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Egypt. J. Bot. **Vol. 60,** No.3, pp. 797-804 (2020)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Fatty Acid Profiles and Fuel Properties of Oils from Castor Oil Plants Irrigated by Microalga-treated Wastewater

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MICROALGAE can function as a bio-fertilizer and a bio-filter for wastewater treatment, thus allowing the re-use of wastewater for the irrigation of plants that produce abundant bio-oils and for the irrigation of low-quality soils. *Chlorella vulgaris* was grown in Om El-Resh drain water as growth medium, after enrichment with nitrogen and phosphorous. Castor seeds were cultivated in a greenhouse at the Algal Biotechnology Unit, National Research Centre, Egypt. Fifteen days later, transplants were irrigated by untreated wastewater (WW); wastewater treated with microalgae (WW+A); or wastewater treated with microalgae followed by removal of microalgae (WW-A). Oil extraction was performed by seed warming and grinding with n-hexane, followed by soaking, filtration, and passage through Silica gel 60. Esterification was performed, and then fatty acid methyl esters (FAMEs) were determined. The results indicated that the wastewater treatment markedly affected seed oil content. The WW+A treatment led to the highest seed oil yield (41.8%), followed WW-A treatment (28.14%), and the WW treatment (25.12%). FAME analysis indicated that the presented fatty acids of castor oil were C16 and C18 and ricinoleic acid (C18:1) was the most abundant (83.1 to 84.63%). In spite of the higher seed oil content when plants were grown in WW+A, there were differences in the fuel proprieties of seed oils in the different groups, based on American Society for Testing and Materials criteria. Our results suggest that wastewater can be successfully used for irrigation of soils that have poor fertility to produce bio-oils during land reclamation.

Keywords: Castor oil, FAME, Fuel properties, Microalgae, Wastewater treatment.

Introduction

The purpose of biological wastewater treatment is to stimulate the consumption of organic carbon by aerobic bacteria by supplying abundant oxygen, typically using an energy-intensive mechanical aeration process. This treatment removes harmful pathogens, thus protecting public health, and removes nutrients, thus protecting fresh water systems from eutrophication. Reuse of pathogen-free wastewater can increase agricultural productivity and reduce the need for chemical fertilizers. Microalgal cultivation plays an important role in tertiary bio-treatment of wastewater, and also produces biomass that consumes inorganic nitrogen and phosphorus for growth and removes pollutants. Reuse of pathogen free wastewater can increase agricultural productivity and reduce the need for chemical fertilizers (Delrue et al., 2016).

Treated wastewater can be an important source of water and nutrients for crops, but also has adverse effects on soil properties and seed production in crops used for bioenergy production. Different plant species can also change soil properties and factors related to seed and biodiesel yield (Chatzakis et al., 2011). Microalgal-based wastewater treatments can be applied in tropical regions, where it is critical to control many environmental factors (Rajesh et al., 2018).

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Received 25/1/ 2020 ; Accepted 7/7/ 2020
DOI: 10.21608/ejbo.2020.22970.1444
Edited by: Prof. Dr. Mostafa M. Elsheekh, Faculty of Science, Tanta University, Tanta, Egypt.

Organic matter in wastewater can enhance soil aeration, infiltration rate, water storage, and cation exchange capacity (CEC); it can also decrease the soil erosion and increase the populations of soil organisms that promote plant growth. In fact, this practice is an efficient method of controlling waste and stopping the introduction of harmful effluents into bodies of fresh water (Valipour & Singh, 2016).

Microalgal-based wastewater treatments are highly suitable for tropical regions because of their high temperatures and abundant sunlight. Many environmental factors must be considered when cultivating microalgae. Maintenance of a suitable temperature and irradiation in microalgal ponds is critical, but can be difficult to achieve in practice. Contamination and grazing must also be considered (Emongor & Ramolemana, 2004). Sterilization and ultra-filtration of the culture medium can reduce contamination by bacteria and other microalgae, and chemical treatments can reduce grazing by protozoans and fungal diseases (Toze, 2006).

Certain non-edible plants that produce abundant oils can help to meet the challenge of the energy crisis. Most of these plants are wild and can grow under conditions considered to be nutritionally and environmentally stressful to plants. Thus, these plants can often be cultivated in non-fertile soils using poor-quality water. The castor oil plant (*Ricinus communis*) is a fresh water plant, but can also grow in saline conditions. This plant is more prone to saline stress during the early stages of vegetative growth than during the later stages, when its bean-like seeds are produced. An increasing level of nitrogen does not affect seed production, but the use of saline water for irrigation reduces the oil content and yield (De Lima et al., 2012).

Castor oil is non-edible and mainly used for biodiesel synthesis. Castor oil, like other seed oils, is extracted from ripe or mature seeds after sun drying, and a sequence of seed processing operations that may include de-hulling, pod or seed coat removal, winnowing, sorting, cleaning, grinding or milling, preheating, and other steps. (Alirezalu et al., 2011). The average oil content among different varieties of castor seeds ranges from about 46 to 55% oil by weight (Ogunniyi, 2006). The actual yield depends on many factors, such as seed variety, geographical origin, climatic conditions, and the methods used for oil extraction. The world-wide average yield of castor oil seed is about 1.1 t per ha-1, though yields up to 4.2 t per ha-1 can be obtained. The castor oil plant has the highest oil yield among land plants (Scholz & Silva, 2008).

This study examined the treatment of wastewater from the Om El-Resh drainage system (Egypt) using microalgae, and the reuse of this water in irrigation of the castor plant cultivated in poor soils aiming at the providing of water and fertilizer.

Materials and Methods

Microalgal and growth conditions

Chlorella vulgaris (NRC-Egypt) was from the Algal Biotechnology Unit (ABU) of the National Research Centre (NRC), Cairo, Egypt. Cultures were grown heterotrophically under optimum conditions in BG-11 nutrient solution (Stainer et al., 1971) to obtain inocula. The growth conditions were the same as employed by El-Sayed et al. (2015).

Wastewater and biological treatment

The study used drainage water from Om El-Resh (Port Said Governorate, Egypt). Prior to treatment, water was filtered over white saw dust to reduce odors and remove particulates. The physico-chemical properties of the water were determined as described by Chapman & Pratt (1978). The microalgae were grown in the pre-filtered water with proper inocula in an indoor growth unit with adequate enrichment by nitrogen (17.6mM, from nitric acid) and phosphorus (7.0ppm, from phosphoric acid) in BG-11 medium. Scaling up was performed using 14L fully transparent acrylic cylinders. Microalgae were harvested by precipitation and centrifugation.

Plant cultivation

The study design consisted of three batch experiments in which treatments were developed to optimize plant cultivation and the bio-filter and bio-fertilizer functions of the microalgae. All seedlings were initially grown in artificial soil in a greenhouse at the NRC. After 15 days, they were transplanted and irrigated using one of the following three treatments: WW (Om El-Resh wastewater); WW+A (WW that was treated with *C. vulgaris* for 48hrs); or WW-A (WW that was treated with *C. vulgaris* for 48 h followed by removal of microalgae by centrifugation).

Seeds were grown in pots (40 cm diameter) that contained sandy soil, and each plant received an average of 0.5L of WW per day without any additional fertilizers. Chemical characterization of soil (Table 1) was determined as described by Chapman & Pratt (1978).

Oil extraction

A solvent method (Soxhlet extraction) was used to extract oils from the seeds. In particular, seeds were first flame warmed, grinded in a mortar, and then soaked in 10-fold volume of n-hexane in an orbital shaker (125rpm) overnight. The residue was removed using a vacuum and Whatman 50 filter paper. The solvent-containing oil was then passed through silica gel (60 mesh) and the colored fraction was removed. A rotary evaporator was used to recover the solvent.

Determination and identification of fatty acids

The extraction of fatty acids used a previously described procedure (Porim Official Test Method, 1995), in which fatty acids were converted to fatty acid methyl esters (FAMEs) by methylation using methyl alcohol (3mL of 3% H_2SO_4 + 97mL methanol). Reflux extraction was performed at 90°C for 3hrs, and the solvent extract was exchanged with n-hexane. The extracted oil phases were combined and dried by passing through a glass funnel containing anhydrous sodium sulfate. The oil fraction was then concentrated using a rotary evaporator in preparation for analysis by gas chromatography with a flame ionization detector (GC-FID) under specific conditions. A Perkin Elmer AutoSystem XL that was equipped with an FID and a fused silica capillary column (DB-5, 60 × 0.32mm) was used for analysis. The initial temperature was 150^{o}C, and was programmed to increase up to 240^{o}C at rate of 3^{o}C/min. The injector temperature was at 230°C and the detector temperature was 250°C. Helium was used as the carrier gas (flow rate: 1mL/min).

Fuel properties of castor oil

The following chemical properties of castor oil FAMEs were determined using equations and methods described by Mittelbach & Remschmidt (2004): cetane number (CN), saponification value (SV), iodine value (IV), degree of un-saturation (DU), long-chain saturated factor (LCSF), and cold filter plugging point (CFPP).

Statistical analysis of data

The one way ANOVA between the different type of water compared with the WW water were done. The differences were considered significant at the 95% confidence level ($P \leq 0.05$). The standard deviation of the different treatments was calculated in triplicate samples. All statistical analyses were performed using proper statistical software STATISTICA Ver. 8 (StatSoft, Inc., 2007).

Results and Discussion

Wastewater characteristics

We used Om El-Resh drainage water subjected to three different treatments (WW, WW+A, or WW-A) for the cultivation of castor oil plants in sandy soil (Table 2). Comparing our soil data, and based on FAO (2010) guidelines, the WW+A had the best electrical conductivity; levels of sulfate, sodium, total dissolved salts; and sodium adsorption ratio (SAR).

C. vulgaris utilizes dissolved CO_2 and bicarbonate ions metabolized by carbonic anhydrase into CO_2 as carbon sources for photosynthesis, and uses phosphorus and nitrogen for metabolic growth. The high rate of microalgal biosynthesis leads to increased biomass that can be used to generate methane as an energy source (Molazadeh et al., 2019). When there is low or no microbial growth, microalgae can utilize organic sources of carbon and other nutrients (except organic phosphorus) to produce large amounts of oxygen. In addition, the chemical composition of wastewater (levels of nutrients such as carbon, nitrogen, and phosphorous) affects the growth of microalgae. In particular, sodium can replace potassium, and this ultimately enhances microalgal growth (Abdel-Raouf et al., 2012).

TABLE 1. Physico-chemical properties of soil used for cultivation of castor oil plants.

pH	EC (dS/m)	OM	Clay	Silt	Sand	$CaCO_3$	CO_3	HCO_3
		%						
7.61 ± 0.34	3.02 ± 0.07	0.0 ± 0.00	4.3 ± 0.14	14.4 ± 1.07	81.3 ± 12.56	5.98 ± 0.84	3.9 ± 0.84	46.9 ± 10.21
N	**P**	**K**	**Na**	**Ca**	**Mg**	**Fe**	**Zn**	**Cu**
		ppm						
0.0 ± 0.00	0.0 ± 0.00	8.01 ± 0.94	211 ± 12.34	131.6 ± 12.12	51.03 ± 7.56	18.6 ± 1.94	1.01 ± 0.04	0.7 ± 0.02

Abbreviations here and below: EC: Electrical conductivity; OM: Organic matter. Mean ± SE, n= 3.

TABLE 2. Chemical characteristics of the different wastewaters.

Parameter	Wastewater treatment			
	WW	**WW+A**	**WW-A**	**FAO /2010**
pH	8. 16 ± 0.43	7.98 ± 0.51	8.01 ± 0.49	6.5–8.5
EC (dS/m)	7.20 ± 0.64	5.98 ± 0.83	6.02 ± 0.43	0–3
TDS (ppm)	4608.00 ± 143.31	3808.00 ± 200.63	3852.80 ± 243.76	0–2000
HCO_3 (mg/L)	762.30 ± 10.28	687.30 ± 12.03	684.30 ± 11.73	0–600
SO_4 (mg/L)	723.90 ± 22.65	397.80 ± 8.19	328.80 ± 13.37	0–1000
Cl (mg/L)	2146.80 ± 156.97	2085.60 ± 132.62	221.60 ± 21.03	0–1100
Ca (mg/L)	69.39 ± 8.94	63.90 ± 5.49	58.95 ± 6.83	0–400
Mg (mg/L)	117.42 ± 12.64	71.10 ± 10.33	75.60 ± 10.43	0–60
K (mg/L)	11.91 ± 1.52	6.21 ± 0.58	6.42 ± 0.51	0–2
Na (mg/L)	303.90 ± 16.82	235.20 ± 16.43	209.40 ± 15.74	0–900
PO_4 (mg/L)	0.66 ± 0.10	0.48 ± 0.09	0.57 ± 0.10	0–2
NO_3 (mg/L)	19.56 ± 2.62	15.03 ± 1.33	12.57 ± 1.59	0–10
Total N (mg/L)	17.94 ± 2.63	19.2 ± 2.17	12.03 ± 2.03	0–30
SAR	7.23 ± 0.83	3.36 ± 0.63	6.27 ± 0.67	0–15

Abbreviations here and below: WW: Om El-Resh drainage water; WW+A: Om El-Resh water treated by *C. vulgaris*; WW-A: Om El-Resh treated with *C. vulgaris* followed by *C. vulgaris* removal; TDS: Total dissolved solids; SAR: Sodium adsorption ratio. The data shown are means ± SD, (n = 3).

The WW is inappropriate for plant irrigation due to its high TDS (4608 ± 143.31 ppm), which far exceeds the level recommended by the FAO (2010). Thus, the salinity of this water must be reduced, although several plant species can grow in this high-salinity water. Nonetheless, long term use of such water will greatly increase soil salinity. This problem can be resolved by increasing the organic matter load using irrigation water containing microalgal biomass. However, the nitrogen and phosphorous content of the WW did not reach the nutritional requirements of *C. vulgaris*. Thus, we added nitric and phosphoric acids to enhance microalgal growth and improve the nutritional status water used for fertigation (Gao et al., 2018).

The use of WW+A for castor plant irrigation is safe and supplies these plants with important macronutrients, including nitrogen, phosphorous, and potassium. In this connection, our study results agreed with Hussien et al. (2012), who concluded that the use of primary and secondary effluent for fertigation of castor oil plants improved their growth because it functioned as a natural conditioner that supplies plants with nutrients and organic matter that stimulated growth.

Oil content

Castor oil seeds had an oil content of 28.14% when plants were irrigated with WW and 25.12% when they were irrigated with WW-A ($P< 0.05$) (Fig. 1). These low oil contents were likely because of the consumption of nutrients by the microalgae. On the contrary, irrigation with WW+A led to the highest oil yield (41.84%) ($P< 0.05$). Microalgae support the growth of plants due to their excretion of certain bio-stimulators, such as phytohormones and amino acids, which improve the nutrient balance (Abdel-Maguid et al., 2004; Shaaban et al., 2010; Enan et al., 2016; El-Sayed et al., 2018). Field experiments by Reda (2017) reported slightly different results regarding total oil content and fatty acid profiles of castor oil, possibly due to different soil proprieties and other environmental conditions.

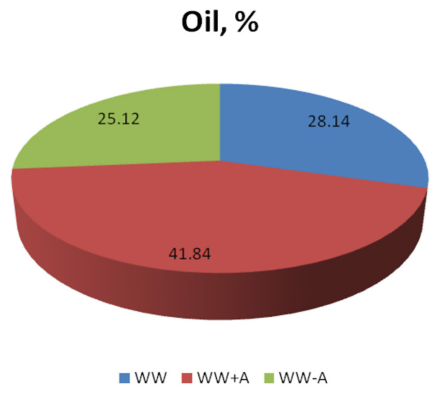

Fig. 1. Total seed oil content when castor oil plants were irrigated with different wastewaters.

The main effect of the WW+A treatment could be ascribed to the increased levels of organic matter, which contains all required nutrients, such as amino acids, organic acids, and growth promoters. In other words, microalgae increase the net seed yield and the total oil content (Öztürk, 2014). Furthermore, our data indicated that the increased seed oil content of plants irrigated with WW+A could be ascribed to increased nutrient availability and absorption from soils, as well as the improvements in soil properties and structure. Future development of the techniques described here may help to reduce the energy crisis by increasing the production of seed oils and simultaneously reducing water shortages. Wastewater can be used for irrigation and improves the quality of soil and thus provides regeneration of poor-quality soils. Importantly, wastewater management and production can be used without the need for external fertilization (Chandra et al., 2018).

Thus, we conclude that wastewater containing microalgae is sufficient to stimulate seed oil production in castor oil plants growing in poor soils, and that addition of external fertilizers is unnecessary. Treatment of wastewater by microalgae promotes plant growth, but may involve an additional cost of harvesting of these microalgae to obtain the treated water. We found that use of WW+A reduced these costs and also improved plant growth.

Fatty acids and biodiesel properties of castor seed oil

Results showed that carbon number of castor oil ranged between C16 and C18.Of these, palmitic acid (C16:0) was accounted by 1.43 to 194 % of total fatty acids and stearic acid (C18:0) represented 1.1 – 2.96 % by the same respect. The major FAME was ricinoleic acid (C18:1- OH), which accounted for 83.1 to 84.63% of the total FAME content (Table 3). It could be mentioned that algal presence affected oil content rather than fatty acid methyl ester fraction. Ricinoleic acid (C18:1-OH) is a long chain fatty acid, the type preferred for biodiesel production. Appropriate percentages of saturated and unsaturated fatty acids are essential when using oil as a biodiesel feedstock (Deng et al., 2011). For instance, the fatty acids produced by *Dunaliella* algae has high levels of palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids, and meet the requirements of European legislation for biodiesel. Results also claimed that castor oil seems to be rich in unsaturated fatty acids (>94%) making it promising in biodiesel production.

It could be mentioned that Plant oils with high oleic acid content have a good fuel balance, including ignition efficiency, combustion heat, cold filter plug point, oxidative stability, viscosity, and lubricity. The addition of oleic methyl ester may improve the oxidative stability and lower the melting temperature (Prabakaran & Ravindran, 2012). Our results indicated a considerable amount of oleic acid in castor oil seeds (>2.0%), making this oil suitable for the production of high-value biodiesel.

Furthermore, results also indicated the FAME profile of castor seed oil varied according to the type of wastewater used for irrigation, However, the fuel properties of all resulting castor oils were within the range approved by the American Society for Testing and Materials (ASTM) D6751 (2012) (Table 4).

TABLE 3. Fatty acid methyl ester (FAME) profiles of seed oils when castor oil plants were irrigated with different wastewaters.

Acid	Formula	Carbons	WW	WW+A	WW-A
Palmitic	$C_{16}H_{32}O_2$	C16:0	1.43 ± 0.16	1.94 ± 0.09^{a}	1.94 ± 0.11^{a}
Stearic	$C_{18}H_{36}O_2$	C18:0	1.1 ± 0.08	1.24 ± 0.07^{b}	2.96 ± 0.23^{a}
Oleic	$C_{18}H_{34}O_2$	C18:1	2.1 ± 0.96	2.86 ± 0.53^{a}	2.86 ± 0.66^{a}
Ricinoleic	$C_{18}H_{34}O_3$	C18:1	83.1 ± 8.73	84.6 ± 10.32^{a}	84.63 ± 9.65^{a}
Linoleic	$C_{18}H_{32}O_2$	C18:2	7.51 ± 0.95	5.85 ± 1.03^{b}	1.73 ± 0.56^{a}
α-Linolenic	$C_{18}H_{30}O_2$	C18:3	2.64 ± 0.87	2.63 ± 0.76^{n}	4.96 ± 0.93^{a}
	TSFA		2.53 ± 0.24	3.18 ± 0.15^{a}	4.9 ± 0.34^{a}
	MUSFA		85.2 ± 9.69	87.46 ± 10.85^{a}	87.49 ± 10.31^{b}
	TUSFA		95.35 ± 11.51	95.94 ± 12.64^{b}	94.18 ± 11.80^{b}
	PUFA		10.15 ± 1.82	8.48 ± 1.79^{a}	6.69 ± 1.49^{a}

- TSFA: Total saturated fatty acids; MUSFA, mono-unsaturated fatty acids; TUSFA, total unsaturated fatty acids; PUFA, poly-unsaturated fatty acids. Mean ± SE, n= 3.

- a: Very highly significance at P< 0.05; b: Highly significance at P< 0.05 and n: None significant. The data shown are means ± SD, (n = 3).

TABLE 4. Some castor oil characteristics as affected by different wastewater.

Treatment	SV	IV	CN	DU	LCSF	CEPP
WW	187	94.7	54.15	105.5	0.693	-14.2
WW+A	189	93.4	54.14	104.5	0.814	-13.9
WW-A	188	91.6	54.68	100.9	0.814	-13.9
EN 14214	---	110.63	51.33	----	---	−8.89
ASTM D6751	---	≤120	≥51	-----	--------	≤−5

SV, saponification value; IV, iodine value; CN, cetane number; DU, degree of unsaturation; LCSF, long-chain saturated factor; CFPP, cold filter plugging point ASTM D6751, American Society for Testing and Materials, biodiesel standard EN 14214, European standard for biodiesel quality.

In particular, we found that the seed oils produced by the 3 different wastewaters had similar saponification values (SVs), although there were differences in the iodine value (IV). Schenk et al. (2008) reported a very low oxidative potential for fatty acids in a ratio of 5:4:1 for C16:1, C18:1, and C14:0, and that other oils should be added to achieve this ratio to improve biofuel quality. The appropriate percentage of saturated and unsaturated fatty acids must be considered when using microalgal oils as a biodiesel feedstock (Deng et al., 2011). The addition of methyl oleate may improve the oxidative stability and lower the melting temperature (Prabakaran & Ravindran, 2012). Our results indicated the dominant FAME was ricinoleic acid (C18:1-OH), which accounted for 81.1 to 84.63% of total fatty acids.

The low IV in our oils (91.6–94.7) are compatible with EN 14214 (110.63) and ASTM D6751 (≤120) standards. The cetane number (CN) ranged from 54 to 56, close to the ASTM criteria. The CN is in indicator of self-ignition quality, knock characteristics, and combustibility of the resulting fuel. A fuel with a high CN may lead to incomplete combustion if the fuel ignites too soon, thus preventing the fuel to mix with air for complete combustion (Okechukwu et al., 2015).The degree of unsaturation (DU) varied slightly according to the type of wastewater used for irrigation, and was lowest in the WW-A group.

There are various specifications for biodiesel fuels, as approved by ASTM D6751, which are based on the composition and structure of the fatty acid esters (Mittelbach & Remschmidt, 2004). These specifications include CN, kinematic viscosity, oxidative stability, and cold-flow properties, such as cloud and pour points (Knothe, 2005, 2008; Ramos et al., 2009). Other chemical properties should also be considered, such as exhaust emissions, lubricity, and heat of combustion (Ramos et al., 2009), although current guidelines do not consider these. Knothe (2005) stated that alcohol-derived ester moieties may also affect biodiesel properties, such as CN, viscosity, and stability to oxidation.

Biodiesel Fuel Specification, Blend Stock (B100) for Middle Distillate Fuels Biodiesel from polyunsaturated fatty acids with 4 or more double bonds are more vulnerable to oxidation, emit more oxides of nitrogen, and have lower thermal efficiency from saturated fatty acids than biodiesels. Thus, microalgal oils with abundant polyunsaturated fatty acids are less acceptable for the production of biodiesel (Chisti, 2007).

Conclusion

The proprieties of castor seed oils varied slightly when plants were irrigated with different types of wastewater, but they were generally within the range of ASTM criteria. Our findings suggest that wastewater containing algae can be safely used for irrigation of soils with low fertility to simultaneously promote the production of castor seed oil and reclaim low-quality soils. Furthermore, microalgae affected the total oil content, rather than fatty acid profile.

Acknowledgment: This research work has been carried out as a part of activities of Algal Biotechnology Unit, National Research Centre, Dokki, Cairo, Egypt, (Prof. Dr. Abo El-Khair B. El-Sayed). The authors express thanks to all other stuff members.

Conflict of interests: The authors declare that they have no conflict of interest.

Authors contribution: All authors contributed equally to this manuscript.

Ethical approval: Not applicable.

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صوره الأحماض الدهنية وخصائص الوقود لنبات الخروع المروي بالطحالب النامية على المياه العادمة

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يمكن أن تعمل الطحالب الدقيقة كسماد ومرشح حيوي لمعالجة مياه الصرف، وبالتالي يمكن إعادة استخدام المياه المعالج لري نباتات تنتج زيوت حيوية وفيرة باستخدام تربة منخفضة الجودة. وعليه تم نمو طحلب الكلوريلا فلجارس باستخدام مياه صرف أم الريش كبيئة للنمو بعد إثرائها بالنيتروجين والفوسفوروتمت زراعة بذور الخروع في الصوب الزجاجية في وحدة بيو تكنولوجيا الطحالب، المركز القومي للبحوث، مصر. بعد 15 يوم، تم ري الشتلات ب: (أ) مياه صرف غير معالج WW، (ب) مياه صرف معالج يحتوي على الكتلة الحيوية للطحالبWW+A، (ج) مياه صرف معالج بعد إزالة الكتلة الحيوية للطحالبWW-A .تم استخلاص الزيت بتسخين البذور وطحنها مع مذيب الهكسان العادى متبوعًا بالنقع و الترشيح والتمرير من خلال سليكا جل 60. تم إجراء الاسترة وتحديد استر ميثيل الأحماض الدهنية. أوضحت النتائج أن محتوى الزيت تأثر بشكل ملحوظ بنوع المياه المستخدمة في الري. حيث أدى الري باستخدام WW+A إلى أعلى إنتاجية للزيت (41.8%)، تليها الريWW-A (28.14%) والري WW (25.12%) . أوضحت تحاليل الأحماض الدهنية أن الأحماض الدهنية الرئيسية كانت C16 وC18 وأن حمض الريسينوليك كان الأكثر شيوعًا (83.1 إلى 84.63%). بغض النظر عن ارتفاع محتوى الزيت في البذور في النباتات التي رويت ب WW + A ، كانت هناك اختلافات في خصائص الوقود المحسوبة لزيوت البذور في النوعين الاخرين، بناءً على معايير الجمعية الأمريكية للاختبار والمواد. تشير النتائج إلى أنه يمكن استخدام مياه الصرف المعالجة بهذه التقنية بنجاح لري التربة ذات الخصوبة المنخفضة لإنتاج الزيوت الحيوية.