Simultaneous Production of Bioethanol and Bioelectricity in a Membrane-Less Single-Chambered Yeast Fuel Cell by *Saccharomyces cerevisiae* and *Pichia fermentans*

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Abstract

Production of bioethanol and bioelectricity is a promising approach through microbial electrochemical technology. Sugars are metabolized by yeast to produces ethanol, CO₂, and energy. Surplus electrons produced during the fermentation can be transferred through the circuit to generate electricity in a microbial fuel cell (MFC). In the present study, a membrane-less single-chambered microbial fuel cell was developed for simultaneous production of bioethanol and bioelectricity. *Pichia fermentans* along with well-known ethanol-producing yeast *Saccharomyces cerevisiae* were allowed to ferment glucose. *S. cerevisiae* demonstrated maximum open circuit voltage (OCV) 0.287 ± 0.009 V and power density 4.473 mW m⁻² on the 15th day, with a maximum ethanol yield of 5.6% (v/v) on the 12th day. *P. fermentans* demonstrated a maximum OCV of 0.318 ± 0.0039 V and power density of 8.299 mW m⁻² on 15th day with an ethanol yield of 4.7% (v/v) on 12th day. Coulombic efficiency (CE) increased gradually from 0.002–0.471% to 0.012–0.089% in the case of *S. cerevisiae* and *P. fermentans*, respectively, during 15 days of the experiment. The result indicated that single-chambered fuel cell can be explored for their potential applications for ethanol production along with clean energy generation.

Keywords *Pichia fermentans* · *Saccharomyces cerevisiae* · Single-chambered MFC · Ethanol production · Coulombic efficiency

1 Introduction

Microbial fuel cell (MFC) is a green technology that requires the utilization of electrogenic biological entities to oxidize a variety of substrates such as glucose, acetate, glycerol, acetic acid, lactic acid, and other biomass-based substrates [1, 2]. Besides this, some inorganic substrates such as sulfides, nitrites for electricity generation have been utilized successfully. The system consists of two electrodes: an anode and cathode being separated by proton exchange membrane or salt bridges or sometimes membrane-less. Whereas, membranes allow protons generated on an anode electrode to be transferred to the cathode electrode. Also, the performance of MFC depends on the type of electrode materials being used, internal resistances, cell configurations, and surface area, etc. [3, 4]. Several pure or mixed cultures of exoelectrogenic biocatalyst have been employed on anode electrodes where they produce electrons and protons through their metabolic activities [5]. The practical application and scale-up of MFC have been progressed in the last decades such as biofuel generation, wastewater treatment, bioremediation processes, etc. [6]. Among them, biofuel generation is emerging as a dynamic approach in the form of gaseous and liquid fuels for the transportation sector. The most important and widely used among liquid fuels is the ethanol being produced from the fermentation of sugars and starches.

The increasing interest in bioethanol production started in the 1980s and hence has been considered as an alternative fuel in many countries. It helps to reduce CO₂ emission up to 80% as compared to using petrol, thus encourages a healthier environment for the future. They are recyclable and contribute to sustainability. Similarly, the production of bioelectricity in a microbial fuel cell (MFC) as a biotechnological system is another developing green approach toward renewable energy from sustainable development [7, 8]. Bioelectricity is produced through a
microbial catalytic activity using various organic sources. In MFC, anode accepts electrons from microbial catabolic activity through oxidation and reduction processes [9]. In general, 2 mol of ethanol is produced from 1 mol of glucose (glucose → Pyruvate → acetaldehyde → ethanol) through NADH-dependant enzyme and alcohol dehydrogenase. This reaction generates two ATP molecules, two H+ ions, and two electrons. These electrons are stored in cells in the form of NADH. An increasing ratio of NADH to NAD+ could help to generate voltage output [10]. Thus the generated electrons from NADH/NAD+ redox cycle may be used in the MFC system [11]. Therefore, the yeast could metabolically convert their energy into heat and efficiently harvested as electricity through a combined approach of MFC during sugar fermentation and ethanol production.

Yeast could potentially be considered as an ideal model organism for MFC applications due to its non-pathogenic nature and being able to utilize a wide-ranging substrate. The most commonly used biocatalyst for fermenting the sugars into ethanol is *Saccharomyces cerevisiae*, but its use is restricted as it cannot ferment xylose and other 5-carbon sugars present in lignocellulosic material. On the other hand, xylose-fermenting yeasts, e.g., *Pichia stipitis*, *Candida parapsilosis*, and *Candida shehatae* [12] may ferment both 5-carbon as well as 6-carbon sugars. *Pichia fermentans* also been reported to produce xylitol using non-detoxified xylose-rich pre-hydrolysate from sugarcane bagasse [13].

Recently published reports suggest that non-pathogenic *Pichia* sp. could be a potential yeast to be used in a microbial fuel cell due to its possible exoelectrogenic property [14]. The signaling molecule produced by one cell and sensed by another to induce oriented growth is considered for cell–cell communication [15]. Most bacterial communities are embedded in structured extracellular polymeric substances (EPS) to survive in a harsh environment [16]. Electroactive biofilms play an important role in the bioelectrochemical system via various electron transfer mechanisms. Yeasts may also produce biofilm to enhance electron transfer mechanism and biofilm dynamics in microbial fuel cells [17].

Most of the work related to MFC has been focused on reactor design, proton exchange membrane, electrolyte development, and modification of electrode design and materials to increase electricity production [18]. There are several advantages in MFC as it can be operated in fed-batch, continuous or batch mode whether single-chambered or double-chambered, with membrane or membrane-less, etc. This paper presents an approach to explore MFC for bioethanol production with simultaneous generation of electricity. The study further evaluates the production of ethanol in MFC by *P. fermentans* and compared its efficiency with a well-known sugar fermenting yeast *S. cerevisiae* under a batch type operation in a single-chambered microbial fuel cell. The electrochemical data was generated by calculating current density, power density, output voltage along glucose consumption for maximum ethanol yield and fermentation efficiency.

2 Experimental Section

2.1 Microorganisms

*Pichia fermentans* was procured from the Microbial Type Culture Collection (MTCC 189) Chandigarh, India, and cultured aerobically in yeast extract and glucose (YG) agar and maintained. Whereas, *Saccharomyces cerevisiae* was purchased from a local market and maintained. Both the yeasts, *P. fermentans*, and *S. cerevisiae* were cultured for 24 h at 30 °C enriched with yeast extract 0.25% (w/v) and Glucose 10% (w/v) in a 1L solution. The same broth medium was used for inoculum preparation to be inoculated in the MFC.

2.2 Single-Chambered MFC Setup

A typical membrane-less single-chambered MFC was constructed using glass flasks (with working volume 100 mL). Bow-shaped Carbon fibers (100 cm length, 7 mm diameter) were used as anode and circular stainless steel wire (as a mesh) (100 cm length, 0.05 mm diameter) as cathode [19]. Both the electrodes were sterilized with ethanol, rinsed with autoclaved distilled water, and treated under UV (254 nm) radiation for 20 min, then dried under aseptic conditions. The flasks contained a production medium (Glucose 10% (w/v), enriched with yeast extract 0.25% (w/v) was sterilized, and then sterilized electrodes were fixed at a vertical distance of 3 cm using rubber corks as a plug to maintain anaerobic condition for fermentation. The flasks were inoculated with either of the yeast (10% (v/v) and incubated at 30 °C for 15 days. All experiments were performed in triplicates and repeated along with suitable controls. The sample (2 mL) was taken out for glucose and ethanol estimations.

2.3 Electrochemical Calculations

The open circuit voltage (OCV) and output voltage were recorded across different external resistors ranging from 1000 {\Omega} to 820 K{\Omega} via a digital multimeter. For the preparation of the polarization curve, the external resistance (Rex) was varied at time intervals. The current (I) was calculated by using Ohm’s law \( V = IR \), where \( V \) represents cell voltage value and \( R \) represents external resistance value. Power was calculated according to \( P = VR \) whereas, current density (j) and power density (p) were calculated using electrode (anode) area (2.2 cm²). The Coulombic efficiency (CE) was calculated using \( CE = CE_x \times 100/C_{TH} \), CEx is the experimental value of total coulombs. The theoretical value of

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coulombs \( (C_{TH}) \) was calculated using \( C_{TH} = \text{FnMV} \), where \( F \) stands for faraday constant \( (96,485 \text{C per mole of electrons}) \), \( n \) represents several electrons produced per glucose unit consumed during fermentation, \( M \) is the glucose concentration and \( V \) represents the reaction volume \( (L) \) \[ 20 \]. Internal resistance \( (R_{int}) \) of the cell was calculated as \( R_{int} = R \times (E/V-1) \) where \( R \) represents external resistance, \( E \) represents OCV (voltage without any resistance), \( V \) represents output voltage with resistors \[ 21 \]. All data presented in the manuscript are average triplicates along with the standard error bars.

### 2.4 Glucose Estimation

The quantifiable reducing sugar content was carried out using the Dinitrosalicylic acid (DNSA) method. The basic principle involves in the method is the interaction of an alkaline solution of DNSA with reducing sugars such as glucose and fructose wherein the aldehyde group of reducing sugar is oxidized to the carboxylic acid and the 3-nitro group \( (\text{NOO}^-) \). The color change was measured at 540 nm \[ 22 \]. The variation of the orange-red color index was the indication of the presence of reducing sugar to a high/low extent.

The concentration of glucose in the cell setup was routinely measured by dinitrosalicylic acid (DNS) method. The quantification was performed according to the step-by-step method \[ 23 \]. DNSA reagent was prepared by dissolving 2 g of DNSA and 60 g of sodium–potassium tartaric acid in 160 mL of 0.5 N NaOH. It was cooled to room temperature and diluted to 200 mL with the help of distilled water. Then 1 mL of DNSA reagent was pipetted out in a test tube containing 0.5 mL of sample \( (1 \text{g/mL}) \) and kept at 100 °C for 5 min. After cooling, 1.5 mL of distilled water was added to the same test tube to stop the further reaction, and absorbance was measured at 540 nm using a UV–VIS spectrophotometer. The glucose concentration was calculated from the standard curve of D-glucose \( (0.1 \text{mg}–1 \text{g/mL}) \), and results were expressed as mg glucose equivalent \( (\text{GE}) \) per mL sample. The ethanol fermentation was confirmed by using High-performance liquid chromatography.

### 2.5 Ethanol Estimation

All parameters were performed with an Agilent HPLC 1260 II INFINITY with autosampler and C18 column \( (5 \mu \text{m}; 25 \times 0.46 \text{cm}) \). All the outputs were processed through Agilent Chemstation-Open laboratory software. All samples were drawn out and recorded on daily basis. The sample was centrifuged at 8000 rpm for 15 min to collect for further process. The total runtime was 6 min with peaks detected with a UV detector. Water was used as a mobile phase with 4% acetone added in an optimized parameter. The standard flow rate was set at 1 mL/min with injection volume of 1 µL and detection was monitored at 235 nm and 25 °C temperature. Standard ethanol solutions were prepared in water ranging from 0.1 to 10% \( (v/v) \).

Ethanol absorbs less UV compared to a high UV absorbing background, which results in the reverse positive or negative peak polarity. Direct estimation of ethanol as a negative peak from samples was confirmed by reversed phase-HPLC and performed same according to \[ 24 \]. In every run, the three peaks were corresponding to acetone, ethanol, or water singly or in combination were detected. Acetone, pure water, and ethanol samples were injected separately for separate verification of retention times into the HPLC system in separate runs. The quantitative analysis of ethanol was calculated from the formula-

$$\text{The concentration of unknown sample} = \frac{\text{Unknown sample area}}{\text{standard solution area}} \times \text{concentration of the standard solution}$$

where peak areas are in arbitrary units and peak heights and area in milli absorbance units \( (\text{mAU}) \). Representative HPLC chromatograms were shown in Figure S1.

### 2.6 EPS Production

*P. fermentans* and *S. cerevisiae* were grown separately in 10 mL sterilized Yeast extract and Glucose \( (\text{YG}) \) broth and incubated for 15 days at 30 °C. The samples were taken out from both the cultures at altered time intervals \( (\text{day 1, day 5, 10 and 15}) \) to estimate EPS. Ten mL of cell culture was centrifuged simultaneously at 8000 rpm for 15 min. The carbohydrate in EPS was analyzed by phenol/sulfuric acid method and glucose was used as the standard \[ 23 \]. Protein was estimated by the Lowry method and bovine serum albumin was used as a standard \[ 25 \].

### 2.7 Fourier Transform Infrared Spectroscopy

Functional groups in EPS were detected by Fourier transform infrared (FTIR) spectroscopy. A drop of EPS solution \( (25–50 \mu l) \) from the samples were placed on a clean and dry glass slide and allowed to dry as a film. The prepared samples were used to analyze FTIR spectrum using Bruker FTIR system.

### 2.8 Scanning Electron Microscopy

The visual appearance of *P. fermentans* and *S. cerevisiae* was evaluated on an anodic surface at 1000X magnification. The sample was prepared by using electrode material from both the cell cultures at different time intervals \( (\text{day 1, 5, 10, and 15}) \) and was air-dried on a glass slide. Dried samples were observed under JEOL make JSM-7610FPlus FESEM. The
surface morphology of biofilm was visualized by using with an accelerating voltage of 5 kV.

3 Results and Discussion

3.1 Performance of MFC

The combination of fermentation and electricity generation demonstrated promising performance in the MFC. Both the yeast *S. cerevisiae* and *P. fermentans* grew rapidly under the experimental setup (membrane-less single-chambered MFC). All the setup depicted a typical behavior of MFC in terms of its electrochemical responses. In the absence of exogenous electron mediators, the intracellular proteins of the model organisms play a major role in electron transfer located inside the cell. This could overcome by adherence of yeast cells to the carbon fiber surface through force-driven interaction such as electrostatic or physical adsorption [26].

3.2 Electrochemical performance

Electrochemical performance of both the MFC setup containing either *P. fermentans* or *S. cerevisiae*, gradually increase was observed after 24 h of inoculation, which was almost stable up to 15 days of the incubation. Then the maximum OCV for *P. fermentans* 0.318 ± 0.0039 V was recorded on the 15th day (Fig. 1a).

Current density also increased gradually from 4.848 mA m⁻² (1st day) to 57.348 mA m⁻² (15th day) (Fig. 2c and d). The power density of the cell also followed a similar pattern with a maximum power density of 8.299 mW m⁻² was recorded on the 15th day. For *S. cerevisiae* also gradual increase was observed after 24 h, with the maximum recorded OCV was 0.287 ± 0.009 V on the 15th day (Fig. 1a) with increasing current density from 1.66 mA m⁻² on 1st day to 43.63 mA m⁻² on the 15th day (Fig. 2a and b). The power density was also recorded with the same increasing pattern out of which the maximum is 4.47 mW m⁻² on the 15th day (Fig. 2a).

The internal resistance (R_{int}) was very high against different *R_{an}*. 1000 Ω-82 KΩ for both the organisms on the 1st day which drastically decreases from the 2nd day onward (Fig. 3a and b). The combination of carbon fiber anode and stainless steel cathode has been explored well in the setup. This indicates the present setup was intact without any physical damage or corrosion and can be reused efficiently after sterilization. MFC appears to be an ecological approach to achieve cost-effective electricity generation. The maximum power density for *P. fermentans* was 8.299 mW m⁻²/100 mL while the current density was 57.348 mA m⁻²/100 mL on the 15th day and 0.318 ± 0.0039 V power output for the same day. For past years, *Pichia* has been explored in the field of MFC such as *Pichia pastoris, Pichia stipitis, Pichia kudriavzevii*, etc., for power generation with genetic modifications and less interest was focused on efficient bioethanol formation [23]. Whereas the *S. cerevisiae* was measured at a power density of 4.473 mW m⁻²/100 mL on the 15th day while the current density was 43.636 mA m⁻²/100 mL on the 15th day and power output was 0.287 ± 0.0094 V.

Among them, *P. fermentans* has resulted in better power, current density, and OCV than *S. cerevisiae*. This is because of the low resistance and large surface area of carbon brush electrodes which makes them ideal features as anodes from small as well as large scale applications of MFCs [27]. Yuan et al. recently published a report on simultaneous power generation systems and bioethanol formation. In this, they used *S. cerevisiae* as a model organism for dual and single-chambered MFC setup with or without Mediator for power generation and ethanol production. This study resulted in high power output (5.2 ± 0.5 W/m²) and high ethanol yield (92.5 ± 2%). There are many factors affecting electron transport such as cell metabolism, pH value, mediator type, yeast cell growth, substrate. But excessive addition of MB for electron transfer could affect the activity of yeast and lasts in unbalancing and blocking of electron transmission.

3.3 Glucose Consumption and Ethanol Production

The sugar consumption and ethanol production by these yeasts was studied in a 100 mL single-chambered cell setup in a sugar medium containing 10% (Glucose) 100 mL Erlenmeyer flasks, supplemented with 0.25% yeast extract, and incubated as described above. The glucose content was calculated from the standardization curve of D-glucose (γ = 6.5333x + 0.0613, R² = 0.9909) and expressed as mg/mL. The glucose was efficiently consumed during the initial days of the incubation period; about 15–20% of glucose was consumed in one day. After 24 h, the glucose concentration reduced from 10% (w/v) to 7.8 ± 0.0004% (w/v) in the reactors inoculated with *P. fermentans* and 8.4 ± 0.0003% (w/v) for *S. cerevisiae*, while the glucose concentration was only 0.2 ± 0.00002% (w/v) at the end of the experiment. A detectable amount of 0.025% (v/v) of ethanol was observed on day 1 for *P. fermentans*, which was maximum on the 12th day 4.7% (v/v), and remain constant on the 13th day and decreased gradually.

Whereas, in the case of *S. cerevisiae* the ethanol concentration measured on day 1 was 0.03% (v/v) with a remaining glucose concentration of 8.4% (w/v). *S. cerevisiae* produced maximum ethanol on the 12th day 5.6% (v/v) and the remaining glucose concentration was left with 0.23% (w/v) at the end of the experiment (Fig. 4a and b). In the figure, it shows alcohol production by yeast cells *P. fermentans* and *S. cerevisiae* on day-wise fermentation analysis.
Fig. 1 Open circuit voltage (a) and columbic efficiency (b) during MFC operation.

Fig. 2 Polarization curve of single-chambered fuel cell with *Saccharomyces cerevisiae*: (a) Current density vs Power density and (b) Current density vs Output voltage; and with *Pichia fermentans*: (c) Current density vs power density and (d) Current density vs Output voltage.

Fig. 3 Internal resistance of *Pichia fermentans* (a) and *Saccharomyces cerevisiae* (b) at different external loads.

Fig. 4 Glucose consumption and ethanol formation of (a) *Pichia fermentans* and (b) *Saccharomyces cerevisiae*.

The 24 hold cells were subjected to fermentation in a 10% glucose solution (10 g/100 mL) and have the ability to ferment glucose to ethanol. The cell setup of *P. fermentans* showed a maximum 4.7% (v/v) ethanol production of theoretical yield (6.41% v/v for 10% glucose) on day 12 and continued to remain constant up to day 13 and gradually decreased on consecutive days [28]. Since 1 mol of glucose produces 2 mol of ethanol and 2CO₂, it is being hypothe-
sized that the fermentation process will not be affected by MFC when using proper operational conditions. Ethanol and power generation occurs concurrently with the process of glucose metabolism through yeast-MFC. By using carbon fiber-based anode electrode for yeast attachment to enhance ethanol fermentation and electricity generation [18, 29]. It has been reported from several studies that under anaerobic conditions, 1 mol of glucose can be converted to give 2 mol of ethanol and 2 mol of electrons. It is evident that under anaerobic conditions, yeast-MFC cannot utilize all the electrons from the complete oxidation of glucose. Another major reason is the direct electron transfer by a yeast cell is very limited, as compared to Shewanella and Geobacter [30]. Therefore, this study focused on the simultaneous production of both bioethanol and electricity generation so that the substrate can be fully utilized for significant benefit. The result of the present study was compared with some of the studies, which demonstrated production of electricity and ethanol in MFC (Table 1).

Performance of MFCs may be influenced by mediator molecule (either indigenously produced or externally added). MFC with methylene blue generated higher current and power density as compared to MFC without mediator. However, the ethanol production by S. cerevisiae using glucose remained unaffected [5]. Bacterium, Zymomonas mobilis in MFC successfully demonstrated the ethanol production. This bacterium owns special Entner–Doudoroff pathway with less ATP and biomass produced and the low-energy coupling respiration. It produced about 35 g/L of ethanol in the fermentation broth [10], which seems to be very close to P. fermentans (37 g/L) used in the present study.

Earlier it has been reported that S. cerevisiae produced ethanol in MFC via a bio-electro-Fenton system. The maximum ethanol production (11.52 g/L) was achieved under anaerobic conditions and the measured maximum power density of 30.46 mW m$^{-2}$, which was much higher in Fe$_2$O$_3$/graphite systems as compared to graphite alone [31]. However, the overall power density was also higher as compared to present study (Table 1).

Based on the stoichiometric information, glucose concentrations were theoretically converted to electron amounts. It was used to calculate the number of electrons passing through the MFC circuit during glucose oxidation [32]. The CE According to the formula for CE, CE = (CE$_X$ × 100/C$_{Th}$), one can calculate how many electrons were involved in electron transfer through the MFC external circuit. Over 24 h, the CE of the proposed MFC setup for P. fermentans was 0.012% and increased up to 0.89% gradually along with the increase in the current density (Fig. 1b). Whereas, the CE after 24 h for the S. cerevisiae was 0.002% and increased to 0.47% on the concluded day. Still, most of the electrons may involve in the ethanol production process. This indicates that the MFC setup had little effect on fermentation efficiency [11, 27]. The carbon fiber anode provides a massive surface area for yeast adsorption on its electrode surface. Hence, the application of yeast cells on carbon fiber anode improved the immobilization effect onto the electrode. A higher glucose concentration would yield more electrons for high electricity generation and ethanol [33, 34]. Whereas, a massive literature survey suggests the ongoing efforts for energy generation and biofuel formation [35]. Still, there is a lot to unravel about the efficient mechanism behind the electron transfer, and different substrates conversion to energy generation requires further consideration.

Therefore, it is possible that yeast metabolic activity, energy production and its conversion into heat could be efficiently harvested as electricity through a combined approach of MFC during sugar fermentation and ethanol production. The findings may be seen as a step toward the production of ethanol from 6C as well as 5C sugars in MFC as P. fermentans may also utilize the 5C sugars present in plant biomass. Thus, the developed system may further be explored for conversion of lignocellulosic biomass into ethanol. However, this

| S. No | Microorganism               | Reactor working volume | Ethanol production | Maximum power generation | Reference  |
|-------|-----------------------------|------------------------|--------------------|--------------------------|------------|
| 1     | Saccharomyces cerevisiae    | 1000 ml                | 5.8 g/L            | 4.48 × 10$^{-3}$ W m$^{-2}$ | [5]        |
| 2     | Saccharomyces cerevisiae    | 800 ml                 | 9.28 g/L           | 5.2 W m$^{-2}$           | [27]       |
| 3     | Zymomonas mobilis           | 140 ml                 | 35 g/L             | 2.0 mW m$^{-2}$          | [10]       |
| 4     | Saccharomyces cerevisiae    | 450 ml                 | 11.52 g/L          | 30.46 mW m$^{-2}$        | [31]       |
| 5     | Saccharomyces cerevisiae    | 100 ml                 | 44 g/L             | 4.473 mW m$^{-2}$        | This study |
| 6     | Pichia fermentans           | 100 ml                 | 37 g/L             | 8.299 mW m$^{-2}$        | This study |
development may require some pre-treatment of biomass for effectively release of 6C and 5C sugars.

### 3.4 Effect of pH

In industrial ethanol production, yeast tolerates a wide range of pH, thus making the whole process less susceptible to contamination. It was observed that higher acidic conditions produce a larger amount of ethanol [12]. At the time of yeast fuel cell setup for \textit{P. fermentans} and \textit{S. cerevisiae}, the initial pH was maintained at 7. In the cells inoculated with \textit{P. fermentans}, the pH decreases to 6.5 after 24 h, and here ethanol was measured is 0.3% (v/v), which was further decreased to 5 and was constant from day 4 to day 7, and during these days the concentration of ethanol ranged from 0.8% to 2.3% (v/v). A decrease in pH and increase in ethanol yield was observed during further incubation. In \textit{S. cerevisiae} after 24 h the pH decreased up to 6.6 and a detectable volume of ethanol was recorded 0.3% (v/v). The pH decreased up to 5 during further 9 days of incubation and increasing ethanol concentration up to 5.6% (v/v). As per the results obtained \textit{P. fermentans} produced maximum ethanol of 4.7% on the 12th day with pH 4.3, while \textit{S. cerevisiae} produced maximum ethanol of 5.6% on the 12th day of incubation with 4.8 pH (Figure S2). The yeast \textit{S. cerevisiae} strains are considered to be pillars of the fermentation industry since then dominated ethanol fermentation due to their low pH tolerance for ethanol formation, organic acids, and low oxygen availability. It is evident that in \textit{P. fermentans} the correlated effect of respiratory and fermentative pathways supports growth and product formation. This yeast ferments glucose or xylene under oxygen-limited conditions [36].

### 3.5 EPS Production

EPS production was observed in both the yeast \textit{P. fermentans} and \textit{S. cerevisiae} along with their growth and colonization on the anode surface. Growing biofilm was observed under scanning electron microscopic images (Fig. 5).

The biofilm was developed on the anode surface as well as on the top of the medium. A significant correlation between EPS production and yeast growth was observed as analyzed on different days (days 1, 5, 10, and 15). The cells grew rapidly along with the biomass accumulation on different time intervals and resulted in a gradual increase in biomass, protein, EPS, and carbohydrate (Fig. 6a and b).

Both the yeast \textit{P. fermentans} and \textit{S. cerevisiae} produced EPS along with a dense biofilm on carbon fiber anode, which showed efficient direct electron transfer. However, its EPS may have boosted the electron transfer via the indirect mechanism [23]. Evident studies suggested that the combination of yeast attached anode improves electron transfer directly creating a synergistic effect [37]. EPS is composed of polysaccharides, extracellular DNA, glycoproteins, glycolipids, and proteins. This EPS plays some significant roles such as microbial cell to cell communication, protection from external and especially extracellular electron transfer [38]. The presence of carbohydrates in EPS carries out specific functions in mat formation.

The protective glycocalyx acts as a mediator in yeast for electron transfer and may be involved in oxidation and reduction reactions. The presence of EPS matrix was confirmed by infrared (IR) spectrum. After 24 h (day 1), the EPS production for \textit{Saccharomyces cerevisiae} and \textit{Pichia fermentans} was minimum but increased gradually during further incubation.
on day 5, day 10, and day 15. The spectrum of purified EPS showed numerous peaks from 3585–502 cm\(^{-1}\) (Figure S3 a and b). The EPS absorption frequency from 3585 to 3174 cm\(^{-1}\) and 3688–3071 cm\(^{-1}\) showed the presence of alcoholic (O–H) group, primary and secondary amine group confirms the polysaccharide nature of EPS produced by \(S.\ ceriseiae\) and \(P.\ fermentans\), respectively. Peaks ranging from 2969–2763 cm\(^{-1}\) and 2956–2763 cm\(^{-1}\) represent saturated aliphatic (alkene/alkyl) with methyl C–H (–CH\(_3\)) asymmetrical/symmetrical stretch and ether and oxy compound with methoxy, C–H stretch (CH\(_3\)-O-) in the case of \(S.\ ceriseiae\) and \(P.\ fermentans\), respectively [39]. The absorption peak at 1628 cm\(^{-1}\) was due to the stretch vibration of a carboxyl group (C = O) in \(S.\ ceriseiae\). Peaks ranging from 1838–1221 cm\(^{-1}\) represent primary amide C–O and secondary amide with N–H stretch bend in EPS produced by \(P.\ fermentans\). The absorption peaks ranging from 1011 to 1036 cm\(^{-1}\) showed alkyl halide and stretching vibrations of the pyranose ring [40]. The peaks between 904–509 and 604 cm\(^{-1}\) showed a stretch of alkyl halides and d (C–O–C) glycosidic linkage, respectively [23].

4 Conclusion

In this study, a system for simultaneous power generation and ethanol production was evaluated. The study demonstrated ethanol production by \(Pichia\ fermentans\) and \(Saccharomyces\ ceriseiae\), which reached up to 4.7% and 5.6% v/v of the theoretical yield (6.41% (v/v)), respectively, for 10% glucose in yeast microbial fuel cell. Analysis of EPS showed the presence of polysaccharides, a protein having several functional groups like C = O, -CONH\(_2\), -CH\(_3\), and OH, of \(P.\ fermentans\) and \(S.\ ceriseiae\). Another important aspect of the present study is the application of \(P.\ fermentans\) for ethanol production in a fuel cell, which was not explored yet. \(P.\ fermentans\) turned out as an efficient yeast for further microbial fuel cell application. The metabolic co-generation of bioethanol, bioelectricity, and other by-products, each stage requires application of engineering to scale up the production in a healthy and productive meet. Also, the advent of new metabolic tools and new organisms can potentially serve as concurrent bioethanol production and electricity generation in both production capacity and economy for the process to maximize efficiency.

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