

Water Hyacinth as Non-edible Source for Biofuel Production

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Received: 22 August 2016 / Accepted: 20 December 2016 / Published online: 3 January 2017
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Abstract Water hyacinth (*Eichhornia crassipes*) represents a promising source for biofuel production and other bioactive compounds because of their high availability and high biomass yield. The present work aims to determine the possibility for biofuel production and other active compounds from seasonally collected water hyacinth. The water hyacinth samples were collected at four seasons (2014/2015) from River Nile, Giza, Egypt, and the biofuel (biodiesel and bioethanol), lipids, glycerol content, carbohydrates and other biochemical compounds were determined in addition to physico-chemically characterized of produced biodiesel. The obtained results indicated that, water hyacinth samples showed variable lipid contents (6.79–10.45%), which by transesterification produced biodiesels (3.22–6.36%) and sediment (pigments+glycerol). Biodiesel composed either totally of saturated fatty acids (Myristic acid) of winter and autumn samples, However, Myristic and Stearic acids with small proportion of pentadecanoic acid of summer and spring samples by 8.1 and 7.9% respectively. The monounsaturated fatty acid, Oleic, was only recorded in the summer sample by 11.6%. So biodiesels produced from water hyacinth have good stability and acceptability to be used in diesel engines, the co-products (sediment) composed of pigments and glycerol reached to 4.69 mg/g

and 1.05 mmol/L respectively in winter season. Also, pre-treatment of water hyacinth by acid at mild conditions was found to be effective with high yield of fermentable sugars and production of ethanol during 120–180 min for different seasonally collected plants. From the results, we can conclude that the produced biodiesel (from water hyacinth) was within the recommended standards and met the criteria required to be a diesel substitute compared with the Egyptian fuel standards. In addition, it is possible to use its pigments as natural coloring substances in food industry, and glycerol can be incorporated in different petrochemical industries.

Keywords Water hyacinth · Biodiesel · Bioethanol · Biochemical compositions · Four seasons

Introduction

The interest in production of biofuel has started in Egypt in eighties of the last century, and the stages for production of biofuel in Egypt divided to the followings: (1) In 1980, production of biogas from animals waste, (2) production of gas by plants and crops residual burning, (3) Biodiesel production from *Jatropha* during 2004 [1].

First and second generation biofuels derived primarily from agricultural crops, like corn, sugar, oil seed plants, palm oils and residual crop matter and forestry products are limited in their sustainability to achieve the target for petroleum oil substitution. Though their net benefit in terms of the reduction of greenhouse gas emissions and achieving energy balance has been recognized [2–4].

Eichhornia (water hyacinth) has gained attention due to its alarming reproductive capacity, which subsequently

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leads to serious ecological damage of water sources in many eutrophic lakes around the world [5]. In Egypt, water hyacinth grows very fast especially in summer months in all water bodies and cause serious problem to navigation, irrigation and deterioration of drinking water quality. Studies have been directed towards making use of these hydrophytes for the production of bioactive substances, which exhibited antimicrobial, anticancer, antioxidant, and anticorrosion of metals and alloys activities [6–9].

Utilization of lignocellulosic materials along with proper optimization of process is most important for cost efficient ethanol production. Among, various types of lignocellulosic substances, water hyacinth, a noxious aquatic weed, has been found in many tropical regions [10]. *Eichhornia crassipes* represents a promising organism for fuel production because of their high availability and high biomass yield. Moreover, the utilization of water hyacinth as the feedstock for bioethanol production has a number of advantages. Water hyacinth is low in lignin content with high content of cellulose and hemicellulose [11]. Ruan et al. [12] reported that, the combined cellulose and hemicellulose content in water hyacinth reached 58.6%, and the lignin content was very low compared with other biomasses.

The most important and beneficial use of *Eichhornia crassipes* is biofuel production in different countries, mainly in Brazil, India and some African countries. It is found that 1 kg of cellulose yields 1.1 kg of glucose and 1 kg of cellulose yield 0.56 kg of ethanol. Other consumption options including *Eichhornia* sp based power plant energy, compost/fertilizer and animal feed production also make the engineering feature so important in many commercial ways. The focus on outlook should regard *Eichhornia* as power plant energy (as source of biogas, biodiesel, bioethanol—etc). It can help to decrease the use of fossil fuels [13].

Because of the following characteristics of *Eichhornia* sp (Water hyacinth), it can be used as biodiesel source; Ideal Attributes, Wide availability, Ease of harvesting and cultivation, Frequent harvest cycles, No competition with food, Easy to extract, non-expensive, Global invasive nuisance weed, Low-tech processing and Millions of dollars spent each year to remove / dispose [14].

This investigation aims to evaluate biofuel (biodiesel and bioethanol) production and other biochemical compounds including pigments and glycerol from non-edible biomass (water hyacinth) which grow exclusively and extensively in all the Egyptian water bodies and causing serious problems.

Materials and Methods

Materials

Chemicals and Reagents

Pure hexane, chloroform, ethanol, ether, acetone and methanol were purchased from E.Merch Co. (Germany), and distilled before use.

Plant Sample

Water hyacinth was collected seasonally during April (spring), July (summer), October (autumn), and December (winter) of 2014–2015, from El-Zomor canal, Giza, Egypt. The hydrophytes was cleaned from any epiphyte and debris by washing several times with tap water, distilled water, then it was left to air dry at room temperature, ground and kept in glass bottles till use.

Methods

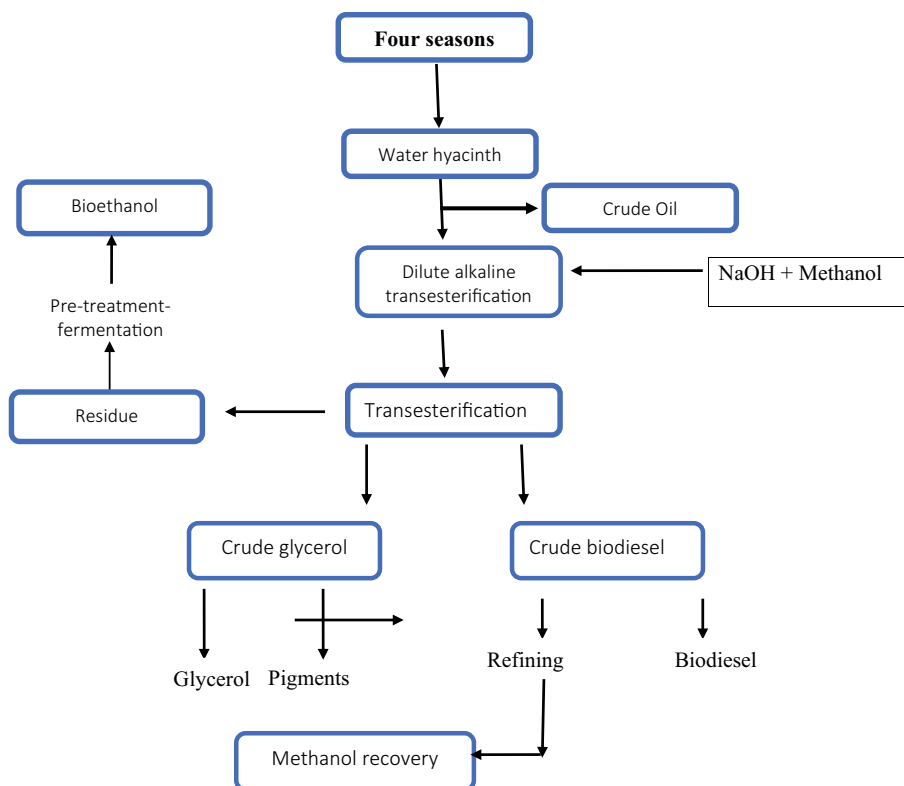
Lipid Extraction

Hundred gram of each ground air dried water hyacinth was mixed separately with the extraction solvent mixture; chloroform/methanol (1000 mL, 2:1, v/v) and sonicated for 20 min (using a microtop of Microson Ultrasonic cell disrupter), followed by the addition of mixture of chloroform/water (500 mL, 1:1, v/v) then, filtration and the sample residue was extracted three times by 1000 mL chloroform at room temperature (25 °C) followed by filtration and then the chloroform layer was separated and dried over anhydrous sodium sulfate. The chloroform extract was evaporated at 40 °C under reduced pressure to dryness and the total lipids were weighted and stored at –20 °C until used (for biodiesel production and fatty acids analysis) according to Bligh and Dayer [15].

Biodiesel Production

The extracted oil was evaporated under vacuum to release the solvent mixture solutions using rotary evaporator at 40–45 °C. Then, the oil produced from Water hyacinth was mixed with a mixture of catalyst (0.25 g NaOH) and 24 mL methanol, with stirring properly for 20 min (Fig. 1). The Mixture was kept for 3 h in electric shaker at 3000 rpm [16]. After shaking the solution was kept for 16 h to settle the biodiesel and the sediment. The biodiesel layer was separated from sediment by flask separator carefully. Biodiesel

Fig. 1 Diagrams illustrating the main steps for production of biofuel and other active ingredients from Water hyacinth



was washed by 5% water many times until it becomes clear then Biodiesel was dried (by using dryer or kept under the running fan for 12 h). The produced biodiesel was measured using measuring cylinder.

Determination of pH

Add five drops of biodiesel to 1 mL of distilled water and mix thoroughly. Using pH meter (Lab 850 BENCHTOP pH METER), then, estimate the aqueous pH of biodiesel.

Physico-chemical Characterization of Produced Biodiesel

The biodiesel product from summer collecting samples was tested for estimating their properties using the standard methods of analysis for fuel product (ASTM Standard Methods) and compared with the Egyptian standards for petro-oil and European and American standards of biodiesel (EN14214 and D-6751, respectively). Shalaby and El-Gendy [17].

Fatty Acid Composition of Biodiesel

The Fatty acids analysis of produced biodiesel was carried out with gas liquid chromatography (GLC) using Agilent 6890 plus, equipped with a HP-50 capillary column

(0.53 nm x 30 m, 0.5 μ m film) and flame ionization detector. Temperature of injector and detector were 250 and 300 $^{\circ}$ C, respectively. The column was hold at 200 $^{\circ}$ C for 3 min then programmed from 200 to 240 $^{\circ}$ C (at rate of 10 $^{\circ}$ C/min). Nitrogen was the carrier gas, hydrogen and air gases were used at flow rates of 30, 33 and 330 mL/min, respectively. The identification of fatty acids was accomplished by comparing the peaks of retention times with those of the corresponding standards.

The quantity of individual compounds was determined by comparing the produced peak area with standard curve of the authentic substances, which expressed the relation between the different concentrations and their peak area [18].

Fourier Transforms Infra-Red Analysis

To investigate the functional groups involved in Transesterification process, FT-IR analysis was carried out using Fourier transform infra-red spectrometer Perkin Elmer FTIR spectra. The spectra were collected within a scanning range of 400–4000/cm.

Sediment Analysis (pigments and glycerol)

Pigment determination. The pigments separated from glycerol layer was determined according to Holden [19]. Ten

mL of the sediment (pigments + glycerol) was mixed with 5 g of active charcoal, after 24 h, the pigments adsorbed on charcoal was separated by filtration. Then, the pigments were eluted from charcoal surfaces by acetone. The pigments were determined spectrophotometrically (CT-2200 spectrophotometer) at the indicated wave lengths and substitute in the equations:

$$\text{chlorophyll a (mg/g)} = 12.3 \times A(663) - 0.8 \times A(645) \times V/A \times 100 \times W$$

$$\text{chlorophyll b (mg/g)} = 19.3 \times A(645) - 3.6 \times A(663) \times V/A \times 100 \times W$$

$$\text{Carotenoids (mg/g)} = 4.57 \times A(452) - 0.22 \times \text{Total chlorophylls}$$

where; A (663), A (645) and A (452) were the absorbance at these wave lengths.

V = Volume in mL.

A = Length of light path in the cell.

W = the fresh weight of sample in gram.

Glycerol Determination

The colorless glycerol left after pigment elimination from the sediment was determined according to Li et al. [20]. One mL (glycerol from the seasonal samples) was mixed with 10 µl of R2 (Free Glycerol Reagent), compared to the blank contained only 1 mL of the coloring reagent (R2) and to the standard (10 µl of the glycerol standard R1 + 1 mL of R2. the developed color (rose-red) after 10 min. of incubation was read at 546 nm and glycerol content was measured according to the following equation:

$$\text{Glycerol content} = (A(\text{sample})/A(\text{standard})) \times 200 = \text{mg/dl} \times 0.0113 = \text{mmol/L.}$$

where A (sample) and A (standard) are the absorbance of the sample and standard at the indicated wavelength (546 nm). where R1 is the glycerol standard, R2 is the Free Glycerol Reagent contain; ATP, Magnesium salt, 4-Aminoantipyrine *N*-Ethyl-*N*-(3-sulfopropyl) *m*-anisidine, sodium salt, Glycerol kinase, Glycerol phosphate oxidase, Peroxidase, Buffer.

Note: Dunstan 's test (Borax-Phenolphthalein test) was used as confirmation test for the presence of glycerol.

Hydrolysable Carbohydrate Content

Total hydrolysable carbohydrate content (%) in the residues of seasonally collected water hyacinth after lipid extraction was determined using Sulfuric acid-phenol 5% method according to Dubois et al. [21].

Bioethanol Production

Carbohydrate extraction. The residues left after lipid extraction from the seasonally collected water hyacinth were separated and extracted according to Gupta et al. [22], briefly, samples were hydrolyzed in dilute 0.70% H₂SO₄ and were heated at 105 °C for 6 h. Then the samples were neutralized

by adding BaCO₃. Samples were centrifuged at 5000 rpm for 10 min. Filtration process carried out to filter the extract.

Fermentation process: To the neutral glucose solution (25 ml), 0.15 g of active dried yeast (*Saccharomyces cerevisiae*) was added at 30 °C and shake for 120 rpm with the collection of sample at intervals (0, 30, 60, 90, 120, 150 and 180 min). The relative percentage of sugar content (%) was determined using Brix meter at time intervals and the change in temperature of the fermented sugar solution during the fermentation period (180 min) was recorded using thermometer.

Confirmation Test of Ethanol Production

Iodoform Test. 2 mL from filtrate in a test tube and 1% iodine solution was added. Then dilute sodium hydroxide was added as a drop until brown color of iodine was discharged. Tube was then gently warmed on a water bath. (Positive result: the appearance of a yellow precipitate) [23].

Statistical Analysis

Data were subjected to an analysis of variance and the means were compared using the least significant difference (LSD) test at 0.05 and 0.01 levels as recommended by Snedecor and Cochran [24].

Results and Discussion

Lipid contents and biodiesel production

Table 1 recorded the lipid content and some physical characteristics of produced biodiesel, which indicated that summer sample, contained the highest lipid content (10.45%) compared to those of the other seasonal samples.

The obtained biodiesel acquired bright yellow color which may be due to the fact that summer environmental

Table 1 Lipid, biodiesel content (%) and biodiesel physical characteristics (color and pH) in the seasonally collected water hyacinth

Seasonal sample	Lipid content (%)	Biodiesel content (%)	Biodiesel color	Biodiesel pH
Summer	10.45	6.36	Bright yellow	5.0
Winter	6.79	3.89	Light yellow	4.0
Spring	8.97	4.27	Light yellow	5.6
Autumn	6.90	3.32	Colorless	4.0
LSD at 0.01	0.0546	0.0470		0.099

Each value is presented as mean of triplet treatments, LSD: Least significantly different at $P=0.01$ according to Duncan's multiple range test

conditions with elevated temperature ($>40^{\circ}\text{C}$) and high light irradiation imposed stress factors on the metabolic pathways of the hydrophytes (water hyacinth) leading to an alteration of the normally produced secondary metabolites and the direction of all carbon structures produced during metabolic processes towards more lipid production.

The obtained results were in agreement with the results reported by Arayana et al. [25] who mentioned that, The lipid contents of the roots, leaf stalks, leaves and flowers of *Eichhornia crassipes* (Mart.) Solms (water-hyacinth) were 1.6, 0.9, 14.9 and 5.7%, respectively, Also, Sagar and Kumari [14] reported that, water hyacinth can be considered as a good tool for cheap industrial production of biodiesel.

Also, data in the same Table 1, reported that, the lowest biodiesel production was observed in autumn samples (3.32%) followed in ascending order by winter samples (3.89%), Spring samples (4.27%) While summer samples produced the highest biodiesel percentages (6.36%).

The bright yellow color of the produced biodiesel maybe due to a higher production of carotenoids in summer season serving as an antioxidant and higher solar protectant against the adverse and harmful effects of Reactive oxygen species (ROS) increment during this season.

A gradual decrease in lipid contents was recorded in other seasonal samples [Spring (8.97), Autumn (6.90) and Winter (6.79%)], which was accompanied with a decrease in the intensity of the yellow color of the produced biodiesel, varying from light yellow (in the Spring sample) to colorless in autumn samples which may be due to a gradual lower carotenoid content in these seasons (Table 1) as a result of changes in the environmental conditions but all biodiesels showed an acidic pH (4–5.6).

These results were in agreement with those reported by Afify et al. [26] and Shalaby et al. [27] who reported that, salt stress and nitrogen starvation conditions induced dramatic increase in total carotenoids of both green microalga *Dictyochloropsis splendida* and the cyanobacterium *Spirulina platensis*.

Also, the lipid content and consequently the biodiesel production was enhanced under salt and nitrogen starvation stress [7].

Fatty Acids Compositions

Fatty acids composition of biodiesel (produced from lipid esterification) revealed the complete absence of polyunsaturated fatty acids in all seasonal samples. Monounsaturated fatty acids represented by Oleic acid (C18:1) was only recorded in the summer sample (11.6%) as shows in Table 2.

Biodiesel of all seasonal samples were composed of saturated fatty acids (Myristic C14:0, Pentadecanoic C15:0 and Stearic C18:0) with variable proportions. Winter and autumn biodiesels were composed of 100% Myristic acid, while summer and spring ones have in addition Pentadecanoic (8.1 and 7.9% respectively) and Stearic acids (68.6 and 46.6% respectively).

Fatty acid synthesis was found to be influenced by the actual temperature and light intensity in any season as reported by Sukenik et al. [28].

The higher proportion of saturated fatty acids in all seasonal samples indicated that the produced biodiesels were so stable and has no susceptibility for auto-oxidation during storage (except the biodiesel from summer sample, which contain small proportion of Oleic acid, 11.6%).

These biodiesels are suitable to be used in diesel engines due to its good stability and acceptability which was found to be more than those produced from the first, second and third generation's feedstock for biodiesel production [29].

Table 2 Fatty acid composition of biodiesel (Fatty acid methyl ester) of seasonally collected water hyacinth samples

Fatty acids	RT	Relative % of fatty acids in the seasonally collected water hyacinth			
		Winter	Summer	Spring	Autumn
Myristic acid (C14:0)	19.61	100	11.7	45.4	100
Pentadecanoic acid (C15:0)	21.45		8.1	7.9	
Stearic acid (C18:0)	26.63		68.6	46.6	
Oleic acid (C18:1)	27.16		11.6		
Total Fatty acids (Saturated + Unsaturated)		100	100	100	100

The obtained results in this investigation concerning biodiesel production from water hyacinth were in conformity with those reported by Sarin et al. [30], Singh et al. [31], Lam and Lee [32] and Shanab et al. [7], who reported that the oil-seed plants, the non-crop plants and algal derived biodiesel contained variable percentage of unsaturated fatty acids which make it susceptible for autoxidation.

Physico-Chemical Characterization of Produced Biodiesel

From the recorded results in Table 3, the produced biodiesel has a density of 0.834 g/cm³, which is near to Egyptian diesel fuel (0.82–0.07 g/cm³). The total acid number represents 0.64, which is higher than that of standard EN14214, and near to that of Biodiesel D-6751. These results indicated that the free fatty acid would not cause any operational problems. The flow properties, including cloud point and pour point, are –1 °C and –5 °C, respectively. The flash point of the produced biodiesel is 191 °C, which is higher than that of Egyptian petro-oil standard (55 °C). This higher value of flash point can prevent auto ignition and fire hazard at high temperature. Viscosity is a significant and important property of any fuel that affects the flow and atomization characteristics of a liquid fuel. The kinematic viscosity of the produced biodiesel is 9.85 cSt at 40 °C. The importance of cetane number of the diesel is considered as an indicator of the ignition quality; a high cetane number for a diesel fuel correlated with a higher performance, shorter ignition delay and duration of the combustion period which led to lower emission of pollutants, besides less occurrence of knocking noise. The obtained results revealed that higher cetane number; 64.9 is comparable to all mentioned standards. Always, the cetane number increases, with the increase of saturated fatty acid concentration in biodiesel oil [33].

Hydrolysable Carbohydrate Content

Figure 2 showed the hydrolysable carbohydrate content, which indicated that summer sample, contained the highest

content (5%) and autumn samples contained the lowest content (2.8%). Moreover, these results were in agreement with those reported by Chand et al. [34].

FTIR Results of Produced Biodiesel

The infra-red spectrum of the produced biodiesel (Fig. 3), showed the IR bands at 1020, 1169 and 1241 cm⁻¹ corresponding to the presence of ester C-O-, 1744 cm⁻¹ for C=O ester, 2854, 2923 cm⁻¹ for (CH₂, CH₃), 3008 cm⁻¹ for (C=C) and 3468 cm⁻¹ corresponding to the presence of alkynes group. The presence of ester group and absence of hydroxyl peak can be correlated to the transesterification process of Eichhornia crude oil.

Sediment (pigments and glycerol) Determination

Pigments content transesterification of the extracted lipids from *Eichhornia crassipes* produced an upper biodiesel layer and a sediment (pigments and glycerol) lower layer.

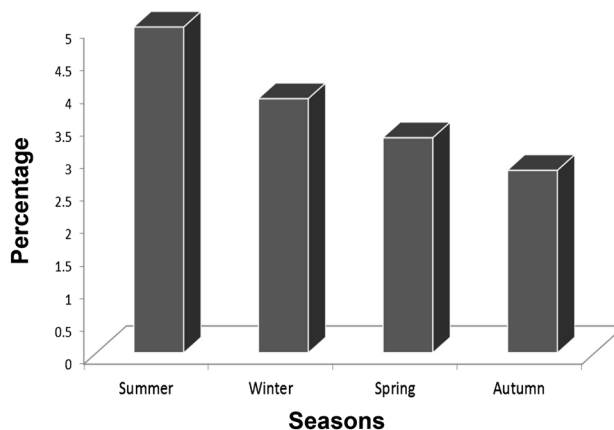


Fig. 2 Hydrolysable carbohydrate content (%) of Water hyacinth from different seasons

Table 3 Physico-chemical properties for biodiesel produced from *Eichhornia crassipes* collected during summer compared to the Egyptian standards and two international biodiesel standards

Test	Produced Biodiesel (from summer season)	Egyptian Diesel oil	Biodiesel (EN14214)	Biodiesel D-6751
Flash point °C	191	>55	>101	>130
Density g/cm ³ @ 15.56 °C	0.834	0.82–0.87	0.86–0.9	–
Kinematic Viscosity cSt @ 40 °C	9.85	1.6–7	3.5–5	1.9–6
Total acid number (mg KOH/g)	0.64	Nil	<0.5	<0.8
Cloud point °C	–1	–	–4	–
Pour point °C	–5	4.5–15	–	–
Cetane number	64.9	Min 55	>51	>47
Iodine number mg I ₂ /100 g	89	–	120	–

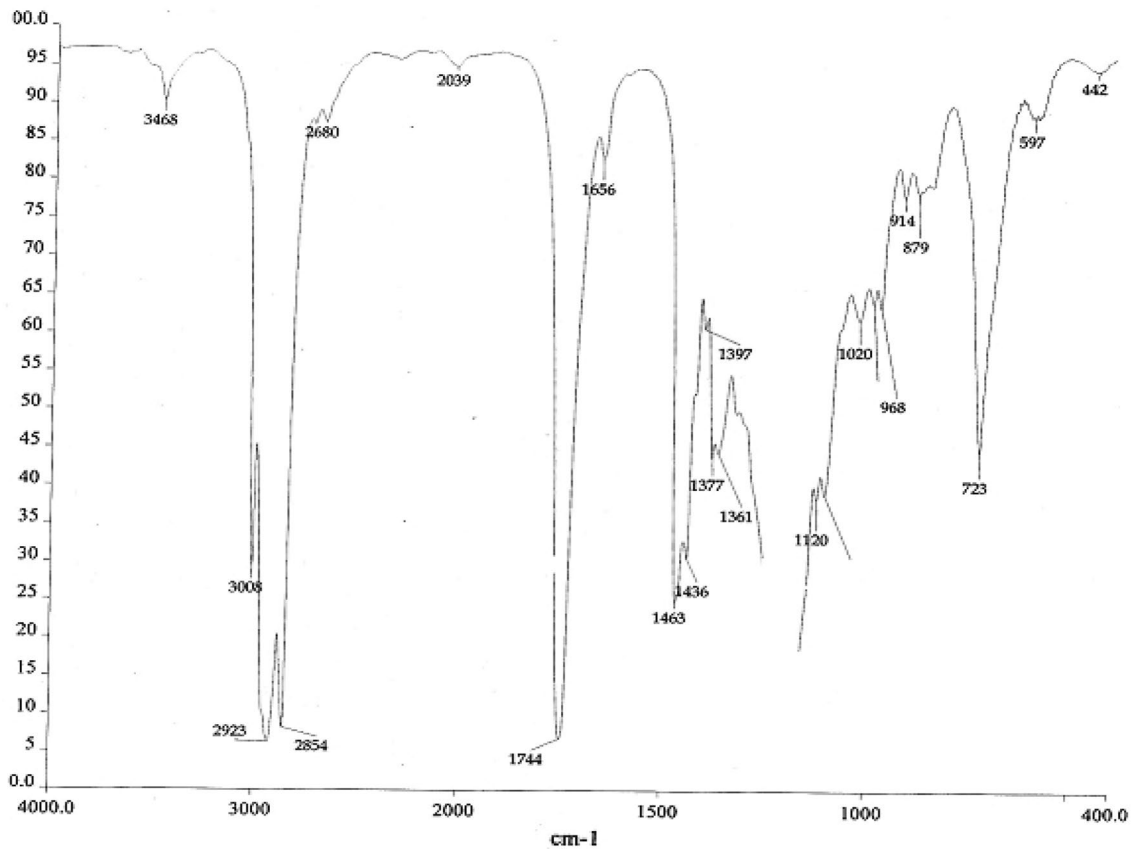


Fig. 3 Infra-red spectra of methyl ester of produced biodiesel (summer samples)

In order to make use of all the transesterification products, separation of each layer and studying their characteristics were performed.

The sediment lower layer contains both the pigments (chlorophylls and carotenoids) and glycerol.

Total chlorophylls (a and b) and total pigment content (chlorophylls and carotenoids) are shown in Table 4, followed the order, winter > summer > spring > autumn (4.69, 3.97, 2.80 and 1.60 mg/g respectively), while the carotenoid contents showed some changes. Spring samples recorded the highest carotenoid content (1.72 mg/g), followed in descending order by (winter, summer) and autumn (1.56, 1.55 and 1.2360 mg/g respectively). These results may be due to the favorable environmental conditions as biotic and a biotic stress in spring season only for carotenoid biosynthesis, while the lower or higher temperature and light intensity in other seasons were inhibitory to carotenoid pigment synthesis (but not for chlorophylls) [35].

Autumn seasonal sample recorded the lowest pigment content, which may be due to the unfavorable environmental conditions for pigment biosynthesis during this season (low temperature and low light intensity).

Glycerol Content

Table 5 demonstrated that winter sample recorded the highest glycerol content (92.66 mg/dl, 1.05 mmol/L). The same season showed the greatest pigment content (Table 4). This was followed in descending order by spring (49.72 mg/dl, 0.56 mmol/L), autumn (36.16 mg/dl, 0.41 mmol/L) and summer (22.6 mg/dl, 0.255 mmol/L).

Based on the results, the increased production of glycerol in winter season may be due to different metabolic pathway: one using photosynthesis products and other via the catabolism of starch molecule (the storage product in *Eichhornia* sp) or due to external osmotic pressure [36].

From the Table 5 obtained results, we can conclude that the climate conditions introduce significant changes in chemical compounds produced by *Eichhornia* sp such as glycerol, pigments, lipid compositions—etc and these changes may be due to gene expression in plant cells.

Bioethanol Production

For the production of bioethanol from the seasonally collected water hyacinth samples, the residues left after lipid

Table 4 Pigment content (Chlorophyll a, b and carotenoids) in the sediment (lower layer) of transesterified lipid contents in the seasonally collected water hyacinth

Seasons	Seasonal pigment content (mg/g) in sediments of <i>E. crassipes</i>				
	Chlorophyll a	Chlorophyll b	T. Chlorophyll	T. Carotenoids	Total pigment content
Summer	2.14	0.28	2.42	1.55	3.97
Winter	1.58	1.55	3.13	1.56	4.69
Spring	0.94	0.14	1.08	1.72	2.80
Autumn	0.22	0.15	0.36	1.23	1.60
LSD at 0.01	0.0196	0.017	0.0196	0.0196	0.0519

Each value is presented as mean of triplet treatments, *LSD* Least significantly different at $P=0.01$ according to Duncan's multiple range test

Table 5 Glycerol content in the sediments (lower layer) of the transesterified seasonally collected water hyacinth

Seasons	Glycerol content	
	mg/dl	mmol/L
Summer	22.6	0.255
Winter	92.66	1.05
Spring	49.72	0.56
Autumn	36.16	0.41
LSD 0.01	0.0597	0.0170

Each value is presented as mean of triplet treatments, *LSD* Least significantly different at $P=0.01$ according to Duncan's multiple range test

extraction constitute the feedstock for bioethanol production after its hydrolysis to the simple fermentable monosaccharide glucose. Fermentation of glucose was performed using the yeast *Saccharomyces cerevisiae*.

The gradual decrease of glucose content was accompanied by an increase in bioethanol production during the fermentation period.

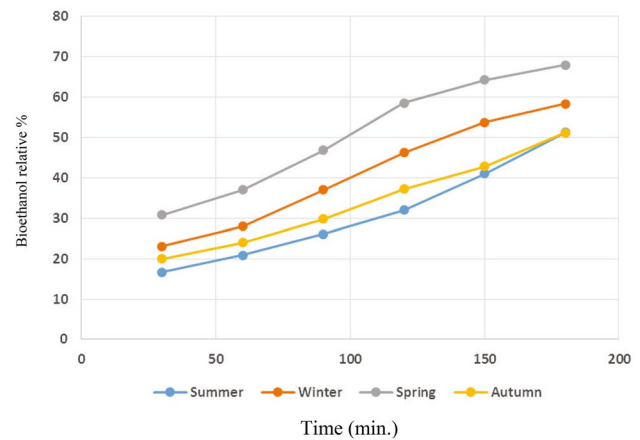
Hydrolysis of carbohydrates in the residues (by dil. H_2SO_4), left after lipid extraction, produced variable glucose contents (% by Brix-meter) in the seasonally collected *Eichhornia* samples.

In first 30 min of fermentation process, about 16.7–33.3% of the glucose content was fermented.

The gradual fermentation of glucose (decrease) during the fermentation period was accompanied by a gradual increase in bioethanol content.

Glucose content in spring sample was highly fermented in 2 h (120 min) giving 58.6% bioethanol production as relative percentage. Followed by winter, autumn and summer seasons (46.25, 37.25 and 32.0% respectively) as seen in Fig. 4 and Table 6.

These results were in agreement with the results obtained by Yan et al. [37]. Who mentioned that, pretreatment of

**Fig. 4** Relative percentage of bioethanol production of glucose from water hyacinth during fermentation time

water hyacinth increased the production of bioethanol by 0.43 g ethanol/g glucose.

The results also revealed that, the temperature of produced bioethanol ranged between (28.5–29.0 °C) in all sampled after 150 min of fermentation.

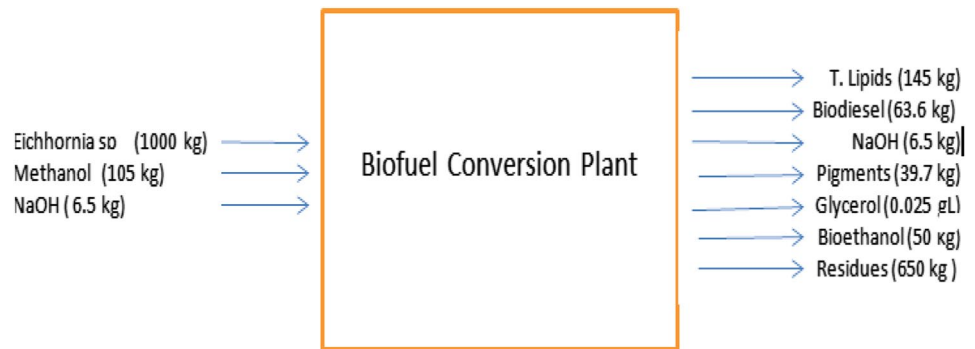
Material balance of biofuel product. Biomass conversion plant has many components, which are connected to each other. Materials and energy flow among the components, therefore we should grasp the detail of the balance (Fig. 5). If there is a choke point, the flow stagnation lead to the troubles of operation and low efficiency of the performance.

Conclusions

From our results we can conclude that water hyacinth is a promising source for production of biofuel (as biodiesel and biofuel), glycerol, pigments and other active compounds, the high biofuel production from water hyacinth due to its high content from lipids and carbohydrates compounds and

Table 6 Bioethanol production (%) during the fermentation period of the seasonally collected water hyacinth

Seasons	Produced bioethanol (as relative percentage) during the fermentation time (min)						
	Zero time	30 min	60 min	90 min	120 min	150 min	180 min
Summer	0.0	16.7	20.87	26.1	32.0	41.0	51.25
Winter	0.0	23.08	28.01	37.06	46.25	53.8	58.4
Spring	0.0	30.77	37.0	46.9	58.6	64.3	68.0
Autumn	0.0	20.0	24.0	29.8	37.25	42.8	51.2

Fig. 5 Materials and energy balance in biofuel production from *Eichhornia crassipes*

the obtained biodiesel properties within the recommended standards and met the criteria required to be a diesel substitute compared with the fuel standards, these pronounced results encourage the rapid announcement for a country-wide project in different governorate of the country mainly to produce biodiesel and bioethanol with high amount. Moreover, Possibility for use the pigments produced from water hyacinth residues as natural coloring substances in food industry without cytotoxicity and Glycerol may be incorporated in different petrochemical industries.

Acknowledgements This work was fully supported by a grant from the Science and Technology Development Fund (STDF-Project ID: 312), Cairo, Egypt.

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