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Solar-Energy Driven Simultaneous Saccharification and Fermentation of Starch to Bioethanol for Fuel-Cell Applications

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A solar reactor was designed to perform the conversion of starch to ethanol in a single step. An aqueous starch solution (5 wt%) was fed into the reactor bed charged with Baker's yeast (*Saccharomyces cerevisiae*) and amylase, resulting in approximately 2.5 wt% ethanol collected daily (ca. 25 mL day^{-1}). A significant amount of ethanol (38 g) was collected over 63 days, corresponding to 84% of the theoretical yield. The production of ethanol without additional energy input highlights the significance of this new process. The ethanol produced was also demonstrated as a potential fuel for direct ethanol fuel cells. Additionally, the secondary metabolite glycerol was fully reduced to a value-added product 1,3-propanediol, which is the first example of a fungal strain (Baker's yeast) converting glycerol in situ to 1,3-propanediol.

Introduction

Overconsumption of petroleum-derived products, especially transportation fuels, results in environmental deterioration and threatens the sustainability of humankind. Bioethanol, which is renewable and environmentally friendly, is one of the best alternatives to petroleum. Its production has increased over the last 25 years, with a sharp increase from the year 2000 onwards.[1] Ethanol also offers an attractive alternative as a fuel in low-temperature fuel cells because it can be produced in large quantities from agricultural products and it is the major renewable biofuel from the fermentation of biomass.[2] Currently, the global ethanol supply is produced mainly from sugar and starch feedstock.[3] Developing technologies that can convert cellulosic materials into motor fuels (ethanol) were a worldwide goal of governments and private industries for the last three decades; however, cellulosic ethanol production is still in the exploratory stage.[4–6]

Starch is an excellent carbon source and a major storage product of many economically important crops. Local cultivation of renewable starch sources, such as potato and tapioca, make its use economically attractive; however, starch must be hydrolyzed to glucose before it is fermented to ethanol.[7] Five groups of enzymes, which comprise 30% of the world's enzyme production, play a key role in the hydrolysis of starch.[8] α-Amylases derived from microbial sources replaced the chemical hydrolysis of starch in starch processing industries.[9] Thermal stability and alkaline characteristics are important features of amylase isolated from alkalophilic organisms.[10] The microbial α-amylases for industrial processes are derived mainly from *Bacillus subtilis, Bacillus amyloliquefaciens*, and *Bacillus licheniformis*.[11]

Conventionally, gelatinization and liquefaction of starch is carried out enzymatically at high temperatures of 90–130 °C for 15 min followed by enzymatic saccharification to glucose.[7] The glucose is subsequently converted to ethanol by fermentation using yeast. This two-step process involving consecutive enzymatic hydrolysis and fungal fermentation can be made much more economical by coupling the enzymatic hydrolysis of starchy substrates and fungal fermentation of the derived glucose, into a single step by a simultaneous saccharification and fermentation (SSF) process. The SSF process was originally developed to combine the enzymatic hydrolysis of cellulose with the simultaneous fermentation of the sugars obtained to produce ethanol.[12] In the SSF process, the stages are the same as in separate hydrolysis and fermentation systems, except that both are performed in the same reactor.[13] Therefore, the yeast, together with the cellulolytic enzymes, reduce the accumulation of sugars within the reactor, increasing the ethanol yield and saccharification rate compared to separate saccharification and fermentation processes.[14] Another advantage of this approach is that a single fermentor, which decreases the investment costs, is used for the entire process.[10] In addition, the presence of ethanol in the culture medium causes the mixture to be less vulnerable to undesired microor-

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ganism invasion.[15] Various economic analyses identified the SSF operation as the major contributor (>20%) to the cost of ethanol production from biomass. Leor et al. performed the SSF process using the seaweed *Ulva rigida* under mild sonication conditions for fast production of ethanol. In addition to being fast (3 h), the process involved only a single stage of sonication for the release of glucose from algae by the action of enzymes and the simultaneous fermentation of glucose to ethanol.[6] The main disadvantage of the SSF process lies in different temperature optima for saccharification (50 °C) and fermentation (35 °C).[16]

It is important to note that, the use of bioethanol as an alternative fuel is still not economically competitive to petroleum based fuel. The main strategies to increase the competitiveness of bioethanol as an alternative fuel include finding a substrate that is cheap and abundantly available, and developing a method or technology that is more efficient and productive to produce bioethanol.[17] Evaluation and economic optimization of SSF in the production of ethanol require knowledge on the chemical and biological processes involved. Although SSF is investigated extensively, there are still no guidelines for the optimal operating conditions for SSF of softwoods (such as pine and spruce).[18] Softwoods are more difficult to utilize than hardwoods (such as oak, aspen, and poplar) because softwoods are more resistant to hydrolysis.[18,19]

Solar energy could be an important alternative energy source, even if only a portion of it is harnessed for heating applications. For efficient utilization of solar energy and for making the SSF process cost-effective, our work focuses on using solar energy for conversion of starch to ethanol in a single-step process. A solar reactor was designed and manufactured to perform the fermentation of starch. This is the first literature report on the utilization of solar energy for driving the fermentation reaction leading to ethanol formation. Moreover, during this process, the common secondary metabolite glycerol is converted in situ to 1,3-propanediol, a value-added product. The ethanol produced in the solar reactor was separated from the broth soon after its formation by evaporation–condensation in the fermentor. The bioethanol produced from this SSF process is demonstrated as a potential sustainable fuel for direct ethanol fuel cells. From the green chemistry point of view, a solar-irradiation-driven SSF process has numerous advantages such as (1) short reaction time (by coupling the hydrolysis and fermentation stages into one stage), (2) more economical (hydrolysis and fermentation stages are in the same reactor; no need for an external source of heating, and no additional energy input for ethanol separation), and (3) no polluting effluent is produced in the process.

Results and Discussion

Simultaneous saccharification and fermentation of starch

The conversion of potato starch to ethanol through a SSF process involves the simultaneous hydrolysis of starch to glucose by the action of amylase and fermentation of glucose by yeast. The first biomass conversion step usually involves a hydrothermal pretreatment before enzymatic hydrolysis;[20] however, in the current study, no pretreatment was applied before the SSF process. Enzymatic hydrolysis of starch is widely investigated by various research groups.[20–25] The chemical structure of starch comprises amylose (20–25%, 1,4-α-linked glucosyl units in linear form, water insoluble) and amylopectin (75–80%, 1,6-α-linked branched, water soluble). α-amylase cleaves the 1,4-α-linked glucosyl units of amylose yielding glucose. Likewise, amyloglucosidase (γ-amylase) cleaves the 1,6-α-linkages in amylopectin as well as the terminal 1,4-α-linked glucosyl units producing glucose. The glucose thus formed from starch is metabolized in situ by *Saccharomyces cerevisiae*.

The process was monitored for over two months (63 days) in the solar reactor at 30–35 °C. The analytes collected at regular time intervals were analyzed for quantification of ethanol using ^{1}H NMR spectroscopy and gas chromatography (GC). The amount of ethanol as a function of time is depicted in Figure 1; a steady increase was observed in ethanol amount collected over time. The concentration of ethanol varied in the range of 1.8–2.6 wt% over the course of study (Figure 1). On the 63rd day, 38 g of ethanol were collected in total, which corresponds to 84 wt% of the theoretical yield of ethanol from starch. The detailed mass-balance calculations are shown in the Supporting Information.

^{1}H NMR analysis of the SSF product

Qualitative and quantitative analyses of the SSF reaction product were performed using ^{1}H NMR spectroscopy. ^{1}H NMR spectra of the analytes collected on 7th, 14th, 21st, and 28th day of

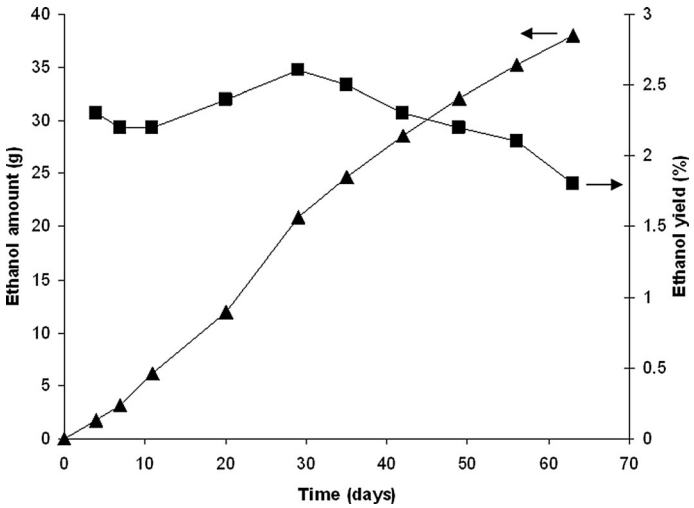

Figure 1. Solar energy driven bioethanol production from starch as a function of time.

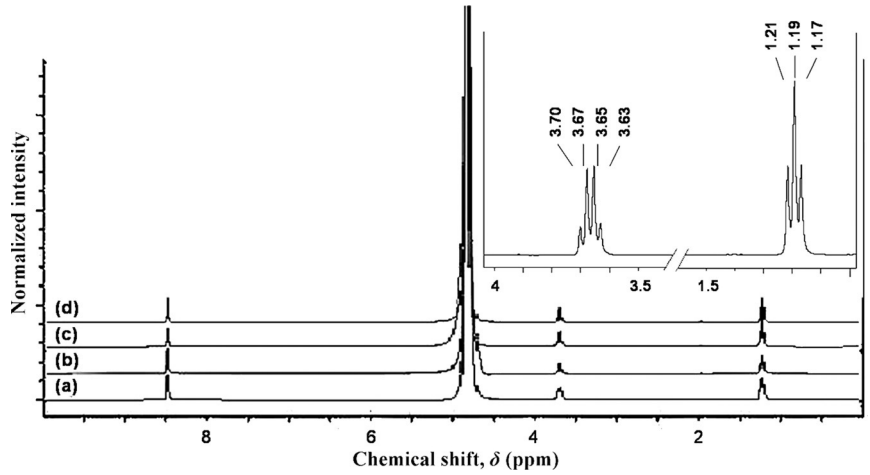

Figure 2. [1]H NMR spectra of the starch fermentation product on the (a) 7th, (b) 14th, (c) 21st, and (d) 28th day. Inset shows the ethanol peaks—a 3H (t) at 1.2 ppm and a 2H (q) at 3.7 ppm. The singlet peak at 8.4 ppm is the internal standard, HCOONa, and the peak at 4.8 ppm is the solvent.

the SSF process are shown in Figure 2. Signature peaks of ethanol at approximately 1.2 ppm (3H, t) and at 3.7 ppm (2H, q) were observed in all four analytes. The sharp singlet signal at 8.4 ppm originates from HCOONa which was the internal standard used for ethanol quantification. The broad peak at 4.8 ppm corresponds to the solvent. The detailed methodology used for the quantification of ethanol is described in the Supporting Information. No other reaction by-products (glycerol or acetic acid) were observed in the analytes indicating the purity (only aqueous ethanol) of the process. [1]H NMR spectra of authentic glycerol (peaks at 3.45 and 3.60 ppm) and acetic acid (peak at 1.24 ppm) are shown in Figure S1 in the Supporting Information.

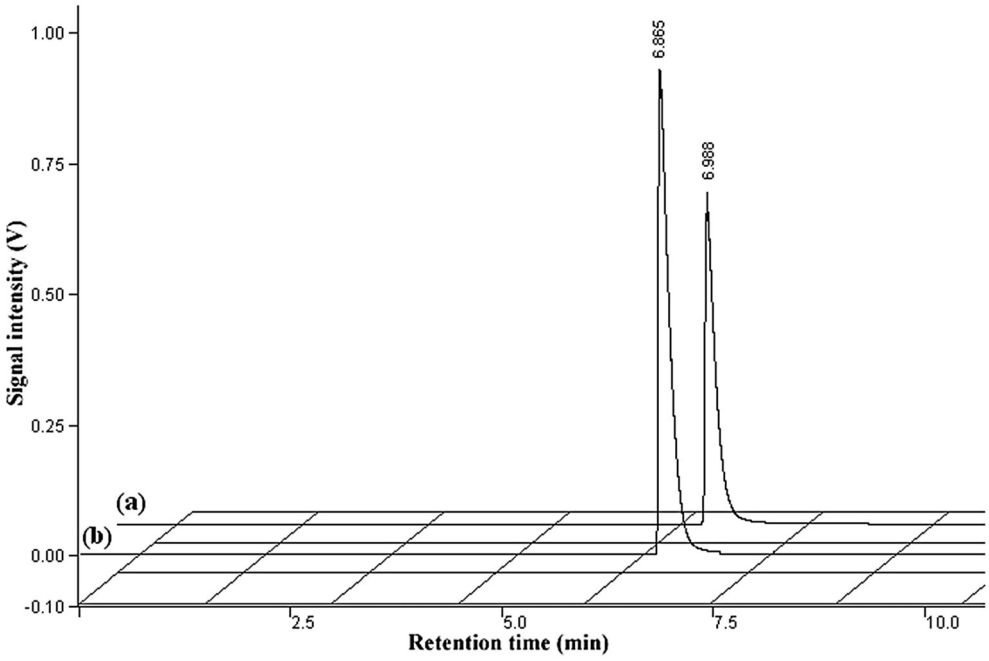

Figure 3. Gas chromatograms of (a) ethanol produced from the SSF of starch (collected on 21 st day, 0.6 M) and (b) authentic ethanol (standard, 0.5 M).

Gas chromatography analysis of the SSF product

In addition to [1]H NMR spectroscopy, the SSF reaction product was also analyzed by GC. As a representative, the gas chromatogram of the reaction product collected on the 21st day of the SSF process is shown in Figure 3a. The peak at approximately 6.9 min retention time corresponds to ethanol as evident from the retention time of the peak corresponding to authentic ethanol (ca. 6.9 min, Figure 3b). In addition, the concentration of the analyte was calculated as 0.6 M (2.77 wt%) using both GC and [1]H NMR spectroscopy. The concentrations of the analytes determined from GC analyses agreed well with [1]H NMR analyses confirming the authenticity of the methodology used for ethanol estimation (Table S1).

[13]C NMR analysis of the SSF product

[13]C NMR spectra of the SSF reaction product collected on the 7th, 14th, 21st, and 28th day are shown in Figure 4. Intense signals seen in all four samples at 17 and 58 ppm are characteristic of ethanol. No signals other than ethanol were observed in the reaction products. This indicates that the reaction product is pure aqueous ethanol and is devoid of the reactant (starch), reaction intermediate (glucose), and usual secondary metabolites of fermentation (glycerol and acetic acid). [13]C NMR spectrum characteristics of authentic ethanol (peaks at 17 and 58 ppm), glucose (peaks at 61.2, 69.9, 71.7, 73.1, 74.4, 76.0, 92.0, and 96.0 ppm), starch (peaks at 61.1, 71.9, 72.2, 74.0, 77.3, and 100.6 ppm), glycerol (peaks at 62.8 and 72.3 ppm) and acetic acid (peaks at 19.6 and 175.6 ppm) are also depicted in Figure S2 and Figure S3 for comparison.

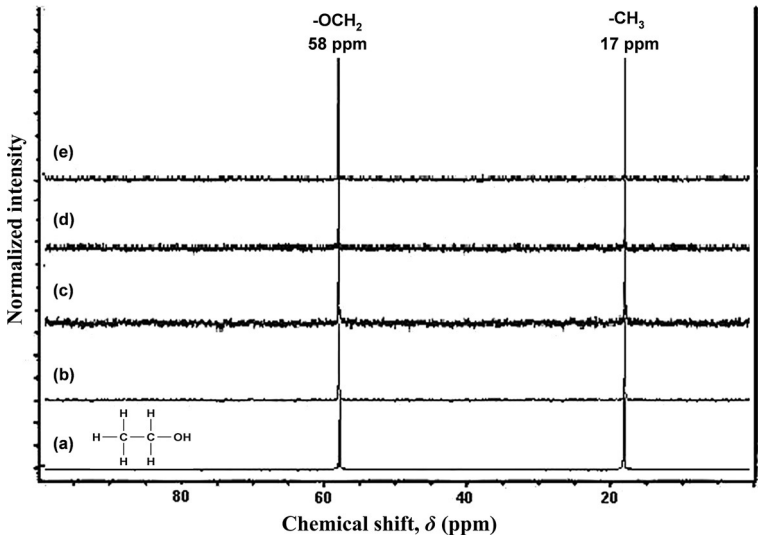

Figure 4. ^{13}C NMR spectra of (a) authentic ethanol and the starch fermentation product on the (b) 7th, (c) 14th, (d) 21st, and (e) 28th day.

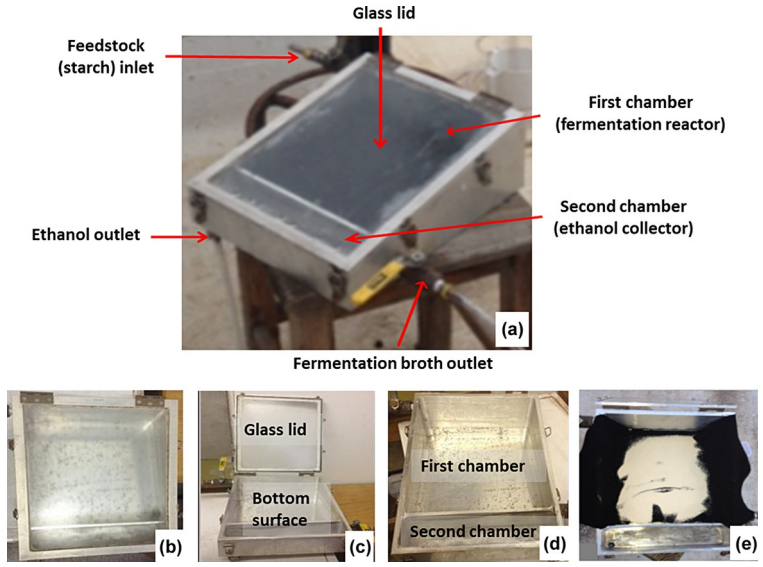

Figure 5. (a) The design of the solar reactor (whole system); (b) closed reactor, (c) open reactor, (d) bottom surface with two chambers, and (e) first chamber with Baker's yeast on activated carbon cloth.

In situ reduction of glycerol to 1,3-propanediol by Baker's yeast

In order to monitor the SSF process of starch and to know about the reaction intermediates and by-products, analytes were collected from the fermentation broth (first chamber of the solar reactor, Figure 5) on the 7th and 60th day. The ^{13}C NMR spectra of these samples are shown in Figure 6. As expected, glucose, glycerol, and ethanol were present in the sample from the 7th day (Figure 6a). Glucose is the reaction intermediate of the SSF process formed from the saccharification of starch by the action of enzymes and glycerol is the secondary metabolite formed during the fermentation of glucose to the reaction product ethanol.

The chemical composition of the analyte from the 60th day was quite surprising as no trace of glycerol was observed. Interestingly, instead of glycerol, its reduced product 1,3-propanediol was observed (Figure 6b). The ^{13}C NMR spectrum of the authentic 1,3-propanediol is shown in Figure S4 for comparison. 1,3-Propanediol is a value-added product usually produced from the bioreduction of glycerol.[26] Although many microorganisms are able to metabolize glycerol in the presence of external electron acceptors (respiratory metabolism), few are fermentative (i.e., in the absence of electron acceptors). The fermentative metabolism of glycerol was studied in great detail for several species of the *Enterobacteriaceae* family (such as *Citrobacter freundii* and *Klebsiella pneumoniae*). Dissimilation of glycerol in these organisms is strictly linked to their capacity to synthesize the highly reduced product 1,3-propanediol.[27] However, to the best of our knowledge, this is the first study reporting on the potential of Baker's yeast to convert glycerol to 1,3-propanediol under the anaerobic conditions maintained in the solar reactor. In addition to 1,3-propanediol, the SSF reaction product ethanol was also observed in the analyte (Figure 6b). It is important to note that, no traces of starch (reactant, Figure S2c) or glucose (the reaction intermediate, Figure S2b) were observed in the sample from the 60th day, indicating the complete conversion of starch to ethanol.

Electrochemical and fuel cell performances

Linear sweep voltammograms recorded with a Pt/C (E-TEK) catalyst in 0.5 M H_2SO_4 + 1.3 M ethanol at a scan rate of 25 mV s^{-1} are shown in Figure 7a. A detailed description of the characterization of the electrocatalyst (Pt/C) is shown in Figure S5. A well-defined peak at +0.75 V corresponding to ethanol oxidation was observed. No additional peaks corresponding to impurities were observed. The shape of the voltammograms recorded using as-produced bioethanol and commercial ethanol was similar and the peak current was comparable (ca. 310 mA mg$_{Pt}^{-1}$, Figure 7a). These aspects clearly indicate the high level of purity of the as-produced bioethanol from starch.

Single-cell DEFC performance with the as-produced 1.3 M bioethanol fuel was tested at different temperatures and *I–V* performance curves are presented in Figure 7b. The open-circuit potential (OCP) of the cell was found to be approximately 0.75 V (65% thermodynamic efficiency) and the effect of temperature on the OCP was small. However, an increase in cell performance with temperature was observed, which is attributed to the enhanced kinetics of ethanol oxidation at the anode and oxygen reduction at the cathode. At operating temperatures of 303, 333, and 363 K, the cell exhibited limiting current

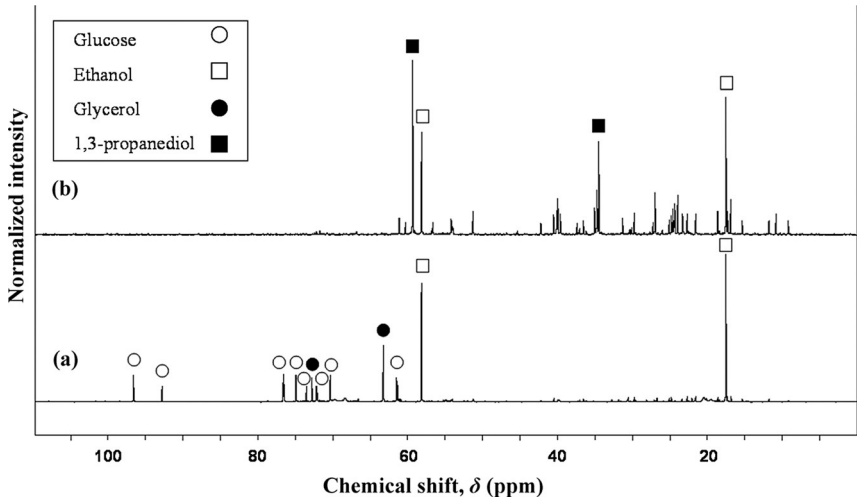

Figure 6. ^{13}C NMR spectra samples collected from the broth on the (a) 7th day and (b) 60th day.

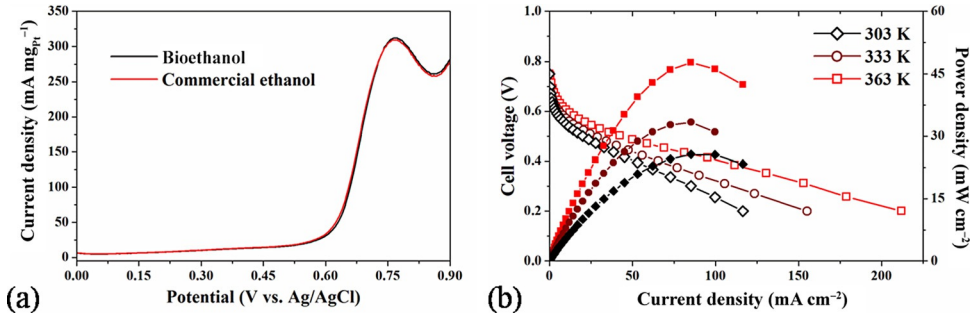

Figure 7. (a) Linear sweep voltammograms of the Pt/C catalyst for the ethanol oxidation reaction in 0.5 M H_2SO_4 + 1.3 M C_2H_5OH (scan rate 25 mV s^{-1}) and (b) polarization and power density curves at 2 mg cm^{-2} catalyst loading for Pt/C (40 wt%, E-TEK) on both the anode and cathode at different temperatures. Anode feed: 1.3 M bioethanol at 1 mL min^{-1}. Cathode feed: pure humidified oxygen at 200 mL min^{-1}.

densities of 116, 155, and 212 mA cm^{-2}, which correspond to maximum power densities of 25.6, 33.3, and 47.7 mW cm^{-2}, respectively.

Conclusions

Successful utilization of solar energy, which is renewable, abundant, and cheap, for bioethanol production from biomass has potential to improve the fuel shortage problem. This research focuses on using solar energy for the conversion of starch to ethanol in a single step using a simultaneous saccharification and fermentation (SSF) process. The solar energy is used as a heating element for the catalyst and the reaction volume, replacing an oven or heating plate. In the same way, the distillation step is also aided by this heating element. The ethanol produced in the reactor was separated from the broth soon after its formation by an evaporation–condensation process. Water and ethanol have vapor pressures at the reaction temperatures (30–35 °C), but to condense their vapors, a cold surface would be required. Under solar radiation, the condensation takes place at room temperature. The solar reactor designed to perform the fermentation of starch yielded 84 wt% ethanol. The ethanol produced from starch was demonstrated as a potential and sustainable fuel for direct ethanol fuel cells. Moreover, glycerol, the secondary metabolite of glucose fermentation, was converted in situ to a value-added product, 1,3-propanediol, by the action of yeast under anaerobic fermentation conditions maintained in the solar fermentor. In conclusion, productive utilization of solar energy for driving the SSF process, as well as the special design of a reactor that facilitates in situ separation of ethanol from the fermentation broth, make the current process economically feasible and environmentally friendly, which is industrially appealing and adoptable.

Experimental Section

Materials

Starch from Potato (product no. S2004), D-Glucose (product no. G8270), Amyloglucosidase from *Aspergilus niger* (≥300 U mL^{-1}, product no. A7095), α-amylase from *B. amyloliquefaciens* (≥250 U mL^{-1}, product no. A7595), and 1,3-propanediol (98%, product no. P50404) were purchased from Sigma Aldrich, Israel. The yeast *Saccharomyces cerevisiae* was purchased from the local supermarket (Instant Baker's yeast). Absolute ethanol (AR, Cat. No. 05250502) and glycerol (AR, Cat. No. 07120501) were purchased from BioLab, Israel. The materials were used as received without any pretreatment.

Gelatinization and liquefaction of starch

A homogeneous aqueous suspension (5 wt%) of starch was prepared using a high-speed stirrer ultraturrax device (Leroy Somer, Digidrive SK, make ESCO-LABOR). In a typical batch, starch (80 g) was added slowly to distilled water (1.6 L) under stirring (5000 rpm) and heating (100 °C) for 15 min. The starch solution obtained was used as a feedstock for ethanol production.

Fabrication of the solar reactor

The solar reactor was designed to perform the SSF process of starch and to continuously separate the aqueous ethanol solution from the yeast bed by in situ evaporation–condensation process. The reactor was fabricated using aluminum blocks so that it is lightweight and non-corrosive. A nearly right-angle triangular geometry (with a base of 275 mm and a height of 127 mm, side view) was selected. The height of the reactor was kept much lower than the base to facilitate the condensation of ethanol vapor (from

the first chamber) onto the top glass surface. Such geometry facilitates free flow of the condensate from the top surface of the reactor to the second chamber, where ethanol is collected. A detailed design and depiction of the components of the reactor are described in Figure 5. The principle of operation of the reactor is described in the Supporting Information (Figure S6).

Simultaneous saccharification and fermentation

The solar fermentor consists of two chambers (Figure 5d). The first chamber, where the SSF process takes place, has Baker's yeast (75 g of *Saccharomyces cerevisiae*) covered with an activated carbon cloth (Kynol, 90 g m^{-2}, 0.43 mm thickness, $>$1800 m^2 g^{-1} specific surface area). Into this chamber, the starch solution (1.6 L, 5 wt%) was added (Figure 5e). In addition to yeast and starch, enzymes amyloglucosidase (2.5 mL) from *Aspergilus niger* and α-amylase from *Bacillus amyloliquefaciens* (2.5 mL) were also added to the first fermentation chamber. The yeast was not supplemented with any other additional nutrients. The ethanol produced evaporated to the top flat glass surface of the reactor, which allowed the solar radiation into the bed (Figure 5a–c). The ethanol droplets that condensed on the glass plate were collected in the second chamber of the reactor, which had an outlet for ethanol collection. The SSF process was scaled up to 15 wt% starch to produce higher concentrations of bioethanol that could be evaluated as a fuel in direct ethanol fuel cell (DEFC) tests.

NMR analyses

Aliquots were collected from the reactor at regular time intervals and analyzed by ^{13}C NMR and ^{1}H NMR spectroscopy. D_2O was used as a solvent and the spectra were recorded using a Bruker Avance DPX 300. HCOONa was used as an internal standard in ^{1}H NMR spectroscopy for ethanol quantification.

Gas chromatography analysis

The quantification of ethanol present in the product was performed by GC according to the following conditions: a Varian 3900 gas chromatograph equipped with a Varian Chrompack capillary column (25 m $\times$ 0.63 mm $\times$ 10 μm) and a flame ionization detector (FID). The detector temperature was fixed at 200 °C and helium was used as a carrier gas. The initial oven temperature was 80 °C for 0.5 min, reaching 160 °C with a rate of 20 °C min^{-1} and remaining at this temperature for 10 min. The column oven end time was 14.5 min. The injection temperature was 140 °C (with a split ratio of 50 and column flow 2.0 mL min^{-1}). The sample (10 μL) was injected into the chromatograph during each analysis. The chromatograms were recorded and the peak responses were measured. The peaks were identified according to the retention time. The ethanol yield was calculated from the calibration plot deduced from standard ethanol.

Electrochemical measurements

Purity of the as-produced bioethanol was checked by performing electrochemical measurements. A three-electrode, one-compartment electrochemical glass cell, assembled with a glassy carbon disk as the working electrode, Ag/AgCl as the reference, and Pt as the counter electrodes, was used for the experiments. A cell containing 0.5 M H_2SO_4 + 1.3 M ethanol was used as the electrolyte. The working electrode was fabricated by coating an ultrasonically dispersed suspension of Pt/C, 5 wt% Nafion, and isopropanol on the polished glassy carbon disk electrode (0.071 cm^2). The electrode contained about 14 μg_{Pt} cm^{-2}.

Direct ethanol fuel-cell tests

The as-produced bioethanol was used as a fuel in DEFCs and measurements were performed to evaluate power density at different temperatures (303, 333, and 363 K). DEFC measurements were performed using an in-house built fuel-cell test station. Commercially available 40 wt.% Pt/C (E-TEK) was used as the electrocatalyst (both anode and cathode), and gas diffusion electrodes (GDEs) were fabricated according to the procedure reported in the literature.[28–29] A homogeneous slurry, consisting of the Pt/C catalyst, 33 wt% Nafion binder, and isopropanol, was spray coated on the carbon fiber paper (Toray, Japan) layer-by-layer to reach a Pt loading of approximately 2 mg cm^{-2}. It was then dried in a vacuum oven at 343 K for 2 h. Thereafter, the membrane-electrode assembly (MEA) was fabricated by sandwiching a Nafion 117 (Du Pont, USA) membrane between the anode and cathode by hot pressing at 398 K and 50 kg cm^{-2} for 2 min. The geometric area of the electrodes was 4.62 cm^2. The fuel cell testing was carried out at different temperatures by pumping 1.3 M ethanol using a peristaltic pump at the anode side and humidified oxygen gas at the cathode side. The flow rate of ethanol and humidified oxygen gas was maintained at 1 and 200 mL min^{-1}, respectively. The MEA was conditioned at a constant current density of approximately 10 mA cm^{-2} until the open circuit voltage (OCV) became steady, then, the steady-state polarization curves were recorded by applying a potential from 0.75 to 0.2 V.

Acknowledgements

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Keywords: bioethanol · fermentation · fuel cells · hydrolysis · solar energy

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