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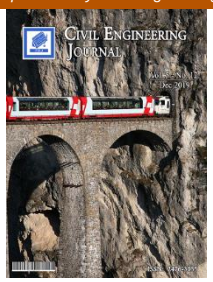

Photosynthetic Microbial Desalination Cell to Treat Oily Wastewater Using Microalgae *Chlorella Vulgaris*

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Abstract

Microbial desalination cell (MDC) offers a new and sustainable approach to desalinate saltwater by directly utilizing the electrical power generated by bacteria during organic matter oxidation. In this study, we used microalgae *Chlorella Vulgaris* in the cathode chamber to produce oxygen as an electron accepter by photosynthesis process for generate bioelectricity power and treat oil refinery wastewater by microorganisms in both anode and cathode.

The power density generated by this Photosynthetic Microbial Desalination Cell (PMDC) with 1KΩ external resistance at the first 4th hr. of operation period was 0.678 W/m^3 of anode volume and 0.63 W/m^3 of cathode volume. It increased after one day to a peak value of (4.32 W/m^3 of anode volume and 4.013 W/m^3 of cathode volume). The microalgae growth in the biocathode chamber followed in terms of optical density. The optical density increased from 0.546 at the beginning of the system operation to 1.71 after 24 days of operation period. The percentage removal of chemical oxygen demand (COD) of oil refinery wastewater was 97.33% and 79.22% in anode and cathode chamber, respectively. The microalgae in the biocathode were able to remove volatile compounds causing odor from the influent wastewater. TDS removal rate 159.722 ppm/h with initial TDS in desalination chamber of 35000 ppm.

Keywords: PMDC; Oil Refinery; *Chlorella Vulgaris.*

1. Introduction

Industrial wastewater generated from the oil industry generally characterized by its high concentration of pollutants such as organic compounds, heavy metals, and chemicals, which may cause adverse public health and environmental problems [1]. Conventional techniques (chemical precipitation, membrane filtration, electrolytic processes, and adsorption) have widely used for the treatment of such wastewater. However, these techniques present many disadvantages, such as high cost, intensive energy requirements, and considerable sludge generation [2]. Moreover, wastewater treatment and reuse have become an essential issue with the increasing population and depletion of freshwater resources in many regions of the world.

Microbial fuel cell (MFC) is a promising technology that has obtained a significant interest in recent years. This technology offers the possibility of treating a wide range of wastewaters with soluble organic pollutants and gaining electrical current simultaneously [3]. MFC technology based on the electrogenic nature of specific bacteria that use electrode (anode) as an electron acceptor instead of dissolved oxygen while treating wastewater anaerobically. The electron transferred to the cathode through an external electric circuit at which the reduction reaction occurs [4].

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Microbial desalination cell (MDC), consists typically of an anode, desalination and cathode chambers, is an amendment of MFC which featured with wastewater treatment, desalination (brackish water and seawater), and bioelectricity production simultaneously [5]. However, MFC and MDC also suffer from low power densities due to power losses in electron transfer and release mechanisms, especially in the cathode chamber. To the performance of the MFC improves, cathodes often painted with noble catalysts such as platinum in case of air-cathode MDC with external aeration provided or using chemical agents such as ferricyanide to achieve faster redox kinetics [6-9]. High cost and toxicity problems when using noble catalysts and chemical electrolytes. So, to avoid that, biocathodes can be used as an alternative to abiotic cathodes [10-14]. Another advantage of using different biocathodes is that the active microbial metabolism can be used to produce beneficial products [15] or eliminate nutrients from wastewaters, such as nitrate and contaminants such as heavy metals [16, 17]. Different microbial populations can be used as biocatalysts in biocathodes such as nitrifying and denitrifying bacteria or microalgae to produce electron acceptors required for reduction reaction at the cathode [18, 19]. Microalgae *Chlorella Vulgaris* was utilized by many studies in biocathode microbial desalination cells to generate oxygen (electron acceptor) by the photosynthetic process. For example, Bahareh and Veera [10] used microalgae *C. Vulgaris* in biocathode photosynthetic microbial desalination cell (PMDC) for clean energy, water, and biomass production. Thomas and Veera [20] used sodium bicarbonate as an inorganic carbon source for microalgae *C. Vulgaris* in biocathode PMDC and studied its effects on the system performance. In addition to oxygen generation microalgae such as *Chlorella sp.* can achieve elimination nitrogen, phosphorus, CO_2, and toxic metals from various types of wastewaters, making them an attractive alternative for wastewater treatment [21, 22].

Many studies investigated the ability of microalgae *Chlorella Vulgaris* to treat different types of wastewater. For example, Madadi et al. [23] studied the biological treatment of petrochemical wastewaters by microalgae *Chlorella Vulgaris*. The results of this study showed that a combination of surfactants and *Chlorella Vulgaris* is an efficient approach for pre-treatment of wastewaters and can use for the removal of nutrients from petrochemical wastewaters. Microalgae biomass harvesting from microalgae bioreactor treating textile wastewater studied by Hala and Laila [24]. This work revealed the adaptability of the microalgae *C. Vulgaris* in textile wastewater and its ability to mitigate the waste effluent by elimination color and Chemical Oxygen Demand (COD) and the ability of the bioreactor to produce biomass. Liang Wang et al. [25] studied the cultivation of green algae *Chlorella sp.* in various wastewaters from municipal wastewater treatment plants and their ability to remove nitrogen, phosphorus, COD, and heavy metals from the wastewaters. Luz T. et al. worked to improve a procedure for biological treatment of wastewater from ethanol and citric acid production industry using the microalga *Chlorella Vulgaris* and the macrophyte *Lemna minuscule* [26]. Biocathode microbial fuel cell was used by Ronald et al. [27] for biotreatment of oils and fats from wastewater of a chocolate factory in *Chlorella Vulgaris* microalgal cathode chamber and removal of blue dye brl in the anode chamber of the MFC by the bacterial community.

This study aimed 1st to use microalgae *C. Vulgaris* in the cathode chamber as an oxygen generator and 2nd to treat oil refinery wastewater in both anode and cathode chambers of a PMDC and to investigate its performance in terms of wastewater treatment, salty water desalination, and bioelectricity generation. PMDC performance was evaluated in terms of COD removal percent, desalination rate, and electricity production. The maximum power densities profiles and desalination rates were derived from the experimental results.

2. Materials and Methods

2.1. PMDC Configuration

The PMDC used in this work consisted of three chambers (anode, desalination, and cathode) of plexiglass with an internal cross-section area 10x10 cm and length of 7, 3, 7 cm for the anode, desalination, and cathode chamber, respectively as described in (Figure 1-A). Cation exchange membrane (CEM, CMI 7000, Membranes international) separated the cathode and the desalination chambers while an anion exchange membrane (AEM, AMI 7001, Membranes international) separated the anode and the desalination chambers. Both membranes were preconditioned by submerging in a 5% NaCl solution for 24 h and rinsed with deionized water before use, to allow for membrane hydration and expansion as recommended by the supplier. Graphite plate electrodes were used in anode and cathode chambers with dimensions 9x9 cm and thickness 2mm. The electrodes were installed at a distance of 1 cm from the respective ion exchange membranes. Before use, the electrodes were drenched in deionized water for a period of 24 hr. The copper wires were used to establish contact between the electrodes, and these contacts were sealed with epoxy.

Each of the three chambers has two ports (inlet and outlet) with 1cm diameter except the inlet port in the cathode chamber with 3cm diameter suitable for dissolved oxygen (DO) measurement by a portable meter. The anode chamber was closed with a screw to ensure an anaerobic microenvironment. The working volume of anode, desalination, and cathode chambers were 650, 300, 700 mL, respectively. Illumination on the algae cathode chamber was provided by white light at 23W. The PMDC illustrated in (Figure 1-B)

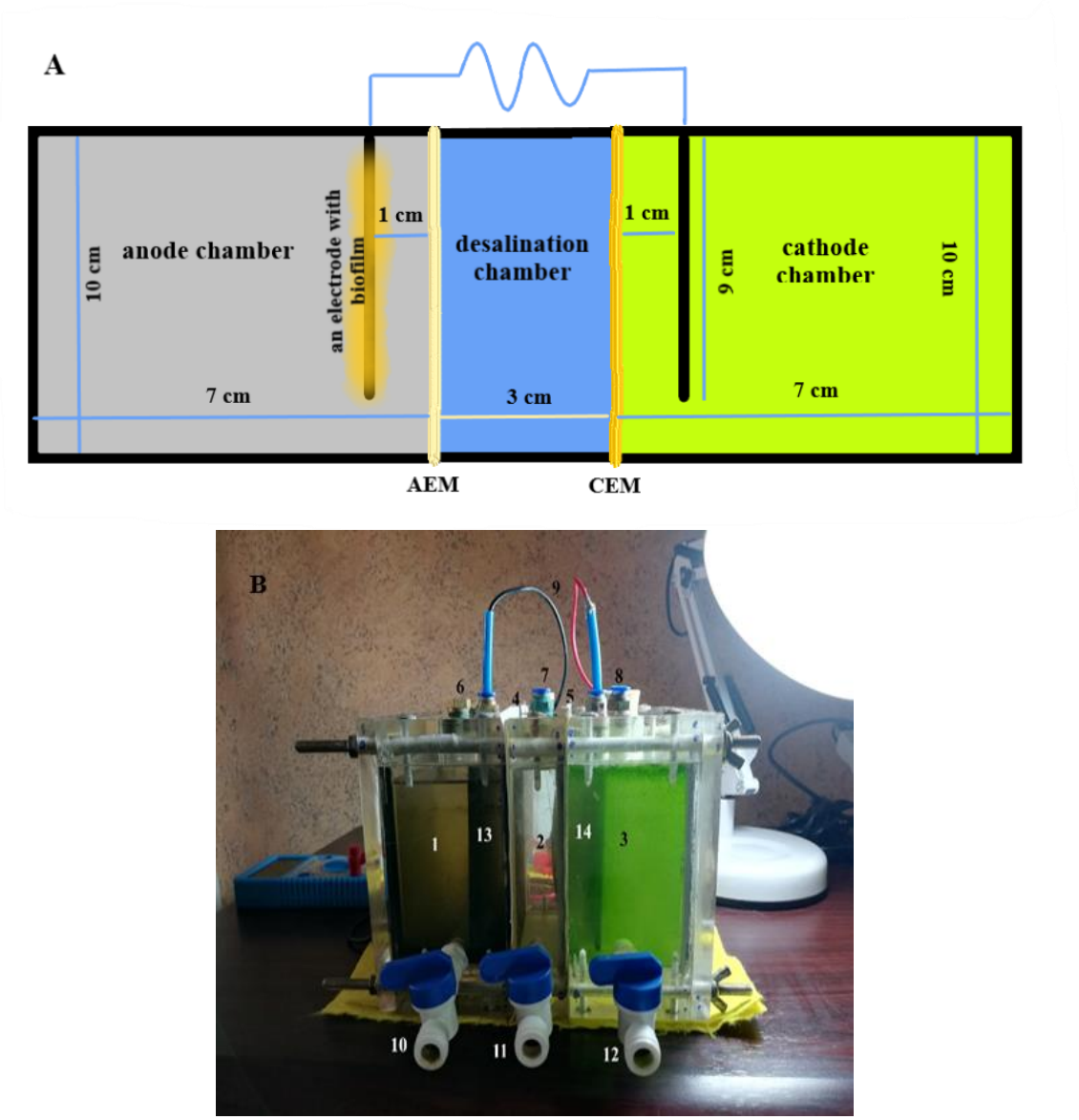


Figure 1. A- Schematic diagram of PMDC and B- PMDC configuration (1. Anode chamber, 2. Desalination chamber, 3. Cathode chamber, 4, 5. The membranes (AEM and CEM, respectively), 6, 7, 8. The inlet ports, 9. Copper wires, 10, 11, 12. The outlet ports, and 13, 14. The electrodes).

2.2. Inoculation of microorganisms in PMDC

The experimental work in this study comprised of three stages, 1st and 2nd stages for two purposes:

1. Acclamation PMDC (biofilm creation on electrodes from anaerobic bacteria in anode and microalgae C. Vulgaris in cathode)
2. Test the ability of system for cultivation biomass of microalgae.

In the 3rd stage, the PMDC used to treat oil refinery wastewater in both anode and cathode, all three stages illustrated in (Figure 3.). So, 1st and 2nd stages have the flowing:

1. Microbial consortium

The microbial consortium in the anode chamber was collected from the activated sludge basin of the wastewater treatment plant of Al-Doura refinery in Baghdad city. The sludge was allowed to acclimatize to anaerobic conditions in synthetic wastewater containing 500 mg/L of COD for 24 days. The acclimation process is done in the MDC to enhance the formation of the biofilm layer on the anode. The synthetic wastewater was consisted of : 500 (mg/l) of sodium acetate in buffer solution of KH_2PO_4 (40 mg/l), NH_4Cl (200 mg/l), $CaCl_2$ (40 mg/l), $MgCl_2$ (40 mg/l), KCl (40 mg/l) and 10 ml of trace mineral solution.

2. Microalgae *C. Vulgaris*

The microalgae Chlorella Vulgaris (Suncoast marine aquaculture, SCMAlabs.com) used in the cathode compartment was examined by microscope (Figure 2.) and was grown in the following mineral solution for 24 days to cultivation algae and form biofilm in cathode chamber: CaCl2 (0.025 g/l), NaCl (0.025 g/l), $NaNO_3$ (0.25 g/l), $MgSO_4$ (0.075 g/l), KH_2PO_4 (0.105 g/l), K_2 $HPO_4.3H_2O$ (0.075 g/l), and 3 ml of trace metal solution with the following concentration was added to 1000 mL of the above solution: $FeCl_3$ (0.194 g/l), $MnCl_2$ (0.082 g/l), $CoCl_2$ (0.16 g/l), $Na_2MoO_4.2H_2O$ (0.008 g/l), and $ZnCl_2$ (0.005 g/l) [11] with buffer solution : KH_2PO_4 (2.25 g/l), K_2HPO_4 .$3H_2O$ (2 g/l), NH_4Cl (0.31 g/l) and KCl (0.13 g/l) and $NaHCO_3$ (500 mg/l) as an inorganic CO_2 source [20].

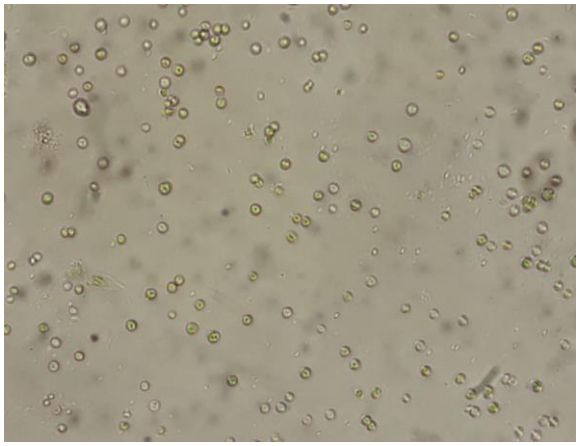


Figure 2. Micrograph of microalgae C. Vulgaris using an optical microscope

3. Saltwater

Saltwater in the medial chamber had a different concentration of NaCl (15 g/l) for the 1st stage and (35 g/l) for the 2nd stage to investigate different behaviours of PMDC with varying concentrations of salt.

After 1st and 2nd stages (acclimation period), 3rd stage started (application period). In this stage, actual wastewater treated in both anode and cathode chamber. The actual wastewater was the effluent of dissolved air flotation (DAF) unit in the wastewater treatment plant of Al-Doura refinery in Baghdad city with COD (75 mg/l) and oil content of (28.1 mg/l). The wastewater was added to anode and cathode at the same time. In the cathode chamber, wastewater was mixed with 10% of algae solution. Salt concentration in the desalination chamber for this stage was (35 g/l).

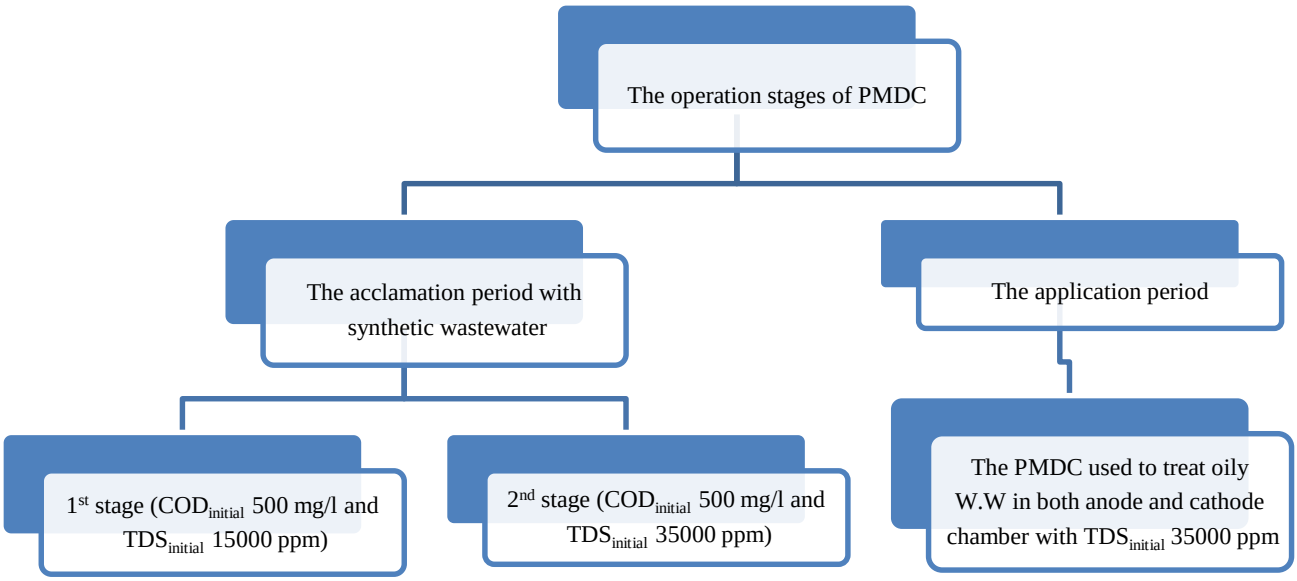


Figure 3. Flow chart of the operation stages of PMDC

2.3. Analyses and Calculations

The voltage was recorded using a digital multimeter (Aswar, DT860D) with a 500 Ω external resistor at the acclimation period (1st and 2nd stages) and 1KΩ at the application period (3rd stage) connecting the anode and the cathode was used in closed circuit tests. The current was determined using Ohm's law, I = Voltage/R. The power density was

calculated as per the anode/cathode chamber volume or the electrode surface area. Coulombic efficiency (CE), defined as the partial recovery of electrons from the substrate, was calculated by using Equation 1 [28]:

$$CE=\frac{M.\int_0^t I\,dt}{n.F.V_a.(COD_0-COD_t)}\times 100 \quad (1)$$

Where (M) is the molecular weight of oxygen, (I) is the current, (F) is Faraday's constant, n = 4 is the number of electrons exchanged per mole of oxygen, and V_a is the anolyte volume. COD_o represents the influent wastewater chemical oxygen demand to the anode chamber, and COD_t is the COD value after the time (t).

After attaining steady-state operation, polarization curves were obtained by changing the external resistance from 10 kΩ to 100 Ω in steps (about 15 min for each step to reach a steady-state). COD tests were executed using standard methods [29]. Electrical conductivity, TDS removal, pH of the samples and DO in the cathode chamber were recorded using a conductivity meter, pH meter and dissolve oxygen meter with temperature meter by the same multimeter (Lovi Bond Senso Direct 150), respectively. The desalination rate was calculated using Equation 2:

$$TDS_{removal\ rate}(ppm/h)=\left(\frac{C_0-C_t}{t}\right) \quad (2)$$

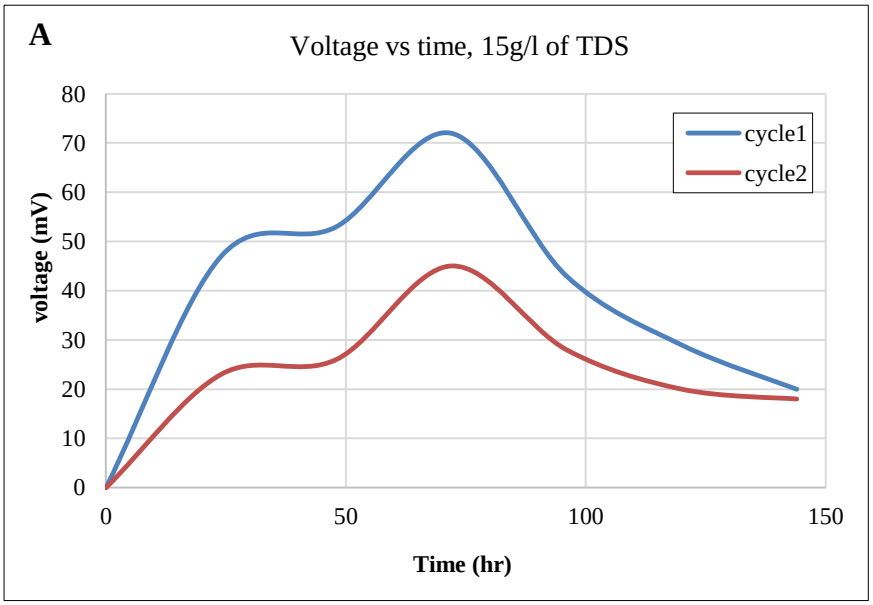
Where, C_0 and C_t are the initial and the final TDS of saltwater in the desalination chamber over a batch cycle of time t. Microalgae growth was observed by measuring the optical density of the microalgae suspension with a UV 2300 spectrophotometer (Techcomp, Spain) at a wavelength of 620 nm [10].

3. Results and Discussion

3.1. Power Production

The voltage generation between anode and cathode in the 1st and 2nd stages with two operation cycles to every stage were illustrated in Figure 4. (A and B). At the beginning of each cycle in the 1st stage the anode chamber was fed with influent wastewater with 500 mg/l of COD, the desalination chamber was filled with salty water with 15 g/l of TDS, and the cathode chamber was fed with microalgae. The absorbance for microalgae suspension was 0.546. While, 500 mg/l of COD, 35 g/l of TDS was used at the beginning of the 2nd stage. The absorbance for microalgae suspension at the beginning of the 2nd stage (resulted from the 1st stage) was 1.061. No additional algae suspension was added. It was clear that the maximum voltage difference across the external 500Ω electrical resistance between anode and cathode was 72 mV and 45 mV (1st stage), and 127 mV and 83 mV (2nd stage) for cycles 1 and 2, respectively; during six days for each cycle. The maximum voltage with open circuit mode varied between 581 mV and 568 mV (1st stage) and 706 mV and 597 mV (2nd stage) for cycles 1 and 2, respectively.

It should be noted that the electricity generation activity has decreased in the second cycle for the two stages which might be attributed to the decreasing of conductivity in the middle chamber, so the ions transferring between compartments has declined and decreased in power generation activity. At the same time, the electricity generation activity has improved in the 2nd stage comparable with that in the 1st stage, as evidenced by the values of maximum voltage as shown in (Figure 4). That is due to the biofilm formation on the electrode in anode chamber and increase in biomass of algae in cathode chamber (increasing in oxygen generation) in addition to the increase of conductivity in the middle chamber that improved ion exchange process between compartments and rising power generation activity.



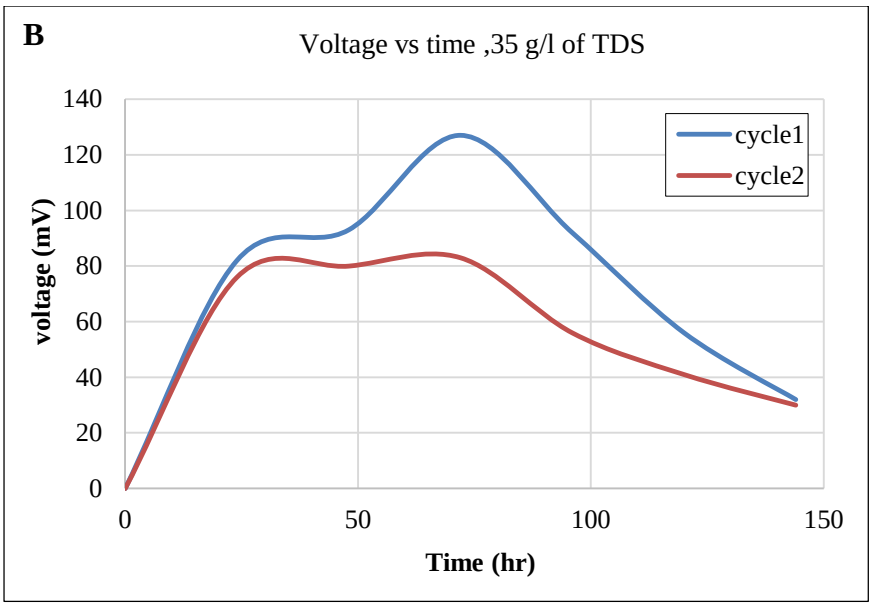


Figure 4. A) Voltage generation in 1st stage and B) voltage generation in 2nd stage

3.2. Microalgal Growth of *C. Vulgaris* on the Cathode

The growth of microalgae *C. Vulgaris* monitored by measuring the optical density at the beginning of the acclimation period, at the end of the 1st stage (with 15g/l of TDS) and the end of the 2nd stage (with 35g/l of TDS). The optical density was 0.546, 1.061 and 1.71 at the beginning, end of the 1st stage, and end of 2nd stage, respectively. These values are shown in (Figure 5.). The growth of algae in this study was higher than that in the previous study [10] the algae growth presented as optical density increased from 0.401 to 0.63 due to the difference of catholyte volume and in this study was used sodium bicarbonate as an inorganic carbon source that enhanced algae growth. That meaning this system was active in the cultivation of algae that can be used as biofuel.

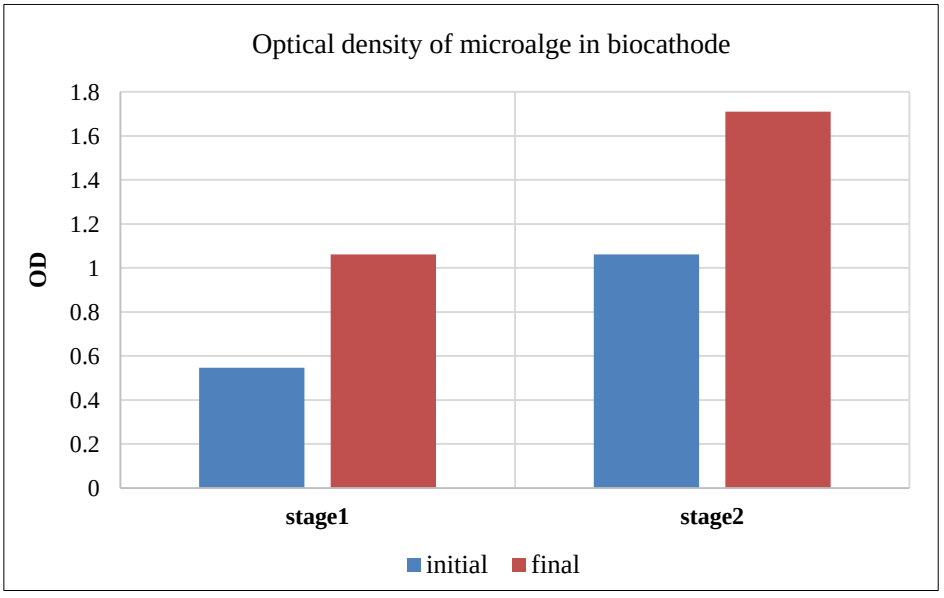


Figure 5. Optical density values of microalgae on biocathode

3.3. Polarization Analysis

After the acclimation period, the PMDC was used to treat oil refinery wastewater in both anode and cathode compartment. After many cycles of operation in this mode, the power production from actual wastewater treatment was illustrated in the polarization curve shown in (Figure 6). The operation conditions were as follows: 75 mg/l of COD (anode chamber), 35 g/l of TDS (desalination chamber), 10% for microalgae suspension to wastewater in biocathode chamber, light is on the maximum power density was 277.8 mW/m^2 with maximum current density of 18.52 mA/m^2.

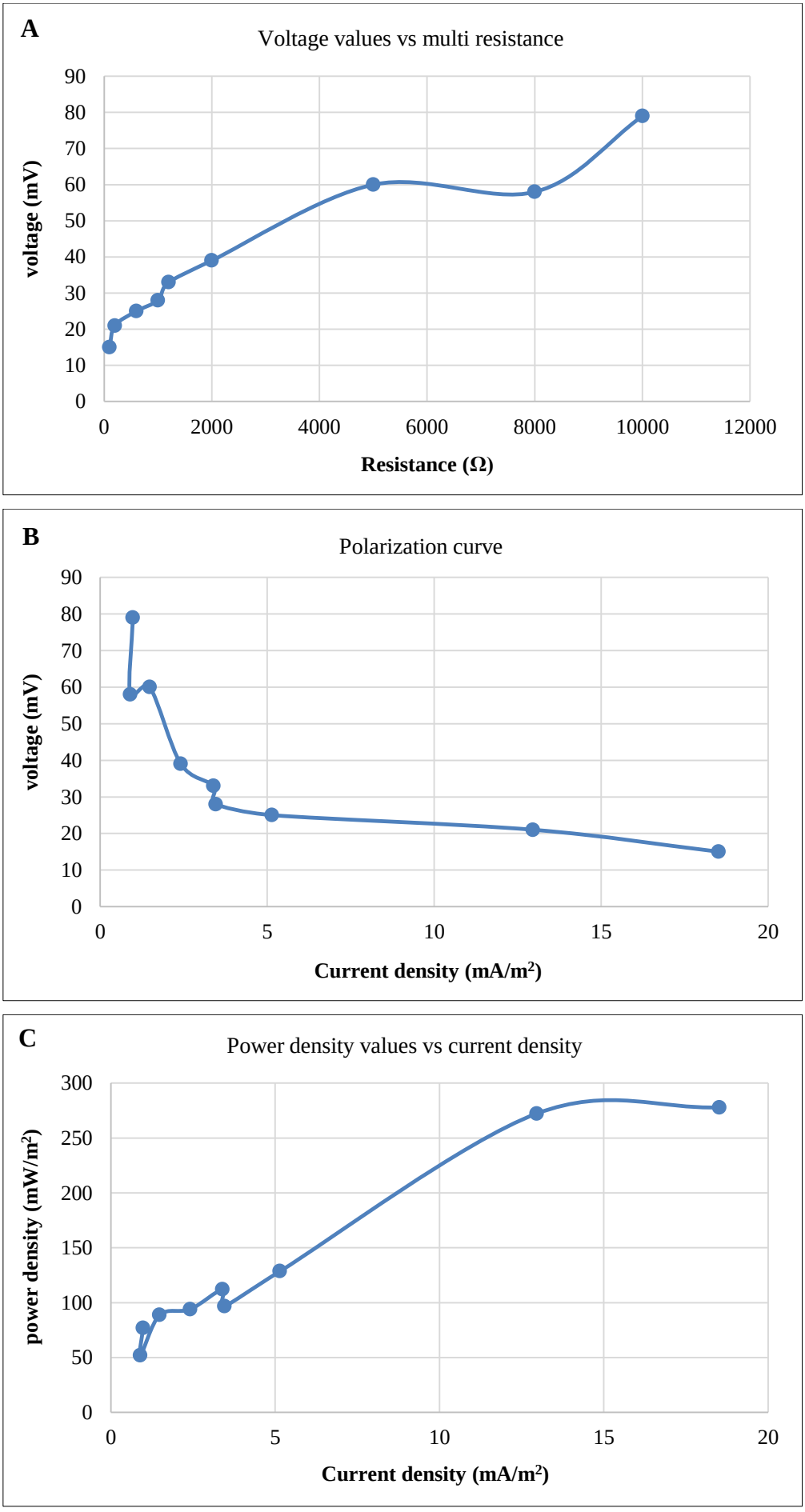


Figure 6. A- Voltage values with multi- resistance, B- Polarization curve, and C- power density versus current density

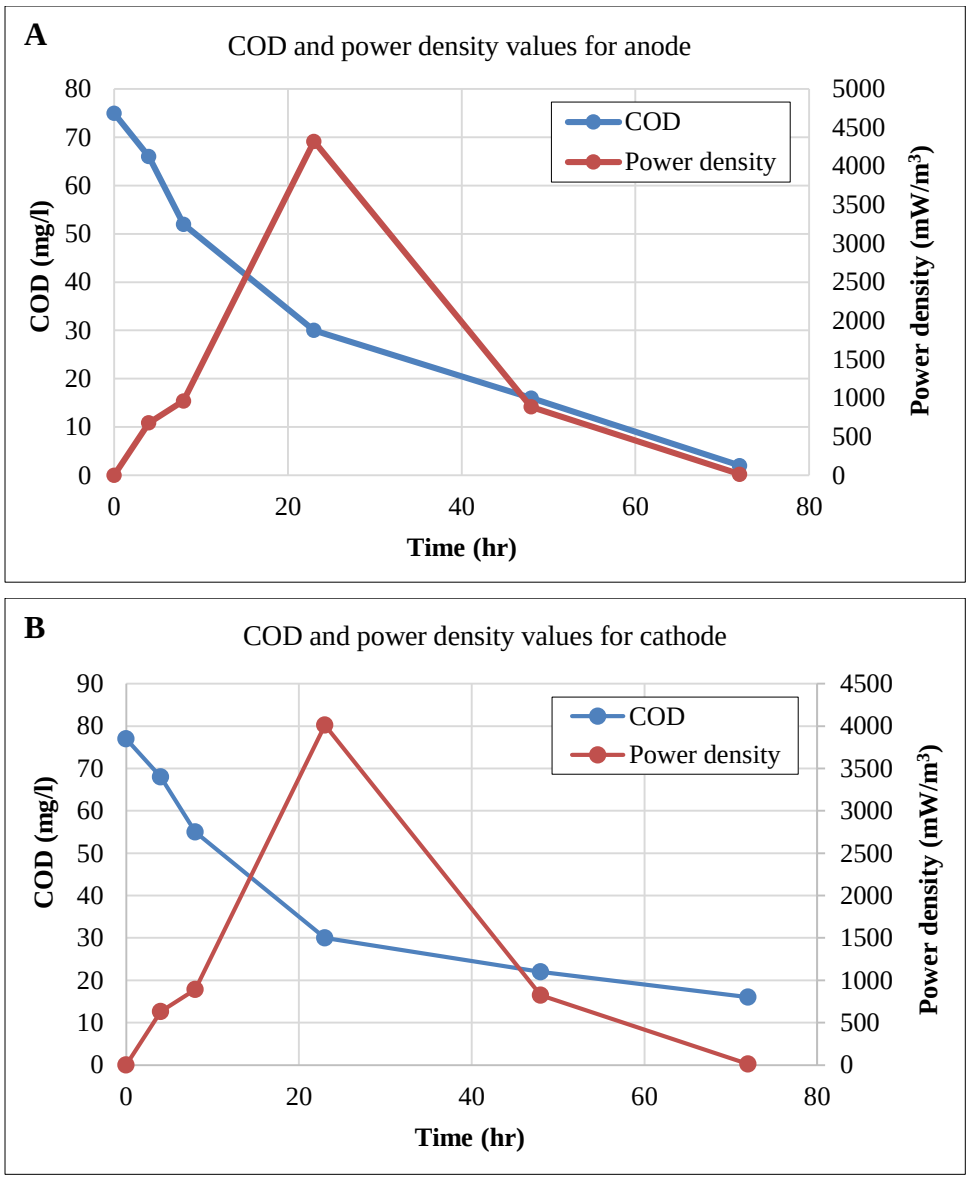


Figure 7. Values of the power density concerning anode volume (A) and cathode volume (B) at 1KΩ resistance with COD values

For a typical cycle, the power density produced with 1KΩ external resistance between anode and cathode at first 4th hr of the run period was 0.678 W/m^3 of anode volume and 0.63 W/m^3 of cathode volume. It increased after one day to the peak (4.32 W/m^3 of anode volume and 4.013 W/m^3 of cathode volume) are shown in (Figure 7). The peak values of power density in this study more than values that were presented by previous studies for PMFC [27] used algae in biocathode to treat actual wastewater which might be attributed to different system configuration.

3.4. Performance of PMDC to Remove COD

The percentage of COD removal of oil refinery wastewater by anode and cathode was 97.33% and 79.22%, respectively during the cycle of three days as showed in (Figure 8-A). That is evidence of the good ability of microalgae *C. Vulgaris* in biocathode to treat this kind of wastewater. So, in addition to using microalgae in biocathode as a source of oxygen by the photosynthetic process, it can be used to treat oil refinery wastewater also. Therefore, the PMDC can be used to treat wastewater in both anode and cathode for more sustainability. The values of COD reduction shown in (Figure 7). Our observations noticed the diminishing of oil odor from cathode effluent. This observation was not seen in the anode chamber, although it treated the same wastewater. Therefore, the algae might be able to adsorption the volatile compounds responsible for the odor in oil refinery wastewater. Previous studies used algae to treat multi kinds of industrial wastewater with different percentage of COD removal for every case. The study [25] used algae *C. Vulgaris* in municipal wastewater treatment plant (MWW) for cultivation algae, the maximum COD removal percent 83% for MWW by algae. The study [28] used algae *C. Vulgaris* in biocathode MFC for producing electrical energy and treat oils and fats of chocolate factory wastewater, the COD removal percent (cathode 78.6%). The algae *C. Vulgaris* was used for bioremediation of textile waste effluent in the previous study [24] and COD removal percent 17.5%. Treatment of

Petrochemical Wastewater by algae *C. Vulgaris* during the last study [23] the maximum COD removal was 38%. By comparing between this study and previous studies COD removal percent in this study butter than other studies except for the research of using microalgae for treating MWW [25].

The values of COD reduction in the anode with columbic efficiency (CE) values versus time illustrated in (Figure 8-B). The maximum values of CE were 1.244%, and this value lower than the value presented by the previous study [11] (17.2%). That might be attributed to the big difference between the initial value of COD in both studies (75 mg/l for the present study and more than 1000 mg/l for the previous study).

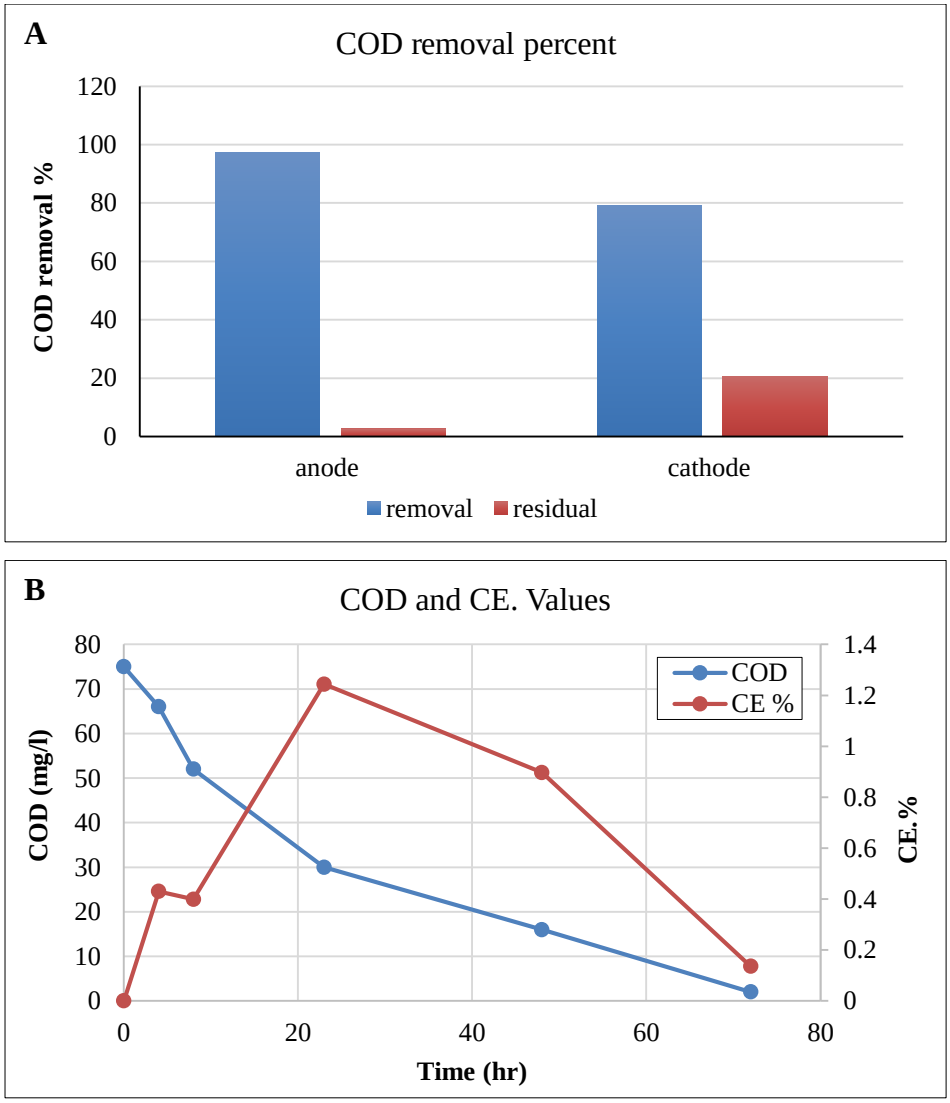


Figure 8. A) COD removal percent of anode and cathode and B) the values of COD and CE. Versus time

3.5. TDS Removal Rates During the Operation of the Different Cases

For different stages of operation, different TDS removal rates achieved. They were 22.153, 65.625, 159.722 ppm/h for 1st, 2nd, and 3rd stage with initial TDS in desalination chamber 15000, 35000, 35000 ppm, respectively as illustrated in (Figure 9-A). Regarding the 1st and 2nd stages, the difference between the concentrations of salt in the desalination chamber should cause variation in the TDS removal rate due to ion diffusion and osmotic process. In (Figure 9-B), the initial conductivity of anode and cathode chambers for 2nd stage were 2.2 and 10.43 mS/cm, respectively. On the other hand, the initial conductivity of anode and cathode for 3rd stage 1.433 and 1.993 mS/cm, respectively, which explained the significant difference of the TDS removal rate between these two stages for the same initial salt concentration in the desalination chamber. The lower conductivity in anode and cathode chambers should improve the ability of ions transfer outside the desalination chamber. The changing of pH values of cathode and anode in the 3rd stage from initial value approximately 7 in both anode and cathode to 6 for the anode and 9 for the cathode was due to the desalination process were shown in (Figure 9-C). The pH in the anode chamber reduced due to a cumulation of H^+ ions produced from the organic material biodegradation process by bacteria in the anode chamber. The AEM has prevented H^+ ions from transporting to the desalination chamber; instead, it was allowed chloride ions transferring from the desalination chamber

to the anode chamber. On the other hand, the pH in the cathode chamber increased due to the depletion of H^+ ions due to stimulating them with oxygen. CEM prevented OH^- ion to transport to desalination chamber; instead, it was allowed sodium ions transferring from desalination chamber to the cathode chamber.

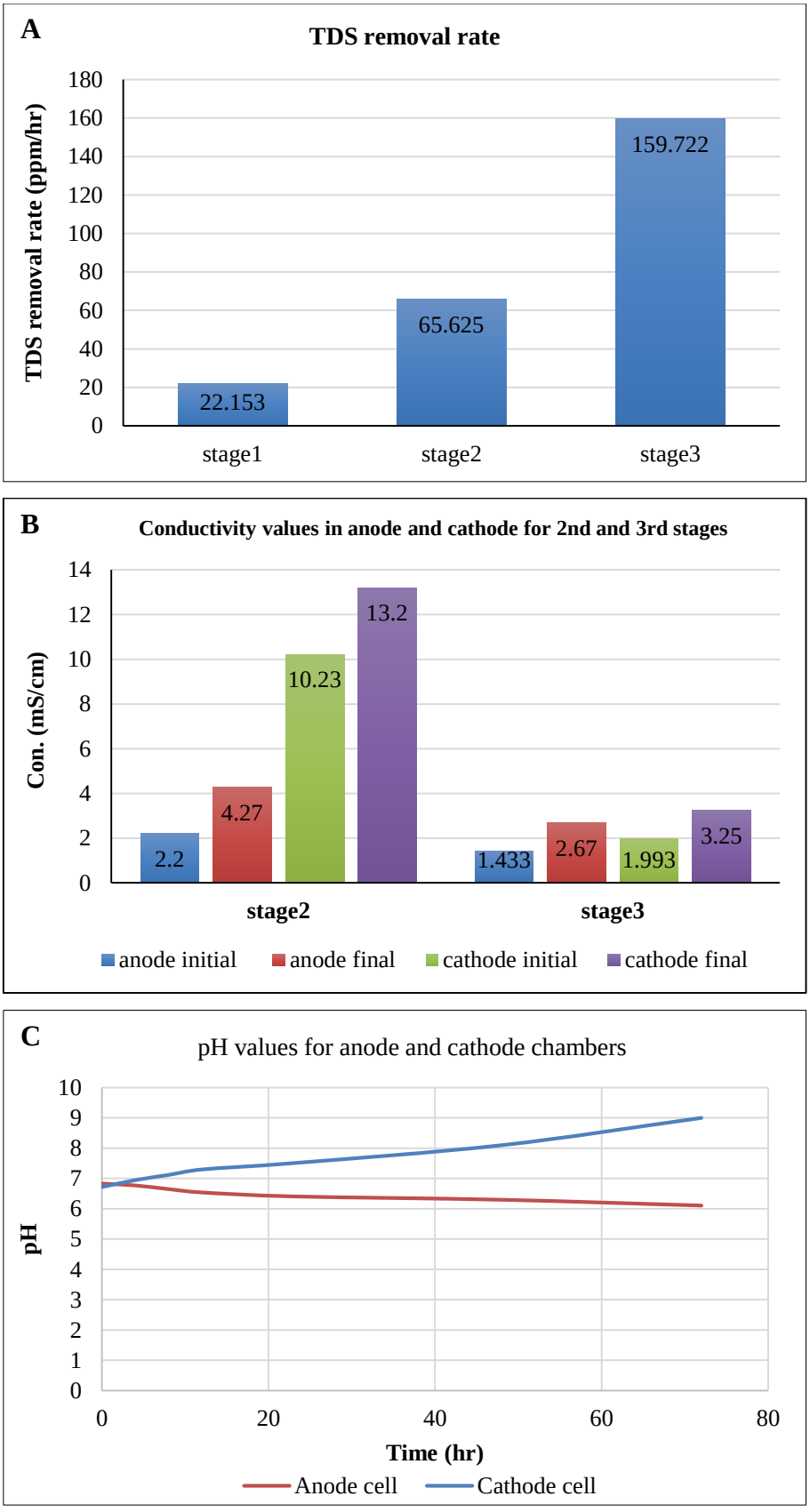


Figure 9. A) The TDS removal rate for the three stages, B) the conductivity values in the anode and cathode chambers for 2nd and 3rd stages (the run period for 2nd stage 12 days and 3rd stage 3 days), and C) pH values of anode and cathode during the 3rd stage

3.6. Dissolve Oxygen Production by Algae *C. Vulgaris* in Biocathode PMDC

Dissolve oxygen production by the photosynthetic process of algae in biocathode during the illumination period (12h) is illustrated in (Figure 10-A) by initial, highest, and final values of DO for 1st stage, 2nd stage, and 3rd stage. DO was varied between (6.8-4), (8.5-6.1) and (7.1-5.5) for 1st stage, 2nd stage, and 3rd stage, respectively. It is worth noting the higher DO production in 2nd stage might be due to an increase in the biomass of algae represented by optical density and biofilm formation inside the biocathode chamber. DO values in the 3rd stage lower than 2nd stage because of the quantity of algae solution so tiny just 10% of cathode volume and that values of DO produced from the biofilm layer inside the biocathode chamber. At the end of every stage, the value of DO decreased. That might be due to the declining of the substrate in cathode that algae feeding on it. In comparison with previous studies [10, 11], DO concentrations in this work were approximately the same values presented by these studies.

Dissolve oxygen production should control the power generation because it acts as an electron acceptor required for completing the redox reaction in the PMDC. (Figure 10-B) Shows the variation of power generation between light and dark during the operation period (12 to 12 light and dark cycle) for treatment oil refinery wastewater (3rd stage) in anode and cathode by measuring the voltage and dissolve oxygen every four hours. The effect of illumination on the DO production and power generation was apparent, as shown in (Figure 10-B). DO, and voltage was improved during the light period and declined during the dark period. For the first day, the highest amount of voltage (53 mV) with the highest value of DO production (7.6 mg/l) and after that the voltage decline to (25 mV) with DO reduction to (4.8 mg/l).

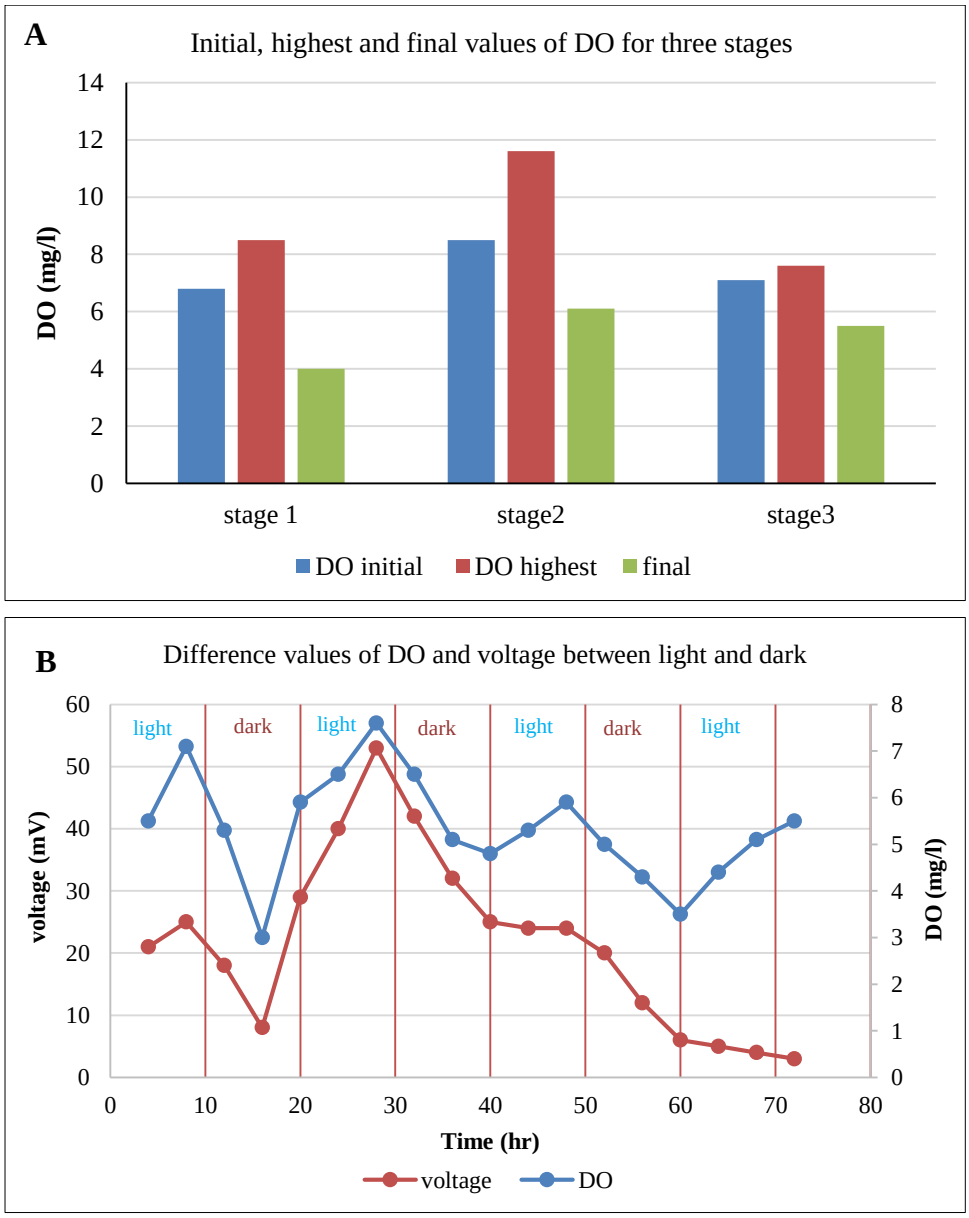


Figure 10. A) Initial, highest, and final values of DO for 1st stage, 2nd stage, and 3rd stage and B) The variation of DO and voltage between light and dark period for the 3rd stage

4. Conclusion

In this work a PMDC in which microalgae were used in the cathode chamber to produce oxygen by photosynthesis process instead of an air pump or chemical materials like ferricyanide in air cathode MDC and this type called photosynthesis biocathode MDC. The peak of power density generated by this Photosynthetic Microbial Desalination Cell (PMDC) with 1KΩ resistance was (4.32 W/m^3 of anode volume and 4.013 W/m^3 of cathode volume). The microalgae growth in the biocathode chamber presented by the optical density was increased from 0.546 at the beginning of the system operation to 1.71 after 24 days of operation period. The percentage removal of chemical oxygen demand (COD) of oil refinery wastewater was 97.33% and 79.22% in anode and cathode chamber, respectively. TDS removal rate 159.722 ppm/h with initial TDS in desalination chamber of 35000 ppm. This study proved the possibility of use PMDC for treatment actual oil refinery wastewater in both anode and cathode chambers by using different microorganisms, anaerobic bacteria to treat the wastewater in the anode chamber and microalgae *Chlorella Vulgaris* to treat the wastewater in the cathode chamber. This method is featured with sustainability because it provides many things at the same time with environment-friendly manner:

- Treat actual oil refinery wastewater in anode and cathode chambers.
- Produce oxygen as an electron accepter by photosynthesis process of microalgae without any bad residual in spite of chemical materials are used in air cathode MDC.
- Generate electricity power.
- Improve the growth of microalgae in the cathode chamber and that use to generate microalgae that can be used to produce biofuel.

5. Conflicts of Interest

The authors declare no conflict of interest.

6. References

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