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RESEARCH ARTICLE

A HYBRID BIOREACTOR WITH INTEGRATED BIOETHANOL-BIODIESEL-MICROBIAL FUEL CELL UTILIZING YEAST AND PHOTOSYNTHETIC ALGAE

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ABSTRACT

Bioenergy as a source of sustainable energy production from organic wastes has exaggerated research in this area in the past few years. Bio-ethanol, bio-diesel, and bio-electricity produced from a hybrid bioreactor consisting a combination of the fermentor, photobioreactor, and microbial fuel cell, has shown a promising result in producing eco-friendly fuel with economical benefits. Apart from the energy production, these systems have been used in waste water treatment, feeds for animals, beverages, and production of commercial gases like biogas (methane from anaerobic digester), oxygen, nitrogen etc. by employing advanced technology. However many drawbacks are also there in the system which can be resolved by producing genetically engineered strains and use of advanced techniques in the processing like pretreatment and isolation of the bioenergy from the system and use of advanced probes for better conduction of the current in microbial fuel cell. We conclude that for economical and effective alternative source of sustainable energy, the hybrid bioreactor can be a potential future with further more advancement.

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INTRODUCTION

Utilization of all the products and byproducts produced by fermentors and photo-bioreactors for economical and biological uses has become the major concern of our society. Depletion of natural energy sources needs to be supported by other renewable energy sources such as bioethanol which can be easily produced from biomass mostly via fermentation process of sugar (sugar cane, sugar beet), starch (corn) and cellulose and provide society with raw materials in a sustainable way (Hahn-Hägerdal, 2006). After the first oil crisis of 1973 in Brazil, bioethanol as a fuel received attentive recognition which led the country to launch a national program to replace part of gasoline by ethanol (Basso, 2008). It has taken up much resourcefulness in Brazil, US, and some European countries as it can be blended with petrol and even can be used as neat alcohol for the engines and is expected to be one of the dominating biofuels in upcoming 20 years in transport sector (Hahn-Hägerdal, 2006). Utilization of free cells of *Saccharomyces cerevisiae* is one of the traditional methods of bioethanol production (Rivaldi, 2008). In Brazilian fermentation processes >90% of yeast cells are used from one fermentation cycle to next causing high cell density inside the fermentor (10-17% w/v, wet basis) contributing to very short

fermentation time (Basso, 2008). Another alternative to fossil fuels might be Algae biofuels required it must overcome a number of difficulties before it can compete in the fuel market. Algae are responsible for more than 40% of the global carbon fixation majorly from marine microalgae. Algae has the efficiency to produce biomass very rapidly creating a big option in the production of biofuel (Hannon, 2010). Some algae species have the ability to accumulate very large amounts of triacylglycerides (TAGs), which are the major feedstock for biodiesel production and even it does not require high-quality agricultural land to grow biomass (Scott, 2010).

As a raw organic material, biomass is used to generate a variable number of energy sources such as heat, liquid and gaseous fuels and electricity. The electricity generated can be sent to energy consumers via electric transmission systems. Apart from bioethanol, Microbial fuel cell (MFC) has also pulled a lot of attention in recent years as they are the major type of bioelectrochemical systems (BESs) which can potentially convert biomass into electricity through the metabolic activity of the microorganisms (Pant, 2010). It uses the electrons released in the oxidation-reduction reactions of microbial metabolism to generate electricity (Powell, 2009). The breakthrough that microbial metabolism could supply energy in electrical current form has drawn a lot of attention in the field of MFC research. In this present experiment, we will be demonstrating the use of all the products and byproducts

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produced during the biological process in a hybrid bioreactor for the benefit and economical aspect for mankind.

MATERIALS AND METHODS

As shown in the figure 1 we have conjoined the fermenter and photobioreactor together to form and the hybrid bioreactor with and an supplementary microbial fuel cell.

Bioethanol fermenter: 5L plastic bottle was used as a prototype model for the fermenter. We used ($\frac{1}{2}$ kg) potato as the source for the production of bioethanol, which was washed and boiled till it became tender and then few potato along with the boiled water was poured into the fermenter. Remaining ingredient such as stabilizer and sugar were also added. Commercially available wine yeast was added after 24hrs of incubation and allowed it to ferment inside a dark chamber. The CO₂ produced during this process was stored and further used in photo-bioreactor.

Photo-bioreactor: Similar to the fermenter, 5L plastic bottle was used as a prototype model for photo-bioreactor. Algae were collected from nearby pond bearing algae bloom and grown in media containing malted barley, milk solids, sugar, wheat flour, malted wheat, minerals, protein isolate, emulsifier, salt, acidity regulator, vitamins. (Horlicks energy drink). We recycled CO₂ produced during fermentation process as the source for the algae to grow using a carbon dioxide delivery system made by connecting the pipe source from the fermenter to the air mixing valve from the air pump and then supplied it to the photo-bioreactor as a sparger as shown in fig1A. The photo-bioreactor was then allowed to incubate with the exposure of sunlight. An outlet was made on the top of the reactor to collect the gases from the photo-bioreactor (mainly O₂).

Microbial fuel cell: The experimental microbial fuel cell (MFC) was set up using the fermenter (yeast) and photo-bioreactor (algae), which was prepared as mentioned above. A salt bridge (length = 30 cm; diameter = 1.5 cm) was prepared in a plastic straw using tissue paper and sodium chloride in deionized water (4%-0.68M) connecting the anode (fermenter) and cathode (photo-bioreactor) horizontally.

The salt bridge was replenished at regular interval of time to maintain constant salt concentration, neglect the effect of salt concentration in the generation of electricity. Galvanized iron and copper naked wire were used as an electrode and were connected to the multimeter. The open circuit voltage (OCV) was measured every 2nd day from the day of inoculation of yeast and algae.

RESULT AND DISCUSSION

Bio-Ethanol production from yeast and its future aspects:

The yeast cell mass was calculated using the spectrophotometer and the graph between the times and OD₆₀₀ was plotted (Figure 2). The growth curve of *S. cerevisiae* was observed according to the standard growth curve and has attained the stationary phase within the 24 hrs of inoculation. The ethanol concentration and substrate concentration was not calculated due to the limitation of the lab and also because our main focus is on the cell mass of *S. cerevisiae* for the microbial fuel cell. The CO₂ produced during this fermentation process was validated by passing the gas evolved during the fermentation through limewater and the solution turned cloudy white, confirming the presence of CO₂ gas. Use of bioethanol as biofuel has already been initiated on a big scale in the USA, Brazil, and certain European nations, and we anticipate that within the upcoming 20 years it will be overshadowing renewable biofuels in the transportation area as it can be unified with petrol or used as neat alcohol in specially designed engines, taking advantage of the higher octane number and higher heat of vaporization; additionally, it is an outstanding fuel for forthcoming innovative flex-fuel hybrid vehicles (Hahn-Hägerdal, 2006). The global market for bioethanol has entered a phase of rapid, transitional growth because of depleting crude oil reserves (Sarkar, Nibedita, 2012) Many investigations are being done in order to enrich and surge the ethanol production. However, in 2007 the world bioethanol production was 13.12 billion gallons which have been increased to 25.68 billion gallons in 2015, with USA and Brazil producing around 85% of the world's ethanol (<http://www.ethanolrfa.org/resources/industry/statistics/#1460745352774-cd978516-814c>).

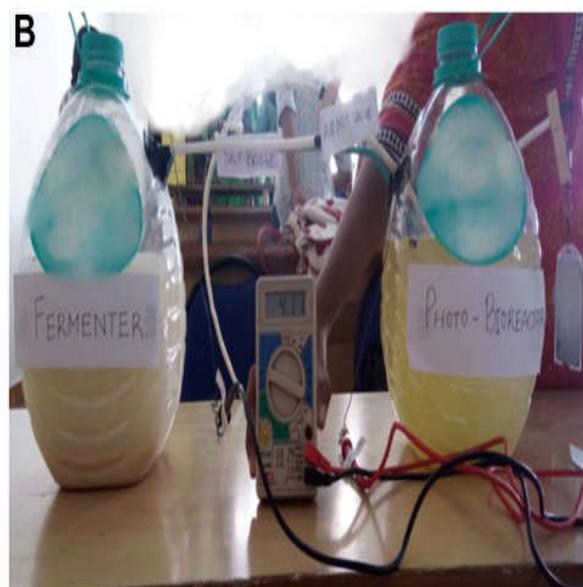
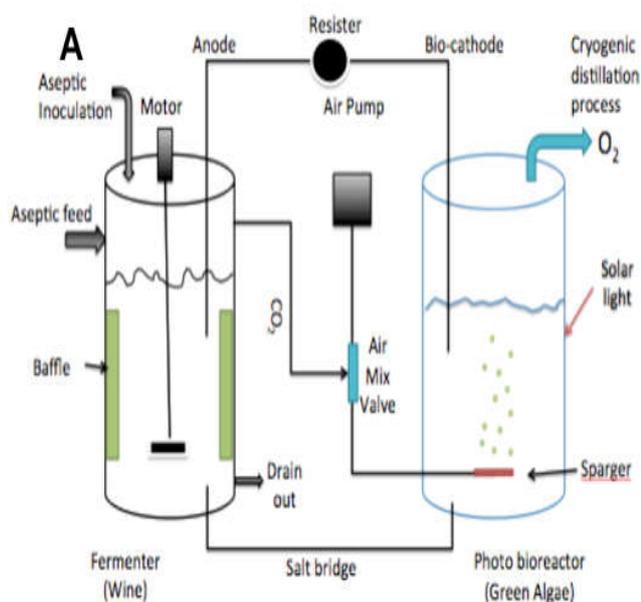


Figure 1. (A) Flow chart of hybrid bioreactor. (B) Prototype designed for the hybrid bioreactor

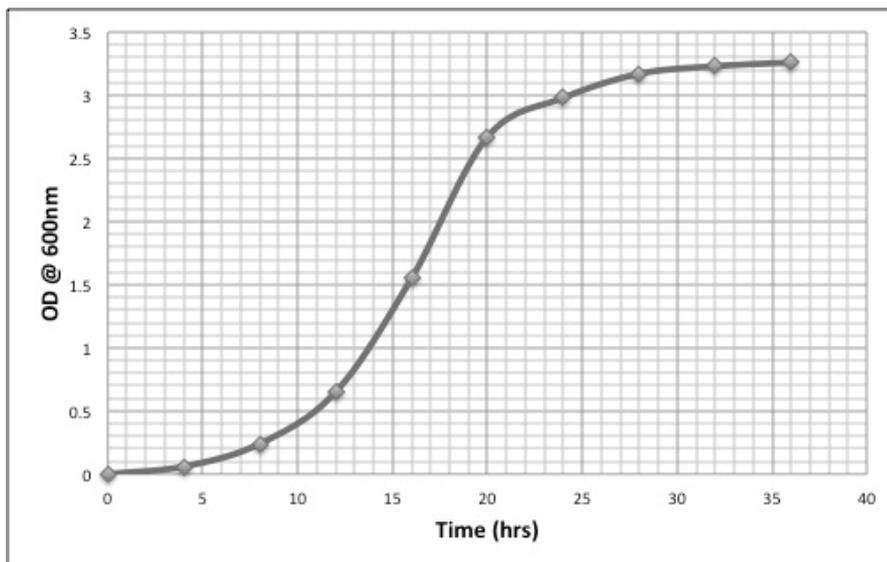


Figure 2. Time versus optical density measured at 600nm for the fermentor

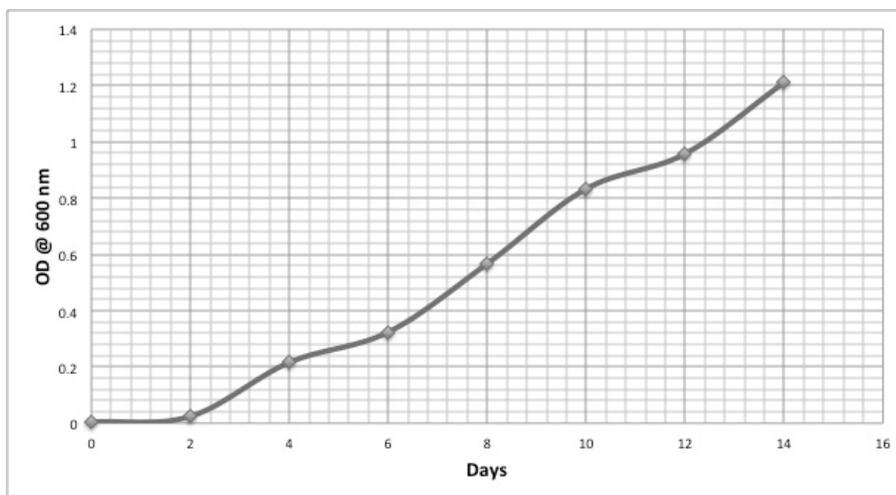


Figure 3. Days versus optical density measured at 600nm for photobioreactor

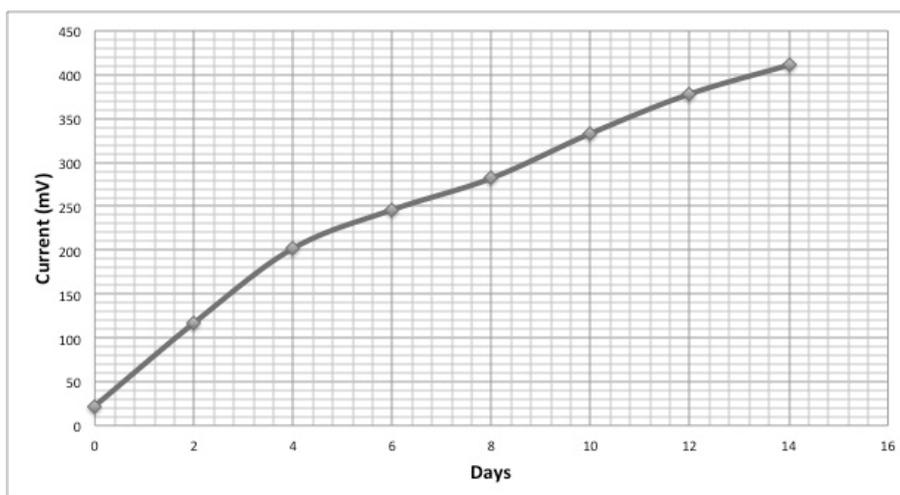


Figure 4. Days versus current (mV) produced by MFC

The prevailing antagonism of food versus fuel triggered by grain-based bioethanol production was avoided by the green gold fuel from lignocellulosic wastes (Bjerre, 1996). It has been calculable that 116.7 billion gallons of bioethanol will be generated from lignocellulosic biomass and overall crop residues and wasted crops will generate 129.7 billion gallons of bioethanol each year, approximately five times beyond the

particular world bioethanol production (Bjerre, 1996). Lignocellulosic materials are renewable, low value and are extravagantly in the market. It includes crop residues, wood chips, sawdust, grasses, etc. Intensive analysis has been performing on bioethanol production from lignocellulosic within the past twenty years (Cadoche, 1989 and Binod, 2010). Thus bioethanol production might be the means of efficient use

of agro-wastes. Rice straw, sugarcane pulp, wheat straw, and corn straw are the main agricultural wastes in terms of the amount of biomass on the market. Pretreatment is one of the major area being researched to minimize the resistance generated by the native lignocellulosic materials against the enzymatic attack and also to reduce the economic cost (Taherzadeh, 2008). Apart from pretreatment, development of robust fermenting organisms and enhanced enzymatic hydrolysis by proficient enzymes can reduce production cost (Chisti, 2007).

Biofuel production from algae and its future aspects

Algae was allowed to grow in the nutrient rich media along with the continuous supply of CO₂ from the fermentation process. The growth curve of the culture was made by plotting the graph between the days versus OD600 (Figure 3). The gas evolved during this process was collected in a balloon separately which can be further processed to extract oxygen liberated during the photosynthesis of the algae. Microalgae mature below measured conditions can produce more than 20 times a lot of oil per hectare than native seed crops like canola and soy (Chisti, 2008). In disparity to the oil-yielding crops, microalgae have potential to supersede bioethanol without adversely impacting stocks of food and other agro-products. The biomass remains that leftovers after oil extraction from microalgae could be used fairly as high-protein feed for animals and, perhaps, as a resource of small amounts of other high-value microalgal products (Chisti, 2006). Though, the greater part of algal biomass remains from oil extraction is likely to go through anaerobic digestion to generate biogas, which can serve as primary energy source for production and processing (Chisti, 2008).

Microbial Fuel Cell

We evaluated the current production by the MFC every 2nd day from the day of inoculation. Initially, the voltage drop remained negative at 2mV for the first 30 min then increased to 23 mV within the 24hrs of inoculation of the organisms. However there was an increase in the current generated with respect to the increasing time (Figure 4), a maximum of 411mV was observed at the 14th day which was greater compared to other previous experiments by E.E Powelle *et al* (Powell, 2010). The MFC is considered as a clean, efficient process and reliable source of alternate energy generation, which employs renewable processes and generates no toxic by-product. A synergistic connection between photosynthetic algae and *S. cerevisiae* may endure during the electricity-generating activity (He, 2009). *S. cerevisiae* within the anode compartment generates protons and electrons throughout oxidization as a part of their organic process. The electrons are forced out of solution within the anode and placed onto a conductor. The electrons are then guided into the cathode compartment by the cathode conduction by means of an external circuit. The protons pass through the proton exchange layer within the anode to comply with the electrons at the cathode (Li, 2013). A major disadvantages of MFCs are low power density, inadequate surface area of the probes where microorganisms adhere, which can be resolved by either generating engineered strains showing large electron transmission rates or by isolation of effective microorganisms that can proficiently transference electrons to anode and use of advanced technology like air cathodes, cloth electrode assemblies and stacked reactors respectively (Chaturvedi,

2016). However, large scale MFCs assembly with high power generation and constant performance is still a challenge which is needed to be resolved.

Conclusion

By extracting bioenergy in the form of bio-ethanol, bio-diesel and bio-electricity in an closed controlled system, the hybrid bioreactor demonstrations a capable prospective for alternative energy source for the world. Also use of this bioenergy can reduce the carbon emission into the environment and provide a sustainable and efficient energy source with lots of other combined economical advantages.

Conflict of Interest

No conflict of interest declared

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