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Carbon dioxide sequestration and sustainable domestic sewage bioremediation by *Microcystis aeruginosa* and *Chlorella vulgaris*

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ABSTRACT

Microcystis aeruginosa and *Chlorella vulgaris* was isolated from domestic sewage and grown in presence of sodium bicarbonate as carbon source at 0.5, 1.0 and 2.0 gl^{-1}. Highest specific growth rate (0.665 μd^{-1}) was obtained with 1 gl^{-1} bicarbonate on *M. aeruginosa* culture. The biomass productivity was in range of 0.281-0.525 $gl^{-1}d^{-1}$on *M. aeruginosa* and 0.217-0.486 $gl^{-1}d^{-1}$ on *C. vulgaris*. At 1 gl^{-1} sodium bicarbonate concentration, maximum CO_2 fixation (0.172 $gl^{-1}d^{-1}$) was found on *M. aeruginosa* culture. The present study also aimed to investigate the potential applications of *Microcystis aeruginosa*, *Chlorella vulgaris* and their consortium for bioremediation of domestic sewage with regard to their pollutant removal and improve water quality for reuse in irrigation. The results showed that both the monocultures and their consortium were quite effective and having capacity to reduce pH, conductivity, turbidity, total dissolved solids (TDS), biochemical oxygen demand (BOD), NO_3-N, PO_4-P, NH_4-N, alkalinity, salinity and chlorides. The consortium cultures shows higher removal efficiency in pH (6.80 ± 0.26%), conductivity (23.48 ± 0.01%), BOD (82.35 ± 0.10%), PO_4-P (55 ± 1.20%), salinity (40.52 ± 0.03%) and chlorides (34.48 ± 0.03%), *C. vulgaris* in turbidity (81.73 ±0.07%), TDS (18.68 ± 0.03%) and NO_3-N (49.48 ± 0.59%) while *M. aeruginosa* in NH_4-N (42.10 ± 1.82) and alkalinity (35.50 ± 0.04). The concentration of all the parameters in effluent provided by all the treatment complied with different standards for irrigation and drinking water with the exception of *M. aeruginosa* in chlorides for irrigation water and all the treatment in conductivity, turbidity and NH_4-N for drinking water.

***Key words**: Bioremediation, Monoculture, Consortium, Domestic Sewage, Removal Efficiency*

Introduction

CO_2 is one of the air pollutants that have a major impact on the greenhouse effect.Various CO_2 mitigation measures have been developed in response to global warming, which is mostly linked to rising CO_2 levels in the atmosphere. It is vital to develop sustainable alternatives to chemical reaction-based CO_2 capture since it is rather expensive and energy-intensive. In addition to produce biomass energy, biological CO_2 mitigation is a substitute method of CO_2 fixation through photosynthesis (de Morais and Costa, 2007). Microalgae play a crucial role in preventing an increase in the concentration of CO_2 in the atmosphere because they are more effective at photosynthesizing ambient air than terrestrial plants. It recycles CO_2 by converting it into biomass energy. Microalgal culturing and CO_2 sequestration together minimize carbon emissions and promote a sustainable ecosystem (Demirbas, 2004).

The increasing urbanization and population growth worldwide have propelled domestic sewage generation to unprecedented levels, necessitating innovative and efficient approaches to wastewater treatment. Wastewater evacuated from homes, hotels, restaurants, retail centres and schools is known as domestic sewage. It has an enormous number and variety of sources. There is an increasing amount of sewage discharge that needs to be treated due to the economy's rapid development and citizen's rising standards of life. Large amounts of organic stuff (such as protein, starch, fat, and urea) harmful microbes and suspended particles are characteristics of this type of sewage (Jiao, 2021). Over 80% of home wastewater is discharged into aquatic bodies without sufficient treatment on a global scale. Numerous contaminants, including nutrients, oils and fats, detergents, bio wastes, household chemicals, heavy metals, salts, pathogens, pharmaceutical ingredients, and soluble and particulate organic matter, are found in domestic wastewater. The contaminants change the water's pH, hardness, inorganic content, and pathogen load in addition to raising the levels of chemical oxygen demand (COD) and biological oxygen demand (BOD). Waterborne and water-related infections are caused by untreated home waste water, which also poses a threat to aquatic and marine life and crops (Agarwal *et al*., 2022). By reducing the demand on natural resources, maximizing energy recovery, and assisting agriculture, wastewater treatment promotes sustainable resource management. One of the most environmentally friendly methods for producing electricity, conserving water, and increasing agricultural productivity is wastewater treatment (Silva, 2023). A popular and effective cleaning method for getting rid of toxic waste from contaminated areas is bioremediation. Through the universal action of microorganisms, bioremediation is highly involved in the degradation, eradication, immobilization or detoxification of various chemical wastes and physical harmful elements from the surrounding environment. The fundamental idea is to breakdown and transform contaminants into less harmful forms (Sharma, 2020). Utilizing elements of the natural environment and naturally existing microorganisms, bioremediation removes nutrients from wastewater. Comparing bioremediation to other approaches for hazardous waste cleaning, it may end up being less expensive (Vidali, 2001). It is widely accepted that algae are essential to the natural process of purifying water (Olguýìn, 2003). Furthermore, ignoring the shortcomings in wastewater treatment and failing to consider the possibility of reusing treated water already equates to wasting scarce water supplies. Only by lowering wastewater treatment plant loads, increasing treatment efficiency, and recycling wastewater will protect and sustain water resources (Sisman-Aydin, 2022). Therefore, it is inevitable that research will create new strategies and tactics to improve the effectiveness of water treatment. Bioremediation by algae is particularly useful in the treatment of domestic sewage because they provide sustainable, economic and eco-friendly with the environment (Wang *et al*., 2017).

Both municipal and industrial wastewaters may contain different amounts of nitrogen and phosphorus, inadequate light penetration, and metal concentrations inappropriate for algal growth, therefore it is crucial to select the most convenient algal species to treat wastewater effectively. Different species have different growth strategies when exposed to pollutants on a regular basis (Spatharis *et al.,* 2007). Because of already adapted to the biotic and abiotic stresses in the medium, isolating the microalgae species that will be used in phycoremediation systems directly from wastewater or polluted areas will be advantageous in providing higher nutrient removal efficiency (Lima *et al*., 2020).

Cyanobacteria such as *Microcystis aeruginosa* have several benefits over other soil-isolated microorganisms as bio remediating agents. Among these include the fact that they are photoautotrophic and that certain species can fix nitrogen from the atmosphere. Because of this, they may become producers and their development and upkeep are less expensive (Castenholz, 1981). *Microcystis* is a genus of cyanobacteria that generate blooms and are commonly found in eutrophic freshwater lakes. They are extremely tolerant of Fe^{3+} and Cu^{2+} in both laboratory and field settings, but nothing is known about how resistant they are to other common contaminants like zinc and cadmium (Singh *et al*., 1998). *M. aeruginosa* also showed high potential in reduction of wastewater nutrients such as nitrate, phosphorus and ammonia by 75.73%, 92.73% and 93.03% respectively (Badamasi, 2023). In wastewater treatment, *Chlorella vulgaris*, a green microalgae, is also the most studied eukaryotic algal species (Chiu *et al*., 2015). Common eukaryotic microalgal species *C. vulgar* is may be found in a variety of natural freshwater and soil settings. The cells of *C. vulgaris* is thin, the growth rate is rapid, and the cell life is brief. This strain of algae is resilient and adapts well to shifting physico-chemical environments. *C. vulgaris* frequently stores a large amount of lipids as a result of stress and food constraint. Because of these characteristics, this microalgae may be grown in wastewater and used for wastewater treatment (Mahdy *et al.*, 2015). It has been noted that *Chlorella* species can remove phosphate and nitrogen with great efficiency (Chen *et al.*, 2018; Chiu *et al.*, 2015).

Several researches used mixed cultures of *Chlorella* and bacteria to investigate the effectiveness of municipal wastewater treatment. As compared to individual axenic culture, the co-culture system demonstrated symbiotic augmentation in the removal of nutrients and organic carbon using synthetic urban effluent. Over the course of four days, the co-culture system demonstrated 80% elimination of ammonium and chemical oxygen demand (COD) (Mujtaba *et al.*, 2015). Furthermore, it is unclear if mixed algal cultures may outperform algal monocultures in terms of treatment efficiency because, there aren't many research on the combination of unicellular microalgae for the treatment of municipal wastewater. In addition to the species of algae, another significant biotic element that affects nutrient removal rates, biomass growth rates, and settleability is the concentration of the algal inoculum (Guieysse *et al.*, 2002), these factors should be thoroughly studied for mixed cultures.

The main objectives of this study is to established the growth of *Microcystis aeruginosa* and *Chlorella vulgaris* in a medium where sodium bicarbonate serves as a source of inorganic carbon by assessing growth rate, biomass productivity and CO_2 fixation for CO_2 sequestration and to analyze and examine the most recent developments in bioremediation of domestic sewage using microalgae biotechnology. The specific goals are to assess, enhance and compare the capabilities of *Microcystis aeruginosa* and *Chlorella vulgaris* monoculture and their mixed culture for bioremediation of domestic sewage. Furthermore, the goal was to assess the final effluents after bioremediation for reuse in terms of sustainability. The results of this study will support the application of biological techniques based on microlagae for the treatment of domestic wastewater in the future.

Materials and Methods

Domestic sewage

The domestic wastewater was collected from Tuikual river located at Tuikual, Aizawl, Mizoram. The initial physical and chemical parameters of the collected wastewater were determined by using standard methods (APHA (American Public Health Association), 1998).

Microalgae

The microalgae *Microcystis aeruginosa* and *Chlorella vulgaris* were collected and isolated from the domestic wastewater at Tuikualriver. Both the species were isolated using serial dilution; standard plating and colony isolation. Agar plating was used in order to isolate distinct species. The cultures were maintained in Chu10 medium (Chu, 1942).

Growth under different Sodium Bicarbonate Concentrations

Batch cultures (250 ml) of *M. aeruginosa* and *C. vulgaris* (Chu10 medium; n=3) were grown under different levels of Sodium bicarbonate concentrations (0.5, 1.0 and 2.0 gl^{-1}) during 20 days culture period, where samples were taken for growth rate, biomass productivity and CO_2 fixation analyses.

Biomass concentration and productivity

Homogeneous cell suspension (1 ml) was collected and centrifuged at 5000 g for 15 min, at 4 °C. The pellet was washed with distilled water twice and dried in a pre-weighed aluminium foil till a constant weight was obtained. The biomass concentration and productivity were calculated as per Eqs. (1) and (2), respectively.

$$\text{Biomass concentration } (gl^{-1}) = (W_2 - W_1)/\text{volume} \quad .. (1)$$

$$\text{Biomass productivity } (gl^{-1}day^{-1}) = (X_f - X_i)/t \quad .. (2)$$

The Specific growth rate was calculated as follows (Eq. (3)):

$$\mu = (\ln X_2 - \ln X_1)/(t_2 - t_1) \quad .. (3)$$

Where, W_2 and W_1 are final and empty weight of aluminium foil. These weights were used to measure biomass concentrations (mg mL^{-1}) at the beginning (t_1) and end (t_2) of the cultivation period; X_i and X_f are the biomass concentration (gl^{-1}) at the beginning and end of growth period (t) respectively; X_2

and X_1 are the final and initial biomass concentration (gl^{-1}) at the end (t_2) and beginning (t_1) respectively.

CO_2 fixation rate

The CO_2 fixation by the microalgae to convert the inorganic CO_2 into organic compound was calculated by Eq. (4) (Dineshkumar *et al.*, 2015).

CO_2 fixation rate ($gl^{-1}d^{-1}$) = carbon content (%) x biomass productivity ($gl^{-1}d^{-1}$) x 44/12 (4)

Treatment of domestic wastewater by microalgae

Experimental layout

To study the capabilities of microalgae in wastewater treatment the following technique was employed; sewage (100% domestic wastewater), sewage treated with *Microcystis aeruginosa* (95% sewage: 5% *M. aeruginosa* inoculum), sewage treated with *Chlorella vulgaris* (95% sewage: 5% *C. vulgaris* inoculum) and sewage treated with both microalgae (95% sewage: 2.5+2.5% inoculum). The specimen were furthermore incubated at a temperature of 25°C and given light/dark photoperiods of 12/12 hours respectively for a period of 20 days.

Table 1. Summary of experimental details of experiment

Treatment	Duration of experiment (Days)	Inoculum
S (without algae)	20	N/A[a]
S_M	20	5%
S_C	20	5%
S_{M+C}	20	2.5 + 2.5%

[a]N/A : non-applicable, S: Sewage without treatment, S_M: Sewage treated with *M. aeruginosa*, S_C: Sewage treated with *C. vulgaris*, S_{M+C}: Sewage treated with mixed inoculum of *M. aeruginosa* and *C. vulgaris*.

Studies of growth, pigment and biochemical concentrations of *M. aeruginosa* and *C. vulgaris* in different treatment of wastewater

Growth was analysed at a regular intervals of 4 days for a period of 20 daysby taking the OD value of the sample and specific growth rate (μ) was determined by using the following formula: $\mu (d^{-1}) = (\ln N_2 - \ln N_1)/(t_2-t_1)$, where N_1 and N_2 represent the OD value at times t_1 and t_2 respectively. The pigment concentration, chlorophyll a and carotenoids and the biochemical concentration, protein, carbohydrates and lipids were determined at the end of the experiment. Cells were extracted using filtration (Whatman filter paper) for determination of dry weight.

Analytical procedures of water samples

Samples were routinely examined for each physicochemical parameters such as pH, conductivity, turbidity, total dissolved salts (TDS), alkalinity, salinity, chlorides, NH_4-N, NO_3-N, PO_4-P, dissolved oxygen and biological oxygen demand (BOD) using standard methods (APHA (American Public Health Association), 1998).

Statistical analysis

One way analysis of variance (ANOVA) was performed to compare the variation between the means of the sample. The significant level of $p \leq 0.05$ was used.

Results and Discussion

Characteristics of domestic sewage

One of the river that flows through Aizawl, Mizoram is called the Tuikual River, and it has many sentimental meanings. The location where it meets the Tlawng River is called Tuithum Lui. It is one of the main tributaries of the Tlawng River, which supplies water to Lunglei, Aizawl, and other nearby towns.The discharge of undesired substances and the concentration of contaminants contaminate the river water. It delivers pollutants from several areas surrounding Aizawl, including wastewater drains, residential trash, city rubbish, and municipal waste (Ngente and Mishra, 2023). Pollution of watercourses comes from a wide range of sources, varying in volume and severity. The technology and ways of life used in the producing civilization are reflected in the makeup of wastewater (Gray, 2004). It is a sophisticated blend of synthetic, natural organic and inorganic compounds. Nearly 99.9% of domestic sewage is made up of water, with comparatively little amounts of dissolved or suspended organic and inorganic particles (Gautam *et al.*, 2013). A particular physical and chemical aspects of wastewater include its pH, dissolved oxygen (DO), chemical and biological oxygen demand, suspended and dissolved particles, nitrogen (nitrite, nitrate, and ammonia), phosphate and metals (Larsdotter, 2006). The analytical parameters of the initial investigated domestic wastewater such as pH, electrical conductivity, turbidity, total dissolved solids (TDS), alkalinity, salinity, chlorides, NH_4-N, $N0_3$-N, PO_4-P, dissolved oxygen (DO) and biological oxygen demand (BOD) are represented in Table 2. The concentration of hydrogen ions is an important quality measures that applies to both natural and wastewaters. It is used to characterize the acidic or basic qualities of the wastewater. Industrial wastes and incompatibility with biological processes are indicated by pH values less than 5 and more than 10, respectively, while a pH of less than 7 in wastewater influent indicates septic conditions. The pH concentration range, usually between 6 and 9, is extremely narrow for biological life to survive.In biological treatment units, an indicator of extreme pH is known to harm biological processes (Gray, 2010). The initial pH of the present study of wastewater is 7.78 which state that the wastewater is suitable for biological life. Conductivity, TDS and turbidity were 724µS, 427 ppm and 33NTU respectively. The initial Alkalinity, salinity and chlorides of the investigated domestic sewage were ranged between 230 mg/l to 250 mg/l. Nutrients such as ammonium, nitrogen nitrate and phosphorus and dissolved oxygen and BOD of the initial value of investigated wastewater were 1.89mg/l, 0.95 mg/l, 0.24 mg/l, 1.3 mg/l and 1.8 mg/l respectively. Similar observations were recorded for the physico-chemical characteristics of urban municipal wastewater by (Odjadjare and Okoh, 2010).

Table 2. Characteristics of domestic sewage used in this study

Parameter	Value
pH	7.78
Conductivity (µS)	724
Turbidity (NTU)	33
Total Dissolved Solids (ppm)	427
Alkalinity (mg/l)	249
Salinity (mg/l)	235
Chlorides (mg/l)	237
NH_4-N (mg/l)	1.89
$N0_3$-N (mg/l)	0.95
PO_4-P (mg/l)	0.24
Dissolved Oxygen (mg/l)	1.3
BOD (mg/l)	1.8

Effect of Sodium bicarbonate on growth

The effect of sodium bicarbonate on specific growth rate (µ) of *M. aeruginosa* and *C. vulgaris* is shown in Fig. 1 and 2. *M. aeruginosa* (0.665 d^{-1}) and *C. vulgaris* (0.593 d^{-1}) shows the highest specific growth rate at 1 gL^{-1} bicarbonate.

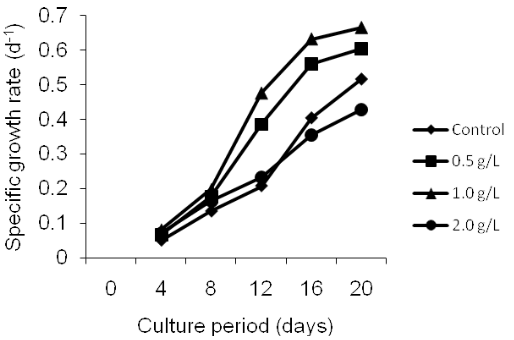

Fig. 1. Specific growth rate of *M. aeruginosa* culture with different levels of bicarbonate

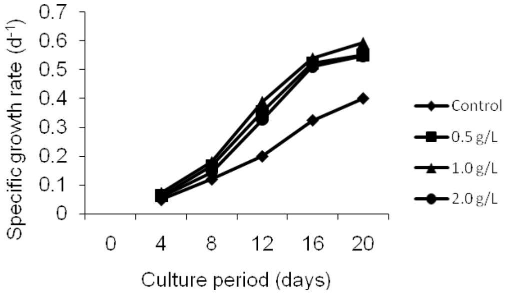

Fig. 2. Specific growth rate of *C. vulgaris* culture with different levels of bicarbonate

Effect of sodium bicarbonate on biomass productivity

The addition of bicarbonate had significant effects on biomass productivity in *M. aeruginosa* ranged from 0.281-0.525 $gl^{-1}d^{-1}$ and *C. vulgaris* ranged from 0.217-0.496 $gl^{-1}d^{-1}$

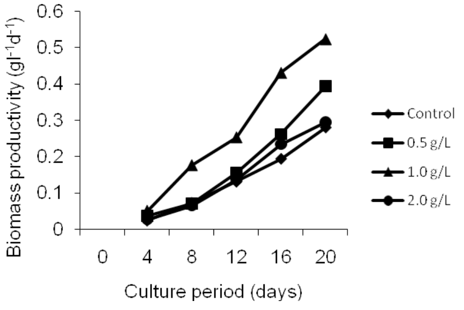

Fig. 3. Biomass productivity of *M. aeruginosa* culture with different levels of bicarbonate

CO_2 fixation rate

At 1 gl^{-1} of sodium bicarbonate concentration on *M. aeruginosa* culture, CO_2 fixation was 0.172 $gl^{-1}d^{-1}$. The CO_2 fixation on *C. vulgaris* culture was 0.159 $gl^{-1}d^{-1}$ at

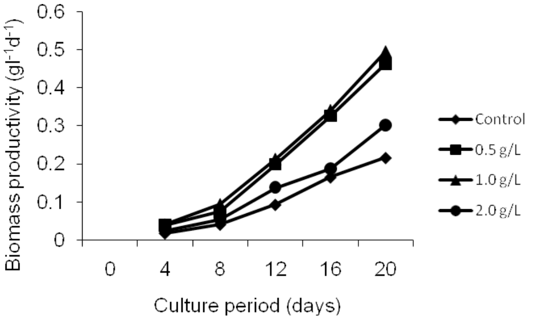

Fig. 4. Biomass productivity of *C. vulgaris* culture with different levels of bicarbonate

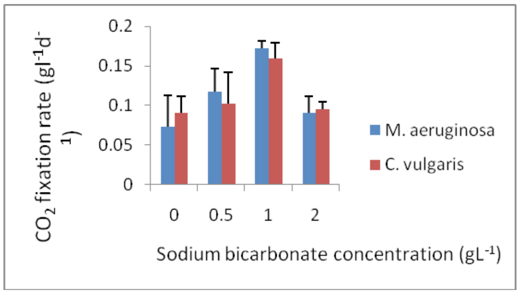

Fig. 5. CO_2 fixation rate in *M. aeruginosa* and*C. vulgaris* culture with different levels of bicarbonate

1 gl^{-1} of bicarbonate.

Growth, pigment and biochemical concentrations of *M. aeruginosa*, *C. vulgaris* and their consortium in different treatment of wastewater

Both the microalgae with single inoculum treatment of each species and their consortium treatment can grew successfully in wastewater. *M. aeruginosa* shows highest specific growth rate with 0.25±0.04 μd^{-1} followed by consortium and *C. vulgaris* with 0.23±0.12 μd^{-1} and 0.20±0.07 μd^{-1} respectively. Badamasi, (2023) also agrees the growth of *M. aeruginosa* and *C. vulgaris* in domestic wastewater. This study employed the spectrophotometric method to measure specific growth rate and pigment concentration by measuring the amount of optical density (Dziosa and Makowska, 2016). Among the two monoculture and the consortium cultures tested, the consortium showed highest in chlorophyll a concentration with 3.24±0.09 (μg/ml) followed by *M. aeruginosa* and *C. vulgaris* with 3.05±0.04 and 2.80±0.13 (μg/ml) respectively. *M. aeruginosa* showed highest carotenoids content followed by consortium and *C. vulgaris* (Table 4). *C. vulgaris* showed highest protein and carbohydrate concentration while consortium showed highest lipids concentration (Table 3).

Physico-chemical analysis of water sample

pH value

Biological instability or the addition or removal of chemicals from the treatment chain can cause pH fluctuations in the wastewater treatment process, which can then modify the properties of the wastewater. The balance of organic and inorganic elements, whether dissolved or particulate, found in wastewater can affect how suspended solids agglomerate and deposit during depth filtration. pH variations in wastewater have the ability to alter the matrix and surface characteristics of the medium, which can have an impact on aggregation and deposition during wastewater filtration (Ncube *et al.*, 2018). The initial wastewater was slightly alkaline with the value of 7.78 which is mostly equivalent by the report of previous study in domestic wastewater (Badamasi, 2023). Following the algal treatment, there was a noticeable reduction in pH levels of wastewater which kept the pH value close to neutral value. *M. aeruginosa*, *C. vulgaris* and synergistic culture reduced the pH value of wastewater from

Table 3. Summary of the growth parameters based on specific growth rate (μd-1), dry weight, final pigment concentration and biochemical content. The cultures were grown for 20 days. Data are represented as mean ± standard deviation (n=3).

Treatment	Dry weight (mg/L)	Specific growth rate (μd^{-1})	Pigment concentration (μg/ml)		Biochemical Content (% dry weight)		
			Chlorophyll a	Carotenoids	Protein	Carbohydrate	Lipids
S (without algae)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
S_M	304.66±4.27	0.25±0.04	3.05±0.04	0.48±0.11	41.32±0.34	30.49±0.08	8.34±0.20
S_C	258.33±2.52	0.20±0.07	2.80±0.13	0.36±0.05	42.95±0.22	35.32±0.15	8.12±0.07
S_{M+C}	280.00±2.31	0.23±0.12	3.24±0.09	0.44±0.04	42.01±0.16	32.43±0.10	9.93±0.19

S :Sewage without treatment, S_M:Sewage treated with *M. aeruginosa*, S_C: Sewage treated with *C. vulgaris*, S_{M+C}: Sewage treated with mixed inoculum of *M. aeruginosa* and *C. vulgaris*.

7.81±0.45, 7.75±0.10 and 7.79±0.05 to 7.33±0.07, 7.40±0.22 and 7.26±0.13 which is 6.14±0.94%, 4.51±0.68% and 6.80±0.26% reduction respectively (Table 4). Similar pH value reduction observation was recorded by (El-Sheekh *et al.*, 2016).

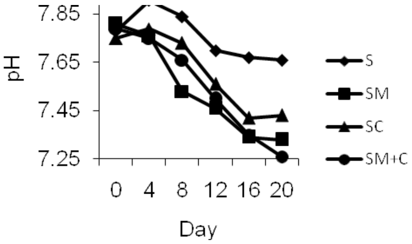

Fig. 6. Changes in pH values of domestic sewage before and after treatment with algae

Conductivity

According to (Levlin, 2007), biological nutrient removal is the principal mechanism that lowers wastewater conductivity, which can be produced by fluctuations in ion concentration (hydrogen H^+, hydroxide OH^-, and nutrients like phosphate and nitrate). Electrical conductivity of the initial investigated wastewater was 724 µS where measurements of conductivity can be used to monitor the wastewater treatment procedures that alter the flow of water. Conductivity was reduced to 12.48±0.04%, 20.78±0.02% and 23.48±0.01% by *M. aeruginosa*, *C. vulgaris* and mixed culture respectively (Table 4). This reduction in conductivity may be due to photosynthesis by microalgae. The process of photosynthesis involves the elimination of ions and carbon dioxide, which lowers conductivity (Falkowski and Raven, 2013).

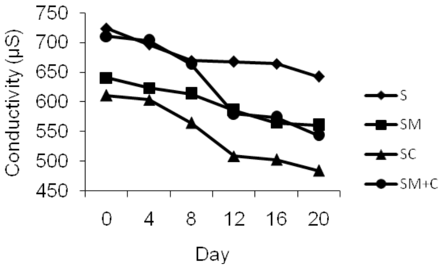

Fig. 7. Changes in conductivity of domestic sewage before and after treatment with algae

Turbidity

The degree to which suspended particles cause water to lose its clarity is measured as turbidity. The water appears murkier and has a greater turbidity level when there are more total suspended solids in it. Trash discharge, urban runoff, bottom feeders (e.g., carp) that agitate sediments, and domestic pets playing in the water are some of the factors that can contribute to turbidity. As suspended particles absorb solar heat, turbid waterways heated and lose oxygen because warm water contains less oxygen

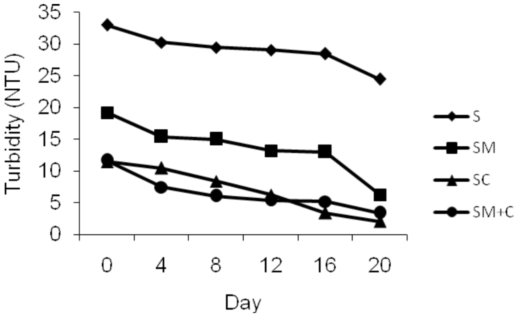

Fig. 8. Changes in turbidity of domestic sewage before and after treatment with algae

Table 4. Reduction of pollutants by different treatments grown in domestic sewage. The cultures were grown for 20 days.

Pollution parameters	S_M			S_C			S_{M+C}		
	Initial	Final	Reduction (%)	Initial	Final	Reduction (%)	Initial	Final	Reduction (%)
pH	7.81±0.45	7.33±0.07	6.14±0.94	7.75±0.10	7.40±0.22	4.51±0.68	7.79±0.05	7.26±0.13	6.80±0.26
Conductivity (µS)	641±2.53	561±2.75	12.48±0.04	611±1.04	484±2.72	20.78±0.02	711±1.62	544±2.15	23.48±0.01
Turbidity (NTU)	19.2±0.15	6.3±0.09	67.18±0.01	11.5±0.52	2.1±0.24	81.73±0.07	11.7±0.14	3.4±0.08	70.94±0.02
TDS (ppm)	400±3.05	344±1.08	14.00±0.05	412±2.25	335±1.45	18.68±0.03	409±0.77	357±1.26	12.71±0.02
BOD (mg/l)	1.6±0.56	0.60±0.06	62.50±0.66	1.6±0.17	0.5±0.07	68.75±0.19	1.7±0.09	0.30±0.10	82.35±0.10
NO_3-N (mg/l)	0.89±0.07	0.77±0.11	13.48±1.08	0.97±0.25	0.49±0.06	49.48±0.59	0.81±0.21	0.41±0.18	49.38±0.73
PO_4-P (mg/l)	0.21±0.03	0.11±0.08	47.61±0.86	0.19±0.17	0.09±0.04	52.63±0.89	0.20±0.08	0.09±0.11	55.00±1.20
NH_4-N (mg/l)	1.90±1.20	1.10±0.66	42.10±1.82	1.87±0.93	1.29±0.15	31.01±1.69	1.84±0.05	1.42±0.15	22.82±0.37
Alkalinity (mg/L)	200±1.22	129±2.90	35.50±0.04	218±2.76	151±3.55	30.73±0.06	244±1.63	176±1.85	27.86±0.00
Salinity (mg/l)	231±2.60	172±1.31	25.54±0.05	229±1.08	150±1.12	34.49±0.02	227±1.77	135±2.03	40.52±0.03
Chlorides (mg/L)	199±2.00	149±1.53	25.12±0.05	195±3.55	140±4.71	28.20±0.10	203±1.05	133±2.36	34.48±0.03

than cool water (Patel and Vashi, 2015). After treating wastewater samples with algae, the turbidity was considerably reduced. Treatment with *C.vulgaris* reduce 81.73±0.07% of turbidity followed by 70.94±0.02% and 67.18±0.01% of treatment with *M. aeruginosa* and mixed culture respectively (Table 4). The reduction of turbidity of wastewater by different treatments are represented in Fig. 3.

Total dissolved solids

Total dissolved solids (TDS) originate from sewage, natural sources, urban runoff, industrial effluent, and chemicals employed in the lengthy water treatment process. A rise in TDS concentration can be attributed to several natural environmental factors, including as mineral springs, salt and carbonate deposits, chemicals, and runoff from agricultural operations (O'Donnell, 2021). The TDS was slightly reduced in all the treatments of wastewater with 18.68±0.03% reduction by *C. vulgaris* followed by 14.00±0.05% and 12.71±0.02% by *M. aeruginosa* and mixed culture treatment (Table 4). The utilization of dissolved solids for growth and metabolism through the processes of bio-absorption and adsorption was the cause of TDS decrease in wastewater. Numerous studies have documented the reduction of TDS when employing microalgae to treat wastewater (Azarpira, 2019).

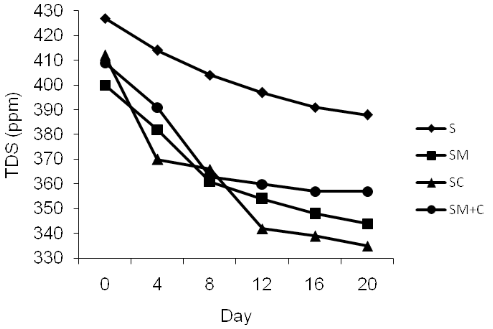

Fig. 9. Changes in TDS of domestic sewage before and after treatment with algae

Alkalinity

The data presented in Fig. 5 clearly shows that the addition of algae to wastewater samples resulted in significant decreases in their alkalinity. Highest alkalinity reduction is shown by *M.aeruginosa* with 35.50±0.04% followed by *C. vulgaris* and mixed culture with 30.73±0.06% and 27.86±0.00% respectively (Table 4).

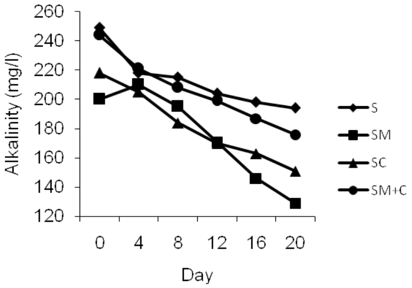

Fig. 10. Changes in alkalinity of domestic sewage before and after treatment with algae

Salinity

The amount of salts, such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl and Na_2CO_3 in water is salinity (Tavakkoli *et al.*, 2010). As microalgae grows, they will utilize the salts in the wastewater as a source of nutrients. The salinity will reduce as they use the salts (Hwang *et al.*, 2016). Result shows that the consortium treatment in wastewater reduce salinity upto 40.52±0.03% during 20 days of experiment (Table 4).

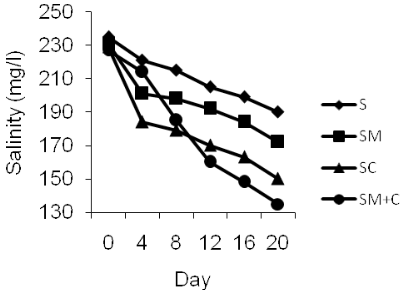

Fig. 11. Changes in salinity of domestic sewage before and after treatment with algae

Chlorides

Part of the main inorganic components of water, which is used in many different applications nowadays, are chloride salts. Although freshwater normally has far lower concentrations of these salts than ocean water, groundwater and surface water still contribute to the presence of chloride in freshwater (Auer *et al.*, 2010). Result shows that the microalgal monoculture and their consortium has the capability to reduce chlorides from wastewater as the consortium treatment in wastewater reduce chlorides to 34.43±0.03% followed by 28.20±0.10 and 25.12±0.05% by monoculture with *C. vulgaris* and *M. aeruginosa* respectively (Table 4).

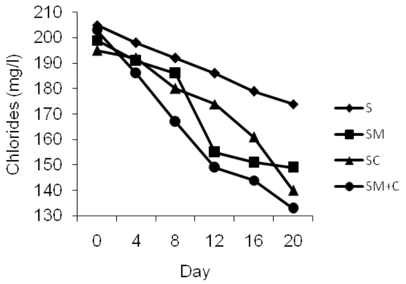

Fig. 12. Changes in chlorides of domestic sewage before and after treatment with algae

Ammonium

Growth of microalgae reduces ammonium resulting in bioremediation of domestic wastewater. There was 42.10±1.82% reduction of ammonium in wastewater treated with *M. aeruginosa*. The concentration of ammonium has been decreased from 1.90±1.20 mg/l to 1.10±0.66 mg/l (Table 3), which is followed by 31.01±1.69% and 22.82±0.37% reduction of ammonium by *C. vulgaris* and consortium respectively (Table 4).

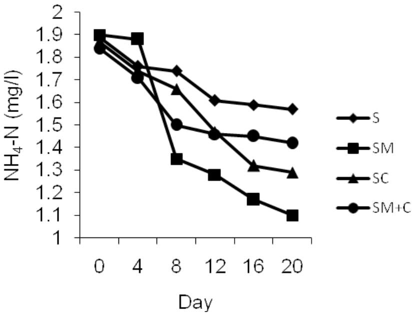

Fig. 13. Changes in ammonium of domestic sewage before and after treatment with algae

Nitrate

There is remarkable reduction of nitrate content in wastewater with 49.48±0.59% and 49.38±0.73% by treatment with *C. vulgaris* and consortium respectively. However less reduction is shown by *M. aeruginosa* with 13.48±1.08% (Table 4).

Phosphates

It is evident from the data in Fig. 10 that, following a 20-day treatment period, the application of algal treatment reduced the phosphate contents in wastewater samples. The removal efficiency of consortium, C. vulgaris and M. aeruginosa was 55.00±1.20, 52.63±0.89 and 47.61±0.86% respectively (Table 4).

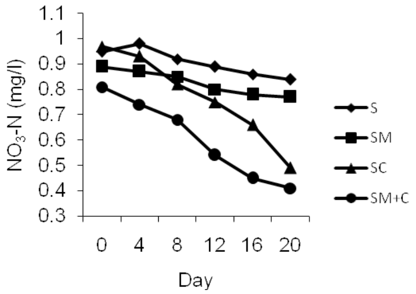

Fig. 14. Changes in nitrate of domestic sewage before and after treatment with algae

Dissolved oxygen (DO) and Biological oxygen demand (BOD)

Dissolved oxygen in the wastewater sample treated with consortium had been found increasing by 423.07±0.12% and treated with *C. vulgaris* and *M. aeruginosa* was observed as 278.57±0.95% and 186.66±0.40% respectively. The amount of dissolved oxygen in the water is reduced by the respiration of the aquatic plants and animals. It increases in tandem with algal photosynthetic activity (V.J. *et al.*, 2009). The amount of organic contaminants in wastewater is indicated by BOD. Aquatic organisms have less access to dissolved oxygen in wastewater with elevated BOD levels. Therefore, before releasing wastewater into freshwater bodies, its BOD loads should be decreased. In present study, it was discovered that the consortium greatly reduced BOD, which increased the DO content of the sewage effluent. The consortium reduces 82.35±0.10% BOD followed by 68.75±0.19% and 62.50±0.66% by *C. vulgaris* and *M. aeruginosa* (Table 4).

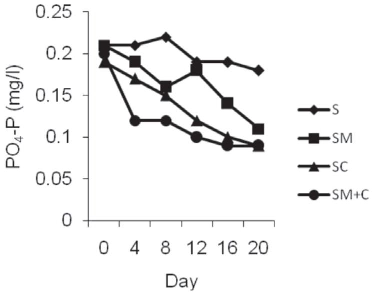

Fig. 15. Changes in phosphate of domestic sewage before and after treatment with algae

Potential reuse of the wastewater after bioremediation

Western Europe has the highest rates of wastewater collection and treatment (88.6% and 86%, respec-

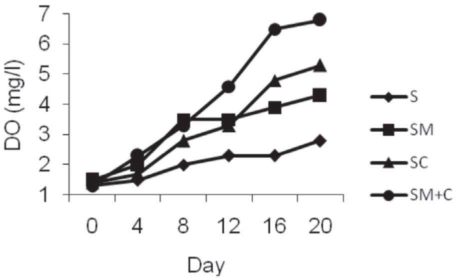

Fig. 16. Changes in dissolved oxygen of domestic sewage before and after treatment with algae

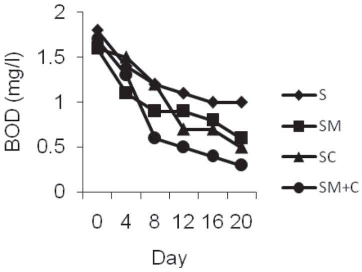

Fig. 17. Changes in BOD of domestic sewage before and after treatment with algae

tively), whereas South Asia and sub-Saharan Africa have the lowest rates (31.1% and 16.6%, respectively). In the East Asia and Pacific area, wastewater collection is noticeably low, where wastewater output totals is high. Significantly, South Asia, the Caribbean, and Latin America have far lower rates of wastewater treatment than wastewater collection, which may be a sign of the significant rates of reuse of untreated wastewater in these areas. The Middle East and North Africa are the regions where treated wastewater reuse is most often used; in the United Arab Emirates, Kuwait, and Qatar, over 80% of the wastewater generated is recycled. In regions like South Asia and sub-Saharan Africa with poor wastewater treatment rates, treated-wastewater reuse is prohibitively low (Jones *et al.*, 2021). Comparing the characteristics of the effluents from phycoremediation using *M. aeruginosa* and *C. vulgaris* monocultures and their consortium to the permissible limits of treated sewage for irrigation water suggested by different standards (CPCB, CPHEEO 1993, (Table 5), shows that the acquired results comply with the requirements of the standards. The final effluents obtained from *M. aeruginosa*, *C. vulgaris* and their consortium treatment can be reuse for irrigation water with the exceptions of chlorides parameter by *M. aeruginosa* monocultures that does not comply with the requirements of the standard. Comparison of the effluents of phycoremediation with the permissible limits for drinking water suggested by different standards shows that the obtained effluents of the tested parameters comply with the requirements of standards. Nonetheless the final effluent obtained from *M. aeruginosa*, *C. vulgaris* and consortium treatment of the parameters including conductivity, turbidity and NH_4-N is not provided requirements of the standards for drinking water. All of these standards also include pathogen limit values, thus disinfecting the final effluent before reuse is advised.

Based on the findings, it was clear that sodium bicarbonate addition had significant effects on growth, biomass productivity and CO_2 fixation.

Table 5. Recommended guidelines for discharge of treated sewage into inland surface waterby CPCB, CPHEEO, Jordan and Turkey and the standards of permissible limits for drinking water by BIS, EU and WHO.

Pollution parameters	Permissible limits of treated sewage for irrigation water	Standards	Permissible limits for drinking water	Standards
pH	6.5-9	CPCB	6.5-8.5	BIS, 2012
Conductivity (µS)	750	CPHEEO, 1993	250	EU, 1998
Turbidity (NTU)	10	Jordan, 2002	1	BIS, 2012
TDS (ppm)	500	CPHEEO, 1993	500	BIS, 2012
BOD (mg/l)	20	CPCB	5	WHO, 1999
NO_3-N (mg/l)	10	CPCB	45	BIS, 2012
PO_4-P (mg/l)	5	CPCB	1	WHO, 1999
NH_4-N (mg/l)	5	CPCB	0.5	BIS, 2012
Alkalinity (mg/l)	600	CPCB	200	BIS, 2012
Salinity (mg/l)	175	Turkey, 1991	200	BIS, 2012
Chlorides (mg/l)	142	CPCB	250	BIS, 2012

Microalgae exposed to 1 gl-1 concentration of sodium bicarbonate expressed higher productivity as compared to other treatments. It was also determined that phycoremediation with various algae species, such as *M. aeruginosa*, *C. vulgaris* and their consortium can recycle and reuse diverse combinations of water samples. The results of the present study showed that both algae species had a high potential for lowering the pollutant levels of every physico-chemical parameter. The microalgal species with monocultures or in consortium produced domestic wastewater with a relatively high ratio of pollutant removal to growth performance. Furthermore, it was discovered that the final effluent of *M. aeruginosa*, *C. vulgaris* and their consortium treatment can be reuse for irrigation in accordance with legal regulations. Wastewater reuse is advocated but the practical uses currently are less because of the expensive initial investment and ongoing operational expenses. Therefore, it would seem to be a more cost-effective and environmental friendly solution to replace the current treatment systems with phycoremediation technology or integrate it into the traditional systems wherever possible.

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Conflicts of interest

The authors state that they do not have any known competing financial interests or personal ties that could appear to have influenced the work disclosed in this study

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