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Multi Response Optimization of Waste Activated Sludge Oxidation and Azo Dye Reduction in Microbial Fuel Cell

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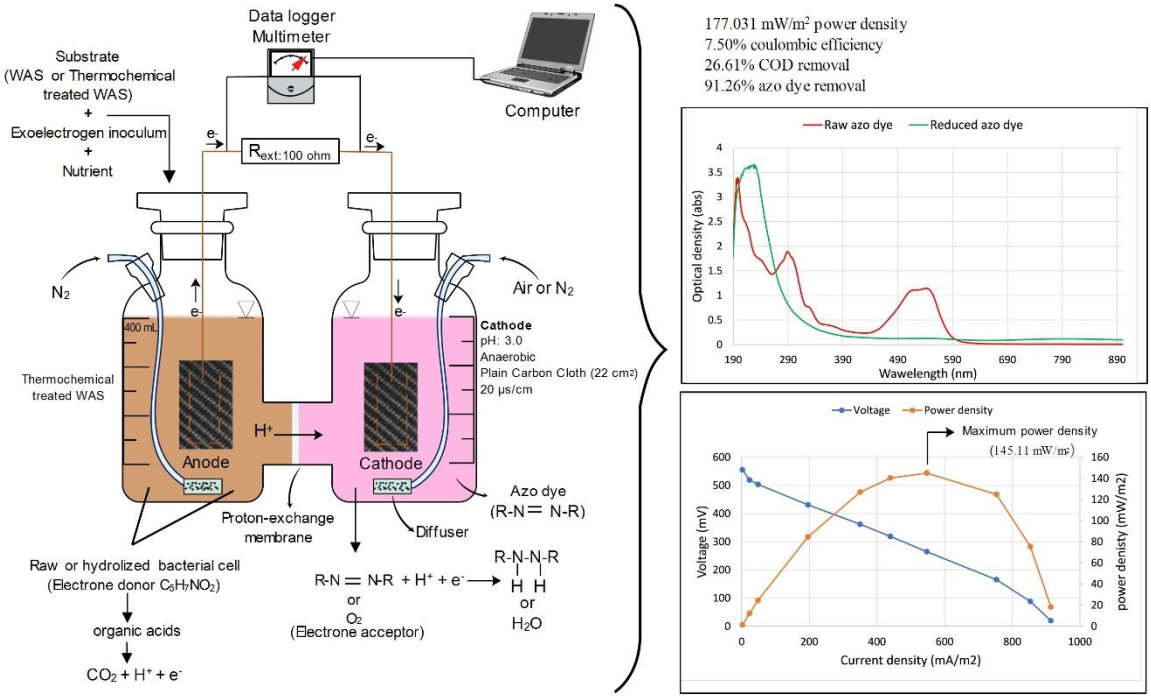
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Abstract

Microbial fuel cell technology draws attention with its ability to directly recover electrical energy from various organic materials. In this study, the operating conditions affecting the oxidation-reduction and electricity generation efficiency of MFC were optimized using the Taguchi Experimental Design model. Optimization was carried out for maximum power density, coulombic efficiency, azo dye removal, and COD removal. With the determined optimum conditions (cathode pH of 3.0, cathode oxygen status of anaerobic, anode substrate of pre-treated, external resistance of 100 Ω, cathode electrode type of plain carbon, cathode electrode surface of 22 cm^2, cathode conductivity of 20 µs/cm), 177.03 mW/m^2 power density, 7.50% coulombic efficiency, 91.26% azo dye removal efficiency and 21.61% COD removal efficiency were obtained. By Pareto analysis, it was determined that the power density, coulombic efficiency and COD removal efficiency were most affected by the substrate type at the anode, and the azo dye removal was most affected by the catholyte pH. The maximum power density and internal resistance of the MFC operated under optimum conditions were determined as 145.11 mW/m^2 and 243.30 Ω, respectively by the polarization curve. Cyclic voltammetry was also performed for the electrochemical characterization of MFC operated under optimum conditions. An anodic peak at −183.2 mV and a cathodic peak at −181.2 mV was visible in the CV curve.

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Keywords: Activated sludge oxidation, azo dye removal, bioelectricity, microbial fuel cells, optimization

1. INTRODUCTION

In recent years, finding renewable, cost-effective and eco-friendly technologies for energy production and waste remediation has been seen as the most viable approach. The microbial fuel cell (MFC) is a bio-catalyzed technology for electricity generation from organic matter. MFC is a low carbon footprint system that generates electrical energy directly from waste at low temperatures and is therefore an advanced clean technology with high potential to solve energy needs and waste disposal issues. The remediated waste residue formed after energy generation has high stability and is considered environmentally safe.

The main components of MFCs are electrodes, membranes, and electron donor/acceptors. The most essential features of electrodes used in MFC are good biocompatibility, conduction, chemical stability, and efficient electron transfer between bacteria and electrode surface. The membrane acts as a physical separator between the anolyte and the catholyte, transferring the protons developed at the anode to the cathode [1]. While external resistance regulates current and voltage, it also indirectly affects the anode potential. The anode potential is an important indicator of energy production in the MFC [2].

Electron acceptors can be used for bio-energy production in MFCs as well as for bioremediation at the cathode. Electron acceptors with high redox potential, fast kinetics, economical and easily available are preferred. Oxygen is the most preferred electron acceptor

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in terms of sustainability, easy availability, cost-effectiveness and scaling of MFCs [3]. The cathode is suitable for the oxygen reduction reaction as well as for the reduction of biotic or abiotic pollutants. The reduction of oxygen to water in air-cathode MFCs is achieved successfully, besides, the use of strong oxidizers is less sustainable as it will damage the electrode and membrane. Various electron acceptors have been used; ferricyanide [4], dichromate, persulfate [5], nitrogenous compounds [6], metal ions [7], and azo dyes [8]. When azo dye is used as an electron acceptor in the catholyte, the chromophoric bond in the structure of azo dyes can be broken by the electrons accepted from the anode chamber. Colorless and easily biodegradable aromatic amines can be formed as a result of the reduction of azo dye [9].

Some pollutants cannot be decomposed by oxidation in the anode chamber due to their high redox potential, in which case they can be removed by reduction in the cathode chamber. Thus, they contribute to the ultimate treatment of wastewater and the development of MFC technology. Since many contaminants such as nitro and chlorinated aromatic compounds have positive redox potential, they can be good alternatives as electron acceptors in the cathode chamber [10].

MFCs have limited commercial applications as the power density achieved is generally low due to the high ohmic losses associated with loss of concentration, activation, bacterial metabolism, and resistance to ion flow through the ion exchange membrane. A high-performance anode material (with excellent electrical conductivity, large surface area, and biocompatibility) is essential to improve the power generations of MFCs [11].

Oxidation of various wastes and wastewaters in MFCs has been studied: domestic wastewater [12], dairy wastewater, sewage sludge [13], brewery wastewater [14], food processing wastewater [15], swine wastewater [16]. Waste activated sludge (WAS) is an important source of electrons for use in MFCs.

In conventional wastewater treatment plants, 70-100 kg organic sludge/ML (megaliters) organic sludge is produced in semi-solid form, rich in nutrients and high in decomposition potential [17]. The selection of the appropriate disposal method is an important issue in the management of WAS. The use of WAS for renewable energy generation is considered an appropriate approach. Anaerobic digestion is a suitable sludge management process applied for both in energy production and waste stabilization. MFC's can be a potential alternative to the conventional anaerobic digestion. The output of the anaerobic digestion is in the form of methane and hydrogen gas but almost 65% of the energy is lost in the process of biogas combustion and conversion into electricity. However, MFC provides direct conversion of

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chemical energy into electricity, resulting in less energy loss [18]. MFC behaves like a chemical fuel cell and is able to produce energy from low-quality waste. Numerous studies have been carried out on energy production from domestic and industrial-origin liquid, semi-liquid, and solid organic wastes at MFC. There are studies showing that waste activated sludge with high organic content is used as an important electron donor in the MFC process [19,20].

Real-scale applications of MFCs are limited due to their relatively high investment cost, low power generation, and high internal resistance, and they are complex bioreactors that need to be optimized for higher performance in terms of electricity generation and waste treatment. Therefore, a statistical modeling approach is recommended for improving MFC performance and optimizing waste degradation and power generation simultaneously [21].

MFC stands out as an energy production process and is accepted as a clean technology because it also performs waste remediation. While the stabilization/decomposition of the wastes is achieved by oxidation and reduction reactions, electricity is produced simultaneously. There are many variables that affect system performance in MFCs. Optimization of all these variables, which affect the anode and the cathode separately, is crucial for the system to reach maximum performance. There are a limited number of studies in the literature in which electricity generation and remediation efficiencies are evaluated simultaneously and process variables are optimized. In this study, activated sludge as electron donor and azo dye as electron acceptor were used in the biotic anode and abiotic cathode chambers, respectively. Process variables (pH, conductivity, oxygen state of catholyte, substrate type of the anolyte, cathode electrode surface area, cathode electrode type, and the external resistance) are optimized with Taguchi experimental design for high electricity generation and remediation efficiency. The individual effect of each variable on the process performance was revealed by Pareto analysis.

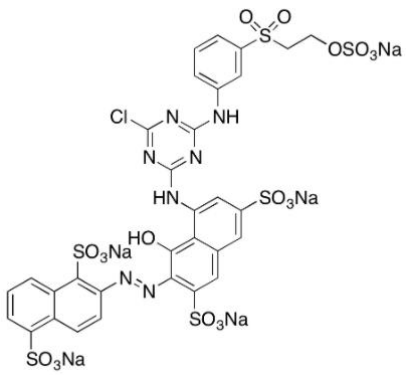
2. MATERIAL AND METHOD

WAS and anaerobic sludge were obtained from the wastewater treatment plant in the factory that produces baker's yeast (PAKMAYA A.Ş Türkiye). WAS was taken from the return sludge line of the activated sludge process and used as a substrate in the anode chamber and used after increasing the solid content by sedimentation. Anaerobic sludge from the fixed bed anaerobic reactor was used to obtain exoelectrogen-rich inoculum. Sunfix Red S3B (SR-S3B-Reactive anionic azo dye) solution was used as an electron acceptor in the cathode

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chamber without any pre-treatment. The characteristic properties of WAS and anaerobic sludge and the molecular structure of azo dye are given in Table 1.

Table 1 The properties of sludge and azo dye used in the study

WAS	Anaerobic sludge	The molecular structure of SR-S3B Azo dye
COD :5100 mg/L MLSS: 21186 mg/L MLVSS:16533 mg/L pH: 7.4	COD:1880 mg/L MLSS: 4853 mg/L MLVSS:2940 mg/L pH: 7.1	

Experiments were conducted in a dual-chamber, 500 mL volume H-type glass MFC. WAS as substrate and exoelectrogenic bacteria-rich mixture as inoculum was used at a ratio of 8/1 (v/v) for each experimental run. During the experiments, WAS was used as raw and pre-treated substrate. The thermochemical pre-treatment process was carried out by adjusting the pH of the WAS to 10 with NaOH and boiling the WAS for 15 minutes. After pre-treatment, the pH of WAS was adjusted to around 7.4. Raw and pre-treated sludge were used with the addition of inoculum and nutrient solution. KCI was used to increase conductivity in experiments with high conductivity according to the experimental design.

In all experiments, O_2 has swept away by purging N_2 gas through the anode chamber. According to the experimental design, in certain experiments, the cathode chamber was also swept with N_2 to remove O_2. When N_2 was passed through the cathode and anode chambers, the chambers were closed throughout the experimental run. In the experiments where the cathode was aerobic, oxygen was supplied to the cathode chamber with the Tetra Aps 400 Aquarium Air pump. When air is pumped into the cathode, oxygen and azo dye co-exist as electron acceptors, whereas when oxygen is not present and an anaerobic environment is formed, only azo dye becomes electron acceptor. Such a factor was included to determine the effect of azo dye and oxygen competing as electron acceptors on the system.

The azo dye solution was used as a catholyte in MFC. The anode and cathode chambers are separated by a proton-permeable membrane (DupontTMNafion® 117) with an area of 9.62 cm^2. The membrane was treated for 24 hours at 40 ^0C with 30% H_2O_2 and preserved in ultrapure water. Plain carbon cloth electrode with an area of 22 cm^2 was used in the anode

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chamber, and either a plain carbon cloth electrode (CC) or a 0.3 mg/cm² 40% platinum coated vulcan carbon cloth electrode (Pt CC) was used in the cathode chamber according to the experimental design.

Obtaining exoelectrogenic bacteria rich inoculum and biofilm formation on the electrode surface were explained in supplementary materials.

The circuit between the electrodes is closed using conductive copper wire. The anode electrode was externally connected with the cathode electrode through the resistor. Since maximum azo dye removal and stabilization of MFC voltage were observed in the first 3 days in the experimental runs, all studies were run for approximately 3 days. The MFC setup is shown in Figure 1.

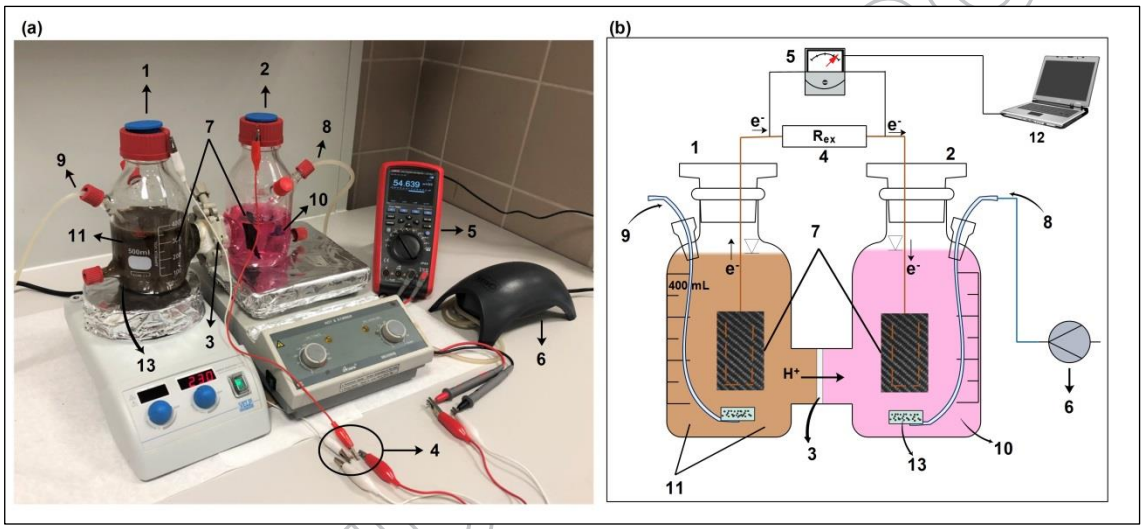


Fig. 1. Double chamber MFC setup, (a) picture of the MFC, (b) schematic representation (1:anode chamber, 2: cathode chamber, 3:Proton exchange membrane, 4: External resistance, 5: Datalogger multimeter, 6: aquarium pump, 7: electrodes, 8: air/nitrogen input, 9: nitrogen input, 10: azo dye solution, 11:WAS/pre-treated WAS, 12: data storage computer, 13: diffuser)

Analysis: Oxidation efficiency were determined by COD and volatile solid analysis in the anode compartment, and reduction efficiency were determined by azo dye analysis in the cathode compartment. Also, control trials were carried out outside the MFC at the same catholyte conditions to determine the possible azo dye removal mechanisms (such as adsorption on the carbon electrode surface) other than the electrochemical reduction of azo dye. The electricity generation efficiency of the MFC was determined by the columbic efficiency and power generation. In addition, cyclic voltammetry was performed for the electrochemical analysis of the cell. Information about the analytical measurements performed is given in the supplementary materials.

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2.1. Optimization study

In this study, the levels of operating conditions affecting oxidation-reduction efficiency and electricity generation potential in the MFC process were optimized using the Taguchi Experimental Design model. Design Expert (version 11) software was used for optimization. Taguchi experimental design methodology is widely used as an advantageous experimental design method, especially due to obtaining fewer experiments. The Taguchi design uses the loss function to measure performance characteristics that deviate from the set value. The value of the loss function is converted to the signal-to-noise (S/N) ratio. Optimum operating conditions are calculated from the S/N ratio of the results obtained from the studies designed with the Taguchi [22]. More than one S/N can be defined in the Taguchi Experimental Design Method. In this study, the S/N ratio, which expresses the "higher is better" situation, was used for all responses Eq. (1) [23].

$$\frac{S}{N} = -log10\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (1)$$

y_i is the performance characteristic observed in the ith experiment for a given combination of control factor levels and n is the number of repetitions. The power density, azo dye removal efficiency, coulombic efficiency (CE) and COD removal efficiency were determined as response parameters. Optimization of the four determined response parameters was performed together as a multi-optimization. The importance weights of all response parameters were accepted as equal. The factors and their levels affecting the MFC were determined by considering the data in the literature and preliminary experiments [24–26]. The factors and levels used in the experimental design are given in Table 2.

Table 2 Factors and levels determined for Taguchi experimental design

Code	Parameter	Level 1	Level 2
A	Cathode pH	3.0	6.05 (pH of raw dye solution)
B	Cathode Oxygen State	Anaerobic	Aerobic
C	Anode Substrate	Pre-treated WAS	WAS
D	External Resistance	100 Ω	1000 Ω
E	Cathode electrode type	Pt catalyzed carbon cloth (Pt-CC)	Plain carbon cloth (CC)
F	Cathode Electrode surface area	11 cm^2	22 cm^2
G	Cathode conductivity	20 µs/cm (Conductivity of raw dye solution)	1600µs/cm

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3. RESULTS AND DISCUSSION

3.1. Optimization of factors affecting the MFC

In MFCs, the oxidation of the pollutant in the anode chamber and the reduction of the pollutant in the cathode chamber are equally important as the electricity generation efficiency. There are many operating parameters that affect the electricity production and the treatability of the wastes. In this study, the electricity generation potential was evaluated by calculating the CE and power density, and the treatability of the wastes was evaluated by measuring the azo dye and COD removal in catholyte and anolyte, respectively. The Taguchi L8 experiment design, which was created for the optimization of the operating parameters that will provide high waste treatability and high electricity production together, and the response parameters (Power density, CE, azo dye removal and COD removal) obtained from each run are given in Table 3.

Table 3 Taguchi experimental design and obtained response parameters

Run	A	B	C	D	E	F	G	Power Density (mW/m^2)	CE (%)	Azo Dye Removal (%)	COD Removal (%)
1	3	Aerobic	WAS	100	Pt-CC	22	1600	1.55	1.25	69.40	15.06
2	6	Aerobic	Pre-treated WAS	1000	Pt-CC	11	1600	100.84	1.79	8.57	23.10
3	6	Anaerobic	WAS	100	CC	11	1600	3.584	1.52	65.76	16.09
4	3	Anaerobic	Pre-treated WAS	1000	CC	22	1600	92.94	1.77	85.41	22.41
5	6	Aerobic	Pre-treated WAS	100	CC	22	20	109.64	8.08	49.95	18.84
6	6	Anaerobic	WAS	1000	Pt-CC	22	20	8.79	0.89	13.48	15.86
7	3	Aerobic	WAS	1000	CC	11	20	4.99	0.42	81.52	15.0
8	3	Anaerobic	Pre-treated WAS	100	Pt-CC	11	20	127.38	6.66	73.18	24.61

As can be seen from Table 3, depending on the experimental conditions, the maximum values of power density, CE, azo dye and COD removal efficiencies were 127.38 mW/m^2, 8.08(%), 85.41(%) and, 24.61(%), respectively. Optimization was performed to determine the operating conditions in which the four response parameters were simultaneously highest.

In order to understand the effect of the variables and to analyze them statistically, ANOVA was performed and coefficients such as F and P were examined (Table 4). The F values of the models were determined to be high enough as 267.42, 97.91, 82.83, 34.34 for power density, CE, azo dye removal, and COD removal, respectively. The P values of the models were

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determined as 0.0037, 0.0101, 0.0120, and 0.0077 for power density, CE, azo dye removal and COD removal, respectively. P-value > 0.10 means the model and factor terms are not significant, while 0.05< P-value shows the significance of the factor and model terms [27]. Response parameters are explainable by obtained models if the models are statistically significant. The anode substrate and cathode conductivity for the power density, the anode substrate, cathode conductivity and the external resistance for the CE, the cathode pH, the external resistance and the cathode electrode type for azo dye removal, the anode substrate for COD removal had significant effects. High correlation coefficients were obtained from each model. The correlation coefficient (R^2) and adjusted correlation coefficient (Adj. R^2) values for all response parameters were determined to be over 95%. The high R^2 values shows the agreement between the predicted values and experimental data [28]. The adequate precision is a measure of the range in the predicted response relative to the error of interest and is desired to be greater than 4 [29]. Adequate precision values for all response parameters were determined to be higher than 4, indicating an adequate signal. In order for the created statistical model to be compatible, it is desired that the R^2 value should be compatible with the adjusted R^2 and there should not be more than 0.2 difference between the adjusted R^2 and the predicted R^2 [30]. It can be interpreted that R^2, predicted R^2 and adjusted R^2 values are compatible.

The models are presented in Table 4. The positive values of the coefficients in model equations represent synergistic effects, whereas the negative values represent an antagonistic effect. In the model equation, it is seen that all parameters have opposite effects on power density. According to the model equations, cathode pH and cathode electrode surface area had a synergistic effect on CE. Substrate type and cathode electrode type showed a synergistic effect on azo dye removal efficiency.

Table 4 ANOVA results obtained for the determined response parameters

	Source	F-value	P-value	Significance	Fit Statistics
Power Density	Model	267.42	0.0037	Significant	R^2= 0.9985 Adj. R^2= 0.9948 Predicted R^2=0.9761 Adeq Precision=35.0700 Power density (mW/m^2):56.22-51.48×C-4.32×D-3.43×E-2.98×F-6.49×G
	C	1297.25	0.0008	Significant	
	D	9.15	0.0942		
	E	5.75	0.1387		
	F	4.36	0.1722		
	G	20.59	0.0453	Significant	
CE	Model	97.91	0.0101	Significant	R^2= 0.9959 Adj. R^2= 0.9858 Predicted R^2=0.9349 Adeq Precision=25.6918 CE (%) :2.80+0.2713×A-1.78×C-1.58×D+0.1996×F-1.22×G
	A	4.97	0.1555		
	C	213.37	0.0047	Significant	
	D	168.69	0.0059	Significant	
	F	2.69	0.2426		
	G	99.84	0.0099	Significant	

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Azo Dye Removal	Model	82.83	0.0120	Significant	R^2= 0.9952 Adj. R^2= 0.9852 Predicted R^2=0.9231 Adeq Precision=23.83 Azo dye removal (%) :55.91-21.47×A-3.55×B+1.63×C-8.66×D+14.75×E
	A	248.25	0.0040	Significant	
	B	6.78	0.1213		
	C	1.44	0.3536		
	D	40.44	0.0238	Significant	
	E	117.24	0.0084	Significant	
COD Removal	Model	34.34	0.0077	Significant	R^2= 0.9786 Adj. R^2= 0.9501 Predicted R^2=0.8480 Adeq Precision=14.5084 COD removal efficiency (%) :18.88-0.8721×B-3.37×C-0.7866×E-0.8282×F
	B	7.79	0.0684		
	C	116.22	0.0017	Significant	
	E	6.33	0.0864		
	F	7.02	0.0770		

The values obtained for each response parameter and the predicted values of the model are compatible. Graphs showing the relationships between the actual values and the values predicted by the model are given in the supplementary information (supplementary information Fig. S1).

Optimum operating conditions determined as cathode pH of 3.0, cathode oxygen status of anaerobic, anode substrate of pre-treated, external resistance of 100 Ω, cathode electrode type of plain carbon, cathode electrode surface of 22 cm^2, cathode conductivity of 20 µs/cm. The presence of O_2 together with the azo dye solution in the cathode chamber, which has a higher redox potential than the azo dye, causes the transfer of electrons to O_2 instead of azo bonds. In the absence of oxygen, the transfer of electrons to the azo bonds in the cathode chamber is facilitated and higher color removal is achieved. The determination of anaerobic condition for cathode by optimization also confirms this. A confirmation experiment was performed to verify the optimum MFC operating conditions. With the confirmation experiment, 177.03 mW/m^2 power density, 7.50% CE, 91.26% azo dye removal efficiency, and 21.61% COD removal efficiency were obtained. It was determined that all response parameters, except power density, were within the 95% confidence interval (Table 5). The fact that the power density is above the confidence interval is evaluated positively in terms of MFC performance. The voltage plot measured against 100 Ω resistance at optimum MFC conditions is given in the supplementary information Fig. S2.

Table 5 Response parameter values obtained under optimum operating conditions, and predicted confidence intervals.

Response	Predicted Mean	95% confidence interval	Actual data
Power density (mW/m^2)	112.10	97.03-127.16	177.03
CE (%)	7.303	6.02-8.58	7.50
Azo dye removal efficiency (%)	102.71	88.35-117.07	91.26

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COD removal efficiency (%)	21.50	19.27-23.72	21.61

Yusoff et al. obtained a higher COD removal efficiency of up to 85% at the end of 10 days of operation with microwave pre-treated sludge. In the same study, power density and CE were obtained as 42±3 and 6.3 %, respectively [31]. Jayashree et al. improved COD removal up to 54% by applying low-temperature thermochemical pre-treatment to dairy WAS. The power density of the MFC had increased from 0.5 W/m^3 (raw sludge) to 0.71 W/m^3 (pre-treated sludge) [32]. Ayol et al., on the other hand, obtained a power density of 312.98 mW/m^2 in an enhanced sludge stabilization coupled with a microbial fuel cell with graphite electrodes [19]. The reason for the relatively low values obtained in this study can be explained by the application of more moderate operating conditions. In addition, the optimum conditions determined are those that maximize the electricity generation efficiency and the treatability of the waste in the anode/cathode chamber simultaneously.

3.2. Pareto Analysis

In MFC, the operating conditions in the anolyte and catholyte affect the CE, power density, azo dye and COD removal. The effects of the factors on the response parameters are different. The Pareto chart checks for statistical significance by presenting the effect of factors on responses. The Pareto graphs plotted with the t-values of the effects versus the parameters affecting each response are shown in Fig. 2. The t values represent the square root of the F values in the ANOVA. There are two different t-value of effects which are the Bonferroni limit line and the t-value limit line. Factors whose effect is above the Bonferroni line are considered to be extremely significant, while those whose effect is between the Bonferroni line and the t-value limit are considered likely to be significant. Factors whose effect is below the t-value limit are considered statistically insignificant [33].

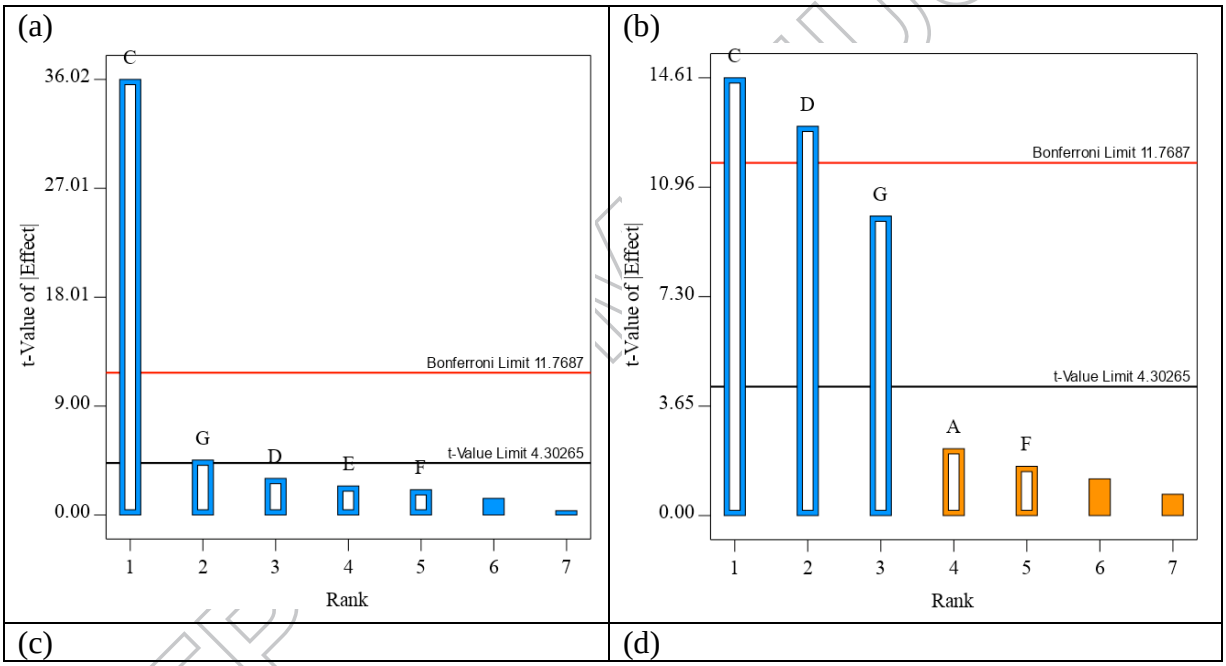
In Pareto analysis, orange-colored bars indicate positive effects of factors, and blue-colored bars indicate negative effects of factors [34]. For power density, the t-value of the substrate type factor was above the Bonferroni limit line, which indicates that the substrate type has the highest effect. If the substrate is used without pre-treatment, the power density will decrease. The t value of all other factors is below the t- value limit line, indicating that these factors are statistically insignificant (Fig. 2. (a)).

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For CE, the t-values of both anolyte substrate and external resistance factors are above the Bonferroni line which indicated that both factors were extremely significant factors. The use of the substrate without pre-treatment and the increase in external resistance negatively affect the CE. The t-value of the catholyte conductivity factor is between Bonferroni and the t-value limit line, indicating that conductivity is an important factor for CE (Fig. 2 (b)).

As seen from the graph, pH shows to have the highest effect on the azo dye removal, followed by electrode type and external resistance. The blue color of the pH effect indicates that an increase in pH from 3.0 to 6.05 will decrease the dye removal efficiency. At the same time, it was determined that higher azo dye removal efficiency would be achieved by using the plain carbon electrode (Fig. 2 (c)).

It was determined from Fig. 2 (d) that the most effective parameter on COD removal was the substrate type. Pre-treatment of the substrate increased the COD removal efficiency.



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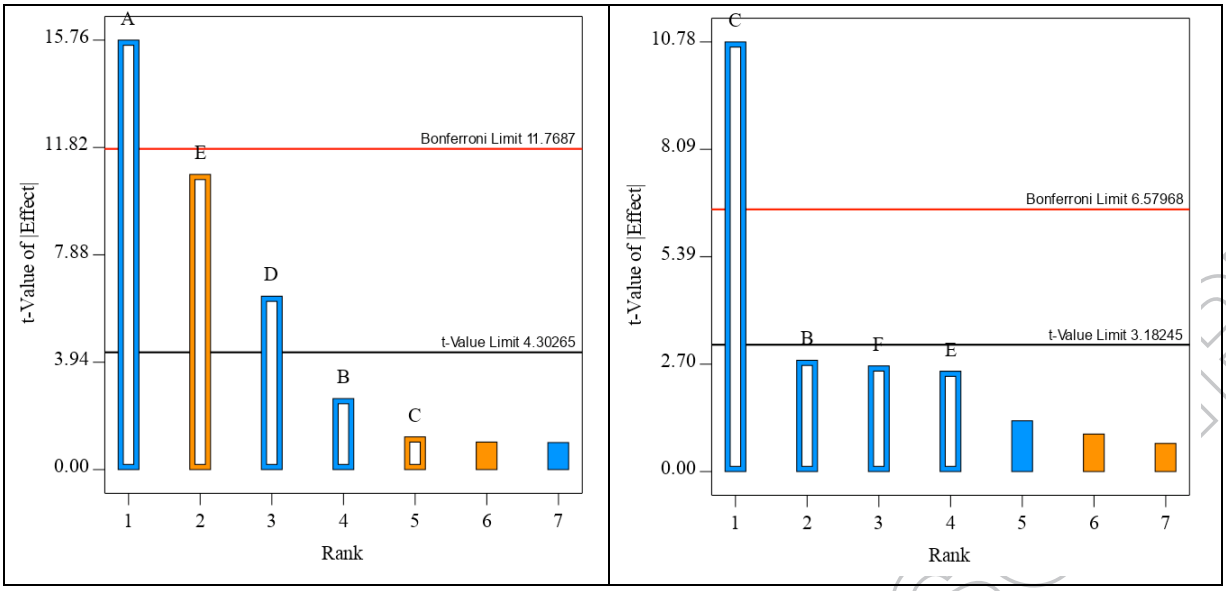


Fig. 2 Pareto graphs of response parameters (Power density (a), CE (b), azo dye removal (c), COD removal (d))

3.3. Evaluation of three-dimensional (3D surface) graphics

The effects of operating parameters on response parameters were evaluated with 3D graphics. Thermochemical pre-treatment of WAS in the anode chamber increased the power density from 1.55 mW/m^2 to 127.38 mW/m^2 when the cathode was anaerobic, the external resistance was 100 ohms, 22 cm^2 PtCC and the cathode conductivity was 20 µs/cm (Fig. 3 (a)). It also increased COD removal from 15% to 24.6% when the cathode was anaerobic, the external resistance was 100 ohms, 22 cm^2 CC and the cathode conductivity was 20 µs/cm (Fig. 3 (d)). Chae et al. (2009) stated in their study that methanogenesis is compatible with higher resistance conditions, because methanogens are more likely to use the substrate when exoelectrogen activity is reduced. Reducing the external resistance from 600 Ω to 50 Ω increased the CE from 32% to 42%. Therefore, lowering the external resistance may be a way to improve MFC performance as it can increase CE by reducing electron losses [35]. In this study, it was observed that the decrease in external resistance increased the CE (Fig. 3. (b)). However, it was observed that the pH change in the cathode chamber did not affect the power density, CE, and COD removal. The conditions with the highest azo dye removal in the cathode chamber were characterized as plain carbon cloth electrode and pH 3. The azo dye removal decreased from 85.41% to 24% when the pH was increased with the cathode being anaerobic, the anode substrate was pre-treated, external resistance was 1000 Ω, 12 cm^2 Pt-CC and the cathode conductivity was 20 µs/cm (Fig. 3(c)).

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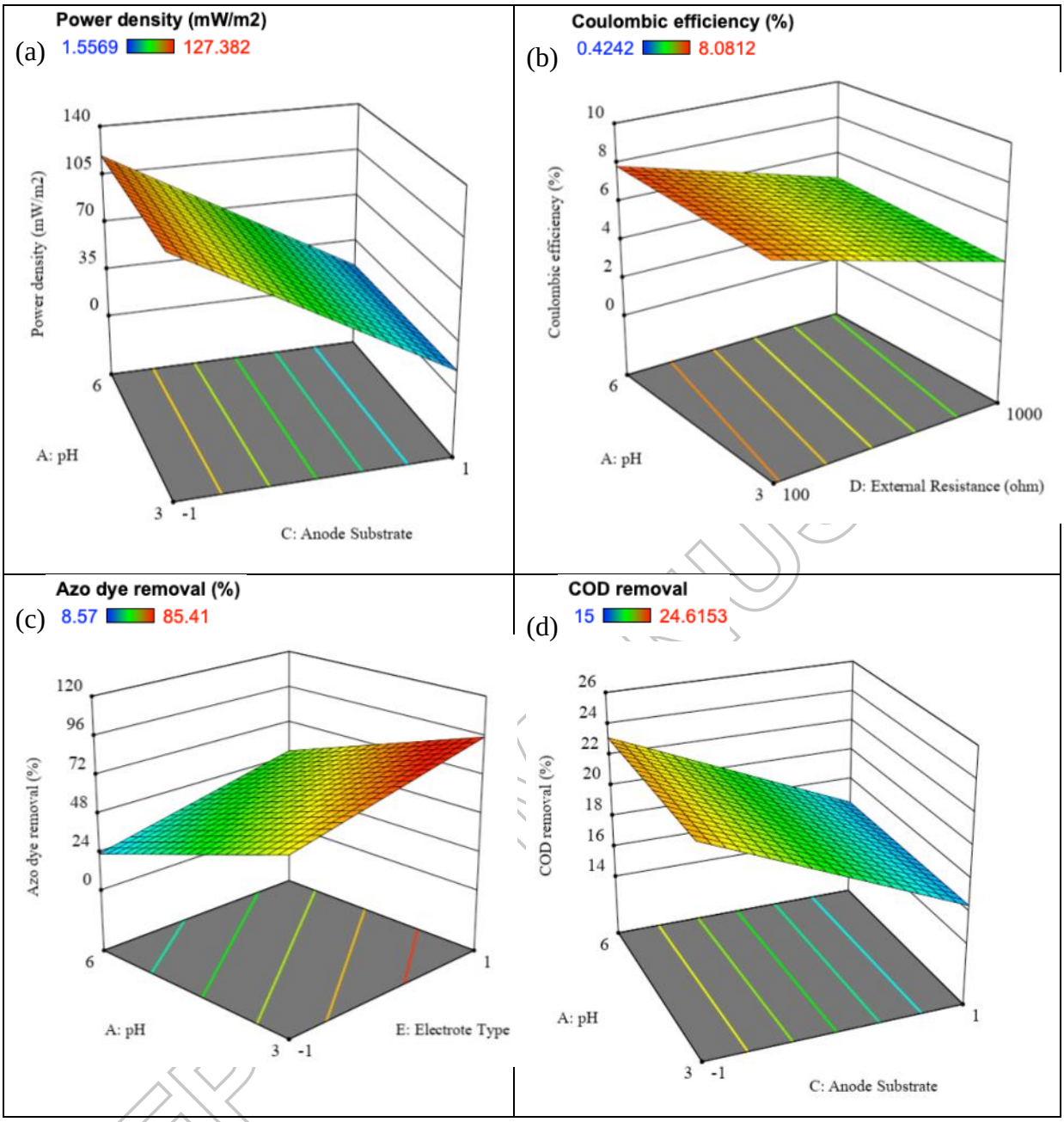


Fig. 3 3-D graphics of Power density (a), CE (b), azo dye removal (c), COD removal (d) (for anode substrate -1 refers to pre-treated and 1 refers to raw WAS, for electrode type -1 refers to Pt catalyzed carbon cloth and 1 refers to plain carbon cloth).

3.4. Evaluation of catholyte reduction

In addition to the anolyte and catholyte conditions, the surface properties of the electrode used to affect the reactions that will take place in the catholyte. It has been extensively accepted that surface-active oxygen functional groups on carbon felt are effective in catalyzing redox reactions of active species and increasing the wettability of carbon felt

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[36]. The reduction potential of the cathode chamber was evaluated by the removal of azo dye. The catholyte of the MFC operated under optimum conditions was compared with the control trial. The ultraviolet-visible (UV-VIS) spectrum of the catholyte at the optimum conditions and the control trial show the same trend (Fig.4)

Aromatic amines and azo bonds are the two main components in azo dyes. In the spectrophotometric analysis, the absorption peaks in the VIS region correspond to the azo bond, while the absorption peak in the UV region shows the aromatic compounds. Peak variation of aromatic compounds and azo bonds in the UV-VIS spectra indicates the decreasing peak of the azo bond and the increase in aromatic amines. Possible intermediates produced after disruption of the azo bond may be benzene, naphthalene-based sulfonated compounds, and triazine-based compounds [37].

The peak seen at 190 nm in the raw azo dye shifted towards 250-260 nm at the end of 70th hour in the control and MFC process. The peak seen at 290 nm in the raw dye disappeared completely. While the azo dye concentration at the end of the MFC process at 541 nm decreased by 91.26%, a decrease of 49.69% was observed in the control trial. After decolorization, the peak at 247 nm, suggesting that azo dye was reduced to its hydrazine derivative [9]. $-N=N-$ double bond was reduced to hydrozo or amine Eq. (2)-(3) [10].

$$-N=N-+2e^{-}+2H^{+}\rightarrow -NH-NH- \qquad (2)$$

$$-N=N-+4e^{-}+4H^{+}\rightarrow -NH_2+NH_2- \qquad (3)$$

An increase in absorbance density was observed at 265 nm. This indicates that the reduction product (sulfonic acid) associated with the degradation of azo dyes may be present [10].

In the light of this information, it is thought that the change in absorbance of the control experiment may be caused by the reactions of active functional groups on the carbon cloth. In the MFC environment, electrons transmitted from the anode chamber were also effective in the azo dye reduction process.

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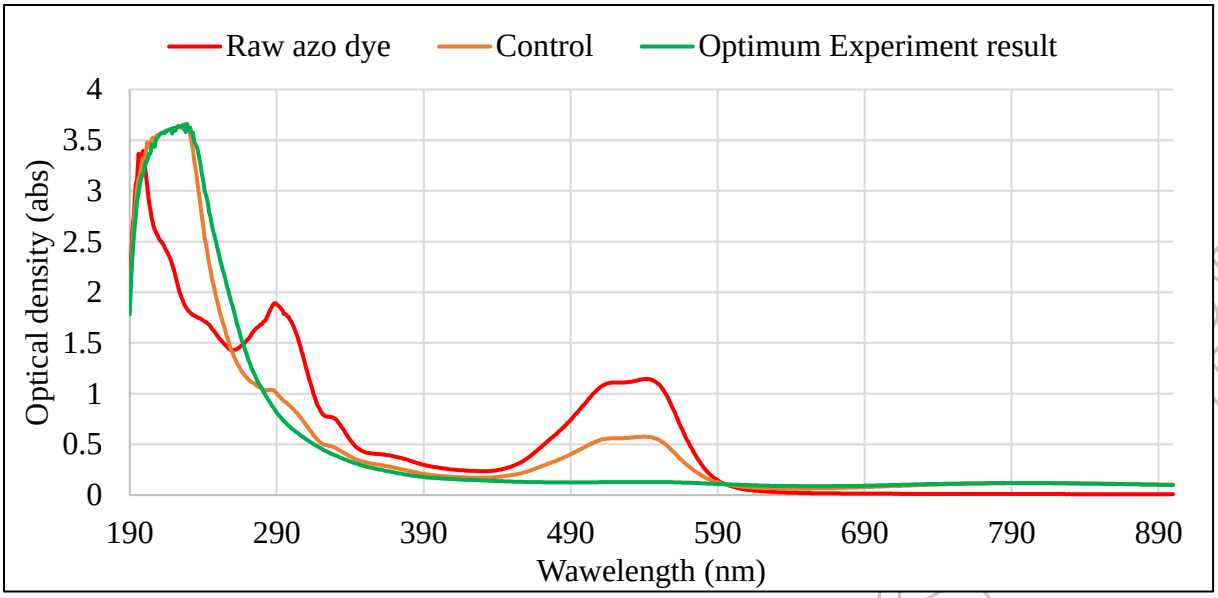


Fig. 4 Wavelength scans of raw azo dye, reduced dye (after MFC experiment operated under optimum conditions) and control dye

3.5. Polarization curve and cyclic voltammetry evaluation

The electricity generation potential varies with the characteristics of the electron donor, electron acceptor, electrode material, and catholyte used. The polarization curve is plotted when stable voltage is obtained in the optimum experiment. The curve was obtained with voltage values across 10 Ω to 100000 Ω external resistor, as shown in Figure 4. The maximum power density under optimum conditions was obtained as 145.11 mW/m^2. The internal resistance of the MFC was obtained as 243.3 Ω. Xiao et al. found the internal resistance to be 371 Ω in the two-chamber MFC using sewage sludge as a substrate [38].

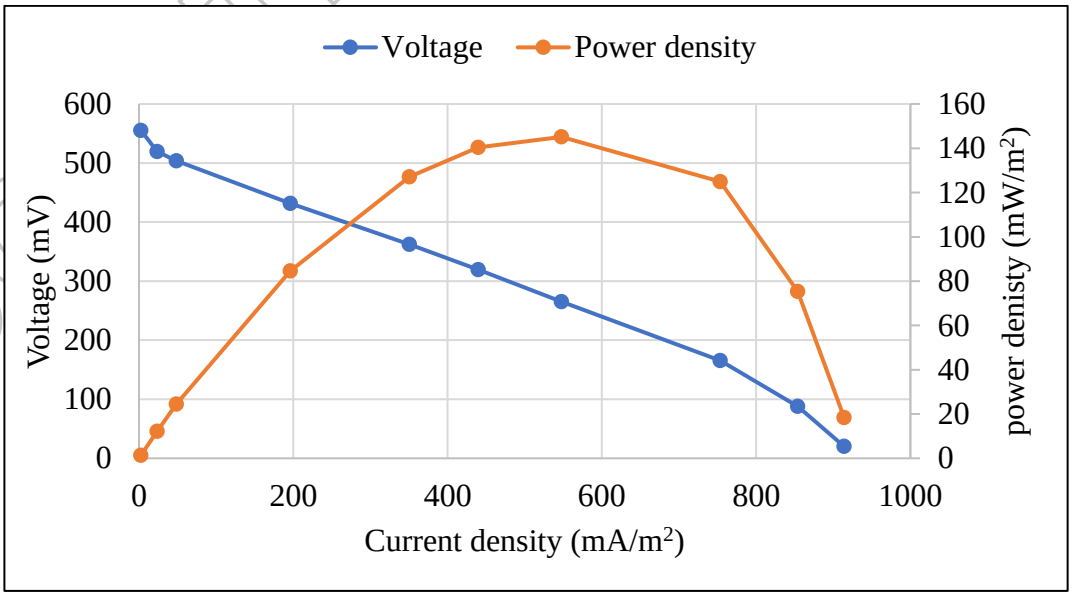


Fig. 5 Polarization and power curve of optimum MFC experiment

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Ayol et al. (2021) obtained a maximum power density of 56.63 mW/m^2 with carbon cloth electrodes in a dual-chamber MFC in their study using WAS as the substrate [19]. In this study, the obtained power density was higher due to the pretreatment applied to the substrate. Neşeli et al. (2022) optimized the electricity generation performance of activated sludge and sediment sludge in MFC. Using Taguchi's orthogonal design and response surface methodology, mathematical models were derived by optimizing with the parameters of resistance, temperature, volumetric flow rate and area. They showed that the models obtained were in good agreement with the experimental data [26].

Maximum power density is not the main goal of MFCs. In addition, it is aimed to ensure Maximum power density is not the main goal in MFC's. In addition, it is aimed to ensure the effective waste removal. The high internal resistance obtained may be due to the use of WAS as a substrate instead of a easly degradable substrate and the high density of the sludge increasing electron transport losses compared to MFCs using wastewater.

Cyclic voltammogram is an electrochemical technique used to describe electron transfer interactions at the electrode-biomass/biofilm-liquor interface in the anode chamber [39]. In this study, the CV measurement was carried out under optimum experimental conditions where the MFC produces a stable voltage. A redox cycle was obtained in the voltammogram shown in Fig. 6, confirming the important role played by the anaerobic microorganism in transferring electrons to the anode. The current density data were correlated with the oxidation and reduction reactions occurring at the working electrode. The highest anode potential was associated with the largest values of current density corresponding to the oxidation of waste activated sludge. The highest cathode potential corresponded to the lowest negative current density values associated with water reduction and hydrogen gas production. An anodic peak at −183.2 mV and a cathodic peak at −181.2 mV were visible in the CV curve. In addition, the small size of the peaks in Fig. 6 may be due to the fact that no redox mediator was used in this study. Mediators have an important effect on the anodic or cathodic current in MFC. Certain microorganisms may also support the electron transfer process [40].

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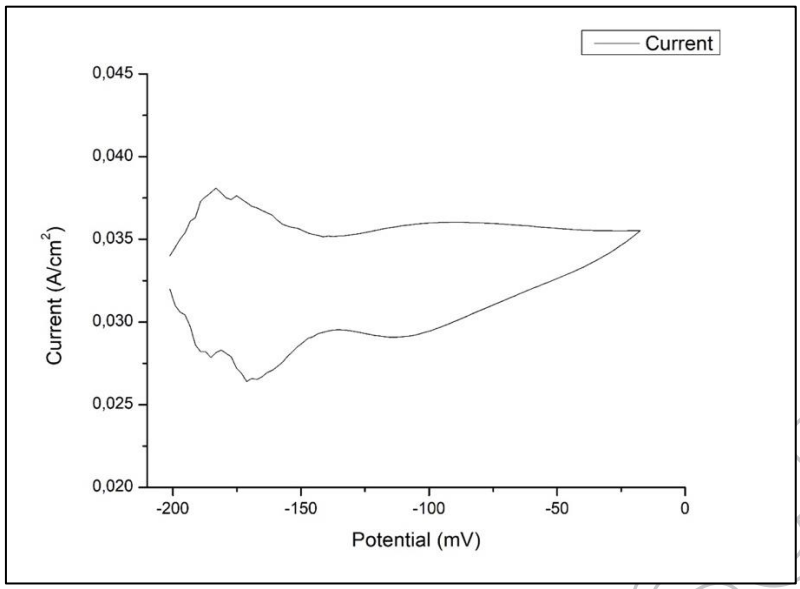


Fig. 6 Cyclic voltammogram of the MFC experiment operated under optimum conditions

4. CONCLUSIONS

In this study, operating conditions for high-level electricity generation and waste remediation are optimized in MFC where sewage sludge is used as electron donor and azo dye is used as electron acceptor. Taguchi Experimental Design approach was used for optimization. The results obtained are summarized below;

- The optimum operating conditions were obtained as follows: the anaerobic oxygen status of cathode, pH of 3.0, conductivity of 20 µs/cm of the catholyte, pre-treated anode substrate, external resistance of 100 Ω, cathode electrode type of plain carbon (surface area of 22 cm^2). With the confirmation experiment performed under optimum conditions, power density of 177.031 mW/m^2, CE of 7.50 %, azo dye removal of 91.26 %, and COD removal of 21.61 % were obtained.
- With the UV-VIS spectrum, it was observed that the azo bonds of the azo dye, which peaked in the visible region, were broken and the aromatic compounds increased in the UV region.
- In Pareto analysis, it was determined that the anode substrate type was the most effective parameter in power density, CE, and COD removal. Also, the most effective parameter in the removal of azo dye was determined as the pH of the catholyte.
- With the polarization curve, the maximum power density and internal resistance were determined as 145.11 mW/m^2 and 243.3 Ω, respectively. The cyclic voltammogram confirmed the oxidation and reduction reactions.

Availability of data and materials

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowlegment

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Confict of interest

The authors declare no conflicts of interest.

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Multi Response Optimization of Waste Activated Sludge Oxidation and Azo Dye Reduction in Microbial Fuel Cell

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Obtaining exoelectrogenic bacteria rich inoculum and biofilm formation on the electrode surface

The anaerobic sludge and WAS (waste activated sludge) were used both as an inoculum for the enrichment of exoelectrogenic bacteria on surface of the electrode and as an electron donor substrate, respectively. In order to increase the MLSS content of the inoculum to be used in MFC experiments, WAS was added to the anaerobic sludge. For the formation of an enriched mixture with exoelectrogenic bacteria and biofilm formation on the electrode surface, the anode chamber consists of a 400 ml mixture of 40% (v/v) WAS, 50% (v/v) anaerobic sludge, and 10% (v/v) anaerobic sludge supernatant was fed with sodium acetate (625 mg/L) and nutrient solution (1 mL of NH_4CI (193.75 mg/L), 1 ml of $MgCI_2$ (62.5 mg/L), 0.5 mL of KCI (81.25 mg/L), 1 mL of NaCI (72.5 mg/L), 5 mL of K_2HPO_4 (2125 mg/L), 8 ml of KH_2PO_4 (2600 mg/L) every 2 days for 30 days.

In addition, 0.5 mL of K_2HPO_4 (212.5 mg/L) and 20 mL of KH_2PO_4 (6500 mg/L) were added to the cathode chamber as a buffer in each experiment. Solution containing azo dye of 15 mg/L was used as the catholyte medium. A certain amount of supernatant from the mixture was removed then nutrients and acetate were added. Biofilm-covered electrodes were used in each experimental run in the experimental design.

Analysis: Oxidation of the sludge mixture in the anode chamber was monitored by COD removal (%). Volatile solids and COD analysis were performed according to standard methods of 2540 and 5220 respectively (APHA and and WEF 2005). The reduction of azo dye and the generation of aromatic compounds in the cathode chamber were determined by spectrophotometer (Hach Lange DR-6000). The wavelength at which the maximum

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absorbance is observed, 541 nm, was used for azo dye determination. The calibration curve was created with standard azo dye solutions in the range of 2 - 30 mg/L, the correlation coefficient (R^2) value of the curve was obtained as 0.9998. The removal efficiency of both COD and azo dye was calculated as shown in the Eq. (1).

$$Removal\ Efficiency\ (\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

where C_0 (mg/L) is the initial COD or azo dye concentration, and C_t(mg/L) is the COD or azo dye concentration at reaction time, t (h).

The output voltage (V) was measured with a digital multimeter (UT181A True RMS Data logging Multimeter) at 10-minute intervals with the software connected to the computer. Voltage is measured over a fixed external resistor *(R_{ext})*, while current (I) is calculated from Ohm's law according to Eq. (2) (Logan et al. 2006).

$$I = E_{cell}/R_{ext} \quad (2)$$

Where E_{cell} is the voltage measured over an external resistance (R_{ext}), I is the electrical current.

The power (P) generation is calculated according to the Eq. (3) and was normalized by the surface area of the electrode (I, in square meter).

$$P = I * E_{cell} \quad (3)$$

The Coulombic Efficiency (CE, %) is expressed as the ratio of the total Coulombs transferred from the substrate to the anode, to the maximum Coulombs that can be transferred. The total Coulombs obtained are determined by integrating the current over time, so that the Coulomb efficiency for a batch mode MFC operation, evaluated over a period t_b, is calculated according to the Eq. (4).(Logan et al. 2006; Jia et al. 2008)

$$CE\ (\%) = \frac{8\int_0^{t_b} Idt}{FV_{an}\Delta COD} \times 100 \quad (4)$$

where F is the Faraday's constant, $\Delta\ COD$ is the change in COD over time t_b and V_{an} is the working volume of the anode chamber.

A power curve describing the power density as a function of current density is calculated from the polarization curve. The polarization curve was determined by the voltage drop by different external resistance between 100000 and 10 Ω.

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Electrochemical characterization of MFC operated at optimum conditions was performed by cyclic voltammetry using a potentiostat (Gamry Reference 3000 Potentiostat). The CV (cyclic voltammetry) measurement was performed during the period when the highest rates of biodegradation and electricity generation occurred. The reference electrode was placed in the anode chamber and the anode electrode was used as a working electrode against an Ag/AgCl reference electrode while the cathode electrode worked as a counter electrode. CV measurement was performed at 25 mV.

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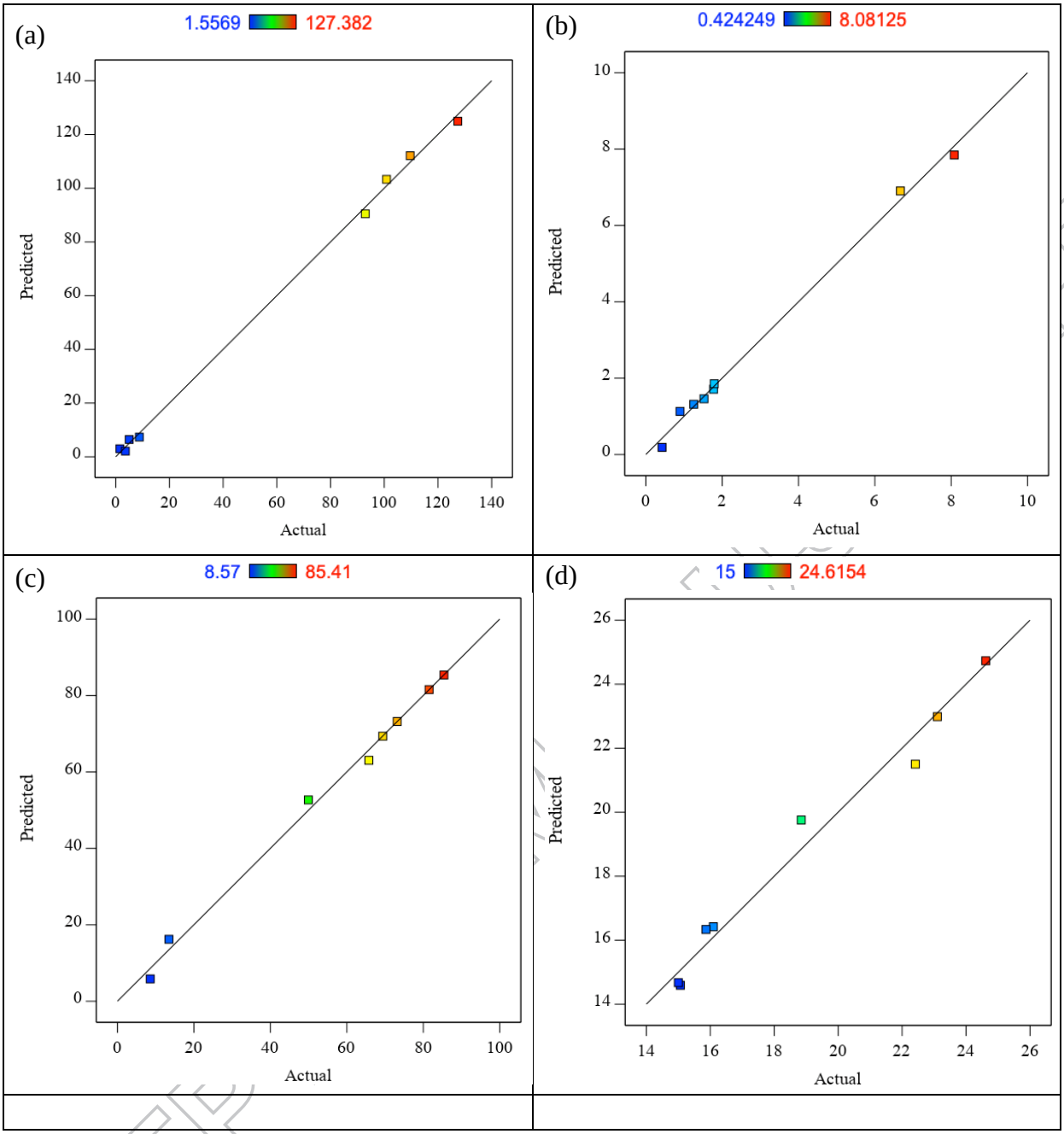


Figure S1. Actual data versus predicted responses for Power density (a), coulombic efficiency (b), azo dye removal (c), COD removal (d)

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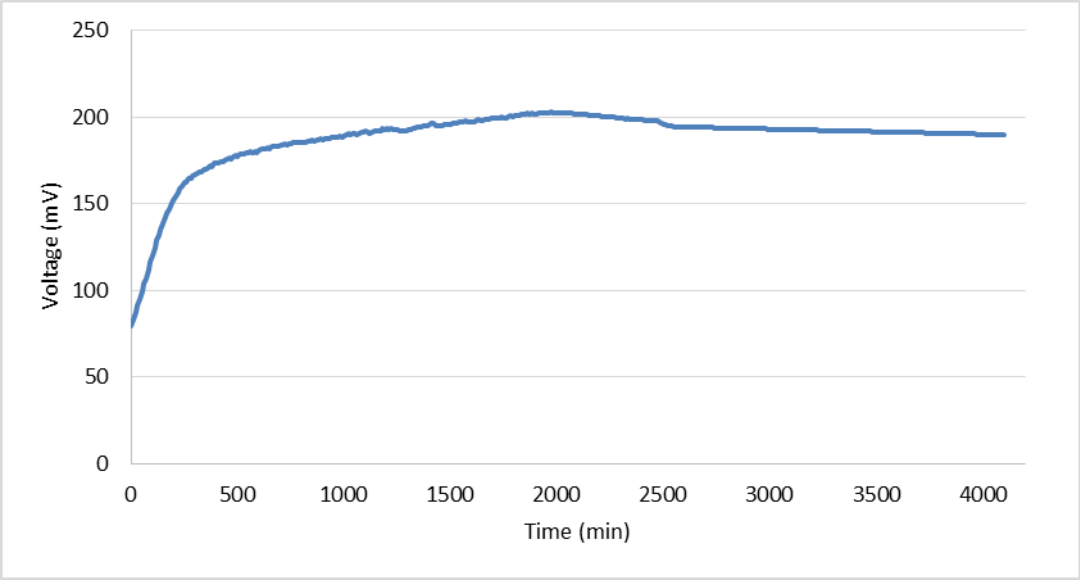


Figure S2. Voltage graph obtained from optimum experiment across 100 Ω external resistance (b)

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