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Feasible design for electricity generation from *Chlorella vulgaris* using convenient photosynthetic conditions

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Abstract: Many recent studies are concerned with low cost, easy to handle and alternative renewable energy as a feasible solution for the upcoming crisis of energy shortage. Microalgae are unicellular entities the can only depend on CO2, water and solar power to cover their nutritional needs. The current study is concerned with using algal cells in a polymeric hydrogel, as a cheap source of energy for electricity generation. *Chlorella vulgaris* has been proved to be a promising algal species for electricity generation, as compared with *Micractinium reisseri.* PVA hydrogel has been used for the immobilization of both algal species in order to protect them from the adverse surrounding conditions in addition to its ability to slowly release the required water molecules according to needs. Under these conditions, *C. vulgaris* showed the ability to generate 60 mV compared with 15 mV generated by *M. reisseri*. Scanning electron micrographs showed nano-threads that bind the *C. vulgaris* cells to each other, indicating the ability of algae to create nanowires that facilitate the electron transfer among algal cells and from cells to the nearest electrode. However, we would expect an increase in the produced potential with simultaneous amendment of environmentally polluted water, such as sewage or waste water. Both of FTIR and raman spectroscopy proved the presence of the characteristic groups of PVA hydrogel and proved the proper integration of the algal cells inside the hydrogel cavities.

Introduction

The need for energy has been increasing exponentially with the increase in the world's population. Energy supply is dependent mostly on fossil fuels, which affects the climate due to CO_2 emissions (Brecha, 2008). Nowadays, interest is growing in exploring low-cost, low-CO_2-emitting, and sustainable means of energy generation (Rashid *et al.*, 2013).

Microbial fuel cell (MFC) has gained widespread attention as a promising renewable energy source. MFC is a bioelectrical chemical system for power generation based on the exploitation of biocatalytic reactions with active microbial fuels (Rashid *et al.*, 2013).

The use of algae to produce energy has many advantages such as the usage of different substrates such as CO_2 or wastewater as fuel and high growth rates (Velasquez-Orta *et al.*, 2009). Microalgae contain large amounts of carbohydrates, proteins and lipids (Schenk *et al.*, 2008). These rich contents encouraged scientists to use them as raw materials for bio-oil production (Li *et al.*, 2007), methane production, (Minowa and Sawayama, 1999), and hydrogen production (Kim *et al.*, 2006; Turner *et al.*, 2008).

Polyvinyl alcohol (PVA) was first synthesized in 1924 by Hermann and Haehnel and was later used to obtain a synthetic fiber with excellent mechanical properties (Finch, 2013). It is also the raw material of Vinylon, the first high-strength and high-modulus synthetic fiber. PVA hydrogel (PVA-H) has been one of the sub-products that are prepared from PVA with higher strength and mechanical properties. PVA is not only used for the manufacture of high-strength, high-modulus fiber, but also serves as a raw material for films and acetal resins, as a textile processing agent, adhesive agent, polyvinyl chloride polymerization stabilizer, inorganic binder, etc. Since the excellent biocompatibility of PVA-H is widely known, this has raised great expectations for its use as

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a biomaterial (Kobayashi and Hyu, 2010).

Generally, hydrogels are characterized by containing water but not being water-soluble. Therefore, PVA-H was at first considered to be a strong gel with turbidity. Then, the freeze-thawing method was developed (Hyon *et al.*, 1989; Cha *et al.*, 1996). These authors made a homogenous PVA solution by heating a mixture of PVA and water/dimethylsulfoxide (DMSO), agitating it under a nitrogen air current, leaving the solution at -20°C) for 10 h, thus promoting the crystallization and cross-linking of PVA molecules. This frozen gel was brought into contact with water to exchange DMSO in the PVA gel with water. After repeating this cryogel-formation process by freeze-thaw cycling, a PVA-H with high mechanical strength, high water content, and excellent transparency was successfully produced.

The procedure we are reporting was aimed to enhance the electricity generation by algae, using PVA-H immobilized algal cells in an easy-made system. The used hydrogel would store and/or release water according to the algae requirements. The produced electrons during algal metabolic pathways would be captured and transferred externally using two immersed copper electrodes.

Materials and Methods

Materials

Polyvinyl alcohol (PVA) with an average molecular weight of 72,000 g/mol was purchased from Merck, Germany.

Algal species

Two species in the Chlorellaceae family, *Chlorella vulgaris* and *Micractinium reisseri*, were kindly provided by Department of Biopharmaceutical Product Research, GEBRI Institute, City of Scientific Research and Technological applications. They were separately kept in an algal culture broth (see below), at 80 rpm, and under a photoperiod of 12 h of fluorescent lighting (8 W) and 12 h of darkness.

Algal culture broth

The algal culture broth was composed of (g/l): Sodium nitrate 1.000; Di potassium phosphate 0.250; Magnesium sulphate 0.513; Ammonium chloride 0.050; Calcium chloride 0.058; Ferric chloride 0.003; and the final pH was adjusted at 7.0±0.2.

Methods

Design and dimensions of prepared algal solar cell

Three simple reactors were fabricated from glass Petri dishes with dimensions 80×12 mm (Anumbra, Czech Republic). Copper wires of 70 mm length and 1mm diameters were used as cell electrodes and were fixed in parallel lines inside the glass plates while separated by a distance of 20 mm. Each cell was filled with 20 ml of either plain PVA hydrogel (1 cell) or PVA-immobilized algae (2 cells), separately. All cells were separately controllable, and were exposed to the 12 h light/12 h darkness photoperiod. The output potential for the three designs was measured with a digital AVOmeter. The diagrammatic illustration and photos of the actual cells are shown in Figs. 1A and 1B, respectively.

Preparation of PVA-H-algal composites

PVA aqueous solution of 12% was added into the prepared algal suspensions of *M. reisseri* and *C. vulgaris* separately, with 20 ml as the total volume for each. At first, the PVA solution was put into a plate and was frozen at -20°C for 12 h and then thawed at 25°C for 12 h. This treatment was repeated three times, and hydrogel disks of about 3 mm in thickness were obtained. Two equal volumes that contain cell dry weight of 0.8 g/l of both algal species *M. reisseri* and *C. vulgaris* were collected from the algal broths, centrifuged at 6000 rpm for 10 min, and each of them was mixed separately with 20 ml of PVA hydrogel. The three prepared hydrogels, two with and one without algal cells, were poured into the designed cells with the aforementioned conditions to measure the electrochemical activity that accompanied the metabolic activities of the algae and were tracked through voltage variations.

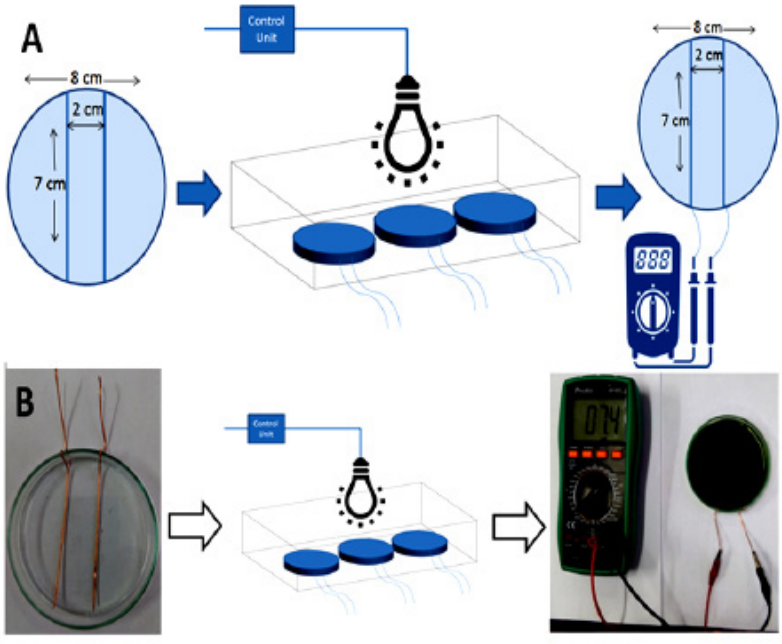

FIGURE 1. Laboratory designed algal solar cells. A. diagrammatic illustration of the design. B. the actual system.

Characterization

SEM

Microscopic analysis and surface characterization of gold coated algal cells was carried out using energy-dispersive analysis in a scanning electron microscope (Jeol Jsm 6360 LA, Japan).

FT-IR

Analysis of I.R. spectroscopic charts investigating the chemical structure of plain PVA, PVA-*M. reisseri,* and PVA-*C. vulgaris* composite hydrogels was carried out using Fourier Transform Infrared Spectrophotometer (Shimadzu FTIR-8400 S, Japan).

Raman scattering spectroscopy

Raman technology was employed to obtain broader information spectra illustrating the chemical structures of the prepared polymeric substances. Time-dependent Raman scattering spectra of the system were recorded using a laser Raman scattering spectrometer (SENTERRA-Bruker, Germany) equipped with a Leica microscope. The instrument was equipped with a 785-nm diod Ar laser, 2 gratings, and an air cooled CCD detector. The laser power of the sample was 50 mW for 1 s. Several areas of each sample were scanned at a resolution of 1 cm^{-1}, where the confocal aperture used (50 mm), gave an approximate 1 mm (x, y) and 2 mm (2) sample volume. The Raman collected using the venture Raman system. The integration times for spectral collection were 20 s per acquisition (Hasan *et al.*, 2015).

Results and Discusson

Electricity generation

The electrochemical behavior of the two tested algal species has been traced as mentioned in the Methods section. As shown in Fig. 2A, the obtained potential in mV for the plain hydrogel, *M. reisseri* and *C. vulgaris* has been detected over time intervals in darkness and light. Overall, it can be seen that *C. vulgaris* was the only one that showed a remarkable stepwise elevated potential as compared with *M. reisseri* and non-inoculated polymeric hydrogel, which shows they were metabolically active and were resilient to the hydrogel preparation procedure. The data depicted in Fig. 2A shows that the electrochemical behavior of *C. vulgaris* has followed the normal growth curve pattern starting with a lag phase followed by an exponential phase followed by a stationary phase. It spent almost 18 h in a lag phase with the subsequent 40 h of its electrochemical life cycle in the exponential phase. However, it spent almost 24 h of its life cycle in the stationary phase followed by a dramatic decrease in the measured potential till the 118th h where the measured potential was close to zero (Fig. 2 B). This would be reasonably expected if we take in account that water is a vital requirement for growth and metabolic activity of algal cells. It has been observed that, during the lag phase, the measured potential was in the range of 9 mV to 15 mV. However, this potential was gradually increased from 20 mV to 60 mV during the exponential phase. The maximum potential was observed through the late lag-beginning of stationary phase after 50 h from the beginning of the experiment, where the cells achieved 60 mV. On the other hand, *M. reisseri* failed to generate electricity under the same conditions. As shown in Fig. 2 A, this algal species followed the same behavior of the blank hydrogel, as the measured potential did not exceed 15 mV. These results matched those of Velasquez-Orta *et al.* (2009), who reported that *C. vulgaris* gave the highest energy generation per gram of substrate, while the macro algae *Ulva lactuca* was able to degrade the supplemented pollutants more efficiently in microfibrillar complexes.

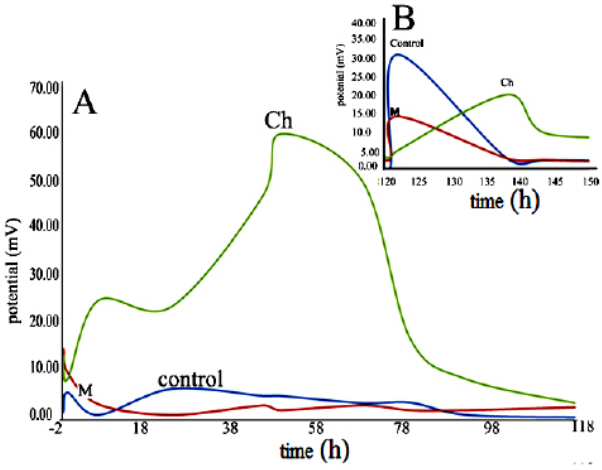

FIGURE 2. Electrochemical behavior of PVA-H (control), PVA-H-*C. vulgaris* (Ch), and PVA-H-*M, reisseri* (M) composites. A: from the beginning of the experiment; B: after addition of 1 ml water to each composite.

SEM

The morphological appearance of the electrochemically most active algal species (*C. vulgaris*) was checked using SEM, as shown in Fig. 3. The cells appeared as intact round structures (black arrow). The most significant observation was the presence of fine elongated nanothreads ('nanowires') that connected the algal cells to each other (white arrow). This may be the first demonstration of the ability of algae to create interconnecting nanowires, which are similar to those created by bacteria used to generate electricity in microbial fuel cells (Schröder, 2007). We attribute the electrochemical activity of *C. vulgaris*, not only to its resiliency to the hydrogel production procedure, but to the species ability to create nanowires that interconnect the algal cells, hence facilitating electron transfer and improving the overall electrochemical behavior.

Fourier transform infrared spectroscopy

FTIR spectroscopy was utilized for the identification of the functional groups involved in the formation of PVA-H algal composites. Fig. 4, shows the FTIR spectra of PVA-H and PVA-H algal composites. PVA exhibited a characteristic broad band OH group signal at 3568-3037 cm^{-1}. The bands at 2914 cm^{-1} are ascribed to symmetric stretching of CH_2 (Rosi *et al.*, 2014), while the bands at 1236 cm^{-1} and 1159 cm^{-1} are due to CH_2 and CH wagging vibrations, respectively (Paipitak *et al.*, 2011; Abu-Saied *et al.*, 2017).

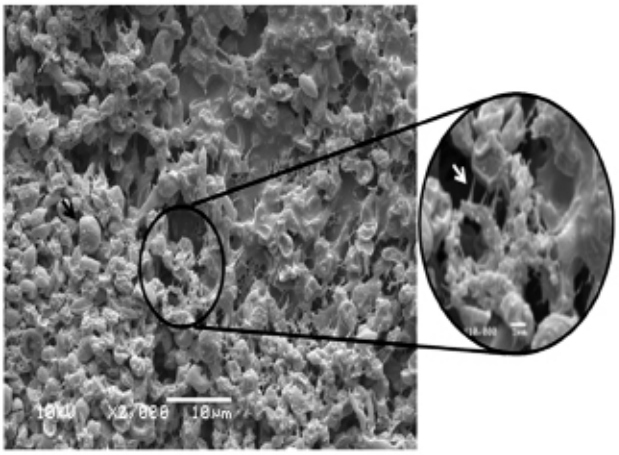

FIGURE 3. Morphological characteristics of *C. vulgaris* strain using SEM.

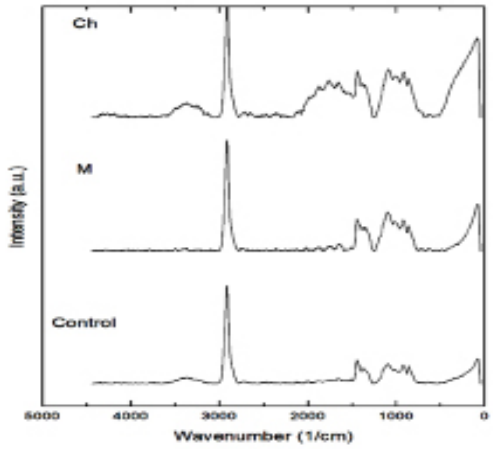

FIGURE 4. FTIR of PVA (control), PVA-H-*C. vulgaris* (Ch), and PVA-H-*M. reisseri* (M) composites.

Raman scattering spectroscopy

It is well known that the vibrational spectra of PVA polymers are related to the vibrational modes of the (-CH_2-CHOH-) monomer, which is expected to have 17N modes of vibrations (Krimm *et al.*, 1956). Because this monomer lacks a center of symmetry, the 17 modes are expected to be active in both Raman and IR. Moreover, PVA is considered to be disordered and, consequently, the degree of disorder and polymerization may cause small shifts in band positions between IR and Raman spectra. The Raman spectra of PVA-H, and PVA-H-algal composites are shown in Fig. 5.

From the comparison of IR and Raman spectra of PVA-H (Figs. 4 and 5), it could be concluded that a pendular mode was not observed. According to Li *et al.* (1998) fan and twist modes took place at 1150–1350 cm^{-1} for hydrocarbons. Thus, the bonds observed here at 914 and 848 cm^{-1} can be attributed to one of these vibrations. According to Cooney *et al.* (1994) a band at 1144 cm^{-1} serves as a measure of PVA crystallinity (Fig. 1). According to the absorption bands with maximums at 1094 $cm^{-1}$and 1330 cm^{-1}, this can be attributed to O-H deformation and C-O valence bond vibrations of secondary alcohols to which PVA belongs.

The Raman band at 2912.5 cm^{-1} of pure PVA-H, which is assigned as *vs.* (CH_2), (Cooney *et al.*, 1994) showed an abnormal behavior for the PVA-H- algal composite samples, where its intensity was found to increase with the increase of the amount of algae added to PVA-H. It was expected that this band would suffer some kind of reduction in its intensity as the PVA-H matrix is diluted by algae. It is thought here that the addition of PVA to the algae matrix caused the CH_2 to form some kind of clustering, and the size of these clusters increases as the concentration of algae in the matrix increases. In conclusion, algal cells can be used for electricity generation. The current results are showing the ability of *C. vulgaris* to generate electricity under conditions of immobilization in a hydrogel matrix that can provide both protection and a slow release of water molecules according to the cellular needs.

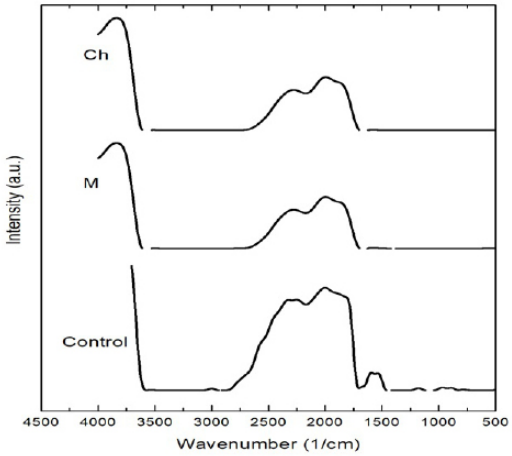

FIGURE 5. Raman spectroscopy of PVA-H (control), PVA-H-*C. vulgaris* (Ch), and PVA-*M. reisseri* (M) composites.

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References

Abu-Saied M, Wycisk R, Abbassy MM, El-Naim GA, El-Demerdash F, Youssef M, Bassuony H, Pintauro PN (2017). Sulfated chitosan/PVA absorbent membrane for removal of copper and nickel ions from aqueous solutions-fabrication and sorption studies. *Carbohydrate polymers* **165**: 149-158.

Brecha RJ (2008). Emission scenarios in the face of fossil-fuel peaking. *Energy Policy* **36**: 3492-3504.

Cooney T, Wang L, Sharma S, Gauldie R, Montana A (1994). Raman spectral study of solid and dissolved poly (vinyl alcohol) and ethylene-vinyl alcohol copolymer. *Journal of Polymer Science Part B: Polymer Physics* **32**: 1163-1174.

Cha WI, Hyon SH, Oka M, Ikada Y (1996). Mechanical and wear properties of poly (vinyl alcohol) hydrogels. *Macromolecular Symposia* **109**: 115-126.

Finch CA (2013). *Chemistry and technology of water-soluble polymers*. Springer Science & Business Media.

Hasan M, Banerjee AN, Lee M (2015). Enhanced thermo-optical performance and high bet surface area of graphene@ PVC nanocomposite fibers prepared by simple facile deposition technique: N2 adsorption study. *Journal of Industrial and Engineering Chemistry* **21**: 828-834.

Hyon SH, Cha WI, Ikada Y (1989). Preparation of transparent poly (vinyl alcohol) hydrogel. *Polymer Bulletin* **22**: 119-122.

Kim MS, Baek JS, Yun YS, Sim SJ, Park S, Kim SC (2006). Hydrogen production from chlamydomonas reinhardtii biomass using a two-step conversion process: Anaerobic conversion and photosynthetic fermentation. *International Journal of Hydrogen Energy* **31**: 812-816.

Kobayashi M, Hyu HS (2010). Development and evaluation of polyvinyl alcohol-hydrogels as an artificial atrticular cartilage for orthopedic Iimplants. *Materials* **3**: 2753-2771.

Krimm S, Liang C, Sutherland G (1956). Infrared spectra of high polymers. V. polyvinyl alcohol. *Journal of Polymer Science Part A: Polymer Chemistry* **22**: 227-247.

Li X, Xu H, Wu Q (2007). Large-scale biodiesel production from microalga chlorella protothecoides through heterotrophic cultivation in bioreactors. *Biotechnology and Bioengineering* **98**: 764-771.

Li Y, Takata M, Nakamura A (1998). Size-dependent enhancement of nonlinear optical susceptibilities due to confined excitons in cubr nanocrystals. *Physical Review B* **57**: 9193.

Minowa T, Sawayama S (1999). A novel microalgal system for energy production with nitrogen cycling. *Fuel* **78**: 1213-1215.

Paipitak K, Pornpra T, Mongkontalang P, Techitdheer W, Pecharapa W (2011). characterization of pva-chitosan nanofibers prepared by electrospinning. *Procedia Engineering* **8**: 101-105.

Rashid N, Cui YF, Rehman MSU, Han JI (2013). Enhanced electricity generation by using algae biomass and activated sludge in microbial fuel cell. *Science of the Total Environment* **456**: 91-94.

Rosi M, Iskandar F, Abdullah M, Khairurrijal (2014). Hydrogel-polymer electrolytes based on polyvinyl alcohol and hydroxyethylcellulose for supercapacitor applications. *International Journal of Electrochemical Science* **9**: 4251-4256.

Schenk PM, Thomas-Hall SR, Stephens, E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008). Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Research* **1**: 20-43.

Schröder U (2007). Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. *Physical Chemistry Chemical Physics* **9**: 2619-2629.

Turner J, Sverdrup G, Mann MK, Maness PC, Kroposki B, Ghirardi M, Evans RJ, Blake D (2008). Renewable hydrogen production. *International Journal of Energy Research* **32**: 379-407.

Velasquez-Orta SB, Curtis TP, Logan BE (2009). Energy from algae using microbial fuel cells. *Biotechnology and Bioengineering* **103**: 1068-1076.