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Efficient use of liquid digestate in microalgae cultivation for high biomass production and nutrient recovery

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ABSTRACT

In this study, different pre-treatment methods were applied to liquid digestate (LD) for the growth of the mixed microalgal culture. In addition, nutrient removal in the LD was investigated. Dilution, filtration, and centrifugation were applied as pre-treatment methods. Microalgal growth was evaluated as dry weight (DW) and nutrient removal was investigated with the analysis of ammonium (NH_4^+-N) and phosphate (PO_4^{-3}-P). Microalgal DW constantly increased throughout the experiment and reached the maximum values of 4.24, 4.02, and 1.47 g/L in centrifuged, diluted, and filtered medium, respectively. Maximum NH_4^+ (97.7%) and PO_4^{-3} (50.4%) removal efficiency was observed in the centrifuged LD. Based on the results of this study, the optimum growth medium for microalgal growth was centrifuged LD but this culture can be cultivated in diluted LD up to 40-fold. Results also showed that using LD for microalgae production is a suitable application for both nutrient supply for microalgal growth and nutrient recovery from LD.

Key words: anaerobic digestion, biogas, microalgal growth, nitrogen, phosphorus

HIGHLIGHTS

- Dilution, filtration, and centrifugation were performed as pre-treatment applications to liquid digestate.
- Maximum biomass (4.24 g/L) was provided in the centrifuged medium after 29 days of cultivation.
- The maximum removal of ammonium (97.7%) and phosphate (50.4%) was observed in the centrifuged liquid digestate.

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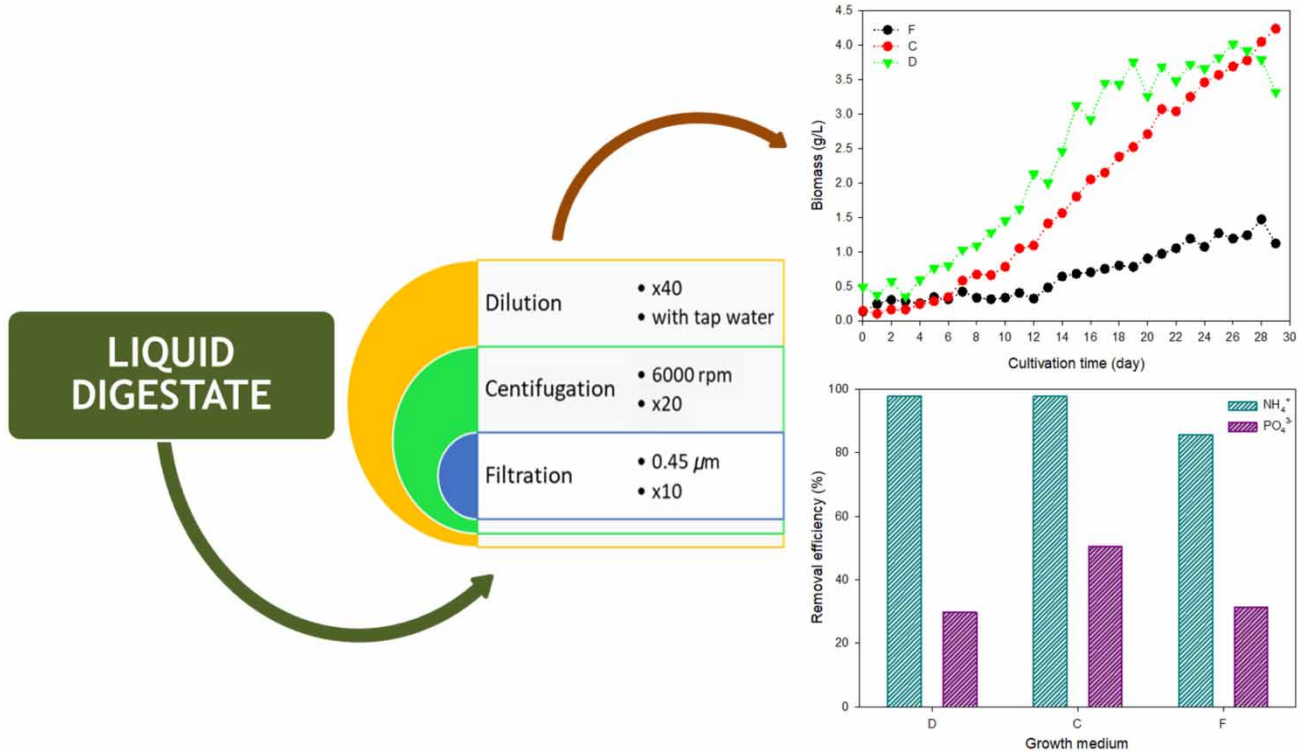
GRAPHICAL ABSTRACT



INTRODUCTION

Currently, most of the energy requirement in the world is covered by fossil fuels. However, fossil fuels have rapidly depleted and their environmental impacts have reached a hazardous level for human beings. Besides, the need to reduce foreign dependency in terms of energy resources and raise awareness of global warming, climate change, and carbon emissions has led to an interest in renewable energy resources. In recent years, anaerobic digestion of biomass (agricultural wastes, animal wastes, forestry wastes, domestic sewage sludge, micro-, and macroalgae) has become a common treatment method due to its high biogas potential. Therefore, the number of anaerobic digestion facilities in European countries has increased (Marcilhac *et al.* 2015). It is estimated that biogas energy potential in the European Union would be 1.200–2.300 PJ/year in the year 2030 (Meyer *et al.* 2018).

Biogas, solid and liquid digestate (LD) are products of anaerobic digestion. Solid digestate (SD) is more stable than LD and it can be easily handled (Xia & Murphy 2016). On the other hand, the amount of LD is very high when compared to that of SD and it is difficult to transport and process (Xia & Murphy 2016). Due to the low C/N/P ratio, anaerobic treatment is not sufficient to remove nutrients. For this reason, the nutrient content of LD is quite high. Therefore, a rich nutrient output occurs which must be properly disposed of or used. Direct application of untreated LD to the soil may result in some harmful impacts on the environment such as nutrient runoff, eutrophication (Massa *et al.* 2017), surface and groundwater pollution, ammonia volatilization (Marcilhac *et al.* 2015), and human health risk (Logan & Visvanathan 2019). Therefore, various treatment methods are applied to minimize the negative effects of LD (Razzak *et al.* 2013). One of the alternative solutions for the proper management of LD is microalgae cultivation.

Microalgae, defined as third-generation biofuel sources, are photosynthetic microorganisms with higher growth rates and production potential compared to other biofuel sources. Microalgal biomass can be transformed into renewable energy sources such as biodiesel, biogas, and biohydrogen and it is a carbon-neutral energy source (Chisti 2010). Therefore, microalgae are seen as prospective fuel sources for sustainable development. Currently, however, the high production cost of biofuels from microalgae is a significant disadvantage. Moreover, low biomass concentration increases the cost of microalgae harvesting.

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Microalgae growth is directly related to light intensity, presence of nutrients, pH, temperature, and initial algae biomass (Wang *et al.* 2010). Other important factors of microalgae production are nutrient input (Singh *et al.* 2011; Zuliani *et al.* 2016) and growth medium (Jiang *et al.* 2011). By using wastewater with high nutrient content, cost reduction and wastewater treatment can be provided. Liquid digestate is one of the types of wastewater used as a growth medium and nutrient source for microalgae cultivation (Marcilhac *et al.* 2015; Massa *et al.* 2017; Jiang *et al.* 2018). Over the last decades, investigations on digestate treatment and/or reuse with microalgae have received increasing attention (Li *et al.* 2017). Researches on digestate treatment with microalgae suggest that using this medium without pre-treatment is not suitable for biomass production due to high ammonium, turbidity, and bacteria concentration (Xia & Murphy 2016; Zuliani *et al.* 2016). It is stated that the most effective methods to reduce ammonia toxicity and turbidity are dilution and filtration (Marazzi *et al.* 2017). However, high dilution rates may reduce the nutrient concentration while dilution water requirement may cause problems in terms of sustainability and cost (Franchino *et al.* 2016). In this respect, it is necessary to optimize the pre-treatment methods to increase microalgal biomass production by reducing the dilution factor and cost.

In recent years, the number of biogas plants where wastes can be evaluated as a source of biofuel has increased. Consequently, significant increases are expected in digestate formation, which must be managed appropriately due to its potential environmental adverse effects. From a circular bioeconomy point of view, digestate has significant potential due to its high nutrient content, especially phosphorus (López-Sánchez *et al.* 2022). The aim of wastewater treatment should not only be the recovery of clean water but also the recovery of valuable components in wastewater (Duque *et al.* 2021). In this context, digestate has high potential, and studies on resource recovery from digestate have increased in recent years (Stiles *et al.* 2018). By using microalgae in digestate treatment and resource recovery, carbon dioxide removal, oxygen release and biomass production with added value that can be used in many areas can be achieved. Due to factors such as operating conditions in biogas plants and raw material properties, the digestate character is highly variable and the microalgae biomass production also shows differences (Kisielewska *et al.* 2022). Since it is not possible to apply a single method in the disposal of wastewater with a complex structure such as digestate, it is necessary to increase the number of studies to determine the appropriate conditions for digestate management in laboratory conditions before moving to real-scale applications (Bauer *et al.* 2021). As far as we have examined the literature, no study has been found in which there is a high biomass production with such a high COD concentration digestate. In addition, studies on digestate and microalgae have been carried out with certain species, and studies with mixed cultures are quite limited. In studies where digestate is evaluated as a nutrient source for microalgae, the dilution factor is very important (Malhotra *et al.* 2022). Low dilution may cause inhibition, while high dilution factors may cause nutritional deficiencies (Malhotra *et al.* 2022). Therefore, filtration and centrifugation methods were also used in this study to optimize the effect of dilution. In light of the literature studies given above, it is understood that they mainly focused on one or two of the pre-treatment applications and rarely implemented three pre-treatment methods together. It is also seen that organic matter and nutrient contents of the digestate used in literature studies were generally at the level of moderate and low. Studying the digestate including a high level of organic matter and nutrient contents can provide a new contribution to the subject of microalgae and nutrient removal. In this study, in addition to the high dilution alone application, filtration, and centrifugation pre-treatment applications were carried out for microalgae biomass production and nutrient removal. The primary aim of the applied pre-treatment methods is to reduce the dilution water requirement of LD. The main parameters assessed were biomass production along with NH_4^+-N and PO_4^{-3}-P removal.

MATERIALS AND METHODS

Digestate characterization

The LD used in this study was obtained from the decanter discharge of a biogas plant within the renewable energy complex of an energy firm. The renewable energy complex is an integrated bio-crude oil, biogas, and organic fertilizer production plant and treats 400 tons/day of organic wastes including materials from cattle and poultry farms, slaughterhouses, agricultural enterprises, and food factories in the immediate vicinity. Samples were immediately transported to the laboratory and stored at 4 °C until analysis. Physicochemical characteristics of LD used in this study are given in Table 1.

Microalgae cultivation and experimental set-up

Mixed microalgae culture obtained from a local pond was used in this study. The stock culture of microalgae used as inoculum in the experiments was obtained in Bold's Basal Medium (Bohutskyi *et al.* 2016). The growth conditions were as follows:

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Table 1 | Characterization of LD used in experiments

Parameter	Unit	Value
pH	–	8.69
Turbidity	NTU	14,300
Conductivity	mS/cm	75
Alkalinity	mg/L	8800
Chloride	mg/L	4799
COD	mg/L	102,080
PO_4^{3-}-P	mg/L	400
NH_4^+-N	mg/L	2400
NO_3^--N	mg/L	150

cool white led lamp light with a light intensity of 17,000 lux, temperature of 25 ± 1 °C, 16/8 h light-dark cycle, and the media were continuously shaken with an orbital shaker at 100 rpm.

Three types of growth medium were used for the experimental procedure. The first medium (D) was prepared as a 40-fold dilution of raw LD with tap water. For the second medium (C), LD was centrifuged for 15 min at 6000 rpm and diluted 20-fold. The third medium (F) was gathered with filtering of LD through a 0.45 μm filter and diluting 10-fold (Figure 1). Since pre-treatment of digestate avoids turbidity, lower dilutions were applied to filtered and centrifuged mediums to reduce the use of clean water to dilute the medium.

All experiments were carried out in batch mode for 29 days using 500 mL Erlenmeyer flasks with a working volume of 400 mL. For each treatment, 400 mL of growth medium was inoculated with a sufficient amount of stock culture to provide 0.1 g/L of initial biomass concentration. Mediums were continuously mixed with an orbital shaker at 100 rpm. Light intensity was set to 17,000 lux provided by white aluminum led lamps with the photoperiod of light/dark cycle of 16/8 hours. All experiments were carried out in duplicate at a controlled room temperature. The medium temperature was monitored with the temperature probe of the JBL Proflora control device and average values were reported in the results.

Analytical methods

The growth of the microalgae was determined based on dry weight (DW) according to standard methods (SM 2540D). To analyze DW, 5 mL of samples were taken and filtered through a glass fibers filter paper. The filter papers were then dried

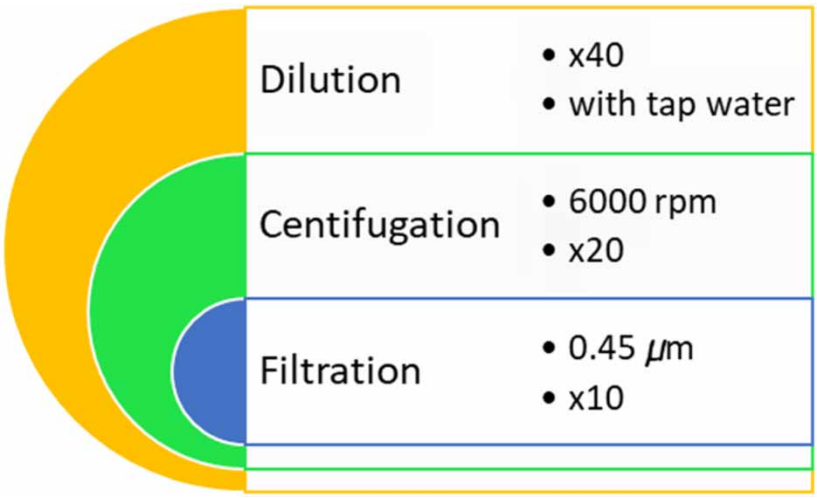

Figure 1 | Pre-treatment applications and dilution factors.

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in an oven at 105 °C for 1 hour. After 30 min cooling in a desiccator, the filter papers were weighed and DW was calculated through the weight difference.

Monitoring of the pH and temperature values along with CO_2 dosing was done using the JBL Proflora control device. Nutrient consumption was evaluated by the difference on the first and last day of the experiments. NH_4^+-N, PO_4^{3-}-P, and NO_3^--N were measured using spectrophotometric (PhotoLab 6600 UV-VIS) test kits (Merck, Germany, M114544, M114543, and M114542 respectively), on 0.45 μm filtered samples.

RESULTS AND DISCUSSION

Microalgae growth

Microalgal growth as indicated by dry weight (DW) measured at D, C, and F mediums are shown in Figure 2. DW constantly increased throughout the study and reached the maximum values of 4.24, 4.02, and 1.47 g/L in C, D, and F, respectively. The mixed microalgae culture survived in all media and approximately 3 days of lag phases occurred. After the 3rd day, the growth curve of microalgae in D and C started to level up and doubled nearly by the 15th day of treatment. The culture growth in F picked up in the latter part of the treatment and it reached the exponential phase almost 2 weeks later. However, the biomass in F could never reach the levels of the biomass in D and C. It is possible to conclude that the adaptation time to relatively high concentrations of nutrient values is longer with a 10-fold dilution. This lag phase duration can be shortened by the use of LD-adapted culture.

As shown in Figure 2, the adaptation time is shortened as the dilution factor increases. The longer duration of the lag phase in C is thought to be caused by high turbidity and bacteria due to the low dilution factor. On the other hand, while growth was continued in C because of available nutrients depending on the dilution factor, D was passed to the death phase as a result of a decrease in nutrients. Besides, as the microalgae biomass concentration increases at the end of the experimental study, the growth may be inhibited by the self-shading phenomenon, so it is difficult to determine whether growth is limited by the nutrient or light limitation (Marcilhac *et al.* 2015). Since filtration of the LD removes bacteria, lower biomass production as dry

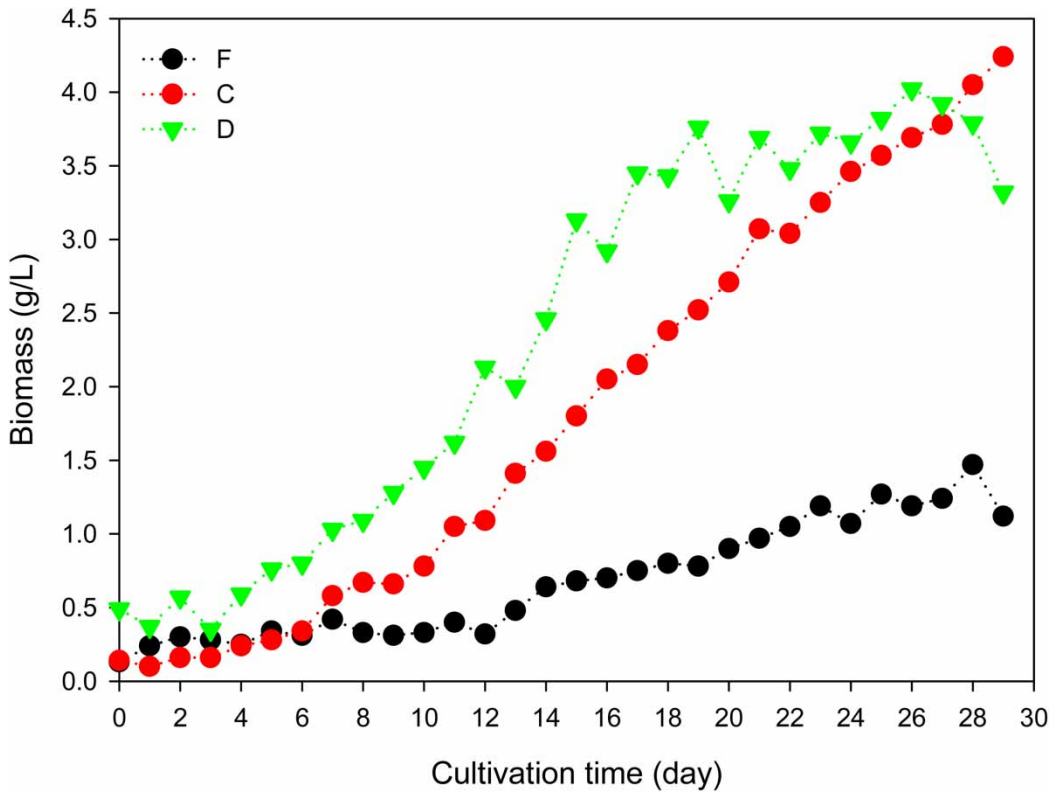

Figure 2 | Growth curves of microalgae culture with different media as recorded by dry weight.

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weight in F compared to C and D resulted because of the lack of bacteria. This is in agreement with Marcilhac *et al.* (2015) who indicated that an increasing amount of dry weight during the microalgae production in digestate was not only caused by microalgae, but also by bacteria. The effect of bacterial growth on biomass concentrations in C and D is confirmed by nutrient analysis results. On the other hand, a lower dilution factor of F can make it less suitable for microalgal growth for a series of possible reasons, namely nutrient concentration, initial color (Marcilhac *et al.* 2015), lower light transmittance, and presence of other toxic compounds (Franchino *et al.* 2016).

We also noticed that F reached the stationary phase almost by the 3rd week possibly due to higher initial nutrient concentrations, which may inhibit microalgae growth. Nevertheless, it can be concluded from these results that the optimum growth medium for microalgal growth was C but that this culture can be cultivated in D. Another indication of the increase in biomass is color. As described in previous studies (Abu Hajar *et al.* 2017; Praveen *et al.* 2018) turning from the characteristic black-brown color of digestate to green shows algal growth. The color change was observed in C and D about 5 days after the beginning, while the change in F was almost after the 10th day. Similarly, the color change due to the increase in biomass was observed by Praveen *et al.* (2018) on the 7th day.

A comparison of biomass production and nutrient removal by microalgae in liquid digestate is given in Table 2. The direct comparison of microalgae growth data with other studies is not exactly possible due to the use of different microalgae species, cultivation conditions, and LD characteristics that can affect biomass production. Cheng *et al.* (2015) used a mutant Chlorella culture in their study and higher biomass production was reported than found in our study. Uggetti *et al.* (2014) used a mixed microalgae culture for biomass production in diluted liquid digestate and obtained 2.6 g/L biomass. Approximately 1.5 g/L biomass was reported by researchers who applied dilution to digestate (Wang *et al.* 2010; Xu *et al.* 2015; Franchino *et al.* 2016; Jiang *et al.* 2018). Comparatively higher biomass production was reported in starch processing wastewater using *Chlorella pyrenoidosa* (Tan *et al.* 2014; Yang *et al.* 2015). The microalgal growth in this study can be considered relatively high if compared to a few previous studies carried out with different types of digestate (Table 2).

Nutrient removal

Nutrient removal efficiency in D, C, and F mediums are shown in Figure 3. The NH_4^+-N removal efficiency was 97.7, 97.7, and 85.6% for D, C, and F, respectively. Besides, 29.7, 50.4, and 31.3% of PO_4^{3-}P were eliminated in D, C, and F, respectively. Anaerobic digestate contains a high amount of nutrients, even if it is diluted excessively (Zuliani *et al.* 2016). It is very difficult to define the transformation-exchange mechanisms due to the alkaline pH (Logan & Visvanathan 2019), high nutrient content (Logan & Visvanathan 2019), and extremely complex structure of digestate. The characteristics of digestate vary not only with the raw material used in anaerobic digestion but also with the operational and seasonal factors. Even the properties of digestate obtained from the same plant may alter with time. Therefore, several chemical and biological variables affect nutrient removal mechanisms.

Microalgal assimilation and different mechanisms can be effective in nutrient removal (Franchino *et al.* 2013; Marcilhac *et al.* 2015). High nutrient uptake rates can be explained by the bacteria and microalgae consortium (Gonçalves *et al.* 2017). Consequently, one of the issues to be considered in this study is that digestate contains microorganisms such as bacteria and protozoa. Abu Hajar *et al.* (2017) mentioned that filtration is one of the prevention methods for microalgae from other microorganisms. Therefore, it is expected that the bacterial effect is eliminated by the filtration pre-treatment. However, since bacteria cannot be removed by centrifugation and dilution methods, there is an interaction between microorganisms and mixed microalgae strains. In particular, it is not possible to provide axenic conditions in a complex medium such as digestate (Gonçalves *et al.* 2017). In this medium, the microorganisms' consortium should be taken into account as well as the microalgae mixture (Franchino *et al.* 2013; Marcilhac *et al.* 2015). In this study, microalgal growth is estimated to be the main nutrient removal mechanism. It is suggested that the difference in the amount of produced biomass is one of the main reasons for the obtained nutrient removal efficiencies.

Ammonium is preferred primarily as a nitrogen source because it is easier to be assimilated by microalgae (Franchino *et al.* 2013; Praveen *et al.* 2018). However, it is stated that high ammonium concentrations cause inhibition (Abu Hajar Riefler & Stuart 2017; Praveen *et al.* 2018) as a result of the conversion to free ammonia due to temperature and pH. On the other hand, it is also stated that nitrate may be preferred as a nitrogen source due to the lack of inhibitory effect (Praveen *et al.* 2018). In this study, during 29 days of the batch experiment, although the ammonium was removed with a high yield, the nutrient limitation was not observed in C. Due to the low N/P ratio of digestate, the ammonium removal efficiencies were found to be quite higher compared to the phosphorus removal efficiencies. All the ammonium present in C and D was

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Table 2 | Comparison of biomass production and nutrient removal by microalgae in liquid digestate

Type of liquid digestate	Pre-treatment	Microalgae culture	Biomass (g/L)	Nutrient removal (%)		Reference
				NH_4^+	PO_4^{-3}	
Piggery digestate	Dilution	*Chlorella vulgaris*	1.47 ± 0.08	>90	>90	Franchino *et al.* (2016)
Digested and undigested dairy manure	Filtration and dilution	Chlorella sp.	1.47–1.71	100 (NH3)	62.5–74.7	Wang *et al.* (2010)
Piggery wastewater	Dilution	*Scenedesmus obliquus* (FACHB-31)	1.5–2.3	58.4–74.6 (TN)	70.1–88.8 (TP)	Xu *et al.* (2015)
Pig farm digestate	Dilution	Chlorella sp. with a bacterial consortium	1.10	30.75	–	Jiang *et al.* (2018)
Poultry litter	Centrifugation and dilution	*Arthrospira platensis* and *Chlorella vulgaris*	0.829–1.52	>95– > 99	>96– > 99	Markou (2015)
Starch processing and alcohol wastewater	Filtration, sterilization	*Chlorella pyrenoidosa*	3.0 ± 0.15	91.6 ± 4.58 (TN)	90.7 ± 4.62 (TP)	Yang *et al.* (2015)
Starch processing wastewater	Filtration, precipitation	*Chlorella pyrenoidosa*	2.05 ± 0.03	83.1 (TN)	96.7 (TP)	Tan *et al.* (2014)
Swine manure and sewage	Centrifugation, autoclave	Chlorella PY-ZU1 mutant	4.81	73	95 (TP)	Cheng *et al.* (2015)
Poultry litter	Centrifugation, dilution	*Chlorella minutissima*, *Chlorella sorokiniana*, *Scenedesmus bijuga*, and their consortium	0.313–0.612	16–49 (TN)	50–100 (TP)	Singh *et al.* (2011)
Piggery farm digestate	Filtration, dilution	Desmodesmus sp.	1.039	91.1–92.7	88.7–100	Ji *et al.* (2015)
Pig farm digestate	Filtration, dilution	Desmodesmus sp.	0.412	~100	51.2– 100	Ji *et al.* (2014)
Liquid digestate	Dilution	Mixed microalgae dominated by Scenedesmus sp.	2.6	43–100	–	Uggetti *et al.* (2014)
Cattle manure	Dilution	*Chlorella sorokiniana* CS-01, UTEX 1230 and UTEX 2714	0.280	65.0–74.7	47.0–57.7	Kobayashi *et al.* (2013)
Agro-zootechnical digestate	Dilution and centrifugation	*Neochloris oleoabundans*, *Chlorella vulgaris* and *Scenedesmus obliquus*	0.20–0.26 (g/L.day)	83.7–99.9	94.4–97.3	Franchino *et al.* (2013)
Organic waste digestate	Centrifugation, filtration, dilution	Mixed microalgae	1.47–4.24	85.6–97.7	29.7–50.4	*This study*

almost consumed, indicating a limiting role in growth. When each pre-treatment is evaluated independently, nutrient removal mechanisms are considered different for each method. Low nutrient removal efficiencies in F may be due to the presence of more nutrients than the need for a microalgae-based dilution rate. Since bacteria were removed with filtering in F and microalgal growth is lower than C and D, it is estimated that removal was not only caused by microalgae but also by struvite precipitation and/or stripping. Despite different initial nutrient concentrations of C and D, ammonium removal rates showed similar trends. For these pre-treatment methods, it can thus be assumed that the proposed mechanism of nutrient degradation in addition to the aforementioned mechanisms was the removal by non-microalgae mechanisms such as assimilation by the bacteria with nitrification and denitrification.

During anaerobic treatment, nitrogen is generally converted to ammonia and nitrate formation is low in digestate because of an oxygen-free environment. Due to the presence of oxygen produced by microalgae and the existence of COD, alkalinity, and bacteria in the medium, it is hypothesized that the nitrification process takes place here. On the other hand, the detection of nitrates in C and D samples confirmed this hypothesis. While ammonium concentrations decreased, in contrast, nitrate

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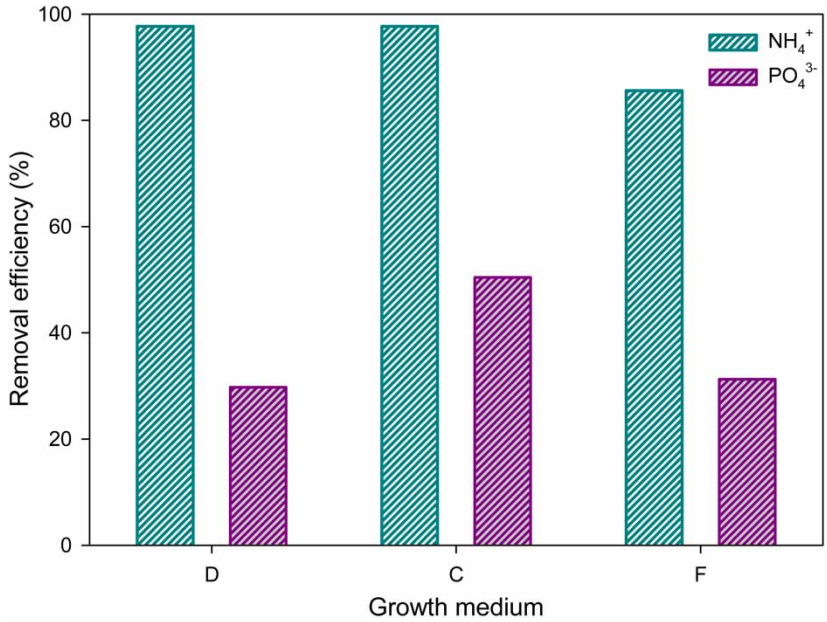

Figure 3 | Nutrients removal efficiencies of mixed culture in D, C, and F mediums.

concentration increased 5.8 times in C and 2.6 times in D. Meanwhile, there was no change in the nitrate concentration in F. Therefore, based on these results, it can be inferred that ammonia removal was not only the result of microalgae uptake but also bacteria with nitrification process. This is in agreement with Praveen *et al.* (2018) who indicated that another ammonium removal reason was nitrification. Consequently, high nutrient removal may not always be explained by the biological assimilation of microalgae (Molinuevo-Salces *et al.* 2016). In this study, it is estimated that some part of ammonium is removed by the stripping process. However, as can be seen from the increase in biomass, it is expected that there is no inhibition due to ammonium stripping. Franchino *et al.* (2013) stated that only 4% of the ammonia removal was carried out by stripping. On the other hand, they also pointed out that after sterilization of digestate, even at pH 7.65, about 60% ammonium is removed by stripping. Bohutskyi *et al.* (2016) indicated that inhibition occurred when ammonium concentration reached 100 mg/L, while Praveen *et al.* (2018) stated that there was no inhibition at 300 mg/L concentration.

Phosphorus is an essential nutrient for microalgae and especially orthophosphate, which is the preferred phosphate form, (Gonçalves *et al.* 2017) plays a significant role in microalgae growth. However, most of the phosphorus forms in the digestate are not available for algal growth (Zuliani *et al.* 2016). pH is an unstable variable due to photosynthesis reactions (Jiang *et al.* 2018). The concentration of dissolved phosphorus also varies considerably depending on pH (Marcilhac *et al.* 2015; Gonçalves *et al.* 2017). Because of the alkaline pH of digestate, a large part of the phosphorus is in particulate form as insoluble metal salts (Sforza *et al.* 2017). The other form of deposition may be in the form of struvite crystals (Marcilhac *et al.* 2015). Many mechanisms, especially precipitation and dissolution reactions, are highly affected by the change in pH. Therefore, as long as there is particulate phosphorus in the digestate, it can be transformed due to dissolution by pH change (Marcilhac *et al.* 2015; Gonçalves *et al.* 2017). During the experiments, microalgal growth was not limited by phosphorus due to the high initial phosphorus concentration. On the other hand, low phosphorus removal in D can be explained by the increase of dissolved phosphorus concentration due to pH change. The dissolution of particulate phosphorus form may have occurred due to pH being reduced from alkali values to neutral values by the addition of carbon dioxide.

Total phosphorus concentration decreases due to the separation of solid liquid by filtering and partly by centrifuging pretreatment methods. On the other hand, there is only a reduction in dilution as much as the dilution factor. Besides, although the concentration of dissolved phosphorus in F is high due to the dilution factor, conversion of particulate phosphorus in C and D to dissolved phosphorus is possible. Furthermore, for C and D, part of the phosphorus may also be removed by possible bacterial assimilation, albeit low. Results of REs showed that differences in phosphorus removal of F and D were not significant. The maximum PO_4^{-3} removal was achieved in C (50.4%) while approximately 30% of REs were gathered in D and F. Although,

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these results were lower than some published studies (Table 2), they are higher than those of Marcilhac *et al.* (2015) who found the REs in the range of 15–38% depending on the operating conditions and explained this with high initial concentrations. At the end of the experimental study, it can be concluded that microalgae did not suffer from phosphorus starvation.

CONCLUSION

In this study, various pre-treatment applications were carried out to reduce the amount of water used for dilution. Microalgae growth and nutrient removal efficiencies were investigated in filtered, centrifuged, and diluted liquid digestate. Under all conditions, mixed culture survived but the highest biomass (4.24 g/L) was provided in the centrifuged medium after 29 days of cultivation. Biomass production results showed that centrifuged digestate is suitable for microalgae cultivation. Maximum removals of PO_4^{-3} (50.4%) and NH_4^{+} (97.7%) were observed in centrifuged LD. While high nutrient content with a low dilution factor may adversely affect microalgae growth in LD, it can be concluded that the effect in this study was only to extend the adaptation period. Future research should include finding the most suitable microalgae species, optimum production conditions, and viable integrated cultivated systems with waste treatment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Abu Hajar, H. A., Riefler, R. G. & Stuart, B. J. 2017 Cultivation of Scenedesmus dimorphus using anaerobic digestate as a nutrient medium. *Bioprocess and Biosystems Engineering* **40** (8), 1197–1207. doi:10.1007/s00449-017-1780-4.

Bauer, L., Ranglová, K., Masojídek, J., Drosg, B. & Meixner, K. 2021 Digestate as sustainable nutrient source for microalgae – challenges and prospects. *Applied Sciences* **11** (3), 1056. MDPI.

Bohutskyi, P., Kligerman, D. C., Byers, N., Nasr, L. K., Cua, C., Chow, S., Su, C., Tang, Y., Betenbaugh, M. J. & Bouwer, E. J. 2016 Effects of inoculum size, light intensity, and dose of anaerobic digestion centrate on growth and productivity of Chlorella and Scenedesmus microalgae and their poly-culture in primary and secondary wastewater. *Algal Research* **19**, 278–290. doi:10.1016/j.algal.2016.09.010.

Cheng, J., Xu, J., Huang, Y., Li, Y., Zhou, J. & Cen, K. 2015 Growth optimisation of microalga mutant at high CO2 concentration to purify undiluted anaerobic digestion effluent of swine manure. *Bioresource Technology* **177**, 240–246. Elsevier Ltd. doi:10.1016/j.biortech.2014.11.099.

Chisti, Y. 2010 Fuels from microalgae. *Biofuels* **1** (2), 233–235. doi:10.4155/bfs.10.9.

Duque, A. F., Campo, R., Val del Rio, A. & Amorim, C. L. 2021 Wastewater valorization: practice around the world at pilot-and full-scale. *International Journal of Environmental Research and Public Health* **18** (18), 9466. MDPI.

Franchino, M., Comino, E., Bona, F. & Riggio, V. A. 2013 Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate. *Chemosphere* **92** (6), 738–744. Elsevier Ltd. doi:10.1016/j.chemosphere.2013.04.023.

Franchino, M., Tigini, V., Varese, G. C., Sartor, R. M. & Bona, F. 2016 Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. *Science of the Total Environment* **569–570**, 40–45. Elsevier B.V. doi:10.1016/j.scitotenv.2016.06.100.

Gonçalves, A. L., Pires, J. C. M. & Simões, M. 2017 A review on the use of microalgal consortia for wastewater treatment. *Algal Research* **24**, 403–415. doi:10.1016/j.algal.2016.11.008.

Ji, F., Liu, Y., Hao, R., Li, G., Zhou, Y. & Dong, R. 2014 Biomass production and nutrients removal by a new microalgae strain Desmodesmus sp. in anaerobic digestion wastewater. *Bioresource Technology* **161**, 200–207. Elsevier Ltd. doi:10.1016/j.biortech.2014.03.034.

Ji, F., Zhou, Y., Pang, A., Ning, L., Rodgers, K., Liu, Y. & Dong, R. 2015 Fed-batch cultivation of Desmodesmus sp. in anaerobic digestion wastewater for improved nutrient removal and biodiesel production. *Bioresource Technology* **184**, 116–122. Elsevier Ltd. doi:10.1016/j.biortech.2014.09.144.

Jiang, L., Luo, S., Fan, X., Yang, Z. & Guo, R. 2011 Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO2. *Applied Energy* **88** (10), 3336–3341. Elsevier Ltd. doi:10.1016/j.apenergy.2011.03.043.

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Jiang, Y., Wang, H., Zhao, C., Huang, F., Deng, L. & Wang, W. 2018 Establishment of stable microalgal-bacterial consortium in liquid digestate for nutrient removal and biomass accumulation. *Bioresource Technology* **268** (June), 300–307. Elsevier. doi:10.1016/j.biortech.2018.07.142.

Kisielewska, M., Dębowski, M., Zieliński, M., Kazimierowicz, J., Quattrocelli, P. & Bordiean, A. 2022 Effects of liquid digestate treatment on sustainable microalgae biomass production. *BioEnergy Research* **15** (1), 357–370. Springer.

Kobayashi, N., Noel, E. A., Barnes, A., Watson, A., Rosenberg, J. N., Erickson, G. & Oyler, G. A. 2013 Characterization of three Chlorella sorokiniana strains in anaerobic digested effluent from cattle manure. *Bioresource Technology* **150**, 377–386. Elsevier Ltd. doi:10.1016/j.biortech.2013.10.032.

Li, R., Duan, N., Zhang, Y., Liu, Z., Li, B., Zhang, D., Lu, H. & Dong, T. 2017 Co-digestion of chicken manure and microalgae Chlorella 1067 grown in the recycled digestate: nutrients reuse and biogas enhancement. *Waste Management* **70**, 247–254. Elsevier Ltd. doi:10.1016/j.wasman.2017.09.016.

Logan, M. & Visvanathan, C. 2019 Management strategies for anaerobic digestate of organic fraction of municipal solid waste: current status and future prospects. *Waste Management and Research* **37** (1_suppl), 27–39. SAGE Publications Ltd. doi:10.1177/0734242X18816793.

López-Sánchez, A., Silva-Gálvez, A. L., Aguilar-Juárez, Ó., Senés-Guerrero, C., Orozco-Nunnelly, D. A., Carrillo-Nieves, D. & Gradilla-Hernández, M. S. 2022 Microalgae-based livestock wastewater treatment (MbWT) as a circular bioeconomy approach: enhancement of biomass productivity, pollutant removal and high-value compound production. *Journal of Environmental Management* **308**, 114612. Elsevier.

Malhotra, M., Aboudi, K., Pisharody, L., Singh, A., Banu, J. R., Bhatia, S. K., Varjani, S., Kumar, S., González-Fernández, C., Kumar, S., Singh, R. & Tyagi, V. K. 2022 Biorefinery of anaerobic digestate in a circular bioeconomy: opportunities, challenges and perspectives. *Renewable and Sustainable Energy Reviews* **166**, 112642. Elsevier.

Marazzi, F., Sambusiti, C., Monlau, F., Cecere, S. E., Scaglione, D., Barakat, A., Mezzanotte, V. & Ficara, E. 2017 A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing. *Algal Research* **24**, 19–28. Elsevier B.V. doi:10.1016/j.algal.2017.03.014.

Marcilhac, C., Sialve, B., Pourcher, A. M., Ziebal, C., Bernet, N. & Béline, F. 2015 Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate. *Bioresource Technology* **175**, 224–230. Elsevier Ltd. doi:10.1016/j.biortech.2014.10.022.

Markou, G. 2015 Fed-batch cultivation of Arthrospira and Chlorella in ammonia-rich wastewater: optimization of nutrient removal and biomass production. *Bioresource Technology* **193**, 35–41. Elsevier Ltd. doi:10.1016/j.biortech.2015.06.071.

Massa, M., Buono, S., Langellotti, A. L., Castaldo, L., Martello, A., Paduano, A., Sacchi, R. & Fogliano, V. 2017 Evaluation of anaerobic digestates from different feedstocks as growth media for Tetradesmus obliquus, Botryococcus braunii, Phaeodactylum tricornutum and Arthrospira maxima. *New Biotechnology* **36**, 8–16. Elsevier B.V. doi:10.1016/j.nbt.2016.12.007.

Meyer, A. K. P., Ehimen, E. A. & Holm-Nielsen, J. B. 2018 Future European biogas: animal manure, straw and grass potentials for a sustainable European biogas production. *Biomass and Bioenergy* **111**, 154–164. Elsevier Ltd. doi:10.1016/j.biombioe.2017.05.013.

Molinuevo-Salces, B., Mahdy, A., Ballesteros, M. & González-Fernández, C. 2016 From piggery wastewater nutrients to biogas: microalgae biomass revalorization through anaerobic digestion. *Renewable Energy* **96**, 1103–1110. doi:10.1016/j.renene.2016.01.090.

Praveen, P., Guo, Y., Kang, H., Lefebvre, C. & Loh, K. C.2018 Enhancing microalgae cultivation in anaerobic digestate through nitrification. *Chemical Engineering Journal* **354** (June), 905–912. Elsevier. doi:10.1016/j.cej.2018.08.099.

Razzak, S. A., Hossain, M. M., Lucky, R. A., Bassi, A. S. & De Lasa, H. 2013 Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing – a review. *Renewable and Sustainable Energy Reviews* **27**, 622–653. Elsevier. doi:10.1016/j.rser.2013.05.063.

Sforza, E., Barbera, E., Girotto, F., Cossu, R. & Bertucco, A. 2017 Anaerobic digestion of lipid-extracted microalgae: enhancing nutrient recovery towards a closed loop recycling. *Biochemical Engineering Journal* Elsevier B.*V.* **121**, 139–146. Elsevier B.V. doi:10.1016/j.bej.2017.02.004.

Singh, M., Reynolds, D. L. & Das, K. C. 2011 Microalgal system for treatment of effluent from poultry litter anaerobic digestion. *Bioresource Technology* **102** (23), 10841–10848. Elsevier Ltd. doi:10.1016/j.biortech.2011.09.037.

Stiles, W. A., Styles, D., Chapman, S. P., Esteves, S., Bywater, A., Melville, L., Silkina, A., Lupatsch, I., Grünewald, C. F., Lovitt, R., Chaloner, T., Bull, A., Morris, C. & Llewellyn, C. A. 2018 Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities. *Bioresource Technology* **267** (June), 732–742. Elsevier. doi:10.1016/j.biortech.2018.07.100.

Tan, X., Chu, H., Zhang, Y., Yang, L., Zhao, F. & Zhou, X. 2014 Chlorella pyrenoidosa cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. *Bioresource Technology* **170**, 538–548. Elsevier Ltd. doi:10.1016/j.biortech.2014.07.086.

Uggetti, E., Sialve, B., Latrille, E. & Steyer, J. P. 2014 Anaerobic digestate as substrate for microalgae culture: the role of ammonium concentration on the microalgae productivity. *Bioresource Technology* **152**, 437–443. Elsevier Ltd. doi:10.1016/j.biortech.2013.11.036.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J. & Ruan, R. R. 2010 Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource Technology* **101** (8), 2623–2628. Elsevier Ltd. doi:10.1016/j.biortech.2009.10.062.

Xia, A. & Murphy, J. D. 2016 Microalgal cultivation in treating liquid digestate from biogas systems. *Trends in Biotechnology* **34** (4), 264–275. Elsevier Ltd. doi:10.1016/j.tibtech.2015.12.010.

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Xu, J., Zhao, Y., Zhao, G. & Zhang, H. 2015 Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digestate liquid treatment. *Applied Microbiology and Biotechnology* **99** (15), 6493–6501. doi:10.1007/s00253-015-6537-x.

Yang, L., Tan, X., Li, D., Chu, H., Zhou, X., Zhang, Y. & Yu, H. 2015 Nutrients removal and lipids production by Chlorella pyrenoidosa cultivation using anaerobic digested starch wastewater and alcohol wastewater. *Bioresource Technology* **181**, 54–61. Elsevier Ltd. doi:10.1016/j.biortech.2015.01.043.

Zuliani, L., Frison, N., Jelic, A., Fatone, F., Bolzonella, D. & Ballottari, M. 2016 Microalgae cultivation on anaerobic digestate of municipalwastewater, sewage sludge and agro-waste. *International Journal of Molecular Sciences* **17** (10). doi:10.3390/ijms17101692.

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