrate concentration also showed a negligible value (<0.05 mg/l). However, after this beginning period, the nitrifiers showed good nitrification process in the following days. Nitrate concentration in the effluent has shown that when the first period had a dominance of bacteria in the microbial consortium, it showed better nitrification than the second one. Accordingly, the average removal efficiency of ammonium in the 3 h settling period was 42%, which is significantly higher than the 28% of the 0.5 h settling period. In the 3-h settling period, if ammonium removal took place by nitrification process of AS, it is uptake into biomass by microalgae in the 0.5 h settling time. In microalgae-bacteria systems, microalgae mainly provide oxygen for bacteria to remove organic substances and actively uptake nutrients [34]. However, without atmospheric CO_2, the photosynthesis of microalgae was limited as the only CO_2 supply was from AS, which leads to longer time for ammonium accumulation into microalgal biomass. At the end of the experiment, ammonium removal efficiency

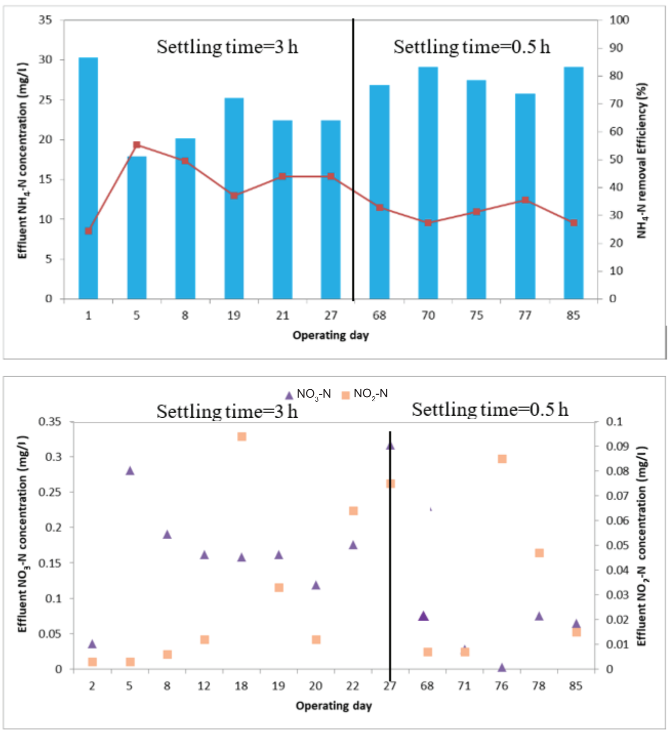

Fig. 2. Ammonia removal (top) and nitrogen compounds (bottom) in the PBR with different settling time.

reduced to lower than 30%, and this demonstrated a solution to increase the ammonium removal ability of co-culture that should be investigated in future studies.

On the other hand, the study by C.S. Lee, et al. (2015) [24] showed that nitrogen removal efficiency was positively related to light intensity and microalgal biomass. In this study, total biomass reached its highest concentration of 2.509±0.212 g/l on day 67 of the operation but tended to decrease to approximately 2.200 g/l by the end of the experiment. An increase of biomass concentration could lead to reduced light transmission, which may significantly reduce nutrient removal performance.

Phosphorus removal

Phosphorous in wastewater is primarily removed by biomass uptake and precipitation [35]. Throughout our experiment, the pH value remained in the range of 6.5-8.5, which means that phosphorous removal by precipitation was negligible. It can thus be confirmed that the phosphorus removal mechanism principally depends on biomass uptake capacity. Fig. 3 shows that after only 2 days of experiment, TP removal efficiency reach over 90%, which indicated that co-culture in PBR systems have a good ability for P uptake without aeration provision. During the whole experiment, TP removal efficiency varied from 90-97% without any significant fluctuation implying that there was no strong relationship between TP removal and settling time. Moreover, it seemed that bio-flocculation did not affect the phosphorus and nitrogen removal either. One possible explanation is due to the fact that nutrient removal is dependent on the O_2 generation rate and coupling with CO_2 from AS biodegradation, which was initially proportional to the algal density within a certain range. However, when the biomass concentration inside the PBR overcame the maximum supply of light, the nutrient removal efficiency rapidly reached its limit. After the microalgae concentration reached that value, the nutrient uptake rate possibly remained at a certain level, without any increase, following the growth of microalgae [36]. The remark from the results was the bio-flocculation could have occurred under stress condition (light-shading, high COD/N ratio, without aeration) and not coupling with high nutrient removal efficiency.

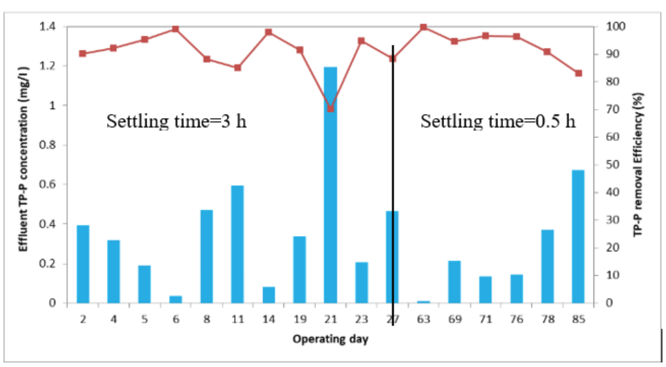

Fig. 3. Total phosphorus removal performance in the PBR with different settling time under low agitation speed.

COD removal

Concluding comprehensibly, AS treats organic matter in wastewater and produces CO_2 and inorganic nutrients required by microalgae, while microalgal photosynthesis generates O_2 back for the AS thus forming the synergistic relationship in co-culture systems [37]. The analysis of COD in effluent was done during the experiment (Fig. 4) with initial concentrations of 400 mg/l for a 2 day HRT. After 4 days of experiment, microbes in the co-culture removed 70% of COD, which showed that bacteria in activated sludge has a good ability to adapt to the new environment with microalgae. When the settling time was 3 h, there was a small fluctuation in COD removal efficiency of co-culture system. However, the COD removal efficiencies during that time were always above 70%, and gradually increased to over 90% when the settling time was reduced from 3 to 0.5 h. In another previous study, microalgae were demonstrated to play a dominant role in nitrogen removal via biological assimilation while activated sludge was responsible for improving COD removal [25]. In the first period in which AS was dominant, the COD removal efficiency was lower than when microalgae became dominant as bio-flocculant. These results imply that the COD removal by co-culture is accomplished by symbiotic interactions between activated sludge and microalgae, which help each other to enhance overall COD removal. The possible interactions are enhanced while the bio-flocculation was configured, and bacteria concentration remained low as well. At the end of experiment, while the bacteria took a small fraction in total biomass, the co-culture still had a good ability to remove COD up to 95.3% (day 85), which indicated the potential of applying co-culture in wastewater treatment using aPBR - an energy-saving system.

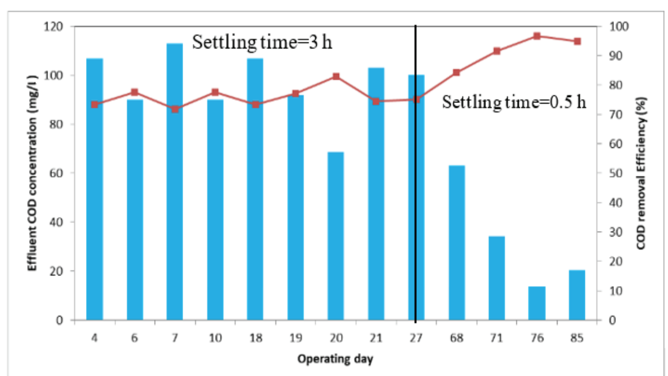

Fig. 4. COD removal performance in the PBR with different settling time.

Conclusions

Under the following typical conditions such as COD/N (10:1) in raw wastewater and a light:dark cycle of 14:10, the results obtained indicate the vital role of microalgae-AS co-culture in treating wastewater with a low agitation photobioreactor. The findings also suggest that the short settling time of 30 min could enhance the bio-flocculation of the microalgae-AS biomass. Besides, short settling times can be considered as an effective and easy strategy in co-culture operations to promote heavy floc formation. This would create an advantage in microalgae harvesting through bio-flocculation. However, due to the low nitrogen removal in this study, further research should be taken to overcome this current disadvantage.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] S. Longo, et al. (2016), "Monitoring and diagnosis of energy consumption in wastewater treatment plants. A state of the art and proposals for improvement," *Applied Energy*, **179**, pp.1251-1268.

[2] G. Quijano, J.S. Arcila, G. Buitrón (2017), "Microalgal-bacterial aggregates: Applications and perspectives for wastewater treatment", *Biotechnology Advances*, **35(6)**, pp.772-781.

[3] A. Ruiz-Martinez, N.M. Garcia, I. Romero, A. Seco, J. Ferrer (2012), "Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent", *Bioresource Technology*, **126**, pp.247-253.

[4] M.L. Menegazzo, G.G. Fonseca (2019), "Biomass recovery and lipid extraction processes for microalgae biofuels production: A review", *Renewable and Sustainable Energy Reviews*, **107**, pp.87-107.

[5] F.G. Acién Fernández, C. Gómez-Serrano, J.M. Fernández-Sevilla (2018), "Recovery of nutrients from wastewaters using microalgae", *Frontiers in Sustainable Food Systems*, DOI: 10.3389/fsufs.2018.00059.

[6] R. Honda, Y. Teraoka, M. Noguchi, S. Yang (2017), "Optimization of hydraulic retention time and biomass concentration in microalgae biomass production from treated sewage with a membrane photobioreactor", *Journal of Water and Environment Technology*, **15(1)**, pp.1-11.

[7] T.T. Nguyen, et al. (2021), "Nutrient recovery and microalgae biomass production from human urine source by membrane photobioreactor at low biomass retention time", *Science of the Total Environment*, **785**, DOI: 10.1016/j.scitotenv.2021.147423.

[8] R. Xing, W.W. Ma, Y.W. Shao, X.B. Cao (2018), "Growth and potential purification ability of Nitzschia sp. benthic diatoms in sea cucumber aquaculture wastewater", *Aquaculture Research*, **49(3)**, DOI: 10.1111/are.13722.

[9] D. García, C. Alcántara, S. Blanco, R. Pérez, S. Bolado, R. Muñoz (2017), "Enhanced carbon, nitrogen and phosphorus removal from domestic wastewater in a novel anoxic-aerobic photobioreactor coupled with biogas upgrading", *Chemical Engineering Journal*, **313**, pp.424-434.

[10] Y. Chisti (2007), "Biodiesel from microalgae", *Biotechnology Advances*, **25(3)**, pp.294-306.

[11] P.M. Schenk, et al. (2008), "Second generation biofuels: High-efficiency microalgae for biodiesel production", *BioEnergy Research*, **1**, pp.20-43.

[12] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux (2011), "Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production", *Applied Energy*, **88(10)**, pp.3411-3424.

[13] O. Tricolici, C. Bumbac, V. Patroescu, C. Postolache (2014), "Dairy wastewater treatment using an activated sludge-microalgae system at different light intensities", *Water Science and Technology*, **69(8)**, pp.1598-1605.

[14] S. Zhu, L. Qin, P. Feng, C. Shang, Z. Wang, Z. Yuan (2019), "Treatment of low C/N ratio wastewater and biomass production using co-culture of *Chlorella vulgaris* and activated sludge in a batch photobioreactor", *Bioresource Technology*, **274**, pp.313-320.

[15] C.C. Tang, W. Zuo, Y. Tian, N. Sun, Z.W. Wang, J. Zhang (2016), "Effect of aeration rate on performance and stability of algal-bacterial symbiosis system to treat domestic wastewater in sequencing batch reactors", *Bioresource Technology*, **222**, pp.156-164.

[16] T.L. Pham, M.H. Bui (2020), "Removal of nutrients from fertilizer plant wastewater using Scenedesmus sp.: Formation of bioflocculation and enhancement of removal efficiency", *Journal of Chemistry*, **2020(809272)**, pp.1-9.

[17] C. Liu, Y. Hao, J. Jiang, W. Liu (2017), "Valorization of untreated rice bran towards bioflocculant using a lignocellulose-degrading strain and its use in microalgal biomass harvest", *Biotechnology for Biofuels*, **10(1)**, pp.1-12.

[18] C. Safi, B. Zebib, O. Merah, P.Y. Pontalier, C. Vaca-Garcia (2014), "Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review", *Renewable and Sustainable Energy Reviews*, **35**, pp.265-278.

[19] H.C. Bold (1949), "The morphology of *Chlamydomonas chlamydogama*, Sp. Nov.", *Bulletin of the Torrey Botanical Club*, **76(2)**, pp.101-108.

[20] H.W. Bischoff, H.C. Bold (1963), *Some Soil Algae from Enchanted Rock and Related Algal Species*, University of Texas Publications, 95pp.

[21] American Water Works Association (2012), *Standard Methods for Examination of Water and Wastewater*, 1796 pp.

[22] C.C. Tang, et al. (2018), "Enhanced nitrogen and phosphorus removal from domestic wastewater via algae-assisted sequencing batch biofilm reactor", *Bioresource Technology*, **250**, pp.185-190.

[23] R.E. Hecky, H.J. Kling (1981), "The phytoplankton and protozooplankton of the euphotic zone of Lake Tanganyika: Species composition, biomass, chlorophyll content, and spatio-temporal distribution", *Limnology and Oceanography*, **26(3)**, pp.548-564.

[24] C.S. Lee, S.A. Lee, S.R. Ko, H.M. Oh, C.Y. Ahn (2015), "Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater", *Water Research*, **68**, pp.680-691.

[25] T.T.D. Nguyen, et al. (2020), "Co-culture of microalgae-activated sludge for wastewater treatment and biomass production: Exploring their role under different inoculation ratios", *Bioresource Technology*, **314**, DOI: 10.1016/j.biortech.2020.123754.

[26] A.L. Gonçalves, J.C.M. Pires, M. Simões (2016), "Wastewater polishing by consortia of *Chlorella vulgaris* and activated sludge native bacteria", *Journal of Cleaner Production*, **133**, pp.348-357.

[27] I. de Godos, et al. (2014), "Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases", *Bioresource Technology*, **153**, pp.307-314.

[28] R. Ramanan, B.H. Kim, D.H. Cho, H.M. Oh, H.S. Kim (2016), "Algae-bacteria interactions: Evolution, ecology and emerging applications", *Biotechnology Advances*, **34(1)**, pp.14-29.

[29] O. Tiron, C. Bumbac, E. Manea, M. Stefanescu, M.N. Lazar (2017), "Overcoming microalgae harvesting barrier by activated algae granules", *Scientific Reports*, **7(1)**, DOI: 10.1038/s41598-017-05027-3.

[30] X. Liu, S. Sun, B. Ma, C. Zhang, C. Wan, D.J. Lee (2016), "Understanding of aerobic granulation enhanced by starvation in the perspective of quorum sensing", *Applied Microbiology and Biotechnology*, **100(8)**, pp.3747-3755.

[31] A.K. Lee, D.M. Lewis, P.J. Ashman (2009), "Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel", *Journal of Applied Phycology*, **21**, pp.559-567.

[32] M. Xu, P. Li, T. Tang, Z. Hu (2015), "Roles of SRT and HRT of an algal membrane bioreactor system with a tanks-in-series configuration for secondary wastewater effluent polishing", *Ecological Engineering*, **85**, pp.257-264.

[33] Z.C. Wang, et al. (2015), "Effect of hydraulic retention time on performance of an anoxic-aerobic sequencing batch reactor treating saline wastewater", *International Journal of Environmental Science and Technology*, **12(6),** pp.2043-2054.

[34] N.G.A.I. Karya, N.P. van der Steen, P.N.L. Lens (2013), "Photo-oxygenation to support nitrification in an algal-bacterial consortium treating artificial wastewater", *Bioresource Technology*, **134**, pp.244-250.

[35] I. de Godos, S. Blanco, P.A. García-Encina, E. Becares, R. Muñoz (2009), "Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates", *Bioresource Technology*, **100(19)**, pp.4332-4339.

[36] Y. Su, A. Mennerich, B. Urban (2012), "Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: Influence of algae and sludge inoculation ratios", *Bioresource Technology*, **105**, pp.67-73.

[37] G. Mujtaba, K. Lee (2017), "Treatment of real wastewater using co-culture of immobilized *Chlorella vulgaris* and suspended activated sludge", *Water Research*, **120**, pp.174-184.