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Wastewater valorization adopting the microalgae accelerated growth

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ABSTRACT

Microalgal biomass cultivation as a byproduct of wastewater treatment represents an interesting opportunity for wastewater valorization. Several studies analyzed the growth of microalgae in urban and agricultural wastewaters, evaluating the potential of microalgae strains to remove organic pollutants. To assess the actual environmental impact of such an integrated system, life cycle assessment (LCA) provides the proper tools for a comprehensive and effective analysis. In this study, olive mill wastewaters (OMW) are chosen and the selected microalgal strains are *Chlrorella vulgaris* and *Scenedesmus quadricauda*. Technical activities were carried out to obtain, starting from OMW, a cultivation medium with the same composition of the synthetic substrate (BG 11) used to grow the selected microalgal strains. Then, by means of LCA, a comparison between the environmental burden of the different scenarios was performed. Particular attention was devoted to the environmental indicators and a sensitivity analysis was performed to account for the transportation of OMW from olive mills to a centralized OMW treatment plant. The results show that the wastewater valorization can bring about an environmental benefit if the treatment plant is properly located. This is largely due to the avoided impact of the OMW purification treatment.

Keywords: Olive mill wastewater; Microalgae; Wastewater valorization

1. Introduction

Recently, some new and innovative wastewater treatment processes are emerging based on the exploitation of microalgae which allows for economic and environmental benefits. A number of studies have been conducted worldwide in order to develop a more economical mass cultivation method, making microalgal biomass production more attractive. An interesting opportunity is given by microalgal biomass cultivation as a byproduct of wastewater treatment, that has been long time promoted [1], using low-quality water as a nutrient solution containing organic carbon and inorganic nitrogen (N) and phosphorus (P). Such treatment is based on the interaction between the microalgae and the aerobic bacteria responsible for "digesting" the organic matter present in the sewage.

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By means of photosynthesis, microalgae are able to consume part of the nutrients (e.g. N and P) present in the wastewater stream, to fix carbon dioxide (CO_2), and to increase the oxygen (O_2) level in the water. At the same time, the metabolic respiration of the bacteria consumes O_2 and produces CO_2 [1].

Many species of microalgae are able to grow in wastewater, e.g. *Chlorella* sp., *Scenedesmus obliquus*, *Selenastrum capricornutum*, and *Ochromonas danica*, that are also able to degrade a variety of xenobiotics [2], including polyaromatic hydrocarbons [3], phenolic compounds [4], and pesticides [5]. Several studies have analyzed the growth of microalgae in urban and agricultural wastewaters because these wastes are more available and uniform in composition than the variable constituents of other wastewaters, e.g. agro-industrial [2,6–10] and treated landfill leachate [11]. These studies have evaluated the potential of microalgae strains to remove N and P. Unicellular chlorophytic microalgae showed to be particularly tolerant to many wastewater and growing conditions and they have a high nutrient/pollutant accumulation [12–14]. *Chlorella* and *Scenedesmus* genera are particularly tolerant to sewage effluent conditions; therefore, most studies have examined the growth of these two microalgae genera [14–18]. Moreover, microalgae biomass represents one of the most attractive and innovative feedstock source and they are characterized by high growth rate, high lipids/carbohydrates content, CO_2 sequestration capacity, and limited land use [19]. To assess the actual environmental impact of such an integrated system, life cycle assessment (LCA) provides the proper tools for a comprehensive and effective analysis. Furthermore, LCA allows evaluating the environmental credits deriving from byproducts valorization, i.e. the savings coming from the integration of the growth of microalgae together with the exploitation of integrated energy systems.

The aim of this work was to identify the most suitable microalgal strains for the treatment of a selected wastewater and to assess the environmental performance of the whole system.

2. Methods

For the selection of the most suitable wastewater, the Mediterranean basin, and in particular Sicily, was taken as the case study. Olive oil production represents a relevant agro-food industry in this area. In fact, around 97% of the world's olive oil production (ca. 3 million of tonnes in 2011) is located in the countries facing the Mediterranean Sea, among which Spain, Italy, and Greece play the lion's share [20]. One of the main olive oil production wastes is represented by the olive mill wastewater (OMW) derived both by traditional (pressure) or most recent (centrifugation) oil extraction methods. The OMW is characterized by a high pollutant load, due to the presence of not readily biodegradable organic compounds. In particular, phenolic compounds represent a major hazard because of their significant phytotoxic effect. Thus, if OMW is released into the environment without the adoption of good agronomic practices, it can be harmful, for example to crops [21].

OMW consists of the water contained in the drupe, the water used to wash olives and treatment plants and, in three stages continuous plants, also of the water used for dilution of olive paste. The held water of the olives amounts to 40–50% by weight of the drupe, the washing water corresponds to about 5% of the weight of the olives processed, while the cleaning water of the plants represents the 5–10%. Therefore, the OMW produced in the process of traditional extraction (discontinuous pressure) corresponds to 50–65% of the weight of the drupe machined. In the three-stage continuous process, the water necessary to fluidify the olive paste during the centrifugal extraction also has to be considered. This water is used to facilitate the outflow of the oil and causes an increase in the wastewater production up to 90–120% [22].

In order to use microalgae to depurate OMW, the first step is the identification of microalgal strains which allow the reduction of the polyphenols content in OMW. In particular, two green microalgae, *Ankistrodesmus braunii* and *Scenedesmus quadricauda*, are reported to be able to remove, in five days, more than the 50% of polyphenols from diluted OMW, starting from an initial content of $0.4\,g\,l^{-1}$ [8] or $1.5\,g\,l^{-1}$ [9]. Cicci et al. [22] grew *Scenedesmus dimorphus* in ultrafiltration pre-treated OMW with an initial polyphenols content of about $1.3\,g\,l^{-1}$: they reported a reduction of about 60% in the polyphenols content at the end of the experiment [23]. From the investigations carried out on the OMW produced by the mills in the area of southeastern Sicily, the polyphenols content is on average $2.6\,g\,l^{-1}$, thus between approximately two and six times greater than the data reported by Pinto et al. Such high OMW content in polyphenols could completely inhibit the microalgal growth, and consequently prevent microalgae from playing their role in the detoxification process. This problem can be avoided by pre-treating OMW with activated carbon (AC) that, adsorbing part of the polyphenols, reduces their content in the wastewaters and allows their subsequent removal by means of filtration. The amount of AC to be used depends on its typology, varying in the range of $60–100\,g\,l^{-1}$. It is also possible to act on the wastewater pH in order to improve the adsorption process [24,25]. Hodaifa et al. [2] with a batch experiment, on laboratory scale, tested the breeding of *S. obliquus*, microalga tolerant to phenolic compounds, into OMW diluted with freshwater to 2.5, 5, and 10%. The major difficulties in the microalgae growth are related to the lack of some nutrients, the presence of fatty substances, and the dark color of undiluted OMW which hinder the passage of light radiation. The OMW dilution prior to microalgae inoculation is also proposed by Sanchez et al. [7] with a trial on *Chlorella pyrenoidosa*. It is reported to be necessary also a correction of the pH which ranges from moderately acid to sub-acid according to the state of fermentation of the OMW.

According to the literature, the most studied microalgal strains for the intended application belong to the *Chlorella* and *Scendesmus* genera. For this reason, *Chlorella vulgaris* and *S. quadricauda* were chosen for this study.

2.1. Experimental setting

The growth of these two microalgal strains was carried out and monitored in a synthetic growth medium, BG-11 [26]. In order to evaluate their growing capability, the selected microalgae were cultivated in continuously aerated flasks and incubated in light and dark conditions (14 h vs. 10 h) for 21 days at a temperature of 20 ± 2°C.

The experimental activities were set according to the following scheme:

(1) OMW pre-treatment by means of AC, aimed at the reduction of polyphenols content and at the flocculation of the suspended solids;
(2) OMW filtration to remove the solid phase; and
(3) pH adjustment.

The goal of these activities is to obtain a cultivation medium for the microalgae with the same composition of the synthetic substrate (BG 11) used to grow the selected microalgal strains.

Following this procedure, we were able to perform a comparison between the environmental burden deriving from using the synthetic medium BG11 and the "adjusted" OMW.

2.2. Life cycle assessment

LCA is a comprehensive analysis of the environmental impact of a product (or a service) throughout its life cycle, from raw materials extraction to final disposal. LCA methodology is standardized according to ISO 14040 [27].

All energy and mass flows, as well as the related environmental impacts, are reported on the basis of a reference unit which is selected coherently with the system analyzed; such unit is called functional unit (fu). Furthermore, the choice of the processes to be included in the assessment and of those to be cut off brings about the definition of the system boundaries.

In the present work, the main goal of the LCA study is the assessment of the potential environmental benefit deriving from the use of wastewater, in place of a synthetic medium, to grow microalgae. The functional unit is set to be 1 m^3 of culture medium.

The system boundaries include the preparation of the culture medium BG-11, starting from well water and synthetic additives (SCENARIO A) or from pretreated OMW, properly diluted with well water and integrated with the lacking substances (SCENARIO B). For both scenarios, the quality of the culture medium is assumed to be equal, with no influence on the microalgal biomass growth rate.

In the LCA model, the composition of the OMW is considered constant during the oil production season. This is a reasonable assumption since industrial wastewaters are not subject to significant compositional variations in time, differently from what happens, for example with municipal wastewater. Furthermore, the pretreatment of OMW with AC is not included within the system boundaries due to the lack in primary data.

For a consistent comparison between the two scenarios, a system expansion was performed. In order to account for the wastewater treatment, in SCENARIO A, OMW were considered to be treated in a separate and traditional depuration process ("Waste water-untreated, slightly organic contaminated EU-27 S", ELCD). This approach is intended to include the potential environmental benefit of integrating wastewater valorization in the production of the culture medium for microalgal growth.

The analysis is performed assuming that the treatment with microalgae has a negligible environmental impact, because the contextual production of biomass can be exploited to produce energy for supplying the treatment plant. Such system is energetically autonomous and does not have significant exchanges with the environment. Furthermore, the microalgae-based treatment is the same for both SCENARIO A and B; therefore, in a comparative study, it is irrelevant and it is excluded from the system boundaries.

Fig. 1 gives an overview of the model built for the LCA analysis.

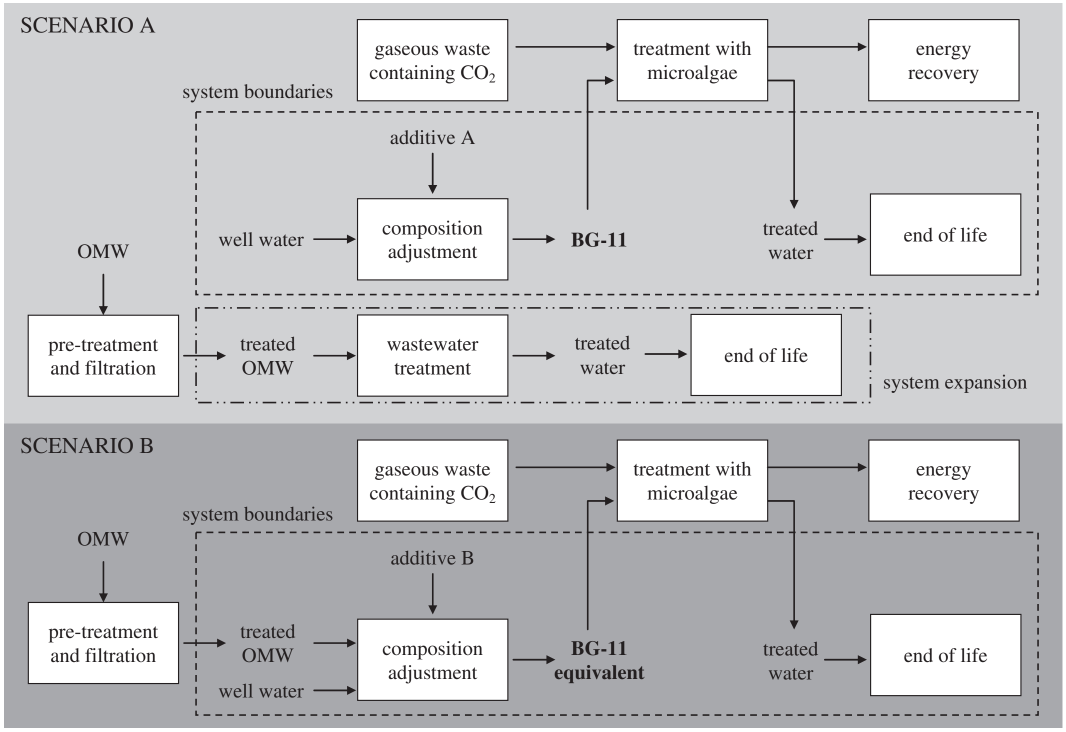

Fig. 1. Process flowchart.

In SCENARIO A, the LCA system boundaries cover the life cycle from cradle to grave for the primary system, while the system expansion includes the OMW treatment from gate to grave. On the other hand, in SCENARIO B, the analysis is performed from gate to grave. It can be assumed that the OMW entering the systems, both in SCENARIO A (system expansion) and in SCENARIO B, has no influence on the overall impact of the compared systems because the OMW is the same.

Finally, a sensitivity analysis was performed in order to evaluate the transportation of OMW from olive mills to the wastewater treatment plant, drawing final remarks about logistics management.

2.2.1. Impact categories and impact assessment method

As far as wastewater treatment is concerned, the assessment of the water streams within the system is of major interest. Water footprint (WF) is an environmental impact indicator which takes into account the direct and the indirect water use related to a product throughout its life cycle. Usually, three contributors to WF are identified and distinguished. Blue water is the freshwater coming from both the surface and groundwater resources, green water refers to rainwater (directly collected or stored in the soil as moisture), and gray water is associated with pollution. In particular, gray water is defined as the freshwater needed to dilute wastewater in order to restore its quality (lowering contaminants' concentration), according to the agreed standards, before its release into the environment [28].

For this study, the WF is represented by two contributors: blue water and treated water. The former represents the well water entering the system (positive value of the indicator), while the latter accounts for the water going out during the water treatment processes (negative value of the indicator).

Together with the WF, the impact categories considered are cumulative energy demand (CED), global warming potential (GWP), acidification potential, and eutrophication potential.

The assessment method, specifically developed for this work, is presented in Table 1.

2.2.2. Life cycle inventory

The primary data collection was focused on the characterization of the culture medium BG-11 and of the pretreated OMW, derived from multi-variety extra virgin oil extracted by means of centrifugation method (Table 2).

Table 1
Assessment method

Impact category	Description
Global warming potential (IPCC 2007)	IPCC 2007 GWP 100a 1.02 (single issue in SimaPro methods), excluding the emission of biogenic methane (CH_4) that is accounted in the indicator GWP bio
GWP bio air emission	It accounts the total biogenic CO_2 and CH_4 (conversion factor 25 kg CO_2 eq/kg) as air emission and returns a positive value
CO_2 uptake	It accounts the total carbon dioxide sequestered from air, as raw material, and returns a negative value
Cumulative energy demand (CED)	Cumulative energy demand 1.08 (single issue in SimaPro methods). Divided in renewable (R) and non-renewable (NR) resources
Blue water	Blue water, Water footprint indicator
Acidification	From the method EPD 2008 1.03, SimaPro
Eutrophication	From the method EPD 2008 1.03, SimaPro

Table 2
Characterization of pre-treated OMW (personal authors communication of 2009–2011 average data)

	Pre-treated OMW
Dry matter (%)	6.6
pH	4.1
EC ($dS\,m^{-1}$)	7.3
Density ($g\,cm^{-3}$)	1.05
Polyphenols ($g\,l^{-1}$)	2.8
COD ($g\,l^{-1}$)	51

The collected data refers to the OMW fraction deriving from drupe. The olive mill from which the waters were sampled is a continuous three-stage centrifugal extractor. The analyzed wastewater is the most concentrated one obtainable, not containing the rinse water, and it is equal in quantity to about 85% of the processed olives.

According to the literature previously examined, the polyphenols content in the pre-treated OMW is too high to be tolerated by microalgae. Cicci et al. [23] reported that, although *S. dimorphus* growth rate is reduced proportionally to the extent of phenolics disappearance during the growth itself, microalgae can grow in OMW with a total polyphenols content of $1.3\,g\,l^{-1}$. Starting from this data, OMW are diluted at 50% with well water in order to reach a polyphenols content (about $1.4\,g\,l^{-1}$) acceptable for microalgae cultivation.

Starting from these preliminary data, two assemblies were considered (Table 3):

- *BG-11*, containing all the substances to be added to pure water in order to have the right synthetic culture medium;
- *BG-11 eq.*, containing all the substances to be added to treated OMW to have a culture medium equivalent to the synthetically produced BG-11.

The effect of internal water recycling is also explored: the LCA model includes the cases in which all the well water required as input is provided by the treated water in output. As a result, four scenarios are considered (Table 4).

3. Results

The evaluation of the four different scenarios previously described (A1, A2, B1, and B2) produced significant results (Fig. 2). For a consistent analysis, SCENARIO A1 is compared with SCENARIO B1 (not including water recycling), while SCENARIO A2 can be related to SCENARIO B2 (including water recycling). In both cases, the use of wastewater for the production of the microalgae culture medium has been proven to result in an environmental benefit. This result does not take into account the burden arising from the microalgae treatment which, on first approximation, has been considered "environmentally neutral."

Moreover, the water internal recycle leads to a significant reduction of the environmental burden both in the case of the synthetic medium (A2) and in the case of OMW valorization (B2).

As far as WF is concerned, in SCENARIO A2 and B2, the internal recycling of water results in a negligible consumption of water as input (blue water). On the contrary, the treated water for these two scenarios is not null due to the system expansion in case A2 and to the non-recycled water in case B2.

Table 3
Assemblies

Component	BG-11 (mg/l)	BG-11 eq. (mg/l)	SimaPro	Data source
$NaNO_3$	14.96	0	Sodium nitrate	Raw material
$MgSO_4 \cdot 7\ H_2O$	74.93	0	Magnesium sulfate, at plant/RER U	Ecoinvent unit process
K_2HPO_4	30.48	0	Dipotassium phosphate	Modeled on stoichiometry
$CaCl_2 \cdot 2\ H_2O$	25.73	0	Calcium chloride, $CaCl_2$, at plant/RER U	Ecoinvent unit process
Citric acid	5.99	0	Dummy citric acid	Modeled as "Chemicals inorganic, at plant/GLO U", Ecoinvent unit process
Ferric ammonium citrate	7.86	0	Dummy ferric ammonium citrate	Modeled as "Chemicals inorganic, at plant/GLO U", Ecoinvent unit process
Na_2EDTA	0.93	0	EDTA, ethylenediaminetetraacetic acid, at plant/RER U	Ecoinvent unit process
Na_2CO_3	15.69	0	Sodium carbonate from ammonium chloride production, at plant/GLO U	Ecoinvent unit process
H_3BO_3	0.06	0.06	Boric acid, anhydrous, powder, at plant/RER U	Ecoinvent unit process
$MnSO_4 \cdot H_2O$	0.03	0.03	Dummy manganese(II) sulfate	Modeled as "Chemicals inorganic, at plant/GLO U", Ecoinvent unit process
$ZnSO_4 \cdot 7\ H_2O$	0.29	0.29	Zinc monosulfate, ZnSO4 · H2O, at plant/RER U	Ecoinvent unit process
$CuSO_4 \cdot 5\ H_2O$	0.003	0.003	Dummy copper(II) sulfate	Modeled as "Chemicals inorganic, at plant/GLO U", Ecoinvent unit process
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.01	0.01	Dummy ammonium molybdate	Modeled as "Chemicals inorganic, at plant/GLO U", Ecoinvent unit process

Table 4
Scenarios description

SCENARIO	Input water	OMW treatment	End of life
A1	100% well water	System expansion	100% sewer system
A2	100% recycled water	System expansion	100% recycled
B1	50% OMW 50% well water	Integrated	100% sewer system
B2	50% OMW 50% recycled water	Integrated	50% sewer system 50% recycled

3.1. Transportation sensitivity analysis

OMW need to be collected from the mills spread around the territory and the wastewater transportation may represent a significant contributor to the environmental impact of SCENARIO B1 and B2. Thus, a sensitivity analysis was carried out in a specific geographic area to assess the influence of transportation on the overall environmental balance of the system. In Sicily, the geographical context of reference, there are almost 700 olive mills, evenly spread on hills and planes of the territory. Porto and Vinciprova [29] reported an average amount of OMW produced for each mill varying between 170 and 350 m^3, in relation to the olive annual production, with a regional amount comprised of between 100,000 and 200,000 $m^3\ year^{-1}$. The processing of olives for oil production has a total duration of about 100 days. In each areal production, in relation to the soil and climatic conditions, the season of harvesting and milling usually does not exceed 45 days. Therefore, mills are geographically distributed according to the duration of the harvesting season. In such context, for an effective transfer of OMW and for a continuous production of microalgal biomass, the location of the wastewater treatment plant has to be carefully chosen in order to supply the OMW in the optimized system.

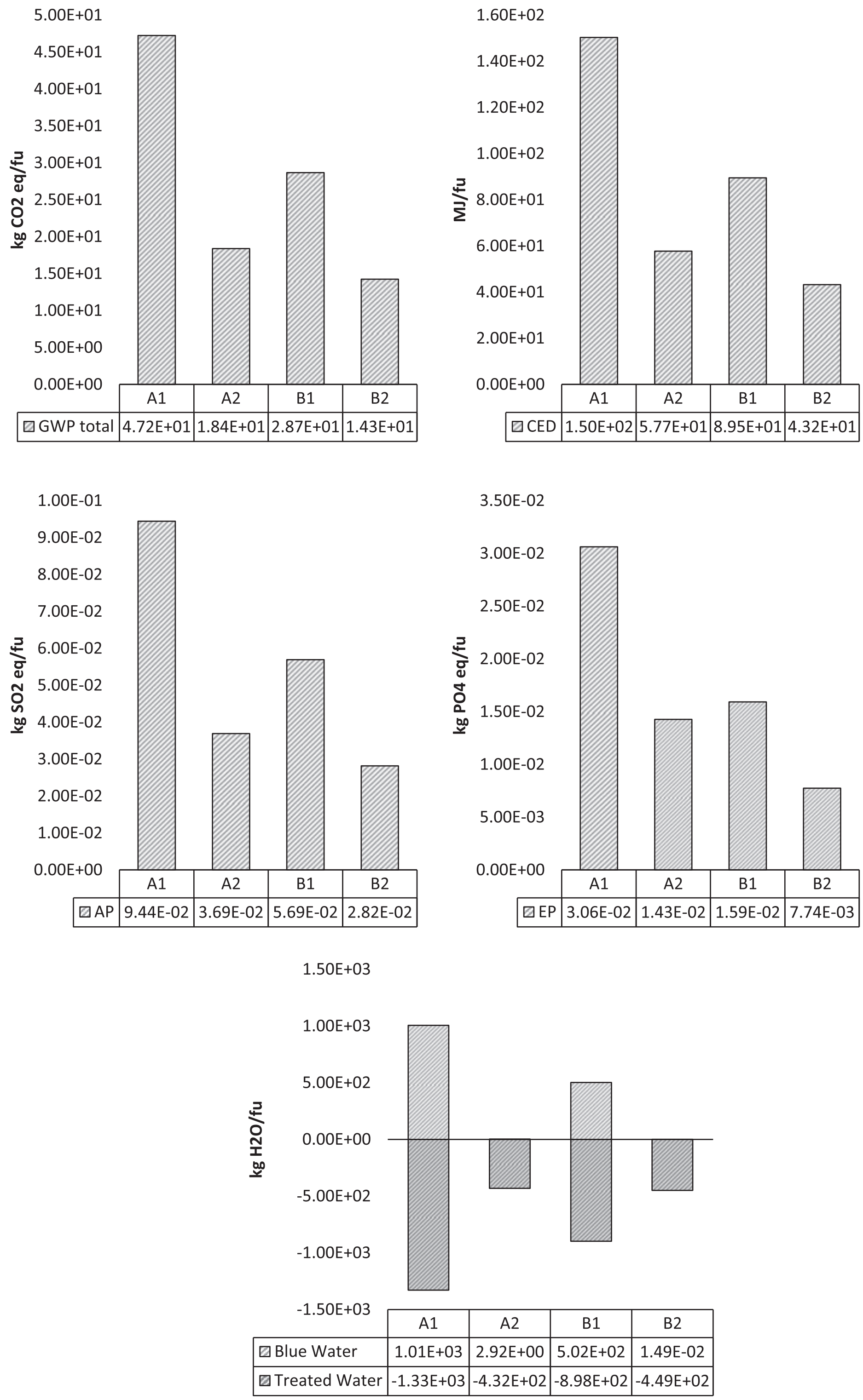

Fig. 2. Results for the three scenarios A, B1 and B2.

For analysis' consistency, comparison is made between SCENARIO A1 and B1. Starting from these considerations, a threshold distance, beneath which it is still environmentally favorable to transport and treat OMW, has been identified, taking into account separately, CED or GWP. The wastewater is transported by means of road trucks with a capacity of maximum 7.5 tonnes ("Transport, lorry 3.5–7.5t, EURO3/RER U", Ecoinvent unit processes) or 32 tonnes ("Transport, lorry 16–32t, EURO3/RER U", Ecoinvent unit processes). The maximum distances (respectively, d_{CED} and d_{GWP}) environmentally acceptable before reaching the breakeven point between SCENARIO A1 and B1 are reported in Table 5.

It is clearly shown that the most limiting indicator is CED, because the d_{CED} value is by far lower than d_{GWP}. If d_{CED} is considered, a maximum environmentally acceptable area for the OMW transportation is determined.

The logistics has been identified, focusing on the area near Catania and Ragusa and taking into account the actual distribution of olive mills. Then, two circular areas were defined:

- the treatment plant location area of radius r, inside which the plant can be placed (r is a

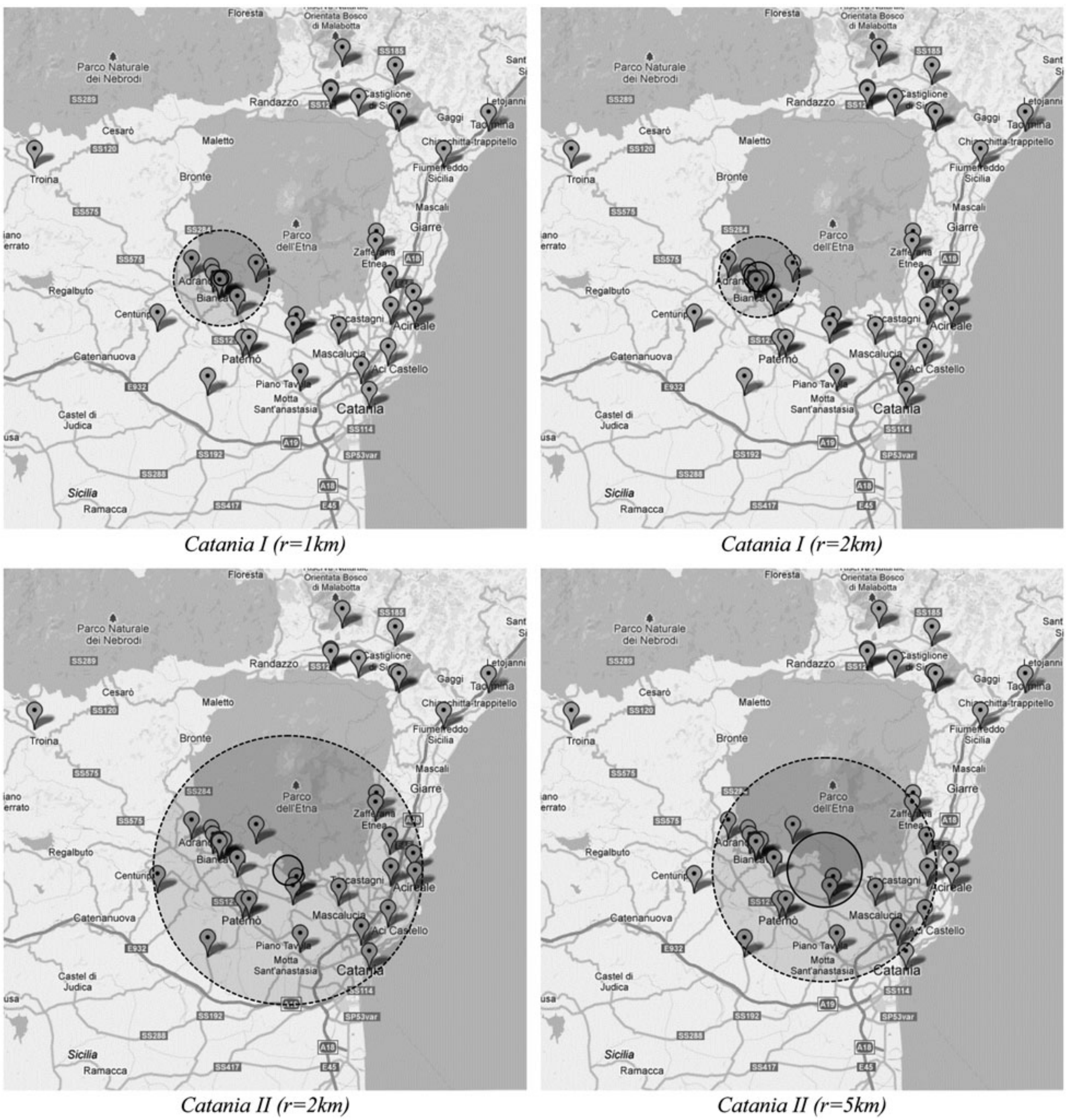

Fig. 3. Treatment plant localization. The pins indicate the olive mills present in the area. The continuous line circle refers to the plant's location and the dotted line circle indicates the area reachable by the collection system in SCENARIO B1.

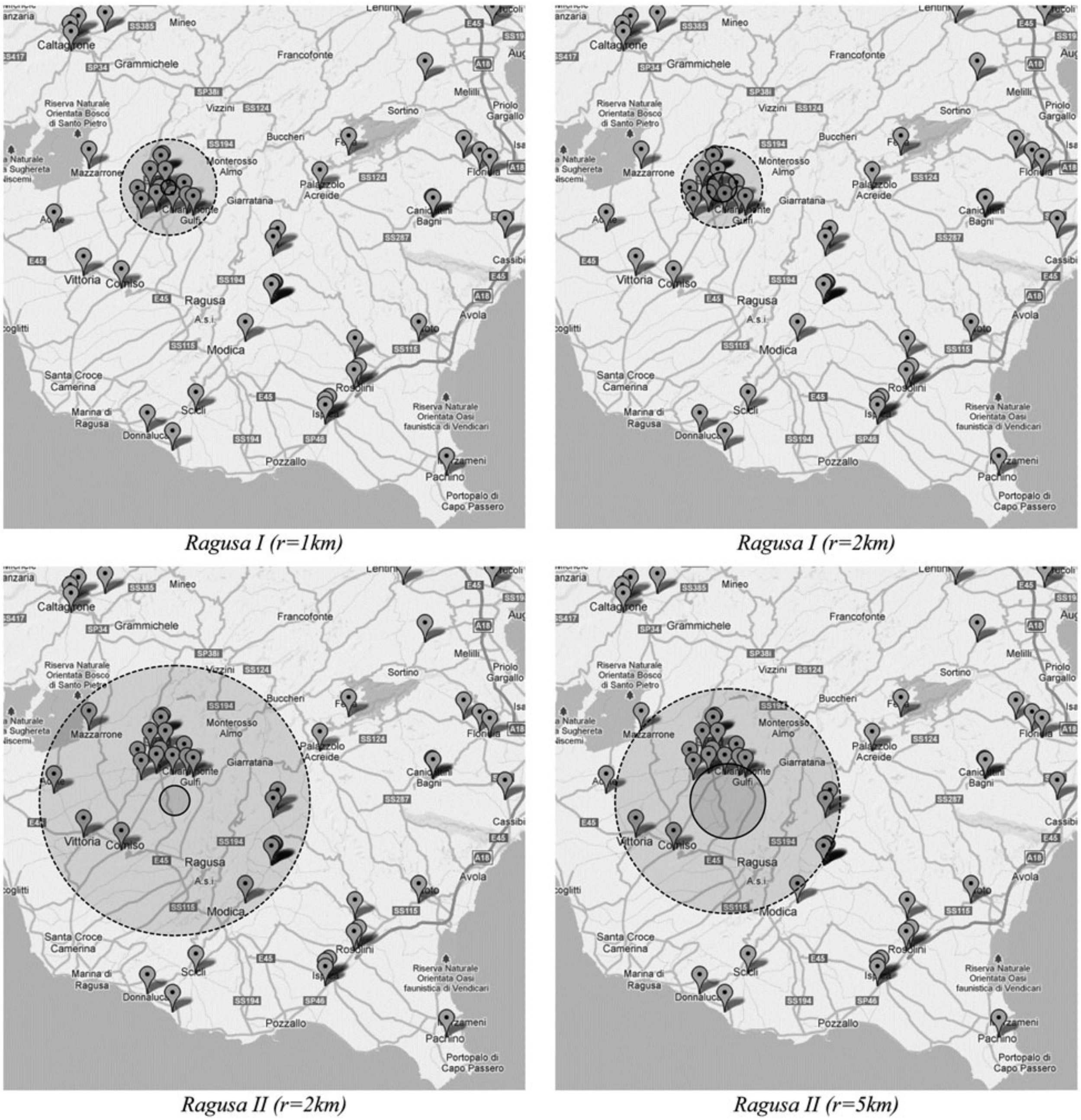

Fig. 3. (*Continued*)

function of the uncertainty linked with the urbanization policies).

- the collection area of radius R, inside which there are the olive mills that can be served by the OMW collection service before the breakeven point is reached.

The threshold distances previously computed are related to these variable by the equation $R + r = \frac{d_{\mathrm{CED}}}{2}$. Thus, as the uncertainty of the plant location increases ($r \uparrow$), the servable area decreases ($R \downarrow$).

By way of example, two cases were considered:

- Case I: using a 7.5 tonnes lorry, the treatment plant location is assumed to be confined within an area of r equal to 1 or 2 km;
- Case II: using a 32 tonnes lorry, the treatment plant location is assumed to be confined within an area of r equal to 2 or 5 km.

Table 5
Maximum transportation distances

	d_{CED} (km/fu)	d_{GWP} (km/fu)
7.5 tonnes	14.8	76.4
32 tonnes	40.0	200.5

Table 6
Treatment plant location and transportation

	r (km)	R_{CED} (km)
Case I	1	6.4
	2	5.4
Case II	2	18
	5	15

Table 6 presents the maximum radii of the environmentally sustainable collection areas for the OMW according to CED indicator.

In Fig. 3, the two cases above described are visually represented on the map of the areas of Catania and Ragusa.

From the results of this analysis, some considerations can be drawn. For all the cases considered, the largest area of the treatment plant localization leads to a reduction of the environmentally favorable OMW collecting area; as a result some olive mills are excluded. Moreover, the capacity of the lorry plays a significant role: it is evident that a larger size allows a wider collection, area and therefore is more indicated where olive mills are more spread (Catania area). On the contrary, small size transportation is advisable only where it is possible to identify a confined cluster of olive mills (Ragusa area).

4. Conclusions

The results obtained show that the use of wastewater to grow microalgae can bring about an environmental benefit. This is largely due to the OMW valorization and to the avoided impact of the purification treatment.

The internal water recycling has a large positive effect on the overall environmental burden, even if the hypothesis of a complete water reuse needs further investigations. However, also a partial recycling would result in an environmental benefit.

Since the transportation phase has a non-negligible impact within the life cycle of the OMW treatment process, using wastewater in place of synthetic media is highly environmentally favorable if the treatment plant is properly located. LCA results were used in this context to define good practices for the localization of the centralized microalgae cultivation plant for two different contexts in Sicily.

Furthermore, to ensure a regular water supply to the microalgae treatment plant over time, the treatment plant should be designed in order to be able to receive agro-industrial wastewaters of different origin in an integrated system. This way it is possible to overcome the problem of olive oil production seasonality and to guarantee a constant production of microalgal biomass all year round. For this reason, it is advisable to extend the analysis to other kinds of agro-industrial wastewater (e.g. citrus industry).

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