ORIGINAL PAPER

Wastewater treatment and bioelectricity production in microbial fuel cell: salt bridge configurations

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Abstract

This study obtained the maximum pollutant reduction and power production from the dairy industry wastewater using double-chambered salt bridge microbial fuel cell by the Taguchi method. The maximum removal of chemical oxygen demand from dairy industry wastewater was found to be 89.7% in double-chambered salt bridge microbial fuel cell. Similarly, the current, voltage, power, current density and power density obtained in double-chambered salt bridge microbial fuel cell from dairy industry wastewater were 17.28 mA, 815.32 mV, 14.09 mW, 1309.09 mA/m^2 and 1067.33 mW/m^2, respectively. The maximum removal of chemical oxygen demand and power production was observed for the process parameters viz., 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge. It may be pointed out from the analysis of variance that the order of prevailing process parameters was agarose concentration followed by KCl molar concentration and salt bridge length for getting the maximum pollutants reduction and power production from dairy industry wastewater using double-chambered salt bridge microbial fuel cell. The other pollutants viz., TSS, TDS, BOD, COD, nitrate, phosphate, sulphate, chloride, ammonia, and oil and grease in a dairy industry wastewater also reduced to the maximum for the best-optimized process parameters of 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge. The regression model obtained in this study was utilized to select the appropriate combination of process parameters for obtaining the required maximum reduction of pollutants and simultaneous power production. Thus, this study suggested that double-chambered salt bridge microbial fuel cell can be performed well for maximum pollutant reduction and simultaneous power production for the appropriate process parameters value from any wastewater.
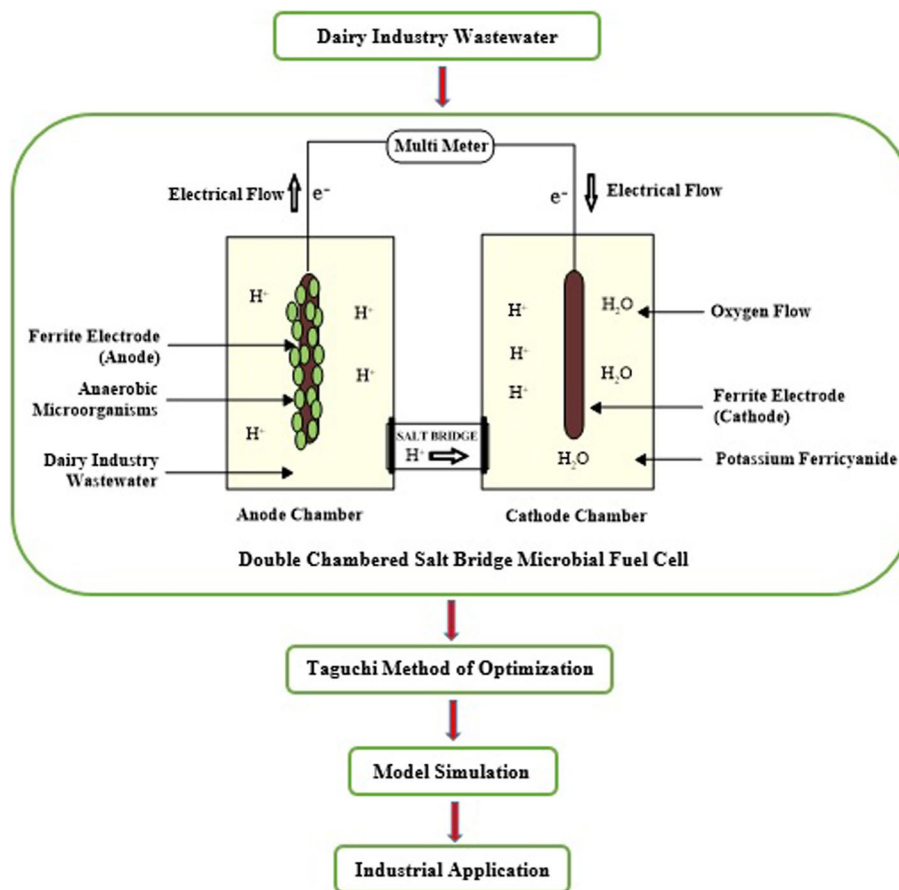
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Graphic abstract

Double Chambered Salt Bridge Microbial Fuel Cell

Abbreviations

A	Surface area
ANOVA	Analysis of variance
APHA	American Public Health Association
BOD	Biochemical oxygen demand
CD	Current density
C_i	Initial concentration
C_o	Final concentration
COD	Chemical oxygen demand
DCSB-MFC	Double-chambered salt bridge microbial fuel cell
HCl	Hydrochloric acid
I	Current
$K_3Fe(CN)_6$	Potassium ferricyanide
KCl	Potassium chloride
KNO_3	Potassium nitrate
MFC	Microbial fuel cell
NaCl	Sodium chloride
NaOH	Sodium hydroxide
P	Power
PD	Power density
R^2	Coefficient of determination
S/N ratio	Signal-to-noise ratio
TDS	Total dissolved solids
TSS	Total suspended solids
V	Voltage

Introduction

Microbial fuel cell (MFC) is a reactor in which microorganisms convert chemical energy possessed by the wastewater into electrical energy. In MFC, microorganisms use the organic matter of wastewater as food for their survival and produce electrons and protons through an external circuit, yielding power generation (Rahimnejad et al. 2014).

MFC also acts as a reactor for treating the wastewater (Singh et al. 2019), since the organic matter in wastewater used as food for the microorganism metabolism results in a reduction in organic matter as microorganisms grow in MFC (Shankar et al. 2015). The performance of MFC depends on various factors viz., the selection of electrodes, electrode surface area, the distance between anode and cathode electrodes, electron donor, electron receiver, pH, type of proton exchange medium, external circuits (Sun et al. 2014). The optimum selection of those factors yields the maximum current, voltage, and power output along with the maximum reduction of pollutants from any selected wastewater as substrate using MFC. There are three types of losses found usually during the operation of MFC, viz., (1) the electron from the microbes takes more energy from the cell wall to move towards anode electrode surface during the oxidation and reduction processes, resulting in more activation losses, (2) microorganisms took food for their survival during metabolisms from the substrate, resulting in more concentration losses, and (3) either an exchange membrane or salt bridge between the anode and cathode chambers abstract the movement of a proton between anode and cathode chambers results in more ohmic losses (Patel et al. 2019). To mitigate the above three losses, the MFC must be managed with the optimum pH conditions of both anolyte and catholyte in anode and cathode chambers, respectively. So, the selection of process parameters determines the maximum output either in terms of reduction of pollutants or in terms of power production from the selected wastewater using MFC.

MFC was operated with a batch flow type (Parkash et al. 2015) and continuous type (Sowmyashree and Udayashankara 2018). Single-chambered (Khare and Bundela 2013) and double-chambered MFCs were utilized for the treatment and simultaneous power production by the previous researchers from whey wastewater (Aishwarya et al. 2011), red cibacron-2G dye solution (Khan et al. 2012), potato processing wastewater with vermicompost (Khare and Bundela 2013), sago wastewater (Muthukumar et al. 2014), distillery industry wastewater (Jatoi et al. 2016), dye effluent (Patade et al. 2016), secondary clarifier of wastewater treatment plant (Amrita et al. 2017), and livestock wastewater (Lai et al. 2018). Different electrode materials like aluminium (Amrita et al. 2017), graphite (Khan et al. 2012; Jatoi et al. 2016; Patade et al. 2016), carbon (Dalvi et al. 2011; Khare and Bundela 2013; Shashikant and Vandana 2017), and manganese dioxide coated on carbon felt (Zang et al. 2015) were used in the anode and cathode chambers of MFC for the power production from wastewater. The Nafion 117 proton exchange membrane (Fatemi et al. 2012), Nafion cation exchange membrane (Zerrouki et al. 2018), anionic membrane Neosepta AMX (Asensio et al. 2018), ceramic membrane (Jimenez et al. 2019), and agar salt bridge (Mahendra and Mahavarkar 2013) were used for transferring the protons between the anode and cathode chambers.

The traditional methods used for finding the optimal values of selected process parameters to achieve the maximum output may not give the interactions between the selected process parameters. The statistical-based methodology provides more effective optimal solutions for obtaining the maximum outputs. One such method is the Taguchi design of experiments. The Taguchi method of approach provides the optimum value of process parameters with minimum experimental runs. Kumar and Mungray (2016) studied the effectiveness of the Taguchi method to optimize the pH of anolyte and catholyte in MFC for producing maximum power. Boas et al. (2015) performed MFC against the operating parameters and using pure culture of *Lactobacillus pentosus* for removing COD and producing simultaneous power from dairy industry wastewater. The effect of pH also studied MFC using food waste leachate (Li et al. 2013), domestic wastewater (Kishimoto et al. 2013) for bioelectricity production. Sarma et al. (2019) studied the performance of MFC using Taguchi method against the salt bridge dimensions.

Double-chambered salt bridge microbial fuel cell (DCSB-MFC) was used in this study for the reduction of pollutants and simultaneous power production from dairy industry wastewater. The Taguchi method of design of the experiment (Kishimoto et al. 2013; Kumar and Mungray 2016) was deployed in this study, to obtain optimum values of the selected process parameters. The selected parameters for this study were the molar concentration of KCl, agarose concentration, and salt bridge length. L_9 orthogonal array was implemented according to three levels and three process parameters. The relationship between selected parameters and their performance was tested with the signal-to-noise (S/N) ratio (Li et al. 2013). Analysis of variance (ANOVA) was used in this study to identify the most influencing parameter among the process parameters (Boas et al. 2015; Sarma et al. 2019). The optimum value of parameters was used only for conducting the control experiment to check the maximum output. The regression model was developed to fit the experimental data on COD removal and power production from dairy industry wastewater using DCSB-MFC. This study was undertaken in the Environmental Engineering Laboratory of Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Chennai, Tamil Nadu, India, during January 2019.

Materials and methods

Collection of wastewater and inoculum

The dairy industry wastewater was collected from Ambattur Dairy Products in Chennai, Tamil Nadu, with the help of

an airtight sterilized bottle. The collected wastewater was kept in a refrigerator at 4 °C to avoid contamination. The refrigerated dairy industry wastewater as a substrate was used in DCSB-MFC for pollutant reduction and simultaneous power production. Quantity of milk processed and milk products produced decide the characteristics of dairy industry wastewater (Kolev Slavov 2017). The characteristics of dairy industry wastewater (average of three experimental test results) are presented in Table 1. The sludge as inoculum obtained from the primary clarifier of the dairy industry wastewater treatment plant was filled in the anode chamber.

Experimental setup

The schematic diagram of DCSB-MFC used in this study is shown in Fig. 1. It consists of anode and cathode chambers connected with KCl and agarose salt bridge. Five-litre

Table 1 The characteristics of dairy industry wastewater

Sl.no.	Parameters	Values
1.	pH	8.5–9.8
2.	Colour	White colour
3.	Total suspended solids (mg/L)	1565 ± 40
4.	Total dissolved solids (mg/L)	3875 ± 90
5.	BOD_5 (mg/L)	2350 ± 76
6.	COD (mg/L)	3654 ± 115
7.	Nitrate (mg/L)	47 ± 8
8.	Phosphate (mg/L)	65 ± 11
9.	Sulphate (mg/L)	855 ± 25
10.	Chloride (mg/L)	760 ± 22
11.	Ammonia (mg/L)	87 ± 12
12.	Oil and grease (mg/L)	16 ± 2

capacities of anode and cathode chambers with a working volume of 4.75 L were used in this study. Ferrite circular electrode surface area of 0.0132 m² (length 0.2 m, diameter 0.02 m) was used in both anode and cathode chambers because of its high strength and good electrical conductivity properties. The anaerobic and aerobic conditions were maintained in anode and cathode chambers, respectively.

The anode chamber was connected with the cathode chamber through an external copper wire electrical circuit. The current (I) in amps and voltage (V) in volts were monitored with a digital multimeter. The power, current density, and power density were calculated using Eqs. 1–3, respectively.

$$\text{The power} (P) = I \times V \tag{1}$$

$$\text{The Current Density} (CD) = \frac{I}{A} \tag{2}$$

$$\text{The Power Density} (PD) = \frac{P}{A} \tag{3}$$

in which 'I' is current in A, 'P' is power in W and 'A' is surface area in m² of electrode used. The various characteristics such as pH, colour, TSS, TDS, BOD, COD, nitrate, phosphate, sulphate, chloride, ammonia and oil and grease were determined as per the procedure stipulated in APHA (2017). The concentration reduction percentage was calculated (Sivakumar 2015) for all selected pollutants from dairy industry wastewater follows Eq. 4.

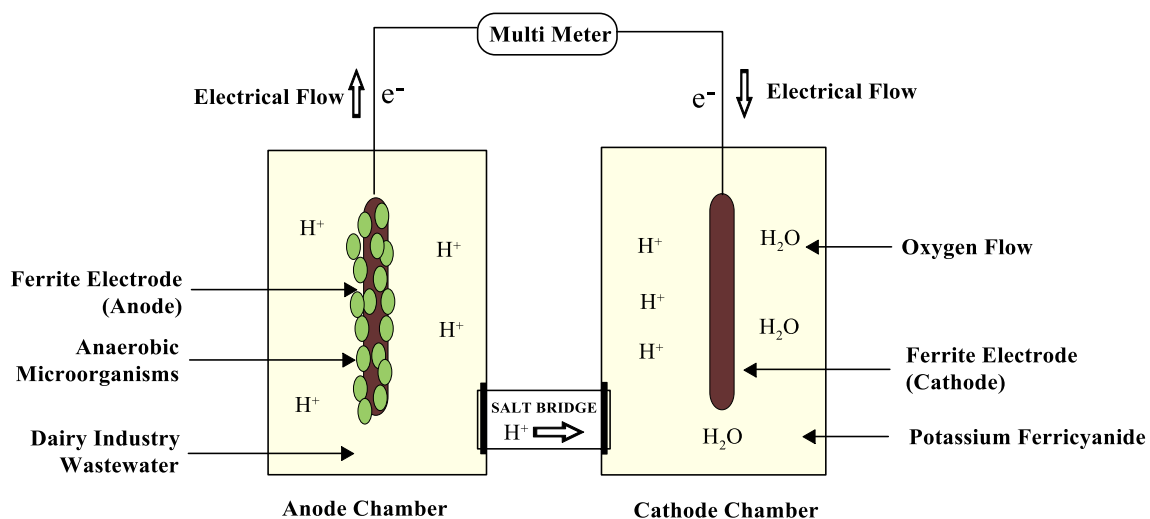$$\text{The Percentage Reduction} = \frac{C_i - C_o}{C_i} \times 100 \tag{4}$$

Fig. 1 Schematic diagram of double-chambered salt bridge microbial fuel cell

in which C_i is the initial concentration of pollutants and C_o is the final concentration of pollutants in dairy industry wastewater after treating with DCSB-MFC.

Experimental procedure

DCSB-MFC was operated for 10 days in a batch method. In this study, the pollutant removal and power production values were obtained after taking the average of three trials of the experimental data. The pH of the dairy industry wastewater was adjusted for each trial until it gets stabilized. The neutral pH for the substrate was maintained in this study by adding both 0.1 M of NaOH and 1 M of HCl solutions. The current and voltage were observed from the digital multimeter and calculated power, current density and power density by using Eqs. 1–3. Furthermore, wastewater samples were analysed between 1 and 10 days as per the procedures stipulated in APHA 2017.

Taguchi method

L_9 (3×3) orthogonal array was used for this study. The experimental investigations were conducted at three levels using three parameters (KCl molar concentration, agarose concentration, and salt bridge length) for reducing the pollutants and producing the power from dairy industry wastewater using DCSB-MFC. The selected three levels for the parameters were 1, 2, and 3 M KCl concentration, 5, 10, and 15% agarose concentration and 5, 10, and 15 cm salt bridge length.

Signal-to-noise (S/N) ratio

The *S/N* ratio is used to determine the controllable and uncontrollable factors that influence the performance of MFC. *S/N* ratio can be categorized as larger the better, smaller the better and nominal the best. In this study, the optimization was decided based on the larger the better category, since the objective of this study is to maximize the pollutants reduction and power production from dairy industry wastewater using DCSB-MFC. The large the better category condition was calculated from Eq. 5 (Kumar and Mungray 2016; Sarma et al. 2019; Sivakumar 2020).

$$\frac{S}{N} = -10 \times \log 10 \left(\frac{1}{n} \sum_{i=1}^{n} \frac{1}{y_i^2} \right) \tag{5}$$

Analysis of variance

Analysis of variance (ANOVA) is used to know the effect of process parameters and percentage contribution to the performance of DCSB-MFC. The Minitab 18 (version 18.1)

package was used for analysing the data obtained from the experimental investigations. The optimal values of the selected process parameters from the Taguchi methods were verified with the control experiments.

Statistical analyses

The statistical analyses were performed using SPSS Statistics version 26 software for all measured and calculated values from the optimized and other process parameters used in the DCSB-MFC from the dairy industry wastewater.

Results and discussion

The experimental investigations were conducted using DCSB-MFC for removing various pollutants and producing power from the dairy industry wastewater. DCSB-MFC was operated with ferrite electrode surface area of 0.0132 m², the dilatation ratio of '0', no mediator, no additional electron transfer microorganisms, an oxygen flow rate of 10 m³/h, $K_3Fe(CN)_6$ electron acceptor and KCl salt bridge at room temperature (28 °C). The parameters and their levels were chosen for this study are presented in Table 2.

The experiments were conducted in DCSB-MFC form dairy industry wastewater using three process parameters viz., KCl molar concentration, agarose concentration, and salt bridge length at three different levels. The Taguchi orthogonal arrays were framed according to the selected three process parameters at three levels. The parameters and levels assigned to the L_9 orthogonal arrays and are presented in Table 3. The experiments were conducted three times to make the system consistency and the average values only represented as the maximum value obtained from this study. The lager-the-better category was implemented in this study to get the optimized parameters at various levels.

Figures 2, 3, 4, and 5 show the COD reduction percentage, current, voltage, and power observed in DCSB-MFC using dairy industry wastewater for Run 1 to Run 9 against the contact time from day 1 to 10. From Figs. 2, 3, 4, and 5, it may be observed that the maximum COD reduction, current, voltage, and power production in MFC from dairy industry wastewater was found to be 86.3%, 15.91 mA, 734.61 mV, and 11.68 mW (Run 2). Thus, 1 M KCl concentration, 10%

Table 2 Process parameters and their levels

Process parameters	Levels		
	1	2	3
KCl molar concentration (*A*)	1M	2 M	3 M
Agarose concentration (*B*)	5%	10%	15%
Salt bridge length (*C*)	0.05 m	0.10 m	0.15 m

Table 3 Taguchi orthogonal arrays (L_9)

Experimental runs	A	B	C
1	1	1	1
2	1	2	2
3	1	3	3
4	2	1	3
5	2	2	1
6	2	3	2
7	3	1	2
8	3	2	3
9	3	3	1

agarose concentration, and 0.10 m salt bridge length (Run 2) found in this study were considered as the optimum process parameters value than other combinations (Run 1–9 except Run 2) for the treatment and power production from dairy industry wastewater using DCSB-MFC. The corresponding maximum current density and power density in DCSB-MFC from dairy industry wastewater were obtained as 1205.30 mA/m^2 and 885.43 mW/m^2, respectively (Table 4).

Furthermore, it may be noted from Figs. 2, 3, 4 and 5 that the minimum treatment efficiency of COD and minimum power production from the dairy industry wastewater using DCSB-MFC were observed for the combination of 3 M KCl concentration, 5% agarose concentration, and 0.10 m salt bridge length (Run 7). The minimum COD reduction, current, voltage and power, current density, and power density obtained in MFC from dairy industry wastewater as

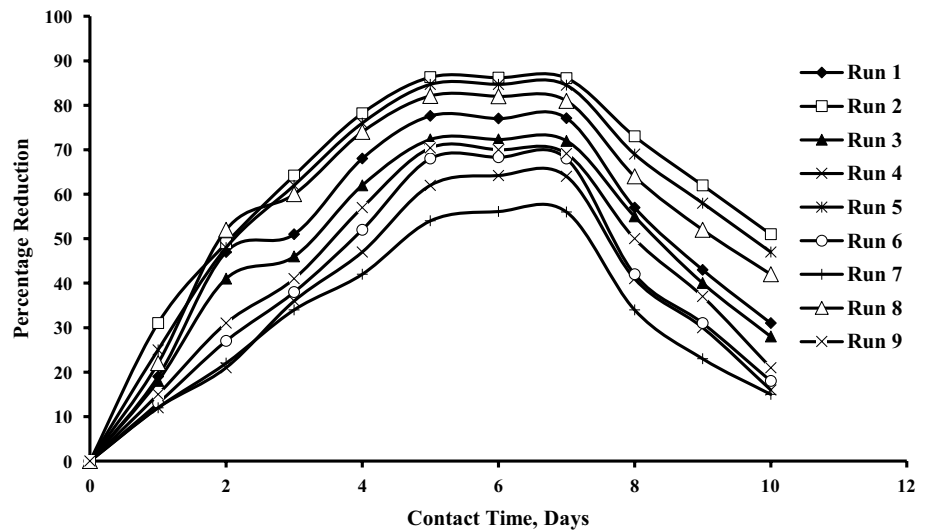**Fig. 2** The COD reduction percentage from dairy industry wastewater using DCSB-MFC

Fig. 3 The current production from dairy industry wastewater using DCSB-MFC
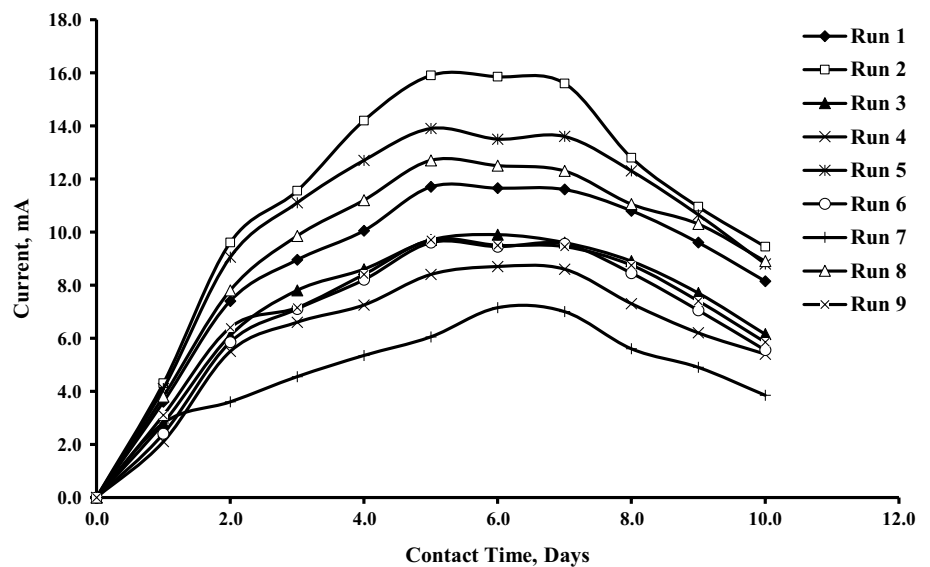
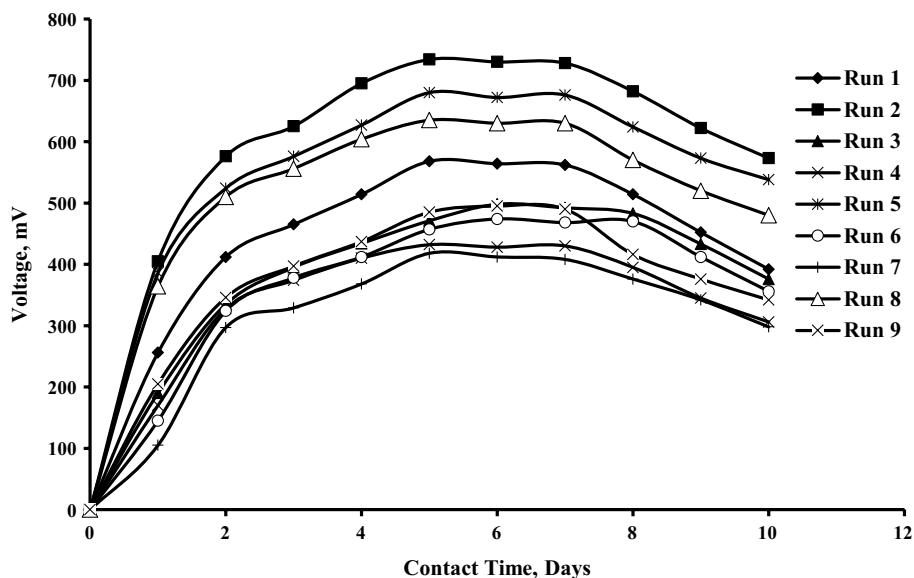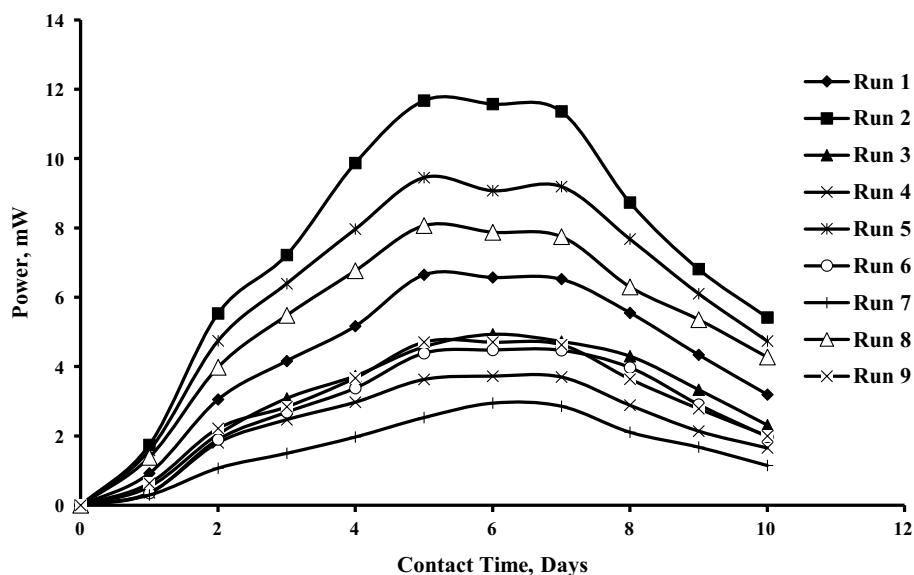**Fig. 4** The voltage production from dairy industry wastewater using DCSB-MFC

Fig. 5 The power production from dairy industry wastewater using DCSB-MFC

56.1%, 7.15 mA, 418.31 mV, 2.99 mW, 541.67 mA/m^2, and 226.58 mW/m^2, respectively (Run 7). The order of maximum COD reduction and power production from dairy industry waster using DCSB-MFC against various Runs was found to be Run 2 > Run 5 > Run 8 > Run 1 > Run 3 > Run 9 > Run 6 > Run 4 > Run 7 (Table 4).

Figures 2, 3, 4, and 5 indicated that the continuous output was obtained after 2 h from the commencement of the experiments and the trend of the COD reduction percentage, current, voltage, and power production variation against the different contact time was the same. The trend of COD reduction percentage, current, voltage, and power variation was similar to the trend of the bacterial growth curve against time. The maximum value of percentage reduction of COD, current, voltage, and power production was obtained for the period between 5 and 7, which was corresponding to the stationary phase of microbial growth in the substrate because the biofilm was fully developed at the anode electrode during the stationary phase period. After 5 days, the percentage reduction of COD, current, voltage, and power production attain their maximum value and maintain the same maximum COD reduction, current, voltage, and power production for successive 3 days (small fluctuations occurred during those 3 days due to variation in metabolism of microorganisms). There was a decline after 7 days, because of the death of microorganisms in the substrate (dairy industry wastewater). Beyond the stationary phase period, current and voltage values started to drop, which is corresponding to the decline phase of the microbial growth curve. The dead microorganisms in the dairy industry wastewater as substrate contribute

Table 4 L_9 orthogonal designs, levels of three factors and experimental results obtained from the DCSB-MFC using dairy industry wastewater

Experimental runs	Experimental design			Experimental results						S/N ratio	
	A	B	C	COD removal (%)	Current (mA)	Voltage (mV)	Power (mW)	Current density (mA/m²)	Power density (mW/m²)	Power	COD removal
1	1	5	0.05	77.6	11.72	568.42	6.66	887.88	504.69	16.47	37.80
2	1	10	0.10	86.3	15.91	734.61	11.69	1205.30	885.43	21.35	38.72
3	1	15	0.15	72.3	9.97	498.13	4.97	755.30	376.24	13.92	37.18
4	2	5	0.15	64.2	8.73	432.67	3.78	661.36	286.15	11.54	36.15
5	2	10	0.05	84.7	13.98	680.52	9.51	1059.09	720.73	19.57	38.56
6	2	15	0.10	68.3	9.64	474.25	4.57	730.30	346.35	13.20	36.69
7	3	5	0.10	56.1	7.15	418.31	2.99	541.67	226.58	9.52	34.98
8	3	10	0.15	82.1	12.71	635.77	8.08	962.88	612.17	18.15	38.29
9	3	15	0.05	70.4	9.78	495.39	4.84	740.91	367.04	13.71	36.95

to current and voltage reduction. The less reduction percentage of COD and power generation were observed for the Run 7 is due to poor transfer of electrons and protons between the anode and cathode chambers, the less microbial activity of the substrate in the anode chamber and less H_2 liberation activity of electrolyte in the cathode chamber. Thus, the reduction of COD, current, voltage, and power production depends on the microbial activities on the substrate in the anode chamber and electrolyte in the cathode chamber. The high the microbial activities in the anode chamber and high liberation activity of electrolyte in the cathode chamber, more the voltage produced in DCSB-MFC.

The typical anode electrode and cathode electrode reaction in DCSB-MFC is presented in Eqs. 6 and 7, respectively.

Anodic reaction : Biodegradation material
+ microorganisms → CO_2 + H^+
+ e^- (anaerobic conditions) (6)
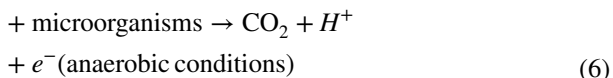
Cathodic reaction : $4H + 4e^- + O_2 → 2H_2O$ (aerobic conditions) (7)

The electrons generated (Eq. 6) in the anode chamber of DCSB-MFC travels across the external circuit to the cathode chamber. In the cathode chamber, the electrons from the external circuit were joined with protons (H^+) from the anode chamber through a salt bridge and oxygen (O_2) to produce water (Eq. 7), completing the half-reaction (Chae et al. 2008). The mixed culture, including bacteria, fungi, and yeast species, in the dairy industry wastewater was identified for the production of electrons in the anode chamber. Though the bacteria, fungi, and yeast species available in the dairy industry wastewater, the species like *Escherichia coli*, *Bacillus subtilis*, *Clostridium butyricum*, and *Saccharomyces cerevisiae* were dominated to produce more electrons

in the anode chamber of DCSB-MFC (Garcha et al. 2016; Al-Wasify et al. 2017).

Taguchi L_9 orthogonal arrays and response for S/N ratio

In this study, experiments were conducted using DCSB-MFC according to Taguchi L_9 orthogonal arrays and responses (COD removal percentage, current, voltage, and power) corresponding to various combinations of process parameters (Run 1–Run 9) was obtained. The L_9 orthogonal arrays consist of minimum experimental runs with various combinations of process parameters. L_9 orthogonal designs, levels of three factors, and experimental results obtained from the DCSB-MFC using dairy industry wastewater are presented in Table 4.

The output of L_9 orthogonal arrays was optimized based on the S/N ratio. The S/N ratio was assessed to know the effect of process parameters on the removal of pollutants and power production from the dairy industry wastewater. This study optimized the process parameters based on the larger the better category (Eq. 5) since the objective of this study is to maximize the pollutants reduction and power generation from the dairy industry wastewater using MFC.

The response (maximum power and COD reduction percentage) for the S/N ratio from Run 1 to Run 9 at various levels is presented in Table 5. The average S/N ratio for each process parameter in each level is shown in Figs. 6 and 7. According to the average maximum S/N ratio (Table 5), level 1, level 2, and level 1 exhibited the maximum value against the KCl molar concentration, agarose concentration, and salt bridge length, respectively, for both maximum power production and COD reduction percentage from dairy industry wastewater using DCSB-MFC.

Table 5 The response for *S/N* ratio from run 1 to run 9

Levels	KCl molar concentration (A)	Agarose concentration (B)	Salt bridge length (C)
Maximum power (mW)			
1	17.25	12.51	15.94
2	14.77	19.69	15.53
3	13.79	13.61	14.33
Delta	3.46	7.18	1.61
Rank	2	1	3
COD reduction (%)			
1	37.90	36.31	37.59
2	37.13	38.52	37.27
3	36.74	36.94	36.91
Delta	1.16	2.21	0.68
Rank	2	1	3

The maximum *S/N* ratio yielded for the power produced by the KCl molar concentration, agarose concentration, and salt bridge length against various levels of the maximum power production was found to be 17.25, 19.69, and 15.94 (Fig. 6). Similarly, the maximum *S/N* ratio yielded for the

COD reduction percentage by the KCl molar concentration, agarose concentration, and salt bridge length against various levels for the maximum COD reduction percentage were found to be 37.90, 38.52, and 37.59 (Fig. 7). It may also be observed that the maximum delta variation exhibited by the agarose concentration has a high influence on the performance of DCSB-MFC for treating the dairy industry wastewater and simultaneous power production followed by KCl molar concentration and salt bridge length (Table 5). According to the Taguchi approach, agarose concentration is the most dominant and salt bridge length is the least dominant parameter in the production of power and reduction of pollutants from dairy industry wastewater.

Analysis of variance (ANOVA)

The analysis of variance (ANOVA) was used in this study to know the process parameters were contributed significantly to obtain the maximum removal of pollutants and power production from the dairy industry wastewater using MFC. Hence, along with the *S/N* ratio, ANOVA was done in this study. The ANOVA for all process parameters is presented in Table 6. From Table 6, it may be noted that the order of
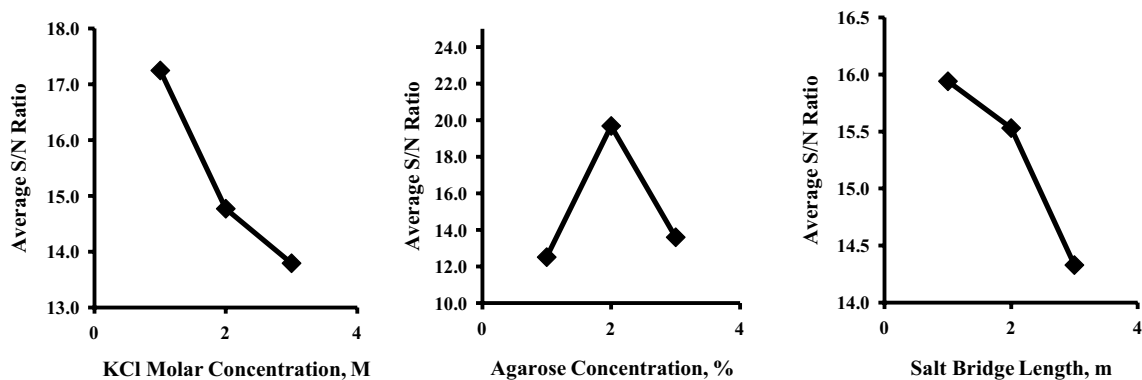

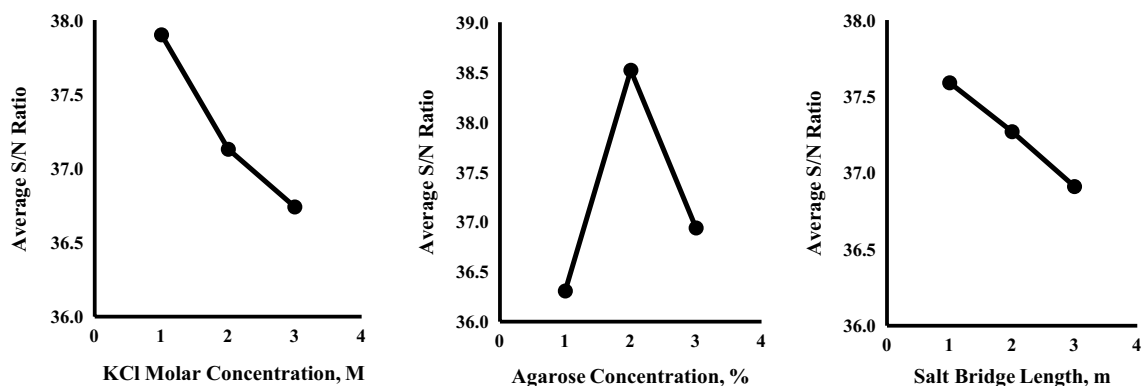**Fig. 6** Average *S/N* ratio for maximum power

Fig. 7 Average *S/N* ratio for COD reduction percentage

Table 6 Analysis of variance (ANOVA) for maximum power and COD reduction

Source	Degree of freedom	Sum of square	Average of squares	F-value	P value	Percentage contribution
Maximum power (mW)						
A	2	9.836	4.918	7.11	0.123	14.72
B	2	52.682	26.341	38.08	0.026	78.83
C	2	2.936	1.468	2.12	0.320	4.39
Error	2	1.383	0.612			2.07
Total	8	66.836				100.00
COD reduction (%)						
A	2	132.97	66.48	1.61	0.384	16.47
B	2	554.56	277.28	6.70	0.130	68.69
C	2	37.03	18.51	0.45	0.691	4.59
Error	2	82.80	41.40			10.26
Total	8	807.36				100.00

observed prevailing process parameters as agarose concentration > KCl molar concentration > salt bridge length. The results of ANOVA (Table 6) were a good agreement with the results of the response for the *S/N* ratio (Table 5).

Confirmation test

The best possible results obtained from the experimental investigations conducted at different process parameters with different levels are called optimization. According to Taguchi, the best possible results (optimized values) were observed for the experimental Run 2 (Table 4), but it is not giving the true optimum results. Taguchi gives the best results at each level for different parameters. Hence, confirmatory experimental investigations were conducted using the optimum values of each parameter selected from different levels. The selected best-optimized parameters for each level were found to be KCl molar concentration of 1 M, an agarose concentration of 10%, and salt bridge length of 0.05 m (Table 5) for the maximum power production (Fig. 6) and maximum COD reduction (Fig. 7) from dairy industry wastewater using DCSB-MFC.

The maximum COD removal, current, voltage, power, current density, and power density for the combination 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge from control experiments were found to be 89.7%, 17.28 mA, 815.32 mV, 14.09 mW, 1309.09 mA/m^2, and 1067.33 mW/m^2, respectively, from dairy industry wastewater using DCSB-MFC. The results of the confirmation experiments showed that there was an increment of 3.94, 8.61, 10.99, 20.52, 8.61, and 20.52% for COD removal, current, voltage, power, current density, and power density, respectively, against the selected optimum values of process parameters for each level (Table 5). Thus, the 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge were considered as the best-optimized parameters for

treating and producing power from dairy industry wastewater using DCSB-MFC at maximum level.

Verification test

In this study, 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge (confirmation test results) used for reducing maximum COD and producing the maximum power from dairy industry wastewater using DCSB-MFC. To check the performance of DCSB-MFC for removing other pollutants from dairy industry wastewater, verification experiment was conducted against the best-optimized parameters 1 M KCl concentration, 10% agarose concentration and 0.05 m salt bridge (from confirmation test) along with other process parameters viz., ferrite electrode, the ferrite electrode surface area of 0.0132 m^2, the dilatation ratio of '0', the operating temperature of 28 °C, no mediator, no additional electron transfer microorganisms, an oxygen flow rate of 10 m^3/h, K$_3$Fe(CN)$_6$ electron acceptor.

The maximum removal percentage of TSS, TDS, BOD, COD, nitrate, phosphate, sulphate, chloride, ammonia, and oil and grease before and after treatment with DCSB-MFC using best-optimized parameters are presented in Table 7. As similar to COD reduction and power production from dairy industry wastewater using DCSB-MFC, the other parameters in a dairy industry wastewater also reduced to the maximum at the best-optimized process parameters of 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge. The pH found in a dairy industry wastewater after treatment with MFC was 7.4 ± 0.6.

The performance of DCSB-MFC for the maximum reduction of pollutants and power production using dairy industry wastewater against the selected process parameters and optimized parameters of this study was compared with the other studies. The results of this study and other studies on current, voltage, power, current density, and power density

Table 7 The characteristics of treated dairy industry wastewater using MFC

Sl.no.	Parameters	Raw wastewater (mg/L)	Treated wastewater (mg/L)	Reduction (%)
1	Total suspended solids	1565 ± 40	135 ± 6	91.4
2	Total dissolved solids	3875 ± 90	597 ± 12	84.6
3	BOD_5	2350 ± 76	181 ± 16	92.3
4	COD	3654 ± 115	376 ± 23	89.7
5	Nitrate	47 ± 8	6 ± 1	87.1
6	Phosphate	65 ± 11	9 ± 2	86.3
7	Sulphate	855 ± 25	177 ± 6	79.3
8	Chloride	760 ± 22	135 ± 2	82.3
9	Ammonia	87 ± 12	5 ± 1	94.6
10	Oil and grease	16 ± 2	2 ± 0	87.5

from various industry wastewaters using MFC are presented in Table 8.

The previous study used the combination of various slats for manufacturing the salt bridge with agar, which can be connected between anode and cathode chambers of MFC (Khan et al. 2012). Khan et al. (2012) performed the salt bridge MFC for decolourization along with power production from the Red Cibacron-2G Dye solution. The salt bridge was prepared with 100 g/L agar and 0.5 g each of KCl, NaCl, and KNO_3. The electrode used in this study was the graphite rod of surface area 6.031 cm². The maximum current and voltage production from MFC was found to be 5.2 mV and 5 μA, respectively. Furthermore, the maximum power density and current density were recorded as 100 mW/m² and 1.04 A/m², respectively, in 24 h of operation (Khan et al. 2012).

Khare and Bundela (2013) studied the single-chambered MFC for generating the power and treating the wastewater blended with vermicompost. MFC was made with 1 M KCl and 3% agarose salt bridge, carbon electrodes as the anode and cathode, and methylene blue as the mediator between anode and cathode chambers. The maximum voltage produced in MFC from blended potato processing wastewater with vermicompost was found to be 292 mV, and a mixture of cow dung with vermicompost was found to be 232 mV. The maximum voltage was obtained for 5 and 4 days, respectively, for the blended potato processing wastewater with vermicompost and mixture of cow dung with vermicompost.

Mahendra and Mahavarkar (2013) studied the dairy industry wastewater treatment and power generation using mediator less single- and double-chambered MFC with an 8-litre working volume of the substrate using a graphite rod as an electrode, agar salt bridge as proton exchanger between the anode and cathode chambers and KCl as an electrolyte in the cathode chamber. The maximum current obtained from the single- and double-chambered MFC was found

to be 1.02 mA and 0.58 mA, respectively (Mahendra and Mahavarkar 2013) for 12 days. Graphite sheet of 0.027 m² was used for producing a current density of 223.3 mA/m² and a power density of 134.6 mW/m² from sago wastewater in double-chambered MFC (Muthukumar et al. 2014). The maximum voltage (603 mV) and current (6.03 mA) produced after 18 days by Muthukumar et al. (2014) against the KCl and agar salt bridge between anode and cathode chambers, and cathode chamber filled with phosphate buffer solution and 50 mM NaCl.

Patade et al. (2016) and Khan et al. (2012) used dye effluent instead of wastewater from the wastewater treatment plants for producing electrical power and simultaneous wastewater treatment. 10% KCl and 7% agar salt bridge of length 0.12 m and 0.02 m diameter was connected between the anode and cathode chambers of MFC for the power generation (Patade et al. 2016). The maximum current of 0.64 mA was obtained by not adding molasses as inoculum with dye effluent. The maximum current of 0.768 mA was observed against 20% molasses as inoculum with dye effluent (Patade et al. 2016) and graphite electrode.

Kumar and Mungray (2016) obtained the maximum current density of 2914.29 mA/m³ and the power density of 1270.12 mW/m³ from the working volume of 350 ml glucose synthetic water against the external resistance of 220 Ω with anolyte pH of 8 and catholyte pH of 2. Kishimoto et al. (2013) obtained the maximum current from the restaurant wastewater, and it was found to be 0.678 ± 0.297 mA. According to the Taguchi method, the current production was 2.9 times higher than the control experiments when MFC was conducted with an air cathode pH of 7 (Kishimoto et al. 2013).

Amrita et al. (2017) produced the maximum voltage and current density of 0.145 mV and 0.06 A/m², respectively, using MFC with 1 M KCl and 3% agar salt bridge and aluminium mesh as an electrode. But, 1 M KCl and

Table 8 Comparison of current, voltage, power, current density and power density from various industry wastewaters using MFC

Sl.no.	Type of MFC	Substrate	Electrodes	Mediator	Voltage	Current	Power	Current density	Power density	References
1	Double chambered	Whey wastewater	Carbon rod	Mediator less	350 mV	–	–	–	–	Aishwarya et al. (2011)
2	Double chambered	Red cibacron-2G dye solution	Graphite rod	Mediator less	5.2 mV	5 µA	26 mW	1.04 A/m^2	100 mW/m^2	Khan et al. (2012)
3	Single chambered	Potato processing waste water	Carbon rod	Methylene blue mediator	292 mV	–	–	–	–	Khare and Bundela (2013)
4	Double chambered	Sago wastewater	Graphite sheet	Mediator less	603 mV	6.03 mA	3.63 mW	223.3 mA/m^2	134.6 mW/m^2	Muthukumar et al. (2014)
5	Double chambered	Distillery industry wastewater	Graphite rod	Mediator less	435 mV	43 mA	18.750 mW	–	–	Jatoi et al. (2016)
6	Double chambered	Dye effluent	Graphite rod	Mediator less	–	0.64 mA	–	–	–	Patade et al. (2016)
7	Double chambered	Restaurant waste-water	Carbon fibre brush	Mediator less	0.435 mV	0.678 mA	0.295 mW	2914.29 mA/m^3	1270.12 mW/m^3	Kumar and Mungray (2016)
8	Double chambered	Wastewater	Aluminium mesh	Mediator less	0.145 V	–	–	0.06 A/m^2	–	Amrita et al. (2017)
9	Single chambered	Blended dairy and distillery industries wastewater	Stainless steel	Mediator less	6.54 V	7.99 mA	0.052 mW	707.07 mA/m^2	4.58 W/m^2	Shashikant and Vandana (2017)
10	Double chambered	Dairy industry wastewater	Ferrite rod	Mediator less	815.32 mV	17.28 mA	14.09 mW	1309.09 mA/m^2	1067.33 mW/m^2	This study

10% agarose salt bridge of length 5 cm and diameter 2 cm produced the voltage and current of 435 mV and 43 mA, respectively, using MFC from distillery industry wastewater against the external resistance of 10 Ω and graphite electrode (Jatoi et al. 2016). Shashikant and Vandana (2017) produced the current, voltage, and power generation of 7.99 mA, 6.54 V, and 4.58 W/m^2, respectively, in the batch flow type single-chambered MFC for treating the dairy and distillery blended wastewater and producing electricity using agar salt bridge in 7 days operation.

As similar to Jatoi et al. (2016) and Amrita et al. (2017), 1 M KCl and 5% agar salt bridge and carbon electrode were used for producing a voltage of 350 mV from whey wastewater along with treatment of whey wastewater (Aishwarya et al. 2011) in MFC. The external resistance of 10 Ω was used for producing the maximum power from wastewater using MFC by Aishwarya et al. (2011) and Jatoi et al. (2016), but Muthukumar et al. (2014) used 100 Ω used for producing the power and simultaneously treating the wastewater using MFC.

The maximum COD removal was obtained as 85.4% at the anolyte pH of 7.6 using two-chambered MFC from the food waste leachate for bioelectricity production (Li et al. 2013). Mahendra and Mahavarkar (2013) observed that the maximum COD removal of 86.42% and 84.8% from the single- and double-chambered MFC, respectively, using dairy industry wastewater. Besides, Mahendra and Mahavarkar (2013) found that the maximum reduction occurred for the 100% substrate concentration.

The COD removal from the dairy industry wastewater using two-chambered MFC was between 56 and 61% when the MFC was operated with proton exchange membrane, graphite brush with filaments of carbon fibre as anode and plain carbon paper with platinum black as cathode from the dairy industry wastewater (Boas et al. 2015). The COD removal was achieved by about 84.77% and 77.25%, respectively, for single- and double-chambered MFCs (Shashikant and Vandana 2017). The corresponding TDS removal was about 73.51% and 72.23%, respectively, for single- and double-chambered MFC (Shashikant and Vandana 2017). The removal efficiency of both MFCs was achieved from the 2-litre blended dairy and distillery wastewater, agar salt bridge, and 7 day operation period.

Though the membrane produced more power than a salt bridge (Shashikant and Vandana 2017), salt bridge produces the power equivalent to the power produced by membrane against the suitable combination of various parameters value using MFC from wastewater (Li et al. 2013; Muthukumar et al. 2014) and also the cost of power production using MFC from wastewater is less compared to the cost involved in MFC with a membrane. The experimental and model results of this study suggested an idea to make DCSB-MFC batch bioreactor into continuous flow DCSB-MFC bioreactor with further modification, and DCSB-MFC could be more helpful for the industry to reduce the power consumption at the site.

Mathematical model

Regression analysis is used to fit the mathematical model using experimental data obtained from the best-optimized process parameters. In this study, the coefficients of the regression model for maximum reduction of COD and maximum power output from dairy industry wastewater in DCSB-MFC were obtained using the software Minitab 18 (version 18.1) and the regression equation is expressed by Eq. 8.

$$\begin{aligned} Y_{COD} \text{ (or) } Y_{Power} = {} & A_0 + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_{11} X_1 X_1 \\ & + A_{22} X_2 X_2 + A_{33} X_3 X_3 + A_{12} X_1 X_2 \\ & + A_{23} X_2 X_3 + A_{31} X_3 X_1 + A_{123} X_1 X_2 X_3 \end{aligned}$$

(8)

in which, A_0 is the interception coefficient, A_1, A_2 and A_3 are the linear coefficients, A_{11}, A_{22} and A_{33} are the one-way interaction coefficient, A_{12}, A_{23} and A_{31} are the two-way interaction coefficient, and A_{123} is the three interaction coefficient, and X_1, X_2, and X_3 are the independent variables viz., KCl molar concentration, agarose concentration, and length salt bridge.

As per Taguchi L_9 orthogonal array, a total of 9 experimental Runs were performed in DCSB-MFC using dairy industry wastewater for maximum reduction of COD and power generation using various process parameters and against different KCl molar concentration, agarose concentration, and length salt bridge combinations (Runs 1–9). But, the model was developed for Run 2, because Run 2 (experiments conducted at 1 M KCl molar concentration, 10% agarose concentration and 0.05 m salt bridge length) produced the maximum reduction percentage of COD, current, voltage and power production from dairy industry wastewater using DCSB-MFC.

Validation test

To validate and know the best fit of the proposed mathematical model with experimental data, the regression coefficients and the coefficient of determination (R^2) were evaluated from the regression equation (Sivakumar 2016). The regression equation for the maximum COD reduction and power production from dairy industry wastewater using DCSB-MFC is presented in Eqs. 9 and 10, respectively.

$$Y_{COD} = 65.93 - 21.470X_1 + 11.662X_2 - 410.33X_3 + 1.733X_1X_1 - 0.6013X_2X_2$$
$$+ 1473.3X_3X_3 + 0.52X_1X_2 + 0.023X_2X_3 + 47.33X_3X_1 + 0.011X_1X_2X_3 \tag{9}$$

$$Y_{Power} = -5.903 - 4.802X_1 + 3.853X_2 + 55.532X_3 + 0.585X_1X_1 - 0.212X_2X_2$$
$$- 233.303X_3X_3 + 0.189X_1X_2 + 0.02X_2X_3 + 6.667X_3X_1 + 0.01X_1X_2X_3 \tag{10}$$

The observed and predicted values (from Eqs. 9 to 10) for maximum COD reduction and maximum power production from dairy industry wastewater using DCSB-MFC are presented in Table 9. The correlation between the experimental results and model results for COD reduction percentage (Table 9) and power production (Table 9) in DCSB-MFC from dairy industry wastewater is shown in Fig. 8a and b, respectively.

The R^2 value of 0.998 and 0.996, respectively, for the COD reduction (Fig. 8a) and power production (Fig. 8b) from dairy industry wastewater using DCSB-MFC between experimental and model results indicated that the model prediction is highly significant with the experimental results. Thus, the selected optimum value of

1 M KCl concentration, 10% agarose concentration, and 0.05 m length salt bridge from the Taguchi approach is the best value for the reproduction of experimental investigations to get the maximum COD reduction and power production using DCSB-MFC.

Conclusion

This study performed the DCSB-MFC with ferrite electrode, the ferrite electrode surface area of 0.0132 m², the dilatation ratio of '0', the operating temperature of 28 °C, no mediator, no additional electron transfer microorganisms, an oxygen flow rate of 10 m³/h, $K_3Fe(CN)_6$ electron acceptor against

Table 9 Experimental results and model results for COD reduction and power production from dairy industry wastewater using MFC

Experimental runs	X_1 (A)	X_2 (B)	X_3 (C)	COD reduction percentage		Power production	
				Observed	Predicted	Observed	Predicted
1	1	5	0.05	77.6	77.58	6.66	6.67
2	1	10	0.10	86.3	86.31	11.69	11.72
3	1	15	0.15	72.3	72.35	4.97	5.03
4	2	5	0.15	64.2	64.21	3.78	3.81
5	2	10	0.05	84.7	84.70	9.51	9.53
6	2	15	0.10	68.3	68.35	4.57	4.62
7	3	5	0.10	56.1	56.11	2.99	3.02
8	3	10	0.15	82.1	82.16	8.08	8.15
9	3	15	0.05	70.4	70.43	4.84	4.87

Fig. 8 Relationship between experimental and model results of COD reduction and power production from dairy industry wastewater using DCSB-MFC

(a) Maximum COD Reduction

(b) Maximum Power Production
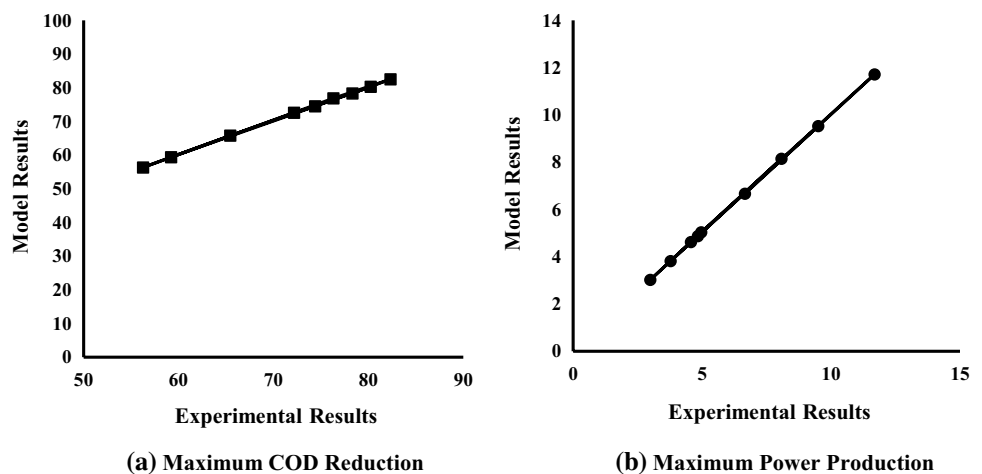
different KCl molar concentration, agarose concentration, and length salt bridge. From Taguchi L_9 orthogonal array optimization, the maximum COD reduction and simultaneous power production were obtained for the 1 M KCl concentration, 5% agarose concentration, and 0.05 m salt bridge length. However, the response for S/N ratio and confirmation test indicated that the best-optimized parameters that influence the performance of DCSB-MFC were 1 M KCl concentration, 10% agarose concentration, and 0.05 m salt bridge length. In this study, the maximum influencing parameter was agarose concentration followed by KCl molar concentration and salt bridge length (ANOVA). As similar to COD reduction and power production from dairy industry wastewater, DCSB-MFC also reduced the other pollutants to the maximum level for the best-optimized value of process parameters. The regression model developed in this study could be to predict the best combination of process parameters against the required output power and COD reduction from the dairy industry wastewater. Thus, this study suggested that DCSB-MFC may be used for reducing the pollutants and power generation from any wastewater for the appropriate process parameters value.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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