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Original scientific paper

Screening technique on the selection of potent microorganisms for operation in microbial fuel cell for generation of power

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Abstract

This paper focuses on determination of the influence of electrochemically active microorganisms on the transmission of electrons from the respiratory enzymes to the electrode and assembling of exoelectrogens to the simulated wastewater medium. In this study, the total of eight microorganisms were experimentally tested to exhibit growth and high iron-reducing ability in the absence of mediators. A major connection was observed between the growth and iron-reduction ability of the microorganism. The growth and iron-reduction ability were monitored experimentally over time. Based on output data, the screening was done among eight different microorganisms, where Escherichia coli -K-12 was chosen as the most potent microorganism for its wide application in a microbial fuel cell (MFC). In the present study, various biochemical process factors were optimized statistically using Taguchi methodology for the rapid development of growth and iron-reducing assay conditions. The design of various experimental trials was carried out using five process factors at three levels with orthogonal arrays (OA) layout of L_{18}. Five process factors, including quantity of lactose, volume of trace element solution, inoculum percentage, pH, and temperature, were taken into consideration as imperative process factors and optimized for evaluation of growth of bacteria and iron reduction ability. The larger-is-best signal to noise (S/N) ratio, together with analysis of variance ANOVA, were used during optimization. Anticipated results demonstrated that the enhanced bacterial growth of 124.50 % and iron reduction ability of 112.6 % can be achieved with 8 g/L of lactose, 2 ml of trace element solution, 4 % (v/v) of inoculum, pH 7, and temperature of 35 °C. Furthermore, the growth and iron reduction time profiles of Escherichia coli-K12 were performed to determine its feasibility in MFC.

Open circuit voltage of 0.555 V was obtained over batch study on a single chamber microbial fuel cell (SCMFC).

Keywords
Bioelectricity; exoelectrogenic bacteria; bacterial growth; iron-reducing ability; Taguchi optimization; batch operation

Introduction

It is a conventional statement that renewable energy sources are urgently necessary. The present necessity for fossil fuels is unsustainable due to their toxic waste and restricted supply [1]. One of the main strategies of using renewable energy sources and applying power-saving programs does not decrease focus on cutting the energy demand but increases the demand for stand-alone systems as alternates. Therefore, use of renewable energy in the world increases in order to have a more supportable energy mix, which reduces greenhouse gas emissions, and also allows lower dependency on fossil fuels [2]. Although much research is conducted in favor of renewable energy sources as alternative solutions, no one can completely replace fossil fuels. This proves that different alternative renewable sources are greatly needed to meet the energy demand.

Due to the recent invention that microorganisms which are exoelectrogenic in nature can be used to generate electricity from wastewater, and this organic matter has gained much attention in the present days. Recently, the increased interest in microbial fuel cell (MFC) technology was highlighted as one of the most imperative renewable sources [3]. MFC not only generates a power, but at the same time stimulates bioremediation of the polluted sediments [4]. Therefore, MFC can be used for detoxification to treat industrial wastewater containing heavy metals [5]. The innovation that microorganisms can be used to produce electrical current has led to increasing attention and the number of publications and research in the field of MFC [6,7]. This is mostly due to high importance of understanding the performance of MFC for continuous energy production and especially the role of various process factors on its performance [8,9].

In this study, the experiment was carried out with dairy wastewater taken as the substrate in MFC. Since wastewater contains various organic substances, it was considered as an organic source. Initially, the media was designed in the presence of simulated wastewater for the appropriate screening of the best microorganisms related to their growth and iron-reducing ability without any mediator. Up to now, several bacteria like *Geobacter spp*, *Rhodoferax sp*., *Klebsiella sp*., *Rhodopseudomonas sp*., and *Dessulfobulbus sp*., were found capable of transferring electrons without any mediator [10-12].

A total of eight microorganisms (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Pediococcus acidophilus*, *Staphylococcus aureus*, *Zymomonas mobilis*, *Klebsiella oxytoca*, *Enterobacter aerogenes* and *Escherichia coli -K-12*) were chosen among potent catalysts for the generation of power in MFC. Firstly, from the screening of their growth and iron-reducing ability, the best microorganisms among eight were selected for further statistical analysis. Here, *Escherichia coli -K-12* proved to be the most potent microorganism with high growth and iron-reducing ability, although there was no normal protocol to check the growth and iron-reducing ability of bacteria which can be used commercially [13]. Different investigations in laboratories have standardized regular procedures for the valuation of iron reduction. Since the proper iron-reducing ability testing is a long time and costly procedure, here we used the recently developed speedy protocol [14] for checking the iron-reducing ability of *Escherichia coli* -K-12. The growth of bacteria is linked with iron reduction ability and thus, the process factors such as lactose, trace element, inoculum percentage, pH, and temperature, would participate in the measurement of iron-reducing ability of microorganisms [15].

There is an enormous number of statistical methods carried out using the change of one variable per time (COVT), but these methods are generally exhausting, time consuming and high-priced. Szöllosi *et al.* [16] have reported a novel protocol for screening of biocatalyst, where COVT method was used to control all parameters used in MFC. COVT needs the maximum experimental number and so, an interaction effect among the process factors cannot be obtained. At the same time, it highlights the average performance of any process [17].

Here, Taguchi methodology (TM) is applied for optimization of biological and chemical reaction factors, specifically for *Escherichia coli -K-12*. TM deploys a particular set of independent parameters, which are controllable and non-controllable over an accurate area of importance. Hwang *et al.* [18] have already applied TM for engineering optimization. In the current work, various biological and chemical process factors were optimized using TM for the quick rise of growth and iron reduction profile of *Escherichia coli -K-12* [13]. *Escherichia coli -K-12* might be a potential candidate for a single chamber microbial fuel cell (SMFC) for renewable energy applications [19].

The process efficiency of MFC can be affected by numerous factors such as type of microorganisms, MFC design, membrane type (PEM), electrodes used, and several other factors. Among all these, microbial activity is the essential factor, so the proper selection of the potential species is particularly important [20]. Numerous experiments proved different methods to screen the exoelectrogenic bacteria [21], micro-fabricated MFC arrays [22], or using tungsten-oxide nanocluster as the probe [23]. These methods, however, are time-consuming and lengthy (5–6 days) to offer assessable information about the transfer of electrons by the microorganisms [24]. Another limiting factor of these methods is the requirement for expensive equipment and materials. Therefore, there is an increasing demand for novel and rapid screening methods in both research and development of MFCs. The main aim of this study was to develop a simple, affordable, and high sample throughput method for the screening of microorganism strains for MFC application.

Experimental

Culture management

Bacillus subtilis, *Lactobacillus acidophilus*, *Pediococcus acidophilus*, *Staphylococcus aureus*, *Zymomonas mobilis*, *Klebsiellaoxytoca*, *Enterobacter aerogenes* and *Escherichia coli -K-12* (MTCC-1302) are eight microorganisms that were purchased from IMTECH Chandigarh, India and examined in the present research. The growth of microorganisms was done on Luria broth. The broth was set aside at pH 7.4 before sterilization. The incubation temperature was maintained at 35 ^{o}C. The microorganisms were repeatedly sub-cultured on the Luria agar plate in seven days intervals.

Growth media

The media for seed culture of all eight microorganisms was prepared [25]. Briefly, the media was ready with 10 g/L of lactose, 0.2 g/L of NH_4Cl, 0.15 g/L of $CaCl_2{\times}2H_2O$, 0.33 g/L of KCl, 0.3 g/L of NaCl, 3.15 g/L of $MgCl_2$, 1.26 g/L of K_2HPO_4 and 0.42 g/L of KH_2PO_4. In this work, the media of 50 ml was kept in 250 ml of Erlenmeyer flask with a screw cap. The trace element solution was prepared with 20 g/L of $ZnSO_4{\times}7H_2O$, 10 g/L of H_3BO_3, 5 g/L of $MnCl_2{\times}7H_2O$, 5 g/L of $FeSO_4{\times}7H_2O$, 1.5 g/L of $CoCl_2{\times}5H_2O$, 1.5 g/L of $CuSO_4{\times}5H_2O$ and 1 g/L of $Na_2MoO_4{\times}4H_2O$. 1 ml of trace element solution was mixed with 50 ml of media, and pH was adjusted to 7.4 in the media. The conical flasks were plugged strongly with a screw cap to maintain oxygen absence in the media, placed inside incubator for 24 h and maintained at temperature of 35^{o}C for all eight microorganisms.

Microbial iron (III) reduction study

The iron reduction study for eight microorganisms was performed using the oversaturated solution of iron(III)-citrate (5 g/L), supplemented with different broths, and used as the electron acceptor substance [26]. In this case, no methylene blue was added to the media as an electron shuttle (mediator). The anaerobic state was maintained in the media to provide the electron-acceptor role of Fe(III) ions by using a screw cap. To seal the cap, parafilm was used on the cap and incubated at 35°C for all eight microorganisms. Each day up to seven days, the samples were taken, and pH of samples was changed to pH 2 by addition of sulfuric acid. Then, a coloring agent of ammonium-thiocyanate (NH_4SCN) (50 g/L) was added to the solution [27]. The final part of the sample was 200 times diluted. After thorough mixing, absorbance (*A*)of the solution was measured using spectrophotometer, covering the absorbance maximum of iron(III)–thiocyanate complex within 300–600 nm range.

Statistical analysis for growth and iron-reducing ability of Escherichia coli -K-12

The study was carried out using Taguchi methodology (TM) and *Escherichia coli -K-12* was selected among eight microorganisms [28]. Further analysis was based on the growth and iron reduction ability which were determined experimentally [29]. The assessment was done to understand the connection between the growth and iron reduction ability of *Escherichia coli -K-12* through active analysis. In this analysis, orthogonal arrays (OA) exhibit the changed experimental situation with the smallest amount of error. Also, TM provides an advanced competence and parameters reproducibility for a range of experiment trials, and at the same time it reduces noise throughout optimization or analysis [30]. TM follows 4 individual steps, which are step by step described in Figure 1.

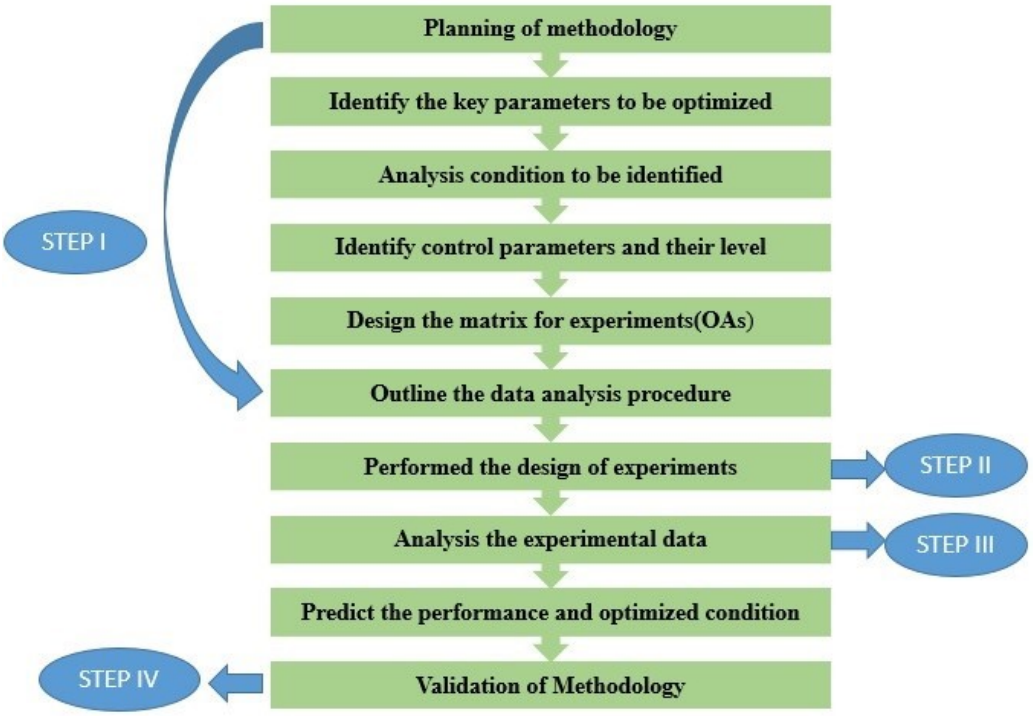

Figure 1. *Four steps of experimental optimization included in Taguchi methodology.*

The first step is the planning of experiments, the second step is experimenting, the third step is analysis of results, and the fourth step is validation of methodology. To bring about the wide-ranging of optimization, each step is interrelated with each other.

Investigational plan (step I)

In this case, different process factors were selected, depending on the growth and iron reduction ability of *Escherichia coli -K-12*. Five different biological and chemical process factors are lactose, trace element, inoculum percentage, pH, and temperature, recognized firstly from seed culture [31]. The planning matrix was executed with suitable OAs for the nominated factors with their consequent three levels [25]. Here, three levels (low, mid and high) of five parameters were designed in the experiments for *Escherichia coli -K-12.* Data are summarized in Table 1.

Table 1. *Trial range of five process factors (A-E) considered using Taguchi methodology.*

Factor code	Name	Range of variables		
		Low (1)	Mid (2)	High (3)
A	Lactose concentration, g/L	6	8	10
B	Volume of trace element solution, ml	1	2	3
C	Content of inoculum, % v/v	4	7	10
D	pH	6	7	8
E	Temperature, ^{o}C	30	35	40

Experiments (step II)

A variety of trials with process factors of different range arrangement are given in Table 2 for *Escherichia coli -K-12*, for which intensity of bacterial growth and iron reduction potential were calculated individually [32]. In every trial, the culture media of 50 ml was used. In Table 2, the planning of the matrix was done with orthogonal array of five factors and three levels (3^5) which gives layout of L_{18}.

Table 2. *L_{18} OA (3^5) of design trials for five process factors (A-E) for growth and iron-reducing ability for* Escherichia coli -K-12*.*

Number of trials	A	B	C	D	E	Growth of bacteria, CFU/ml × 10^{11}	$A_{460\ nm}$ (Iron reducing ability)
1	1	1	1	1	1	0.81	0.211
2	1	2	2	2	2	1.52	0.415
3	1	3	3	3	3	0.74	0.191
4	2	1	1	2	2	1.98	0.505
5	2	2	2	3	3	1.22	0.306
6	2	3	3	1	1	0.89	0.221
7	3	1	2	1	3	0.77	0.212
8	3	2	3	2	1	1.11	0.299
9	3	3	1	3	2	1.16	0.312
10	1	1	3	3	2	1.09	0.289
11	1	2	1	1	3	1.01	0.269
12	1	3	2	2	1	1.00	0.256
13	2	1	2	3	1	0.94	0.234
14	2	2	3	1	2	1.44	0.378
15	2	3	1	2	3	1.49	0.381
16	3	1	3	2	3	1.10	0.282
17	3	2	1	3	1	0.86	0.221
18	3	3	2	1	2	0.89	0.242

The cell growth and iron reduction ability were determined separately after seven days of each trial for the microorganism, as per the protocol reported in the previous study [16,33]. All trials were performed 3 times, and mean values are shown in Table 2.

Analysis of experimental data (step III)

To treat the obtained results, Qualitek-4 software (Nutek Inc., MI, USA) was used. In step III, the evaluation was done on bacterial growth and iron-reducing ability individually for the microorganism to examine the effects of individual process factors, and their interactive influence. Moreover,

the performance of the total process, as well as the analysis of optimum conditions were done. The performance was analysed based on the *larger-is-better* S/N ratio for each trial. Here, standard deviation (SD) is noise, and the anticipated value is signal [34]. Hence, the ratio between anticipated and SD values defines signal-to-noise (S/N) ratio. Thus, defined S/N ratio is used to calculate the quality characteristics of the output, represented by a deviancy from the anticipated value. Loss function [$L(y)$] was used to calculate the quality characteristics of the output [35], which is computed by $L(y)=k(y-m)^2$, where k = proportionality constant, m = target value and y = experimental data collected from each trial. The measurement of the loss function is performed using $L(y) = k(1/y^2)$ for quality appearances of output from each trial. Here, the *larger-is-better* concept of S/N ratio is applied and hence, the projected loss function was set by

$$E\left[L(y)\right]=kE\left(\frac{1}{y^2}\right) \tag{1}$$

where $E(1/y^2)$ was computed from n number of trials and written as

$$E\left(\frac{1}{y^2}\right)=\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y^2} \tag{2}$$

Here, the mean square deviation (MSD) represents the mathematical appearance for *larger-is-better* S/N ratio, which was computed through the deviance from the target value:

$$\frac{S}{N}=-10\log\,(\text{MSD})=\text{-}10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y^2}\right) \tag{3}$$

Validation of Taguchi methodology (step IV)

To authenticate the methodology assumed by predicted optimized conditions, the experiments were additionally performed individually for each microorganism. Through TM, the results were then compared with predicted outputs known independently for each microorganism.

Wastewater

The dairy wastewater used in our experiments was collected from Gomati cooperative milk producers union limited, Agartala. After the collection of dairy wastewater from Gomati, storage of wastewater was done under the refrigerator for further use in the experiment. To achieve various chemical oxygen demand (COD) for the real dairy wastewater (RDW), it was further diluted with distilled water. The COD range in these experiments was fixed to 8000 mg/L after the statistical analysis. The standard methods were performed to achieve the correct COD measurement [36].

MFC setup and operating procedures

Batch study was conducted on 300 ml MFC with a working volume of 200 ml (Figure 2).

The compartment was acrylic having an anode, a membrane, and the cathode [37,38]. The anode was carbon cloth with a carbon-coat of 0.5 mg/cm^2 and surface area of 50 cm^2. Nafion 117 (Sainenergy fuel cell, Chennai, India) was used as a membrane, and the electrode spacing was kept about 157 μm, *i.e*. thickness of the membrane. The cathode (Pt/C) was reinforced on carbon cloth with the loading of 0.5 mg/cm^2 (Sainenergy fuel cell, Chennai, India). The cell was arranged in the order anode-membrane-cathode. The stainless-steel wire was attached to connect the electrodes, while digital multimeter is used to record the cell voltage (V) of SCMFC.

***Figure 2**. Batch operation on a single chamber microbial fuel cell (SCMFC)*

Results and discussion

Experimental results for growth and iron-reduction ability

The experiments for growth and iron-reducing ability were done for all eight microorganisms. To understand the strength of all eight microorganisms, dilution plating was done, and strong iron-reduction ability was checked in absence of a mediator for screening purposes [39]. The exception was found in the case of *Escherichia coli -K-12* [40]. As shown in Table 3, *Escherichia coli -K-12* was found to have the highest growth as well as iron-reducing ability in the absence of mediator. Thus, it proves to be an exoelectrogenic bacteria which indicates the production and secretion of exoelectrogens in the medium [41]. The most potent microorganisms were thus selected, and further statistical analysis was done based on their growth and iron-reducing ability tested experimentally.

***Table 3.** Growth and iron(III)-reduction ability of different microorganisms.*

S. N.	Microorganism	Bacterial growth	Iron(III)-reduction without mediator
1.	*Bacillus subtilis*	++	+
2.	*Lactobacillus acidophilus*	+++	---
3.	*Pediococcus acidophilus*	+	---
4.	*Staphylococcus aureus*	++	---
5.	*Zymomonas mobilis*	++	---
6.	*Klebsiella oxytoca*	+	---
7.	*Enterobacter aerogenes*	+	---
8.	*Escherichia coli -K-12(MTCC-1302)*	+++	+++

Bacterial growth: (++) minimum, (+++) maximum; Iron(III) reduction: (–) the change in absorbance was not detectable, (+) change less than 0.1, (++) change between 0.1 and 0.2, (+++) change more than 0.2.

Optimization outcome

The optimization was done with the most potent microorganism among the selected eight. The estimation of growth and iron-reduction ability was done by the planning of individual process factors at their consigned levels [41]. Data for *Escherichia coli -K-12* are shown in Table 4.

***Table 4.** Main effects of particular parameters for growth and iron-reducing ability of Escherichia coli -K-12*

Serial No.	Factor code	Effect of individual factors in growth by S/N ratio				Effect of individual factors in iron reduction ability by S/N ratio			
		Level-1	Level-2	Level-3	L2-L1	Level-1	Level-2	Level-3	L2-L1
1.	A	-0.019	2.101	-0.285	2.119	-11.626	-9.829	-11.785	1.796
2.	B	0.455	1.346	-0.005	0.891	-11.253	-10.262	-11.726	0.99
3.	C	1.248	0.234	0.313	1.014	-10.444	-11.395	-11.401	-0.951
4.	D	-0.504	2.438	-0.139	2.942	-12.081	-9.243	-11.916	2.837
5.	E	-0.654	2.271	0.18	2.924	-12.481	-9.239	-11.521	3.241

Influence of each factor

The influence of individual process parameters at their consigned level used for evaluation of growth and iron-reduction ability, has been reported in Table 2. Results show that bacterial growth and corresponding iron reduction ability depend on all assigned process factors chosen in the present study. From Table 4, it is observed that pH, temperature, lactose, and inoculum percentage are incredibly significant at Level 2 among all designated biochemical process factors, and the trace element solution is maximum at Level 1. It is also clear from Level 3 that growth, as well as iron reduction ability is declined with an additional enrichment of all selected parameters except inoculum percentage and does not extensively affect the enrichment of trace element. Therefore, the relative effect of individual factors was measured from (L2-L1). The better average magnitude in a variation of their effects designates a stronger influence in output.

Moreover, degradation of complex substance to simple products happens through the complex biochemical process, where terminal acceptor of an electron is ferric ion (Fe^{3+}) [17]. Since pH of media controls the growth of bacteria, the iron reduction ability is indirectly controlled by the level of pH too. The second important parameter following pH, is temperature. Generally, at a higher temperature, enzyme present inside the microbial system becomes deactivated, which indirectly inhibits the growth of bacteria. Since growth and iron reduction capacity are interlinked to each other, temperature indirectly controls both [13].

In the present study, lactose is the sole carbon source, and the third important factor which supplies energy for the growth of bacteria. The destruction of growth, however, is found at higher and lower concentrations of lactose, what may be due to catabolic repression of monosaccharides [10]. The fourth important parameter is the inoculum percentage. The optimum inoculum percentage is required to attain maximum biomass after completing of a biochemical process. As the bacterial growth depends on the substrate accessibility in media, the suppressive growth was observed when a higher percentage of inoculum was added on it, what may be due to competitive inhibition [33]. Thus, the enhancement of tracer amount in media does not significantly affect the growth and iron-reducing ability of the bacteria, and so, their requirement in media is low in comparison with other media components [16].

Interaction between two factors

Severity index (SI) was calculated using the TM individually for *Escherichia coli -K-12* [43]. The assessment of the interactive effect between two factors was carried out at different levels [44]. The results for *Escherichia coli -K-12* after the analysis are reported in Table 5. Interaction pairs are exposed in downward order of their SI (0-100 %). SI indicates the maximum angle along with all probable combinations of the line segments for interaction involving pairs of 3 level factors. Thus, the distribution of the interacting process factors for which they are responsible is indicated in the columns shown in Table 5. The 90^{o} angle involving the lines for the parameters defines 100 % SI interaction, while parallel lines stuck between them signifies 0 % SI interaction. Based on the first two levels, the best possible values are represented by the level of factors.

It is evident from Table 5 that the combination of trace element solution and inoculum percentage shows the highest interaction for both, growth and iron reduction ability. This combination is followed by the combination of lactose and inoculum percentage, trace element solution and pH, lactose and trace element solution, inoculum percentage and temperature, lactose and temperature, inoculum percentage and pH, pH and temperature, trace element solution and temperature and finally, lactose and pH. Table 4 indicates that pH is designated as the highest impact parameter,

followed by temperature (high impact parameter), lactose (moderate impact parameter), inoculum percentage (less impact parameter) and trace element solution (least impact factor) for iron reduction ability. It is evident that trace element solution and inoculum percentage are least and less impact parameters (Table 4) but illustrates the highest interaction SI in combination with them. The results of variance analysis demonstrate that yield of cell number and iron-reducing ability is dependent on the interaction of process factors for *Escherichia coli -K12* and quite independent of individual effects [45].

Table 5. *Interactions estimated by severity index (SI)*

Growth					
S. No	Factors	Columns	RC	SI (100 %)	Levels
1	Volume of trace element solution × Inoculum content	3 × 4	7	75.05	2, 2
2	Lactose concentration × Inoculum content	2 × 4	6	53.57	2, 1
3	Volume of trace element solution × pH	3 × 5	6	44.06	1, 2
4	Lactose concentration × Volume of trace element solution	4 × 6	2	34.76	1, 2
5	Inoculum content × Temperature	2 × 3	1	33.14	2, 1
6	Lactose concentration × Temperature	2 × 6	4	19.07	2, 2
7	Inoculum content × pH	4 × 5	1	16.63	1, 2
8	pH × Temperature	5 × 6	3	14.75	2, 2
9	Volume of trace element solution × Temperature	3 × 6	5	10.02	2, 2
10	Lactose concentration × pH	2 × 5	7	7.31	2, 2
Iron reduction ability					
S. No	Factors	Columns	RC	SI (100 %)	Levels
1	Volume of trace element solution × Inoculum content	3 × 4	7	69.13	2, 2
2	Lactose concentration × Inoculum content	2 × 4	6	61.10	2, 1
3	Volume of trace element solution × pH	3 × 5	6	41.39	1, 2
4	Lactose concentration × Volume of trace element solution	2 × 3	1	35.47	2, 1
5	Inoculum content × Temperature	4 × 6	2	28.79	1, 2
6	Lactose concentration × Temperature	2 × 6	4	19.37	2, 2
7	Inoculum content × pH	4 × 5	1	18.62	1, 2
8	pH × Temperature	5 × 6	3	11.07	2, 2
9	Volume of trace element solution × Temperature	3 × 6	5	9.46	2, 2
10	Lactose concentration × pH	2 × 5	7	7.83	2, 2

Analysis of variance (ANOVA)

To improve the relative effect and comparable interactions of the process factors within the variation of results, trial data were examined by the analysis of variance, ANOVA [35]. After ANOVA analysis reported for *Escherichia coli -K12,* the percentage of contribution of each process factor is presented in Table 6.

Table 6. *Analysis of variance (ANOVA).*

Factor	DOF	Growth			Iron reduction ability		
		Sum of squares	Variance	Contribution, %	Sum of squares	Variance	Contribution, %
Lactose concentration, g/L	2	20.514	10.257	22.383	14.169	7.084	15.140
Volume of trace element solution, ml	2	5.659	2.829	5.814	6.697	3.348	6.846
Inoculum content, % v/v	2	3.819	1.909	3.762	3.645	1.822	3.458
pH	2	30.861	15.430	33.923	30.456	15.228	33.216
Temperature, °C	2	27.246	13.623	29.891	33.278	16.637	36.348
Other/Error	7	1.559	0.222	4.227	1.850	0.264	4.992
Total	17	89.661		100.000	90.099		100.000

Conversely, when these factors act jointly, they influence the maximum output. The on top of results are due to the outcome of several parameters collectively.

Optimal state

Maximum growth and iron-reducing ability are attained by the optimum values of selected parameters, which are for *Escherichia coli -K12* [31] reported in Table 7. Based on the excellence [46], quality selected for the analysis of the optimum situation was resolute. Table 7 includes the ordinary presentation in calculating only considerable factors [47]. Results illustrate that pH has maximum impact on the growth, while temperature achieves the maximum impact on the iron reduction of *Escherichia coli -K-12.*

Table 7. *Optimum conditions and performance of growth and iron reduction ability for Escherichia coli -K12*.

Factor code	Factor	Value	Level	Contribution for growth from S/N ratio	Contribution for iron reduction ability from S/N ratio
1	Lactose concentration, g/L	8	2	1.501	1.251
2	Volume of trace element solution, ml	2	2	0.747	0.818
3	Inoculum content, v/v %	4	1	0.649	0.636
4	pH	7	2	1.839	1.837
5	Temperature, ^{o}C	35	2	1.671	1.841
Total contribution from all factors				6.407	6.383
Current grand average of performance				0.599	-11.080
Expected result at optimum condition				7.006	-4.697

Validation of experiments

The frequency distribution plots for bacterial growth, as well as iron reduction ability for the current and improved conditions are shown in Figures 3A and 3B, respectively. The yield of bacterial growth is increased from 0.98×10^{11} CFU/ml (S/N ratio is 0.599) to 2.2×10^{11} CFU/ml (S/N ratio is 7.006) after optimization of process factors. In general, 124.50 % increase of yield of bacterial growth is shown in Figure 3A. TM also calculates that enhancement of iron reduction capacity may be carried out from 0.300 to 0.638 of absorbance as S/N ratio increases from -11.080 to -4.697. It is shown in Figure 3B that 112.6 % of iron reduction ability is enhanced under the optimized condition.

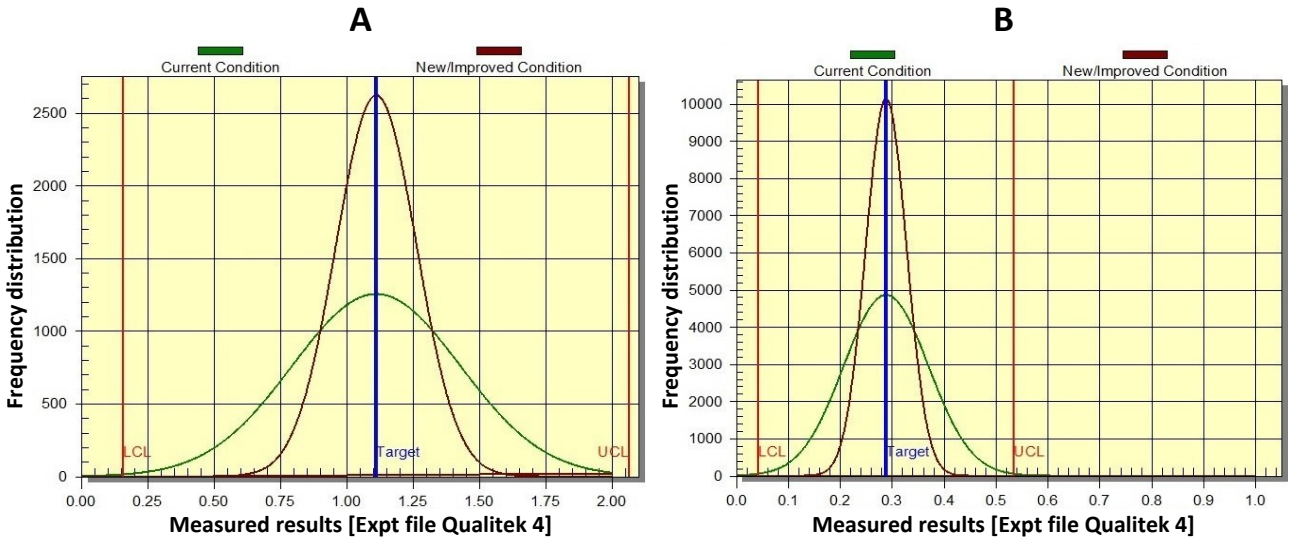

Figure 3. *Performance distribution of improved and current condition of Escherichia coli -K-12 for (A) bacterial growth and (B) iron-reducing ability*

To validate the experimental methodology, the experiments were carried out under predicted optimized conditions. It is found that the yield of bacterial growth is increased to 2.2×10^{11} (124.50 %), while iron reduction capacity is increased to -4.697 of absorbance (112.6 %) under predicted optimized condition. The variation of predicted data about bacterial growth and iron reduction ability from experimental data was found within the range of validation [49].

In Figures 4A and 4B, time profiles of growth and iron-reducing ability of *Escherichia coli-K-12* are illustrated. Figure 4A shows that in the growth of Escherichia coli -K-12 there are three distinct phases [42]. At this point, three unconnected phases of iron-reducing consider absorbance changes at 460 nm (A_{460nm}) for which, the raised time profile is found (Figure 4B). Therefore, Figure 4B illustrates that up to 24 h of incubation, the iron-reducing ability of bacterial is almost constant with the samples collected. However, an important change of absorbance is found from 24 h (1 day) to 120 h (5 days) within the collected samples [50].

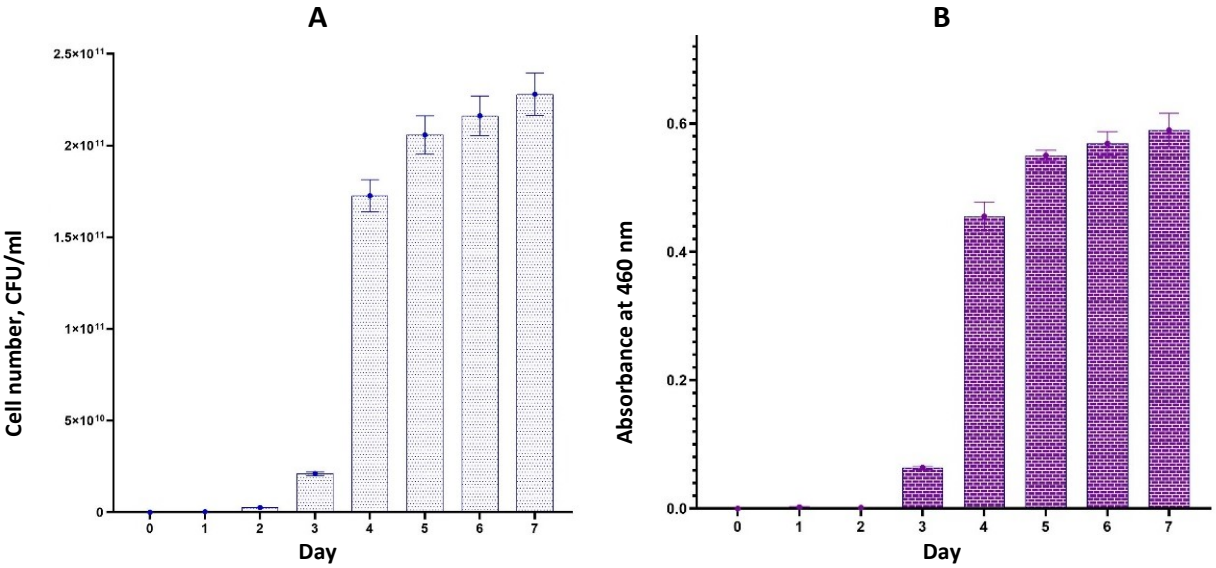

Figure 4. A - growth and B - iron reduction time profiles of *Escherichia coli-K-12 under optimized condition*

The lower rate of shifting the absorbance is noticed from 120 h (5 days) to 168 h (7 days) of incubation for sample withdrawal. The present finding may be due to exhaustion of the main source of energy in the media *i.e*. lactose. In MFC, bacteria receive energy from carbon sources *i.e*., lactose or any organic substance in anaerobic condition, and transmission of electrons takes place from the anode to cathode which acts as an electron acceptor in presence of oxygen. Microorganism usually develops -320 mV in the form of NADH in the anode, and +840 mV is gained by the cathode suitable for the shuttling of an electron.

Batch study for voltage generation

The collected dairy wastewater was tested to find the chemical oxygen demand (COD), using the existing protocol [36] and found equal to 8010 mg/L. The value of COD obtained for simulated dairy wastewater (SDW) having 0.8 % (w/v) of lactose is equivalent to the real dairy wastewater (RDW). A little dilution of COD is done with distilled water with RDW and kept at 0.8 % (w/v). Therefore, for real RDW, the value of COD was kept at 0.8 % (w/v) for batch experiments (up to 360 h) for 15 days. The highest OCV after 96 h of incubation was measured as 555 mV (Figure 5). After a certain interval of time, the OCV is gradually decreased and the value of OCV was found to be lower after 180 h of MFC operation, *i.e*. equal to 500 mV [51].

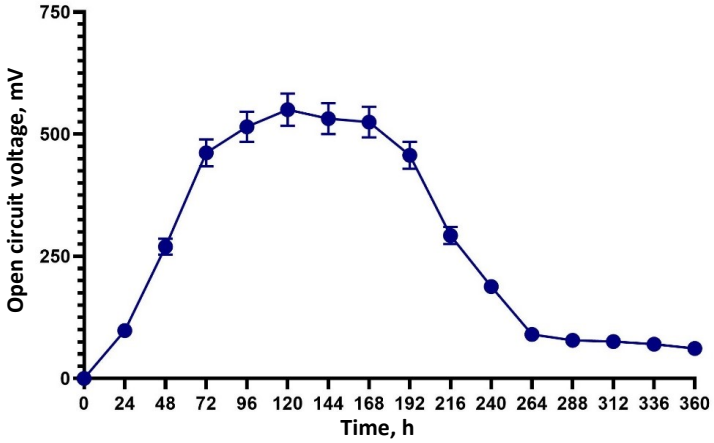

Figure 5. *Maximum open-circuit voltage obtained during the batch process in SCMFC*

The accessibility of substrate, pH of media, and incubation temperature are responsible for higher multiplication rate of bacteria [52]. The accessibility of substrate in MFC encourages bacterial growth and thus transfer of electrons in the system. Since *Escherichia coli -K-12* is an exoelectrogenic bacteria, no mediator was used additionally [53]. As microorganisms need energy source, they use the substrate directly or indirectly. During batch operation, however, a huge amount of substrate hampers OCV generation. Therefore, for further improvement of OCV, a systematic feeding strategy should be implemented to reach a constant and stable voltage in a microbial fuel cell.

Conclusion

The current study investigates optimization of process factors, to get the progress of quick growth and iron reduction assay for *Escherichia coli -K-12* using Taguchi methodology. The optimal value for each of five process factors has been projected using Taguchi methodology by the experimentation with 18 trials. S/N ratio of *larger-is-better* concept was used to evaluate the main and interaction effects of process factors. It is also proven that iron reduction ability is associated with growth in the case of *Escherichia coli -K-12*. Lastly, it also proves to have a noteworthy growth rate in anaerobic conditions along with the presence of a strong oxidizing agent. Thus, *Escherichia coli -K-12* was signified to be the most potential biocatalyst to produce energy in a single chamber-MFC. The highest OCV of 555 mV was measured with batch operation in SCMFC for 15 days at pH 7, 35 ^{o}C of temperature, 8 g/L of lactose, 2 ml of trace element solution, and 4 % (v/v) of inoculum percentage. The yield of bacterial growth is increased to 2.2×10^{11} CFU/ml (S/N ratio is 7.006) and iron reduction ability has been carried out from -11.080 to -4.697 for *Escherichia coli -K-12* after optimization of process factors. In general, 124.50 % increase of yield of bacterial growth and 112.6 % of iron reduction ability is enhanced under the optimized condition.

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