Nutrients removal and biomass production from anaerobic digested effluent by microalgae: A review

Gang Li[1], Xue Bai[1], Huan Li[2], Zhitao Lu[1], Yuguang Zhou[2,3,4], Yingkuan Wang[5], Jinxin Cao[6], Zhigang Huang[1*]

(1. *School of Material Science and Mechanical Engineering, Beijing Technology and Business University, Beijing 100048, China*; 2. *Laboratory of Bioenergy and Environment Science & Technology, College of Engineering, China Agricultural University, Beijing 100083, China*; 3. *Key Laboratory of Clean Production and Utilization of Renewable Energy, Ministry of Agriculture and Rural Affairs, Beijing 100083, China*; 4. *National Center for International Research of BioEnergy Science and Technology, Ministry of Science and Technology, Beijing 100083, China*; 5. *Chinese Academy of Agricultural Engineering Planning and Design, Ministry of Agriculture and Rural Affairs, Beijing 100125, China*; 6. *Inner Mongolia University, Hohhot 010070, China*)

Abstract: This article summarizes the recent progress of nutrient removal from wastewater via microalgae. Removal of nitrogen and phosphorous compounds from wastewater are of great importance, while those compounds are suitable for growth of some microalgae species. Such a combination provides more opportunities for anaerobic digestion facilities, which are producing large amount of wastewater with high nitrogen and phosphorous contents. However, in order to optimize and maximize the performance and durability of the nutrient removal process, it is suggested that the basic principles about nitrogen and phosphorous migration should be investigated thoroughly, especially from the fundamentals of substance transfer mechanism between water environment and algal cells.

Keywords: microalgae, biomass accumulation, nutrient removal, anaerobic digested effluent (ADE), pollution control
DOI: 10.25165/j.ijabe.20191205.3630

Citation: Li G, Bai X, Li H, Lu Z T, Zhou Y G, Wang Y K, et al. Nutrients removal and biomass production from anaerobic digested effluent by microalgae: A review. Int J Agric & Biol Eng, 2019; 12(5): 8–13.

1 Introduction

With the rapid increase of globally energy demands and fossil fuel crisis, more attention has been paid on the production of the substitute energy sources[1]. Microalgae, especially unicellular eukaryotic algae has higher lipid production yield than that of the best oilseed crops in terms of land area that required for cultivation[2,3]. It has the advantages of high photosynthesis efficiency, high growth rate, high lipid content, no occupation of farmland and high added value[4]. Therefore, energy from microalgae could be a potential bioenergy in the future[5,6].

Microalgae is the most primitive and widely distributed biological species on the earth, which can carry out photosynthetic autotrophic, organic heterotrophic or polyculture of lower biomass[7,8]. It is widely distributed in fresh water, salt water and sea water, with wide temperature, pH and nutritional adaptability[9,10]. Based on the existing data of lipid-rich microalgae, Hu et al.[11] summarized the lipid-producing characteristics of the main microalgae species. Under normal cultural conditions, the lipid content is about 25.5% dry weight for green algae, 22.7% dry weight for diatoms, and 27.1% dry weight for other lipid-rich algae. As for the absence of nitrogen or other stimulating conditions such as strong light, the lipid content will be higher (45.7% dry weight for green algae, 37.8% dry weight for diatoms, 44.6% dry weight for other lipid-rich algae on average). Cyanobacteria generally has relatively low oil content (about 9.8%), even in the external stimulating conditions.

A large quantity of water and considerable nutrient input are consumed during microalgae cultivation, and water consumption could account for 10%-20% of the total cost[12,13]. Wastewater, which is rich in nitrogen, phosphorus and other minerals, has potential as a substrate for microalgae cultivation[14-19].

The most important work of wastewater treatment is to remove or reuse the use nitrogen, phosphorus, other nutrients, heavy metals and other substances[20-24]. Wastewater treatment methods are mainly physical, chemical and biological treatments or those combinations. Physical and chemical methods are usually with relatively high cost, and easily to cause secondary pollution, whereas biological treatment could avoid those problems much better[25-27]. As early as the 1970s, algae began to be the third stage of wastewater treatment process, and it has been reported that microalgae can also grow in high concentrations of inorganic wastewater contained nitrogen, phosphorus and some metal elements[28-30]. The cultivation of microalgae in wastewater can effectively repair the environment of eutrophic nitrogen and phosphorus elements while accumulating biomass for further

Received date: 2018-09-20 **Accepted date:** 2019-09-07
Biographies: Gang Li, PhD, Associate Professor, research interests: microalgae conversion and bioenergy, agricultural product processing engineering, Email: ligang@btbu.edu.cn; **Xue Bai,** Master student, research interests: agricultural engineering, Email: bai_xue0903@163.com; **Huan Li,** research interests: bioenergy clean utilization, Email: huanli828@sina.com; **Zhitao Lu,** Master student, research interests: bioenergy engineering, Email: luzztao@163.com; **Yuguang Zhou,** PhD, Associate Professor, research interests: bioenergy clean utilization, Email: zhouyg@cau.edu.cn; **Yingkuan Wang,** PhD, Professor, research interests: agricultural engineering, Email: wangyingkuan@163.com; **Jinxin Cao,** PhD, Professor, research interests: logistic analysis for biomass Email: imucjx@163.com.

***Corresponding author: Zhigang Huang,** PhD, Professor, research interests: agricultural product processing engineering. School of Material Science and Mechanical Engineering, Beijing Technology and Business University, No. 11 Fucheng Road, Haidian District, Beijing 100048, China. Tel/Fax: +86-10-68985249, Email: huangzg@btbu.edu.cn.

energy utilization[31-35].

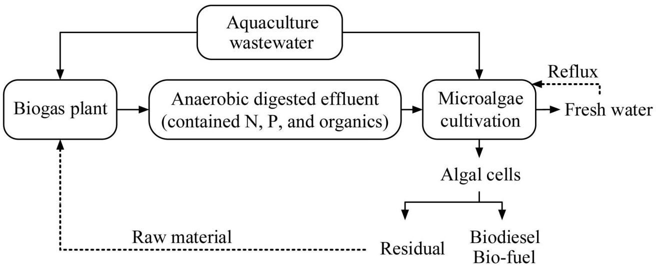

Figure 1 Flow chart of the coupling system that combining microalgae production with effluent treatment

As shown in Figure 1, the harvested algae products can be used as feed, fertilizer, and even for the production of useful chemicals[36,37]. Co-locating algae production with wastewater could make the cost of the biofuel and the level of environmental pollution much lower and this bioremediation also improves the thermodynamic cycle in a further more sustainable way[38-40].

2 Nutrient removal and biomass production by microalgae cultivated in ADE

This part mainly discusses performance of microalgae cultivation and nutrient removal from agricultural wastewater, especially anaerobic digested effluent (ADE).

The nitrogen and phosphorus contents of agricultural wastewater are higher compared with municipal wastewater, and most of the nitrogen is present in ammonium form[41,42]. The traditional way to deal with agricultural wastewater is to produce farmland fertilizer. However, depending on the types of animals, the time of feeding, the composition of the feed, the mode of management and location of the farm, the composition and content of the agricultural wastewater are also quite different[28]. At the same time, due to the quite different needs of nutrients from diverse crops, and incomplete consumption of nitrogen and phosphorus in agricultural wastewater, continuous disposal for long period will lead to the accumulation of those ingredients that sediment into soil, which causing a potential threat to the environment.

As shown in Table 1, ADE contains a large number of nitrogen, phosphorus, potassium, as well as amino acids, vitamins, protein, long prime, carbohydrate, nucleic acid, gibberellin and other substances that can regulate growth and development of crop and livestock. Because the anaerobic digestion process converts the carbon contained in the waste into CH_4, the carbon content of the ADE is lower compared to the traditional municipal wastewater, agricultural wastewater and industrial wastewater[43]. The contents of total nitrogen (TN) and ammonium nitrogen were relatively high; the content of total phosphorus (TP) were relatively lower; and the chemical oxygen demand (COD) content was quite different according to the category of raw material.

Besides, there are also various ions that stimulate the germination and growth of crops, such as calcium, phosphorus, iron, zinc, etc (Table 2). If those elements are used rationally to cultivate microalgae, it is possible to obtain cheap raw materials while treating ADE[45], this is of great significance to discharge the ADE and reduce the carrying pressure of the environment[59-61].

However, there are still some problems to be solved about ADE as the substrate for microalgae cultivation. The vast majority of the nitrogen in the ADE is ammonium nitrogen[43]. It has been reported that free ammonia obtained from the conversion has a serious toxic effect on the microalgae[62]. Besides, high turbidity of ADE will inhibit the photosynthesis of microalgae, further affect the utilization of nutrients and biomass accumulation[63], so the dilution treatment of wastewater can be a beneficial way for microalgae cultivation[64]. It's worth noting that the dilution treatment also increases the water cost, and the cost of microalgae harvesting process (including sedimentation[65], centrifugation[66], drying[67] and so on) will increase proportionally with the dilution ratio. Literature results showed the microalgae dehydration is the most energy-consuming process in the life cycle of microalgae, and accounts for about 20%-30% of the total cost[66]. For economic reasons, the appropriate dilution is required to ensure the growth of microalgae while keeping production costs in mind.

Table 1 Chemical composition of ADE in different types used for the growth of microalgae (mg/L)

Type	pH	COD	NH_4^+-N	TN	TP	Ref.
Pig [b]	8.5±0.2	280±14	60.5±0.5	-	45.0±1.2	38
Pig [b]	9.18	6900±53	824.55±4.2	928.46±4.64	45.72±0.55	44
Pig [b]	-	120.77±15.09	135.5-143	146.64±8.12	2.18	45
Starch [c]	7.1-7.3	926.3±65.2	240.88±18.9	265.10±19.1	28.34±1.2	46
Dairy [b]	-	32650	1620	2371	240	41
Pig [a]	-	1200-1350	960-1000	1050-1011	25-26.5	47
Pig [b]	6.3±0	485.2±3.5	219.5±11.3	277.6±6.1	30.2±0	48
Pig [a]	-	-	1317±10	-	20.2±0.5	49
Pig [b]	7.05±0.0	6470±140	101.2±5.21	172.7±7.11	113.8±2.12	50
Pig [b]	7.0±0.03	18600±200	255.4±4.9	463.0±6.7	113.3±7.0	51
Pig [b]	8.33	793.57±7.65	-	453.62±5.83	56.8±1.35	52
Pig [b]	7.7	332	233	348	101.4	53
Dairy [a]	-	23760	2232	3456	249.7	54
Poultry [b]	-	-	1143	1570	-	55
Pig [b]	6.1±0.1	3700±51	-	162.0±8.0	209.0±5.5	56
Pig [b]	8.0±0.3	813.12±39.83	-	421.5±9.19	5.0±0.28	57
Pig [b]	6.3±0.1	3402.53±48.12	-	138.83±17.03	185.37±7.85	58
Dairy [b]	8.67	10320	1554	1722	111.6	59

Notes: [a] untreated and undiluted ADE; [b] pretreated ADE; [c] pretreated anaerobic digested starch wastewater.

Table 2 Several ions of ADE in different types (mg/L)

Type	K	Mg	Fe	Cu	Ref.
Pig [b]	4.2381	0.9429	0.2345	-	45
Starch [c]	174.47±10.7	181.16±9.34	32.86±1.54	-	46
Pig [a]	530-545	165-175	8.5-9.5	-	47
Pig [b]	1.65±0.08	1.33±0.09	1.29±0.06	5.18	48
Pig [a]	-	-	-	7.3±0.5	49
Pig [b]	-	-	4.58±0.03	2.53	50
Poultry [b]	1632	21.5	22.4	10.4	55
Pig [b]	-	-	1.29±0.07	0.26	57

Notes: [a] untreated and undiluted ADE; [b] pretreated ADE; [c] pretreated anaerobic digested starch wastewater.

Recently, many studies have been focusing on the topic of nutrients removal based on biomass accumulation and lipid production[68-70]. Table 3 shows the summary of major nutrient removal capabilities and biomass production, lipid accumulation by microalgae cultivation in various ADE conditions. The raw material of ADE consisted of pig manure, dairy manure and others, among which of pig manure was mostly studied. The algae strains were composed of *Chlorella zofingiensis*, *Chlorella* sp., *Chlorella sorokiniana, Chlorella vulgaris, Chlorella pyrenoidosa*, *Desmodesmus* sp., *Scenedesmus dimorphus* and *Scenedesmus obliquus* and so on. Along with the growth of microalgae, the COD removal could be up to 32%-96%, the NH_4^+-N removal of

22.9%-100%, the TN removal of 23%-93%, and the TP removal of 20%-98% based on the diverse conditions. The biomass accumulation also reached the 0.6-2.9 g/L with the specific growth rate of 0.12-2.73 d^{-1}, and the lipid content in the range of 9.0%-31.6% with the lipid productivity of 30.0-110.56 mg/L/d.

Additionally, some studies also investigated the chemical compositions in the microalgae from the ADE media, such as pigments, carbohydrate, protein and lipids, especially the fatty acids profiles which could be a potential way to determine some important properties of biodiesel[57,72]. Deng et al.[72] investigated the potential biodiesel feedstock production and nutrient removal of *Chlorella vulgaris* (UTEX 2714) cultivation in the anaerobically digested swine manure. The fatty acids profiles were different with the change of dilution multiple of ADE. The predicted properties of biodiesel fit for the biodiesel standards of ASTM D6751-08 and EN 14214[72]. Chung et al.[68] cultivated *Spirulina* sp. with 10% ADE and $NaHCO_3$. The yield of *Spirulina* sp. was up to 5 g/(m^2·d) and nitrogen assimilation efficiency was up to 76%. The protein of such *Spirulina* sp. was employed for feeding mice and did not show any toxic reactions[68].

Table 3 Summary of major nutrient removal capabilities and biomass production by microalgae cultivation in various ADE conditions

Type	Strain	Time /d	Biomass /g·L^{-1}	Biomass productivity /mg·L^{-1}·d^{-1}	Specific growth rate /d^{-1}	Lipid content/%	Nutrient removal				Ref.
							COD	NH_4^+-N	TN	TP	
Pig [a]	*C.zofingiensis*	10	2.962±0.192	296.16±19.16	0.34±0.001	-	79.84%	-	82.7%	98.17%	56
Dairy [b]	*Chlorella* sp.	21	1.57	-	0.282 [c]	9	38.4%	100%	82.5%	70.1%	54
Pig [d]	*C.sorokiniana*	5	1.33±0.14	617.1±0.18	2.73±0.02	-	-	54.23±3.13%	50.06±0.41%	-	45
Pig [d]	*C. vulgaris*	5	0.89±0.1	504.2±20.73	2.67±0.05	-	-	80.74±1.85%	65.87±4.71%	-	45
Pig [d]	*C. vulgaris* ESP-6	5	1.36±0.22	643.6±89.39	1.59±0.11	-	-	29.43±1.67%	28.28±4.84%		45
Pig [d]	*Desmodesmus* sp F51	5	1.51±0.02	268.35±62.69	2.2±0.0	-	-	57.68±0.14%	51.35±2.17%	-	45
Pig [d]	*S. dimorphus*.	5	0.95±0.09	538.56±20.05	1.11±0.02	-	-	60.28±1.77%	64.38±0.36%	-	45
Pig [e]	*C. vulgaris*	7	1.1	140	-	17.4±1.89	32.9%	99.8%	55.4%	20.0%	51
Pig [f]	*C. vulgaris*	7	1.68	234.1	-	31.6±0.26	950 mg/L/d	22.9 mg/L/d	23.4 mg/L/d	7.68 mg/L/d	50
Pig [g]	*C. vulgaris*	7	1.26±0.09	155±7	-	-	96±0%	100±0%	93±1%	91±2%	48
Pig [h]	*C. vulgaris*	7	2.42	440.3	1.00	22.3±1.61	34.4%	98.8%	51.3%	38.4%	51
Pig [i]	*C. pyrenoidosa* [j]	10	1.31	150	0.821	-	55.1%	100%	51.9%	60.5%	71
Pig [k]	*C. vulgaris*	7	0.616±0.04	-	0.12±0.03	21.95±1.05	85.94±1.7%	-	72.48±10.5%	86.93±2.49%	57
Pig [l]	*S. obliquus*	7	-	311.28±12.36	0.382±0.008	-	75.29±5.89%	-	74.63±6.94%	81.73±7.28%	58

Notes: [a] the initial concentration of COD was 1900 mg/L; [b] 10*dilution of ADE; [c] the average specific growth rate in the first 7 days; [d] 8-fold diluted ADE; [e] 3*ADE; [f] the initial concentration of NH_4^+-N was about 100 mg/L; [g] the initial concentration of NH_4^+-N was 110 mg/L; [h] 3*diluted ADE with pretreated centrate; [i] 4* diluted ADE with sterile water; [j] *C. pyrenoidosa* sparged with simulated flue gas; [k] cultivation in the flat plate photobioreactors of 75 L outdoor; [l] the initial concentration of COD was 1600 mg/L.

3 Mechanism of nitrogen and phosphorous removal from wastewater by microalgae

Xue et al.[73] and Huntley et al.[74] have proposed that the empirical formula $C_{106}H_{263}O_{110}N_{16}P$ of algae which has been widely recognized. The molecular formula shows that nitrogen accounts for about 6.3% of the whole algal cells, with a ratio nitrogen: phosphorus ratio of 7:1. Assuming that nitrate is a nitrogen source and phosphate hydrate is a phosphorus source, the algae photosynthesis reaction equation is as follows:

$$106CO_2+16NO_3^-+HPO_4^{2-}+122H_2O+18H^+ \xrightarrow{\text{Light}} C_{106}H_{263}O_{110}N_{16}P+138O_2$$

In the process of growth, the nitrogen and phosphorus elements contained in organic matter such as urea, amino acids and other organic substances can be converted into self-synthetic substances by inorganic ions such as nitric nitrogen, ammonia nitrogen and orthophosphate in the sewage process. In addition, some algae species (such as *cyanobacteria*) can directly fix nitrogen in the air. Therefore, it is one of the effective ways to solve the problem of water eutrophication by applying algae to the absolute demand of nitrogen and phosphorus and its resistance to water pollution.

3.1 Mechanism of nitrogen removal

Nitrogen is a kind of necessary element for all living organisms. All organic nitrogen sources are inorganic nitrogen, and microbial life process can be a perfect embodiment of inorganic nitrogen into organic nitrogen important role. Microalgae can use nitrogen sources not only inorganic nitrogen sources but also organic nitrogen sources (urea, amino acids, etc.). Inorganic nitrogen in water often exists in ammonia nitrogen (including ammonium nitrate, NH_4^+, and free ammonia, NH_3), nitric nitrogen (NO_3^-) and nitrite nitrogen (NO_2^-). Actually, many studies have shown that in the presence of multiple inorganic nitrogen sources, microalgae will give priority to the use of ammonium nitrogen[75,76]. Some microalgae cells cannot use nitrate nitrogen due to the lack of nitrate reductase. Xin et al.[77] suggested that ammonia nitrogen could be better for microalgae during the growing period, and as for the stable period, the nitrate and urea suit for microalgae growth mechanism.

The nitrate nitrogen absorption using microalgae is a positive absorption. Nitrate is reduced to nitrite under the catalysis of nitrate reductase, and further to ammonium nitrogen using 6 electrons for the next step amino acid synthesis. For algae, nitrate is a more stable source of nitrogen, which was used as the dominant nitrogen source by sodium nitrate in green algae culture of BG11 medium. Therefore, the concentration of nitric acid in water generally does not affect the growth of microalgae[78].

Differently, the ammonia nitrogen absorption using microalgae has both active and passive transport, which depends on the ammonia concentration and microalgae glutamine synthetase activity. As shown in Figure 2, ammonium nitrogen can be directly converted to amino acids by amination or transamination. Free ammonia (NH_3) could through into the cell membrane by the passive diffusion of free penetration. The ratio of the two forms of ammonia nitrogen is determined by the pH value, which is a dynamic equilibrium relationship. Free ammonia is the main substance that inhibits the growth of microalgae[79]. When the pH is higher (pH 8 and the concentration of ammonia nitrogen of 2.0 mmol/L), the free ammonia content that dissolved in water will cause fatal damage to microalgae. In addition, the oxygen concentration in the water body will decrease rapidly, cause the inhibition of the photosynthesis and growth of microalgae. Some scholars believe that once the ammonia concentration exceeds 1.2 mmol/L, microalgae photosynthesis will be affected[80]. With the advantages of high fat-soluble, easy to pass through the algae cell membrane, and inhibiting the respiratory process of electron transport system and so on, the toxicity of free ammonia for microalgae is much greater than that of ammonium. When the free ammonia in water environment increases, excess free ammonia will enter the living cells and has significant impacts on the stability of the biological cell membrane and the enzymatic hydrolysis reaction, also inhibit the activity of the enzyme (especially for the antioxidant enzyme) and decrease the intracellular and extracellular oxygen content. The research results showed that the concentration of ammonia was about 120 mg/L at pH 8.5 and 25°C[81]. Therefore, in order to ensure the normal growth of microalgae, ammonia concentration in the medium should be controlled at 120 mg/L or less.

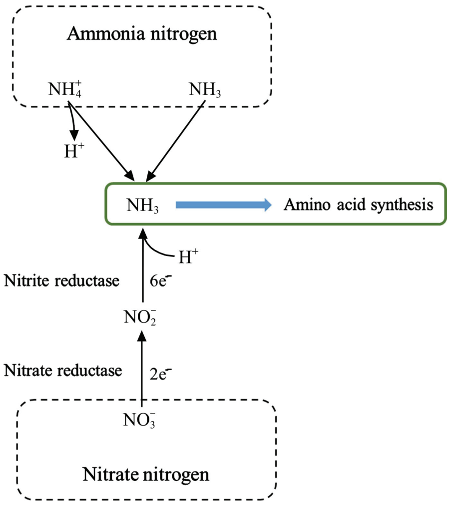

Figure 2 Schematic diagram of transformation of nitrogen elements in the adsorption process of microalgae

3.2 Mechanism of phosphorous removal

Phosphorous is an essential element for the growth of microalgae, and is involved in the synthesis of adenosine triphosphate (ATP), nucleic acid, phospholipid and coenzyme, which plays an important role in cell metabolism. The phosphorus element in the water can be absorbed by microalgae cells in the form of soluble phosphate such as hydrogen phosphate and dihydrogen phosphate, and then be converted into microalgae by hydrolysis such as phosphatase[82]. When the phosphorus deficiency is absent in the culture medium and without phosphorus starvation treatment, microalgae cells will absorb excess phosphate (i.e. excess intake) and rapidly store the soluble phosphate in algal cells in the form of polyphosphoric acid particles. Once the phosphorus in the medium is lack, cells can use the stored phosphate to maintain cell division and growth. In the phosphorus-rich medium, the absorption of phosphorus by microalgae can be maintained at a high level, which is twice more than that of the phosphate-free medium, but the difference in the daily biomass yield is not obvious[83].

4 Conclusions

Microalgae can utilize nutrients such as nitrogen or phosphorous contents in wastewater. This study summarized some available results and found that some microalgae could tolerate high organic compound concentration in different types of wastewater, especially in ADE. However, the basic principles about nitrogen and phosphorous migration are still not so clear as the booming demands in real applications of improving the performance and durability of the nutrient removal process. It is necessary to conduct much more in-depth investigations on the substance transfer mechanism of the process so that the relationship between biomass accumulation and nutrient removal could be better interpreted.

Acknowledgements

This research was supported by Chinese National 13th five-year Plan for Key R & D Projects (2018YFD0400804); the Beijing Municipal Education Commission General Project (KM201810011002); the Research Foundation for Youth Scholars of Beijing Technology and Business University (PXM2018_014213_000033); the research was also supported by the National Natural Science Foundation of China (Grant No. 51806242) and the National Natural Science Foundation of China (No. 71961024); the Chinese Universities Scientific Fund (No. 2019TC010); and the Chinese Universities Scientific Fund - Special Project of “Agricultural Mechanization and Equipment Engineering” for “Double First-Class” Initiative of College of Engineering, China Agricultural University.

[References]

[1] Li G, Ji F, Xiang S N, Zhou Y G, Jiang M M, Huang Z G. Analytical study on pyrolyzed products of *Desmodesmus* sp. cultivated in BG11. Inter J Agric & Biol Eng, 2017; 10(3): 218–226.
[2] Li G, Xiang S N, Ji F, Zhou Y G, Huang Z G. Thermal cracking products and bio-oil production from microalgae *Desmodesmus* sp. Inter J Agric & Biol Eng, 2017; 10(4): 198–206.
[3] Brennan L, Owende P. Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews, 2010; 14(2): 557–577.
[4] Stephens E, Ross I L, King Z, Mussgnug J H, Kruse O, Posten C, et al. An economic and technical evaluation of microalgal biofuels. Nature Biotechnology, 2010; 28: 126–128.
[5] Wijffels R H, Barbosa M J. An outlook on microalgal biofuels. Science, 2010; 329(5993): 796–799.
[6] Park J B, Craggs R J, Shilton A N. Wastewater treatment high algal pond for biofuel production. Bioresource Technology, 2011; 102: 35–42.
[7] Shen Y, Yuan W, Pei Z, Wu Q, Mao E. Microalgae mass production methods. Transactions of the ASABE, 2009; 52: 1275–1287.
[8] Smith V H, Sturm B S M, deNoyelles F J, Billings S A. The ecology of algal biodiesel production. Trends in Ecology & Evolution, 2010; 25: 301–309.
[9] Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976; 72(1): 248–254.
[10] Falkowski P G, Raven J A. Aquatic photosynthesis. New Jersey: Princeton University Press, 1997; 375 p.
[11] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal, 2008; 54: 621–639.
[12] Subhadra B G. Water management policies for the algal biofuel sector in the Southwestern United States. Applied Energy, 2011; 88(10): 3492–3498.

[13] Sander K, Murthy G S. Life cycle analysis of algae biodiesel. International Journal of Life Cycle Assessment, 2010; 15(7): 704–714.
[14] Chang H X, Fu Q, Huang Y, Xia A, Liao Q, Zhu X. Improvement of microalgae lipid productivity and quality in an ion-exchange-membrane photobioreactor using real municipal wastewater. Inter J Agric & Biol Eng,. 2017; 10(1): 97–106.
[15] Franchino M, Comino E, Bona F, Riggio V A. Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate. Chemosphere, 2013; 92(6): 738–744.
[16] Mallick N. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. Biometals, 2002; 15(4): 377–390.
[17] Dianursantia, Rizkytataa B T, Mohamad T G, Taufik H A. Industrial tofu wastewater as a cultivation medium of microalgae *Chlorella vulgaris*. Energy Procedia, 2014; 47: 56–61.
[18] Gentili F G. Microalgal biomass and lipid production in mixed municipal, dairy, pulp and paper wastewater together with added flue gases. Bioresource Technology, 2014; 169: 27–32.
[19] Prajapati S K, Choudhary P, Malik A, Vijay V K. Algae mediated treatment and bioenergy generation process for handling liquid and solid waste from dairy cattle farm. Bioresource Technology, 2014; 167: 260–268.
[20] Ji F, Zhou Y, Pang A P, Ning L, Rodgers K, Liu Y, et al. Fed-batch cultivation of *Desmodesmus* sp. in anaerobic digestion wastewater for improved nutrient removal and biodiesel production. Bioresource Technology, 2015; 184:116–122.
[21] Hu Z, Houweling D, Dold P. Biological nutrient removal in municipal wastewater treatment: new directions in sustainability. Journal of Environmental Engineering, 2011; 138(3): 307–317.
[22] Li Y C, Chen Y F, Chen P, Min M, Zhou W G, Martinez B, et al. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal waste-water for nutrient removal and biodiesel production. Bioresource Technology, 2011; 102(8): 5138–5144.
[23] Chi Z Y, Zheng Y B, Jiang A P, Chen S L. Lipid production by culturing oleaginous yeast and algae with food waste and municipal wastewater in an integrated process. Applied Biochemistry and Biotechnology, 2011; 165(2): 442–453.
[24] Larsdotter K, Jansen J. Phosphorus removal from wastewater by microalgae in Sweden a year round perspective. Environmental Technology, 2010; 31: 117–123.
[25] Perez-Garcia O, de Bashan L E, Hernandez J-P, Bashan Y. Efficiency of growth and nutrient uptake from wastewater by heterotrophic, autotrophic and mixotrophic cultivation of *Chlorella vulgaris* immobilized with *Azospirillum brasilense*. Journal of Phycology, 2010; 46: 800–812.
[26] Yun Y S, Lee S B, Park J M, Lee C I, Yang J W. Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology and Biotechnology, 1997; 69: 451–455.
[27] Wang B, Lan C Q. Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent. Bioresource Technology, 2011; 102(10): 5639–5644.
[28] Cai T, Park S Y, Li Y. Nutrient recovery from wastewater streams by microalgae: status and prospects. Renewable and Sustainable Energy Reviews, 2013; 19: 360–369.
[29] Caporgno M P, Taleb A, Olkiewicz M, Font J, Pruvost J, Legrand J, et al. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. Algal Research, 2015; 10: 232–239.
[30] Chen R, Li R, Deitz L, Liu Y, Stevenson R J, Liao W. Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass & Bioenergy, 2012; 39: 128–138.
[31] Ge S, Champagne P. Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Research, 2016; 88: 604–612.
[32] Gonzalez C, Marciniak J, Villaverde S, Garcia-Encina P A, Munoz R. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. Applied Microbiology and Biotechnology, 2008; 80(5): 891–898.
[33] Song M, Pei H, Hu W, Ma G. Evaluation of the potential of 10 microalgal strains for biodiesel production. Bioresource Technology, 2013; 141: 245–251.
[34] Huang G H, Chen F, Wei D, Zhou X W, Chen G. Biodiesel production by microalgal biotechnology. Applied Energy, 2010; 87(1): 38–46.
[35] Han T, Lu H F, Ma S S, Zhang Y H, Liu Z D, Duan N. Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review. Inter J Agric & Biol Eng,, 2017; 10(1): 1–29.
[36] Debowski M, Rusanowska P, Zielinski M, Dudek M, Romanowska-Duda Z. Biomass production and nutrient removal by *Chlorella vulgaris* from anaerobic digestion effluents. Energies, 2018; 11: 1654.
[37] Luo L, He H J, Yang C P, Wen S, Zeng G M, Wu M J, et al. Nutrient removal and lipid production by *Coelastrella* sp. in anaerobically and aerobically treated swine wastewater. Bioresource Technology, 2016; 216: 135–141.
[38] Li Y, Zhou W, Hu B, Min M, Chen P, Ruan R R. Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains screening and significance evaluation of environmental factors. Bioresource Technology, 2011; 102: 10861–10867.
[39] Liu R, Yu Q Q, Guo Q Q, Zheng W, Wang G R, Chen L J. Raceway pond cultivation of a new *Arthrospira* sp. ZJWST-S1 in digested piggery wastewater treated by MBR and ozonation. Inter J Agric & Biol Eng, 2017; 10(1): 115–124.
[40] Chinnasamy S, Bhatnagar A, Claxton R. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresource Technology, 2010; 101: 6751–6760.
[41] Mulbry W, Wilkie A C. Growth of benthic freshwater algae on dairy manure. Journal of Applied Phycology, 2001; 13(4): 301–306.
[42] Mulbry W, Kondrad S, Buyer J. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. Journal of Applied Phycology, 2008; 20(6): 1079–1085.
[43] Hyenstrand P, Burkert U, Pettersson A, Blomqvist P. Competition between the green alga *Scenedesmus* and the *cyanobacterium Synechococcus* under different modes of inorganic nitrogen supply. Hydrobiologia, 2000; 435(1-3): 91–98.
[44] Ji F, Liu Y, Hao R, Li G, Zhou Y G, Dong R J. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresource Technology, 2014; 161: 200–207.
[45] Tan F, Wang Z, Zhouyang S Y, Li H, Xie Y P, Wang Y P et al. Nitrogen and phosphorus removal coupled with carbohydrate production by five microalgae cultures cultivated in biogas slurry. Bioresource Technology, 2016; 219: 749–752.
[46] Yang L B, Tan X B, Li D Y, Chu H Q, Zhou X F, Zhang Y L, et al. Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater. Bioresource Technology, 2015; 181: 54–61.
[47] Eltanahy E, Salim S, Vadiveloo A, Verduin J, Pais B, Moheimani N. Comparison between jet and paddlewheel mixing for the cultivation of microalgae in anaerobic digestate of piggery effluent (ADPE). Algal Research, 2018; 35: 274–282.
[48] Zheng H L, Wu X D, Zou G Y, Zhou T, Liu Y H, Roger R. Cultivation of *Chlorella vulgaris* in manure-free piggery wastewater with high-strenth ammonium for nutrients removal and biomass production: Effect of ammonium concentration, carbon/nitrogen ratio and pH. Bioresource Technology, 2019; 273: 203–211.
[49] Li X, Yang W L, He H J, Wu S H, Zhou Q, Yang C P, et al. Response of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. Bioresource Technology, 2018; 251: 274–279.
[50] Deng X Y, Gao K, Addy M, Li D, Zhang R C, Lu Q, et al. Cultivation of *Chlorella vulgaris* on anaerobically digested swine manure with daily recycling of the post-harvest culture broth. Bioresource Technology, 2018; 247: 716–723.
[51] Deng X Y, Gao K, Zhang R C, Addy M, Lu Q, Ren H Y, et al. Growing *Chlorella vulgaris* on thermophilic anaerobic digestion swine manure for nutrient removal and biomass production. Bioresource Technology, 2017; 243: 417–425.
[52] Wang M Z, Yang Y, Chen Z H, Chen Y Z, Wen Y M, Chen B. Removal of nutrients from undiluted anaerobically treated piggery wastewater by improved microalgae. Bioresource Technology, 2016; 222: 130–138.
[53] Marjakangas J M, Chen C Y, Lakaniemi A M, Puhakka J A, Whang L M, Chang J S. Selecting an indigenous microalgal strain for lipid production in anaerobically treated piggery wastewater. Bioresource Technology, 2016; 191: 369–376.
[54] Wang L, Li Y C, Chen P, Min M, Chen Y F, Zhu J, et al. Anaerobic

digested dairy manure as a nutrients supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Bioresource Technology, 2013; 101: 2623–2628.
[55] Singh M, Reynolds D L, Das K C. Microalgal system for treatment of effluent from poultry litter anaerobic digestion. Bioresource Technology, 2011; 102: 10841–10848.
[56] Zhu L D, Wang Z M, Shu Q, Takala J, Hitunen E, Feng P Z, et al. Nutrient removal and biodiesel production by integration of freshwater alage cultivation with piggery wastewater treatment. Water Research, 2013; 47: 4294–4302.
[57] Sun Z, Sun L, Chen G. Microalgal cultivation and nutrient removal from digested piggery wastewater in a thin-film flat plate photobioreactor. Applied Biochemistry and Biothechnology, 2019; 187: 1488–1501.
[58] Xu J, Zhao Y J, Zhao G H, Zhang H. Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digested liquid treatment. Applied Microbiology and Biotechnology, 2015; 99: 6493–6501.
[59] Wang L, Wang Y K, Chen P, Roger R. Semi-continuous cultivation of *Chlorella vulgaris* for treating undigested and digested dairy manures. Applied Biochemistry and Biotechnology, 2010; 162(8): 2324–2332.
[60] Barlow E W R, Boersma L, Phinney H K, Miner J R. Algal growth in diluted pig waste. Agriculture and Environment, 1975; 2: 339–355.
[61] Shen Y, Yuan W, Pei Z, Mao E. Culture of microalga *Botryococcus* in livestock wastewater. Transactions of the ASABE, 2008; 51: 1395–1400.
[62] Chen C Y, Yeh K L, Su H M, Lo Y C, Chen W M, Chang J S. Strategies to enhance cell growth and achieve high-level oil production of a *Chlorella vulgaris* isolate. Biotechnology Progress, 2010; 26(3): 679–686.
[63] Chen R, Li R, Deitz L, Liu R, Stevenson R J, Liao W. Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass and Bioenergy, 2012; 39: 128–138.
[64] Halim R, Gladman B, Danquah M K, Webley P A. Oil extraction from microalgae for biodiesel production. Bioresource Technology, 2011; 102(1): 178–185.
[65] Shen Y, Fan Z, Chen C, Xu X. An auto-flocculation strategy for *Chlorella vulgaris*. Biotechnology Letters, 2015; 37(1): 75–80.
[66] Zhou W, Chen P, Min M, Ma X, Wang J, Griffith R, et al. Environment-enhancing algal biofuel production using wastewaters. Renewable and Sustainable Energy Reviews, 2014; 36: 256–269.
[67] Shen Y, Yu T, Xie Y P, Chen J F, Ho S H, Wang Y X, et al. Attached culture of *Chlamydomonas* sp. JSC4 for biofilm production and TN/TP/Cu(II) removal. Biochemical Engineering Journal, 2019; 141: 1–9.
[68] Chung P, Pond W G, Kingsbury J M, Krook L. Production and nutritive value of arthrospira platensis: a spiral blue-green alga grown on swine wastes. Journal of Animal Science, 1978; 47: 319–330.
[69] Zhu Y H, Jiang J G. Continuous cultivation of *Dunaliella salina* in photobioreactor for the production of β-carotene. European Food Research and Technology, 2008; 227(3): 953–959.
[70] Sánchez J F, Fernández J M, Acién F G, Rueda A, Perez-Parra J, Molina E. Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Process Biochemistry, 2008; 43(4): 398–405.
[71] Zhang W G, Li J Y, Zhang Z H, Fan G P, Ai Y C, Gao Y, et al. Comprehensive evaluation of a cost-effective method of culturing *Chlorella pyrenoidosa* with unsterilized piggery wastewater for biofuel production. Biotechnology for Biofuels, 2019; 12(1): 69.
[72] Deng X Y, Gao K, Min M, Chen P, Li D, Zhang R C, et al. Growing *Chlorella vulgaris* on mix wastewaters for biodiesel feedstock production and nutrient removal. Journal of Chemical Technology& Biothechnology, 2018; 93(3): 2748–2757.
[73] Xue H B, Stumm W, Sigg L. The binding of heavy metals to algal surfaces. Water Research, 1988; 22(7): 917–926.
[74] Huntley M E, Redalje D G. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigation and Adaptation Strategies for Global Change, 2007; 12(4): 573–608.
[75] Przytocka-Jusiak M, Duszota M, Matusiak K, Mycielski R. Intensive culture of *Chlorella vulgaris* as the second stage of biological purification of nitrogen industry wastewaters. Water Research, 1984; 18(1): 1–7.
[76] Lin L, Chan G, Jiang B L, Lan C Y. Use of ammoniacal nitrogen tolerant microalgae in landfill leachate treatment. Waste Management, 2007; 27(10): 1376–1382.
[77] Li X, Hu H Y, Ke G, Jia Y. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. Ecological Engineering, 2010; 36(4): 379–381.
[78] Van Eykelenburg C. Ecophysiological studies on *Spirulina platensis* effect of temperature, light intensity and nitrate concentration on growth and ultrastructure. Antonie Van Leeuwenhoek, 1980; 46(2): 113–127.
[79] Källqvist T, Svenson A. Assessment of ammonia toxicity in tests with the microalga, *Nephroselmis pyriformis*, *Chlorophyta*. Water Research, 2003; 37(3): 477–484.
[80] Yen H W, Brune D E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology, 2007; 98(1): 130–134.
[81] Li Y, Han D, Sommerfeld M, Hu Q. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (Chlorophyceae) under nitrogen-limited conditions. Bioresource Technology, 2011; 102(1): 123–129.
[82] Yu R, Wang W. Biokinetics of cadmium, selenium, and zinc in freshwater alga *Scenedesmus obliquus* under different phosphorus and nitrogen conditions and metal transfer to *Daphnia magna*. Environmental Pollution, 2004; 129(3): 443–456.
[83] Wu Y, Yu Y, Li X, Hu H Y, Su Z F. Biomass production of a *Scenedesmus* sp. under phosphorous-starvation cultivation condition. Bioresource Technology, 2012; 112: 193–198.