Environmental Technology & Innovation 19 (2020) 100926

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

# Enhancing the synergistic interaction of microalgae and bacteria for the reduction of organic compounds in petroleum refinery effluent

V.R.V. Ashwaniy [a], Muthiah Perumalsamy [a,*], Sivakumar Pandian [b]

[a] Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli 620015, India
[b] School of Petroleum Technology, PanditDeendayal Petroleum University, Gandhinagar 382007, India

ARTICLE INFO

*Article history:*
Received 28 March 2020
Received in revised form 15 May 2020
Accepted 23 May 2020
Available online 26 May 2020

*Keywords:*
*Scenedesmus abundans*
Biocathode microbial desalination cell
Desalination
Petroleum refinery effluent
Reverse osmosis

ABSTRACT

Biocathode microbial desalination cell represent an energy yielding technology for reducing the organic compounds of petroleum refinery effluent (PRE) coupled with sea water desalination and bioelectricity production. The synergistic interaction between the microalgae *Scenedesmus abundans* and the bacteria played the major role in reduction of organic compounds present in PRE. It was observed that microalgae grown in 50% PRE produced maximum growth absorbance of 2.058, chlorophyll and carotenoid concentration of 2.78 and 1.365 $\mu g\ mL^{-1}$ respectively. The presence of PRE in cathode chamber significantly increased the bioelectricity generation, ionic separation rate and microalgae growth and also reduced the addition of BG11 commercial media to greater extent. The maximum power density of 0.573 $Wm^{-3}$ was generated during the single batch operation using algae grown in PRE as biocathode. The study analyzed the desalination efficiency using microalgae grown in PRE as an electron acceptor and the ability to act as bioresource for bacterial growth in anode. The net energy output of the present study reveals that the microbial desalination cell (MDC) can save 1.245 $kWhm^{-3}$ of power.


## 1. Introduction

Petroleum refineries carry out multiple operations depending on the type of crude to be refined and the desired product. A petrochemical refinery can be a large consumer of water depending on its size, the volume of crude to be processed, the valuable products (more than 2500 products) and the complexity of operations. Many processes in the refinery not only utilize the raw and treated water but also reuse the water. Different processes such as cooling systems, distillation, hydro-treating and desalting consume a significant amount of water in the refinery (Benyahia et al., 2006). Petrochemical wastes and wastewater originating from different processes such as crude refining, manufacturing fuels and petrochemical intermediates contain complex mixtures of organic and inorganic chemicals (Freeman, 1995). High concentration of nitrogen and phosphorous levels in petrochemical effluent causes eutrophication effect and has been observed to be a significant cause of aquatic environmental pollution.

A typical refinery effluent treatment process involves a primary treatment that combines the physicochemical process to remove oil and suspended particles followed by the secondary treatment process (Diyauddeen et al., 2010). The biological treatment process plays a vital role in handling the waste materials effectively and thus helps in maintaining

* Corresponding author.
*E-mail address:* mpsamy@nitt.edu (M. Perumalsamy).

https://doi.org/10.1016/j.eti.2020.100926
2352-1864/

the disposable law standards. Recently, microalgae for wastewater treatment are gaining attention because of its photosynthetic capabilities, production of valuable products (biofuels and bioproducts) and the nutrition removal (Noue and Pauw, 1988). Similarly, the use of microalgae biomass as biosorbents for removing the heavy metals has been found to be economical, ecologically safe and an effective way to remove the pollutants from different wastewaters (Mata et al., 2009). Talebi et al. (2016) have presented an integrated strategy to reduce the operating expenses of petroleum wastewater treatment process along with a sufficient improvement in biodiesel production with regard to quality and quantity, using the microalgae *Duneliella salina*. *Scenedesmus abundans* is exhibited to be an efficient microalga in the treatment of wastewaters such as rice mill effluent, domestic wastewater, and municipal wastewater (Abinandan et al., 2015; Lekshmi et al., 2015; Sivakumar and Kumar, 2017). Further, it is also used in the biosorption of cadmium and copper from contaminated water (Terry and Stone, 2002). The successful growth of algae in chemically different wastewaters proves the potential scalability of algal based wastewater treatment. Besides, the effective utilization of *Scenedesmus abundans* as the passive cathode is seen in the biocathode MDC (Ashwaniy and Perumalsamy, 2017).

MDC is a green, environment friendly and innovative technology that integrates desalination, wastewater treatment and bioelectricity generation in a single reactor (Cao et al., 2009). Among the different configurations of MDC design such as air cathode MDC, bipolar membrane MDC, capacitive MDC, biocathode MDC, decoupled MDC, osmotic MDC, stack MDC, upflow MDC, etc., biocathode MDC is considered to be an emerging and a promising technology because of the application of photosynthetic microorganisms and also because of its potential to replace expensive catalyst such as platinum. Algae are considered to be the primary producers of oxygen because of the oxygenic photosynthesis process and also because they eliminate the external aeration cost. The photosynthetic energy conversion process in microalgae is carried out by internal molecules such as membrane bound proteins and small mobile molecules (Allen and Stanier, 1968). Kokabian and Gude (2013) have been the first to report the utilization of microalgae, *Chlorella vulgaris* as passive biocathode attaining 40% desalination efficiency compared to air cathode. In a recent study, the biocathode MDC has been coupled with a hollow fiber microfiltration membrane to enhance the real time wastewater purification process (Zuo et al., 2018). The process has recovered a part of nitrogen, 98.7% phosphorous, and also has reduced the initial conductivity of the wastewater by 6.7 times.

The present study investigated the activity of different inoculums size of *Scenedesmus abundans* in variable ratios of PRE for the following reasons: 1. to improve the biomass production by utilization of nutrients present in PRE would produce increased concentration of dissolved concentration under insitu conditions; 2. decreased commercial media utilization in microalgae growth; 3. simultaneous waste removal process. The present study reports the activity of algae grown in PRE as electron acceptor (MDC1) and the ability to act as a substrate for bacterial growth in anode (MDC2) to enhance the desalination, petroleum refinery effluent treatment and power generation.

## 2. Materials and methods

### 2.1. Sample collection

The PRE for the experiment was collected from the petroleum refinery: Chennai Petroleum Corporation Limited, Tamilnadu, India. The raw sample of effluent treatment plant at the initial collection point was used for the analysis. The physiochemical compositions were analyzed by following standard methods.

### 2.2. Cultivation of microalgae scenedesmus abundans

The stock culture of *Scenedesmus abundans* was maintained in the BG11 media at ambient temperature. The experiments were carried out in 250 mL Erlenmeyer flasks and the stock culture in exponential phase was taken for bioremediation of petroleum effluent. Three different inoculums size of microalgae samples such as 5, 10, and 15 mL (i.e. 5, 10, and 15 volume %) from the stock culture was inoculated in different ratios of PRE and BG11 media. Blank experiments (wastewater without inoculums) were maintained in the same conditions. The experiments were carried out in triplicates. Table 1 represents the algae growth in different proportions of PRE in the BG11 Medium.

Analyses of samples were done on a daily basis to determine the microalgae growth and bioremediation efficiency in PRE. The optimized inoculum size of microalgae inoculated in a particular ratio of PRE and BG11 media was identified and further used in the biocathode MDC.

### 2.3. Biocathode MDC setup

The laboratory scale biocathode MDC made of acrylic sheets encompassed the anode, middle desalination and biocathode chambers. The anion exchange membrane is made of polystyrene cross-linked with divinyl benzene with quaternary ammonia separated the anode and desalination chamber. The cation exchange membrane made of polystyrene cross-linked with divinylbenzene with sulphonic acid separated the desalination chamber and biocathode. The highly conductive graphite rods were used in anode and cathode chambers to acquire and transfer the electrons across the external circuit. The volumetric ratio of biocathode MDC resulted in 1:0.5:1, after the insertion of graphite electrodes. The middle desalination chamber containing 35 g $L^{-1}$ of real time sea water, the anolyte concentration of 500 mg $L^{-1}$ and the external resistance of 100 Ω were used for the present study.

**Table 1**
Different ratio of PRE and BG11 medium used for algae growth.

| Concentration of PRE (Vol. %) | BG11 media (mL) | PRE (mL) | Inoculum (mL) |
|---|---|---|---|
| 0% | 95 | 0 | 5 |
| 10% | 85 | 10 | 5 |
| 30% | 65 | 30 | 5 |
| 50% | 45 | 50 | 5 |
| 70% | 25 | 70 | 5 |
| 100% | 0 | 95 | 5 |
| 0% | 90 | 0 | 10 |
| 10% | 80 | 10 | 10 |
| 30% | 60 | 30 | 10 |
| 50% | 40 | 50 | 10 |
| 70% | 20 | 70 | 10 |
| 100% | 0 | 90 | 10 |
| 0% | 85 | 0 | 15 |
| 10% | 75 | 10 | 15 |
| 30% | 55 | 30 | 15 |
| 50% | 35 | 50 | 15 |
| 70% | 15 | 70 | 15 |
| 100% | 0 | 85 | 15 |

### *2.4. Analysis of PRE and performance of desalination*

All the experiments were carried out at the ambient temperature of 30 ± 2 °C. The water analysis kit (Eutech Instruments, Cyber scan series 600) was used to measure the pH, electrical conductivity, Total Dissolved Solids content (TDS), NaCl content in the saline solution of biocathode MDC and treated PRE. The Chemical Oxygen Demand (COD) were analyzed based on the standard procedure of American Public Health Association (Greenberg et al., 1992). The suspended solids, the sulfide content and the phosphorous content in the treated PRE were measured by the spectrophotometer Hach 2800. The chlorophyll and the carotenoid estimation of algae grown in the PRE were carried out along with the growth estimation by measuring the optical density at 680 nm in a UV double beam spectrophotometer (Shimadzu, Japan) as suggested by Mackinney (1941). The total phosphorous content was measured using PhosVer® 3 phosphate reagent powder pillows with acid persulfate digestion method (USEPA Method 8190). The sulfide content was measured by Methylene Blue Method (USEPA Method 10254). The voltage across the 100 Ω resistor in biocathode MDC was measured periodically by the digital multimeter to determine the power density of the system. The biofilm activity was measured indirectly in terms of potential difference between the electrodes ie., continuous monitoring of voltage across the anode and cathode. The volume of the anode solution had eventually normalized the power density.

## 3. Results and discussion

### *3.1. Optimization of algae growth in PRE*

Different inoculums size of microalgae *Scenedesmus abundans* was selected for treatment of different concentrations of PRE samples. The differential activity of algae in the effluent can be identified by varying the inoculums size. The growth parameters such absorbance at 680 nm, chlorophyll content and carotenoid contents of biomass was measured on a daily basis. The physicochemical properties such as changes in pH, COD removal, reduction in phosphorous, sulfide content, suspended solids, Total Suspended Solids (TSS), NaCl content and DO concentration in the microalgae grown in different concentrations of PRE was also analyzed to identify the growth of algae and the nutrient uptake capacity. In the present study, the algae were allowed to grow for 26 days in the PRE in which 22 days was considered to be ideal growth period and the algae completely utilized the available nutrients. Beyond 22 days the growth declined and effluent color treated with *Scenedesmus abundans* turned to dark green.

#### *3.1.1. Growth rate and chlorophyll content of microalgae*

The applicability of microalgae in PRE treatment can bypass the need for the additional treatment processes. The microalgae *Scenedesmus abundans* had exhibited a marked tolerance to the different concentrations of PRE in comparison with its growth on BG11 media. The study also elucidated the photosynthetic activity of algae growing in PRE by estimating the chlorophyll and carotenoid content.

In the present study, different algal inoculums size in the variable concentration of PRE had undergone exponential growth accompanied by stationary growth and finally the cell death. It had been observed that the microalgae growth had shown the typical microorganism growth pattern, where algae growth increased with progression in time for different concentrations of PRE starting from 0 to 50%. Afterwards, 70 and 100% of PRE affected the growth of algae culture for a different inoculums size of microalgae. The same pattern was observed in the pigment concentrations. Besides, the

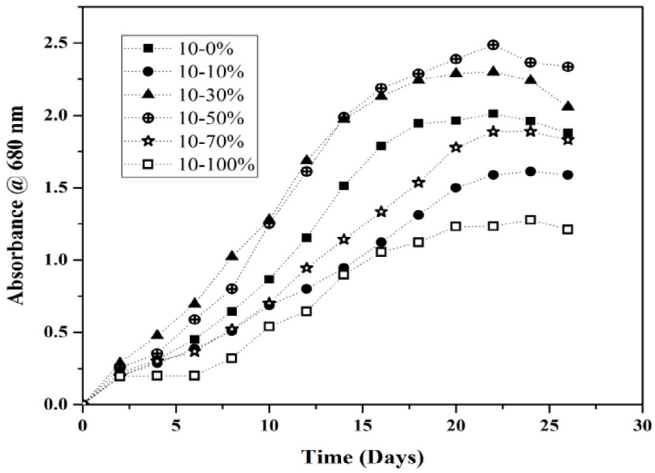



**Fig. 1.** Growth of microalgae in different concentrations of PRE.

decrease in pigments may also be due to the dilution of growth media present in various concentrations of PRE. The synthesis of carotenoids in green algae occurs by the acetate-mevalonate pathway and the phosphoglyceraldehyde-pyruvate pathway along with the components synthesized from isopentenyl diphosphate and its isomers (Cunningham, 2002). It was observed that the increase in pigment production in algae may be due to the presence of phosphorous, nitrogen and other chemical compositions in PRE, which aptly agrees with the findings of Khuantrairong and Traichaiyaporn (2011). It was reported that the phosphate levels have positively influenced the $\beta$ carotene, lutein and zeaxanthin in Cladophora sp.

In the presence of 5 mL inoculums size, the microalgae yielded a maximum absorbance of 1.932 in 30% concentration of PRE in which the salt concentration was reduced from 425 to 364 ppm. The absorbance values of other PRE concentrations such as 0, 10, 50, 70 and 100% were 1.461, 1.514, 1.73, 1.616, and 1.001 respectively. The maximum chlorophyll and carotenoid concentration for 5 mL in 30% concentration of PRE were 1.874 and 0.745 respectively. It was observed that the 10 mL inoculum size in different concentrations of PRE had quite shorter lag phase when compared to the other inoculums size of 5 and 15 mL. The shorter lag phase indicates the required amount of carbon dioxide generation by bacteria in the initial days of the growth period. Secondly, bacteria have helped in the degradation process of pollutants and nutrients in the wastewater and have transformed the complex substrates into small organic molecules that in turn can be used easily by algae (Zhang et al., 2012).

The 10 mL algae inoculum size showed greater adaptability and viability specifically on 50% concentration of PRE due to the presence of wide variety of chemical composition of PRE and had exhibited higher growth absorbance of 2.336. The absorbance values of microalgae grown in other PRE concentrations such as 0, 10, 50, 70 and 100% were 1.88, 1.587, 2.058, 1.83 and 1.211 respectively. Fig. 1 represents the growth rate of 10 mL inoculums size in different ratios of PRE. The 10 mL algal inoculums size cultivated in 50% of PRE had produced very high concentrations of chlorophyll and carotenoids, i.e., 2.78 $\mu g\ mL^{-1}$ of chlorophyll and 1.365 $\mu g\ mL^{-1}$ of carotenoid respectively. Fig. 2 represents the pigment levels of 10 mL microalgae inoculums size in different concentrations of PRE. The chemical composition in PRE had supported the additional growth of microalgae when it was combined with BG11 media.

It was observed that the 15 mL algal inoculums size showed variable and irregular growth pattern in different concentrations of PRE. The increase in algae concentration had caused the substrate limitation and a decrease in the algal activity after 21 days of cultivation. The maximum growth absorbance of 1.61 was seen in 50% of PRE concentration in the presence of 15 mL algal inoculums size. The salt concentration for 15 mL inoculums size in 50% PRE reduced from 510 to 437 ppm. The absorbance values of other PRE concentrations such as 0, 10, 50, 70 and 100% are 1.56, 1.2, 1.584, 1.51, and 1.084 respectively. The chlorophyll and the carotenoid concentration were 1.687 and 0.598 $\mu g\ mL^{-1}$. Similar to *Scenedesmus* sp. the growth of green algae *Chlorella vulgaris* has been seen successfully in various dilutions of PRE and has alleviated the contaminants to a greater extent (Madadi et al., 2016). Fig. 3 represents the experimental results of three inoculation volumes in respective PRE concentration.

The typical optimum temperature of 28 $\pm$ 2 °C, neutral pH, the hydrocarbons as the source of carbon, other PRE nutrients such as phosphorous and sulphides promoted the effective growth of *Scenedesmus abundans*. In the current study, the salt concentration in 10 mL of inoculums size in 50% of PRE concentration had been reduced from 532 to 325 ppm. It was due to the osmotic and cellular ionic stress in the cells of algae due to the selective ion permeability of the cell wall (Moheimani, 2005; Salama et al., 2014). Increase in the DO concentration throughout the experimental period of different inoculums size grown in PRE indicates the photosynthetic activity of algae over heterotrophic carbon-oxidation and nitrification reactions.

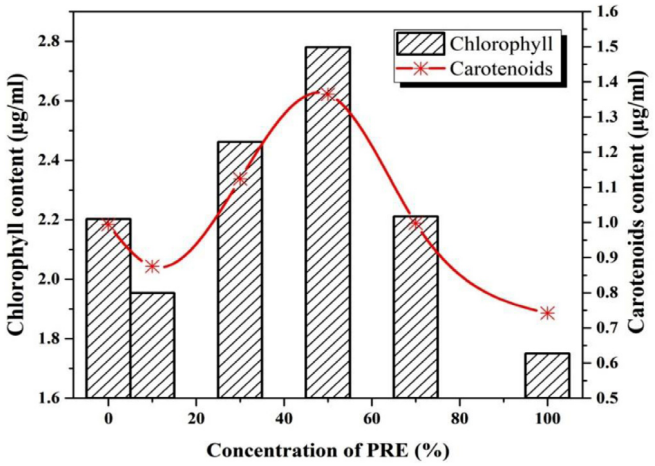



**Fig. 2.** Concentration of pigment in different concentrations of PRE.

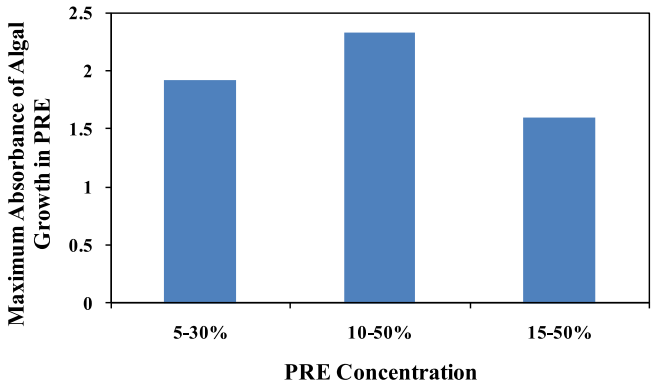



**Fig. 3.** Results of three inoculation volumes in respective PRE concentration.

**Table 2**
Physicochemical parameters in initial and final PRE.

| Parameters | Initial | Final |
|---|---|---|
| pH | 8.1 | 7.6 |
| COD (mg $L^{-1}$) | 610 | 164.7 |
| $BOD_5$ (mg $L^{-1}$) | 321 | 64.2 |
| TDS (mg $L^{-1}$) | 580 | 191.4 |
| TSS (mg $L^{-1}$) | 340 | 129.2 |
| Phosphorous (mg $L^{-1}$) | 1.7 | 0.544 |
| Sulphide (mg $L^{-1}$) | 15 | 5.85 |

### *3.1.2. Effect of scenedesmus abundans on nutrient removal*

Culturing the different inoculums size of microalgae in variable ratios of PRE had resulted in the removal of COD and other nutrients. All the physicochemical parameters such as pH, $BOD_5$, COD, phosphate, sulfate, TSS, salinity, TDS, conductivity change and DO was quantified regularly with a time gap of 5 days throughout the experimentation. In the present study, the nutrient removal percentages had directly influenced the growth of microalgae in PRE. Thus, 10 mL inoculums size in 50% of PRE concentration removed the pollutants effectively when compared to the other inoculums sizes grown in PRE. The initial and final levels of physicochemical parameters in 50% ratio of PRE are given in Table 2.

It is observed from Table 2 that the harmonious relationship along with the complementary relationship between the algae and the bacterial community has significantly reduced the COD levels in the PRE. Higgins et al. (2018) have explained that the organic carbon produced by the algal photosynthate has effectively increased the bacterial population and has initiated the algal–bacterial synergy in the treatment of winery wastewater treatment and in turn has reduced the soluble COD to a greater extent. Moreover, in the present study, the adaptation of bacterial consortium present in different ratios of PRE to the particular photosynthate secreted by microalgae *Scenedesmus abundans* could be the reason for the

reduction in COD levels. The best COD removal efficiencies among the inoculums size were 73% (10%–50% ratio of PRE), 51% (5%–30% ratio of PRE) and 42% (15%–50% ratio of PRE) respectively. The BOD level is the indicator of the biological organic breakdown of materials by consuming DO. The visibility of increase in DO concentration by algae throughout the experimental period has significantly enhanced the BOD removals when compared to the COD removal efficiencies. The BOD removal for 10 mL inoculum size treated in 50% of PRE is 80% whereas removal percentages for 5 mL inoculums size in 30% PRE and 15 mL inoculums size in 50% PRE were 63 and 55%, respectively. The rate of COD and BOD increased continuously and been maintained at a constant level until 21 days, after which a gradual decrease was observed until the end of the experimental period.

Phosphorous and sulfur are essential macronutrients that help in the critical metabolism of microalgae. Long-term deprivation in nutrient content can cause a self-degrading process called autophagy process followed by cell death (Cakmak et al., 2012). In the present study, consumption of phosphorous and sulfides from different ratios of PRE was observed along with the microalgae *Scenedesmus abundans* growth. Phosphorous has undergone biogeochemical cycle in algae and has been considered to be a critical macronutrient for its biomass production. Phosphorous forms a significant constituent of phospholipids in cell membrane and nucleic acid which acts as an intermediate for carbon metabolism in microalgae (Cai et al., 2013; Cunningham et al., 2010). The macronutrient also helps in conversion of adenosine diphosphate to adenosine triphosphate through phosphorylation mechanism (Martínez et al., 1999). Sulfur plays the pivotal roles in cell physiology of algal cells and maintains the overall metabolism of cells. The deprivation in sulphur impacts photosynthesis, and carbon, nitrogen metabolism. Different inoculums size had removed the phosphorous and the sulfides at different rates. 10 mL inoculums size in 50% ratio of PRE has effectively eliminated 68% of phosphorous and 61% of sulphide.

The general quality of PRE had been checked by measuring the TDS and TSS concentration. TDS represents the total composition of positive ions and negative ions present in the wastewater. The present study had revealed a relatively good negative correlation between TDS and DO content ($y = -17.546x + 583.54$; $R^2 = 0.9246$) that is in congruence with the study carried out by Muigai et al. (2002). Increase in DO levels by *Scenedesmus abundans* in PRE over a period also decreased the TDS and TSS concentration. Low DO levels in the initial stages of experiments were associated with high organic matter content (Belal, 2019). The essential contributing factor of biological treatment by algae is the analysis of DO concentration that reveals the difference between the untreated wastewater containing organic and inorganic matter and the treated effluent. The uniform increase in DO and TSS concentration reduction has been observed in all the inoculums size treated PRE. The 10 mL inoculums size in 50% of PRE ratio has been found to be more effective in decreasing the levels of the solid. The maximum reduction was observed in 22 days of operation, i.e. 67% reduction of TDS and 62% reduction of TSS.

The microalgae *Scenedesmus abundans* had readily decreased the salinity and conductivity of PRE to some extent. Different ranges of saline concentration in the variable ratios of PRE have also influenced the microalgae growth, i.e., increase in biomass content and removal of nutrients. The disturbance in the osmotic balance between the cells and surrounding environment led to the electrochemical gradient that has forced the water efflux and influx of inorganic ions into the cell thus leading to the decrease in salt content (Fisher et al., 1997; Kinraide, 1999). 10 mL inoculums size in 50% of PRE decreased 42% of salt concentration throughout the 26 days of experimental time.

### 3.2. Performance of biocathode MDC

Biocathode MDC utilizes innovatively sustainable microbial catalysts i.e. electro active bacteria and algal species (*Chlorella vulgaris* and *Scenedesmus abundans*) as biological cathodes to obtain efficient desalination process (Ashwaniy and Perumalsamy, 2017; Kokabian and Gude, 2013; Wen et al., 2012; Zamanpour et al., 2017). The desalination performance and bioelectricity generation of biocathode MDC was studied using optimized inoculums size of microalgae grown in optimized concentration of PRE. The ability of microalgae to act as an electron acceptor (MDC1) and as a bioresource for bacterial growth in MDC anode (MDC2) was analyzed.

#### 3.2.1. Desalination performance

The desalination performances of MDC1 and MDC2 were estimated through the change in electrical conductivity and NaCl removal. The whole operation is depicted in Fig. 4. In the initial days of the operation (0–15 days), MDC had enhanced the desalination process thereby removing more than 47% of salt from MDC1 and 45% from MDC2. A relative stable increase period followed by reduction efficiency period have constituted the total operation time of 23 days. The replenishment of substrate in the anode chamber has improved the desalination process significantly. In general, the PRE has lower electrical conductivity (1.056 mS $cm^{-1}$) when compared with the saline solution (46.01 mS $cm^{-1}$).

In MDC1, the variable difference in the anolyte (1.056 mS $cm^{-1}$), catholyte (1.265 mS $cm^{-1}$) and saline solution electrical conductivity and along with the effective synergetic interaction between the microalgae–bacterial communities in PRE of cathode chamber had created a high potential difference that in turn, had increased the passive movement of ions from the desalination chamber leading to the desalination process. The variation in pH of anolyte (decreased from 6.32 to 5.12) and catholyte (increased from 6.78 to 8.64) is due to the movement of sodium and chloride ions. Moreover, these changes also determine the removal efficiency and the hydraulic retention time of the system. A higher salinity removal efficiency of 62% was achieved using the microalgae grown in PRE in the cathode chamber compared to the

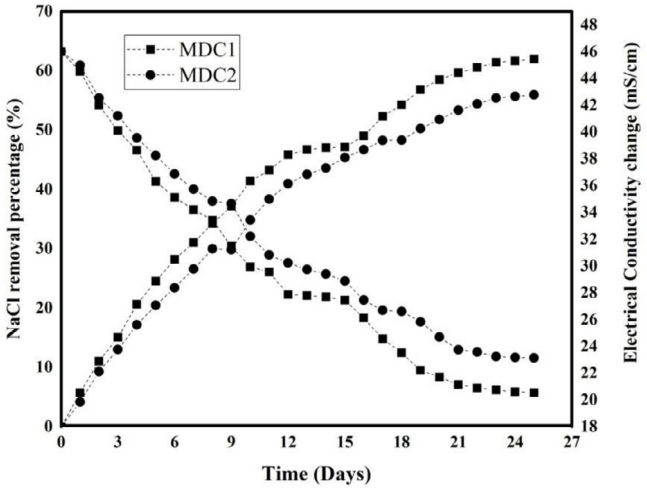



**Fig. 4.** Desalination performance of MDC1 and MDC2.

microalgae that has been grown only in BG11 media where the removal efficiency of 55% was obtained by Ashwaniy and Perumalsamy (2017). Live and dry algal biomass has been reported as a substrate for electricity generation in the microbial fuel cells (Xiao and He, 2014).

In MDC2, the potential difference created between the chambers was low when compared with MDC1. This change was due to the insufficient activity of microalgae as electron donor in the anode chamber thus producing 56% of salt removal efficiency. The initial and the final conductivities of the anolyte solution was 1.256 and 4.52 mS $cm^{-1}$ whereas for catholyte solution was 1.256 to 6.345 mS $cm^{-1}$. In MDC 2, the anolyte pH decreased from 6.54 to 5.28 and catholyte pH increased from 6.51 to 8.46.

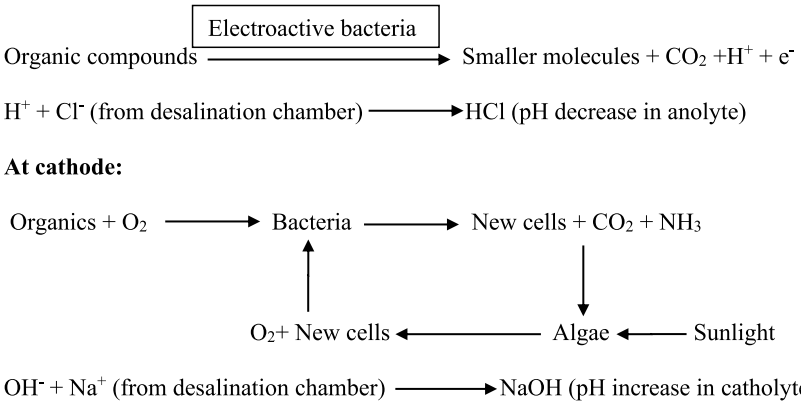
In both MDC1 and the MDC2, the higher salt concentration (i.e., 35 mg $L^{-1}$) had decreased the internal resistance of the system and had enhanced the salt removal rate. The water volume in the middle chamber increased from 150 to 170 mL in MDC1 and 150 to 165 mL in MDC2. The transport of water in the present system was due to the difference in the ion concentration between the middle chamber and the electrolytes present in the anode and cathode chamber. The same scenario has been reported by Kim and Logan (2013) wherein 15.4 mL (i.e., 18 to 33.4 mL) water volume rise has been observed in the middle desalination chamber. The desalination efficiency had decreased over a period due to the biofouling and the scaling of membranes.

*3.2.2. Anode substrate utilization and power generation*

The MDC1 and 2 showed the different pattern of COD removal and bioelectricity generation. The present study has utilized 500 mg $L^{-1}$ of initial anode concentration and a lower resistance of 100 Ω.

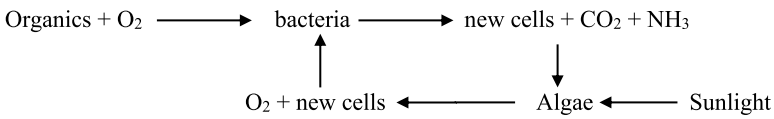
In MDC1, under anaerobic condition, the exoelectrogens present in the anode effectively oxidized the nutrient rich (phosphorous, sulphur, nitrogen, hydrocarbons, etc.) PRE and produced carbon dioxide, protons, electrons and other end products. The electrons were cascaded over solid graphite and had been transferred via external resistance to produce bioelectricity. Higher insitu oxygen generation by the photosynthetic activity of algae had decreased the lag phase duration and had increased the electron mobility leading to a higher power production. At the initial stage of operation, maximum voltage was generated with a sufficient organic matter supply. After 12 days of operation, the voltage had dropped to 50 mV and the power density had reduced to 0.042 W $m^{-3}$. Further, the replenishment of the anode substrate had increased the power density to 0.325 W $m^{-3}$. The substrate degradation percentage per batch of operation was as high as 69% in MDC2 when compared to the 63% degradation in MDC1. The complementary cycle between the bacterial and the algal community in the anaerobic environment of the anode chamber had actively participated in the consumption of organic matter of PRE thus, reducing the COD levels. The algae effectively acted as a substrate along with the PRE for the growth of bacteria in MDC2 and hence the increase in COD reduction levels was observed when compared with MDC1. However, the power production had decreased in MDC2, since most of the electrons produced were consumed by the algae to produce biomass and the oxygen for the bacterial community and only the remaining electrons had produced bioelectricity through the closed circuit. The maximum power density of 0.674 W $m^{-3}$ has been generated during the single batch operation of MDC1. The chloride ion migration to the anode chamber had affected both MDC1 and 2 by increasing the conductivity and decreasing the pH of the anolyte solution. The formation of hydrochloric acid by microbes had increased the acidophilic bacteria growth thus, affecting the COD removal efficiencies. This effect can be reduced by recirculation of electrolytes and thus wastewater pre-treatment can be done to improve the organic matter degradation in the anode chamber (Luo et al., 2011). Biochemical mechanisms of biocathode MDC in shown in Schemes 1 and 2.

**At anode:**




**Scheme 1.** Mechanism of MDC1.

**At anode and cathode:**




**Scheme 2.** Mechanism of MDC2.

*3.2.3. Algae growth in the cathode chamber*

In the present study, successful growth of algae was seen in the MDC1 compared to the MDC2 and the control (in BG11 media). The growth absorbance for MDC1, MDC2, and control for 23 days of operation was 2.845, 2.601 and 2.487 respectively. The bacteria decomposed the organic compounds under aerobic condition produces new cells, carbon dioxide and ammonia. The *Scenedesmus abundans* present in the cathode absorbed carbon dioxide and ammonia produced by bacteria for its cell multiplication and oxygen in the presence of light. The cycle repeated continuously until the end of experimental period. Generally, the carbon dioxide rich environment and incomplete metabolites produced by bacteria favors the growth of algae whereas the bacteria consume the vitamins and growth factors released by algal cells (Humenik and Hanna, 1971).

The results obtained indicates that the transfer of sodium ions across the cation exchange membrane has the influential role in algal growth rate in MDC1 and MDC2 rather than the control samples in which the interaction of sodium ions is negligible (Brownell, 1980). The sodium ion is essential in the carbon and nitrogen assimilation process of autotrophic microorganisms to maintain the nature of viable cells under the alkaline conditions. Sodium ions also act as the primary coupling ions in the cellular energy transformation processes by the generation of trans-membrane potential difference (Skulachev et al., 2013). The increased transfer of electrons from anode via the external circuit along with the migration of sodium ions in MDC1 significantly increased the growth of microalgae in the cathode compartment rather than in MDC2. Thus the microalgae growth is interdependent on the movement of ions due to the potential difference across the compartments and the effective transfer of electrons from the anode chamber along with the external environmental conditions.

*3.2.4. Energy production in biocathode MDC*

The net energy output determination in bioelectrochemical systems can be expressed regarding the power density or the current density and thus it helps in MDC scale up studies. The overall energy production rate, i.e., the total energy production and consumption can be determined with the help of the electrode area or volume of the electrolyte.

In the present study, the energy profile of the experimental biocathode MDC had been compared with the theoretical energy values of the reverse osmosis process as shown in Fig. 5. In general, it requires 3–5 kWh $m^{-3}$ for desalination of seawater (Shannon et al., 2008). Thus, to desalinate 150 mL of seawater, the reverse osmosis process requires an average energy input of 0.6 kWh $m^{-3}$.

In biocathode MDC, the energy input can be expressed regarding the light intensity. Thus, the biocathode MDC requires 0.0006354 kWh $m^{-3}$ of energy consumption via light intensity for the complete operation. The total energy produced has been calculated by the total desalinated water volume and per kg of COD removal. In theory, the conventional anaerobic wastewater treatment requires 1.2 kWh $kg^{-1}$ COD removal as the energy input. The current biocathode study

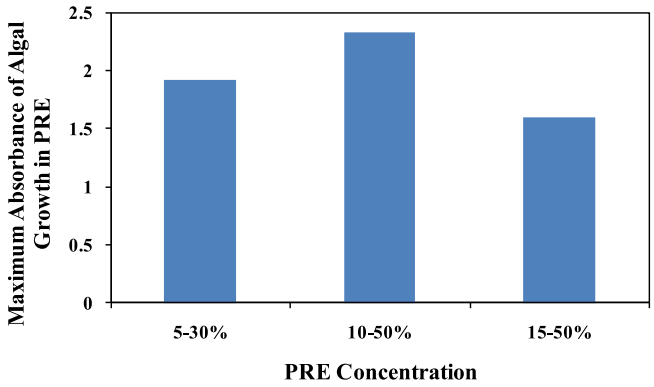



**Fig. 5.** Comparison of energy required for reverse osmosis process and bio-cathode MDC.

has produced the highest energy output of 0.40026 kWh $kg^{-1}$ COD across 100 Ω resistance. Thus, 33.3% of energy could be saved by biocathode MDC process compared to the conventional wastewater treatment process. The highest power output of 0.505 kWh $m^{-3}$ has been achieved for the total desalinated water volume. On an average, the net energy output of the present study reveals that the biocathode MDC can save 0.6455 kWh $m^{-3}$ of power. As a whole, biocathode MDC can save 1.245 kWh $m^{-3}$ of power in addition with the energy spent by reverse osmosis to treat 150 mL of sea water. The energy benefits could be even higher when the microalgae energy content in terms of dry weight (5–8 kWh $kg^{-1}$) was considered in the form of biofuels.

## 4. Conclusion

In the present work, the microalgae cultivation of different inoculums size on different ratios of PRE was studied along with the nutrient removal efficiencies. High growth rate and nutrient depletion were seen in 10 mL inoculums size grown in 50% ratio of PRE. The findings of the present study showed the reduction of 70% COD, 81% BOD, 67% phosphorous, 61% sulfide, 67% TDS and 62% TSS which in turn, demonstrated the beneficial use of photosynthetic microorganisms in the wastewater treatment. The higher desalination performance and power density of MDC1 (algae grown in PRE as electron acceptor) were observed compared to MDC2 (algae grown in PRE acted as substrate for bacterial growth). Thus, the proposed system of utilizing algae cultured in PRE as biocathode can increase the production of clean energy, biomass cultivation and can replace the energy intensive nutrient removal processes. The study also proved that the biocathode MDC was energy efficient in comparison with the commercial reverse osmosis process.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

Abinandan, S., Bhattacharya, R., Shanthakumar, S., 2015. Efficacy of chlorella pyrenoidosa and scenedesmus abundans for nutrient removal in rice mill effluent (Paddy soaked water). Int. J. Phytoremediat. 17, 377–381.
Allen, M.M., Stanier, R.Y., 1968. Growth and division of some unicellular blue–green algae. J. Gen. Microbiol. 51, 199–202.
Ashwaniy, V.R.V., Perumalsamy, M., 2017. Reduction of organic compounds in petro-chemical industry effluent and desalination using Scenedesmus abundans algal microbial desalination cell. Biochem. Pharmacol. 5, 5961–5967.
Belal, A.A.M., 2019. Spatial and temporal changes in the population of macro-benthic invertebrates exposed to oil spillage in Suez Bay, Red Sea, Egypt. Egypt. J. Aquat. Res. 45, 353–358.
Benyahia, F., Abdulkarim, M., Embaby, A., Rao, M., 2006. Refinery wastewater treatment : a true technological challenge. In: Seventh Annual U.A.E. University Research Conference. U.A.E. University Research Conference, Al Ain, UAE, pp. 1–8.
Brownell, P.F., 1980. Sodium as an essential micronutrient element for plants and its possible role in metabolism. Adv. Bot. Res. 7, 117–224.
Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew. Sustain. Energy Rev. 19, 360–369.
Cakmak, T., Angun, P., Ozkan, A.D., Cakmak, Z., Olmez, T.T., Tekinay, T., 2012. Nitrogen and sulfur deprivation differentiate lipid accumulation targets of Chlamydomonas reinhardtii. Bioengineered 3, 343–346.
Cao, X., Huang, X., Liang, P., Xiao, K., Zhou, Y., Zhang, X., Logan, B.E., 2009. A new method for water desalination using microbial desalination cells. Environ. Sci. Technol. 43, 7148–7152.
Cunningham, F.X., 2002. Regulation of carotenoid synthesis and accumulation in plants. Pure Appl. Chem. 74, 1409–1417.
Cunningham, M., Heim, C., Rauchenwald, V., 2010. Algae Production in Wastewater Treatment: Prospects for Ballen. Ballen.
Diyauddeen, H.B., Daud, W.M.A.W., Aziz, A.R.A., 2010. Treatment technologies for petroleum refinery effluents : A review. Process Saf. Environ. Prot. 9, 95–105.

Fisher, M., Gokhman, I., Pick, U., Zamir, A., 1997. A structurally novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga Dunaliella salina grown in high salinities. J. Biol. Chem. 272, 1565–1570.
Freeman, H.M., 1995. Industrial Pollution Prevention Handbook, first ed. McGraw Hill. Inc., New York, USA.
Greenberg, A.E., Clesceri, L.S., Eato, A.D. (Eds.), 1992. Standard Methods for the Examination of Water and Wastewater, eighteenth ed. American Public Health Association, Washington, USA.
Higgins, B.T., Gennity, I., Fitzgerald, P.S., Ceballos, S.J., Fiehn, O., VanderGheynst, J.S., 2018. Algal–bacterial synergy in treatment of winery wastewater. npj Clean Water 1, 1–10.
Humenik, F.J., Hanna, G.P., 1971. Algal–bacterial symbiosis for removal and conservation of wastewater nutrients. Water Pollut. Control Fed. 43, 580–594.
Khuantrairong, T., Traichaiyaporn, S., 2011. The nutritional value of edible freshwater alga Cladophora sp. (Chlorophyta) grown under different phosphorus concentrations. Int. J. Agric. Biol. 13, 297–300.
Kim, Y., Logan, B.E., 2013. Simultaneous removal of organic matter and salt ions from saline wastewater in bioelectrochemical systems. Desalination 308, 115–121.
Kinraide, T.B., 1999. Interactions among Ca2+, Na+ and K+ in salinity toxicity: Quantitative resolution of multiple toxic and ameliorative effects. J. Exp. Bot. 50, 1495–1505.
Kokabian, B., Gude, V.G., 2013. Photosynthetic microbial desalination cells (PMDCs) for clean energy, water and biomass production. Environ. Sci. Process. Impacts 2178–2185.
Lekshmi, B., Joseph, R.S., Jose, A., Abinandan, S., Shanthakumar, S., 2015. Studies on reduction of inorganic pollutants from wastewater by Chlorella pyrenoidosa and scenedesmus abundans. Alexandria Eng. J. 54, 1291–1296.
Luo, H., Jenkins, P.E., Ren, Z., 2011. Concurrent desalination and hydrogen generation using microbial electrolysis and desalination cells. Environ. Sci. Technol. 45, 340–344.
Mackinney, G., 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140, 315–322.
Madadi, R., Pourbabaee, A.A., Tabatabaei, M., Zahed, M.A., Naghavi, M.R., 2016. Treatment of petrochemical wastewater by the green algae chlorella vulgaris. Int. J. Environ. Res. 10, 555–560.
Martínez, M.E., Jiménez, J.M., El Yousfi, F., 1999. Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga Scenedesmus obliquus. Bioresour. Technol. 67, 233–240.
Mata, Y.N., Blázquez, M.L., Ballester, A., González, F., Munoz, J.A., 2009. Biosorption of cadmium, lead and copper with calcium alginate xerogels and immobilized Fucus vesiculosus. J. Hazard. Mater. 163, 555–562.
Moheimani, N.R., 2005. The Culture of Coccolithophorid Algae for Carbon Dioxide Bioremediation. Murdoch University.
Muigai, P., Shiundu, P.M., Mwaura, F.B., Kamau, G.N., 2002. Correlation between dissolved oxygen and total dissolved solids and their role in the eutrophication of Nairobi Dam, Kenya. Int. J. Biochem. 5, 18–37.
Noue, J.D.L., Pauw, N.D., 1988. The potential of microalgal biotechnology. a review of production and uses of microalgae. Biotechnol. Adv. 6, 725–770.
Salama, R., Sadaie, M., Hoare, M., Narita, M., 2014. Cellular senescence and its effector programs. Genes Dev. 28, 99–114.
Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marĩas, B.J., Mayes, A.M., 2008. Science and technology for water purification in the coming decades. Nature 452, 301–310. http://dx.doi.org/10.1038/nature06599.
Sivakumar, T., Kumar, P.S., 2017. Treatment of municipal wastewater using scenedesmus abundans and studies on saccharification of grown biomass using ultrasound assistance. Desalination Water Treat. 89, 94–100.
Skulachev, V.P., Bogachev, A.V., Kasparinsky, F.O., 2013. Principles of Bioenergetics, first ed. Sprringer-Verlag, Berlin, Germany.
Talebi, A.F., Dastgheib, S.M.M., Tirandaz, H., Tirandaz, H., Alaie, E., Tabatabaei, M., 2016. Enhanced algal-based treatment of petroleum produced water and biodiesel production. RSC Adv. 6, 725–770.
Terry, P.A., Stone, W., 2002. Biosorption of cadmium and copper contaminated water by Scenedesmus abundans. Chemosphere 47, 249–255.
Wen, Q., Zhang, H., Chen, Z., Li, Y., Nan, J., Feng, Y., 2012. Using bacterial catalyst in the cathode of microbial desalination cell to improve wastewater treatment and desalination. Bioresour. Technol. 125, 108–113.
Xiao, L., He, Z., 2014. Applications and perspectives of phototrophic microorganisms for electricity generation from organic compounds in microbial fuel cells. Renew. Sustain. Energy Rev. 37, 550–559.
Zamanpour, M., Kariminia, H., Vosoughi, M., 2017. Electricity generation, desalination and microalgae cultivation in a biocathode-microbial desalination cell. J. Environ. Chem. Eng. 5, 843–848.
Zhang, Y., Su, H., Zhong, Y., Zhang, C., Shen, Z., Sang, W., Yan, G., Zhou, X., 2012. The effect of bacterial contamination on the heterotrophic cultivation of Chlorella pyrenoidosa in wastewater from the production of soybean products. Water Res. 46, 5509–5516.
Zuo, K., Chen, M., Liu, F., Xiao, K., Zuo, J., Cao, X., Zhang, X., Liang, P., Huang, X., 2018. Coupling micro filtration membrane with biocathode microbial desalination cell enhances advanced purification and long-term stability for treatment of domestic wastewater. J. Memb. Sci. 547, 34–42.