ORIGINAL RESEARCH ARTICLE

Biological treatment of agro-processing industrial effluents from tannery, coffee and dairy plants using green algae (*Chlorella Ssp*.) cultured in a photo bioreactor

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ABSTRACT
Due to increased environmental pollution as a result of high emission rates from agro-processing industries, the effluents must be cleaned up before being released into the environment. This study outlines the use of green algae for nutrient removal from agro-processing effluents discharged from three agro-industries (namely coffee, dairy, and tannery) in Kenya and how they can be used for the propagation of microalgae for biofuel production. Green algae were grown inside a photobioreactor containing the three agro-industrial effluents as nutrient media for 21 days. Thereafter, the algae were harvested and evaluated for biofuel production. The effectiveness of green algae (*chlorella ssp*) to extract the cations from the various agro-processing effluents was used to gauge how well they performed. Additionally, the algae growth rate, quantity of lipids, and biomass generated were used to evaluate the strains' effectiveness in producing biofuel. The results indicate that the highest maximum algae growth rate of 14.528 mg/mL occurred in the dairy effluent. The corresponding values for the coffee and tannery effluents were 13.016 mg/mL and 10.866 mg/mL, respectively. Biochemical analysis was done to establish the amount of biomass in the algae. The results showed that there was higher biomass productivity per day of 293.944, 124.849, and 91.997 µg/mL for the dairy, coffee, and tannery effluents, respectively. The contents of linolenic acid in the chlorella strain in the dairy, coffee, and tannery effluents were 13.21, 12.86, and 15.98%, respectively. The values obtained were slightly above the recommended lower limit value of 12% (EN 14214, 2004) for the production of quality biofuels. The results further show that high chemical oxygen demand removal (maximum of 47.7–67.8%) and total phosphorus removal (maximum of 95%) were achieved in all three effluents. Finally, the fatty acid methyl ester profiles produced indicated that the lipid content of the cultivated green algae was appropriate for the production of biofuel.

Keywords: Agro-processing effluents, biological treatment, cations, green algae, photo bioreactor

1.0 Introduction
The rate at which the environment suffers as a result of high amounts of heavy pollution loads and the availability of harmful components and harmful metallic components emitted from agro-processing industries is alarming (Liang et al., 2019; Alam et al., 2012). The said effluents contain very high environmental pollution abilities in comparison to other sources and, as a

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result, affect living organisms that depend on the water bodies directly or indirectly (Cooman et al., 2003). Different agro-processing effluents have varying levels of nutrient composition that can be eliminated by growing green algae (Kushwaha et al., 2011).

Microalgae are regarded as the most favourable biofuel feedstock that can result in a high amount of biomass and biodiesel production (Chisti, 2007; Ahmad et al., 2011). The growth of microalgae in wastewater from industries has been widely studied (Acién et al., 2018; Qin et al., 2016; Ummalyma & Sukumaran, 2014). During the process of cultivation, the oxygen released during the breakdown of microalgae is utilised by microorganisms to digest the organic matter while at the same time generating carbon dioxide through organic material oxidation that supports the photoautotrophic maturation of microalgae (Shahid et al., 2020). However, studies about green algae cultivation using industrial wastewater are very limited (Khalid et al., 2019). In some other cases, literature indicates that strains of green algae reported an increased rate of nutrient elimination from wastewater (Mukabane et al., 2022; Feng & Zhang, 2011).

In this study, green algae (*Chlorella ssp*.) was cultivated with three different industrial wastewaters inside a photobioreactor to estimate the ability of these strains to treat waste water by relating the ability to remove nutrients and withdrawal efficiency, lipids, biomass, and biofuel production abilities. The agro-processing effluents were effectively treated, and the required information about the quantity, quality, and discrepancies in the components of nutrients discharged was determined.

In this way, the physicochemical properties of waste water are vital in the characterization of the quality of the treatment process. Information on the physicochemical properties provided a crucial approach to the estimation of the quality and extent to which water is contaminated (Salghi, 2006). This study therefore aimed at evaluating how good the green algae are at removing nutrients from effluents from three agro-processing industries (i.e., dairy, coffee, and tannery).

2.0 Materials and methods

2.1 Acquisition and preparation of samples

Agro-processing effluents for this study were gathered from three (3) agro-industries, namely, Kenya cooperative creameries (KCC-Sotik branch), Sasini coffee plants (Kiambu), and the Thika leather industry. The discharges were thoroughly blended to integrate any accessible sediment before collection, and then they were placed in 20-litre jerricans, coded, and preserved. Filtration of the samples using Whiteman 41 filters with a diameter of 125 mm then took place to remove large sediments and take them for further analysis in the laboratory.

2.2 Isolation of green algae (*Chlorella* sp)

The *Chlorella* sp was chosen from 50 microalgae strains separated from Lake Naivasha, Kenya. Repeated dilutions were used to isolate pure colonies, followed by the use of a micromanipulator after the mixed solutions were first cultured with F medium (Pereira et al.,

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2013). The agro-processing effluents used were a modification of an OECD standard wastewater (Zhu et al., 2013).

2.3 Characteristics of agro-processing effluents

The 20-litre samples, as described above, were divided into six different sampling bottles (each holding 500 ml), one for each agro-processing effluent. They were then transferred to a lab, where each sample was split once more into three different proportions. There, they underwent a number of tests, including those for electrical conductivity, pH, biological oxygen requirement, total solids, total suspended solids, and total dissolved solids. A mixture of ascorbic acid and concentrated sulphuric acid were added to the samples, and AAS (Indarti et al., 2005) was able to estimate the quantities of CO_3^{2-}, SO_4^{2-}, PO_4^{3-} and NO_3^- in the discharge (Pereira et al., 2013).

2.4 Sampling and nutrient analysis

Every three days, samples of the effluent were taken to the laboratory for characterization and analysis. BOD and COD were estimated at the soil water engineering labs using the HACH-LDO101-probe and the HI-83399 water and wastewater multi-parameter COD photometer and pH meter, respectively, while nitrates, carbonates, sulphates, and phosphates were estimated using the Shimadzu UV-1800 UV spectrophotometer at the civil engineering laboratories all in JKUAT. The data obtained was used to determine the removal rate (RR) and the removal efficiency (RE, %) for the chlorella strain. Nutrient removal efficiency (RE, %) for the algae strains was evaluated using Equation (4), where N_o represents the nutrient concentration at the beginning of the experiment while N_f is the concentration of the nutrients in mg/L at the end of the analysis.

$$RE(\%) = \left[\frac{N_0 - N_f}{N_0}\right] \times 100 \tag{4}$$

The nutrient removal rate (RR) in mg/L/week for the algae strains was calculated using Equation (5), in which C_o is the concentration of the nutrients in mg/L at start time t_o in seconds and C_i is the concentration of the nutrient at final time t_i.

$$RR = \frac{C_0 - C_i}{t_i - t_0} \tag{5}$$

2.5 Growing of algae

The growth rate of the algae species was measured every day, starting from the first week, with respect to total chlorophyll content using a Shimadzu UV-1800 UV spectrophotometer, which measured the absorbance of ions present. The specific growth rate was computed using Equation (6), in which G_1 and G_2 represent the growth rate per day at times t_1 and t_2, respectively.

$$\mu(day^{-1}) = ln\left(\frac{G_2}{G_1}\right) \times (t_2 - t_1) \tag{6}$$

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2.6 Biomass harvesting

After 21 days, the cultured algae species were harvested for biomass extraction. The filtration method was used during harvesting, where a membrane filter of 5µm was employed. By using a high-precision scale (HTZ, Germany), the weight of each extracted sample was recorded. The effluent left behind after harvesting the algae was later subjected to BOD, COD nitrate, and phosphate analysis.

Biomass productivity per day (P) of the green algae was computed using Equation (7), in which G_i and G_o represent the biomass produced at time t_i and t_o in days, respectively.

$$P(\mu g\, mL^{-1}d^{-1}) = \frac{(G_i - G_0)}{(t_i - t_0)} \tag{7}$$

2.7 Determination of lipid content

A more effective technique was used to assess the total lipid content (Zhu et al., 2013). Samples that had been freeze-dried were extracted after 45 minutes at 45 °C using 2 ml of methanol and 10% dimethyl sulfoxide in a water bath shaker. Centrifugation of the mixture at 3000 revolutions per minute for 10 minutes was then carried out. The remainder was twice extracted using the same methods after the supernatant had been collected. A 4-mL ether-hexane mixture was then used to extract the residue for 60 minutes at 45 °C; the remaining components were then extracted two more times after the remainder of the solution was removed.

The combined extracts were then combined with 6 mL of distilled water. In a pre-weighed glass tube with nitrogen present, the organic phases were combined and evaporated to dryness. The obtained lipids were then subjected to a 24-hour freeze-dried process, after which total lipid contents (TLC) in percentage were calculated using Equation (8), where W_L and W_B represent the weights in grams of the dried algae biomass and the extracted lipids, respectively. Total lipids (TLC) were measured gravimetrically.

$$TLC(\%) = \left[\frac{W_L}{W_B}\right] \times 100 \tag{8}$$

2.8 Analysis of fatty acid methyl ester

The fatty acid methyl esters (FAME) were produced using a single-step extraction-transesterification procedure (Indarti et al., 2005). Shimadzu GC 14B at the Food Science Laboratories in JKUAT was used. Samples of effluent from three agro-processing industries were produced. The samples were homogenised and freeze-dried before being weighed out at 50 mg each. 2 mL of acetone was added, and the mixture was stirred. The extract solutions were then produced by centrifugal separation. The extract solutions were also combined with 2 mL of hexane before the extract solutions were centrifuged out of them. The extract solutions of acetone and hexane were then combined, 2 mL of ion-exchanged water was added, the mixture was agitated and centrifuged, and the upper phase was then collected and dried. The

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fatty acid methylation kit from Nacalai Tesque was used as a pretreatment for the dried material. The extract sample was diluted by a factor of 100 in hexane before being analysed. The percentage FAME composition was calculated using Equation (9). The terms A_r and $A_{i.s}$ in the equation denote the overall peak area, the internal standard peak area (methyl heptadeconatoate), and A_{comp} is the peak areas of each individual FAME profile component, respectively.

$$\text{FAME (\%)} = \left[\frac{\mathrm{A_{comp}}}{A_r - A_{i.s}}\right] \times 100 \tag{9}$$

2.9 Analysis of the individual elements present

Analysis of the individual elements was performed with the help of the ICP-OES Perkin Elmer Optima 8000 located at KEPHIS laboratories in Karen. For this analysis, 10 mg of freeze-dried samples were required. Based on the constitution of the elements, high heating values (HHV) were evaluated using the recommended relationships as illustrated by Friedl et al. (2005) in Equation 10, in which *H*, N, and *C* indicate the mass percentages of hydrogen, nitrogen, and carbon, respectively, within the sample.

$$\text{HHV (MJ kg}^{-1}) = 3.55C^2 - 232C - 2230H + 51.2C \times H + 131N + 20{,}600 \tag{10}$$

3.0 Results and discussion

3.1 Characterization of wastewater

Table 1 displays the physical and chemical characteristics of the three agro-processing effluents as well as the discharge limits stipulated by the National Environmental Management Authority (NEMA) of Kenya.

Table 1: Physical and Chemical Properties of the Agro-processing Effluents

Parameters	Tannery	Coffee	Dairy	NEMA Standards
Temperature (°C)	24±1.20	28±1.20	27±1.20	20-35
TDS (mg/L)	188±10	328±07	123±05	2000
pH	10.5±0.50	2.6±0.20	4.6±0.20	6.0-9.0
EC (mS/cm)	398±1.0	668±1.20	265±10	-
BOD_5 (mg/L)	700±10	800±10	1200±10	500
TSS (mg/L)	1200±10	510±02	420±10	-
COD (mg/L)	128±10	176±10	118±10	1000
DO	5.20±0.20	0.81±0.10	1.24±0.40	-
CO_3^{2-} (mg/L)	287±0.50	80±0.80	28±0.30	-
PO_4^{3-} (mg/L)	0.38±0.06	0.11±0.01	0.37±0.04	30
SO_4^{2-} (mg/L)	49.51±0.50	52.76±0.10	40.80±0.4	-
NO_3^- (mg/L)	7.20±0.01	3.50±0.04	0.49±0.02	100

The results show that the temperatures recorded of 28±1.20, 27±1.20, and 24±1.20°C in coffee, dairy, and tannery effluents, respectively, were within the permissible limits of 20–35 °C by NEMA standards. Previous studies show that agro-processing effluent temperatures depend on

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the industry's production process, and its allowable limits of discharge are 22 to 40°C (Abubakari et al., 2016; Tariq et al., 2005; Khan et al., 2002). The pH ranged from 2.6±0.20 to 10.5±0.50 for the three effluents, which were well below the recommended limits for disposal except for tannery effluents whose pH value was slightly above the allowable limits.

The estimated values of BOD_5 and COD were 700±10 to 1200±10 mg/L and 118±5 to 176±1 mg/L consecutively. The high values of BOD and COD in dairy effluents of 1200±2 and 176±1 mg/L, respectively, were as a result of the presence of organic content brought about by the available carbohydrates and proteins in the effluents. The BOD_5 values ranging from 700±10 to 1200±10 were slightly higher as compared to allowable values before discharging to water bodies. According to Raposo et al. (2008) and Henze & Comeau (2008), the smaller amounts of DO, which were less than 4 mg/L, available in the agro-processing effluents were due to a high level of pollution from natural materials, showing an indisputable degree of BOD_5 in the discharge.

It was observed that the levels of CO_3^{2-} were the highest among the four cations tested, with values of 287±0.5, 80±0.8, and 28±0.3 mg/L in tannery, coffee, and dairy effluents, respectively. In addition, it was noted that the levels of NO_3^- attained in the effluents were below the limits recommended and hence permitted to be disposed of into surface water bodies. The results further show that high levels of PO_4^{3-} were obtained for tannery effluent (0.380 mg/L) as compared to dairy (0.370 mg/L) and coffee (0.11±0.01) effluents. High levels of phosphate ions indicate the likelihood of effluent promoting algal sprouts and resultant damage to aquatic life as high amounts of nutrients are present (Akan et al., 2008). Clearly, there was no significant difference between the estimated values in each agro-processing effluent and those allowed for discharge by NEMA.

3.2 Nutrient removal

Nutrient removal involves the biological remediation process, which involves the use of algae to clean up various contaminants and nutrients. The effectiveness biological remediation was investigated in terms of decreases in the BOD_5, COD, CO_3^{2-}, NO_3^-, PO_4^{3-}, and SO_4^{2-} concentrations. The values were determined at an interval of 7 days for 3 weeks.

3.3 Growth and yield of algae

Figure 1 shows the growth profiles of the green algae in the effluents. It is noted that the dairy effluents presented the greatest growth rate (14.528 mg/mL^{-1}) on the 19th day. The maximum values for the tannery and coffee effluents were 10.866 mg/mL occurring on the 12th day and 13.016 mg/mL occurring on the 11th day, respectively.

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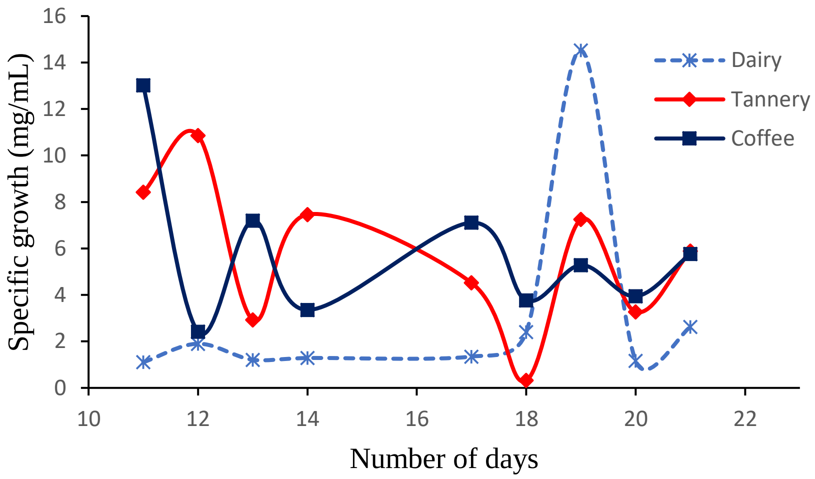

Figure 1: Chlorella vulgaris growth rate for different industrial effluents.

The growth pattern demonstrates that agro-processing effluents can offer a favourable environment for nutrients such as nitrates, carbonates, and phosphate for autotrophic development and COD for heterotrophic growth. These findings are in line with earlier research that suggested increased nutrient concentrations favour microalgae growth, leading to higher growth (Peng et al., 2020; Guo et al., 2017; and Fernando et al., 2017). It was observed that the Chlorella species growing in the neighborhood municipal effluent significantly indicated an increase in concentrations of COD, phosphate, and nitrogen (Li et al., 2011). The results show that wastewater can provide enough nutrients for heterotrophic growth in the form of COD as well as for autotrophic development in the form of phosphates and nitrates in a dark environment. The simultaneous growth acceleration and decrease in the levels of COD show that many microalgae species can survive in low light (Beuckels et al., 2014).

3.4 Production of lipid content and biomass

The content of lipids present in green algae (microalgae) plays a crucial role in the viability and applications that influence biofuel productivity. The rate at which lipid content and its constituent parts are generated is accelerated by factors like temperature and the availability of nutrients (Bellou et al., 2014).

Figure 2 shows the biomass productivity curves for the green algae in the three effluents. The maximum biomass productivity values of 293, 124, and 91 µg/mL for the dairy, coffee, and tannery effluents, respectively, were achieved on the 21st day.

The highest production of lipids (61.9898, 26.300, and 19.401 mg/L/day) was attained in the dairy, coffee, and tannery effluents, respectively, showing a similar profile as that of biomass productivity. The outcomes are consistent with the report by Bellou et al. (2014), Nayak et al. (2016), and Tang et al. (2020) who evaluated the productivity of biomass and lipid potential of various microalgae strains cultured in wastewater.

Dairy effluents had the highest biomass productivity (61.9898 mg/L/day) since the amount of nitrate ions present in the effluent was low (Table 1). The lower levels of biomass productivity

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in tannery effluents could be attributed to the lower values of sulphate ions extracted, indicating that very little sodium sulphide salts are needed for the growth of microalagae (Yan et al., 2013).

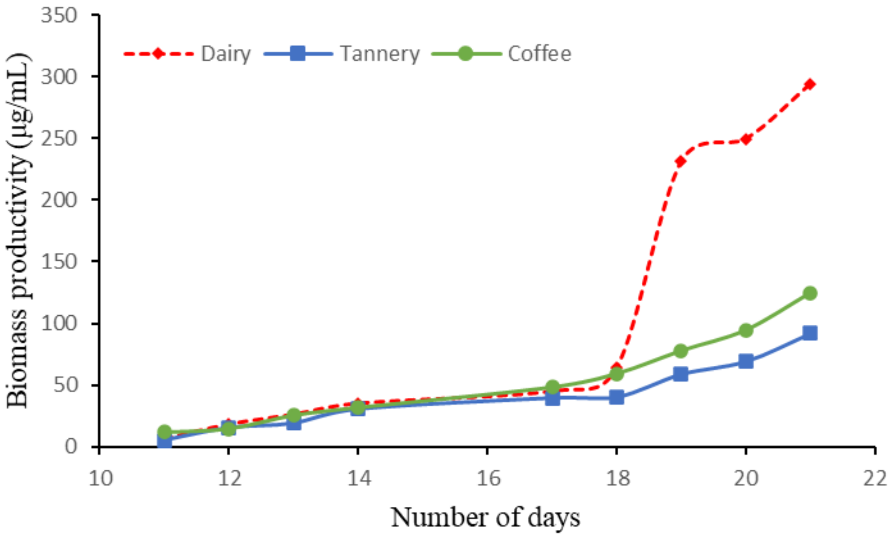

Figure 2: Biomass productivity for the three agro-industrial effluents.

Beuckels et al. (2014) indicated that Chlorella sp. in raw dairy wastewater reported the highest biomass production of microalgae in industrial wastewater at 0.11 g/L/d and 0.26 g/L/d, respectively, in pilot-scale cultures conducted outside. Various amounts of biomass productivity from green algae cultured in industrial effluent have been observed by numerous researchers. Moreover, Kumar et al. (2014) observed biomass productivity for Chlorella saccharophila and Scenedesmus sp. in untreated domestic wastewater of 0.201 g/L/d and 0.211 g/L/d, respectively. While Cheah et al. (2018) claimed that Chlorella vulgaris produced considerably more biomass than the other algae species, in this investigation, Chlorella sp. only produced a maximum of 293.944 µg/mL of biomass for the period of 21 days beyond which the lipid content would have started deteriorating.

The growth rate of the microalgae (which depends on their nutritional availability and metabolic rate), the system's operational circumstances, and the collection of the accumulated biomass for effective nutrient removal were all indirectly or directly accountable for the microalgae's efficiency in the treatment of agro-processing effluents (Cheah et al., 2018; Hena et al., 2015). The better nutrient balance brought on by the microalgae's nutrient needs may be the reason for the greater lipid content in Chlorella sp. (Kumar et al., 2014).

3.5 Fatty acid methyl ester profiles (FAME)

The FAME compositions of Chlorella sp. grown in agro-industrial effluents are summarised in Figure 3. The effluents were found to have a predominant FAME composition of C18:0 (stearic acid), C16:0 (palmitic), C18:3 (linolenate), C16:1 (palmitoleic), C14:0 (myristic acid), C20:0 (arachidic), and C18:2 (linoleic), all of which account for 79–94% of the total FAME content. These results are in agreement with those by Chinnasamy et al. (2010), who found fatty acids

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to be predominant in an algae species cultivated using agro-processing wastewater (Chinnasamy et al., 2010). The composition of linolenic acid of the chlorella strain in dairy, coffee, and tannery effluents was 13.21, 12.86, and 15.98%, respectively.

The values reported in this study compare favourably with those of 14.42 obtained by Bellou et al. (2014). These values are also slightly above the lower limit value of 12% recommended by EN 14214 (2004) for quality biofuel production. Previous studies by Aghbashlo et al. (2021) also show that in biodiesel, oleic, palmitic, stearic, linoleic, and linolenic acids predominate.
The stearic acid amounts of saturated fatty acids of 38.48% in dairy, of 36.21% in coffee, and of 34.18% were predominant in all the effluents than for other components. Monounsaturated fatty acid proportions of oleic acid were the least in all the effluents.

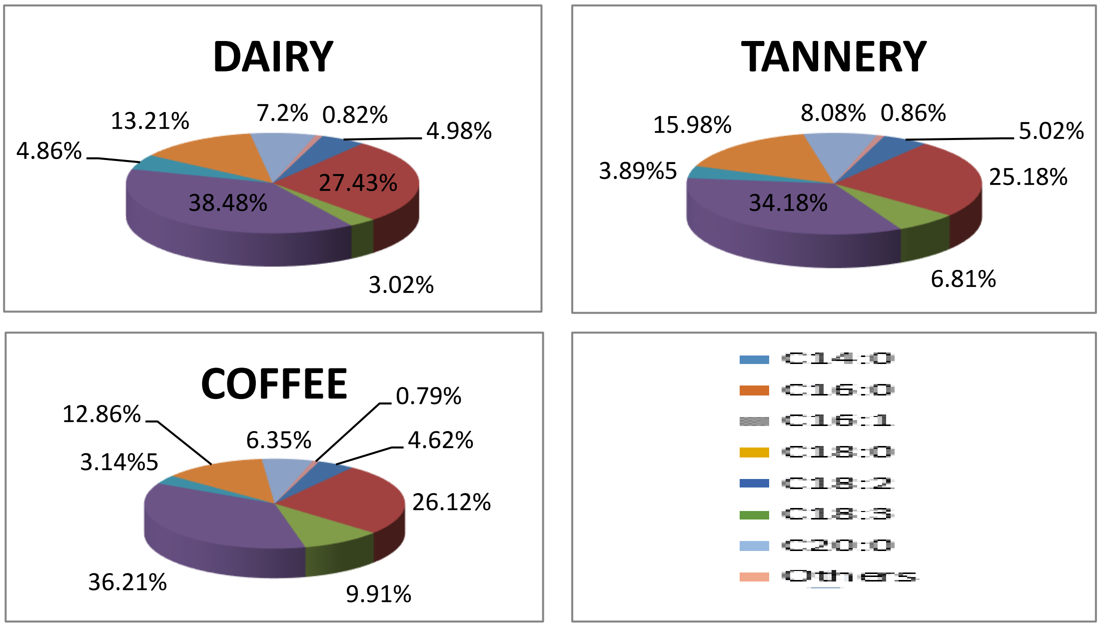

Figure 3: Summary of Chlorella sp. FAME profiles cultivated in three different effluents.

Extremely saturated fatty acids play a crucial role in oxidation resistance and sustainability whenever there are high temperatures, oxygen, light, and metal ions, making them vital components of the feedstock for biofuels (Canakci and Sanli, 2008).

4.0 Conclusions

The study was conducted to establish the feasibility of using green algae for the treatment of agro-industrial effluents from dairy, coffee, and tannery plants. Based on the level of nutrient and nutrient removal rates, growth rate, and productivity of the algae in each of the wastewater streams, the findings showed that there is a relationship between the concentration levels of wastewater nutrients and the final biomass of the cultured algae species. High removal levels of chemical oxygen demand (maximum of 47.7–67.8%) and phosphates (maximum of 95%) were achieved in all three agro-industrial effluents (coffee, dairy, and tannery) in which green algae were cultivated. In addition, biomass productivity levels of 90.4–293.944 mg/L/day for the green algae were obtained. Furthermore, the fatty acid methyl ester profiles for the lipids

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produced for biofuel generation demonstrated a comparison between biomass concentration and lipid content.

The outcomes proved that the growth of microalgae in various wastewaters with known cation concentrations can minimize the presence of impurities in the form of ions by growing only the required algae species in the known wastewater, hence increasing the yield of biomass produced. In addition, the results showed that the *Chlorella species* had the highest removal rate and was more efficient in removing cations from dairy effluents compared to effluents from coffee and tannery plants. In conclusion, further findings on the reasons regarding the superiority of *chlorella sp* and their application in large-scale production are needed.

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5.2 Conflict of interest

None.

5.3 Ethical consideration

None.

6.0 References

Abubakari, A., Oppong-Kyekyeku, A. F., Emmanuel, D., & Adiyiah, J. (2016). The Effect of Industrial Effluents on the Quality of Onukpawahe Stream, Tema-Ghana. *Journal of Environmental Science and Engineering*, *5*(1), 457-475.

Acién Fernández, F. G., Gómez-Serrano, C., & Fernández-Sevilla, J. M. (2018). Recovery of nutrients from wastewaters using microalgae. *Frontiers in Sustainable Food Systems*, *2*, 59-60.

Adeniyi, O. M., Azimov, U., & Burluka, A. (2018). Algae biofuel: current status and future applications. *Renewable and Sustainable Energy Reviews*, *90*, 316-335.

Aghbashlo, M., Peng, W., Tabatabaei, M., Kalogirou, S. A., Soltanian, S., Hosseinzadeh-Bandbafha, H., & Lam, S. S. (2021). Machine learning technology in biodiesel research: a review. *Progress in Energy and Combustion Science*, *85-86*.

Ahmad, A. L., Yasin, N. M., Derek, C. J. C., & Lim, J. K. (2011). Microalgae as a sustainable energy source for biodiesel production: a review. *Renewable and sustainable energy reviews*, *15*(1), 584-593.

Alam MZ, Ahmad S. Toxic chromate reduction by resistant and sensitive bacteria isolated from tannery effluent contaminated soil. *Ann. Microbiol.* 2012; 62:113-21.

Akan, J. C., Abdulrahman, F. I., Dimari, G. A., & Ogugbuaja, V. O. (2008). Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara wastewater channelin Kano Metropolis, Kano State, Nigeria. *European Journal of Scientific Research*, *23*(1), 122-133.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i1.2

Bellou, S., Baeshen, M. N., Elazzazy, A. M., Aggeli, D., Sayegh, F., & Aggelis, G. (2014). Microalgal lipids biochemistry and biotechnological perspectives. *Biotechnology Advances*, *32*(8), 1476-1493.

Beuckels, A., Smolders, E., & Muylaert, K. (2014). Wastewater treatment using microalgae. In VLIZ Young Marine Scientists' Day, Date: 2014/03/07-2014/03/07, Location: Bruges, Belgium.

Mukabane, B. G., Gathitu, B., Mutwiwa, U., Njogu, P., & Ondimu, S. (2022, March). Microalgae Cultivation Systems for Biodiesel Production. In *Proceedings of the Sustainable Research and Innovation Conference* (pp. 17-22).

Canakci, M., & Sanli, H. (2008). Biodiesel production from various feedstocks and their effects on the fuel properties. *Journal of Industrial Microbiology and Biotechnology*, *35*(5), 431-441.

Cheah, W. Y., Show, P. L., Juan, J. C., Chang, J. S., & Ling, T. C. (2018). Waste to energy: the effects of Pseudomonas sp. on Chlorella sorokiniana biomass and lipid productions in palm oil mill effluent. *Clean Technologies and Environmental Policy*, 20(9), 2037-2045.

Chinnasamy, S., Bhatnagar, A., Hunt, R. W., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology*, *101*(9), 3097-3105.

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

Cooman, K., Gajardo, M., Nieto, J., Bornhardt, C., & Vidal, G. (2003). Tannery wastewater characterization and toxicity effects on Daphnia spp. *Environmental Toxicology: An International Journal*, *18*(1), 45-51.

Fernando, Y., & Hor, W. L. (2017). Impacts of energy management practices on energy efficiency and carbon emissions reduction: a survey of Malaysian manufacturing firms. *Resources, Conservation and Recycling*, *126*, 62-73.

Feng, Y., Li, C., and Zhang, D. (2011). Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. *Bioresource Technology*, 102(1), 101-105.

Friedl, A., Padouvas, E., Rotter, H., & Varmuza, K. (2005). Prediction of heating values of biomass fuel from elemental composition. *Analytica Chimica Acta*, *544*(1-2), 191-198.

Guo, G., Cao, W., Sun, S., Zhao, Y., & Hu, C. (2017). Nutrient removal and biogas upgrading by integrating fungal–microalgal cultivation with anaerobically digested swine wastewater treatment. *Journal of Applied Phycology*, 29(6), 2857-2866.

Hena, S., Fatimah, S., & Tabassum, S. (2015). Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. *Water Resources and Industry*, *10*, 1-14.

Henze, M., & Comeau, Y. (2008). Wastewater characterization. *Biological wastewater treatment: Principles modelling and design*, 33-52.

Indarti, E., Majid, M. I. A., Hashim, R., & Chong, A. (2005). Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. *Journal of Food Composition and Analysis*, *18*(2-3), 161-170. 1

Khalid, A. A. H., Yaakob, Z., Abdullah, S. R. S., & Takriff, M. S. (2019). Analysis of the elemental composition and uptake mechanism of Chlorella sorokiniana for nutrient removal in agricultural wastewater under optimized response surface methodology (RSM) conditions. *Journal of Cleaner Production*, *210*, 673-686.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i1.2

Khan, S., Khan, A. M., & Khan, M. N. (2002). Investigation of pollutants load in waste water of Hayatabad Industrial Estate, Peshawar, Pakistan. *Journal of Applied Sciences*, *2*(4), 457-461.

Kumar, K., Dasgupta, C. N., & Das, D. (2014). Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. *Bioresource Technology*, 167, 358-366.

Kushwaha, J. P., Srivastava, V. C., & Mall, I. D. (2011). An overview of various technologies for the treatment of dairy wastewaters. *Critical reviews in food science and nutrition*, *51*(5), 442-452.

Li, Y., Zhou, W., Hu, B., Min, M., Chen, P., & Ruan, R. R. (2011). Integration of algae cultivation as biodiesel production feedstock with municipal wastewater treatment: strains screening and significance evaluation of environmental factors. *Bioresource Technology*, 102(23), 10861-10867.

Liang, L., Wang, Z., & Li, J. (2019). The effect of urbanization on environmental pollution in rapidly developing urban agglomerations. *Journal of cleaner production*, *237*, 117649.

Nayak, M., Karemore, A., & Sen, R. (2016). Performance evaluation of microalgae for concomitant wastewater bioremediation, CO2 biofixation and lipid biosynthesis for biodiesel application. *Algal Research*, *16*, 216-223.

Peng, L., Fu, D., Chu, H., Wang, Z., & Qi, H. (2020). Biofuel production from microalgae: a review. *Environmental Chemistry Letters*, 18(2), 285-297.

Pereira, H., Barreira, L., Custódio, L., Alrokayan, S., Mouffouk, F., Varela, J., & Ben-Hamadou, R. (2013). Isolation and fatty acid profile of selected microalgae strains from the Red Sea for biofuel production. *Energies*, *6*(6), 2773-2783.

Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., & Yuan, Z. (2016). Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. *Environmental Science and Pollution Research*, *23*(9), 8379-8387.

Raposo, F., De la Rubia, M. A., Borja, R., & Alaiz, M. (2008). Assessment of a modified and optimised method for determining chemical oxygen demand of solid substrates and solutions with high suspended solid content. *Talanta*, *76*(2), 448-453.

Salghi R. Différents filières de traitement des eaux. . (2006) Cours. Ecole nationale des sciences appliquées d'AGADIR. Université *Iben Zohir. Roaume Du Maroc*.48-53.

Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S., & Mehmood, M. A. (2020). Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, *704-705.*

Tang, D. Y. Y., Yew, G. Y., Koyande, A. K., Chew, K. W., Vo, D. V. N., & Show, P. L. (2020). Green technology for the industrial production of biofuels and bioproducts from microalgae: a review. *Environmental Chemistry Letters*, *18*, 1967-1985.

Tariq, S. R., Shah, M. H., Shaheen, N., Khalique, A., Manzoor, S., & Jaffar, M. (2005). Multivariate analysis of selected metals in tannery effluents and related soil. *Journal of Hazardous Materials*, *122*(1-2), 17-22.

Ummalyma, S. B., & Sukumaran, R. K. (2014). Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load. *Bioresource Technology*, *165*, 295-301.

Yan, C., & Zheng, Z. (2013). Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae Chlorella sp. *Bioresource Technology*, *139*, 292-299.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i1.2

Zhu, L., Wang, Z., Takala, J., Hiltunen, E., Qin, L., Xu, Z., & Yuan, Z. (2013). Scale-up potential of cultivating Chlorella zofingiensis in piggery wastewater for biodiesel production. *Bioresource Technology*, 137, 318-325.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v23i1.2