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DETERMINATION OF UPTAKE RATE OF NUTRIENT DURING PHOTOAUTOTROPHIC CULTIVATION OF MICROALGAE IN AL- RUSTUMEYAH WASTEWATER TREATMENT PLANT IN BAGHDAD-IRAQ

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Abstract

In that current study microalgae *Chlorella vulgaris* (Beijerinck) and *Chroococcus minor* (Kützing)) locally isolated, were utilized in wastewater treatment to minify the pollutant parameters. Six parameter were thoughtful to determine the competence of *C. vulgaris* and *C. minor* in decrease Nitrate (NO3), nitrite (NO2), ammonia (NH3), (PO4-3), biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) . Dry weight and optical density of microalgae were calculated daily. Wastewater samples were taken from last tank in the Al-Rustumia wastewater treating terminal before it is discharged into the Diyala River. In general, it has been found that the mean values of examined variables were varied significantly in terms of algae species and times. *C. vulgaris* gave better removal result of nutrient and COD, BOD than C. minor. The results showed removal 100% after 4-5 days of treatment of both ammonia and phosphorous at the same period , removal observed of COD was to be 84.8% - 71% and was recorded the removal BOD 93%-67.2% for *C. vulgaris* and *C. minor* , respectively. In addition, that dry weight and optical density have increased significantly as the testing time. Results confirm that wastewater as a cost efficient growth medium for lipid and higher biomass production accompanied for the nutrient removal efficiency of microalgae which in turn reduces Eutrophication.

Key word: Wastewater treatment, Microalgae, Nutrients uptake, biomass

Introduction

Wastewater contains a large number of pollutants rich in nutrients, heavy metals xenobiotics and pathogens they must be removed before being discharged into surface water. Wastewater is released into the water bodies without proper treatment it can cause major and hard environmental and health problems which affects Human activities influence the food chain of aquatic environments through as industrial ,agricultural, municipal and wastewater (Henry *et al*., 2019; Eerkes,et al.,2019;Preisner *et al*., 2021).

In the last years, the traditional treatment processes have been introduced much modifications to get better performance. However, the majority of these modifications are not satisfying due to the high cost and increased complexity in maintaining and operating (Marchello *et al*, 2015). Biological treatment of nitrogen and phosphorus wastewater has been widely studied; microalgae have been used for this purpose (Shahriari *et al*., 2016).Microalgae received considerable attention in the recent years due to potential to remove mineral nutrients such as nitrogen and phosphorus and heavy metals from wastewater, besides other micronutrients and produce valuable biomass

that can be used biomass in different industrial applications such as biodiesel , pharmaceuticals, food processing ,bio fertilizer (Acién et al., 2016 Singh et al, 2017) . Recently studies have indicated that many species of algae were used for uptake organic matter and nutrients from wastewater like Scenedesmus *sp, Chlamydomonas sp. Haematococcus sp, Botryococcus sp., Chlorella sp. Spirulina sp.* (Al Raie *et al*., 2020).

In various types of wastewater the microalgae which can grow. Through a phenomenon called biosorption, the binding of pollutants to the cell surface with functional groups present in the cell wall of algae allows (Spain *et al*., 2021)

This reversible and rapid process is independent on the microalgae's metabolism and ability thus be performed on dead or living biomass (Plöhn *et al*, 2021). The advantages of using algae for this purpose involve: low cost of operation, eco-friendly, More efficient and cheaper than removing nutrients from traditional methods , Drainage of wastewater abundant with oxygen to water bodies and the possibility of recycling phosphorous and nitrogen in the algal biomass, and it can be exploited as bio-fertilizer (Arora *et al*.,2021).

Microalgal biomass is considered a promising energy feedstock for production of sustainable biodiesel,The possibility of microalgae accumulating large amounts of assimilated carbon in the cell in the form of lipids. Microalgae have sundry advantages as a biodiesel feedstock , inclusive faster growth,higher lipid content, higher biomass yield, and a few competition for arable land when compared to used terrestrial plant(Goncalves *et al* ., 2016; Tabatabai *et al* ., 2018)

However, when treatment of wastewater by using microalga would demand: such as (a) mechanisms contributory in microalga for removal pollutants of various types of wastewater (b) Using different methods for cultivating microalgae in wastewater and (c) Thus, the use of algae biomass for a variety of value-added and diverse applications
(Al-Jabri *et al*,2020)

Aimed this study to evaluation the *Chlorella vulgaris* and *Chroococcus minor* for uptake nutrients (nitrogen and phosphate) in wastewater from the Al-Rustumeyah wastewater treatment plan t.

Material and Method

Wastewater treatment with algae

Algae culture of *Chlorella vulgaris* and *Chroococcus minor* strains were obtained from the College of Science department of biology, University of Mustansiriyah . Wastewater samples were taken from the local wastewater station from Al-Rustumiya wastewater treatment plant in Baghdad, Al-rusafa Samples of wastewater were taken from final tank before it is discharged into the Diyala River, was used as cultivation media for species at stable laboratory conditions 16: 8 light: dark ,268 µE / m² / s, 25 ± 2°C, Batch culture was used (Mohammed, 2016). The sample was filtered using vacuum with Whatman filter paper of (0.45µ,) to remove large particles (Tran *et al*., 2020). 900 ml of wastewater was placed in 2.5 liter transparent glass bottles and then add 400 ml inoculated for each species. For chemical tests, 125 ml of samples were taken daily for the duration of the experiment (Al Raie *et al*., 2020).

Chemical characterization of wastewater

Chemical analysis methods were used described by the American Public Health Association (APHA,2017) for Nitrate (NO3), nitrite (NO2), ammonia (NH3), (PO_4^{-3}), biochemical Oxygen Demand (BOD)and Chemical Oxygen Demand (COD).However, The present amount of nutrient content was measured in wastewater samples .

Biomass calculation

Biomass was calculated by dry weight and Optical Density (OD). The dry cell weight of the microalgae biomass was determined by filtering 125 ml wastewater sample using (0.45µm) Wattman filter paper with air vacuum device and left to dry in electric oven at 105-110°C (Fogg , 1975). The dry weight was measured daily using the following formula:

Dry weight mg/l = (A-B) × 1000) / (volume of sample)

A=Paper weight after filtration, B=Paper weight before filtration

Algal cell density was determined by Optical Density (OD) measurement by Spectrophotometer at 540 nm every day for 14 days to all experiment (Miyachi *et al.,* 1964).

Statistical Analysis:

The Statistical Analysis System (2012) program was used to reveal the influence of variance factors in study parameters. lower significant difference –LSD test (ANOVA).

Results and Discussion

The results showed that the high efficiency of *C. vularis* and *C. minor* have reduced nutrient concentrations (ammonia, nitrite, nitrate, Orthophosphates, COD and BOD) from wastewater treated within ten days, Except for pH gradually increases during the trial period (Table 1).The pH of the samples was directly laboratory tested using a pH meter. the results of all treatments It was observed in all treatments a gradual rise in pH .In *C. vulgaris* which ranged between 6.6 -8.5 while the *C.minor* which ranged between 7.1-8.1 during the test period. The pH gradual increase and then remains constant value (Kube *et al*., 2018). The pH is one of the factors that determine the viability of water and the type number of organisms that reside in it. This may be attributed to the photosynthesis process carried out by algae (Al Raie *et al*., 2020). pH plays an important role in the ability of microalgae to assimilate nutrients either by change the shape of the available elements or by change the algal cell physiology., increased algae growth may increase pH due to the use of CO_2 by algae during photosynthesis (Matthew *et al*., 2018)

Table (1): Percentage of pH, EC, N-NH_4, N-NO_3, N-NO_2, p-PO4, COD, BOD of **wastewater before and after using three algae species**

Algae species	Treatments	pH	EC	N-NH_4	N-NO_2	N-NO_3	p-PO_4	BOD	COD
Chlorella vulgaris	Control	6.66	1800	7.85	1.5	5.18	2.54	89.20	125
	2 hours	6.6	1620	6.50	1.5	5.10	2.13	88.50	114
	2 days	7.8	1570	5.10	1.3	5.10	1.27	76.10	98
	4 days	7.5	1560	3.20	1.1	4.22	0.83	54.22	81
	6 days	8.2	1430	1.11	0.9	4.08	0.08	15.12	69
	8 days	8.3	1360	0.02	0.9	3.80	0.00	10.40	46
	10 days	8.5	1293	0.00	0.6	3.20	0.00	6.16	19

LSD value		**0.702 ***	**176.2 ***	**1.64 ***	**0.662 ***	**0.921 ***	**0.569 ***	**8.71 ***	**15.77 ***
Chroococcus minor	Control	7.1	1800	4.31	1.6	5.50	1.59	111.4	150
	2 hours	7.3	1780	4.00	1.57	5.50	1.55	107.54	147
	2 days	7.5	1670	2.50	1.52	5.33	1.16	85.88	130
	4 days	7.6	1554	2.10	1.45	5.01	0.86	62.66	107
	6 days	7.7	1545	1.30	1.34	4.73	0.24	50.45	79
	8 days	8	1358	0.20	0.90	4.50	0.17	44.58	56
	10 days	8.1	1300	0.00	0.71	4.21	0.12	36.47	43
LSD value		**0.966 ***	**193.3 ***	**0.988 ***	**0.659 ***	**1.05 ***	**0.844 ***	**9.73 ***	**15.08 ***

The current study has found significant reduction in NH_4 content when treated by both examined algae species. Also show *C.vulgaris* the best ammonia removal than *C. minor.* about 7.85 -4.3 mg / l ammonia decreased before treatment to 1.11 -1.3 mg / l on the sixth day for *C.vulgaris* and *C. minor*, respectively. The removal rate is 100% on the eighth and tenth day for *C.vulgaris* and *C. minor*, respectively (Figure 1).

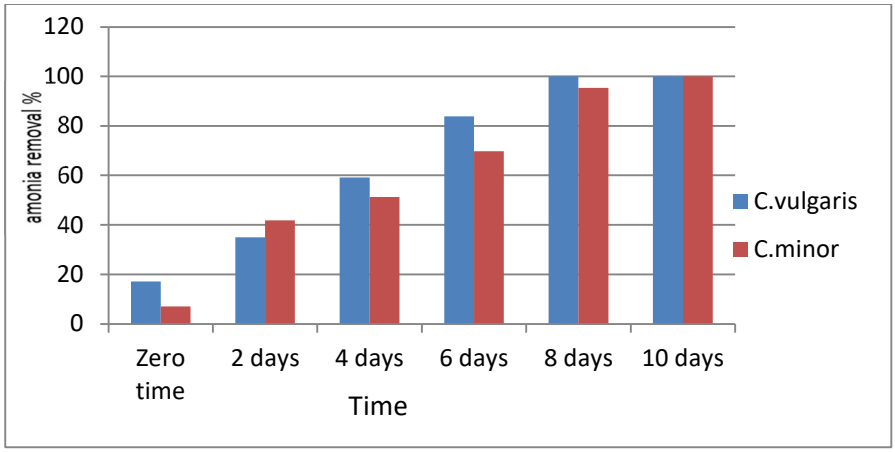

Figure 1. Percentage of removal of ammonia from the wastewater treatment by *C. vulgaris* and *C. minor* .

Microalgae ability use various nitrogen source forms such organic nitrogen, ammonium, nitrate, nitrite, the diverse N-source also ability effect the biochemical structure of the algal cells in in various ways (Ruangsomboon, 2015). In the current study, there was a complete removal of ammonia during the experimental period for both algae because of its easy absorption, which consumes less energy compared to other nitrogen sources. Therefore, the algae prefer ammonia as a nitrogen source. (Cai *et al.,* 2013). The rise pH associated with the process of photosynthesis has also proven to have an effective role in removal nutrients by algae in wastewater (Al-Hassany *et al*., 2021,). in a culture medium, the photosynthesis inhibits in several microalgal species ,when ammonium concentration higher than 100 mg L-1 (Li et al ,2019) but some strains are more

tolerant than others of High concentrations of ammonia (Acién Fernández *et al.,* 2018). The current work has found that there was slight reduction and nitrites and Nitrates content treated by both algae species. Reduction from 1.5-1.6 mg/l before treatment to 0.6 - 0.71 mg/l on the tenth day, for *C.vulgaris , C. minor*, respectively. On the other hand, the removal rate is 60-51% at the tenth day for *C.vulgaris* and *C. minor*, respectively (Figure 2).

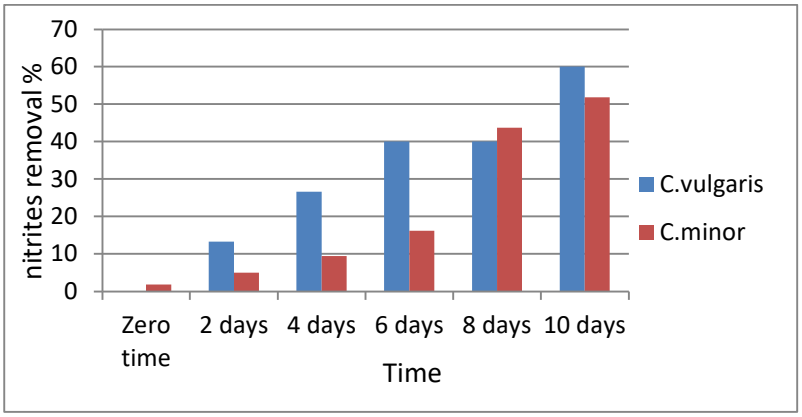

Figure 2. Percentage of removal of nitrites from the wastewater treatment by *C. vulgaris* and C. minor

The same pattern for nitrates, reduction in nitrates ($N\text{-}NO_3$) from 5.18-5.50 mg/l before treatment to 3.20 - 4.21 mg/l on the tenth day for *C.vulgaris* , C. minor, respectively. There was no removal Nitrates during the zero time and then gradually removed, where it reached 38.2% on the tenth day, while removal 23.3% on the tenth day for *C.minor.* Figure 3).

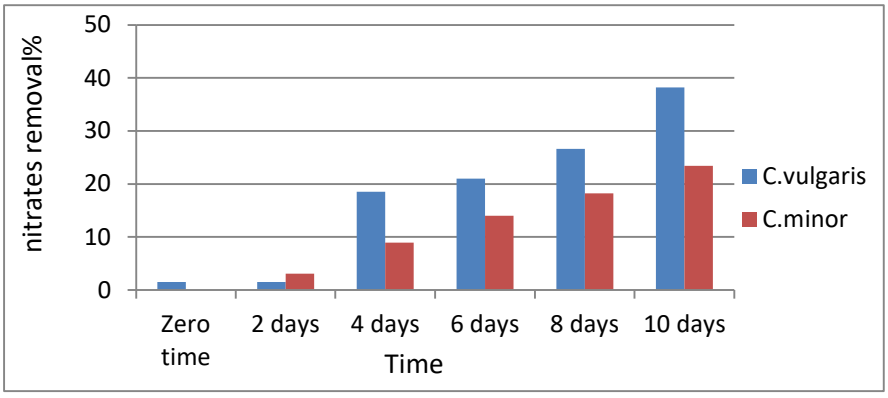

Figure 3. Percentage of removal of nitrates from the wastewater treatment by *C. vulgaris* and C. minor.

To assimilate the inorganic nitrogen, nitrate and nitrite must be reduced to ammonium, via nitrate and nitrite reductase (Jia and Yuan, 2016). the enzyme glutamine synthetize is responsible for nitrogen assimilation , via that glutamate reacts with ammonium to form the amino acid (Markou *et al*., 2014).

During the first days, it was found that *C. vulgaris* had high efficacy in removal orthophosphates from wastewater, the concentration decreased from 2.54 mg/l before treatment to 0.08 on the sixth day for *C. vulgaris*; also it showed decreased from 1.59 mg/l before treatment to 0.12 on the last

day for *C. minor*. Showed a complete removal of 100% in the last days of the experiment for *C. vulgaris* , While the best removal 92.4 % for *C. minor* at the last days (Figure 4).

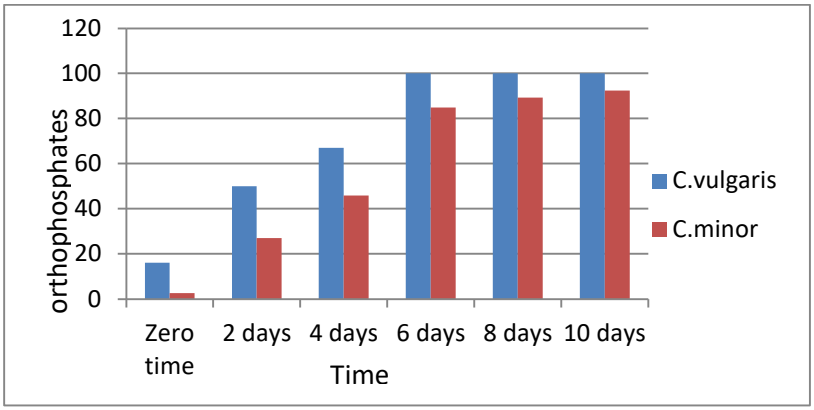

Figure 4. Percentage of removal of Orthophosphates from the wastewater treatment by *C. vulgaris* and *C. minor*

Phosphorous is an important component of microalgae, where it is involved in many metabolic pathways, as well as a structural component of nucleotides, phospholipids and Adenosine triphosphate (ATP) (Borowitzka *et al*., 2016). One of the most bioavailable forms that can be easily assimilate is inorganic phosphorous as $H_2PO_4^-$ or HPO_4^{2-} (Silva *et al*., 2015). Algae cultivated in wastewater, which normally has a concentration of between 10 and 20 mg P L-1, (Larsdotter,, 2006). There is no evidence of the toxicity of phosphorous in wastewater, which is used as a culture medium for algae and perhaps it has low concentrations. The generality pertinent problem concerning phosphorous conduct in microalgae that happens in alkaline conditions the precipitation of calcium phosphate (del Mar Morales-Amaral *et al*., 2015a).

Both species showed the ability to reduction the (COD) in wastewater. But the efficiency of *C. vulgaris* reduction (COD) better than *C. minor*. The chemical oxygen demand was reduced from 125-150 mg / l before treatment to 19-43 mg / L for C. vulgaris and C. minor, respectively, on the tenth day. On the other side, the first-day removal rate was 8.8% and 2 % in both *C .vulgaris* and *C. minor*, respectively. It increased to 84.8% and 71.1% on the tenth day, respectively (see Figure 5). In this study, the successfully of using organic carbon for their metabolisms processes has been proven by both species of algae

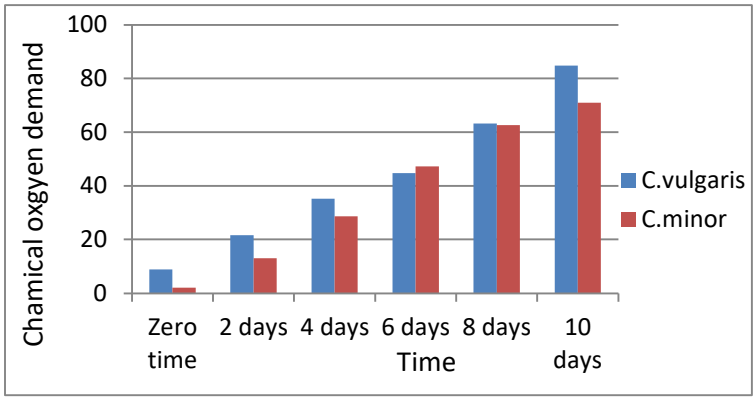

Figure 5. Percentage of removal chemical oxygen demand of from the wastewater treatment by *C. vulgaris* and *C. minor*

During the study period, All the treatment the a decrease in the COD due to the relation amidst microalgae and bacteria, Wastewater borne bacteria break down complex organic compounds into simple molecules, which make nutrients usable for algae, thus removing higher amounts of COD; Through continuous culture aeration, it leads to obtained the highest biomass of algae (Ma, *et al*,2014; Al-Hassany *et al*, .2021). Some studies have shown that microalgae during the first days are able to remove COD, ammonia and phosphorous from wastewater with high organic loads (Lv *et al*.2018; Nagi *et al* ,2020) .In the same pattern, the BOD was reduced from 89.20 -111 mg / l before treatment to 6.16 -36.47 mg / L for *C.vulgaris* and *C.minor*, respectively, on the tenth day. On the other side, the first-day removal rate was 0.87% and 3.4 % in both *C.vulgaris* and *C.minor* , respectively. It increased to 93% and 67.2% on the tenth day, respectively (Figure 6).

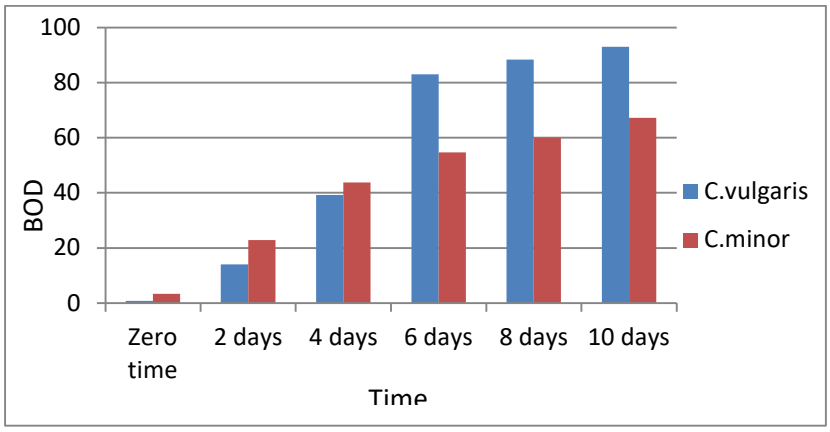

Figure 6.Percentage of removal biological oxygen demand of from the wastewater treatment by *C. vulgaris* and C. minor

The effluent is the main loaded of organic matter contaminant to be removed in wastewater treatment process .BOD test was applied to measure the potency of microalgae to oxide organic substance in the wastewater to water and CO_2, Biochemical oxygen demand is an index and give of materials that can be degraded biologically (Mohammed *et al*., 2016). Though heterotrophic and mixotrophic growth of microalgae, it has been reported that microalgae are unable to degrade large organic molecules, but can degrade low molecular weight such as glucose, glycerol, etc. (Ren *et al*., 2014). processes is not easy when Controlling the microalgae growing in wastewater treatment , but by the amendment of the culture conditions such as nutrients outfit, temperature, pH or favor type of microalgae strain. This microorganism it would be rich in proteins lipids and carbohydrates and these compounds have important properties that are used in many applications. (Acién Fernández *et al*, 2018).

Dry weight and Optical Density of Algae

Microalgae can grow in wastewater and using pollutants as nutrient sources and then produce different amounts of active compounds such as proteins, lipids, fatty acid , vitamins (Goncalves et al., 2017). The result shows a gradual increase in the biomass of the algae which leads to increase in the dry weight and Optical density (OD) of algae, it can be notice from (table 2). The biomass increased continuously and

gradually to the 10th day of treatment found to range from 0.055-0.041at zero time to 0.366-0.160 at 10 th day for *C.vulgaris* and *C.minor*, respectively. Same status the result also shows a gradual increase in the optical density of the algae this lead to increase in the density of algae. Showed the range from 0.055-0.044 at zero time to 0.351-0.171 at 10 th day for *C.vulgaris* and *C.minor*, respectively.

Table2: biomass in terms of dry weight and absorbance of C.vulgaris and C.minor during cultivation time

Time	*C. vulgaris*		*C .minor*	
	dry weight mg /l	absorbance (540 nm)	dry weight mg /l	absorbance (540 nm)
Zero time	0.055	0.055	0.041	0.044
2 days	0.135	0.098	0.062	0.056
4 days	0.187	0.163	0.081	0.083
6 days	0.247	0.227	0.145	0.111
8 days	0.323	0.292	0.155	0.143
10 days	0.366	0.351	0.160	0.171

This result is agreed with Liang (2014) monitored the growth of four microalgae (*Chlorella sp., Scenedesmus sp., Chlorococcum sp.* and *Spirulina sp*) in batch culture and found that the ratio of dry weight to optical density increased with time. Also showed Kumar et al (2019) the dry cell weight of microalgae Chlorella about 0.45, 0.6 g/L ,with CO_2 removal about 28% ,50% in kitchen wastewater and sewage wastewater .respectively. Wastewater rich in nutrients is considered a suitable medium for the growth of microalgae, which has the ability to remove nutrients and increase biomass, which in turn is involved in many applications, including used for energy, animal feed or agriculture uses (yan *et al* 2013).

CONCLUSION

The study appeared when increasing the growth rate of *C. vulgaris* and *C.minor* in wastewater leads to a decrease in the rate of pollutants with nutrients. It was also found that the alga *C. vulgaris*, which is able to removal ammonia, nitrite, nitrate, orthophosphate, and lower concentration COD and BOD, is better than *C.minor*. The unicellular alga *C. vulgaris* can be widely used as it has rapid growth rates and efficient nutrient removal, It also improves the quality of water, that providing an efficient and environmentally acceptable option for wastewater treatment.

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