The Feasibility Study of Green Microalgae Assisted Coal Mine Effluent Desalination

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Abstract. Carbon-neutral sustainable approaches are highly demanding in the coal energy sector. Coal mine effluent disposal is a severe challenge with crucial concern issues of salinity hazard and heavy metal contamination due to long-duration water and coal interaction. The medium to the high salinity of coal mine effluent leads towards irrigation unsuitability due to the negative impact upon infiltration and permeability of nutrients from the soil to plant. Focusing on the international irrigation water quality standards given by the Food and Agriculture Organization (FAO) of the United Nations, most coal mine effluents are considered negatively impacting crops, soil fertility, groundwater, and aquatic life. Therefore, the current study investigates the direct cultivation suitability of *Chlorella pyrenoidosa* to simultaneously treat coal mine effluent for salinity removal and biomass production. Initially, *C. pyrenoidosa* culture adaptation in varying concentrations of coal mine effluents (25%–100%) in coal mine effluent, which are collected from two different points of coal mine named as coal mine effluent 1 (CME1) and coal mine effluent 2 (CME2). Evaluating *C. pyrenoidosa* growth kinetics, it was observed that the doubling time extended from 2.25 days (100% BG-11 as a medium; control) to 4.33 days (100% CME as a medium). Interestingly, the highest value for biomass production was 1.78 ± 0.12 g/ L with 25% CME 1 supplemented with essential growth nutrients; this value lies near 100% BG11 supplemented growth, 1.81 ± 0.05 g/L. In the current study, taking salinity removal as a prime concern, 100% utilization of CME-2 in place of BG-11 medium was very significant for salinity reduction from 4.80 ± 0.50 mS/cm (initial) to 0.98 ± 0.02 mS/cm (final) during 14 days batch growth. In continuation of that, the significant finding was salinity reduction of both samples (50% and 75% sample) to the level of 0.7 mS/ cm, which lies under the FAO guidelines for irrigation. Present findings also revealed an alternative to conventional processes, i.e., thermal and membrane desalination. Microalgae-assisted desalination is a novel, energy-efficient, eco-sustainable, cost-effective, and long-term operational approach. It has good potential to treat medium to sub-optimal salinity of coal mine effluent coupled with high-value biomass production.

Keywords: Carbon neutral · Salinity hazard · Chlorella pyrenoidosa · Biomass

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1 Introduction

The ratio of potable water quantity to worldwide population is depleting very drastically and becoming a challenge for the upcoming generation. Dumped coal mining effluent (CME) at the coal mining site is another primary concern with freshwater scarcity. Poor water quality of CME and drainage challenges are creating difficulty for consistent agriculture water supply in rural mining sites [1]. Although, utilization of wastewater for irrigation purposes is gaining international interest, but the quality of water is key considering factor for food safety [2]. The direct disposal of CME without any treatment can alter soil property and accumulate hazardous trace metals in the soil as well as cultivated crop [3]. In the context of CME, the hazardous impact of coal mine elements remains in the mine's life cycle and decades later, even after the end of mining activity [1]. CME is reported to be primarily saline with an exceeding concentration of boron (B) and heavy metals such as manganese (Mn), cadmium (Cd), chromium (Cr), iron (Fe), nickel (Ni), and copper (Cu) due to long-duration water- coal interaction [4]. Due to these metals, orange, red or yellow deposition can negatively impact groundwater, aquatic life, nearby soil, and the ecosystem [5].

Microalgae, a heterogeneous group of organisms with different sizes and shapes, can absorb carbon dioxide through photosynthesis and produce fatty acids and oxygen [6]. Integrated process technologies considering microalgae biomass production with biofuel development are applied in different energy sectors [7]. More recently, utilization of algae for wastewater management has gained significant attention due to cost reduction for commercial-scale biomass production by direct supplementing wastewater in place of expensive nutrient medium and bulk volume of water requirement [8]. Previously reported studies of microalgae-assisted dairy industry effluent [9], pharmaceutical effluent [10], and distillery effluent [8] treatment showed the potential of microalgae to cultivate in nutrient-rich wastewaters to reduce toxic components along with long term environmental sustainability and carbon neutrality. The application of microalgae to solve the issues concerned with water salination is in a nascent stage with few reported studies. In this context, marine algae species, *Nannochloropsis oculata* and *Dunaliella tertiolecta*, are reported to be applied for desalination of inland brackish water by utilizing ion removal ability to remove nitrate and phosphate from wastewater with concentrate biomass production [11]. Recently, *Chlorella vulgaris* cultivated in the altered BG-11 nutrient medium is reported to reduce the salinity of brackish water by up to 30% [12]. However, elevated NaCl concentration (>30g/L) and Na_2CO_3 (9g/L) are reported to inhibit *C. vulgaris* [13]. Further, microalgae-based phycoremediation technology has been employed to reduce nutrient and salinity levels in lakes [13] as well as seawater [14]. The present study is reported first time to investigate the feasibility of *C. pyrenoidosa* to grow in saline CME for salinity removal due to microalgae potential to grow at medium to suboptimal salinity zone. The quality up-gradation of mining water for further usability as irrigation water with biomass production schematic set up has shown in Fig. 1.

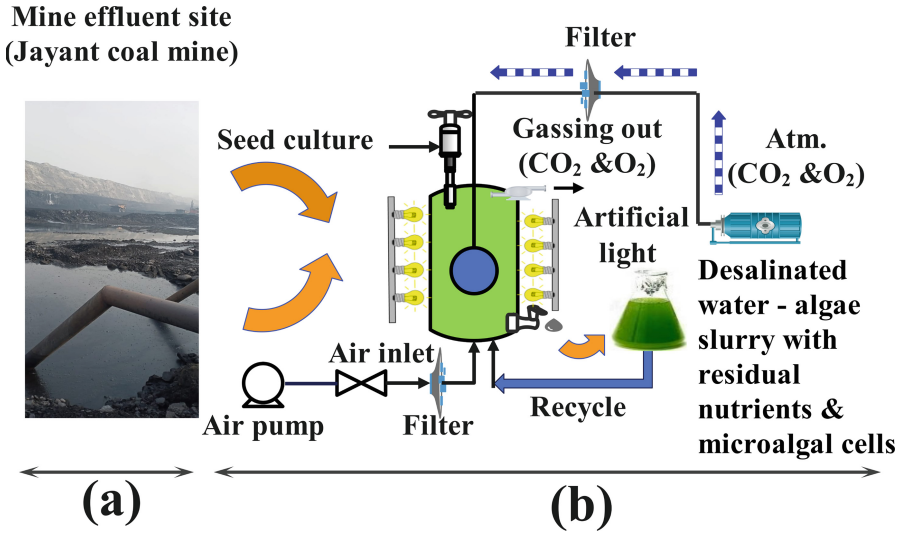


Fig. 1. Novel strategy for coal mine effluent desalination with algae growth (a) Coal mine effluent as a nutrient medium (b) Proposed microalgae cultivation system for desalination.

2 Materials and Methods

2.1 Algae Culture Maintenance

In the present study, pure algae culture, *C. pyrenoidosa* (NCIM 2738), was procured from the Indian Council of Agricultural Research, New Delhi. The microalgae cells were maintained in most suitable BG 11 medium, composed of sodium nitrate (1.5 g/L), magnesium sulphate heptahydrate (0.075 g/L), calcium chloride dehydrate (0.036 g/L), sodium carbonate (0.02g/L), potassium phosphate dibasic trihydrate (0.04 g/L), citric acid with ferric ammonium citrate (0.006 g/L and 0.006 g/L), EDTA Na_2 (0.001g/L) and essential trace metals containing boric acid (2.8 mg/L), manganese chloride tetrahydrate (1.81 mg/L), zinc sulphate heptahydrate (0.22 mg/L), copper sulphate pentahydrate (0.079 mg/L), sodium molybdate dihydrate (0.39 mg/L) and cobalt nitrate hexahydrate (0.0494 mg/L).

A photo cabinet facilitated with a light intensity of 222.00 $\mu mol/m^2sec$ and a temperature of 28 $\pm$ 2 °C is utilized for microalgae cultivation under a light-dark cycle of 16:8 h. During cell growth, the purity of cultures was examined under a fluorescent microscope (Olympus CKX53) during the regular interval.

2.2 Physicochemical Analysis of CME

The effluent sample region is located at Singrauli (*latitude 24.177729°N and longitude 82.65884°E*), Madhya Pradesh, India. CME is sampled from two different points; coal main section (CME1) and effluent treatment plant (ETP) inlet (CME2). Standard water sampling methods: ISO 5667-3:2018 (E) as provided in Water quality-Sampling-Part 3 are followed for sampling [15]. Removal of suspended particles from raw coal

mine effluents (CME1 and CME2) is processed through filtration operation, followed by physicochemical characterization using the standard methods of water and wastewater analysis reported by the American Public Health Association [16]. Further, pH was determined using benchtop pH meter (EUTECH), electric conductivity by a conductivity meter (EUTECH), and COD using portable COD analyzer with digester (UNIPHOS).

2.3 CME Supplemented Growth Medium for *C. Pyrenoidosa*

Initially, the microalgae growth medium, BG-11 media was gradually supplemented with varying concentrations (v/v) of CME. In present study, 0 (control), 25, 50, 75 and 100% of CME1 and CME2 media was utilized to grow culture. Flasks containing 0% CME (100% BG11) were used as positive control and 100% CME (0% BG11) as negative control. The cells were initially grown in 100 ml BG-11 media which was regularly replaced with 10 ml of the CME per 24 h till ten days under the identical condition of temperature (28 ± 2 °C), light intensity (222.0 μmol/m^2/sec) and light-dark cycle (16:8 h) to acclimatize microalgae with saline water [17]. All the experiments were performed in a 250 ml flask with 100 ml working volume in triplicate. All sets of flasks were inoculated with pure grown *C. pyrenoidosa* culture having an optical density of 2.0 at 680 nm with 10% inoculum size [18]. The run time of all experiments was 15 days. After 15 days, the microalgae biomass was harvested through centrifugation at 2549 g rcf (relative centrifugal force) for 8 min, and the biomass pellet was collected. The harvested biomass was then washed thrice with deionized water and dried in a hot air oven (105 °C for 3 h) and stored for future experiments.

2.4 Microalgae Growth Kinetic Study for Biomass Determination

For microalgae growth estimation, UV/Vis spectrophotometer (Agilent) is used to prepare the calibration curve between dry cell weight (DCW) and absorbance at 680 nm (OD_{680}). Further, a correlation equation between DCW and OD_{680} was developed. The gravimetric method was used to measure the DCW followed by sample drying at 60 °C temperature for 24 h time. After completing the batch growth study, the suspended culture was harvested by centrifugation at 2459 g rcf (relative centrifugal force) for 10 min followed by oven drying at 60 °C temperature until the attainment of constant weight. The dry cell weight was determined by using Eq. (1):

$$\text{Dry cell weight(g/L)} = (W_f - W_i)/\text{Sample volume} \tag{1}$$

Where, dry cell weight is given in weight/ volume basis. W_i and W_f are the initial and final weight of filter paper (g), respectively.

Correlation equation between dry cell weight and absorbance for *C. pyrenoidosa* culture was determined by Eq. (2):

$$\text{Biomass conc. (g/L)} = 0.4859 \times OD_{680} - 0.0293 \ (R^2 = 0.9982) \tag{2}$$

Where OD_{680} is the optical density at 680 nm wavelength.

The biomass productivity (BP, g L^{-1} day^{-1}) during the culture period was calculated from the following Eq. (3):

$$\text{Biomass productivity (BP)} = (X_t - X_0) / (t_t - t_o) \quad (3)$$

Where X_t and X_0 are biomass concentration (g/L) at the end of the growth phase (t_t) and at start t_0 (day) respectively.

The following Eq. (4) and (5) were used to calculate the specific growth rate (μ, day^{-1}) and cell doubling time (t_d, day).

$$\mu = \ln (W_t/W_0)/\Delta t \quad (4)$$

$$t_d = \ln 2/\mu \quad (5)$$

Where W_t and W_0 are the DCW at the end and beginning of batch cultivation respectively and Δt represents time difference between end and start of growth phase.

2.5 Estimation of Salinity Reduction

Salinity is considered a key parameter to indicate water quality and its impact upon soil fertility, aquatic life, and groundwater which further decides irrigation suitability of that water. The linear correlation between electrical conductivity and salinity is used to estimate salinity variations during the cultivation period. Therefore, after the adaptation of pure microalgae culture to high salinity, all five sets of samples (0%, 25%, 50%, 75%, and 100%) were monitored at predefined culture conditions for electrical conductivity (EC) variation using Eq. (6):

$$\text{Salinity reduction } \% = 100\,(EC_i - EC_f)/EC_i \quad (6)$$

EC_i indicates electric conductivity at inoculation and EC_f, final electric conductivity after 14 days of culture growth. All set of experiments were performed in duplicate with standard deviation (mean ± SD is <3%).

3 Result and Discussion

3.1 Quality Assessment of Mining Effluent for Irrigation Suitability

Physical, chemical, and nutritional properties of both coal mine effluent samples, CME1 and CME2 were summarized in Table 1. The comparative assessment of present coal mine effluent with other mines indicates high salinity with electric conductivity of 4.8 mS/cm. However, the allowed limit is <0.7 mS/cm (as per given FAO guidelines). Therefore, the quality of present samples lies under the category of 'severe problem for the sensitive plant. 'Opposite to the acceptable limit of nitrate, <5 mg/L, a very high nitrate (NO_3^-) concentration (140–160 mg/L) was observed, which causes eutrophication, lodging, and delayed crop maturity, so not recommended for direct use as irrigation water. Further, total hardness in the form of bicarbonate (HCO_3^-) was also exceeded (8.30–8.40), negatively impacting the crop. Taking FAO guidelines as a benchmark, all exceeded quality parameters of coal mining effluents directly indicate tough decision towards no recommendation for direct utilization of mining effluent as irrigation water.

Table 1. Physical and chemical parameters analyzed in mining effluents with Food and Agriculture Organization (FAO) guidelines for irrigation

Parameters	Units	FAO guidelines for irrigation (Restriction on use)			Lajkura coal mine, Orissa, India [18]	Sao Pedro da Cova coal mine, Portugal [1]	Present study	
		none	slight to moderate	severe			CME 1	CME 2
Salinity								
EC	ms/cm	0.7	0.7–3.0	>3.0	3.25 ± 0.80	0.92 ± 0.18	4.1 ± 0.20	4.8 ± 0.50
Nutrients								
Nitrate (NO_3^-N)	mg/l	<5	5–30	>30	NA	NA	140 ± 0.10	160 ± 0.50
Bicarbonate (HCO_3^-)	meq/l	<1.5	1.5–8.5	>8.5	7.76 ± 0.92	3.37 ± 1.04	8.30 ± 0.25	8.40 ± 0.50
Phosphate (PO_3^-P)	mg/l	0–2			NA	NA	NA	NA
Potassium (K)	mg/l	0–2			1.28 ± 0.86	2.08 ± 0.02	NA	NA
TOC	mg/l	NA			NA	NA	42 ± 0.50	54 ± 0.25
COD	mg/l	NA			NA	NA	250 ± 0.34	264 ± 0.54
pH		6.5–8.4			7.35 ± 0.20	NA	7.8 ± 0.25	7.96 ± 0.43

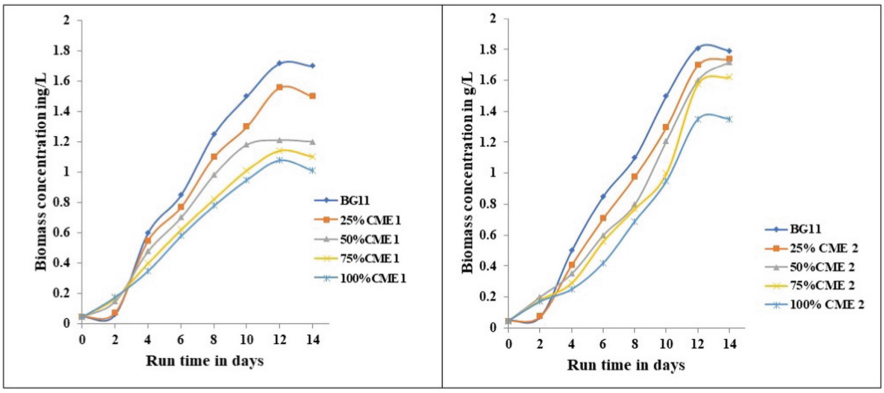
NA: Not available.

3.2 Growth Study of *C. Pyrenoidosa* in Coal Mine Effluent

Five selected doses (0–100%) of the CME 1 and CME 2 showed varying effects on microalgae growth during 14 days batch experiment. The growth curves of microalgae, *C. pyrenoidosa* cultivated in both samples are shown in Fig. 2. During the initial three days (lag phase), no significant biomass growth was observed in all five doses of both samples. However, from the 4^{th} day onwards, significant cell growth increment can be observed until the 12^{th} day.

Overall, during 15 days of growth, the doubling time of *C. Pyrenoidosa* varies from 2.3 days (in most suitable BG 11 medium) to 4.33 days (in CME 1 medium). In the second set of experiments, the doubling time varies from 2.25 days to 3.98 days for BG 11 and CME 2 medium. This data represents slightly better cell growth, 1.81 g/L–1.35 g/L in CME2 in place of 1.72 g/L–1.08 g/L in CME1. One desalination study using microalgae [19] reports *Scenedesmus sp.* And *C. vulgaris* doubling time in the range of 0.42 to 1.2 days and 3.1 to 5.9 days respectively at high salt concentrations (2g/L to 4g/L).

In the present study, 100% supplementation of BG11 with coal mine effluent also showed very significant results, 1.35 g/L biomass concentration in CME 2 and 1.01 g/L in CME 1. However, 25% to 75% CME 1 and CME 2 showed better growth results as 1.56 g/L–1.14 g/L and 1.7 g/L–1.58 g/L, respectively. These results indicate that nutrient concentration in growth media affects microalgae growth. Therefore, modified coal mine effluent supplemented with some essential nutrients can be utilized directly as a growth media for pilot scale and large-scale microalgae cultivation studies. However,



(a) (b)

Fig. 2. Growth profile of *C. pyrenoidosa* in different doses of coal mining effluent (a) CME1 (main section) and (b) CME2 (inlet of coal mine effluent).

optimization of media composition, detailed elemental analysis of coal mine effluent, and investigating its impact upon microalgae growth are crucial steps for high-density algal biomass production.

3.3 Desalination Study in Coal Mine Effluent

Bio-desalination experiments were conducted simultaneously with microalgae growth study during 14 days batch cycle. The high salinity adaptation of microalgae could involve salt uptake and export of cations, sodium (Na^+), and potassium (K^+) by using a redox-driven Na^+ pump. Previously, Na^+ influx in the freshwater cyanobacterium, *Anabaena* L-31, and *Anabaena torulosa* is reported to tolerate high Na^+ concentration at 150 mM [20]. In another study of *Dunaliella salina*, the osmotic pressure balance is assumed by Na^+ and Cl^- accumulation in the vacuole of the cell and K^+ compounds such as proline and glycine accumulation in the cytoplasm of algae [21]. In the present study, bio-desalination of coal mine effluent is assessed by electric conductivity (EC) variation during cell growth (Fig. 3). These measurements showed EC reduction in the range of 3.0 mS/cm–0.7 mS/cm for 50% CME 1 and 3.20 mS/cm–0.72 mS/cm for 75% CME 1 sample. In similar way, EC reduction for 50% and 75% CME 2 was 3.20 mS/cm–0.59 mS/cm and 3.7 mS/cm–0.7 mS/cm. In both studies, remarkable salinity reduction is obtained to the safe limit of 0.7 mS/cm, which lies under the irrigation suitability range as per FAO guidelines. However, final conductivity values for 100% CME 1 and CME 2 samples are 1.49 mS/cm and 0.98 mS/cm, which require additional treatment to reduce salinity to a safe limit. Desalination studies suggest avoiding the microalgae growth till the decline phase as absorbed salt within the cell may be released to the solution due to molecular diffusion [19].

In the present study, bio desalination of coal mine effluent exhibited significant results in both samples by removing salinity 76.67%–63.66% in CME 1 and 81.56%–79.58% in CME 2 (Fig. 3). Recent attempts on microalgae-based desalination are summarized in Table 2, which provides a clear indication that salt concentration declined steadily until

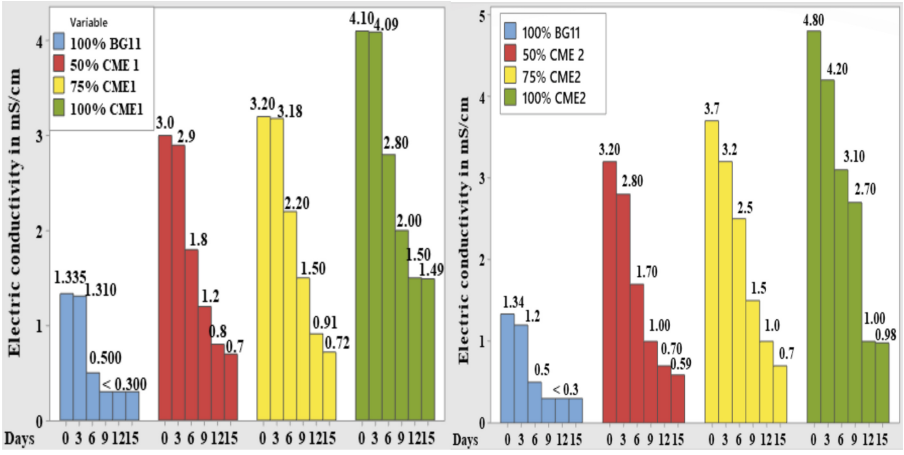
Table 2. Recent attempts made on microalgae-based desalination study

Algae used for desalination	Reactor utilized	Water type/medium	Salinity removal (%) with biomass production	Reference
Halophyte *Chlorella vulgaris*, *Scenedesmus* sp.	Batch photobioreactor	Brackish water	30% with maximum salinity tolerance 20g/l *C. vulgaris* doubling time 3.1–5.9 day and *S.* sp. Doubling time 0.63–1.81 day	[19]
Chlorella vulgaris	Bubble column photobioreactor	Brackish water with modified BG 11 medium	40% -80% with maximum salinity tolerance 1-5g/l	[12]
Nannochloropsis oculata, Dunaliella tertiolecta		Waste concentrate of water desalination units	Na^+ removal yield: 0.1 for *N.oculata* and 0.09 for *D. tertiolecta*	[11]
Desmodesmus subspicatus, Desmodesmus armatus, Dictyosphaerium sp.	Shake flask Study	Seawater	Halotolerant potential with cell concentration 4.86×10^7 cells/ml for *D. subspicatus*, 4.97×10^8 cells/ml for *D. armatus,* 3.25×10^8 cells/ml for *Dictyosphaerium sp.*	[22]
Chlorella sp., Chlorococcum sp., Desmodesmus sp., Scenedesmus sp, Monoraphidium sp.	Shake flask Study	Salt rich wastewater	Conductivity reduction 3% by 10,000 mg/l treated *Scenedesmus sp.*, 39% by 5000 mg/l treated *Desmodesmus sp.* Nitrate removal (more than 90%) and chloride removal up to 39%.	[23]

(*continued*)

Table 2. (*continued*)

Algae used for desalination	Reactor utilized	Water type/medium	Salinity removal (%) with biomass production	Reference
Chlorella pyrenoidosa	Shake flask study	Coal mine effluent	Significant conductivity reduction of coal mine effluent till the level of irrigation suitability for 50% and 75% CME samples (lower limit: 0.59–0.7 mS/cm) with maximum biomass concentration of *C. pyrenoidosa* 1.81 g/L	Present study



(a) **(b)**

Fig. 3. Electric conductivity variation (a) CME1 (main section) and (b) CME2 (inlet of coal mine effluent).

the end of exponential and early stationary phases. However, extended bio-desalination in the death phase of microalgae is reported to show a sudden rise in salinity due to the export of accumulated Na^+ from cell to a solution [19]. Interestingly, high lipid content (21%) was reported for *Scenedesmus obliquus* assisted salinity removal of 8.8g/L NaCl to 1.0 g/L NaCl which leads towards successful application of bio-desalination in brackish water [24]. Similarly, remarkable % salinity removal (63.66%-81.56%) is achieved by present desalination study (Fig. 4).

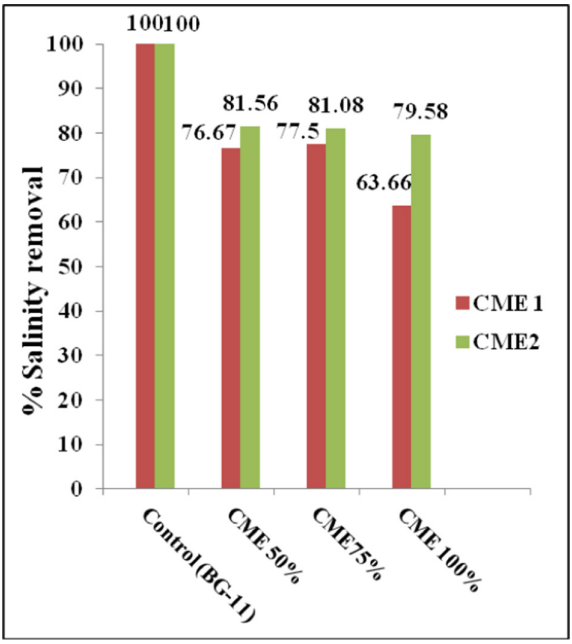


Fig. 4. Salinity removal (%) of coal mining effluent.

3.4 Challenges Concerned with Algae-Water Separation

After completing the bio-desalination experiment, a considerable amount of algae-water mixture separation is needed. High-speed continuous centrifugation application to separate algae cells from the desalinated water is not a very recommendable approach due to high capital and energy cost along with its negative impact upon cells integrity with the chances of absorbed salt release into the desalinated water [21]. Technical and economic challenges are more significant in larger-scale desalinated water separation from an aqueous microalgae suspension.

In the direction of microalgae assisted desalination cell, *Chlorella vulgaris* is reported to generate 0.12 mA/cm^2 current density with 60.15% desalinate efficiency [25]. Further, waste water treatment, brackish water desalination and electric energy production are integrated with key finding that electrochemical performance can be predicted with conductivity variation [26].

High-density microalgae recovery is also needed for further biofuel development. Concerning this challenge, cell harvesting by using alkaline flocculants as $Ca(OH)_2$, $Mg(OH)_2$, KOH, or NaOH is more practicable to allow coalesce of algal cells into a 3D floc formation [19]. In the present study, desalinated CME separation from biomass is concerned with further irrigation utilization of that water and biofuel development. Therefore, study concern with the impact of flocculants upon soil property is also very significant, as toxic flocculants, Alum (hydrated aluminum potassium sulfate), and other aluminum salts may negatively impact the soil and further vegetation quality. Hence,

prosperous and sustainable utilization of bio-desalination is connected with low cost and effective separation of valuable end products, desalinated water, and microalgae cells.

4 Conclusion

Integrated strategy towards bio-desalination with bio-fuel development could address two significant challenges water and energy together. The utilization of microalgae for desalination is a pretty novel strategy that is successfully applied in the present study as a replacement for costly and energy-intensive conventional desalination. At the initial stage, high salinity adaptation of *C. Pyrenoidosa* is achieved with a gradual increment of coal mine effluent concentration from 0% to 100%. Biomass concentration of 1.35 g/L is achieved at 100% coal mine effluent utilization as nutrient media; however, maximum growth (1.81 g/L) is still obtained with BG-11 media. Salinity removal of 50% and 75% coal mine effluents till the irrigation applicable limit (<0.7 mS/cm) may be considered as significant achievement with the aspect to use such after for irrigation purposes in a nearby coal mine. However, fresh microalgae growth inhibition at highly saline water (>20 mg/L), algae-water separation and maintenance of salt absorbed cell integrity are major bottlenecks in bio-desalination studies. Extensive research in the direction of microalgae assisted bio-desalination technology may be used to treat brackish water as coal mine effluent or integrated with conventional desalination as reverse osmosis for sea water pretreatment to develop long term sustainable and innovative water utilization process.

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