# Potentiality of Some Yeast Isolates for Electricity Generation from Sugarcane Molasses

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## Abstract

Microbial fuel cells (MFCs) are considered simple and robust technology in the field of wastewater treatment as well as electricity generation. The middle-term application can be foreseen at market value prices. This technology might become certified as a prospective new technology for the conversion of organic wastes to electricity. In the current study, a total of 40 yeast isolates were collected from 20 samples of sugarcane molasses. Twenty-one yeast isolates were examined for their potential to produce electricity from sugarcane molasses. All isolates had the ability to produce electricity. Four of them (isolates number: MY 9, MY10, MY18 and MY19 were considered as high producers. They yielded power densities of 59.858, 61.25, 52.8125 and 47.432 mW/m$^2$, respectively. Yeast isolate MY10 from sugarcane molasses showed the highest power density (P) (61.25 mW/m$^2$), highest current (I) (0.175mA/m$^2$) and highest voltage (V) (350mV/m$^2$). This isolate was identified based on 18S rRNA sequences as *Meyerozyma guilliermondii* and deposited in the Gene bank nucleotide sequence database under accession numbers KY624417.

***Keywords: Microbial fuel cells; Bioelectricity generation; Sugarcane molasses; Yeasts.***

## Introduction

Fossil fuels including coal, oil and natural gas are currently the world's primary energy source which is formed from organic material over millions of years. Over the past century, fossil fuels have fueled global economic development and it will not appreciably run out for at least 100 years or more and they can also irreparably harm the environment (Goldemberg, 2007). The burning of fossil fuels was responsible for gas emissions. These gases insulate the planet, and could lead to potentially terrible changes in the earth's climate. More sustainable solutions and environmentally safe resources of renewable energy are considered promising for industrial biotechnology.

Among the several renewable energy sources (RES), microbial fuel cells (MFCs) have emerged as one of the most promising solutions. A key reason that could make the MFCs a strategic technology is related to electricity production from biodegradable compounds (Song et al., 2015; Zhou et al., 2015).

Microorganisms can act as a biocatalyst in fuel cells. Cultures of *Firmicutes* (Park et al., 2001) and *Acidobacteria* (Bond and Lovley, 2005) and *Proteobacteria* (Zhang et al., 2008) in addition to the yeast strains of *Saccharomyces cerevisiae* (Walker and Walker, 2006) and *Hansenula anomala* (Prasad et al., 2007) were used to catalyze the transfer of organic matter that present in wastes into electricity through a quite complicated biochemical process (Dumas et al., 2008; Mohan et al., 2008). These microorganisms can oxidize organic wastes to $CO_2$ and produce electrons and protons. Electrons produced by microorganisms are transferred to the anode electrode and current to the cathode linked by a conductive material containing a resistor or operated under a load. Protons go throughout the proton exchange membrane (PEM) and enter the cathode chamber.

Molasses is the by-product from sugar industry and is considered as promising feed stock in the production of biofuel owing to its high sugar content (55%), high volume of production, accessibility and cost-competativeness (Zohri et al., 2014 $_{a\&b}$).

The objective outlined in the present paper aimed to detect the potentiality of yeast isolates recovered from sugarcane molasses as potent electricity producers for generation of bioelectricity from sugarcane molasses.

## Materials and Methods

### Samples Collection

During the harvesting season of 2016, a total of 20 samples (500 g each) of sugarcane molasses were collected from four different sugar factories in Upper Egypt (Abo Qurqas, Nag Hammady, Dishna and Qus). Samples were kept in a refrigerator (4 ˚C) until further microbiological analysis.

### Isolation of yeasts from sugarcane molasses

Yeasts were isolated by transferring 1 ml of sugarcane molasses to 100 ml conical flask containing 25 ml liquid YME medium (contained (g/l):Glucose, 10; Yeast extract, 3; Malt extract, 3; Peptone, 5; and Chloromphenicol, 0.25 (Wickerham, 1951), then the conical flask was incubated at 30 ˚C for 48 hr. After that, 1 ml of the culture was transferred to Petri dishes with solid YME medium and incubated at 30˚C for 48 hr. Single yeast colonies were transferred into plates containing YME for purification. The pure yeasts were preserved in YME slants and kept in a refrigerator (4 ˚C) until further cultivation and utilization as inoculum in the microbial fuel cell (MFCs).

### Screening for electricity production by isolated yeasts

### Inoculum preparation

Twenty one yeast isolates recovered from sugarcane molasses were, individually cultured in 100 ml Erlenmeyer flasks containing 25 ml YME broth medium. Cultures were incubated aerobically in an orbital shaking incubator for 24 h at 120 rpm. Each culture of yeasts was used as an inoculum in the microbial fuel cells (MFC).

**Microbial fuel cells construction**

Different configurations are possible for MFCs. A two chamber MFC (H" shape) inexpensive design was used in this study. It consisted of a dual chamber of two bottles; an anaerobic anode chamber and an aerated cathode chamber, coupled by a tube containing a separator which was a proton exchange membrane (PEM). Which was clamped between the flattened ends of the tube fitted with two rubber gaskets. On each side of the PEM, two electrodes (one anode and one cathode, both 10 cm2) consisting of non-wet-proof carbon paper where provided. All MFC tests were operated at a fixed external circuit resistance (2000Ω, unless stated otherwise).

**Microbial fuel cells operation**

The two chambers were filled with 250 ml of nutrient mineral buffer (NMB) solution consists of (g/L) $NaHCO_3$, 3.13; $NH_4Cl$, 0.31; $NaH_2PO_4.H_2O$, 0.75;KCl, 0.13; $NaH_2PO_4$, 4.22; $Na_2HPO_4$, 2.75; trace metal and vitamin solutions as previously described by (Oh et al, 2004). The anode compartment was inoculated with 1 ml of yeast culture, 1 ml of methylene blue as a mediator and 1 ml of sugarcane molasses. A 50 mM of $K_3Fe(CN)_6$ as the terminal electron acceptor was used as catholyte. Once the MFCs demonstrated a repeatable cycle of power generation, the anode compartment was refilled with fresh NMB solution and molasses for each isolate. The H-type MFC was operated at 30 ˚C for 3 days then the opened circuit voltage (OCV) and closed circuit voltage (CCV) were measured using multimeter (model DT830D).

## Calculations

Cell voltages (V) were measured with an external circuit resistor (2000Ω) for close circuit voltage measurement using a multimeter that recorded every 24 hr. Power (P) was calculated according to $P=IV(I=V/R)$.Where, I (A) is the current, V (V) is the voltage, and R (Ω) is the external resistance. The power was normalized to the anode surface area (Logan et al., 2005).

### Identification of the highly electricity producing yeast isolate

The analysis of 18S rRNA gene was conducted. Sequences of the 18S rRNA of isolate were first analyzed using the advanced BLAST search program at the NCBI website: http://www.ncbi.nlm.nih.gov/BLAST/T in order to evaluate the degree of similarity with closely related strains deposited in the GenBank.

## Results and Discussion

### Isolation of yeasts from sugarcane molasses

A total of 40 yeast isolates were collected from the 20 samples of sugarcane molasses. Yeasts appeared in 13 out of the 20 tested samples matching 65 % of the total samples. Yeast isolates were collected from the four factories on yeast extract malt extract agar medium as follow: thirteen yeast isolates from Abo Qurqas, 2, 5 and 20 isolates from Qus, Dishna and Nag Hammady, respectively (Table, 1). In Egypt, Nor El-Din (2015) recovered sixty nine yeast isolates from 25 samples of sugarcane molasses collected from five sugar factories. In Sri Lanka, Chandrasena et al., (2006) isolated 1000 yeast isolates from various sugar – rich sources.

**Table 1 : Yeast isolates recovered on yeast extract malt extract agar medium from 20 samples of sugarcane molasses collected from four Egyptian sugar factories.**

| Factories | Sample number | Number of isolates | Code number (MY*) |
|---|---|---|---|
| Abo Qurqas | 1 | 7 | MY1, MY2, MY3, MY4, MY5, MY6, MY7 |
| | 2 | - | - |
| | 3 | 2 | MY8, MY9 |
| | 4 | 1 | MY10 |
| | 5 | 3 | MY11, MY12, MY13 |
| Dishna | 1 | 2 | MY14, MY15 |
| | 2 | 2 | MY16, MY17 |
| | 3 | 1 | MY18 |
| | 4 | - | - |
| | 5 | - | - |
| Nag Hammady | 1 | - | - |
| | 2 | 6 | MY19, MY20, MY21, MY22, MY23, MY24 |
| | 3 | 4 | MY25, MY26, MY27, MY28 |
| | 4 | 4 | MY29, MY30, MY31, MY32 |
| | 5 | 4 | MY33, MY34, MY35, MY36 |
| | 6 | 2 | MY37, MY38 |
| Qus | 1 | - | - |
| | 2 | 2 | MY39, MY40 |
| | 3 | - | - |
| | 4 | - | - |
| Total Isolates | 40 | | |

***MY= Molasses Yeast**

**Potentiality of some yeast isolates for electricity production using sugarcane molasses**

In the current study, 21out of the 40 yeast isolates were investigated for their ability to produce electricity from molasses using MFC technology with external resistance of 2000 Ω. Results of the power production by yeasts from sugarcane molasses are shown in Table (2). All tested yeast isolates exhibited various degrees of power activities when used as biocatalysts in MFC with 1ml of sugarcane molasses as substrate. Four out of the twenty one active yeast isolates were considered as highly power producers (First group). The highest production was recorded by MY9, MY10, MY18 and MY19 with power density of 59.858, 61.25, 52.8125 and 47.432 $mW/m^2$, respectively. The second group contains moderate producers of power such as MY4, MY11, MY15 and MY16. The remaining yeast isolates have low production of power as shown in Table (2). Yeast isolate no. MY10 showed the highest power density (P) (61.25 $mW/m^2$), highest current density (I) (0.175$mA/m^2$) and highest voltage (V) (350mV).

According to the obtained data, yeast isolates showed various degrees of power densities with the highest power density (P) (61.25 mW/m2) recorded for yeast isolate MY10. These results revealed the potentiality of yeast isolates as ideal biocatalysts for MFCs; due to their wide substrate range and they are robust, easily cultivated, fast growing. Adaptation of yeasts to a various environmental conditions is the main advantage for yeast-catalyzed fuel cells development (Gunawardena et al., 2008, Schaetzle et al., 2008).

Although, there are numerous scientific reports related to the application of bacteria as a biocatalyst in microbial fuel cell technology, only a few yeast species – *Saccharomyces cerevisiae* (Gunawardena et al., 2008; Rawson et al., 2012; Walker and Walker, 2006), *Hansenula anomala* (Prasad et al., 2007), *Hansenula* *polymorpha* (Shkil et al., 2011), *Arxula adeninivorans* (Haslett et al., 2011) have been studied as biocatalysts in biofuel cells.

Interestingly, our results are quite good compared with other published data. Walker and Walker, (2006) reported that, *Saccharomyces cerevisiae* grown on glucose substrate generate maximum voltage at the open circuit potential (~0.5 V) by using an electron mediator. Moreover, bioelectricity production by *Hansenula anomala* as a biocatalyst by using graphite electrode revealed maximum cell voltage of 0.52V over 74 h and by using graphite felt electrode the highest cell voltage was 0.658V over a period of 96 h (Prasad, et al., 2007). Gunawardena et al., (2008) stated that *S. cerevisiae*-based fuel cell showed enhanced performance when methylene blue mediators and ferricyanide are used and exhibited a maximum power generation of 146.71±7.7 mW/m3 and produced a maximum open circuit voltage of 383.6±1.5 mV under 100 kΩ of external load. Recently, Permana et al. (2015) showed that, the largest power density of MFCs with mediator was 4.48×10-3 W/m2 utilizing *Saccharomyces cerevisiae* as biocatalysts. In our study MY10 yeast – based biofuel cell produce higher power density (61.25 mW/m2) and that is related to the higher efficiency of electron transfer between the mediator molecules and the microorganism's cell wall.

**Table 2 : Potentiality of different yeast isolates to produce electricity**

| Isolates | V (mV) | I (mA) | P (mW/m$^2$) | Remark* |
|---|---|---|---|---|
| MY 1 | 54 | 0.027 | 1.458 | L |
| MY 2 | 60 | 0.03 | 1.8 | L |
| MY 3 | 84 | 0.042 | 3.528 | L |
| MY 4 | 238 | 0.119 | 28.322 | M |
| MY 5 | 64 | 0.032 | 2.048 | L |
| MY 6 | 167 | 0.0835 | 13.9445 | L |
| MY 7 | 195 | 0.0975 | 19.0125 | L |
| MY 8 | 142 | 0.071 | 10.082 | L |
| MY 9 | 346 | 0.173 | 59.858 | H |
| MY10 | 350 | 0.175 | 61.25 | H |
| MY 11 | 246 | 0.123 | 30.258 | M |
| MY 12 | 98 | 0.049 | 4.802 | L |
| MY 13 | 164 | 0.082 | 13.448 | L |
| MY 14 | 102 | 0.051 | 5.202 | L |
| MY 15 | 279 | 0.1395 | 38.9205 | M |
| MY 16 | 246 | 0.123 | 30.258 | M |
| MY 17 | 87 | 0.0435 | 3.7845 | L |
| MY 18 | 325 | 0.1625 | 52.8125 | H |
| MY 19 | 308 | 0.154 | 47.432 | H |
| MY 20 | 109 | 0.0545 | 5.9405 | L |
| MY 21 | 99 | 0.0495 | 4.9005 | L |

***H= High production of power density (more than 40mW/m$^2$); M= Moderate production of power density (20 $\geq$ 40 mW/m$^2$) and L= Low production of power density (less than 20 mW/m$^2$).**

**Molecular identification of the highly electricity producing yeast strain**

The highest electricity producer yeast isolate from sugarcane molasses (MY10) was selected for identification based on phylogenic analysis of 18S rRNA gene sequences and compared with full sequences available in the Gene Bank database using BLAST search from the National Center of Biotechnology Information (NCBI). MY10 isolate had a 99 % similarity sequence with *Meyerozyma guilliermondii* (KP764968),*Meyerozyma guilliermondii* (KP764967) and *Pichia guilliermondii* (AM160625) that available in Genbank database. The nucleotide sequence of yeast strain MY10 *Meyerozyma guilliermondii* was deposited in Gene bank nucleotide sequence database under the accession number KY624417.

## Conclusions

Microbial fuel cells have recently considered as an interesting technology for the production of electricity by microorganisms. The current study revealed the potentiality of yeast isolates as a biocatalyst for power generation from renewable sustainable resources. Yeast strain *Meyerozyma guilliermondii* (accession number KY624417) exhibited a promising exoelectrogenic properties from sugarcane molasses recording the highest power density (61.25 mW/m2), highest current (0.175mA/m2) and highest voltage (350mV/m2).

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الملخص العربي

مقدرة بعض عزالات الخميرة لتوليد الكهرباء من قصب السكر

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**تعتبر خلايا الوقود الحيوية في الآونة الأخيرة تقنية مثيرة لإنتاج الكهرباء بواسطة الكائنات الحية الدقيقة. كشفت هذه الدراسة عن إمكانية بعض عزالات الخميرة والمعزولة من مولاس قصب السكر كمحفز حيوي لتوليد الطاقة الكهربائية من الموارد المستدامة المتجددة مثل مخلفات صناعة السكر. أظهرت سلالة الخميرة *Meyerozyma guilliermondii* ,والمسجلة بالبنك الجينى تحت رقم (KY624417) خصائص كهربائية واعدة من مخلفات صناعة السكر مسجلة أعلى كثافة للطاقة ($61.25\ mW / m^2$)، أعلى شدة تيار ($0.175mA / m^2$) وأعلى جهد ($350\ mV / m^2$ ).**