



On the Performance of a Novel Bio-photovoltaic Hydrogen Cell from Green Leaves

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Abstract: The main objective of this work was the design and construction of a bio-based novel photovoltaic. For this purpose, two prototypes were designed and constructed. The first was an efficient extraction prototype of active pigments, and then the validation of the pureness of the extracted pigments took place by using the Ultra Violet Visible region (UV-V) spectra and Infra-Red (IR) spectrum. And, the second prototype as novel bio-photovoltaic hydrogen cell (NB-PV) was consisting of three glass sections donor, acceptor and membrane. These sections were containing a certain water type dissolved active pigments extracted from spinach (*Spinacia Oleracea*) by using the first prototype. So, both water dissolved chlorophyll a (Chl a) and chlorophyll b (Chl b), and the water dissolved pheophytin (pheo) were contained in the donor and acceptor sections respectively. In addition, the third section enclosed a precipitated ferredoxin (Fd) on rice straw as membrane. The membrane design allowed only electron transportation from donor section to the acceptor section. This second prototype was used also for water dissociation ($2H^+$ and O^-) as byproducts of such novel cell. Hence, the performance of the novel cell was determined based on measuring the average output voltage and lifetime of such cell. The experimental program was designed to investigate different variables that affect the performance of the present cell such as operating pressure, water type and light intensity. The results showed that the average voltage output flux diminishes with any slight increase of the operating pressure above the atmospheric one. The maximum values of the output flux were achieved when the active pigments were dissolved in sea water (SW). The results also, indicated that the light intensity had a minimal effect on the average voltage output flux.

Keywords: Chlorophylls, Pheophytin, Ferredoxin, UV-V Spectra, IR Spectrum, Bio-Photovoltaic, Hydrogen, Photosynthesis

1. Introduction

In the next century, burning hydrocarbons, oil, gas, and coal is going to be a no-no. Worldwide have to stop burning hydrocarbons as soon as possible because they're wonderful raw materials. Human being desperately needs them to make dyes, drugs, T-shirts, chairs and automobiles thus it's crazy to burn them [1]. In addition, fossil fuels are in short supply, and they're contributing to pollution and global warming. Coal, while abundant, is highly polluting of the environment. Wind turbines are hurting picturesque landscapes, and current solar-cell technology is expensive and inefficient. Artificial bio inspired solar photovoltaic can offer a new,

possibly ideal way out of the energy predicament [1]. However, there are really only two clean energy sources: hydrogen and direct generation of electricity. Thus the photosynthesis process is a vital process on the earth where the green leaves can consume sunlight and give electrons, hydrogen (clean energies), and oxygen through the photosystem II (PSII) [1].

The green leaf consists of three complexes; these complexes are photosystem I (PSI), photosystem II (PSII) and the electron transfer complex from PSII to PSI. The PSII is a multi-subunit pigment protein complex embedded in the thylakoid membrane of green plants, algae and cyanobacteria. photochemical charge separation and

secondary electron transport take place in the PSII reaction center (RC), which is surrounded by the core antenna proteins CP47 and CP43 [2].

The primary function of these core antenna proteins is to harvest light and to efficiently deliver the energy of the absorbed light to the RC.

Recently published X-Ray structures reported for cyanobacteria PSII cores [3-5] revealed presence of 8, 14 and 16 chlorophylls and pheophytin molecules within the reaction center [6].

Upon light absorption, an electron is transferred from the primary electron donor P680 to a nearby pheophytin molecule. This charge separation is stabilized by rapid electron transfer to a tightly bound plastquinone molecule, (QA) and finally a mobile plastquinone, (QB) on the stromal/cytoplasmic side of the membrane. After double reduction and protonation, the reduced QB is released from its binding site on the D1 protein and replaced by an oxidized plastquinone molecule. On the donor side, P680 is reduced by a tyrosine residue of the D1 protein, (tyrZ) [3-5], which in turn is reduced by a cluster of four manganese atoms located on the luminal side of PSI. The manganese cluster is the place where the water splitting takes place. In four successive charge separation steps, four positive charge equivalents are accumulated that yield one molecule of oxygen out of two molecules of water. Accordingly, the manganese cluster can assume five different oxidation states, S0-S4, with S4 being the state in which oxygen is released. Because of water is a very stable molecule, its oxidation requires a potential of +1 V. Therefore, PSII is the most oxidizing enzyme in the nature. According to Figure 1, what is happening inside the PSII can be understood rapidly [1].

As stated by Furukawa et al. [7] a novel nanostructure and modified Poly-aniline have been used to enhance the performance of the new bio-fuel cell. The mechanism of the electron transfer process and the electro catalysis of Poly-aniline have been investigated. The developed Direct Photosynthetic/metabolic Bio-Fuel Cells (DPBFC) succeeded in generating peak current density of more than 150 $\mu\text{A}/\text{cm}^2$ with a 100 Ω load, 5.3 $\mu\text{W}/\text{cm}^2$ of max power density (using prototype fuel cell) [7].

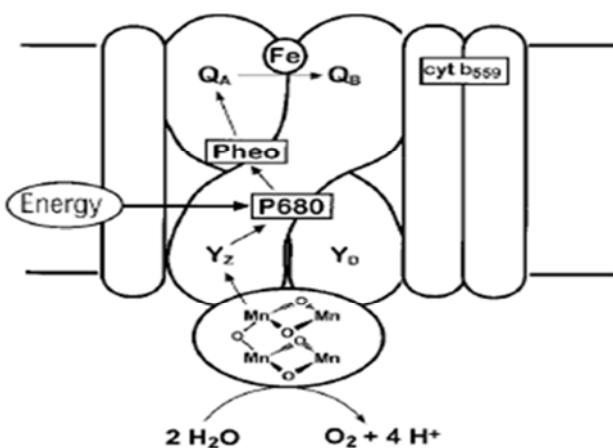


Figure 1. PSII chemical reactions.

Therefore, as shown from Figure 1, PSII is a suitable factory for producing hydrogen as well as electricity as a renewable energy and oxygen as a by-product. Thus, the extraction of the active pigments of the green leaves such as chlorophyll a and chlorophyll b as light harvesting complexes including P680 as an electron donor, and pheophytin as an electron acceptor have to be achieved in pure regards. As a novel approach, such extraction can put the milestone of designing a novel bio-photovoltaic-hydrogen cell (NB-PV) that can work through these active pigments.

2. Experimental Set-up

The main objective of this work was the design and construction of a bio-based novel photovoltaic. For this purpose, two prototypes were designed and constructed. The first was an efficient extraction prototype of active pigments, and the second prototype as novel bio-photovoltaic hydrogen cell (NB-PV) was consisting of three glass sections donor, acceptor and membrane. These sections were containing a certain water type dissolved active pigments extracted from spinach (*Spinacia Oleracea*) by using the first prototype. So, both water dissolved chlorophyll a (chl a), and chlorophyll b (chl b), and the water dissolved pheophytin (pheo) were contained in the spherical glass donor and acceptor sections respectively. In addition, the third section enclosed a precipitated ferredoxine (Fd) on rice straw as membrane. The membrane design allowed only ions transportation from donor section to the acceptor section. This second prototype was used also for water dissociation (2H^+ and O^-) as byproducts of such novel cell.

2.1. Materials

The materials used for extraction of Chl a, Chl b, as an electron donor and Pheo as an electron acceptor were 100 gm. of spinach (*Spinacia Oleracea*) partial dried leaves without stems, 250 ml of petroleum ether, 250 ml of methanol, 250 ml of 30%-70% volumetric petroleum ether-acetone solution, 150 ml of 80% aqueous acetone and powder of silica gel.

The materials used for the Ferredoxin (Fd) extraction were 100 gm of spinach (*Spinacia Oleracea*) (partial dried leaves without stems, 150 ml of ice-cold acetone, 0.7 N hydrochloric acid, distilled water and cotton fabric (*Domoria*). The *Spinacia Oleracea* is used due to its higher content of Fd as mentioned by Osman et al. [8].

2.2. First Prototype for Extraction of Active Pigments

The extraction of the pure active pigments of the PSII was achieved through development and combination of different extraction methods those were stated by Hosikian [9]. Therefore, Figure 2 illustrates the extraction prototype, which was designed and constructed in the Center of Excellency for Research & Development of Bio-Fuel Technology (CER&DBT) at the Faculty of Engineering Mataria, University of Helwan, for achieving the extraction method.

First, such 100 gm partially dried spinach, had to be grinded to small segments of 1 cm x 1cm minimum to avoid the destruction of the plant cells. These grinded leaves were soaked in the soaking chamber as shown in Figure 2 with 250 ml petroleum ether for extraction of the carotenoids, fatty acids, proteins and xanthophyll's; the duration of such step was 48 hours as mentioned by Abdelrazik *et al.* [10]. Thus, these grinded leaves were soaked in 250 ml methanol for 14 hours to get the extract, which is dark green solution, and white tissues. However, The Liquid Chromatography (LC) was packed up with the powder of blue silica gel (Merck Kieselgel 60; 230-400 mesh) and washed with the solution of 30-70% petroleum ether-acetone until obtaining blue column and a layer of such solution was kept to cover the column. Therefore, the previous mentioned dark green extract was poured in the LC until separation of three layers (blue green, olive green and dark grey). Thus, each layer was washed

individually using 50 ml of 80% aqueous acetone and the pigments were stored in -4°C freezer.

The following steps were carried out for extraction of Fd pigment to be precipitated on rice straw and employed as membrane by using the extraction prototype shown in Figure 2:

1. Preparation of spinach extract: 100 grams of spinach leaves were washed and grinded with 150 ml of ice-cold acetone in a blender for 3 minutes.
2. The solution was filtered through a thick cotton fabric (Domoria), and the filtrate was centrifuged for 20 min at 4°C at a speed 4680 rpm in an International Centrifuge.
3. The precipitate was freed from salts by dialysis against distilled water for 48 hrs at 4°C using stirrer. The dialyzed extract was supplemented with 0.7 N hydrochloric acid buffers, pH 7.3.

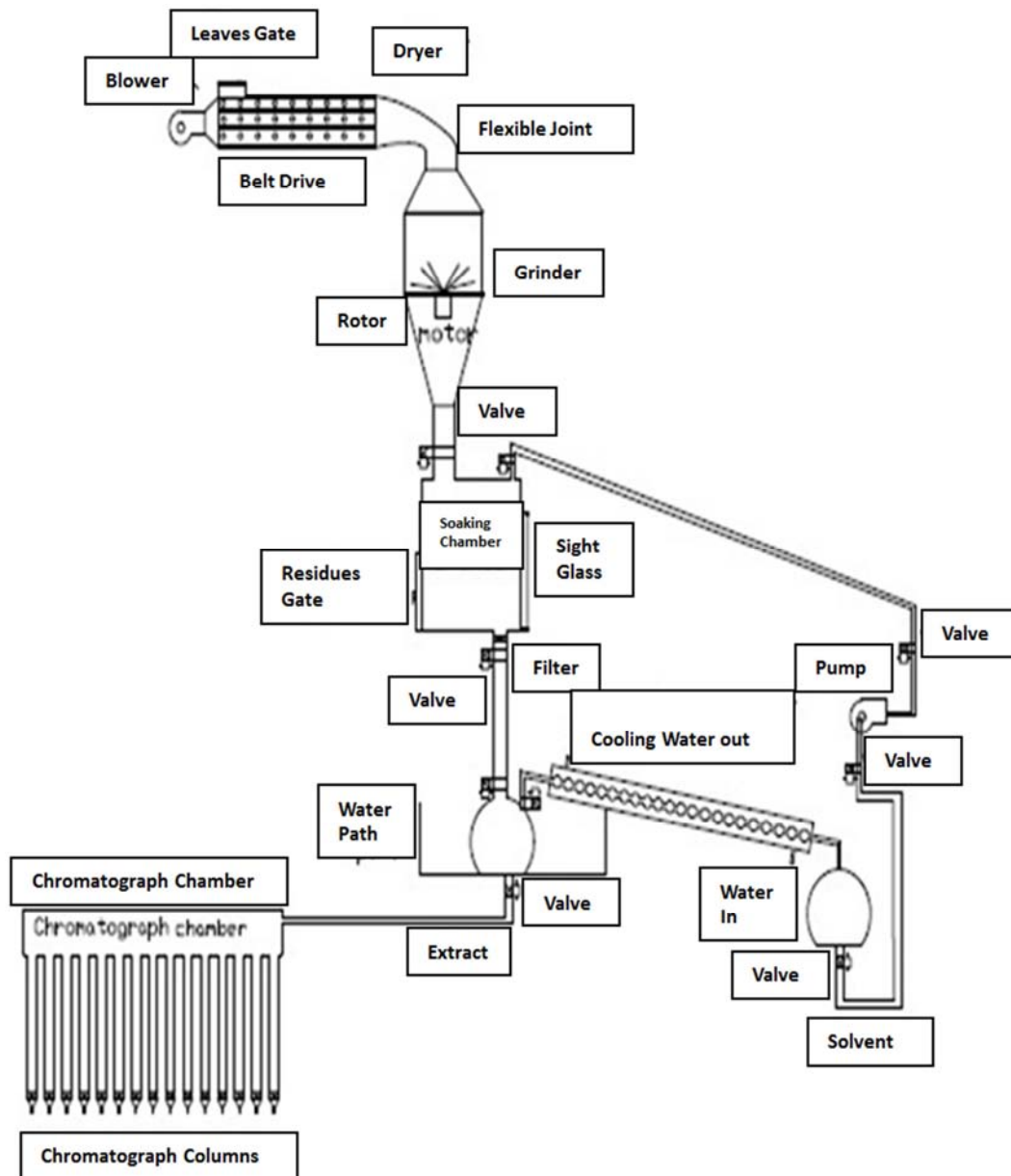


Figure 2. First Prototype for Extraction.

2.3. Second Prototype as Novel Bio-photovoltaic Hydrogen Cell (Design Criteria and Description)

The present prototype could be considered as an energy converter in glass ball shape. Since the surface area is an important factor for harvesting solar energy as well as the volume of the active pigments. So, ball shape is suggested to be used because it has the lowest surface to volume ratio.

The working principle of conventional solar cells is based on the photovoltaic effect, which is the generation of a potential difference at the junction of two different materials in response to visible or other radiation. The basic processes behind the photovoltaic effect are:

- Generation of the charge carriers due to the absorption of photons in the materials that form a junction.
- Subsequent separation of the photo-generated charge carriers in the junction.
- Collection of the photo-generated charge carriers at the terminals of the junction.

In general, a solar cell structure consists of an absorber layer, in which the photons of incident radiation are efficiently absorbed resulting in electron-hole pair creation. In order to separate the photo-generated electrons and holes from each other, the so called “semi-permeable membranes” are attached to the both sides of the absorber. The important requirement for the semi-permeable membranes is that they selectively allow only one type of charge carrier to pass through. An important issue for designing an efficient solar cell is that the electrons and holes generated in the absorber layer reach the membranes. This requires that the thickness of the absorber layer is smaller than the diffusion lengths of the charge carriers. A membrane that let electrons go through and blocks holes is a material, which has a large conductivity for electrons and a small conductivity of holes [11].

Figures 3 and 4 illustrate that the novel bio-photovoltaic NB-PV cell was consisting of two main glass spherical sections; the first one (side A) was called the donor part contained Chl a, and Chl b. And the second part (side B) was termed as the acceptor section holds Pheo. Such two sections are connected by a glass container that contains the membrane, as Fd precipitated on rice straw. The membrane design allows only electron exchange from donor section to the acceptor section. The first and second sections were a glass sphere containing Chl a + Chl b and Pheo dissolved in a certain type of water respectively. It is worth to mention that the present novel membrane has excellent environmental and economical advantage since it consists of only natural materials (Fd precipitated on waste rice straw). The donor and acceptor sections equipped with makeup glass column controlled by a valve and glass vent out of the oxygen and hydrogen produced from the water dissociation. So, this prototype was used also for water dissociation ($2H^+$ and O^-) as byproducts of the novel cell.

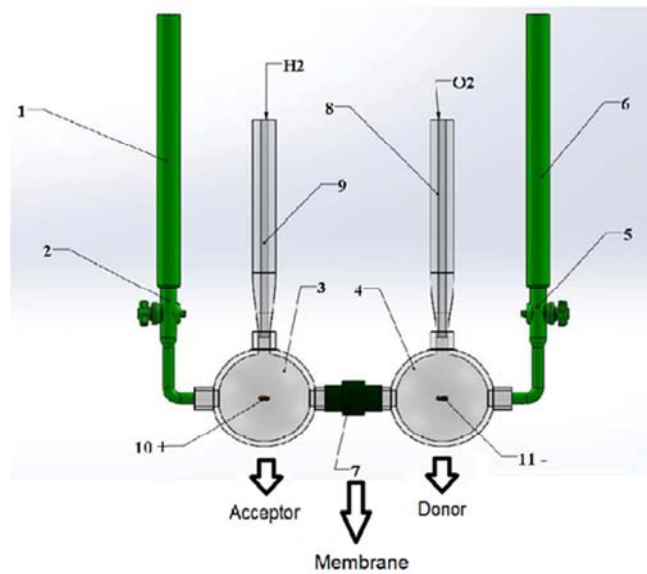


Figure 3. Second Prototype of the Novel Bio-Photovoltaic NB-PV cell.

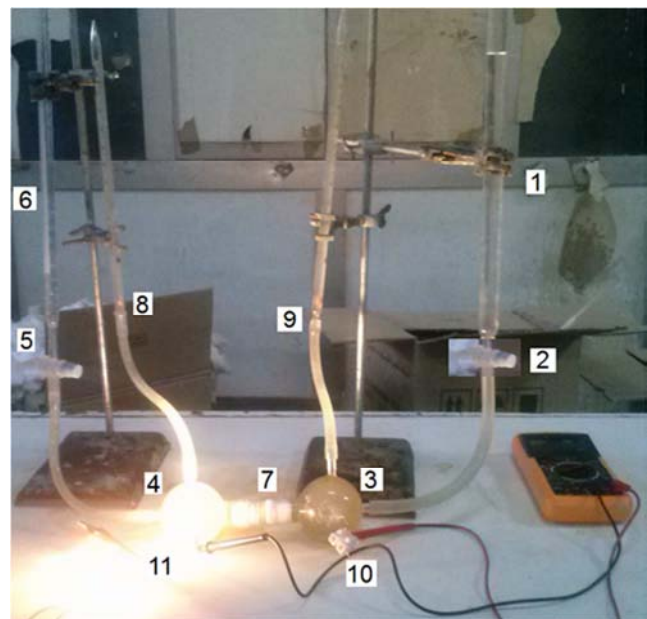


Figure 4. Photograph of the Second Prototype of the NB PV cell.

1- Makeup supplier of Pheo dissolved in a certain type of water; 2- Control valve; 3- Spherical container of Pheo dissolved in a certain type of water; 4- Spherical container of Chl a + Chl b dissolved in a certain type of water; 5- Control valve; 6- Makeup supplier of Chl a + Chl b dissolved in a certain type of water; 7- Electron exchange membrane; 8- Oxygen vent; 9- Hydrogen vent; 10- Aluminum positive electrode; 11- Aluminum negative electrode.

2.4. Measuring Techniques

The Ultra Violet Visible wavelength spectra (UV-V) was recorded on a Pye-Unicam 8700 spectrophotometer (200-900 nm) in 80% aqueous acetone solution for the blue green

layer, olive green layer and dark grey layer, which represented chlorophyll a (Chl a), chlorophyll b (Chl b) and for pheophytin (Pheo) pigment respectively.

Therefore, the pureness of each specimen (pigment soluble in 50 ml of 80% aqueous acetone) can be determined by measuring the absorbance at wave lengths (A_i) of 663.6, 665.4, 653.4, 646.6 and 440.5 nm for Chl a, Pheo a, Pheo b, Chl b and Car respectively as gravimetric of such pigments. Therefore, the pigments contents in each sample are calculated by using the following equations as stated by Yang *et al.* and Dere *et al.* [12, 13] on the basis of $\mu\text{g/ml}$:

$$\text{Chl a} = 12.25A_{663.6} - 2.55A_{646.6} \text{ (}\mu\text{g/ml)} \quad (1)$$

$$\text{Chl b} = 20.31A_{646.6} - 4.91A_{663.6} \text{ (}\mu\text{g/ml)} \quad (2)$$

$$\text{Chl a+b} = 17.76A_{646.6} + 7.34A_{663.6} \text{ (}\mu\text{g/ml)} \quad (3)$$

$$\text{Car} = 4.69A_{440.5} - 0.267\text{Chl a+b (}\mu\text{g/ml)} \quad (4)$$

$$\text{Pheo a} = 22.42A_{665.4} - 6.81A_{653.4} \text{ (}\mu\text{g/ml)} \quad (5)$$

$$\text{Pheo b} = 40.17A_{653.4} - 18.58A_{665.4} \text{ (}\mu\text{g/ml)} \quad (6)$$

And Infra Red spectra (IR) were recorded on a Nicolet 410 IR spectrophotometer ($4000\text{--}400\text{ cm}^{-1}$) using Nujol mulls or liquid films between potassium bromide cells. These results were obtained for validation the efficiency of the extraction method of pigments.

However, the day average open circuit voltage output was

Table 1. Percentages of the total recovery of each solvent.

Solvent Type	Recovery Temperature	Total Recovery Percentage
Petroleum Ether	71°C	95%
Acetone	57°C	87%
Methanol	66°C	65%

The separated active pigments are identified by colors using Liquid Chromatography (LC). These pigments are chlorophyll a (Chl a) blue green, chlorophyll b (Chl b) olive green as stated by Anderson [14] and pheophytin (Pheo) dark grey as mentioned by McLaughlin *et al.* [15]. Therefore, these layers were color-separated then determined and evaluated by using the UV-V and IR spectrum. Thus, each solution contained one pigment could be called specimen.

The pigments contents are summarized in Table 2 and emphasized on the pureness of each specimen. The blue green, olive green and dark grey specimens are contained a mass percentage of 98.97% Chl a, 95% Chl b and 64.75%

recorded by measuring the voltage output of the NB-PV cell through aluminum electrodes. The output signal is fed to a National Instruments NI-DAQmx-USB data acquisition card, 8 analog inputs up to 48 KS/s maximum multi-channel sampling rate. Data acquisition is managed on a laptop computer using LabVIEW software. The output signals are saved and averaged along 24 hours for this experimental work as indoor experiments. The day average outputs were converted to output flux through dividing such average day output by the projected area of the spherical NB-PV cell. The light intensity was measured through the indoor experimental work by lux meter.

3. Results and Discussions of Pigment Extraction

It is worth to mention that, for obtaining an economical extraction development, a recovery process was carried out by using water bath and glass heat exchanger as shown in Figure 2. Such process was applied on the different solvents used for the extraction of active pigments; such solvents as petroleum ether, acetone and methanol to be reused for any further active pigments extraction. Thus, the percentage of recovery of these solvents was 95%, 87% and 65% respectively as shown in Table 1.

Pheo, respectively. Also, the amount of each pigment is agreed with the following yields of these pigments. Thus, the yields from 100 gm of partially dried spinach are 21-22 mg of chlorophyll a (Chl a), 10.5-11 mg of chlorophyll b (Chl b), and 8.4-8.8 mg of pheophytin (Pheo). These yields values were obtained for (Chl a), and (Chl b) by applying equations (1) and (2) respectively, and matched with Dikio and Isabirye [16]. In addition, the yield of pheophytin (Pheo) (obtained in triplicate) was achieved by using the summation of the results of applying equations (5) and (6). These results also are agreed with those of Murata *et al* [17].

Table 2. The pigment contents in ($\mu\text{g/ml}$) and each percentage of each pigment of each specimen.

Specimen	Chl a ($\mu\text{g/ml}$)	Chl a %	Chl b ($\mu\text{g/ml}$)	Chl b %	Car ($\mu\text{g/ml}$)	Car %	Pheo a ($\mu\text{g/ml}$)	Pheo a %	Pheo b ($\mu\text{g/ml}$)	Pheo b %
Blue Green	24.4	98.97%	0.047	0.19%	0.039	0.16%	0.12	0.49%	0.048	0.19%
Olive Green	0.46	2.87%	15.25	95.23%	0.027	0.17%	0.2	1.25%	0.077	0.48%
Dark Grey	9.45	29%	2	6.14%	0.038	0.12%	14.26	43.77%	6.83	20.97

The ambient temperature absorption of the UV-V spectrum of the specimens was characterized by the peaks as shown in Table 3. These peaks were matched with those mentioned by Dikio and Isabirye, Hornero and Minguez,

Watanabe *et al.*, Geferey, Schmid *et al.*, Goedheer, klemov *et al.* and King [16, 18, 19, 20, 21, 22, 23, 24] as concluded in Table 3. Also, they are emphasized on the purity concept of the extracted pigments.

Table 3. Comparison between the absorbed peaks of the UV-V spectra at ambient temperature of each specimen and the literature peaks.

		Absorbed Peaks of the UV-V (nm) at Ambient Temperature					
Chl a	Blue Green Specimen (Present Work)	665 (max) ⁺	618	580	535	433 (max)	412
	Dikio and Isabirye [16]	662 (max)	615	579	535	430 (max)	328
	Hornero and Minguez [18]	662 (max)	617	580	534	431 (max)	327
	Watanabe et al. [19]	662 (max)	617	580	534	431 (max)	327
	Jeffrey [20]	663 (max)	615	580	535	430 (max)	410
	King [24]	614.8 (max)	N/A [*]	574.3	529	429.1 (max)	N/A
Chl b	Olive Green Specimen (Present Work)	645 (max)		598		458 (max)	
	Jeffrey [20]	645 (max)		595		455 (max)	
	Shmidt et al. [21]	645 (max)		N/A		N/A	
	King [24]	645.3 (max)	595.8	550.4	455.5		
Pheo	Dark Grey Specimen (Present Work)	685 (max)	545	518	420 (max)		
	Goedheer [22]	667 (max)	534	505	408 (max)		
	Klemov [23]	685 (max)	545	518	422 (max)		
	King [24]	665.9 (max)	534	505	409.3 (max)		

However, the infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. These differences may be due to the environment as stated by Ramamurthy and Kannan [25]. The interpretation of these IR infrared spectrums were based mainly on the work of Dikio and Isabirye, King, Ramamurthy and Kannan, Holt and Jackobs, Goodwin, Baucher et al., Baucher and Katz, and Ballschmitter and Katz [16, 24, 25, 26, 27, 28, 29, 30]. The region of the greatest significance was the carbonyl region, where most of the legend absorption bands were obtained. These legend absorption bands are more or less independent of the metal ion in the center of the tetrapyrrole ring.

Therefore, the IR spectrum of the first specimen, which represented Chl a, exhibited the characteristic finger print band features. However, the very strong absorption bands at 3080, 3420 and 3434 cm^{-1} were representative of C-H, O-H and N-H stretching vibrations, characteristics of the presence of amino acids, respectively as presented by Ramamurthy and Kannan, and Rao [26, 31]. Thus, the observed spectrum at 3436 cm^{-1} was agreed with the literature values. It was noticed that the bands at 2925 and 2858 cm^{-1} were matched with those at 2918, 2921 and 2928 cm^{-1} ; these were indicative of the chlorophyll groups due to the stretching vibrations of $-\text{CH}_3$ and $-\text{CH}_2$ groups as illustrated by Bellamy [32]. The C-9 ketone ($\text{C}=\text{O}$) band was observed at 1704 and 1642 cm^{-1} , which was reported to appear as two bands at $\sim 1700 \text{ cm}^{-1}$ (normal ketone absorption) and at $\sim 1650 \text{ cm}^{-1}$ (ketone oxygen coordination to magnesium) as mentioned by Boucher et al. [28]. C-N vibrations of the tetrapyrrole ring were observed at 1372 cm^{-1} , which assigned by Boucher and Katz and Ballschmitter and Katz [29, 30] at 1350 cm^{-1} . Otherwise, C-C skeletal vibration was observed at 1089 cm^{-1} . C-O vibration gave a strong sharp band at 1242 cm^{-1} as agreed with Ramamurthy and Kannan [25]. The alkyl C-H anti-symmetric and symmetric deformation was observed at 1430 cm^{-1} as stated by Dikio and Isabirye [16]. Therefore, Table 4 concludes matching between these results and those mentioned in the literature.

Also, the results of the absorption bands of the second specimen, which represented Chl b, It showed the difference

between Chl a and Chl b series pigments. Such difference was the appearance of aldehyde group at C-3; thus, it exhibited bands for an aldehyde C-H stretch at 2750 cm^{-1} and $\text{C}=\text{O}$ aldehyde at 1664 cm^{-1} . These results were agreed with those stated by King and Holt, and Jackobs [24, 26] as concluded in Table 4.

In addition, the IR spectrum of the specimen of the pheophytin pigment contains an additional stretch at 3400 cm^{-1} due to N-H stretching as discussed by Boucher et al. [28]. This band was partially hidden by the O-H stretching of the water absorbance in each spectrum. Since, Chl a and Chl b have magnesium molecule (Mg) in the center of the macro cycle and do not have pyrrolic hydrogens; the N-H stretching band was not presented in these compounds.

In the carbonyl region of the IR spectrum of the third specimen, one stretching band occurred for each carbonyl group in the structure of the compound analyzed. The maximum number of $\text{C}=\text{O}$ stretch seen in the IR spectrum of the Chl b pigment is 3 with only 2 occurring in the IR spectrum of the decarbomethoxylated compound, Pheo. The assignments in the carbonyl region of the IR spectra were based on the explanation of Boucher and Katz and Ballschmitter and Katz [29, 30].

Each absorption in the carbonyl region of the IR spectra of the chlorophyll products could be assigned as resulting from a specific $\text{C}=\text{O}$ in their structure. The stretching vibration of the C-7 propionic ester $\text{C}=\text{O}$ occurred near 1733 cm^{-1} in the IR spectrum of each studied compound. The C-10 methyl ester $\text{C}=\text{O}$ stretch, occurred near 1717 cm^{-1} in the IR spectra of the series and 1700 cm^{-1} in the b series compounds as shown in Table 4. Also, the C-9 ketone $\text{C}=\text{O}$ stretch befall between 1690 and 1700 cm^{-1} in the IR spectrum of all studied pigments. The lower frequency of the $\text{C}=\text{O}$ ketone band was due to conjugation with the aromatic macro cycle in the chlorophyll pigment structure. In the IR spectrum of the b series compound, a fourth band was found near 1660 cm^{-1} .

Thus, Table 4 concludes a good agreement of the obtained IR spectra of the three pigments produced by using present extraction prototype shown in Figure 2 with the literature. Also, it emphasizes on the purity of each pigment produced by the present prototype as matched with the previous results of the UV-V spectra.

Table 4. Comparison between the absorbed peaks of the IR spectra of each specimen and the literature peaks.

C=O									
	Source	Solvent	NH	OH	CH Phytol	CH Aldehyde	Ester	Ketone	Aldehyde Chelate
Chl a	Blue Green Specimen (Present Work)	Acetone		3436	2925-2858			1704-1642	1629
	Dikio and Isabirye [16]	Petroleum Ether			2970-2925-2875			1700-1650	
	King [24]	Acetone			2950-2923-2851			1733-1717-1698	
	Ramamurthy and Kannan [25]	Acetone	3434	3420	2918-2921-2928				1640-1629-1632
	Holt and Jacobs [26]	Nujol	3605	3440	2920		1787	1694	1640
	Goodwin [27]	Petroleum Ether	3400	3360	2960-2925-2875			1702-1642	
	Boucher et al. [28]	Petroleum Ether	3400	3360	2960-2925-2875			1702-1642	
	Boucher and Katz [29]	Petroleum Ether	3400	3360	2960-2925-2875			1695-1698-1652	
Chl b	Ballschmiter and Katz [30]	Petroleum Ether	3400	3360	2960-2925-2875			1695-1698-1652	
	Olive Green Specimen (Present Work)	Acetone		3365	2950-2924-2850	2750		1733-1717	1664 1629
	King [24]	Acetone			2951-2924-2852	2750		1733-1717-1699-1664	
	Holt and Jacobs [26]	Nujol		3360	2924	2710	1738	1701	1664
	Boucher et al. [28]	Petroleum Ether	3400	3360	2951-2924-2852	2720		1733-1717	1664
	Boucher and Katz [29]	Petroleum Ether	3400	3360	2951-2924-2852	2720		1733-1717	1664
	Ballschmiter and Katz [30]	Petroleum Ether	3400	3360	2951-2924-2852	2720		1733-1717	1664
	Dark Grey Specimen (Present Work)	Acetone	3389		2950-2925-2855		1740-1717-1690	1700	
Pheo a	King [24]	Acetone	3400		2950-2925-2856			1739-1717-1693	
	Holt and Jacobs [26]	Nujol	3382	3100-3700	2920		1736	1700	
	Boucher et al. [28]	Petroleum Ether	3400		2950-2925-2856		1735-1715-1695	1698	
	Boucher and Katz [29]	Petroleum Ether	3400		2950-2925-2856		1735-1715-1695	1698	
	Ballschmiter and Katz [30]	Petroleum Ether	3400		2950-2925-2856		1735-1715-1695	1698	

Table 4. Continue.

	C=C	C-H Alkyl	C-N Tetrapyrrole Ring	C-O Vibration	C-C Skeletal Vibration
Chl a	1604	1430	1372	1244 (Strong)	1089
		1429	1370	1242 (Strong)	1089
	1604				
		1434-1411		1232-1216	1095-1101
	1604				
		1429	1370	1242 (Strong)	1089
Chl b		1429	1350	1242 (Strong)	1089
		1429	1350	1242 (Strong)	1089
		1434	1370	1216	1089
	1604				
	1604				
	1609				
Pheo a	1605				
	1605				
	1605				
	1617				
	1617				
	1617				
Pheo b	1617				
	1617				
	1617				
	1617				

4. Results and Discussion of the Novel Bio-Photovoltaic Hydrogen Cell

The indoor experiments were performed at the light intensity of 2.19×10^{-5} and 6.57×10^{-6} W/cm² using indoor artificial full visible light wave lengths. Also, the following results elucidated the effect of operating pressure, and light intensity on the average open circuit voltage output flux as well as the lasting time by using different water types as city water CW, washing water WW and sea water SW as

dissolvers of the active pigments of the NB-PV cell.

4.1. Preliminary Test

A preliminary test was performed at light intensity of 2.19×10^{-5} W/cm² using artificial full visible light wave lengths and city water as a dissolver of the Chl a + Chl b in the donor side as well as Pheo in the acceptor side. This test clarified that the decrement of the water level was due to the dissociation of water into O⁻ and 2H⁺ by burning these gases hence a lifted flame was produced as shown in Figure 5.



Figure 5. Photograph of Lifted Flame Occurred by Combustion of O⁻ and 2H⁺ Resulted from Water Dissociation.

4.2. Influence of the Operating Pressure on the Average Voltage Output Flux

The effect of operating pressure on the average voltage flux is presented in Figure 6. The experiments were performed indoor at the light intensity of 2.19×10^{-5} W/cm² for CW as a dissolver of both Chl a + Chl b and Pheo in the donor and acceptor side respectively. However, the control of the operating pressure was

achieved by plugging both vents out of oxygen and hydrogen and hence moving the makeup supplier upper the cell to increase such pressure and vice versa. It was found that, the increasing of the operating pressure above the atmospheric diminished the output voltage from the NB-PV cell as shown in Figure 6. This behavior may be due to the negative impact of increasing the operating pressure on the active pigments (Chl a, Chl b, and Pheo), which is not allowed in the vivid green leaf.

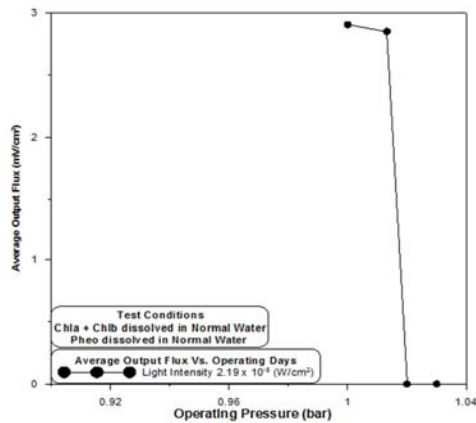


Figure 6. Effect of the Operating Pressure on the Average Output Flux.

4.3. Influence of the Water Type on the Lasting Period of NB-PV Cell

The effect of the different types of water as normal drinking water (NW), washing water (WW), and sea water SW on both the open circuit average voltage output flux and lasting period is illustrated in Figure 7. Six experiments were performed by using different combination of water types as a dissolver of the active pigments. The first, fourth, and fifth experiments used sea water SW, normal drinking water NW, and washing water WW respectively for dissolving both Chl a + Chl b in the donor section and Pheo in the acceptor section. While the second, third, and sixth tests used SW, WW, and CW respectively as a dissolver of Chl a + Chl b in the donor section also, WW, NW, and SW respectively as a dissolver of Pheo in the acceptor section. These experiments were performed indoor using artificial full visible light wave lengths at light intensity of $2.19 \times 10^{-5} \text{ W/cm}^2$.

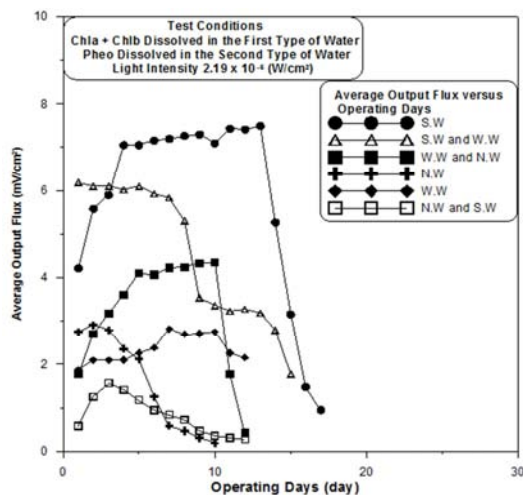


Figure 7. Effect of the Water type on the Lasting Period.

The results shown in Figure 7 elucidate that, the maximum lasting periods was occurred in two cases at the first and second experiments of 10 and 8 days respectively. It was noticed that, the maximum output flux for the first case was obtained after 4 days from starting but such output was obtained at the first day for the second case. These results are stipulated to be due to the

use of SW in the donor section due to high amount of dissolved salts and heavy metals. Also, the both cases exhibited the maximum average voltage output flux of 7.3 and 6.18 mV/cm² for the first and second cases respectively.

4.4. Influence of the Light Intensity on the Average Voltage Output Flux

Figure 8 illustrates the effect of the light intensities of 2.19×10^{-5} and $6.57 \times 10^{-6} \text{ W/cm}^2$ on the average open circuit voltage output flux for different types of dissolving water. The ratio of decreasing the light intensity was 33% while the decrement of the maximum open circuit output fluxes were 13.68, 12.64, 6.13, 1.88, 4.21 and 14.31% for different types of dissolving water NW, WW, NW + WW, SW, SW + WW, and SW + CW respectively. Thus, the minimum effects of the light intensity were occurred in two cases. The first test was using SW for dissolving both Chla + and Chlb and Pheo in the donor and acceptor sections respectively. Whereas the second was using SW for dissolving Chla + Chlb in the donor section and WW as the dissolver of Pheo in the acceptor section. Such increase is stipulated to be due to the fact that, increase the dissolved salts and metals in SW and WW compared with NW.

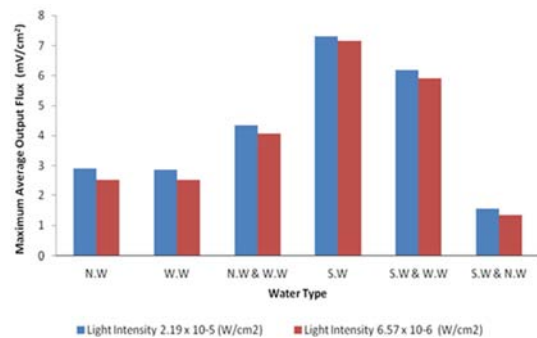


Figure 8. Effect of Light Intensity on the Average Output Flux for Different Types of Water.

Figure 9 illustrates the effect of changing the light intensity along the lasting period of each experiment using SW and WW for dissolving both Chla + Chlb in the donor section and Pheo in the acceptor section respectively.

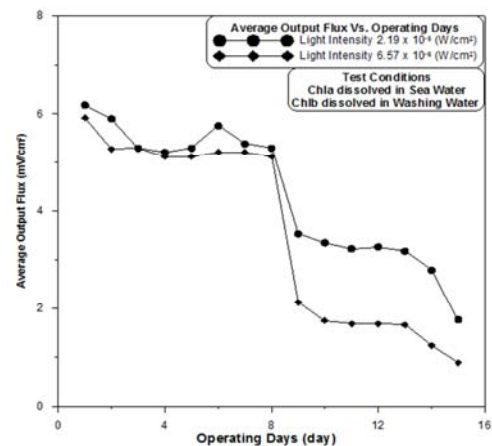


Figure 9. Effect of Changing the Light Intensity Along the Lasting Period by using SW for the Donor Side and WW for the Acceptor Side.

4.5. Influence of the Percentage of Substitute on Renewal of the NB-PV Cell

As presented in the previous sections, the output open circuit voltage was decreased dramatically after a certain number of days called the lasting period. It is noticed also that, the maximum lasting period was 8 days starting from the first day of operation in case of using SW and WW for dissolving both Chla + Chlb in the donor section and Pheo in the acceptor section respectively. Thus, a set of indoor experiments were achieved for investigating the effect of the percentage of substituted solutions (the ratio of new solutions to the existing amount of the solutions in the ball shaped prototype) that could be used for renewing the BNPV. Such effect can be investigated by measuring the open circuit average voltage output flux. These experiments were performed indoor using artificial full visible light wave lengths at light intensity of $2.19 \times 10^{-5} \text{ W/cm}^2$. The results explained that a minimum percentage 50% of substitute solution could be added to the novel cell after its lasting period of 8 days as shown in Figure 10. This supplementary percentage could renew the cell with the same output for another lasting period. These results were thought to be due to the chemical instability of Chl a, Chl b and Pheo. Therefore, from the previous discussion, about 50% of the active pigments of Chl a, Chl b and Pheo are dissociated after ending of its lasting period thus; this substitute can renew such novel cell.

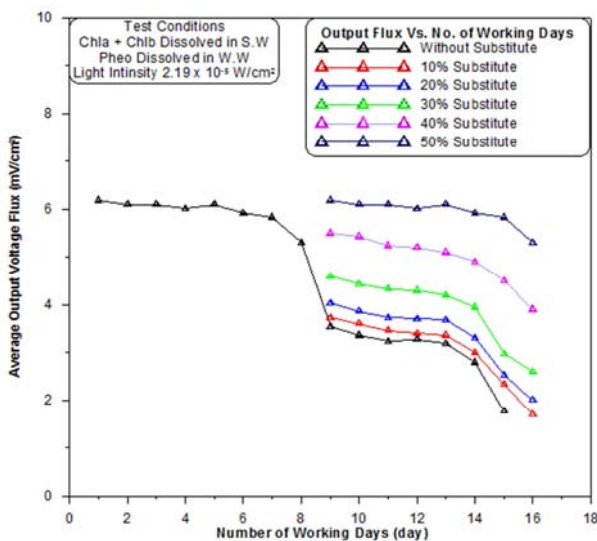


Figure 10. Effect of the Percentage of Substitute on Renewal of the Cell.

5. Conclusions

According to the present work, the following conclusions may be drawn:

- This extraction prototype could establish the economical manufacturing of high pure active pigments from spinach (*Spinacia Oleracea*). Such prototype consists of many sections such as dark drying, grinder, soaking chamber, hot water bath for solvent recovery,

and liquid chromatograph for separation of pigments.

- The extraction process was achieved by using solvents as petroleum ether, acetone and methanol. In regarding to economic issues, such solvents had to be reused by applying a recovery process hence, the percentage of recovery of these solvents was 95%, 87% and 65% respectively.
- The separated active pigments were identified by colors using LC. These pigments are chlorophyll a (Chl a) blue green, chlorophyll b (Chl b) olive green and pheophytin (Pheo) dark grey. The blue green, olive green and dark grey specimens were contained a mass percentage of 98.97% Chl a, 95% Chl b and 64.75% Pheo respectively.
- Both Ultra Violet Visible Spectra (UV-V) and Infra-Red Spectrometry (IR) were very efficient methods for validating the purity of the extracted active pigments.
- The glass sphere novel bio-photovoltaic NB-PV prototype consists of two sections. The first section was the donor, while the second was the acceptor one. Both of these sections were connected to each other by the backed membrane. The extracted active pigments as Chl a, Chl b, Pheo, and Fd were dissolved in different types of water (normal drinking water NW, washing water WW, and sea water SW) to be used for constructing a cell imitating the photosynthesis for producing electricity in addition to hydrogen. The Chl a+Chl b, and Pheo pigments dissolved in a certain type of water could be considered as the donor and acceptor sections of the NB-PV cell respectively. And, the Fd precipitated on waste rice straw could be considered as the membrane allows transport electrons only from the donor to acceptor sections.
- The decreasing of the water level through the operation of the NB-PV cell was due to the water dissociation into O^- and 2H^+ . The evidence of such dissociation was the appearance of a lifted flame by burning the outlet gases.
- The increasing of the operating pressure above the atmospheric diminished the output voltage from the NB-PV cell. This behavior may be due to the negative impact of increasing the operating pressure on the active pigments (Chl a, Chl b, and Pheo), which is not allowed in the vivid green leaf.
- The maximum output voltage flux occurred in two cases. The first and second cases were achieved by using SW as a dissolver of Chl a + Chl b in the donor section as well as Pheo in the acceptor section for the first case but WW for the second case. Such maximum output voltage fluxes were 7.3 mV/cm^2 starting from the fourth day and 6.18 mV/cm^2 starting from the first day of operation respectively. These results are stipulated to be due to the use of SW in the donor section due to high amount of dissolved salts and heavy metals.
- The maximum lasting period occurred in two cases. The first and second cases were achieved by using SW as a dissolver of Chl a + Chl b in the donor section as well

as Pheo in the acceptor section for the first case but WW for the second case. Such lasting periods at the first and second cases were 10 days starting from fourth day and 8 days starting from the first day of the operation respectively. These results are stipulated to be due to the use of SW in the donor section due to high amount of dissolved salts and heavy metals.

- Although the decreasing of the light intensity was 33%, the decreasing of the output voltages were 1.88 and 4.21% for the two above mentioned cases respectively. The first and second cases were achieved by using SW as a dissolver of Chl a + Chl b in the donor section as well as Pheo in the acceptor section for the first case but WW for the second case. Thus, this novel cell has very low sensitivity of the light intensity.
- The percentage of substitute for renewing this novel cell was 50% at the end of its lasting period as eighth day for the case at which Chla + Chlb were dissolved in SW and Pheo was dissolved in WW. These results were thought to be due to the chemical instability of Chl a, Chl b and Pheo. Therefore, from the previous discussion, about 50% of the active pigments of Chl a, Chl b and Pheo are dissociated after ending of its lasting period thus; this substitute can renew such novel cell.

Nomenclature

- A_i : Absorbance at wave length i
- CER&DBT: Center of Excellency for Research & Development of Bio-Fuel Technology
- Chl a: Chlorophyll a
- Chl b: Chlorophyll b
- CP47: Core antenna protein
- CP43: Core antenna protein
- D1: Protein
- Fd: Ferredoxin
- IR: Infra-Red
- LC: Liquid Chromatography
- Mg: Magnesium Molecule
- NB-PV: Novel Bio-Photovoltaic
- NW: normal drinking Water
- P680: Pigment absorbs only wave length of 680nm
- PSI: Photosystem I
- PSII: Photosystem II
- Q_A : Plastquinone molecule A
- Q_A : Plastquinone molecule A
- RC: Reaction Center
- SW: Sea Water
- tyrZ: tyrosine residue of the D1 protein
- UV-V: Ultra Violet Visible
- V: Volt
- WW: Washing Water

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