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Original Research Paper

Phosphorus Removal of Tofu Processing Wastewater in Recirculated Raceway Pond Bioreactor by *Chlorella vulgaris*

Wahyunto A. Nugroho, M. Bagus Hermanto, Mustofa Lutfi and Muhammad Fakhri*
Department of Agricultural Engineering, Brawijaya University, Jalan Veteran 1, Malang, East Java, Indonesia 65145
*Department of Aquaculture, Brawijaya University, Jalan Veteran 1, Malang, East Java, Indonesia 65145
Corresponding Author: Wahyunto A. Nugroho

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ABSTRACT

The objective of this research is to observe the phosphorus elimination from tofu industrial wastewater. *Chlorella vulgaris* as autotrophic microalgae was applied in raceway pond type bioreactor. The reactor volume was 120L, 17m of path length with 10 days of hydraulic retention time. The light source is mainly from the sun during the day without specifically any light added at night. The observed parameters in this study were BOD, phosphorus and ammonium removal, and microalgae growth with its initial density set to be 5,00,000 cells per mL of wastewater. A higher growth rate of microalgae was shown during the batch treatment than the continuous one. The average achievement for the phosphorus, ammonium and BOD removal were 58.6%, 84% and 83 % respectively.

INTRODUCTION

Food processing wastewater contains high organic matter in the solution (Galambos et al. 2004) that need to be treated before it is discharged into the environment. Two main contents of the food wastewater are carbohydrates and protein (Guerrero et al. 1999). Especially for tofu processing wastewater, Kaswirani (2007) reported that protein occupies up to 60% of organic fraction in the solution. This nutrient cannot be eliminated effectively neither during primary nor secondary treatment. To cope up with this problem, a set of tertiary treatment has been developed. In a tertiary biological treatment, nitrogen is commonly removed using nitrification-denitrification processes.

Several ways are applied to eliminate phosphorus content from the wastewater. A chemical treatment is commonly used by adding metal salt in a precipitation mechanism (de-Bashan et al. 2004). Besides costly, the precipitate is usually a complex mixing that contains various salt metals. Recovery of phosphorus into struvite crystal could be one of the promising chemical treatment (Esemen et al. 2003, Molinos-Senante et al. 2011), and the product can be directly used as a high quality fertilizer (Ponce & Garcialopez 2007).

An enhanced biological phosphorus removal is another type of removing the phosphorus by employing the bacteria in the treatment plant. In the process, the removal takes many steps in aerobic and anaerobic conditions by using "release and pick up" process and the phosphorus is finally removed by accumulating it inside the bacterial cell (Buchan 1981, Lotter 1985). The generation of large number of solids, which is heterotrophic bacterial biomass, is a consequence of this treatment. A further effort to treat this biomass is necessary, in order not to give another impact to the environment.

Another way of removing the phosphorus is by applying an autotrophic organism, one of them is microalgae. Phosphorus, together with nitrogen, is a macronutrient that plays a significant role in the growth of algae. The basic removal principle is that the microalgae takes CO_2 as its carbon source and light energy as its energy source in the photosynthesis process. Nitrogen is the main compound to assemble protein that constitute the algal cell, while phosphate is needed to support metabolism process.

The use of microalgae in wastewater treatment gives many benefits. The photosynthetic process is useful for reducing CO_2 produced in anaerobic digestion as well as for nutrient removal in the wastewater (Benemann et al. 2003). Microalgae can also provide several types of renewable energy, such as biodiesel produced from its oil content (Chisti 2007), methane from the anaerobic digestion of its substrate (Mussgnug et al. 2010, Zamalloa et al. 2011), and biohydrogen (Benemann et al. 2000, Melis & Hape 2001). Microalgae is also a protein-rich microorganism that provides nutritious food for human an animals (Becker 2006).

Removal of BOD and nitrogen by autotrophic organisms often meet the expectation, but little efficiency was found in removal of phosphorus using the autotrophic organisms. The objective of the study is to observe the elimination of phosphorus from tofu industrial wastewater by microalgae. In this study, the raceway pond type reactor is used as it is simple in the operation.

MATERIALS AND METHODS

This study used raceway pond for the reactor in a pilot scale with the capacity of 1200L of wastewater. The reactor was made of wooden board with polyethylene as a liner. The total length of the path is 17m. The depth of the pond was set to 28 cm, which was close to suggestion of Chisti (2007) that was about 30 cm deep. Fig. 1 shows the design of the raceway pond used in this study. The pond was located in an open area with a polyethylene as a cover or roof. The aim of the cover was to prevent it from the rain and excessive evaporation during the day. The light source was naturally from the sun during the day.

The wastewater taken for the study was the effluent of anaerobic treatment of tofu industrial wastewater. At the first ten days, the reactor was set in a circulated-batch system. A paddle wheel was used to support this circulation. The aim of this treatment condition was to let the microalgae adapt with its environment first before it grew later. After ten days, the effluent point was opened to set the reactor into re-circulated continuous system. The hydraulic retention time was set up for about 10 days by adjusting the flow rate around 5L per hour and it was working for 24 hours a day. The circulation was given to the reactor after the 10th day. A low rotational speed-paddle wheel was set to support the partial circulation of the algae and water. The temperature of the wastewater naturally was about 22°C to 30°C, which was in the allowable range for the microalgae to grow.

The supply of CO_2 was done using aerator with the gas produced from the anaerobic treatment. The hoses were placed in five points that make it possible for the microalgae had enough carbon source for its photosynthesis.

This study used *Chlorella vulgaris*, which was provided by Brackish Water Aquaculture Center, Situbondo, Indonesia. The initial density of the microalgae, placed on the first day, was about 500,000 cells per mL of water.

The observed parameter of the study was the microalgae growth, biochemical oxygen demand (BOD) and phosphorus content. Since ammonium is a macro-nutrient for the growth of microalgae, the concentration of this compound was also investigated. The samples were taken on the tenth day after the wastewater been re-circulated. The samples were taken from two points, i.e. initial and final point. The density was counted using haemocytometer under a microscope.

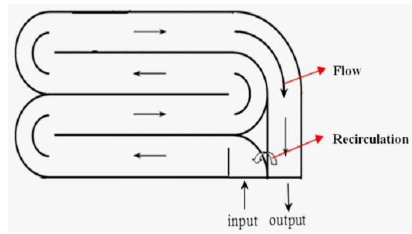

Fig. 1: Recirculated raceway pond bioreactor used in this experiment.

The BOD test was done using standard redox titration method. The phosphorus and nitrate tests were done using standard techniques and measured using spectrophotometer. As the phosphorus used by microalgae is mainly in the form of phosphate, this study did not test the total phosphorus content, but only the phosphate content. This phosphate was tested by colourimetric test using aminonaphtosulfonate. The nitrate test was carried out using UV-Vis spectrophotometer.

RESULTS AND DISCUSSION

After ten days of continuous process, both the wastewater and the microalgae concentrations were analysed. The chemical content, i.e. BOD, phosphate and nitrate, were observed by taking wastewater samples from the influent and effluent wastewater. For observing the microalgae growth, the samples were taken in the initial point, i.e., after the recirculation, and in the effluent wastewater. The samples for observing microalgae growth were taken for five days since the tenth day after the re-circulation.

Growth of microalgae: In the first ten days, the reactor was set in circulated-batch system to let the microalgae doing their adaptation to their environment. After ten days of recirculation, the samples of algae were taken from two points, i.e. the initial point and the effluent. In general, the *Chlorella vulgaris* grew well in the tofu wastewater. During the batch culture, the *Chlorella vulgaris* shows a significant growth with its relative growth rate of about 0.41/day. Its population increased and reached more than thirty-fold (15.5 $\times 10^6$cells/mL) from its initial state (0.5 $\times 10^6$cells/ mL) during the ten days of incubation. Under the continuous conditions, the relative growth rate decreased to be only 0.07/ day. It was found that on the tenth day after the recirculation, the population of the microalgae in the initial point (in front of paddle wheel) was 10.9 $\times 10^6$ cells/mL; while in the effluent water, the population was 18.6 $\times 10^6$ cells/mL. This growth rate was stable for the next four days (Fig. 3).

The growth of microalgae depends on several factors, e.g. nutrients, CO_2 and light intensity. During the circulated

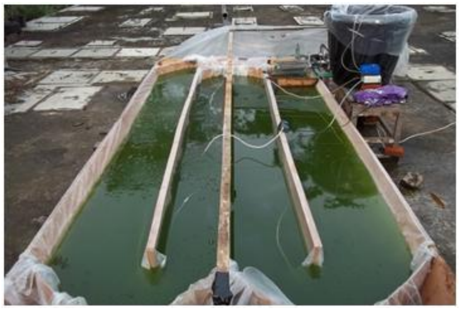

Fig. 2. The paddle wheeled pilot scale bioreactor during the treatment (without cover).

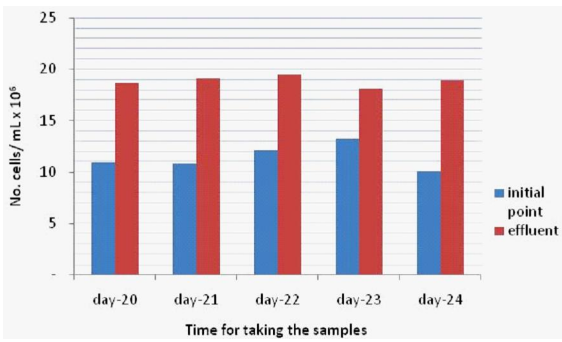

Fig. 3: The growth of *Chlorella vulgaris* in tofu wastewater (Taken after anaerobic wastewater treatment) using re-circulated pond-type bioreactor.

batch culture, there was an abundance of nutrients compared to the slight number of the microalgae. Carvalho & Malcata (2005) studied that the increase of CO_2 had significant impact on the growth of the bacteria. In this study, the CO_2 concentration diluted into the solution was the same between the batch culture and the continuous one even though the number of population of the continuous culture was much higher than that of the batch process. This condition could be another reason of the growth rate decrease during the continuous process.

To promote the growth, microalgae need sufficient light for photosynthesis (Lotter 1985). The increase of concentration of the microalgal cells gave negative impact to the light penetration into the lower layer of cultured microalgae. Such condition could be another factor that makes the overall growth rate of the microalgae in the continuous culture decreased compared to the batch process when the cells concentration was relatively low.

BOD removal: The average BOD content observed in the influent was 246.3 mg/L. It is the typical of BOD content in the food industrial wastewater after the anaerobic treatment. Protein and carbohydrate are two main organic compounds in the tofu wastewater since the soy, material for making tofu, has high content of protein and carbohydrate. These two compounds undergo degradation during the anaerobic treatment before further treatment in recirculated raceway pond reactor. After ten days in the continuous reactor, the BOD decreased with the final value of about 41.48 mg/L or about 83% removal.

Phosphorus removal: The samples of phosphorus removal were taken on the tenth day after the system being re-circulated. Phosphate (PO_4^{3-}) was observed in this experiment as representative of phosphorus since this is the main form of phosphorus in food wastewater.

The experiment shows that about 58.6% of phosphate (from 10.4 mg/L to 4.3 mg/L) was eliminated during ten days treatment. A similar result of phosphorus removal by *Chlorella vulgaris* was reported as well by Gonzales et al. (1997) in a batch culture with synthetic-sterile wastewater as the medium. Larsdotter et al. (2004) reported a nearly complete elimination of phosphorus by using a combination of assimilation-precipitation treatment in suspended microalgae culture in reused water during the summer. A shorter HRT (2 days) was used in this continuous treatment. The report showed that only a third of the phosphorus was removed by absorption mechanism by the microalgae. A lower phosphorus removal was found in a higher HRT. In biological treatment system, there are three possible mechanisms of phosphorus removal, i.e. precipitation (Larsdotter 2007), assimilation for further degradation (Becker 1994) and accumulation in microorganism (de-Bashan et al. 2002). An alkaline pH of wastewater is the best condition for phosphorus precipitation. In a bioreactor, this alkaline pH is due to the release of OH^- by bicarbonate, which is used by microalgae as a carbon source (Borowitzka 1988). A precipitation of phosphorus in a neutral pH could also happen, but only when the phosphorus concentration is above 50 mg/L (Calsson et al. 1997). This experiment did not analyze the precipitate in the reactor. Since this experiment did not meet the above required conditions for phosphorus precipitation, it can be assumed that all the phosphorus was removed by assimilation across the cell surface of the algae.

A significant phosphorus removal was also shown using immobilized *Chlorella vulgaris* in alginate beads that was done by de-Bashan et al. (2002). They reported that about 83% phosphate could be removed in the first 48 hours of semi-continuous treatment. However, a lower removal was achieved in the later cycle using the same beads. A phosphorus accumulation in the beads that might lead to the saturation was suspected to be the reason for this lower phosphorus removal.

Nitrogen removal: Ammonium is a macro nutrient for the plant to grow. As given in Table 1, the ammonium was

Table 1: Concentration of BOD, phosphate and nitrate in the influent and effluent of the raceway pond bioreactor.

S. No.	Parameter	Point	Concentration (mg/L)
1	BOD	Influent	246.34
		Effluent	41.48
2	Phosphate	Influent	10.40
		Effluent	4.30
3	Ammonium	Influent	5.00
		Effluent	0.80
4	Nitrate	Influent	0.018
		Effluent	0.043

significantly removed by about 84%. On the other hand, the nitrate did not experience any removal. Even the concentration was increased after ten days of treatment, i.e. from 0.018 mg/L to 0.043 mg/L. The increasing of nitrate during the treatment was also reported by Gonzales et al. (1997). Nitrate could be generated from an oxidation process of the ammonium. The oxygen produced by the microalgae during the process might support the nitrification of ammonium.

To support the removal of nitrogen, de-Bashan et al. (2004) suggested an addition of growth promoting bacteria. This bacterium is commonly used in terrestrial plant. It works by providing growth hormone for the microalgae (de-Bashan et al. 2008). In their study that employed *Azospirillum brasilense* together with *Chlorella vulgaris*, the ammonium could be removed completely while the nitrate could be eliminate only about 15% compared to 6% removal by the *Chlorella vulgaris* alone.

To complete the overall nitrogen removal, an anoxic process needs to be added to denitrificate the effluent (Borowitzka 1988). Some of the possible options are to re-circulate the effluent into anaerobic reactor or to give additional anoxic treatment for the effluent.

CONCLUSION

The study shows a better growth rate of microalgae in a low algal concentration. The use of *Chlorella vulgaris* in tofu processing wastewater treatment has shown a good result for BOD removal and only about a half removal for phosphorus. A further study to optimize the environmental parameters need to be done to boost the removal through degradation by the microalgae. A combination or additional treatment for removing phosphorus could be done with respect to the environmental issue, e.g. by recovering phosphorus in the form of struvite. The increase of nitrate during the treatment may lead to the low achievement of overall nitrogen removal. An additional anaerobic treatment should be used to complete this nitrogen removal.

Suggestion for future research: Piles of laboratory and pilot scale studies have been done for the nutrient removal that employ autotrophic microorganisms. A few have been done to scale-up the study into full scale treatment. A feasibility study to scale-up the process that consist of techno-economic studies is a challenge to be carried out in the future. It is suggested that the study will consider not only water purification aspect but also a positive energy balance for the overall process.

REFERENCES

Becker, E. W. 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge.

Becker, E.W. 2006. Micro-algae as a source of protein. Biotechnology Advances. 25(2): 207-210.

Benemann, J.R. 2000. Hydrogen production by microalgae. Journal of Applied Phycology, 12(3-5): 291-300.

Benemann, J.R., Van Olst, J.C., Massingill, M.J., Weissman, J.C. and Brune, D.E. 2003. The controlled eutrophication process: Using microalgae for CO_2 utilization and agricultural fertilizer recycling. In: Gale, J. and Kaya, Y. (eds), Proceedings of Conference on Greenhouse Gas Technologies, Kyoto, Japan.

Borowitzka, M.A. 1988. Vitamins and fine chemicals from microalgae. In: Borowitzka, M.A. and Borowitzka, L.J. (Eds). Microalgal Biotechnology, Cambridge University Press, Cambridge, pp. 153-196.

Buchan, L. 1981. The location and nature of accumulated phosphorus in seven sludges in activated sludge plants which exhibited enhanced phosphorus removal. Water SA, 7(1): 1-7.

Carlsson, H., Aspegren, H., Lee, N. and Hilmer, A. 1997. Calcium phosphate precipitation in biological phosphorus removal systems. Water Res., 31(5): 1047-1055.

Chisti, Y. 2007. Biodiesel from microalgae. Biotechnology Advances, 25(3): 294-306.

Carvalho, A.P. and Malcata, F.X. 2005. Optimization of ω-3 fatty acid production by microalgae: crossover effects of CO_2 and light intensity under batch and continuous cultivation modes. Marine biotechnology, 7(4): 381-388.

De-Bashan, L. E., Moreno, M., Hernandeza, J. and Bashan, Y. 2002. Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium A*zospirillum-brasilense*. Water Research, 36(12): 2941-2948.

De-Bashan, L. and de-Bashan, Y. 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003). Water Research, 38: 4222-4246.

De-Bashan, L.E., Hernandez, J., Morey, T. and Bashan, Y. 2004. Microalgae growth-promoting bacteria as "helpers" for microalgae: A novel approach for removing ammonium and phosphorus from municipal wastewater. Water Research, 38(2): 466-474.

De-Bashan, L., Antoun, H. and Bashan, Y. 2008. Involvement of indole-3-acetic acid produced by the growth-promoting bacterium *Azospirillum* spp. in promoting growth of *Chlorella vulgaris*. Journal of Phycology, 44(4): 938-947.

Esemen, T., Rand, W., Dockhorn, T. and Dichtl, N. 2003. Increaseing cost efficiency of struvite precipitation by using alternative precipitants and P-remobilization from sewage sludge. In: Ashley, K., Mavinic, D., Koch, F. (eds). International Conference on Nutrient Recovery from Wastewater Streams. IWA Publishing, pp. 269-280.

Galambos, I., Molinaa, J.M., Járaya, P., Vataia, G. and Bekássy-Molnára, J. 2004. High organic content industrial wastewater treatment by membrane filtration. Desalination, 162: 117-120.

González, L.E., Cañizares, R.O. and Baena, S. 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Bioresource Technology, 60(3): 259-262.

Guerrero, L., Omil, F., Méndez, R. and Lema, J.M. 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. Water Research, 33: 3281-3290.

Kaswirani, F. 2007. Technical study of the treatment of wastewater and solid waste from tofu processing. Thesis, Universitas Diponegoro, Semarang, Indonesia.

Larsdotter, K., Soderback, E. and Dalhammar, G. 2004. Phosphorus removal from wastewater by microalgae in a greenhouse in Sweden. In: Ujang, Z. (Ed.). Environmental Biotechnology: Advancement in Water and Wastewater Applications in the Tropics. IWA Publishing, London, UK.

Larsdotter, K., Jansen, J.L.C. and Dalhammar, G. 2007. Biologically mediated phosphorus precipitation in wastewater treatment with microalgae. Environmental Technology, 28(9), 953-960.

Lotter, L.H. 1985. The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. Water Science & Technology, 17(11-12): 127-138.

Melis A. and Happe, T. 2001. Hydrogen Production. Green Algae as a Source of Energy. Plant Physiology, 127(3): 740-748.

Mussgnug, J.H., Klassen, V., Schlüter, A. and Kruse, O. 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. Journal of Biotechnology, 150(1): 51-56.

Molinos-Senante, M., Hernández-Sancho, F., Sala-Garrido, R. and Garrido-Baserba, M. 2011. Economic feasibility study for phosphorus recovery processes. Ambio: A Journal of the Human Environment, 40(4): 408-416.

Ponce, G. and Garcialopez, M.E. 2007. Evaluation of struvite as a fertilizer: A comparison with traditional P sources. Agrochimica, 51(6): 301-308.

Zamalloa, C., Vulsteke, E., Albrecht, J. and Verstraete, W. 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresource Technology, 102(2): 1149-1158.