Phytoremediation of samples extracted from wastewater treatment plant and their socioeconomic impact

Hayfa Rajhi, Anouar Bardi, Salwa Sadok, Mohamed Moussa and Saifeddine Turki

ABSTRACT

The physico-chemical and bacteriological quality was evaluated in wastewater samples before and after treatment by microalgae enrichment. Three types of wastewater samples – raw water, inlet water and outlet water – were taken directly from the wastewater treatment plant and subjected to microalgae enrichment culture during two months. The main objective of this work was to apply a phytoremediation process based on the use of compulsory microalgae treatment of wastewater from treatment plants compared to other secondary treatments. The biomass of microalgae was extracted to determine the concentrations of phenolic compounds, sugars and especially lipids, which can be subsequently transformed into biodiesel. As a result, the pH showed a significant increase after microalgae proliferation, with values ranging from 9.94 to 10.36. Bacterial community analysis before and after microalgae culture showed a clear shift in biomass content. The total coliform (TC) and the fecal coliform (FC) contents decreased after microalgae enrichment. In addition, the fecal streptococci (FS) and *Pseudomonas* present in the different wastewater samples completely disappeared after treatment. The applied phytoremediation process showed a drop until the disappearance of the contagious microbes – which present a very serious health risk – due to the release of the quinic acid. The quinic acid observed in the treated waters exceeded the content of 464.328 mg/L. This phenolic compound naturally produced during the process demonstrated a very effective antimicrobial power. However, a significant increment of 100% of phenol compound removal was observed after microalgae enrichment. The lipid content in the various studied samples appeared after microalgae culture. In addition, the heavy metals, namely cadmium and chromium, were completely eliminated after the treatment. Several socioeconomic advantages can be achieved by the use of this process, notably the environmental advantages of bioenergetics and economic and social benefits of the non-expensive valorization of wastewaters for irrigation.

Key words | economic and social benefits, microalgae, phytoremediation, wastewater

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HIGHLIGHTS

- The total coliforms (TC) as well the fecal coliforms (FC) contents were decreased. The fecal streptococci (FS) and *Pseudomonas* present in the different wastewater samples completely disappeared after microalgae enrichment.
- A significant increment of 100% for phenol compound removal was observed after microalgae enrichment. The heavy metal contents, namely cadmium and chromes, were completely eliminated after the microalgae enrichment.
- The lipid content in the various studied samples appeared after microalgae culture.
- This result confirms the role of phytoremediation played by microalgae: several socioeconomic advantages can be highlighted during the use of this process, such as

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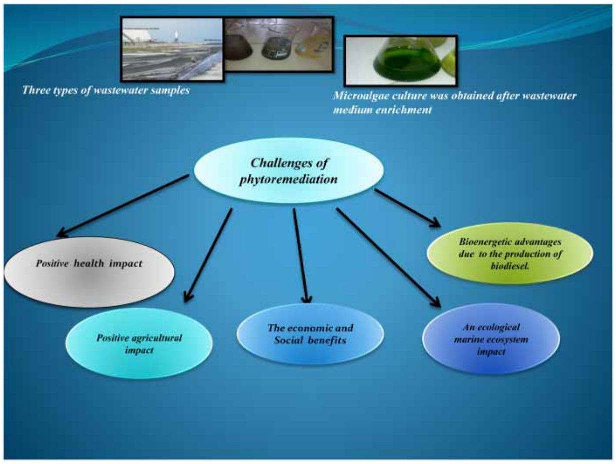
bioenergetics advantages, environmental advantages and an economic and social benefit.

GRAPHICAL ABSTRACT



INTRODUCTION

The depletion in fossil fuel reserves and global climate change have attracted more attention to the search for renewable energy sources. In 2016, the Paris Agreement was signed by more than 170 countries that were committed to implementing measures to reduce greenhouse gas emissions and to promote the use of renewable energies (World Energy Council 2016).

Wastewater treatment is necessary to reduce their contents of contaminants before release into the environment. Several methods, including chemical treatment and the conventional biological method are being used to remove nutrients such as phosphorus and nitrogen from wastewater (Ruzhitskaya & Gogina 2017). However, the high costs of the process and the resulting sludge are the main drawbacks that limit its use. Compared to conventional treatment methods, microalgae may be an alternative for removing nutrients from wastewater (Li *et al.* 2013; Ungureanu *et al.* 2019). In addition, this method helps to fix CO_2 by reducing the emissions of this gas, which is a major contributor to the greenhouse effect (Molazadeh *et al.* 2019). The use of microalgae or macroalgae (algae) for wastewater treatment is called phytoremediation (Wang *et al.* 2010). In fact, the microalgae have a great potential for treatment and could remove several nutrients from the water with greater efficiency than activated sludge (Falkowski & Raven 2007; Raouf *et al.* 2012). It therefore seems economically more attractive to use these microorganisms in secondary rather than tertiary treatment (Tam & Wong 1989). During the photosynthesis, microalgae absorb mineral nutrients such as potassium, sodium, calcium and magnesium, trace elements (molybdenum, zinc, copper) and CO_2 dissolved in water to produce their cellular constituents (Masojidek *et al.* 2004). Microalgae can accumulate, under certain growing conditions, fixed carbon, in the form of lipids called triglycerides. The stored lipids then constitute a carbon reserve for the microalgae. Under normal conditions, these levels remain low between 20 and 50%, and lipids are mainly composed of phospholipids and glycolipids (membrane components). However, some species are able to accumulate up to 80% of their dry weight in lipids (Chisti 2007). These lipids can easily be transformed into biodiesel (3rd generation biofuel) by transesterification (Brennan & Owende 2009). Phytoremediation was also used to decontaminate water charged with organic matter or various contaminants such as the hydrocarbon metal, the organochlorines and the heavy metals (Mata *et al.* 2010). In fact, treatment of wastewater by microalgae is environmentally sound and offers the benefit of a cost-effective means of nutrient removal and biomass production (Mulbry *et al.* 2008; Ungureanu *et al.* 2019).

The objective of this investigation was to study the physico-chemical and microbiological quality of wastewater before and after treatment with microalgae enrichment. Note, in this regard, that this work presents an environmental asset for the Gulf of Gabès region, which is characterized by a climate with very high humidity and salinity and is rich in luminous intensity (a continuous presence of fairly important light throughout the year). In this area, large amounts of partially treated household wastewaters loaded with organic matter are directly discharged into the sea. Also, industrial wastewaters are directly released into sea waters. In addition, the main objective of this work was to apply the phytoremediation process based on the use of compulsory microalgae treatment of wastewater from treatment plants compared to other secondary treatments. In fact, the use of the phytoremediation process as a compulsory treatment in treatment plants can achieve several advantages. (i) First of all, its positive health effect, particularly with regard to the non-proliferation of contagious microbial diseases, which can spread and constitute a threat of a serious pandemic crisis: the phytormediation process when applied can completely eradicate the contagious microbes, which present a very serious danger to public health. (ii) On the agricultural level, wastewater treated by the phytoremediation process can be used for the irrigation of agricultural soils, especially those which are poor in basic nutrients like nitrogen and phosphorus. They can constitute a major asset for the soil fertility and a key solution to the risk of chemical contamination of soils by heavy metals. (iii) In addition, a socio-economic benefit occurs as the reuse of treated water for irrigation can contribute to improving the economic situation of farmers by making significant savings on the price of chemical fertilizers while using the nutrient-loaded wastewater. Likewise, the reuse and development of the agricultural sector would encourage trade in agricultural irrigation equipment and installations in the communes. Regarding social benefits, it should be noted that irrigation would be beneficial for the municipality, since it is likely to create employment, limit rural migration to large cities and improve the quality of life of the commune. (iv) Furthermore, an ecological impact is obtained as the obligation to use this phytoremediation treatment can constitute the only adequate biological treatment that ensures the total removal of heavy metals and therefore would help to protect the marine ecosystem. (v) Finally, significant bioenergy advantages are achieved due to the fact that this phytormediation process can make it possible to obtain a large quantity of lipids useful for the production of biodiesel.

MATERIALS AND METHODS

Experimental setup

Three types of wastewater (raw water, inlet water and outlet water) were sampled directly from the wastewater treatment plant of Gabés. These waters were used as media for microalgae enrichment culture during two months at 25 °C. A light intensity of 100 W during 12 h alternated with 12 h obscurity were applied. Batch experiments were conducted in 250-mL glass Erlenmeyer flask reactors filled with 200 mL of medium. The culture was incubated statically: 3 reactors were flushed with CO_2:N_2 (80:20), gas was renewed weekly; the other three remained as controls. All tests were done in triplicate. The physico-chemical and biological parameters of these water samples (six samples), before and after treatment with microalgae culture were determined.

Sampling and meteorological parameters

Water samples were taken from the wastewater treatment plant located in the region of Gabès (Southern Tunisia, * Longitude: 10° 04′ 7.32″ E * Latitude: 33° 52′ 56.82″ N * Altitude: 21 m). The study zone is located in the southeast of Tunisia. This region has an arid Mediterranean climate with an annual average rainfall of 145.2 mm. Average monthly values of different meteorological factors were detailed by Msadki *et al.* (2017), including monthly temperature (°C), evaporation rate (mm per days), relative humidity (%), wind speed (m per second) and dominant wind direction. The annual temperature and precipitation are 18.56 °C and 12.1 mm per year, respectively. The maximal air humidity and wind speed were around 74.57% and 1.64 m per second, respectively, followed by 114.46° as a maximal value of a wind direction. Average global solar radiation was 207.1 (w/m²). The meteorological conditions described above can facilitate an algal culture that is efficient and natural.

The characteristics of the treatment plant were as follows: domestic nature raw water, the average daily and hourly flows were 17.260 m^3/d and 720 m^3/h, respectively. An equivalent population biological oxygen demand (BOD) of 150.000 PE is produced.

Physico-chemical analysis

Different physico-chemical parameters such as the BOD, the electrical conductivity (EC) and pH were performed

according to *Standard Methods for the Examination of Water and Wastewater*, 20th edition (APHA *et al.* 1998). Total nitrogen (TN) was determined by the Kjeldahl Stander method (APHA *et al.* 1997). Nitrate (NI) was measured using HACH 8037 (cadmium control method). This method consists of reducing nitrate to nitrite by metal cadmium. All reagents are combined into a powder called Nitra ver 5. The nitrate concentration was expressed in mg N/L and determined at a wavelength of 500 nm. A volume of 10 ml of sample was added with a capsule of HR Nitrate (Nitra Ver Nitrate Reagent). The phosphate was measured according to HACH 8178 (amino-acid method) adapted to the 'standard methods'. The orthophosphate concentration of PO_4 was determined at a wavelength of 530 nm. A 10 ml sample was added with a phosphate capsule RGT (phosVer 3 phosphate Reagent). Aluminum, iron, magnesium, cadmium, vanadium, manganese, chromium, and zinc were determined by atomic absorption spectrometry (Avanta, GBC spectrometer, Australia), using air and acetylene as the mode of oxidation.

Germination index evaluation

The phytotoxicity of the water samples was evaluated by the measurement of the germination index according to the method proposed by Zucconi *et al.* (1981). This technique consists of allowing to germinate, in a petri dish, 10 seeds of tomatoes in the different samples studied during one week in darkness at 25 °C. The Control ground is used as a positive control. After incubation, the reading of the result is determined by counting the number of germinated seeds. The length of the roots of the seeds having germinated is measured in mm. The germination index 'GI' is calculated according to the following formula:

$$GI(\%) = \frac{[No\ of\ seeds\ germinated * root\ lenght]}{[Control\ No\ of\ seeds\ germinated * control\ root\ lengthl]} * 100$$

Six wastewater samples (raw, inlet and outlet) before and after algae phytoremediation treatment were tested.

Phenol compounds analysis

High-performance liquid chromatography (HPLC 2010 Plus, Shimadzu) was used to quantify phenol compounds. The mobile phase was an equal mixture of (a) 0.1% of formic acid in water and (b) 0.1% of formic acid in methanol with a flow of 0.4 ml/min. The column TSK (TSK gel with a length and inner diameter of 30 cm and 7.8 mm, respectively) was used (Pérez *et al.* 1990; Kemal 1994). The polyphenols extraction was realized using methanol and the filtrate was stored at 4 °C.

Fatty acid analysis

Free fatty acids were analyzed with a Shimadzu gas chromatograph system (GC–MS GP2010 Ultra) adapted for capillary columns. For the analysis, a fused silica capillary column, 30 m × 0.25 mm × 0.25 μm film thickness was used. The injector and detector temperatures were set at 200 °C with interface of 220. The column temperature was set at 50 °C, then raised to 250 °C with a flow of 50 °C/min, then increased from 200 to 230 °C at the rate of 50 °C/min. Peak heights were determined by integration software (D'Annibale *et al.* 1998). The fat and fatty acid extraction was performed using hexane.

Microbiological analysis

Slantez and Bantley Medium were used to detect the Fecal streptococci at 37 °C during 24 hours. VRBL agar medium-was used to detect the total coliform and fecal coliforms at 44 and 37 °C during 24 hours, respectively. Ceftrimide agar (base) medium was used for *Pseudomonas* incubation in 37 °C during 24 hours.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Version 20.0) was used to perform the statistical analysis. The phenol, fatty acids and mineral compounds values of both samples (before and after treatment) were compared using Student's t test at the '5% ($P = 0.05$)' significance level. Data are presented as a mean standard deviation. Values were obtained from triplicate determinations and the differences were examined using one-way analysis of variance (ANOVA) followed by the Fisher's Least Significant Difference (LSD) post hoc test. Statistical significances of the correlations between datasets were calculated using Pearson's R values. At least three replicates were performed for each laboratory measurement

RESULTS

All the results obtained after analyses of six wastewater samples (raw, inlet and outlet) before and after algae

phytoremediation treatment were studied. A proliferation of microalgae was detected (Figure SI 1, Supplementary Information). No phytotoxicity effect was detected in the different wastewater samples. Therefore, the wastewater illustrated a good medium to support phytoremediation.

Physico-chemical proprieties

The physico-chemical properties of wastewater samples before and after algae phytoremediation are reported in Table 1. The pH of wastewater samples before microalgae culture enrichment was relatively neutral: the pH values varied from 7.68 to 7.93. However, the values showed a significant increase to the basic aspect after microalgae proliferation. Values varied from about 9.94 to 10.36. In fact, the microalgae tend to alkalize the culture medium. However, the values of wastewater electrical conductivity after microalgae enrichment culture treatment showed a slight decrease in the order of 4.52 ms/cm, 4.50 ms/cm and 4.54 ms/cm for raw water, inlet water and outlet water, respectively (Table 1). As for the total nitrogen, values displayed a very remarkable variation. However, the maximum value that was recorded corresponded to the outlet water and the raw water after treatment. Microalgae cultures have the ability to reduce the high levels of phosphorus present in the wastewater. The BOD values showed a failure for raw water and inlet water. In contrast, a negative BOD removal was observed with outlet water.

Bacteriological parameters

As observed in Table 2, bacterial community analysis before and after microalgae culture showed a clear shift in biomass content. The total coliforms (TC) as well the fecal coliforms (FC) content were decreased after microalgae enrichment. In addition, the fecal streptococci (FS) and *Pseudomonas* present in the different wastewater samples completely disappeared after microalgae enrichment.

Phenol compounds

Tables 3–5 summarize the phenol compound concentration found in wastewaters before and after microalgae enrichment. Comparative study of phenol compounds showed a significant difference in the concentration values (Tables 3–5). An important failure of phenol compound concentration rate as well as the efficient removal of a large number of phenol compounds was observed. A significant increment to 100% of phenol compound removal of the highest compound after microalgae enrichment was observed. The major phenol compounds were completely

Table 2 | Microbial analysis, total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), and *Pseudomonas* in the studied water samples

Samples	TC (10^3)	FC	FS	Pseu
R	$302 \pm 2^{\alpha\$@\#\pounds}$	$140 \pm 1^{\alpha\$@\#\pounds}$	$39 \pm 1^{\alpha\$@\#\pounds}$	$60 \pm 1^{\alpha\$@\#\pounds}$
RT	$188 \pm 2^{*\$@\#\pounds}$	$65 \pm 5^{*\$@\#\pounds}$	$0 \pm 0^{*\$\#}$	$0 \pm 0^{*\$\#}$
E	$31 \pm 1^{*\alpha@\#\pounds}$	$36 \pm 3^{*\alpha@\#\pounds}$	$8 \pm 1^{*\alpha@\#\pounds}$	$8 \pm 1^{*\alpha@\#\pounds}$
ET	$0 \pm 0^{*\alpha\$\#\pounds}$	$0 \pm 0^{*\alpha\$\#\pounds}$	$0 \pm 0^{*\$\#}$	$0 \pm 0^{*\$\#}$
EX	$310 \pm 1^{*\alpha\$@\pounds}$	$50 \pm 1^{*\alpha\$@\pounds}$	$5 \pm 1^{*\alpha\$@\pounds}$	$17 \pm 1^{*\alpha\$@\pounds}$
EXT	$56 \pm 5^{*\alpha\$@\#}$	$40 \pm 1^{*\alpha@\#}$	$0 \pm 0^{*\$\#}$	$0 \pm 0^{*\$\#}$

Data are presented as average ± SD. Values were obtained from triplicate determinations and statistical significance was examined by one-way analysis of variance (ANOVA) followed by the Fisher's LSD (Least Significant Difference) *post hoc* test. $^{*}p < 0.001$ as compared to R; $^{\alpha}p < 0.01$ as compared to RT; $^{\$}p < 0.001$ as compared to E; $^{@}p < 0.001$ as compared to ET; $^{\#}p < 0.001$ as compared to EX; $^{\pounds}p < 0.001$ as compared to EXT.

Table 1 | Physical-chemical and biological parameters: pH, electric conductivity (EC), total nitrogen (TN), nitrate (NI), biological oxygen demand (BOD) in the studied water samples. Raw water before treatment (R) and after treatments (RT); the inlet water before treatment (E) and after treatments (ET); the outlet water before treatment (Ex) and after treatments (EXT)

Samples	pH	EC	TN	NI	P	BOD
R	$7.6 \pm 0.1^{\alpha@\pounds}$	$4.75 \pm 0.05^{\alpha\$@\#\pounds}$	$0.14 \pm 0.01^{\alpha\$@\#\pounds}$	$16.79 \pm 0.01^{\alpha@\#\pounds}$	$21.63 \pm 0.03^{\alpha\$@\#\pounds}$	$251 \pm 0.5^{\alpha\$@\#\pounds}$
RT	$9.76 \pm 0.31^{*\$@\#\pounds}$	$4.55 \pm 0.05^{*\$@\#\pounds}$	$37.82 \pm 0.06^{*\$@\#\pounds}$	$452.33 \pm 59.5^{*\$\#\pounds}$	$10.04 \pm 0.1^{*\$@\#\pounds}$	$202 \pm 1^{*\$@\#\pounds}$
E	$7.66 \pm 0.033^{\alpha@\pounds}$	$4.69 \pm 0.01^{*\alpha@\#\pounds}$	$2.37 \pm 0.025^{*\alpha@\#\pounds}$	$25.1 \pm 0.1^{\alpha@\pounds}$	$36.6 \pm 0.26^{*\alpha@\#\pounds}$	$170 \pm 1^{*\alpha@\#\pounds}$
ET	$10.14 \pm 0.033^{*\alpha\$\#}$	$4.48 \pm 0.03^{*\alpha\$\#}$	$1.96 \pm 0.02^{*\alpha\$\#\pounds}$	$429.66 \pm 1.52^{*\$\#\pounds}$	$101.1 \pm 0.1^{*\alpha\$\#\pounds}$	$0 \pm 0^{*\alpha\$\#\pounds}$
EX	$7.87 \pm 0.068^{\alpha@\pounds}$	$4.84 \pm 0.04^{*\alpha\$@\pounds}$	$2.51 \pm 0.01^{*\alpha\$@\pounds}$	$9.43 \pm 0.05^{\alpha\$@\pounds}$	$29.3 \pm 0.1^{*\alpha\$@\pounds}$	$162.5 \pm 0.5^{*\alpha\$@\pounds}$
EXT	$10.35 \pm 0.055^{*\alpha\$\#}$	$4.53 \pm 0.01^{*\$\#}$	$28 \pm 0.1^{*\alpha\$@\#}$	$320 \pm 0.57^{*\alpha\$@\#}$	$28.8 \pm 0.1^{*\alpha\$@\#}$	$257 \pm 1^{*\alpha\$@\#}$

Data are presented as average ± SD. Values were obtained from triplicate determinations and statistical significance was examined by one-way analysis of variance (ANOVA) followed by the Fisher's LSD (Least Significant Difference) *post hoc* test. $^{*}p < 0.001$ as compared to R; $^{\alpha}p < 0.01$ as compared to RT; $^{\$}p < 0.001$ as compared to E; $^{@}p < 0.001$ as compared to ET; $^{\#}p < 0.001$ as compared to EX; $^{\pounds}p < 0.001$ as compared to EXT.

Table 3 | Phenolic compounds behaviour of raw water before (R) and after treatments (RT)

Phenol compounds	R (mg/l^{-1})	RT (mg/l^{-1})	Removal (%)
Gallic acid	0.456[a]	0.000[b]	100
Protocatchuic acid	0.726[a]	0.000[b]	100
1,3-di-O-caffeoyquinic acid	0.003[a]	0.000[b]	100
Hyperoside (quercetin-3-o-galactosid)	0.019[a]	0.000[b]	100
Apegenin-7-o-glucoside	0.001[a]	0.000[b]	100
4,5-di-O-caffeoyquinic acid	0.0016[a]	0.000[b]	100
Cirsiliol	0.074[a]	0.000[b]	100
Quinic acid	0.000[a]	48.881[b]	–
p-coumaric acid	0.000[a]	0.714[b]	–

Student's t test was determined with R and RT ($p = 0.01$, $*p < 0.05$). Mean score in the same line with a different letter ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).
Reduction of single phenolic compound is represented in removal percent.

Table 4 | Phenolic compounds behaviour of inlet water station before (E) and after treatments (ET)

Phenol compounds	E (mg/l^{-1})	ET (mg/l^{-1})	Removal (%)
Hyperoside (quercetin-3-o-galactoside)	0.011[a]	0.000[b]	100
Apegenin-7-o-glucoside	0.002[a]	0.000[b]	100
4,5-di-O-caffeoyquinic acid	0.016[a]	0.000[b]	100
Cirsiliol	0.2[a]	0.000[b]	100
Quinic acid	0.000[a]	71.827[b]	–
Rutin	0.000[a]	0.004[b]	–
3,4-di-O-caffeoyquinic acid	0.000[a]	0.027[b]	

Student's t test was determined with E and ET ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).
Reduction of single phenolic compound is represented in removal percent.

Table 5 | Phenolic compounds behaviour of outlet water station before (EX) and after treatments (EXT)

Phenol compounds	EX (mg/l^{-1})	EXT (mg/l^{-1})	Removal (%)
Hyperoside (quercetin-3-o-galactoside)	0.004	0.000[b]	100
Cirsiliol	0.218	0.000[b]	100
Quinic acid	0.000[a]	464.328[b]	–

Student's t test was determined with Ex and EXT ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared for the same parameters in the two different water samples (before and after treatment).
Reduction of single phenolic compound is represented in removal percent.

removed. However, we note the appearance of quinic acid in the different treated waters.

Lipid analysis

The comparison of fatty acids between the different wastewater samples showed a significant difference in the fatty acid concentration ($p = 0.01$). Lipid extraction analyses of the wastewater samples (raw, inlet and outlet), before and after treatment with microalgae showed that the three types of water before treatment were devoid of lipid constituents. However, the lipid content in the various studied samples appeared after microalgae culture. For raw water-that was rich in lipids, it contained six types of acids at different rates (Table 6). In addition, in the input water, the results of the lipid contents analysis showed a remarkable increase in lipid content after microalgae enrichment (Table 7). Indeed, more than 12 types of lipid constituents were detected after microalgae phytoremediation treatment of inlet water. Notably, no fatty acids were detected in treated outlet water.

Mineral content

The mineral analyses were carried out to determine the mineral and heavy metal contents in the wastewater samples before and after microalgae treatment (Tables 8–10). It was found that the magnesium content was very high in all wastewater samples before their treatment by microalgae enrichment. However, this content was completely reduced after microalgae treatment. Similarly, the contents of aluminum, iron and manganese were very low after microalgae culture treatment, which showed the assimilation of these different minerals by microalgae. In addition, the heavy

Table 6 | Fatty acid concentration rate water studied samples, fatty acid concentration rate behaviour of raw water before (R) and after treatments (RT)

Fatty acids	R(%)	RT(%)
Tetradecanoic acid, Methyl ester	0.000[a]	5.591[b]
Hexadecanoic acid, Methyl ester	0.000[a]	30.42[b]
9-Hexadecenoic acid, Methyl ester, (Z)-	0.000[a]	5.192[b]
Octadecanoic acid, Methyl ester	0.000[a]	19.679[b]
9-Octadecenoic acid, Methyl ester, (Z)-	0.000[a]	29.658[b]
9,12-Octadecadienoic acid (Z,Z)-, Methyl ester	0.000[a]	9.459[b]

Student's t test was determined with R and RT ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).

Table 7 | Fatty acid concentration rate water studied samples, fatty acid concentration rate behaviour of inlet water before (E) and after treatments (ET)

Fatty acids	E(%)	ET(%)
Decanoic acid, Methyl ester	0.000[a]	0.159
Dodecanoic acid, methyl ester	0.000[a]	0.217
Tetradecanoic acid, Methyl ester	0.000[a]	4.918
Pentadecanoic acid, Methyl ester	0.000[a]	0.771
Hexadecanoic acid, Methyl ester	0.000[a]	26.666
9-Hexadecenoic acid, Methyl ester, (Z)-	0.000[a]	4.639
Heptadecanoic acid, Methyl ester	0.000[a]	1.381
cis-10 heptadecenoic acid, Methyl ester	0.000[a]	0.924
Octadecanoic acid, Methyl ester	0.000[a]	16.771
9-Octadecenoic acid, Methyl ester, (E)-	0.000[a]	1.09
9-Octadecenoic acid (Z)-, Methyl ester	0.000[a]	35.084
9,12-Octadecadienoic acid (Z,Z)-, Methyl ester	0.000[a]	4.675
9,12,15-Octadecatrienoic acid, Methyl ester, (Z,Z,Z)	0.000[a]	1.454
Eicosanoic acid, Methyl ester	0.000[a]	0.323
5,8,11,14-Eicosatetraenoic acid, Methyl ester, (ALL-Z)	0.000[a]	0.924

Student's t test was determined with E and ET ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).

Table 8 | Mineral concentration rate behaviour of raw water before (R) and after treatments (RT)

Mineral compounds	R (ppm)	RT (ppm)	Removal (%)
Al	1.18[a]	1.14[b]	3.38
Fe	0.79[a]	0.11[b]	86.07
Mg	148[a]	133[b]	10.13
Cd	0.04[a]	0.01[b]	75
Cr	2.9[a]	0.01[b]	99.65
Mn	0.15[a]	0.06[b]	60
V	0.04[a]	0.000[b]	100
Zn	0.01[a]	0.000[b]	100

Student's t test was determined with R and RT ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).
Reduction of single mineral compound is represented in removal percent.

metal contents, namely cadmium and chromium, were completely eliminated after the microalgae enrichment (Tables 8–10), the removal rate reached 100%. This result confirms the role of phytoremediation played by microalgae.

Table 9 | Mineral concentration rate behaviour of inlet water before (E) and after treatment (ET)

Mineral compounds	E (ppm)	ET (ppm)	Removal (%)
Al	1.7[a]	1.13[b]	33.52
Fe	0.64[a]	0.03[b]	95.31
Mg	123[a]	115[b]	6.5
Cd	0.03[a]	0.01[b]	66.66
Cr	0.03[a]	0.01[b]	66.66
Mn	0.06[a]	0.02[b]	66.66
V	0.02[a]	0.01[b]	50
Zn	1.7[a]	1.13[b]	33.52

Student's t test was determined with E and ET ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).
Reduction of single mineral compound is represented in removal percent.

Table 10 | Mineral concentration rate behaviour of outlet water before (Ex) and after treatment (ExT)

Mineral compounds	E (ppm)	ET (ppm)	Removal (%)
Al	1.39[a]	1.1[b]	20.86
Fe	0.15[a]	0.02[b]	86.66
Mg	112[a]	111[b]	0.89
Cd	0.04[a]	0.02[b]	50
Cr	0.01[a]	0.000[b]	100
Mn	0.01[a]	0.000[b]	100
V	0.000[a]	0.000[b]	0.000
Zn	0.000[a]	0.000[b]	0.000

Student's t test was determined with Ex and EXT ($p = 0.01$, $*p < 0.05$). Mean score in the same line with different letters ('a' and 'b') are significantly different (for $p < 0.05$). The result is compared to the same parameters in the two different water samples (before and after treatment).
Reduction of single mineral compound is represented in removal percent.

DISCUSSION

The decline observed in electrical conductivity (Table 1) can be due to the salt contents decreasing in the water samples. The minerals and heavy metals removal was due to their consumption by microalgae during the phytoremediation process (Traveiso *et al.* 1999). The increase of nitrate content after microalgae enrichment can be explained by the nitrification process in the reactors treated with phytoremediation: the role of microalgae was particularly important for the elimination of aerobic ammonia, because they provide sufficient oxygen levels to justify a complete bacterial nitrification. In addition, a previous work showed that a few microalgae

were capable of a significantly higher rate of nitrate medium uptake (Taziki *et al.* 2015). In addition, our experiments in small batches limited the proliferation of anaerobic bacteria, which were capable of assimilating the nitrate to nitrogen gas (Taziki *et al.* 2015). The detection of microorganisms such as TC/FC/FS/Pseu with higher content, especially in the outlet wastewater, confirmed a bacterial contamination in the studied plant. In fact, the rate of microorganisms mentioned above was not confirmed to the standard norms (ISO 5667-15:2009). Therefore, we note that microorganisms such as fecal streptococci (FS) and *Pseudomonas* disappeared completely after microalgae enrichment. As illustrated in Table 2, the bacterial mesophilic community present in wastewater treatment plant disappeared completely after microalgae culture, even though there was an available substrate. Notably, competing species or new species such as microalgae appeared (Figure SI 1).

Above all, the microalgae community played the role of phytoremediation. In fact, the pH increment could be justified by the microalgae proliferation. It is well known that microalgae are more able to make the pH alkaline than other microorganisms (Maloney 2007). In addition, the failure of bacterial content of CF and TC and the disappearance of certain microorganisms such as fecal streptococci and *Pseudomonas* after phytormediation can be explained by an antibacterial effect caused by the quinic acid. Note in this regard that the quinic acid detected after microalgae phytoremediation was already produced from microalgae. It is well known that quinic acid is extracted from algae and plant sources (Santos *et al.* 2011) and has an important antibacterial effect (Gohari *et al.* 2010). The appearance of the lipid constituents only after microalgae treatment highlighted the importance of the process applied in this research, in particular in the usefulness of this high lipid content in the production of biodiesel later. In fact, triglycerides, which are esters formed by the reactions of three free fatty acids and the trihydric alcohol, glycerol were used in the transesterification process (Chisti 2007; Cadoret & Bernard 2008) as expressed by the following Equation (1).

$$Triglycerides + Methanol \rightarrow Methyl\ esters + glycerol.$$

Equation (1) of transesterification reaction.

The most relevant correlations are shown in Table SI 1 (Supplementary Information). The pH was positively correlated with total nitrogen ($p = 627^{**}$) and nitrate ($p = 928^{**}$). In fact, the nitrogen compounds accumulated in the culture medium can justify the high pH. Previous studies suggested that biological nitrification processes can increase the pH of the medium (Amatya *et al.* 2013). TC was negatively correlated with pH ($p = -516$) and with the P content ($p = -595^{*}$). These medium factors such as pH and P can limit the TC growth. However, significant correlations between FC/TC ($p = 732^{**}$), FC/SF ($p = 833^{**}$), TC/STR ($p = 499^{**}$), FC/FS ($p = 833^{**}$), Pseu/TC ($p = 654^{*}$), Pseu/FC ($p = 843^{**}$), Pseu/SF ($p = 971^{**}$) were observed. These plead in favor of the hypothesis that these microorganisms coexist in the same microhabitat. Iron has a positive correlation with the microorganisms such as FC, FS and Pseu. In fact, we found the following positive correlations: Fe/FC ($p = 502^{*}$), Fe/STR ($p = 608^{*}$) and Fe/Pseu ($p = 530^{*}$). The iron is a micronutrient and cofactor ubiquitous for many bacteria (Py & Barras 2014). Different correlations were reported between the microorganisms such as TC, FC, FS and Pseu with the iron nutriments and mineral nutriment such as Cr, Mg and V the Mg. In fact, we noted the following correlations: Mg/FC ($p = 638^{**}$), Mg/FS ($p = 595^{**}$), the Cr: Cr/FC ($p = 774^{**}$), Cr/FS ($p = 721^{**}$), Cr/Pseu ($p = 692^{**}$), V/FC ($p = 519^{*}$), V/FS ($p = 653^{*}$), V/Pseu ($p = 592^{*}$), Zn/TC ($p = 550^{*}$) Zn/FC ($p = 793^{**}$), Zn/FS ($p = 743^{**}$) and Zn/Pseu ($p = 730^{**}$). These positive correlations quoted above between the microorganisms and the iron nutriments and mineral nutriment were indispensable and can constitute a cofactor of bacteria growth (Samaras *et al.* 2012). However, the free fatty acid level was affected by different parameters. In fact, the BOD, iron and cadmium can decrease the lipid production. We found a negative correlation between FAN/BOD ($p = -575^{*}$), FAN/Fe ($p = -484^{*}$) and FAN/CD ($p = -750^{**}$). In fact, a decrease of BOD to lipid oxidation occurred (Eymard 2003). However, the heavy metals Cd and Fe were identified in previous work as an inhibitor in fatty acid production (Jones 1986). In contrast, the P was a key factor to the fatty acids production. A positive correlation was found with FAN and P ($p = 484^{*}$). The P was implicated in the pentose phosphate pathway in fatty acid production. (Kruger & Schaewen 2003).

Challenges of phytoremedaition

Assessment of the socio-economic impact of microalgae phytoremediation on wastewater. The evaluation of the effect of microalgae phytoremediation on wastewater must focus on three major areas, namely: an agricultural impact, an environmental impact and a socio-economic impact.

Bioenergetics advantages: valorization of fatty acids produced by phytoremediation in biodiesel. The water treated

by phytoremediation showed a very high content of lipid constituents. In fact, the lipid compounds were obtained only after the water treatment by phytoremediation, which highlights the importance of this process to produce a high lipid content in the production of biodiesel (a 3rd generation biofuel). In addition, no fatty acid was detected in the outlet wastewater already treated in the treatment plant. In fact, microalgae have more processing potential than expected and can remove many nutrients from the water, with greater efficiency than conventional wastewater treatment. From an economic point of view, it seems more interesting to use these microorganisms in a secondary treatment rather than a tertiary one. In particular, the energy cost of electricity supplied and usable during secondary treatment in a station can be replaced by the energy produced by the biodiesel (IRENA 2015).

Environmental and agricultural benefits. The purified water already treated by phyotoremediation was free from heavy metals and rich in phosphorus and nitrogen, which constitutes a good irrigation substrate in agriculture and can bring out several positive impacts on the environment, on the economy and on society.

Environmental benefits. The treatment of wastewater by the phytoremediation process can reduce the environmental impact of discharges of excessively loaded polluted wastewater in the Gulf of Gabès. Similarly, this treatment can contribute to improving the quality of bathing water and to the regeneration of the marine ecosystem of the Gulf of Gabès. We can observe a clear fall in the content of minerals and heavy metals including cadmium and chromium, which are completely eliminated after the application of this process. In fact, the elimination rate reached 100%. Again, we observed that the objective of phytoremediation was successfully achieved. In fact, the treatment of wastewater with microalgae is eco-friendly and offers a cost-effective way of eliminating nutrients and producing biomass (Amenorfenyo *et al.* 2019). In particular, the environmental advantages characterizing the region of the Gulf of Gabès, namely a climate with very high humidity and richness in light intensity (a fairly significant light and whose presence was continuous throughout the year). Likewise, the Gabès region has long suffered from a large amount of polluted water heavily loaded with organic matter and which comes from the discharge of partially treated wastewater directly into the sea as well as chemical waste such as phosphogypsum (El kateb *et al.* 2018), which is discharged in large quantities in marine waters. This requires the large-scale recycling of waste from the region, with the resulting environmental and energy benefits. The experimental batch research already carried out in this study was very close to the natural meteorological parameters of the region defined by an annual temperature of 18.56 °C per year and an average global solar radiation of 207.1 (w/m²). Recalling that the experimental batch parameters are defined by a temperature of 25 ° C and a light intensity of 100 W, regarding CO_2 and P, these were the key factors in the production of FAN/P fatty acids (positive correlation $p = 484^*$). These are already involved in the pathway of pentose phosphate towards the production of fatty acids and CO_2 emanating from the industrial zone, which is a few meters from the wastewater treatment station. In fact, the strategic conditions described above can facilitate a very effective and large-scale natural treatment.

Advantages to agriculture. Likewise, the reuse of wastewater well treated by phytoremediation and which is devoid of any chemical contamination (heavy metal content), organic contamination (phenolic compounds) and biological contamination (pathogenic flora) is able to ensure the protection of water resources by reducing water withdrawals from the aquifer, and by making water more available for agricultural purposes at the local level. As a result, this could improve the negative balance caused mainly by excessive water withdrawals during irrigation, while water supplies remain insufficient. In addition, analyses of purified water indicated a nutritional value for agricultural land and plants because of the presence of significant quantities of nitrogen and phosphorus in the final effluent (Belaid *et al.* 2010). Indeed, this could reduce the potential risk of contamination of water bodies vulnerable to the use of mineral fertilizers in agriculture and even improve and preserve the quality of agricultural soil (regulation for water reuse 2020/741).

The economic benefits. The reuse of treated water for irrigation would help improve the economic situation among farmers. In fact, the availability of water would allow farmers to avoid the risk of drought, which often affects the harvest, for what is known as "classic" agriculture based mainly on precipitation, particularly in the arid regions. Another advantage is related to the costs associated with the use of fertilizers. In fact, environmental and economic analyses have shown that this reuse would allow farmers to make significant savings by replacing the purchase of fertilizer by reuse of water, since the latter represent the same agronomic value as that of agricultural amendments (Dany

et al. 2019). Likewise, the reuse and development of the agricultural sector would encourage trade in agricultural irrigation equipment and installations in the commune. According to empirical studies carried out on the irrigation of agricultural land in Tunisia (Neubert & Benabdallah 2003), the use of wastewater treated by chemical processes has a cost which rises to $70–105/ha. This is an advantageous average value for the different types of crops compared to the conventional irrigation type. Farmers using this technique must, due to market gardening restrictions, look for other economically more optimal production plans to produce lower cost crops due to low irrigation load. These costs relating to the use of wastewater treated by chemical processes, namely the technique of irradiation with UV rays and the membrane technique, remain relatively high compared to other biological processes, in this case phytoremediation. Indeed, according to the study by Kibi *et al.* (2000), the cost of treating wastewater by biological processes is one of the cheapest cost category chains. These encouraging results are explained by the fact that phytoremediation of wastewater by microalgae is a technology that adapts better to climatic, geological and socio-economic contexts. It is a cleaner treatment process that can be adopted in lagoon treatment plants.

The impact on health. In terms of health, there has been a significant decrease in the rates of total and fecal germs and the disappearance of pathogenic bacteria. In this regard, it should be noted that the detection of these microorganisms was at a high content, especially in the wastewater already treated in the treatment plant (outlet water), reflecting biological contamination of the station studied. In fact, the wastewater treatment was not efficient and did not comply with the standards and norms. The suggested process can be an effective secondary treatment alternative. Likewise, 100% effective removal of a large number of phenolic compounds (which are toxic and difficult to degrade) after the enrichment of microalgae has been observed. However, the appearance of quinic acid after phytoremediation has a very effective antibacterial effect on pathogenic bacteria (Guil-Guerrero *et al.* 2016), which are easily eliminated from wastewater.

Social benefits. Regarding social benefits, it should be noted that irrigation would be beneficial for the municipality, since it is likely to create employment, limit rural migration to large cities and improve the quality of life of the commune. In fact, it can be expected that the development of irrigation and the increase in agricultural yields would positively influence the standard of living of the population in a commune made up mainly of farmers. This is all the more important since at the macroeconomic level agricultural activity is considered in Tunisia as one of the main engines of growth and economic development, both in terms of GDP formation and in terms of job creation.

An annual contribution of around 15–20% in the national GDP is expected, whereas estimates count on approximately 3–4 million workers to be concerned by these rural agricultural activities and among which 60 100,000–100,000 new jobs can be created in the agrifood sector (FAO 2017). Likewise, a health impact has a significant social impact that can manifest itself by eliminating the spread of contagious diseases. These diseases are of microbial origin and are generally caused by biological contamination in the treatment plant. Note in this regard that the treatment plant studied was located in the vicinity of local communities. A valuation of algal biomass can possibly offer jobs to a local workforce in search of employment in particular through the use of algal biomass after drying in cattle and compost industrial manufacturing.

CONCLUSION

According to results, we noticed a decrease in the quantity of mineral salts and phosphorus in the treated wastewater. These elements were assimilated by microalgae during their growth. In addition, the heavy metal contents were completely eliminated after the enrichment of the microalgae culture. This result is the role of phytoremediation played by microalgae. Likewise, there was a very significant fall in coliforms, fecal streptococci and *Pseudomonas*. This is explained by the antibacterial power of microalgae, marked by the increase in content of phenolic compounds, especially quinic acid. A remarkable increase in lipid contents after treatment was also recorded. Indeed, more than 12 types of lipid constituents were detected after treatment. Therefore, the increase in lipid constituents has highlighted the importance of the process applied in this research, especially as that can be intended for biodiesel manufacture. In addition, several socioeconomic advantages can be highlighted during the use of this process, which consists of comparing the physicochemical and microbiological quality of wastewater before and after a process of phytoremediation by microalgae, namely: (I) bioenergetics advantages: valorization of fatty acids produced by phytoremediation in biodiesel production and (II) advantages to agriculture. Likewise, the reuse of wastewater well treated by

phytoremediation. (III) The economic benefits: the reuse of treated water for irrigation would help improve the economic situation among farmers and (IV) social benefits: it should be noted that irrigation would be beneficial to the municipality, since it is likely to create employment, limit rural migration to large cities and improve the quality of life of the commune.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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