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Cultivation of *Chlorella vulgaris* in Membrane-Treated Industrial Distillery Wastewater: Growth and Wastewater Treatment

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The alcohol industry discharges large quantities of wastewater, which is hazardous and has a considerable pollution potential. Cultivating microalgae in wastewater is an alternative way of overcoming the current high cost of microalgae cultivation and an environmentally friendly treatment method for industrial effluents. The study analyzed the growth and biochemical composition of *Chlorella vulgaris* cultivated in membrane-treated distillery wastewater (MTDW) and nutrients removal efficiency. The results showed biomass productivity of 0.04 g L^{-1} d^{-1} for MTDW with the contents of content of protein, carbohydrate, and lipid at 49.6 ± 1.4%, 26.1 ± 0.6%, and 10.4 ± 1.8%, respectively. The removal efficiencies of TN, TP, and COD were 80, 94, and 72.24% in MTDW, respectively. In addition, removal efficiencies of 100, 85.37, and 42.86% for Ca^{2+}, Mg^{2+}, and Mo^{2-} were achieved, respectively. The study added to our growing knowledge on the cultivation of *Chlorella* with wastewater, suggesting that it was feasible to cultivate *Chlorella* with MTDW and represented an economical and environmentally friendly strategy for microalgae biomass production and reuse of wastewater resources.

Keywords: wastewater treatment, microalgae, *Chlorella*, distiller wastewater, biomass production

INTRODUCTION

Industrial wastewater is one of the main sources of pollution of the water environment, and its production has a serious negative impact on the ecosystem and human life. Therefore, the fight against wastewater has become a major issue in terms of health, environment, and economy (Abdel-Raouf et al., 2012). The quality and quantity of industrial wastewater vary according to the type of industry. The metal processing industry emits chromium, nickel, zinc, cadmium, lead, iron, and titanium compounds. The printing plant releases inks and dyes (Hanchang, 2009). Wastewater from paper mills contains chloride organics and dioxins, as well as suspended solids and organic wastes (Lindholm-Lehto et al., 2015). The petrochemical industry discharges a large number of phenols and mineral oil (Éverton et al., 2018). The content of suspended solids and organic matter in wastewater from food processing plants is very high (Qasim and Mane, 2013). In contrast, distilleries produce large amounts of acidic, stubborn, and colored wastewater with high organic content (Sanjay and Jamaluddin, 2018), which may lead to the destruction of the aquatic environment causing eutrophication, affecting human health and recreational activities (Sanjay and Jamaluddin, 2018; Stutter et al., 2018; Thoré et al., 2021). The alcohol industry discharges up to 0.3 billion m^3 of high-concentration wastewater each year in China (Guo et al., 2006), which has become the second largest

source of organic pollution. Cassava alcohol wastewater is widely produced in cassava-based bioethanol industries (Quan et al., 2014). In general, about 12 tons of wastewater would be generated to produce 1 ton of cassava ethanol (Lin et al., 2016). Industrial wastewater with high organic and acidic substances many contain as high as 40,000~130,000 mg L^{-1} chemical oxygen demand (COD) concentration (Gang et al., 2008).

The source of industrial wastewater comes with different characteristics. Hence, the treatment of industrial wastewater should be specifically designed for specific wastewater. Recently, several technologies such as photocatalysis (Al-Mamun et al., 2019), electrodialysis (Deng et al., 2020; Liu et al., 2020), iron-oxides-doped granular activated carbon catalyst (Deng et al., 2021), and Fe/C galvanic cells strengthened A2O process (Fe/C-A2O) (Peng et al., 2020) have been developed with significant effects for industrial wastewater treatment. Presently, distilleries use a multi-stage strategy in wastewater treatment, including pretreatment, secondary treatment, and tertiary treatment. Pretreatment reduces temperature of the wastewater as well as removes suspended solids of large particles in the wastewater (Yang and Wyman, 2007), while an anaerobic wastewater treatment system removed most of the organic matter in the wastewater (Sanjay and Jamaluddin, 2018). Finally, most of the N and P in the wastewater is removed through advanced treatment such as anaerobic and aerobic processes and membrane bioreactor (MBR) (Noor et al., 2013). However, the wastewater after membrane treatment still contains some amount of organic matter, nutrients, and other substances, which may be harmful to the environment. Conventional treatment methods are extensively used, they are however characterized by excessive use of chemicals, high operational and maintenance cost. These methods also generate huge amounts of sludge, thereby making conventional treatment methods environmentally and economically unfavorable (Amenorfenyo et al., 2020).

The microalgae-based wastewater treatment is an environmentally friendly wastewater treatment method, which is often used to treat secondary or tertiary wastewater and is considered one of the promising technologies for wastewater treatment. As a kind of eukaryotic green microalgae with strong photosynthesis ability, *Chlorella* is considered as one of the easily cultivated microalgae that contains high-quality protein, carotenoids, vitamins, and minerals, it has long been proposed as a healthy food substitute for humans and animals (Liu et al., 2013; Liu et al., 2014; Znad et al., 2018). The use of *Chlorella* for wastewater treatment and nutrient recovery reduces cost of wastewater treatment, and production of useful biomass (Sánchez-Zurano et al., 2021). In recent years, cultivation of microalgae especially *Chlorella vulgaris* in wastewater have attracted more and more attention. According to previous studies, *Chlorella* could remove organic contaminants, and heavy metals from as urban wastewater (Tercero et al., 2014), domestic wastewater (Aziz and Ng, 1992), textile wastewater (Chu et al., 2009), and piggery wastewater (Ji et al., 2012). However, little is known about cultivation of *Chlorella* in membrane-treated distillery wastewater (MTDW) for nutrient recovery and biomass production, and its feasibility.

In this study, we performed the biomass production of *Chlorella* coupled with the treatment of MTDW, which seeks to explore the nutrient removal efficiency, biomass production and productivity, and biochemical content of *C. vulgaris* grown in MTDW. The work sought to give useful information that will lead to the understanding of the cost-effective method of wastewater treatment and microalgae biomass production.

MATERIALS AND METHODS

Collection and Pre-Inoculation of Microalgae

Freshwater algae *C. vulgaris* was acquired from the laboratory of Ecology of Water Area and Aquaculture Environment of Fisheries College, Guangdong Ocean University, South China. The vegetative cells were grown photoautotrophically at 2000 lx (white light). The 7-day-old algal cells were collected and inoculated into 2 L Erlenmeyer flasks filled with 1 L of BG11 medium at an initial optical density of 0.2 (OD_{680}) in FDFF illuminated incubator 2000 lx (white light) and 25°C for 9 days.

Wastewater Collection and Analysis

The wastewater used in this study, MTDW was collected from SDIC Guangdong Bio-Energy Co., Ltd., Zhanjiang, South China. The wastewater sample was collected in a 5 L plastic container that was thoroughly pre-washed with the wastewater from the company. To reduce the decomposition of a substrate, MTDW sample was stored at 4°C before the wastewater characteristics analysis. Then wastewater was pretreated by means of filtration using a glass microfiber filter (934-AH, Whatman, United States) to remove turbidity and large particles. The filtered sample was autoclaved at 121°C for 30 min to eliminate bacteria and other algal growth inhibitors.

Determination of Dry Weight and Chlorophyll *a*

Microalgae biomass concentration were measured every 72 h. Dry weight (DW) was determined by filtering a 10 ml samples of the algal suspension through pre-weighed (m_1) filters (47 mm, 1.2 μm, Whatman). Then drying the filters (105°C, overnight) to a constant weight and weighing with microbalance (m_2). The DW (g L^{-1}) was calculated with **Eq. 1**.

$$\boldsymbol{DW} = (\boldsymbol{m_2} - \boldsymbol{m_1}) \times \mathbf{10^3/10}. \quad (1)$$

Biomass productivity (g L^{-1} day^{-1}) was calculated with **Eq. 2**.

$$\boldsymbol{Biomass\ productivity} = (\boldsymbol{DW_i} - \boldsymbol{DW_0})/(\boldsymbol{t_i} - \boldsymbol{t_0}), \quad (2)$$

where DW_i and DW_0 represent the dry biomass (g L^{-1}) at time t_i and t_0 (day).

The pigment contents of the microalgae thus chlorophyll *a* was analyzed after extraction in 95% ethanol (w/v). Briefly, 5 ml of the suspensions were filtered and freeze-dried at −20°C for 12 h; the dried biomass was suspended in ethanol for 4 h in dark.

The suspensions were later centrifuged at 5,000 rpm for 10 min and the pigment contents of the supernatant were spectrophotometrically measured at 665 and 649 nm. The **Eq. 3** was used to calculate the pigment contents (Hartmut and Alan, 1983).

$$\textit{Chlorophyll a} = (13.95 \times A_{665}) - (6.88 \times A_{649}). \quad (3)$$

Carbohydrate, Protein and Lipid Quantification

Protein extractions were determined according to the modified method described in Barbarino and Lourenço (2005) and Ge et al. (2018). As follows: weigh 30 mg powder of *Chlorella*, and then add 8 ml of distillery water. After soaking for 12 h, centrifuge at 15,000 rpm (4°C) for 20 min to collect the supernatant. Then use 2.0 ml 0.1 N (or 2 M) NaOH to re-extract the concentrated pellets. After centrifugation at 15,000 rpm (21°C) for 20 min, the supernatant was collected and mixed with the previous supernatant. 10 ml of the extract was taken to determine protein concentration with the aid of Bio-Rad DC protein assay (Cat. 500-0111, Bio-Rad Laboratories, Hercules, United States). Anthrone colorimetric approach was used to assess carbohydrate and protein content of the supernatants using a Hach model DR 2800 spectrophotometer, glucose, and serum albumin were used as the standard for carbohydrate and protein.

Ge and Champagne (2016) methods were used to assess lipid content. Briefly, the microalgal suspension was harvested via centrifugation (4°C, 5000 rpm, 10 min), the bio-mass was washed twice with distillery water, and then oven-dried overnight at 60°C. A 0.1 g dry biomass of *Chlorella* was immersed in 3 ml of distillery water and vortexed at 3,000 rpm for 30 s, then placed in a water bath at 90°C for 20 min. Methanol/chloroform (extraction solution) of a proportion of 1:2 v/v was added after it attained room temperature. The lipids of sample were extracted overnight at room temperature after which, 1 ml of distillery water was added. The organic phase was collected by centrifugation (20°C, 10 min) and transferred into a pre-weighted dish. The chloroform was evaporated at 50°C, and then the extracted lipids were subjected to gravimetric analysis.

Nutrients Concentration and Removal Efficiency

All samples were filtered through filter paper (0.22 μm, Whatman) and analyzed for nutrients, TN, TP, and COD. Macro and Micronutrients were tested by Qingdao Sci-tech Innovation Quality Testing Co., Ltd. (China). Total Nitrogen (TN), Total phosphorus (TP), and COD were determined every 3 days starting from the day of inoculation. Persulfate digestion method and acid-persulfate digestion method were used for analyzing TN and TP, respectively. COD concentration was measured with a multi-functional water quality analyzer (LIANHUA, 5B-3B, China).

Nutrient removal efficiency (%) is calculated by the **Eq. 4**.

$$\textit{Nutrient removal efficiency} = \frac{(C_0 - C_1)}{C_0} \times 100\%, \quad (4)$$

where C_0 is the nutrient concentration of the influent and C_1 is the nutrient concentration of the effluent.

FAMEs Test and GC Analysis

A 0.1 g wet sample of *Chlorella* was hydrolyzed and methylated with 2 ml of 100% acetyl chloride in 20 ml of methanol solution at 90°C (Ge et al., 2018). Then, filter the sample with filter paper (90 mm, Whatman) by washing it with 10 ml of methanol. Next, use a rotary evaporator to evaporate the methanol, and then add 10 ml of hexane and vortex the sample for 5 min. Use a glass pipette (Fisherbrand™ Pasteur) to remove the hexane layer and evaporate the hexane, and then analyze the recovered FAME by gas chromatography (GC). Helium was used as a carrier gas. The temperature of the injector and detector is 260°C. The FAME peaks in the samples were identified by comparing their retention times with those of the standards (Supelco TM 37 component FAME mix, Sigma-Aldrich).

RESULTS

Microalgae Growth

C. vulgaris was cultivated in MTDW and BG11 (control) under 30°C, 4,000 lx, 40% Vinoculation/Vmedia growth condition for 15 days to assess biomass and biomass productivity. The growth curve of *C. vulgaris* was shown in **Figure 1A**. The algal biomass in MTDW and control are 0.65 g L^{-1} and 0.26 g L^{-1} with biomass productivity of 0.04 g L^{-1} d^{-1} (MTDW) and 0.02 g L^{-1} d^{-1} (control) respectively, after 15 days. This result was totally higher than the results obtained by Tan et al. (2018). The study showed maximum Chl-*a* (see **Figure 1B**) content in both the MTDW and the control with 6.48 ± 0.67 mg L^{-1} and 1.80 ± 0.65 mg L^{-1} on day 14 and day 8 respectively. However, both media showed a decreasing trend in Chl-*a*, with 5.73 ± 0.94 mg L^{-1} and 0.76 ± 0.16 mg L^{-1} at the end of the cultivation period. Both treatments showed totally different growth patterns. The MTDW experienced a lag phase between day 1 and 2, and the exponential growth phase was experienced in day 3 and lasted for 14 days. The stationary phase set in toward the end of the cultivation period as the nutrient concentration diminished. The control showed steady growth throughout the cultivation period. The lag phase lasted for a day whiles exponential growth begun on the third day and lasted up to day 14.

Biochemical Composition of *C. vulgaris* Cultivated in MTDW and BG11 Media

Table 1 depicts the biochemical composition of *C. vulgaris* biomass cultivated in MTDW and control (BG11) at the end of the treatment. As the results showed, MTDW demonstrated 2-fold higher protein content than the control. The MTDW recorded 49.6 ± 1.4% protein content compared to 22.4 ± 2.3% of the control medium. And 26.1 ± 0.6% and 29.9 ± 1.1%, and 10.4 ± 1.8% and 16.2 ± 0.4% of carbohydrate and lipids for MTDW and the control respectively. Compared with the control, the reason for the lower

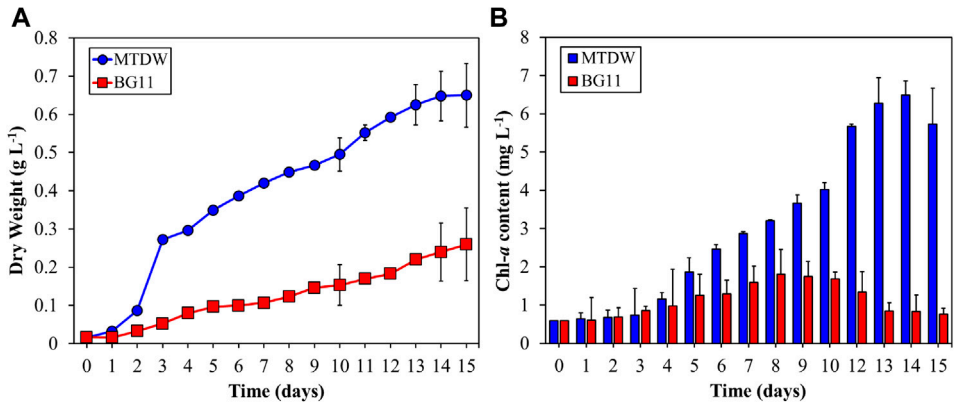

FIGURE 1 | The dry weight **(A)** and Chl-*a* content **(B)** of *C. vulgaris* in the MTDW group and BG11 group.

TABLE 1 | Biochemical compositions of *C. vulgaris* cultivated in MTDW and BG11.

Composition (%)	MTDW	Control
Protein	49.6 ± 1.4	22.4 ± 2.3
Carbohydrate	26.1 ± 0.6	29.9 ± 1.1
Lipid	10.4 ± 1.8	16.2 ± 0.4

TABLE 2 | Amino acids compositions and contents (g/100 g dry biomass) of *C. vulgaris* cultivated in MTDW and BG11.

Amino acids	MTDW	BG11
Aspartate	3.30	2.535
Threonine	1.15	0.75
Serine	1.55	0.85
Glutamate	6.90	4.67
Glycine	3.16	2.31
Alanine	4.16	3.350
Valine	2.89	2.30
Isoleucine	2.12	1.40
Leucine	4.25	3.48
Tyrosine	1.29	0.50
Phenylalanine	2.14	1.35
Lysine	4.46	3.345
Histidine	1.04	0.61
Arginine	5.92	3.900
Proline	2.49	1.21

carbohydrate and lipid content in MTDW may be related to the light intensity. This is in agreement with a similar result obtained by Qiu et al. (2019). It is however clear that MTDW can promote protein production in *C. vulgaris* than BG11 medium. This result was slightly higher than that of Miao et al. (2016). It is documented that higher nitrogen content induces algae growth that could lead to accumulation of amino acid (Martínez et al., 2000). *C. vulgaris* showed higher biomass growth in MTDW than BG11 which translated into higher protein production.

The amino acid content and composition were analyzed in MTDW and BG11 at the end of the cultivation. As shown in **Table 2**, all tested amino acids in the MTDW group were higher than those in the control group.

TABLE 3 | Fatty acid compositions and contents (g/100 g dry biomass) of *C. vulgaris* cultivated in MTDW and BG11.

Fatty acid	MTDW	BG 11
Myristic Acid (14:0)	0.11	0.14
Pentadecanoic Acid (15:0)	0.001	0.001
Palmitic Acid (16:0)	0.139	0.312
Palmitoleic Acid (16:1)	0.012	0.03
Margaric Acid (17:0)	0.001	0.031
Oleic Acid (18:1)	0.43	0.80
Stearic Acid (18:0)	0.016	0.013
Linoleic Acid (18:2, ω-6)	0.137	0.169
Arachidonic Acid (20:0)	0.044	0.036
γ-Linolenic Acid (18:2)	0.003	0.003
α-Linolenic Acid (18:3)	0.249	0.218
Behenic Acid (22:0)	0.004	0.004
Erucic Acid	0.068	0.078
11,14,17-Eicosatrienoic Acid (20:5 ω-3)	0.043	0.053
Arachidonic Acid	0.002	0.002
13-16-Docosadienoic Acid (22:6, ω-3)	0.003	0.003
Lignoceric Acid	0.004	0.004
15-Tetracosenoic Acid	0.002	0.002

GC analysis of fatty acid composition *C. vulgaris* in both MTDW and the control are shown in **Table 3**. The contents of Myristic Acid, Palmitic Acid, Margaric Acid, Oleic Acid, Linoleic Acid, Erucic Acid, and 11,14,17-Eicosatrienoic Acid in the MTDW group were lower than those in the control group, and the contents of Palmitoleic Acid, Stearic Acid, Arachidonic Acid, and α-Linolenic Acid were higher than those in the control group. Pentadecanoic Acid, γ-Linolenic Acid, Behenic Acid, Arachidonic Acid, 13-16-Docosadienoic Acid, Lignoceric Acid, and 15-Tetracosenoic Acid were similar in both MTDW and the control.

MTDW Treatment Using *C. vulgaris*

TN, TP, and COD Removal

The concentration of TN, TP, and COD were presented in **Figure 2**. Nitrogen, is the main component of algal proteins and enzymes catalyst, and are responsible for microalgae

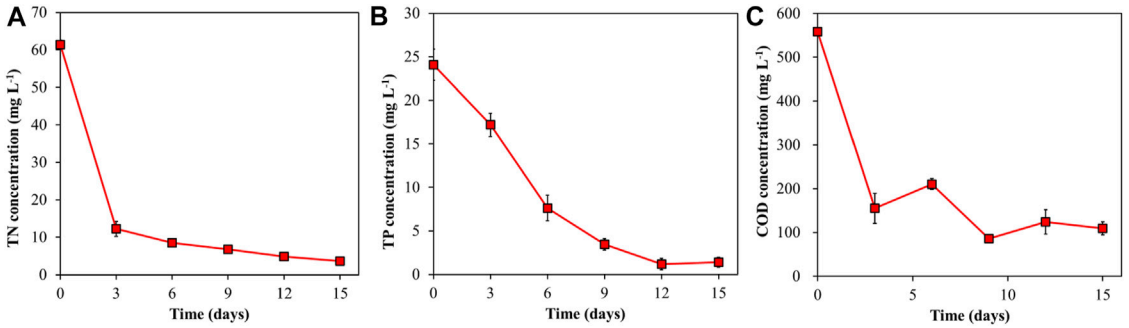

FIGURE 2 | The changes in TN **(A)**, TP **(B)**, and COD **(C)** concentration of MTDW group.

TABLE 4 | Macro/micro nutrient concentrations and RE values in MTDW.

Nutrient	Initial concentration (mg L^{-1})	Final concentration (mg L^{-1})	Removal efficiency (%)
Macro			
Na^{+}	269	253	5.23
K^{+}	1,020	980	3.92
Ca^{2+}	0.01	0.00	100
Mg^{2+}	18.8	2.75	85.37
Micro			
Fe^{3+}	1.8	1.52	15.56
Zn^{2+}	nd	nd	
Mn^{2+}	nd	nd	
As^{3+}	0.03	0.02	33.33
B^{-}	0.3	0.2	33.33
Mo^{2-}	0.07	0.04	42.86
Pb^{2+}	0.03	0.02	33.33
Cu^{2+}	0.18	0.13	27.78

nd: not detected.

growth, photosynthesis and metabolism (Kong et al., 2021). The study showed 80% TN removal efficiency. However, the highest removal rate was observed on the exponential growth phase. This coincides with a study reported by Iasimone et al. (2018).

Phosphorus is another key element for microalgae growth and other cellular activities (energy transfer and biosynthesis of nucleic acids) (Kong et al., 2021). Affected by the utilization of *C. vulgaris*, TP concentration decreased steadily within the first 12 days until stable on day 15. Affected by *C. vulgaris*, 94% TP removal efficiency was recorded at the end of cultivation period. Based on the results, phosphorus (TP) removal could directly be affected by *C. vulgaris* growth due to culture conditions. Asian and Kapdan (2006) reported 78% phosphate removal efficiency for *C. vulgaris* cultivation at 7.7 mg L^{-1} initial concentration, less than 30% removal efficiency at higher concentration, this is clear that cultivation conditions of microalgae could affect phosphorus removal efficiency.

COD concentration (**Figure 2C**) varied during the period of measurement. There was a slide increase in concentration on day 6 and day 9. Again, the highest COD removal was recorded at the exponential growth phase of *C. vulgaris* with 72.24% removal efficiency. It was observed from the results that COD in MTWD was effectively utilized by *C. vulgaris*.

Macro and Micronutrient Removal

As shown in **Table 4**, at the end of the cultivation period, removal efficiency obtained for macronutrients ranged between 3.92 and 100% compared to 15.56–42.86% for micronutrients. Ca^{2+} reduced from the initial concentration of 0.01 mg L^{-1} to 0.00 mg L^{-1} in MTDW with a removal efficiency of 100%. The concentration of Mg^{2+} reduced from 18.8 mg L^{-1} to 2.75 mg L^{-1}, and the removal efficiency reached 85.37%. The concentration of Na^{+} and K^{+} decreased from 269 mg L^{-1} to 1,020 mg L^{-1}–253 mg L^{-1} and 980 mg L^{-1}, and the corresponding removal efficiencies were 5.59 and 3.92%, respectively. For micronutrients, Mo^{2-} had the highest removal efficiency of 42.86%, and the concentration reduced from 0.07 mg L^{-1} to 0.04 mg L^{-1}. Followed by As^{3+}, B^{-}, and Pb^{2+}, their concentrations were reduced from 0.03 mg L^{-1}, 0.30 mg L^{-1}, and 0.03 mg L^{-1} to 0.02 mg L^{-1}, 0.20 mg L^{-1}, and 0.02 mg L^{-1}, respectively, and the corresponding removal efficiencies were all 33.3%. The concentration of Cu^{2+} and Fe^{3+} in MTDW decreased from the initial 0.18 mg L^{-1} and 1.80 mg L^{-1} to 0.13 mg L^{-1} and 1.52 mg L^{-1}, and the removal efficiency reached 27.78 and 15.56%, which were lower than other micronutrients. In

addition, Zn^{2+} and Mn^{2+} were not detected in this experiment.

DISCUSSION

Microalgae is a single-cell bioreactor driven by sunlight that converts carbon dioxide into potential proteins, lipids, carbohydrates, and high-value biological compounds, in the presence of a sufficient amount of nitrogen, phosphorous, and some trace elements. By 2024, the overall market potential of algae-based products expected to reach approximately USD 1.143 billion (Mehta et al., 2018). Meanwhile, the viable market potential of microalgae in the phytoremediation of wastewater and biofuels is currently increasing (Mustafa et al., 2021). Wastewater treatment with microalgae has been considered an environmentally sound bioremediation method and applied for more than 60 years (Jing et al., 2007). Many microalgae can grow effectively under wastewater conditions by utilizing the rich inorganic nitrogen and phosphorus in wastewater, such as *Desmdesmus* sp. (Benítez et al., 2018), *Scenedesmus* sp. (Han et al., 2020), *Acutodesmus dimorphus* (Chokshi et al., 2016), *C. vulgaris* (Lv et al., 2018; Mujtaba et al., 2018), and so on. Due to its rich in protein and other nutrients, bio-safety, and the feasibility of large-scale outdoor cultivation and maintenance, *Chlorella* has become one of the most in-depth studies of microalgae in biomass production and wastewater treatment (Liu and Chen, 2014). Previous studies have shown that *Chlorella* can grow and produce biomass in wastewater such as urban wastewater (Tercero et al., 2014), domestic wastewater (Aziz and Ng, 1992), textile wastewater (Chu et al., 2009), piggery wastewater (Ji et al., 2012), etc. Our study showed that cultivating *Chlorella* with MTDW was also a feasible strategy.

Although *Chlorella* is easily adaptable to different wastewater media, the nutrients in the wastewater significantly affect the growth of microalgae and the production of biomass (Cai et al., 2013). When wastewater is used as a nutrient source for wastewater-based microalgae cultivation, carbon: nitrogen (C: N) and carbon: phosphorus (C:P) ratios could be considered (Chiu et al., 2015). Lee and Lee (2002) pointed out that *Chlorella kessleri* culture could successfully remove high concentrations of nitrogen from wastewater supplemented with glucose, indicating that sufficient carbon source supply was beneficial to the utilization of nitrogen and phosphorus. Chui et al. (2015) suggested that the carbon limitation in wastewater could be overcome by adding waste CO_2, such as flue gas. In addition, the typical N/P ratio for the optimal conditions for microalgal biomass production is 8:1 (Chui et al., 2015). However, the N/P ratio of MTDW is close to 5:2, which means that the nitrogen source in the wastewater would be another limiting factor for microalgae growth. According to Chiu et al. (2015), the biomass productivity of *Chlorella* in different wastewater ranged from 0.029 g L^{-1} d^{-1} to 0.64 g L^{-1} d^{-1}. In contrast, 0.04 g L^{-1} d^{-1} biomass productivity of *Chlorella* cultured in MTDW is not high, indicating that the use of MTDW to cultivate *Chlorella* to produce biomass still has much room for improvement.

Due to microalgae can use nutrients in wastewater to promote their growth, microalgal are particularly useful for reducing the concentration of inorganic nitrogen and phosphorus of wastewater (Ahluwalia and Goyal, 2007). Previous studies have shown that *Chlorella* has a very significant removal effect on nitrogen, phosphorus, and COD in different wastewater (Lam et al., 2017; Benítez et al., 2018; Lv et al., 2018; Mujtaba et al., 2018). However, the nutrient concentration of wastewater from different sources is different, which has a direct impact on the removal of nutrients. Kumar et al. (2019) reported that the nitrogen removal efficiency of *Chlorella* in sewage wastewater (38%) was lower than that of kitchen wastewater (67%), while the removal efficiency of phosphorus (88%) is higher than that of kitchen wastewater (75%). In this study, the removal rate of TP in MTDW by *Chlorella* reached 94%, the removal rate of TN was 80%, and the removal rate of COD exceeded 70%. These results indicate that *Chlorella* was very effective in MTDW treatment to reduce the organic and inorganic nutrients released into natural water, thereby preventing eutrophication problems.

Many studies have shown that utilizing microalgae could effectively remove metal elements from wastewater (Cabanelas et al., 2013; Cho et al., 2013; Zhu et al., 2013). Some metal ions can be attached to the cell surface through one or more surface complexation, ion exchange, and redox (Sheng et al., 2004; Vinod et al., 2010). Biosorption also involves cell metabolism and other processes, during which metal ions enter the cell through metal transporters, and are finally stored in vacuoles or organelles (Mehta and Gaur, 2005; Flórez-Miranda et al., 2017). *Chlorella* has been reported to remove many metals from wastewater, including Al, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Ur, and Zn (Sandau et al., 1996; Lau et al., 1999; Chong et al., 2000; Mehta and Gaur, 2001; Mehta and Gaur, 2005; Wang et al., 2009). Similar to previous studies' conclusions, our study shown that *Chlorella* could effectively remove Ca, Mg, Mo, Fe, As, B, Pb, and Cu in distillery wastewater.

Cultivation of microalgae in large quantities is challenged by the high cost of nutrients and freshwater. According to (Kadir et al., 2018), the cultivation of microalgae in wastewater is an alternative way of overcoming the current high cost of microalgae cultivation. Slade and Bauen (2013) estimated more than 50% reduction in production cost by cultivation microalgae in wastewater as a nutrient, CO_2 and freshwater source. The composition of MTDW is stable and could be used for *Chlorella* cultivation without complicated treatment, which can effectively reduce the cost of *Chlorella* cultivation. On the other hand, *Chlorella* can remove nutrients, organic matter, and metals from MTDW. These substances may lead to the destruction of the aquatic environment causing eutrophication, affecting human health and recreational activities (Stutter et al., 2018). In addition, because biomass is rich in protein and fatty acids, *C. vulgaris* could be cultivated in MTDW as a high-quality protein source in aquaculture.

CONCLUSION

The growth and biochemical composition of *C. vulgaris* cultivated in MTDW and nutrients removal efficiency from the wastewater were analyzed. After cultivated 15 days in MTDW, 0.65 g L^{-1} algal biomass with biomass productivity of 0.04 g L^{-1} d^{-1} were obtained. The protein content, carbohydrate, and lipids reached 49.6 ± 1.4%, 26.1 ± 0.6%, and 10.4 ± 1.8%, respectively. 94% of phosphorus and 80% of nitrogen were removed from MTDW, and the removal efficiency of COD reached 72.24%. In addition, there was the highest removal efficiency of Ca^{2+} in MTDW with recording a 100%. Followed by Mg^{2+}, an 85.37% removal efficiency was reached. The removal efficiency of other nutrients Na^{+}, K^{+}, Fe^{3+}, As^{3+}, B^{-}, Mo^{2-}, and Cu^{2+} obtained ranged 3.92–42.86%. This study proved that it was feasible to cultivate *Chlorella* with MTDW and represented an economical and environmentally friendly *Chlorella* cultivation strategy. There appears to be a great potential for *Chlorella* in the area of tertiary distillery wastewater treatment. The feasibility of applying it to full-scale requires further research in culture strategy to maximize biomass production and improve the removal efficiency of nutrients in wastewater.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

FL: Data curation, methodology, writing-original draft, writing-review and editing; DA: Data curation, investigation, writing-original draft; YZ: Methodology; NZ: Methodology; CL: Project administration; XH: Project administration, resources.

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