Background

I read an article in Washington post that there are about 1.3 billion people living without electricity in this world. Out of that there are 300 million people in India, spread across 80,000 villages living without electricity. This is a very large number and I strongly believe that everyone should have access to electricity. People do not have access to power because it is not produced in enough quantity. I was curious to know if there were any ways through villagers can generate power on their own, that were also environmentally friendly. I have always been fascinated by Microbial Fuel Cell (MFC), as a way to convert household waste into electricity. Bacteria or yeast can be used as the micro organism in microbial fuel cell. MFC is a device which converts chemical energy into electrical energy by respiring anaerobically. Anaerobic respiration, is the release of energy, without the presence of oxygen. The following reaction equation depicts the basic reactors and products obtained during anaerobic respiration.

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2C0_2$

i.e glucose → ethanol + carbon dioxide

Anaerobic respiration, can however, be split into two parts: glycolysis, and fermentation.

Glycolysis is the break down of glucose. It can be summed up in the following [1]

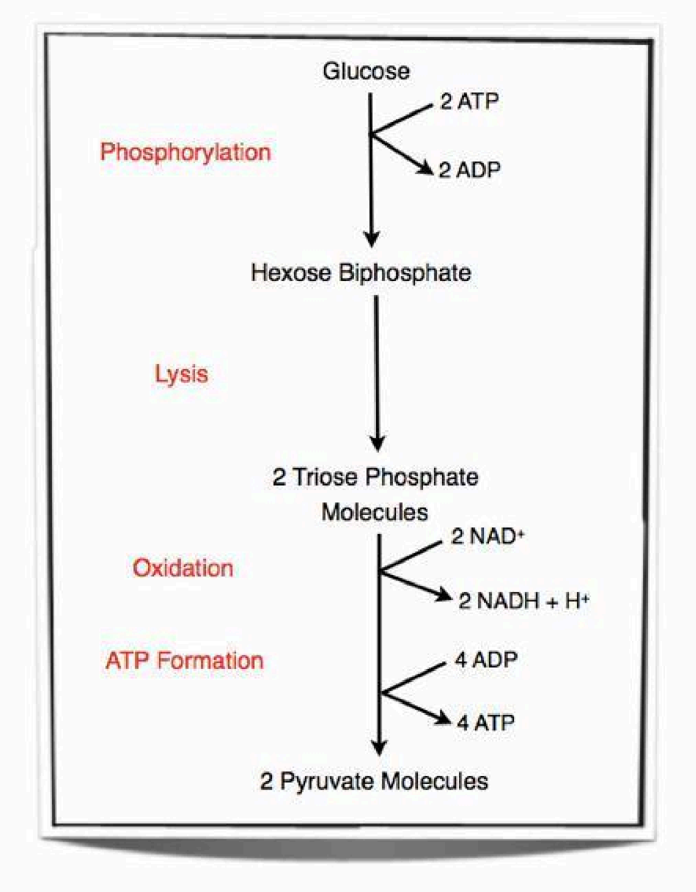


FIGURE 1[1]

[1] ("IB Biology Notes - 8.1 Cell Respiration")

The end products of glycolysis are 2 pyurvate molecules. 2 ATP molecules are used, and 4 ATP molecules are produced (there's a yield of 2 ATP molecules). Two NAD+ are converted into NADH+ H+.

The second part of anaerobic respiration, is fermentation. Due to the lack of oxygen, the following a process called alcohol fermentation occurs, that converts the pyruvate molecules, into NAD and ethanol. The following [2] explains the process:

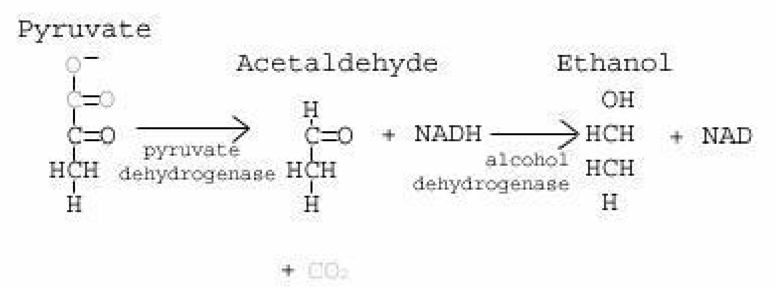


FIGURE 2

The microbial fuel cell then converts the obtained chemical energy from anaerobic respiration, into electrical energy via oxidation reduction reactions (a process which involves a transfer of electrons between two species [3]). The microbial fuel cells use living biocatalysts to facilitate the movement of electrons through their systems instead of the traditional chemically catalysed oxidation of a fuel at the anode, and reduction at the cathode[4].

If MFC can work, then it has the potential of easing electricity gap in villages, as its fuel.

Research Question:

Which of the yeast strains of ***Saccharomyces cerevisiae***: instant yeast, compressed fresh yeast and active dry yeast, produces the highest electrical energy when measured against each other in a microbial fuel cell, using a voltmeter?

Aim

The aim of this biological analysis is to investigate and deduce which strain of the specie of yeast known as ***Saccharomyces cerevisiae***, would produce the most electrical energy in a microbial fuel cell, and thus, be the most advisable one to use, in poorer villages.

Hypotheses

1. The most commonly used ***Saccharomyces cerevisiae*** strain, Instant Yeast, will be the highest producer of electrical energy, compared to the other strains, and thus will produce the highest voltage in the MFC. I assume this as Instant Yeast is the most commonly used form of yeast as, I have noticed that when cooking, the Instant Yeast causes for the dough to rise the fastest. This speed may suggest that it quickly respires anaerobically, and also, the fact that a small amount, can rise a large amount of bread, may mean that it produces a lot of the required alcohol concentration, for a small amount of sugar.

[2] "SparkNote on Glycolysis" (SparkNotes Editors)

[3] "Oxidation-Reduction Reactions" (Chemistry LibreTexts)

[4] “What are Microbial Fuel Cells- How Do Fuel Cells Work, Info On Microbial Fuel Cells.” (Davison)

Strains of *Saccharomyces cerevisiae* used in investigation

Instant Yeast

Active Dry Yeast
Compressed fresh Yeast

(Note: the binomial nomenclature was unable to be found, as these were obtained locally, and with the name specific strain, not mentioned on the packages. Further research into the name, including sources such as the internet, proved unfruitful.)

Safety concerns

Micro organism is also found in other organic sources such as cow dung or soil top. However, these samples may also contain bacteria which can be harmful. Hence, the study was restricted to the most commonly used specie of yeast, ***Saccharomyces cerevisiae (also known as Baker's Yeast)*** only. If the experiment is successful with the yeast it can be replicated for any other material with appropriate safety.

Tools

To make the Microbial Fuel Cell I used the following materials:
Drill press Measuring cylinder

Soldering iron with solder Skewer

Multi-meter
Raw material for the body: 2 Carbon brushes
2 1x1x1 PVC 40 Tee
3 1" PVC Schedule 40 connector

1 1" PVC 40 Slip cap
2 1" PVC 40 Plug Dry sponge Wires

Raw material for contents of anode

Active Dry Yeast/ Instant Yeast/ Compressed Fresh Yeast being tested
Sucrose

Water

Raw material for contents of cathode

NaCl Salt
Water

Salt bridge medium

Unflavored kitchen gelatine (jelly)
NaCl Salt
Coupler
Plastic wrap
Measuring beaker

Rubber band

Materials for sugar source

Sucrose

For the nutrient broth:

Create a nutrient broth that can act as a source of energy and sugar, for the yeast strains, by mixing sucrose with water.

Independent Variable	How I controlled it?	Why I controlled it?
Active Dry Yeast/ Compressed Fresh Yeast/ Instant Yeast	I ensured that I took the same concentration of each strain.	In order to conduct a fair experiment.

Dependent Variable
Voltage produced

Controlled Variable
Amount of nutrient broth each strain recieved
Composition of nutrient broth was kept the same
Temperature of surroundings
Amount/ concentration of each yeast strain inserted into MFC

Method

To build the salt bride:

Take a coupler section and fasten the plastic wrap firmly over one end. Use a rubber band to secure it.
Place the coupler in a bowl, with its open end facing upwards, and pour the unflavored gelatin into it upto three fourth.

Fill rest of the coupler with salt.
Pour mixture into measuring beaker, and then return it back to the coupling, before returning it back to the measuring beaker. This is done to blend the mixture thoroughly.
Add a tablespoon of salt to the mixture.
Use a spoon to stir the mixture.
Pour mixture back into the upright coupler.

Boil water and pour it into a measuring cylinder.
Use the boiled water to fill the salt bridge, gently.
Ensure that the entire column of the mixture in the salt bridge, has been saturated.
11. Allow the mixture to stand for two to three minutes.
Pour off any water that has accumulated at the top.

Place the bowl in a refrigerator for around three to four hours.
Remove the salt bridge from the fridge once assembly of the electrodes of the microbial fuel cell has been completed.

- Assembling the electrodes of the Microbial Fuel Cell:

To Build the Anode assembly:

Take a dry sponge and cut it in such a way that it’s 3/4’’ wide and an inch long. It should be able to easily fit into the mouth of the neck of the screw top.

Remove the sponge, and using a nail create a hole big enough for the wire to pass through the sponge. This allows for the decomposition gases to escape into the atmosphere, and the sponge inhibits air from entering the chamber.
Insert the electrode lead through the sponge and then slide the sponge into the neck of the screw top. The wire must be able to easily pass through the screw cap.
Moisten the sponge with water in order to fill out the neck, and screw the screw top lightly.

To Build The Cathode assembly:

Detach the coil wire from the remaining brush.

Cut out a strip 1" wide and 4" long, from the remaining brush.
Further cut this strip into two strips.
Ensure that the remaining carbon brush remains between two strips, in order to maintain the position of only the top being exposed.
Now insert this into a 1" PVC Schedule 40 connector.
In order to fix the electrode into its position, wet the sponge.

- Assembling the Microbial Fuel cell:

1. Only perform this once the salt bride has cooled and solidified. 2. Insert the end caps completely into the remaining two couplers.

3. Insert the couplers into the base of the two 1x1x1 PVC 40 Tees, as this will provide the foundation for the fuel cell.
4. Fix the salt bride into the centre of the two 1x1x1 PVC 40 Tees. It will be clear that it has been placed properly if the salt bride connector is not visible between the two components.

- Operating and powering the Microbial Fuel Cell:

Pour about 59 ml of warm water into a measuring cup.
Keep adding salt to the water until no more can dissolve, in order to create a saturated solution.
Ensure that the saturated solution has been created by stirring contents of the container and swaying the container from side to side.
Allow for the water to cool thoroughly.
Detach the sponge pieces from the cathode and ensure that they are completely soaked in the salt solution.

Pour off the rest into the cathode, until nothing but salt slush remains.
Place the carbon electrode on a sponge, and gently cover it with the salt slush.
Sandwich the sponge from point 7, by placing the other sponge on top of it.
Attach this back into the connector, and fasten it into the cathode tube. Ensure that the tube doesn't overflow.

The microbial fuel cell is now ready to be used. It looks like following:

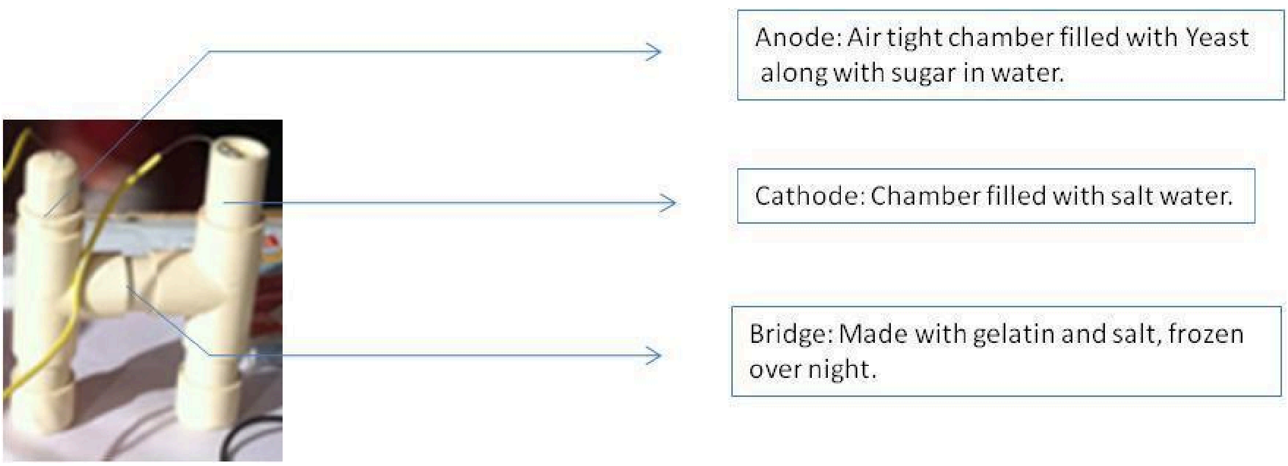


FIGURE 3[1]

Qualitative Observations

As the anaerobic, and oxidation-reduction processes occur inside the MFC, no physical, observe-able changes can be seen. The only physical change that can be seen, is the changing of the voltmeter reading. It was observed that the Active Dry Yeast began to produce a reading first. However, a sharp alcohol scented smell begins to appear for each other the yeast strains, once fermentation has begun. Instant Yeast produced the strongest smell, that lasted the longes

[1] Picture taken of the MFC that I constructed.

Data

Three set of readings of the voltage were taken from each of the yeast. The average was calculated for each of them for the comparison purpose. The readings were taken every 6 hours, during a time span of 72 hours.

I: Trial 1

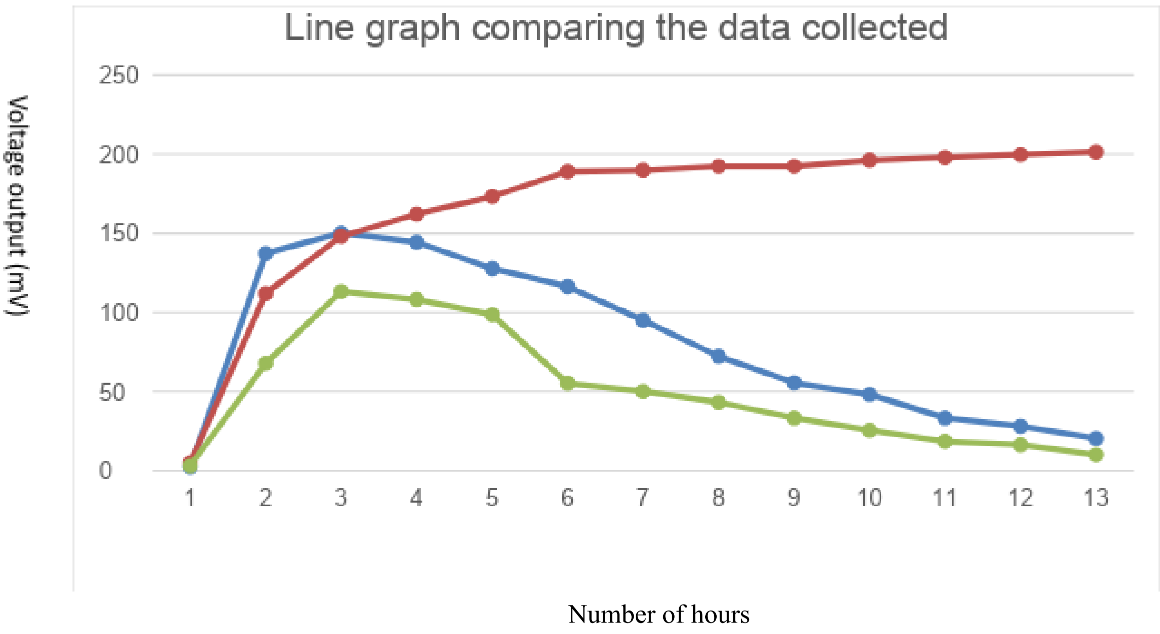
II: Trial 2
III: Trial 3

Active Dry Yeast

Hours	Voltage produced by Active Dry Yeast (in mV)/ no. of uncertainty				
	I	II	III	Average	Standard Deviation
0	2.4	2.4	2.3	2.4	0.06
6	140.3	133.3	138.3	137.3	3.60
12	147.3	151.4	152.0	150.2	2.56
18	146.7	141.3	145.2	144.4	2.79
24	124.5	132.7	126.2	127.8	4.33
30	108.4	127.1	113.7	116.4	9.64
36	85.0	101.0	99.0	95	8.72
42	73.2	72.7	71.0	72.3	1.15
48	55.4	55.3	55.9	55.5	0.321
54	44.9	53.2	46.5	48.2	4.403
60	32.8	33.5	33.6	33.3	0.436
66	27.6	27.8	28.9	28.9	0.700
72	20.4	20.5	20.3	20.3	0.100

Hours	Voltage produced by Compressed Fresh Yeast (in mV)/ no. of uncertainty				
	I	II	III	Average	Standard Deviation
0	3.2	3.1	3.3	3.2	0
6	72.8	61.1	69.5	67.8	6.03
12	108.8	114.8	116.3	113.3	3.97
18	104.8	112.7	107.1	108.2	4.06
24	100.8	98.1	97.0	98.6	1.96
30	57.4	52.3	55.9	55.2	2.62
36	47.9	51.6	51.1	50.2	2.01
42	37.9	40.5	51.2	43.2	7.05
48	33.5	33.5	32.9	33.3	0.346
54	23.2	28.9	24.4	25.5	0.693
60	17.8	19.0	18.7	18.5	0.520
66	15.8	16.1	17.0	16.3	0.693
72	10.1	10.2	10.0	10.1	0.0578

Hours	Voltage produced by Instant Yeast (in mV)/ no. of uncertainty				
	I	II	III	Average	Standard Deviation
0	5.0	5.0	4.9	5.0	0.06
6	116.4	106.4	113.5	112.1	5.14
12	140.4	152.7	151.2	148.1	6.71
18	157.2	168.9	160.5	162.2	6.03
24	169.8	174.2	175.9	173.3	3.15
30	194.8	181.5	191.0	189.1	6.85
36	182.7	194.2	192.8	189.9	6.27
42	185.2	188.8	203.2	192.4	9.52
48	194.7	194.8	193.7	194.4	0.608
54	191.5	203.2	193.9	196.2	6.18
60	191.5	202.5	200.3	198.1	5.82
66	204.5	202.1	192.8	199.8	6.18
72	200.8	200.4	203.4	201.5	1.63



Hours represented as numbers in y-axis=

1 = 6 hour
2= 12 hours
3= 18 hours
4= 24 hours
5= 30 hours
6= 36 hours
7= 42 hours
8= 48 hours
9= 54 hours
10= 60 hours
11= 66 hours
12= 72 hours

Series 1= Active Dry Yeast
Series 2= Instant Yeast
Series 3= Fresh Yeast
Note: Error bars indicated are of standard deviation.
According to the graph, the voltage produced by Instant yeast was the highest. This occurred, although initially Active Dry Yeast rose the fastest (for the first 13 to 14 hours), before it began to produce the same voltage as the Instant Yeast (150 mV at 15 hours), before it began to reduce the voltage. Instant Yeast, however, began to increase its voltage(and thus, it’s respiration rate, and alcohol production) after 15 hours. Fresh Yeast, unlike the other two species, rises at a slower rate until hour 14, before it began to decline. Unlike the other two species, Fresh Yeast produces its highest voltage at around 110 mV, during hour 14. This may be due to the fact that Instant Yeast may have a higher tolerance level[2] for alcohol, and thus able to survive at higher concentrations, than Fresh Yeast and Active Dry Yeast. It could also indicate the amount of time it takes Instant Yeast to begin to metabolise properly, and thus, produce a higher concentration of alcohol.

For the Instant Yeast, at around 6 hours and 72 hours, the error bars don’t overlap.

T-TEST between Instant Yeast & Active Dry Yeast

A t-test was conducted to measure if there was any significant difference between the voltage produced by Instant Yeast, and Active Dry Yeast.

Formula:

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{S_1^{\,2}}{N_1} + \frac{S_2^{\,2}}{N_2}}}$$

Figure 4[3]: T-Test formula

[2] Tolerance level to alcohol of the yeast, here refers to the yeasts ability to survive in higher concentrations of alcohol

[3] (http://ezquants.weebly.com/, n.d.)

In the above formula:

X= mean
S= standard deviation
n= number of trials
N_0 **(Null Hypothesis)** is that there is no significant difference between the voltage produced by Instant Yeast, and Active Dry Yeast.
N_1 (Alternate Hypothesis) is that there is a significant difference between the voltage produced by Instant Yeast and Active Dry Yeast.

Degree of freedom=

df= n_1-n_2-2
df= 4
$t_{critical}$ ($p \leq 0.05$)= 2.776
t_{calc}= 192.08

As the t_{calc} is greater than the $t_{critical}$ the null hypothesis is rejected, and the alternate hypothesis is accepted instead, that there is a significant difference between the voltage produced by Instant Yeast and Active Dry Yeast.

T-TEST between Instant Yeast & Compressed Fresh Yeast

A t-test was conducted to measure if there was any significant difference between the voltage produced by Instant Yeast, and Compressed Fresh Yeast.

Formula:

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{{S_1}^2}{N_1} + \frac{{S_2}^2}{N_2}}}$$

Figure 5: T-Test formula

In the above formula:

X= mean
S= standard deviation
n= number of trials

N_0 **(Null Hypothesis)** is that there is no significant difference between the voltage produced by Instant Yeast, and Compressed Fresh Yeast.
N_1 (Alternate Hypothesis) is that there is a significant difference between the voltage produced by Instant Yeast and Compressed Fresh Yeast.

Degree of freedom=

df= n_1-n_2-2
df= 4
$t_{critical}$ ($p \leq 0.05$)= 2.776
t_{calc}= 203.3

As the t_{calc} is greater than the $t_{critical}$ the null hypothesis is rejected, and the alternate hypothesis is accepted instead, that there is a significant difference between the voltage produced by Instant Yeast and Compressed Fresh Yeast.

Conclusion

In conclusion, it was deduced that the hypothesis, that the most commonly used ***Saccharomyces cerevisiae*** strain, Instant Yeast, will be the highest producer of electrical energy, compared to the other strains, and thus will produce the highest voltage in the MFC, was proven correct. On comparing with the three strains, used for MFC, it was seen that Instant Yeast is the most efficient yeast strain in producing energy using a microbial fuel cell, and was proven using a T-Test. This could be due to the Instant Yeast having a higher tolerance to the alcohol they produced. Another possible reason, is that the metabolic rate of the Instant Yeast may take time to increase. Further on, the lifespan of the Instant Yeast seems longer than the other strains, as it continues to rise in its voltage, and doesn't drop, while the other two do, over the span of 72 hours.

Application

This comparison could help answer the question of, "How can electricity be brought to villages in an economically efficient manner?" as, in place of the yeast, bacteria that is found in compost and waste material, can be used to power the microbial fuel cell. This will help solve the power crisis in numerous villages, while adopting an eco-friendly method to do so.

Possible errors

The possible factors could have created fluctuations in the readings, and thus, act as possible error contributors during the experiment.

Temperature wasn't kept constant, and thus, may have varied between different experiments. This could have either sped up, or slowed down, the anaerobic respiration, and thus, affected the results of the experiment.

As a specific instrument wasn't used to measure the concentration of the yeast strain (a sponge was used to soak in yeast) in each experiment could have differed, and thus, affected the outcome.

Further analysis

I observed that MFC produced very encouraging results. To make it viable option to generate power for large scale usage, more study is needed to be done for the MVFC. Following are the elements which need to be researched:

increasing the research on different kinds of microorganisms that could possibly be used in an MFC.
the research could be carried out with different sugars. I used sucrose for my experiment, but impact of different sugars on the MFC can be measured as well.
the effect of the other variables in the surrounding of the MFC can be measured, and noted down.

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