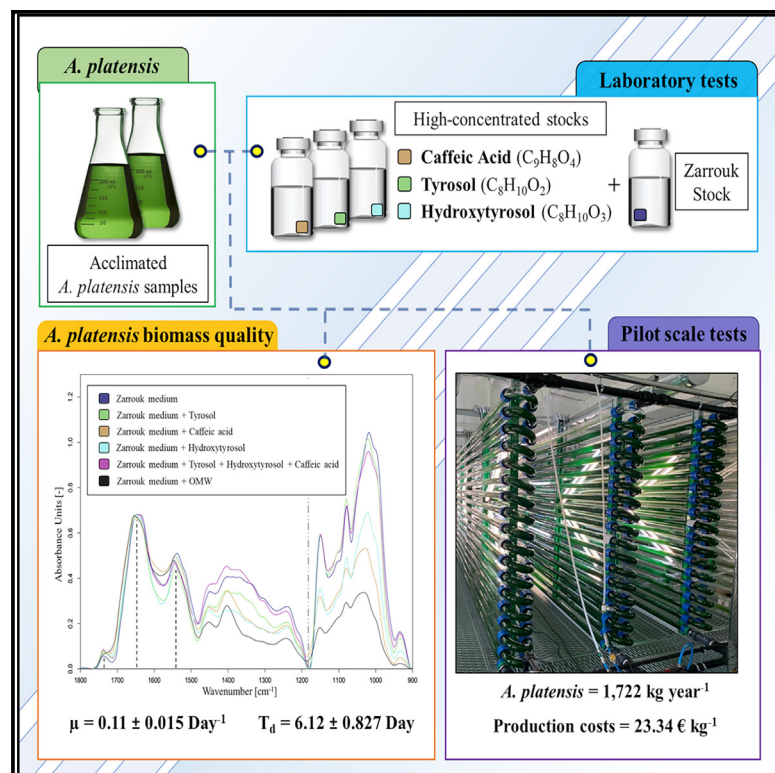


Valorization of olive mill wastewater for *Arthrospira platensis* production

Graphical abstract



Authors

Matteo Moglie, Giovanni Biancini, Alessandra Norici, Alice Mugnini, Luca Cioccolanti

Correspondence

matteo.moglie@uniecampus.it

In brief

This paper investigated the feasibility of an integrated process that recycles olive mill wastewater while concurrently producing *Arthrospira platensis* for food applications. From the experimental results obtained at laboratory and pilot scale, *Arthrospira platensis* produced using olive mill wastewater is feasible with food-grade quality and with a production cost lower than 25 €/kg.

Highlights

- Membrane filtration and *A. platensis* cultivation is effective for the treatment of OMW
- *A. platensis* growth rate is not inhibited by the phenols present in OMW
- Avoided cost for OMW disposal reduces about 70% of the biomass production cost
- The use of olive mill wastewater for microalgae production is potentially profitable



Article

Valorization of olive mill wastewater for *Arthrospira platensis* productionMatteo Moglie,^{1,4,*} Giovanni Biancini,¹ Alessandra Norici,² Alice Mugnini,³ and Luca Cioccolanti¹¹CREAT, Università degli studi e-Campus, Via Isimbardi 10, Novedrate 22060, Como, Italy²Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy³Dipartimento di Ingegneria Industriale e Scienze Matematiche, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy⁴Lead contact*Correspondence: matteo.moglie@uniecampus.it<https://doi.org/10.1016/j.crsus.2024.100017>

SCIENCE FOR SOCIETY Currently, the olive oil industry is one of the most relevant agro-food sectors of the Southern Mediterranean area, but the overall production process generates a huge amount of wastewater with a high polluting and phytotoxic content. To reduce the environmental impact of wastewater generated, we propose a novel integrated treatment to obtain (1) organic fertilizer and clean water for agricultural purposes and (2) inorganic nutrients for *Arthrospira platensis* cultivation. The results of this study show that the integration of the different sub-systems makes it possible to efficiently exploit the inorganic nutrients of the olive mill wastewater to cultivate *Arthrospira platensis*.

SUMMARY

Intending to reduce the related environmental impact of olive oil wastewater while producing new by-products, this research paper proposes an innovative solution for the treatment of wastewater that combines microfiltration and ultrafiltration techniques with microalgae cultivation. Laboratory scale analysis and pilot scale operation have been performed to assess the techno-economic viability of the olive mill wastewater for *Arthrospira platensis* production. More precisely, growth rate, time of division, and characterization (lipids, carbohydrates, proteins, and so forth) of microalgae are evaluated. The results obtained from the techno-economic analysis show that the integration of the systems makes it possible to efficiently exploit the inorganic nutrients of the olive mill wastewater for the cultivation of *Arthrospira platensis*. In particular, the quality of the obtained biomass complies with the food grade regulations, whereas avoided costs for the olive mill wastewater disposal bring a reduction of 70% in the biomass production cost.

INTRODUCTION

The Water Framework Directive (WFD), also known as Council Directive 2000/60/EC revised and amended by Directive 2013/39/EU,¹ affirms that “water supports life” and that water is a crucial resource for humanity, generating and sustaining economic and social prosperity. While the WFD considers all aspects of water use and consumption, good water management must be integrated into all areas of water utilization (i.e., agriculture, industry, and services), reducing the utilization of tap water when possible.² The correct exploitation and management of water-related resources is a strict requirement of any environmental regulation, even more for the achievement of the Sustainable Development Goals (SDGs) set by the United Nations General Assembly. The Responsible Consumption and Productions Patterns goal aims to improve the use of resources and reduce waste production by increasing recycling and reuse.

Focusing on the olive oil industry, which is one of the most important agri-food sectors in the South of Europe,³ the generated wastewater causes a significant environmental burden because the olive waste generated is highly phytotoxic due to considerable concentrations of phenols, lipids, and organic acids. The level of pollution they cause is affected by the method used to extract olive oil, olive cultivar, and other factors related to weather and localization of the orchard.⁴ Therefore, the olive mill wastewater (OMW) cannot be recycled directly but needs a pre-treatment.

Traditional olive oil processing methods are estimated to produce between 500 and 1,100 L of OMW for each ton of processed olives.⁵ Furthermore, the pollution of 1 m³ of OMW has been estimated to be equivalent to 200 m³ of urban sewage⁶ due to its high chemical oxygen demand (COD)—up to 400 g L⁻¹.⁷ Removal of the organic compounds, mostly phenols, from OMW is significantly hard and expensive.⁸ The disposal of OMW can be expensive, and, depending on the disposal



Table 1. Chemical composition of wastewater after MF and UF

Chemical element	Unit	Value	
		MF	UF (5 kDa)
Calcium	mg L ⁻¹	146.20	166.30
Sodium	mg L ⁻¹	35.40	71.90
Potassium	mg L ⁻¹	30,330.00	28,190.00
Copper	mg L ⁻¹	0.90	6.20
Zinc	mg L ⁻¹	7.70	8.60
Phosphates	mg L ⁻¹	130.00	470.00
Chlorides	mg L ⁻¹	762.00	2,019.00
Nitrates	mg L ⁻¹	2,817.00	3,321.00
Phenols (tyrosol)	mg L ⁻¹	3,316.00	6,954.00
Magnesium	mg L ⁻¹	138.00	155.00
Iron	mg L ⁻¹	25.50	4.60
Glucose	g L ⁻¹	<0.10	<0.10
Fructose	g L ⁻¹	<0.10	<0.10
Sucrose	g L ⁻¹	<0.10	<0.10

process, the price varies from a few tens to hundreds € m⁻³. The cheapest and most used technique is spreading OMW on soils, which can increase soil organic matter and nutrient contents, but only in strictly controlled conditions and with a maximal spreading rate of 80 m³ Ha⁻¹ year⁻¹.⁹ An analysis of the literature in the field has revealed that alternative treatment options for OMW exist,¹⁰ which span from biological treatment after coagulation and flocculation¹¹ to co-digestion for biogas production¹² to solar still distillation¹³ and membrane filtration.¹⁴ Vuppala et al.¹¹ studied the coagulation and flocculation pre-treatment process of OMW before treating the wastewater with a continuous oxidating process. This research concluded that if an optimal agent with appropriate process conditions is used, a lower unitary treatment cost for OMW can be obtained compared with wastewater sparging and hazardous water treatment. As discussed by Maragkaki et al.,¹⁵ which conducted different lab-scale experiments on co-digestion, OMW can be used to boost the biogas yield (up to 170%) of sewage sludge when diluted in different concentrations. However, a discussion on an upscaled version is missing. The pilot plant of Mastoras et al.¹³ demonstrated the feasibility of medium-scale OMW treatment systems that use solar distillation for organic chemical recovery in the distillate. The unitary operating cost of the system is low, down to 0.60 €/m³, but there is a relatively high payback period of 16 years for the investment due to high capital costs. A net present value of 25,000 € can be obtained after 25 years, meaning fair profitability for this type of investment. In the review of Hube et al.,¹⁴ numerous direct membrane filtration systems reported in the literature were analyzed. As the authors claim, the use of pressure-driven membranes for pollutant removal and resource recovery may be used for decentralized facilities. Despite some challenges concerning the contamination control on an extended time frame, irreversible membrane fouling, and flow decline, such systems can offer an enhanced permeate quality suitable for markets focused on a circular economy while decreasing the carbon footprint of wastewater treatment with a sustainable realization cost. Elkacmi et al.¹⁶ proposed instead

Table 2. Chemical composition comparison of Zarrouk medium and diluted OMW medium

Chemical element	Unit	Zarrouk	Diluted OMW	Difference
Calcium	mg L ⁻¹	361	178.7	+182.3
Sodium	mg L ⁻¹	5,664	54.1	+5,609.9
Potassium	mg L ⁻¹	673	15,489.0	-14,816.0
Phosphorus	mg L ⁻¹	88	84.1	+3.9
Chlorine	mg L ⁻¹	612	1,109.3	-497.3
Nitrogen	mg L ⁻¹	411	412.3	-1.3
Sulfur	mg L ⁻¹	211	82.5	+128.5
Magnesium	mg L ⁻¹	19	97.2	-78.2
Iron	mg L ⁻¹	1.8	2.5	-0.7

a new and simpler method based on centrifugation and fractionation with urea that can be used for the extraction of oleic and linoleic fatty acids, phenols, biodegradable soap, and high-quality glycerol from OMW. The implementation of this process-based system on a large scale can reduce the overall environmental load compared with traditional discharge on sewers (perfectly represented by a linear economic model) and cover their relevant realization cost, too. Therefore, the intrinsic value of the organic compounds entrained in OMW fosters the circular economy paradigm. This approach pursues optimal resource management through promoting reuse, recycling, or other solutions that move the interest to a more sustainable perspective. As its name entails, this model shifts the supply chain from the traditional “linear” economy toward a zero-emissions framework.¹⁷

Another strategic approach based on the circular economy model is using waste as a culture medium for other microorganisms. In the framework of reduction and recycling of waste, the use of OMW for microalgae production is considered an interesting option to produce new high-value by-products.¹⁸ It has been widely proven that microalgae are an important source of high-value molecules such as pigments (i.e., β-carotene, astaxanthin, and phycocyanin), proteins, and fatty acids, which are increasingly appreciated in the market, especially compared with other synthetic and traditional alternative molecules.¹⁹ Interests in biodiesel production from microalgae are expected to continually rise in the future. In the research work of Hodaifa et al.,²⁰ the authors provided different laboratory analyses regarding the feasibility of a new bio-physicochemical process for OMW treatment that uses the microalga *Scenedesmus obliquus*. The high concentration of carbohydrates and lipids (from 44.9% to 72.5% of dry mass) justifies the scaling up of the process at the industrial level for biodiesel production from a yield perspective (0.21 g L⁻¹). Nonetheless, any economic guidance for the scale-up process is missing. On an industrial design level, microalgae biomass can be converted into biofuels such as bioethanol, biodiesel, biogas, and jet fuel. Chia et al.²¹ reviewed these aspects and concluded that the key points for a successful microalgae-based biodiesel production plant are the robustness of the strain, lipid/carbohydrate productivity, cheap lipid extraction methods, and supporting policies from the authorities. The difficulties encountered during the scaling-up attempts can be partially overcome with closed systems and a slow adaptation of the culture in the growth medium. In this way, the substitute of fossil fuels is not a

Table 3. Values of growth rate (μ) and division time (T_d) for *A. platensis*

Parameters	Zarrouk medium	Zarrouk + tyrosol	Zarrouk + hydroxytyrosol	Zarrouk + caffeic acid	OMW
μ (day ⁻¹)	0.70 ± 0.17 ^a	0.27 ± 0.05 ^b	0.32 ± 0.03 ^b	0.33 ± 0.02 ^b	0.11 ± 0.015
T_d (day)	1.02 ± 0.24 ^a	2.53 ± 0.52 ^b	2.15 ± 0.19 ^b	2.11 ± 0.12 ^b	6.12 ± 0.827

Different letter in the superscript denotes significantly different means across treatments for each parameter ($p < 0.05$).

remote achievement but an alternative solution that can decelerate their depletion²²; however, the economic sustainability of microalgae cultivation still raises some concerns due to the high realization costs, limited productivity, consistent energy demand, sterilization of high volumes, and so forth. As reported by Peng et al.,²² a few large-scale microalgae systems exist. Companies such as Algenol and Sapphire Energy accomplished different facilities with remarkable extension, which can produce multi-stream microalgae products and not only biofuel. At smaller scales, it is seldom preferred to use instead specified microalgae cultures suitable for different markets, such as food or pharmaceutical, as pointed out by the techno-economical study conducted by Mennella et al.²³ The authors pointed out how the selling price significantly influences the economic sustainability. They also assessed that profitability is expected for prices around 35 € kg⁻¹ for their indoor production of *Arthrospira platensis* to be sold in the food sector. Because the price perfectly represents a niche business, with the relevant risk involved, additional sources of revenues should be foreseen. Hence, linking to different markets linked within circular economies could strengthen and improve the economic asset, for example, if the utilization and/or treatment of waste fluxes such as¹⁹ flue gases, wastewaters, and low-grade waste heat²⁴ is pursued. Moreover, an optimal pathway to achieve this goal could be the concurrent production of several by-products extracted from microalgae biomass, leading to the minimization of raw material costs required by microal-

gae cultivation and the maximization of the revenue streams.²⁵ For example, regarding wastewater treatment, marine and freshwater microalgae have been used to treat dairy wastewater,²⁶ municipal wastewater,²⁷ and food processing wastewater.²⁵ In each case, it has been proven that, by using microalgae, more than 90% efficiency in total nitrogen, phosphate, and sulfate removal has been obtained. In summary, to overcome production and economic bottlenecks, the process should be designed to maximize biomass quality, minimize the utilization of raw materials (i.e., nitrogen and carbon dioxide), and utilize local resources.²⁸

Recently researchers and microalgae producers are focused on the production of extracts that can be used in food supplements, nutraceuticals, and biopharmaceuticals.²⁹ Within more than 70,000 isolated species of microalgae, the renowned species available on the market for food or food supplements are *A. platensis*, *Chlorella*, *Aphanizomenon flosaquae*, *Ulkenia*, *Schizochytrium*, and *Haematococcus pluvialis*.³⁰ Among them, *Chlorella* and *A. platensis* are the most produced microalgae in the world.³¹ In particular, *A. platensis* was the first microalgae harvested from the natural environment and used as food. It grows in alkaline lakes with pH up to 11 and salts concentration on the order of 30 g L⁻¹. Being able to grow in extreme conditions, *A. platensis* has been used to simultaneously treat aquaculture effluent while producing biomass to supplement fish diets.³² Lim et al.³¹ reported the utilization of *A. platensis* in numerous wastewater applications ranging from municipal to

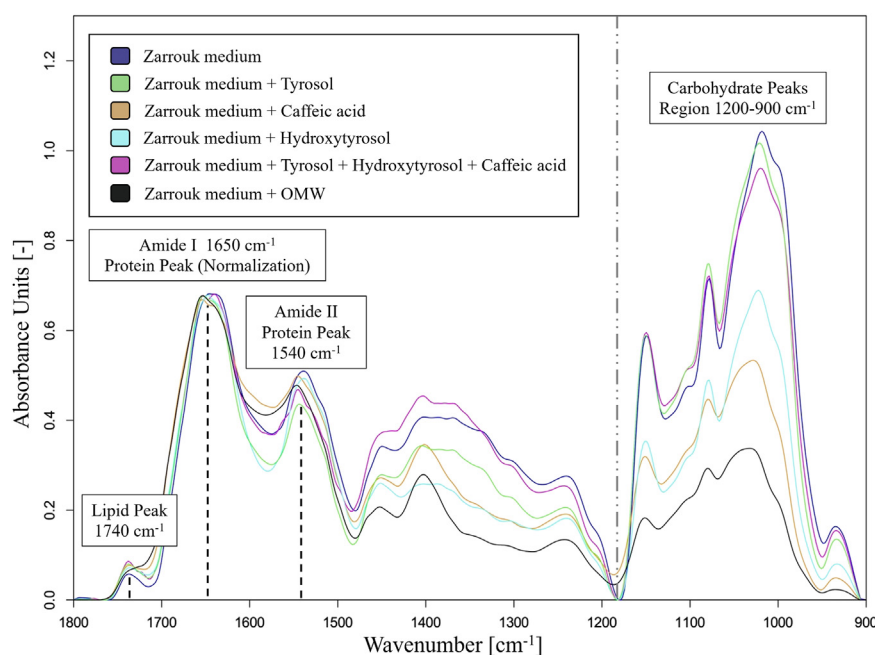


Figure 1. Averaged FTIR spectra of at least 3 distinct ones cut between 1,800 and 900 cm⁻¹, baselined and normalized on the amide I peak

Table 4. Quality control parameters for microalgae for food applications

Parameters		Unit	Limit value	Value obtained	LQ
Heavy metals	lead	mg kg ⁻¹	1.2	<0.002	0.002
	cadmium	mg kg ⁻¹	0.3	0.098	0.01
	mercury	mg kg ⁻¹	0.1	<0.01	0.01
Microbiology	total bacterial count	colony-forming unit (CFU) g ⁻¹	1.0E+5	3.1E+2	–
	yeast	CFU g ⁻¹	1.0E+4	<1.0E+2	–
	<i>E. coli</i>	CFU g ⁻¹	<10	<10	–
	<i>Salmonella</i>	CFU g ⁻¹	negative	negative	–

cow effluents. In all these cases, the final use of microalgae biomass was not intended for food applications but mainly for biofuels or fertilizer production. However, *A. platensis* biomass could be used directly as food or used in nutraceuticals, cosmetic, and functional food applications. The biomass is rich in proteins, phycocyanin, and allophycocyanin, i.e., compounds that can be extracted separately and valorized on the market,³³ thus generating positive cash flows. Markou et al.³⁴ demonstrated that OMW, after treatment with sodium hypochlorite, is suitable as a medium for *A. platensis* production, raising environmental issues on the method used and reducing the application potentials of the produced biomass. Mollo et al.³⁵ demonstrated also that microalgae responses to phenols, which are present in OMW, were species specific and that a scale of toxicity exists on the base of the phenol type.

Literature review showed that to implement wastewater treatment using microalgae, the final biomass must be used as fertilizer or for biofuel application, raising concerns about biomass safety for food or nutraceutical applications. The biomass obtained in this way could be used for food applications, unlocking a new panorama of applications generally precluded to microalgae biomass produced from wastewater. From the olive mill side, olive oil producers can benefit from a new way of OMW treatment, which is cheaper than sewage treatment when spreading on soil is not allowed. For these reasons, in this paper, we perform a preliminary test of a novel integrated OMW treatment process based on membrane filtration and microalgae cultivation that ensures a correct techno-economic feasibility of OMW recycling into raw nutrients used for *A. platensis* production and hence promotes a model of circular economy. Moreover, the characterization of the biomass quality has been addressed to verify its safe designation for food markets as an alternative source of healthy nutrients. Experimental activities are conducted both at the laboratory scale to analyze the influence of phytotoxic compounds of OMW on the microalgae growth and at the pilot scale through an indoor photobioreactor (PBR) to assess the *A. platensis* production from OMW in real conditions. Hence, the main novelty of the work relies on the combined experimental analyses with techno-economic details of the pilot scale plant to evaluate the potential uptake of the proposed technology.

The paper is structured as follows. After this introduction, in the [results and discussion](#) section, the material and methods are described for both experimental facilities. Then, in the [exper-](#)

[imental procedures](#) section, the results of the experimental activities are presented in terms of *A. platensis* macromolecular composition (lipids, carbohydrates, proteins, and so forth), and in the end, the economic analysis is performed. Eventually, the main conclusions are drawn.

RESULTS AND DISCUSSION

In this section, the results of the laboratory and pilot scale experimental activities are presented and discussed. More precisely, the results of the phytotoxicity analysis conducted at laboratory scales were compared with the subsequent pilot scale analysis in real operating conditions. To this aim, all the electricity consumptions of all components of the pilot scale have been monitored as further detailed.

Phytotoxicity and growth rate comparison

The growth rate of *A. platensis* has been assessed in real operating conditions at the pilot scale with the final aim of estimating the potential yearly productivity and the related cost-effectiveness of the proposed OMW valorization process. In the first section, the OMW collected from olive mills was passed through both microfiltration (MF) and ultrafiltration (UF) membranes. Compounds can be recovered down to the threshold of 5 kDa, while permeate passes through. [Table 1](#) details the composition of the permeate from the MF step and of the retentate from the UF step.

The UF retentate was then diluted to reach a nitrogen concentration like Zarrouk medium,³⁶ thus obtaining 1.82 L of growth medium from 1 L of OMW. [Table 2](#) reports the chemical composition of the diluted OMW and its difference compared with the Zarrouk medium chemical composition.

At the laboratory scale, the effect of the phenols content of OMW on the microalgae growth was expressed as the mean \pm standard deviations of the measures on at least three biological replicates. The significance of mean differences was tested with a one-way analysis of variance, followed by Tukey's post hoc test, with a significance threshold of 0.05, using GraphPad Prism 7.0. Regarding the growth rate (μ) and division time (T_d), it was statistically evident that the presence of the phenols reduced the growth rate and division time of *A. platensis*. Such difference due to the cytotoxic effect of phenols on algal cells has an impact in terms of productivity of *A. platensis* cultured with OMW. [Table 3](#) reports growth rate and division time of *A. platensis* in different media. The

Table 5. Direct capital cost (DCC) based on 2018/2019 financial information provided by Greentech Srl

Item	Value (€)	Lifespan (year)	Capital cost (€ year ⁻¹)
Olive mill wastewater treatment			
Microfiltration and ultrafiltration unit	46,500.00	15.00	3,100.00
Polyethylene tanks 1,000 L	390.00	15.00	26.00
Control system for temperature, level, pressure	334.00	15.00	22.27
Piping	1,124.00	10.00	112.40
Electrical panel	1,450.00	15.00	96.67
Total olive mill wastewater treatment	49,798.00	–	3,357.33
Tubular indoor PBR			
Glass tube OD 65 mm, 5.5 m length	41,817.60	50.00	836.35
“U” bends	11,664.00	50.00	233.28
Coupling	4,860.00	50.00	97.20
Support structure	30,201.60	25.00	1,208.06
Led tubes	2,580.48	1.00	2,580.48
Electrical connections	5,520.00	20.00	276.00
Diaphragm pump	10,848.00	10.00	1,084.80
Stainless-steel tanks	7,998.00	25.00	319.92
Control system for temperature, level, pressure	1,800.00	15.00	120.00
Piping	31,944.00	20.00	1,597.20
Compressed air	4,722.00	20.00	236.10
Electrical panel	2,900.00	15.00	193.34
Thermoregulation	30,238.00	15.00	2,015.87
Total tubular indoor PBR	187,093.68	–	10,798.60
Growth medium preparation and supply			
Polyethylene tanks	2,916.00	20.00	145.80
Control system for temperature, level, pressure	900.00	15.00	60.00
Centrifuge pumps	1,020.00	10.00	102.00
Filtration kit 0.2 μm	924.00	15.00	61.60
Sterilization kit UV	1,182.00	15.00	78.80
Piping	1,124.00	10.00	112.40
Mixing station 300 L	1,012.45	15.00	67.50
Mixer 3000 l tank	511.82	15.00	34.12
Electrical panel	1,450.00	15.00	96.67
Total growth medium preparation and supply	11,040.27	–	758.88
Culture harvesting and medium reuse			
Stainless-steel tank 1,000 L	940.00	20.00	47.00
Centrifuge pumps	340.00	10.00	34.00
Control system for temperature, level, pressure	300.00	15.00	20.00
Vibrating screen	10,880.00	25.00	435.20
Piping	1,124.00	20.00	56.20
Drying	8,360.50	15.00	557.37
Electrical panel	1,450.00	15.00	96.67
Total culture harvesting and medium reuse	23,394.50	–	1,246.43
Total	271,326.45	–	16,161.25

Table 6. Electrical energy consumption characterization

Item	Operation time (h year ⁻¹)	Energy(kWh year ⁻¹)	Cost (€)
Electrical energy consumption			
Olive mill wastewater treatment			
Centrifuge pump—400 V, 50 Hz, 3PH, 1,1 kW	4,620.00	5,082.00	1,261.35
Centrifuge pump—400 V, 50 Hz, 3PH, 0,5 kW	660.00	330.00	81.92
Centrifuge pump—230 V, 50 Hz, 0,37 kW	990.00	366.30	90.92
Control system for temperature, level, pressure	7,920.00	435.60	108.12
Total olive mill wastewater treatment	–	6,213.90	1,542.29
Tubular indoor PBR			
Led tubes	3,041,280.00	54,743.04	13,587.72
Control system for temperature, level, pressure	7,920.00	435.60	108.12
Thermoregulation	3,960.00	25,715.27	6,382.53
Compressed air	3,960.00	15,840.00	3,931.49
Total tubular indoor PBR	–	96,733.91	24,009.36
Growth medium preparation and supply			
Centrifuge pump—230 V, 50 Hz, 0.37 kW	2,970.00	1,098.90	272.75
Centrifuge pump—230 V, 50 Hz, 0.37 kW	2,970.00	1,098.90	272.75
Centrifuge pump—230 V, 50 Hz, 0.37 kW	2,970.00	1,098.90	272.75
Mixer—230 V, 50 Hz, 0.18 kW	330.00	59.40	14.74
Mixer—230 V, 50 Hz, 0.37 kW	330.00	122.10	30.31
Sterilization kit UV	2,970.00	163.35	40.54
Sterilization kit UV	2,970.00	163.35	40.54
Sterilization kit UV	2,970.00	163.35	40.54
Control system for temperature, level, pressure	7,920.00	435.60	108.12
Total growth medium preparation and supply	–	4,403.85	1,093.04
Culture harvesting and medium reuse			
Centrifuge pump—230 V, 50 Hz, 0.37 kW	4,290.00	1,587.30	393.97
Control system for temperature, level, pressure	7,920.00	435.60	108.12
Vibrating screen—electric motor. 230/400 V, 50 Hz, 3 kW	4,290.00	1,287.00	319.43
Vibrating screen—pump. 230/400 V, 50 Hz, 1.5 kW	4,290.00	6,435.00	1,597.17
Dryer—400 V, 50/60 Hz, 5.1 kW	3,960.00	20,196.00	5,012.65
Total culture harvesting and medium reuse	–	29,940.90	7,431.33
Total	–	137,292.56	34,076.01

comparison shows that a direct utilization of the retentate from OMW slows down the division time of *A. platensis* and hence a penalization of the productivity expected from the harvesting in pure Zarrouk medium.

Tyrosol, hydroxytyrosol, and caffeic acid have a similar effect on *A. platensis* growth rate and division time, compared with OMW medium, where we have a mix of the three types of phenols at a greater concentration.

As shown by Figure 1, macromolecular composition of *A. platensis* changed with the composition of the growth medium, and the effect was phenol specific. In particular, normalizing all the

spectra on the protein proxy at 1,650 cm⁻¹, the relative abundance of lipids was fairly similar among growth conditions while the relative abundance of carbohydrates differed depending on the treatment. Cells grown in OMW showed a macromolecular profile with the lowest amount of carbohydrates while tyrosol did not affect the relative abundance of such pool as compared with that in control cells. Interestingly, when the three phenols were added to the Zarrouk medium, algal profile of macromolecules was fairly similar to the control one.

The volumetric productivity³⁷ of *A. platensis* obtained on pilot scale where of 0.16 g L⁻¹ day⁻¹, leading to an annual biomass

Table 7. Operating costs (OPEX) characterization

Direct Operating Costs			
Item	OMW treatment [Year ⁻¹]	Microalgae production [Year ⁻¹]	Total [€]
Plant manager	13,750.00	13,750.00	27,500.00
Skilled worker - Biologist	-	35,000.00	35,000.00
Unskilled Worker	23,000.00	23,000.00	46,000.00
Electricity	1,542.29	32,533.72	34,076.01
Consumables		1,174.29	1,174.29
Total Direct Operating costs (TDO)	38,292.29	105,458.01	143,750.30
Indirect Operating Costs			
Maintenance (5% of TDC)	2,489.90	11,076.42	13,566.32
Overhead (10% of TDO)	3,829.23	10,545.80	14,375.03
Administration (10% of TDO)	3,829.23	10,545.80	14,375.03
Total Indirect Operating costs (TIO)	10,148.36	32,168.02	42,316.38
Total Operating Costs	48,440.65	137,626.04	186,066.68

productivity of 1,722 kg, calculated according to the equation as follows,

$$Y = 365 \times V \times b$$

where Y represents the annual productivity (kg year⁻¹), V is the pilot scale volume exposed to light (m³), and b is the volumetric productivity (g L⁻¹ day⁻¹).

Considering an 8-h shift for 330 days of work, about 5,280.00 m³ of OMW would be treated annually by the proposed plant. Considering this input, the filtration system could generate the following products: (1) 686.4 kg of organic fractions (used as fertilizer), (2) 3,168.00 m³ of clean water (for olive mill reuse or conveyed directly to drainage), and (3) 805 kg of nitrogen (for microalgae production). The annual productivity of *A. platensis* grown in whole Zarrouk medium can be estimated around 6,460 kg, considering a volumetric productivity of about 0.6 (g L⁻¹ day⁻¹).³⁸

It is worth noticing that the OMW medium was not optimized for algal growth except for the nitrogen concentration (equal to the one in standard replete Zarrouk medium); balancing macro and micro-nutrient availability is thus possible to improve productivity.^{39,40}

From an environmental point of view, the utilization of this integrated approach could lead to the reduction of land required for spreading the OMW. Taking into account the output of the filtration system studied and the maximal annual rate of 80 m³ Ha⁻¹ year⁻¹, we can save up to 66 Ha of land and up to 805 kg of raw nitrogen for microalgae production.

Next, the biomass produced in pilot scale has been analyzed to check whether the regulatory requirements for the food market are met. Some dried samples of *A. platensis* are sent in an external laboratory. The outcome of the analysis is reported in Table 4. The heavy metal and pathogen contents are far below the acceptable limits prescribed for food consumption.

Economic analysis

The economic feasibility of the system was investigated by quantifying both the direct capital cost (DCC) and the operational

cost of the production throughout the entire lifetime of the plant. The annual cost of *A. Platensis* production has been assessed accounting for the following costs of items: (1) labor cost, (2) electricity consumption, and (3) amount of materials required. Then, the overall production cost is divided by the annual productivity of *A. platensis* biomass and OMW treated. Hence, the production costs have been compared with the selling price of the microalga in the wholesale market.^{41,42} Furthermore, while assessing the overall profitability of the plant, the avoided costs brought by the MF-UF treatment section have also been taken into account.

The DCC of the pilot scale is reported in Table 5, which includes the details of all the equipment required to fully operate the system and their lifespan.

Electricity consumption has been evaluated for each piece of equipment of each section, and an electricity price of 0.24 € kWh⁻¹ was considered for the estimation of the total cost. Regarding the electricity price, it is worth noting that it has been calculated as the mean value of the electricity price in semester one of 2022 for non-household consumers in Italy, Spain, and Greece,⁴³ where olive mills are mostly located.

From the analysis of the values reported in Table 6, the major electrical energy expenditure is due to the *A. platensis* cultivation section of the plant. The most energy-consuming parts of the pilot scale are, indeed, the illumination, the thermoregulation of the PBR, and the drying, which cover more than 70% of total electric energy expenditure. Eventually, the cost of consumables, maintenance, overhead, and administration are reported in Table 7. The labor cost required to run the plant amounts to 108,500.00 €. Salaries were taken from the average labor cost in Italy for each staff category.

OPEX costs have been allocated to the OMW treatment and the microalgae production separately. Regarding the former, given a total operating cost for OMW treatment of 48,440.65 € (Table 7) and a total fixed capital per annum of 3,357.33 € (Table 5), the total annual cost for OMW treatment is 51,797.98 €. Considering an annual amount of treated OMW

Table 8. Olive mill wastewater characterization

Parameter	Value
pH	4.86
Conductivity (mS cm ⁻¹)	3.70
P total (mg L ⁻¹)	37.76
Total Kjeldahl nitrogen (TKN) (mg L ⁻¹)	162.38
Phenols (mg L ⁻¹)	1,925.28
Chemical oxygen demand (COD) (g L ⁻¹)	33.95
Total solids (TSs) (g L ⁻¹)	23.70
Total suspended solids (TSSs) (g L ⁻¹)	0.61
Total dissolved solids (TDSs) (g L ⁻¹)	23.10
Total salts (mg L ⁻¹)	31,508

of 5,280.00 m³ (see section [pilot scale](#)), this comes to a wastewater unitary cost of 9.81 € m⁻³. Regarding *A. platensis* production, instead, the total operating cost is 137,626.04 € (Table 7), whereas the total fixed capital cost per annum is 12,803.92 € (Table 5), which means that a total annual cost for microalgae production is 150,429.96 €. Considering the annual productivity of 1,800 kg, as reported in section [pilot scale](#), the biomass unitary cost is about 83.57 € kg⁻¹, which is higher than the actual selling price of the whole *A. platensis* biomass in the food market (>50.00 € kg⁻¹), as reported by Bataller and Capareda⁴¹ and Santini and Cavicchi⁴²

However, if the wastewater service cost is included in the analysis, considering the actual disposal cost of 30.00 € m⁻³, the service brings to a profit of 106,602.02 € as avoided cost. Therefore, the corresponding total annual cost for *A. platensis* production is significantly reduced, and the corresponding unitary cost decreases to 23.34 € kg⁻¹ with a 72% reduction of its production cost mentioned above.

Conclusions

This paper investigates the feasibility of an integrated process that recycles OMW while concurrently producing *A. platensis* for food applications. From the experimental results, we obtain the following:

- the analysis conducted at laboratory scale has proven that the phenolic compounds present in OMW affect the growth of *A. platensis* in terms of growth rate and division time but do not have a negative impact on biomass quality, thus making possible its valorization on the food market. Further analysis should be performed to understand whether *A. platensis* is mostly influenced by quality or the concentration of phenols in diluted OMW.
- The results obtained at the pilot scale, instead, have shown that the use of the OMW medium for the *A. platensis* cultivation is possible with food-grade quality but with a penalization of the annual productivity (1,722 kg/year) compared with what is achievable with the Zarrouk medium (6,450 kg/year).
- The production cost of *A. platensis* is about 83.57 € kg⁻¹ but could be reduced if the treatment cost of OMW, charged to mills, is considered. Thus, the production costs fall down to 23.34 € kg⁻¹, which is significantly lower than the selling price of *A. platensis* in the market.
- From the environmental point of view, the utilization of this integrated approach could lead to the reduction of land required for spreading the OMW up to 66 Ha for a single mill and the recycling of 805 kg of nitrogen.

Nevertheless, the techno-economic analysis has shown that further optimization is required to improve the profitability of the system and its commercialization. Among the potential actions for improvements, the optimization of OMW culture medium process and renewable energy integration are at the top

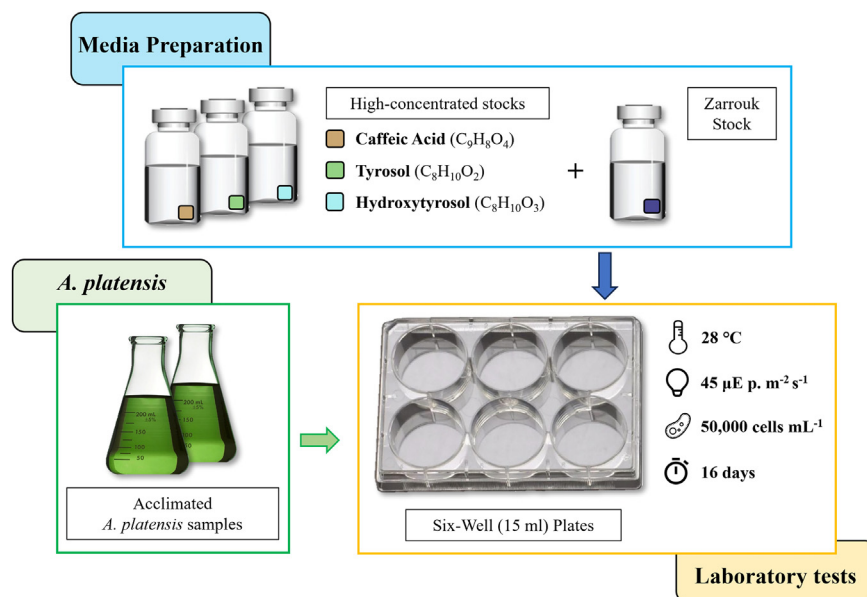


Figure 2. Experimental test bench for *A. platensis* growth rate evaluation with phenols

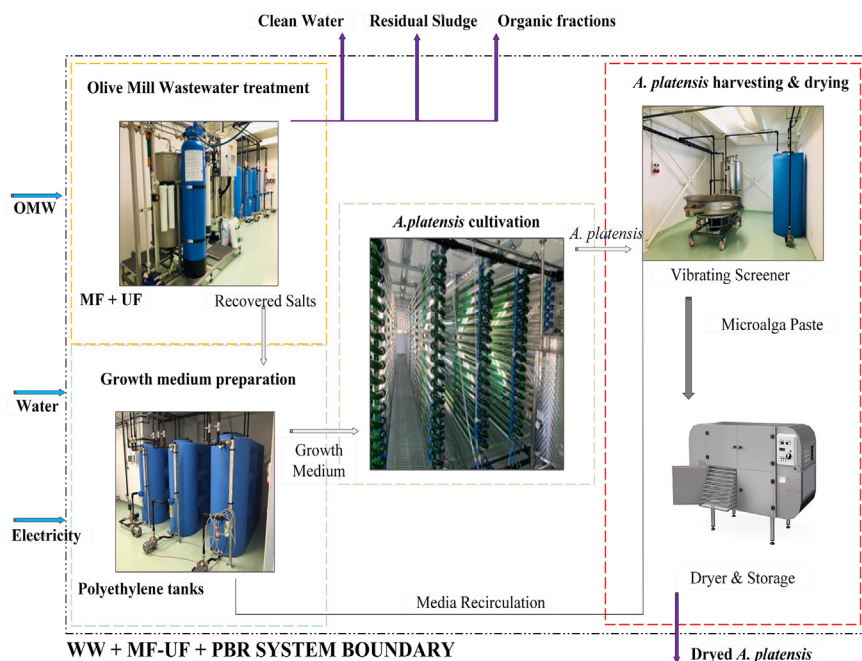


Figure 3. Pilot scale layout of the integrated OMW treatment—*A. platensis* production

of the priority list. The first one is related to the optimization of *A. platensis* growth rate and thus the reduction of division time with possibly an enhancement in the biomass concentration. The second point is related to the reduction of the thermal energy required for culture temperature control by recovering low-grade waste heat from other industrial processes or from renewables.

In conclusion, the use of OMW as an input stream for microalgae production can be considered an interesting option to improve the sustainability of the olive oil sector while promoting the circular economy paradigm.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources should be directed to the lead contact, Matteo Moglie (matteo.moglie@uniecampus.it).

Materials availability

No new materials were generated in this study.

Table 9. Aqueduct water characterization

Chemical element	Aqueduct water (mg L ⁻¹)
Calcium	87.5
Sodium	14.6
Potassium	1.0
Phosphorus	–
Chlorine	0.01
Nitrogen	0.75
Sulfur	82.5
Magnesium	12.0
Iron	–

Data and code availability

The data are derived from existing studies (see [experimental procedures](#)).

To preliminarily assess the suitability of OMW for *A. platensis* cultivation, some samples of OMW were initially collected from an olive oil mill located in central Italy on the Adriatic coast. The extraction of olive oil is performed using a traditional cold press extraction system, using a decanter to obtain olive oil, pomace, and OMW. The treated olives are a mix of Leccino, Raggia, and Frantoio cultivar, traditionally grown in Marche region. Hence, the OMW samples have been analyzed by an independent external certified laboratory to limit the measurement uncertainties related to the complex matrix of the samples. The OMW under investigation has a density of 1,023.7 kg m⁻³, and its chemical characterization is reported in [Table 8](#).

Being its chemical characteristics are in line with the *A. platensis* cultivation, the experimental activities have been conducted as detailed in the following.

Laboratory scale

To evaluate the toxicity of the phenols content of OMW on the *A. platensis* growth and the related biomass quality,³⁸ an experimental test bench has been set up at the premises of Università Politecnica delle Marche, as schematized in [Figure 2](#). In particular, *A. platensis* has been cultivated in different

Table 10. Geometric parameters of the PBR

Parameters	Unit	Value
Tube diameter	M	0.065
Long tube (Lt)	M	9,504.00
Land length (L)	M	15.00
Land width (W)	M	15.00
Surface exposed to light (St)	m ²	1,940.00
Land occupied (S)	m ²	225.00
Volume exposed to light (V)	m ³	29.50
S/V	m ² m ⁻³	5.62
V/S	m ³ m ⁻²	0.17
St/V	m ² m ⁻³	48.5

conditions: whole Zarrouk medium and Zarrouk medium with the addition of the most abundant phenols in OMW at a constant temperature of 28°C and under a constant photon flux density (PFD) of 45 mmol photons m⁻²s⁻¹.

Effect of phenolic compounds on algal growth

In OMW, the three most abundant phenols potentially affecting microalgae growth are tyrosol, hydroxytyrosol, and caffeic.⁴⁴ To test their influence on the *A. platensis* cultivation at laboratory scale, all these phenolic compounds were purchased from Sigma-Aldrich: caffeic acid (C₉H₈O₄), molecular weight (MW) 180.16 g mol⁻¹, purity ≥ 98% (high-pressure liquid chromatography [HPLC]); tyrosol (C₈H₁₀O₂), MW 138.16 g mol⁻¹ purity 98% (HPLC) and hydroxytyrosol (C₈H₁₀O₃), MW 154.16 g mol⁻¹, purity ≥ 98% (HPLC). The three compounds were dissolved in water (tyrosol and hydroxytyrosol) or pure ethyl alcohol (caffeic acid) for the creation of high-concentration stocks to be used for the experiments. Because phenolic compounds are known to be toxic already at low concentrations (for *Chlorella pyrenoidosa* when >5 mg L⁻¹)⁴⁵ and OMW valorization as algal growth medium implies dilution, the concentrations of phenols used in these experiments were five times lower than the common concentrations found in OMW⁴⁴: 40.5 mg L⁻¹ of tyrosol, 63.2 mg L⁻¹ of hydroxytyrosol, 28.1 mg L⁻¹ of caffeic acid.

Cells acclimated to the experimental growth conditions were inoculated into six-well plates to a final density of 50,000 cells mL⁻¹ for a volume of 15 mL per well. Growth was evaluated both in the absence of phenols (control) and in the presence of each phenol. For each condition, three biological replicas were made. Growth was assessed approximately every 2 days for a total period of 16 days (15 days post-inoculation). The growth of *A. platensis* was assessed spectrophotometrically at a wavelength of 670 nm, using Beckman DU 640 spectrophotometer. The absorbance was then converted into the biomass dry weight thanks to a calibration curve where 0.005, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, and 5 mL of exponentially growing cultures were dried on pre-combusted filters and weighted. For dry weight determination, an aliquot of culture was filtered on 0.2 μm filters, washed with deionized water, and dried overnight at 80°C.⁴⁶

Macromolecular composition by FTIR spectroscopy

Due to the complex matrix of microalgae biomass, Fourier transform infrared (FTIR) spectroscopy has been used to profile the relative abundance of carbohydrates, lipids, and proteins in *A. platensis*.

Cells were harvested by centrifugation at 1,500 g for 10 min, washed once with an iso-osmotic solution of ammonium formate to minimize medium carry-over, and re-suspended in a small volume of the latter. Aliquots of 50 μL of this suspension containing intact cells were deposited on silica windows (Crystran, Poole, UK) and desiccated in an oven at 80°C for at least 3 h. Silica windows with 50 μL of iso-osmotic ammonium formate solution were treated as the samples and used as blanks.

FTIR spectra were acquired with a Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany), and at least 3 distinct spectra were averaged and cut between 1,800 and 900 cm⁻¹, baselined and normalized on the amide I peak.⁴⁷ Band assignment was performed according to Giordano et al.³⁹

Pilot scale

Later, the techno-economic feasibility of growing *A. platensis* in OMW was verified through a pilot scale test at the premises of Università Politecnica delle Marche Spin-off GreenTech S.r.l. The pilot scale apparatus was composed of four distinct blocks: (1) OMW treatment, (2) growth medium preparation and supply, (3) *A. platensis* cultivation, and (4) *A. platensis* harvesting and drying. Figure 3 depicts the layout of the process, with an overview of the single unit involved in the system.

The OMW was stored in a 1 m³ polyethylene tank and delivered to the filtration unit using a centrifugal pump. The OMW treatment consists of a two-step filtration, based on MF and UF membranes, which have different pore sizes and employ diverse retention materials to separate bacteria and concentrate macromolecules or colloids from OMW. More precisely, the UF filtration membrane was made of polysulfone with a total filtration area of 12 m². Therefore, in the first step, the organic compounds were removed from OMW while the permeate was delivered to the second filtration step. The permeate from this second filtration step was sent directly to the sewage while the retentate was recovered and used in the medium preparation of *A. platensis*. To evaluate the correct amount of OMW retentate in the medium for microalgae cultivation,

the obtained OMW retentate from the two-step filtration process has been analyzed by an external independent certified laboratory.

The OMW retentate was blended with water from the aqueduct whose chemical composition is reported in Table 9. The tap water was softened, deprived of chlorine and microorganisms, and then conveyed into a polyethylene tank with a capacity of 3 m³. The tank was previously sterilized with a bleach solution of sodium hypochlorite. To prevent microorganism build-up during the storage and then microalgae culture contamination, the stored water was then recirculated through a 0.2 μm filter and UV sterilizer. Then, the water and the OMW nutrient mixture were mixed for 30 min using a mixer. Hence, the prepared medium was continuously recirculated through a 0.2 μm filter and UV sterilized. Once this operation was completed, the medium was delivered to the PBR.

The PBR consists of horizontal tubes with a total volume of 40 m³. It is arranged in 6 modules as depicted in Figure 3. Each module is composed of 288 glass tubes, 144 “U” bend, and 108 couplings and was supported by a metallic structure. The geometric parameters of the PBR are summarized in Table 10. Each module is equipped with a diaphragm pump, a stainless-steel tank, and 128 led tubes for artificial lighting. The illumination system ensures 130 μmol photons s⁻¹ m⁻² and a 12:12 h photoperiod. The plant has a heating and air conditioning system that ensures a constant room temperature of 27°C during the illumination period.

Therefore, the *A. platensis* strain 85.79 SAG Culture Collection of Algae at Gottingen University⁴⁸ was cultured in the PBR with a continuous operation mode. Based on their growth rate, the microalgae were harvested and delivered to a stainless-steel tank, and the equivalent volume of fresh media was supplied back to the PBR. To follow growth rate of *A. platensis*, a daily sample of 30 mL was collected and filtered through a glass fiber filter in a filtration ramp. Once filtered, the sample was washed twice with distilled water, dried overnight at 95°C, and weighted using an analytical scale.⁴⁹

In the harvesting and drying section of the pilot plant, the *A. platensis* was then separated from the liquid medium with a vibrating screener having a screening net of 50 μm and able to treat up to 2 m³ h⁻¹ of the input stream. Then, the microalgae paste obtained from the screener with a moisture content of about 80% was dried with an electric dryer at 40°C overnight. At the end of the drying process, the *A. platensis* biomass reached a moisture content of 10%. To verify the compliance of *A. platensis* biomass produced using OMW medium with food regulations, the dried biomass was then analyzed again by an independent external certified laboratory.^{50,51} The residual medium from the harvesting and drying section was instead sent back to one of the polyethylene tanks of the growing medium section and reused for its preparation.

All the spent medium resulting from the cultivation of *A. platensis* is equivalent to domestic waste water, as defined by Legislative Decree 152/2006,⁵² and can be discarded directly to the sewerage system without any further treatment.

ACKNOWLEDGMENTS

No funding support received. We wish to thank the anonymous reviewers for their valuable comments.

AUTHOR CONTRIBUTIONS

Conceptualization: M.M., G.B., A.N., A.M., and L.C.; methodology: M.M., G.B., and A.N.; formal analysis and investigation: M.M. and A.N.; writing – original draft: M.M., G.B., and A.N.; writing – review & editing: M.M., G.B., A.N., A.M., and L.C.; resources: M.M.; A.N., and L.C.; supervision: M.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: June 13, 2023

Revised: December 15, 2023

Accepted: January 17, 2024

Published: February 6, 2024

REFERENCES

- European Parliament (2020). Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance. *Off. J. Eur. Union* 2013, 1–17.
- Alberti, M.A., Blanco, I., Vox, G., Scarascia-Mugnozza, G., Schettini, E., and Pimentel da Silva, L. (2022). The challenge of urban food production and sustainable water use: Current situation and future perspectives of the urban agriculture in Brazil and Italy. *Sustain. Cities Soc.* 83, 103961.
- Pattara, C., Salomone, R., and Cichelli, A. (2016). Carbon footprint of extra virgin olive oil: a comparative and driver analysis of different production processes in Centre Italy. *J. Clean. Prod.* 127, 533–547.
- Garcia-Castello, E., Cassano, A., Criscuoli, A., Conidi, C., and Drioli, E. (2010). Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system. *Water Res.* 44, 3883–3892.
- Vitolo, S., Petarca, L., and Bresci, B. (1999). Treatment of olive oil industry wastes. *Bioresour. Technol.* 67, 129–137.
- Al-Hmoud, L., Al-Saida, B., and Sandouqa, A. (2020). Olive Mill Wastewater Treatment: A Recent Review. *Jordanian J. Eng. Chem. Ind.* 3, 91–106.
- Amaral, C., Lucas, M.S., Coutinho, J., Crespi, A.L., do Rosário Anjos, M., and Pais, C. (2008). Microbiological and physicochemical characterization of olive mill wastewaters from a continuous olive mill in Northeastern Portugal. *Bioresour. Technol.* 99, 7215–7223.
- Ntaikou, I., Kourmentza, C., Koutrouli, E.C., Stamatelatou, K., Zampraka, A., Kornaros, M., and Lyberatos, G. (2009). Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. *Bioresour. Technol.* 100, 3724–3730.
- Mohawesh, O., Albalasmeh, A., Al-Hamaiedeh, H., Qaraleh, S., Maaitah, O., Bawalize, A., and Almajali, D. (2020). Controlled Land Application of Olive Mill Wastewater (OMW): Enhance Soil Indices and Barley Growth Performance in Arid Environments. *Water Air Soil Pollut.* 237, 214.
- Paraskeva, P., and Diamadopoulos, E. (2006). Technologies for olive mill wastewater (OMW) treatment: a review. *J. Chem. Technol. Biotechnol.* 81, 1475–1485.
- Vuppala, S., Bavasso, I., Stoller, M., Di Palma, L., and Vilardi, G. (2019). Olive mill wastewater integrated purification through pre-treatments using coagulants and biological methods: Experimental, modelling and scale-up. *J. Clean. Prod.* 236, 117622.
- Chrispim, M.C., Scholz, M., and Nolasco, M.A. (2021). Biogas recovery for sustainable cities: A critical review of enhancement techniques and key local conditions for implementation. *Sustain. Cities Soc.* 72, 103033.
- Mastoras, P., Vakalis, S., Fountoulakis, M.S., Gatidou, G., Katsianou, P., Koulis, G., Thomaidis, N.S., Haralambopoulos, D., and Stasinakis, A.S. (2022). Evaluation of the performance of a pilot-scale solar still for olive mill wastewater treatment. *J. Clean. Prod.* 365, 132695.
- Hube, S., Eskafi, M., Hrafnkelsdóttir, K.F., Bjarnadóttir, B., Bjarnadóttir, M.Á., Axelsdóttir, S., and Wu, B. (2020). Direct membrane filtration for wastewater treatment and resource recovery: A review. *Sci. Total Environ.* 710, 136375.
- Maragkaki, A.E., Vasileiadis, I., Fountoulakis, M., Kyriakou, A., Lasaridi, K., and Manios, T. (2018). Improving biogas production from anaerobic co-digestion of sewage sludge with a thermal dried mixture of food waste, cheese whey and olive mill wastewater. *Waste Manag.* 71, 644–651.
- Elkacmi, R., Boulmal, N., Kamil, N., and Bennajah, M. (2017). Techno-economical evaluation of a new technique for olive mill wastewater treatment. *Sustainable Production and Consumption* 10, 38–49.
- Farooque, M., Zhang, A., Thüerer, M., Qu, T., and Huisingsh, D. (2019). Circular supply chain management: A definition and structured literature review. *J. Clean. Prod.* 228, 882–900.
- Premaratne, M., Nishshanka, G.K.S.H., Anthonio, R.A.D.P., Liyanaarachchi, V.C., Thevarajah, B., Nimarshana, P.H.V., Malik, A., and Ariyadasa, T.U. (2022). Resource recovery from waste streams for production of microalgae biomass: A sustainable approach towards high-value bio-refineries. *Bioresour. Technol. Rep.* 18, 101070.
- Chacón-Lee, T.L., and González-Mariño, G.E. (2010). Microalgae for “Healthy” Foods—Possibilities and Challenges. *Compr. Rev. Food Sci. Food Saf.* 9, 655–675.
- Hodaifa, G., Malvis, A., Maaitah, M., and Sánchez, S. (2020). Combination of physicochemical operations and algal culture as a new bioprocess for olive mill wastewater treatment. *Biomass Bioenergy* 138, 105603.
- Chia, S.R., Chew, K.W., Show, P.L., Yap, Y.J., Ong, H.C., Ling, T.C., and Chang, J.S. (2018). Analysis of Economic and Environmental Aspects of Microalgae Biorefinery for Biofuels Production: A Review. *Biotechnol. J.* 13, e1700618.
- Peng, L., Fu, D., Chu, H., Wang, Z., and Qi, H. (2020). Biofuel production from microalgae: a review. *Environ. Chem. Lett.* 18, 285–297.
- Mennella, L., Tosco, D., Alberti, F., Cembalo, L., Crescimanno, M., Del Giudice, T., Galati, A., Moglie, M., Scardera, A., Schifani, G., et al. (2020). Perspectives and challenges of small scale plant microalgae cultivation. Evidences from Southern Italy. *Algal Res.* 45, 101693.
- Bhatt, A., Khanchandani, M., Rana, M.S., and Prajapati, S.K. (2022). Techno-economic analysis of microalgae cultivation for commercial sustainability: A state-of-the-art review. *J. Clean. Prod.* 370, 133456.
- Li, S., Zhao, S., Yan, S., Qiu, Y., Song, C., Li, Y., and Kitamura, Y. (2019). Food processing wastewater purification by microalgae cultivation associated with high value-added compounds production — A review. *Chin. J. Chem. Eng.* 27, 2845–2856.
- Daneshvar, E., Zarrinmehr, M.J., Koutra, E., Kornaros, M., Farhadian, O., and Bhatnagar, A. (2019). Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment and biochemical composition. *Bioresour. Technol.* 273, 556–564.
- Ji, M.K., Abou-Shanab, R.A.I., Kim, S.H., Salama, E.S., Lee, S.H., Kabra, A.N., Lee, Y.S., Hong, S., and Jeon, B.H. (2013). Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO₂ for nutrient removal and biomass production. *Ecol. Eng.* 58, 142–148.
- Paiho, S., Wessberg, N., Pippuri-Mäkeläinen, J., Mäki, E., Sokka, L., Parviainen, T., Nikinmaa, M., Siikavirta, H., Paavola, M., Antikainen, M., et al. (2021). Creating a Circular City—An analysis of potential transportation, energy and food solutions in a case district. *Sustain. Cities Soc.* 64, 102529.
- Abu-Ghosh, S., Dubinsky, Z., Verdelho, V., and Iluz, D. (2021). Unconventional high-value products from microalgae: A review. *Bioresour. Technol.* 329, 124895.
- Prüser, T.F., Braun, P.G., and Wiacek, C. (2021). Microalgae Microalgae as a novel food Potential and legal framework. *Ernaehrungs Umsch. Int.* 68, 78–85.
- Lim, H.R., Khoo, K.S., Chew, K.W., Chang, C.K., Munawaroh, H.S.H., Kumar, P.S., Huy, N.D., and Show, P.L. (2021). Perspective of Spirulina culture with wastewater into a sustainable circular bioeconomy. *Environ. Pollut.* 284, 117492.
- Zhang, F., Man, Y.B., Mo, W.Y., and Wong, M.H. (2020). Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. *Rev. Aquac* 12, 582–599.
- Thevarajah, B., Nishshanka, G.K.S.H., Premaratne, M., Nimarshana, P.H.V., Nagarajan, D., Chang, J.S., and Ariyadasa, T.U. (2022). Large-scale production of Spirulina-based proteins and c-phycocyanin: A bio-refinery approach. *Biochem. Eng. J.* 185, 108541.
- Markou, G., Chatzipavlidis, I., and Georgakakis, D. (2012). Cultivation of *Arthrospira* (*Spirulina*) *platensis* in olive-oil mill wastewater treated with sodium hypochlorite. *Bioresour. Technol.* 112, 234–241.
- Mollo, L., Drigo, F., Moglie, M., and Norici, A. (2023). Screening for tolerance to natural phenols of different algal species: Toward the phycoremediation of olive mill wastewater. *Algal Res.* 75, 103256.
- Zarrouk, C. (1966). Contribution a l'etude d'une cyanophycee: Influence de divers facteurs physiques et chimiques sur la croissance et

- photosynthese de *Spirulina maxima* (Setch et Gardner) Geitler. PhD thesis (Université De Paris).
37. Dixit, G., Kiran Kumar, A., Shah, E., Patel, A., and Sharma, S. (2017). Dairy Effluent to Irrigation Water: A Holistic Bioremediation Process Using Microalgal Treatment. *Int. J. Res.* *4*, 895–902.
 38. Clippinger, J., and Davis, R. (2019). Techno-Economic Analysis for the Production of Algal Biomass via Closed Photobioreactors: Future Cost Potential Evaluated Across a Range of Cultivation System Designs. <https://www.nrel.gov/docs/fy19osti/72716.pdf>.
 39. Gerotto, C., Norici, A., and Giordano, M. (2020). Toward Enhanced Fixation of CO₂ in Aquatic Biomass: Focus on Microalgae. *Front. Energy Res.* *8*, 213.
 40. Norici, A., Gerotto, C., Beardall, J., and Raven, J.A. (2022). Environmental variability and its control of productivity. In *Blue Planet, Red and Green Photosynthesis: Productivity and Carbon Cycling in Aquatic Ecosystems* (Wiley).
 41. Batailler, B.G., and Capareda, S.C. (2022). Preliminary Techno-economic and Sensitivity Analysis of *Spirulina* Powder Production Using a Short-tank Internally Illuminated Concentric-tube Airlift Photobioreactor. *Chem. Eng. Trans.* *92*, 619–624.
 42. Santini, C., and Cavicchi, A. (2023). Organic and Made in Tuscany *Spirulina*: the story of Severino Becagli. In *Case Studies on the Business of Nutraceuticals, Functional and Super Foods* (Springer), pp. 161–174.
 43. Eurostat (2022). Eurostat: Statistics Explained. Electricity price statistics. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics.
 44. Deeb, A.A., Fayyad, M.K., and Alawi, M.A. (2012). Separation of Polyphenols from Jordanian Olive Oil Mill Wastewater. *Chromatogr. Res. Int.* *2012*, 1–8.
 45. Malvis, A., Hodaifa, G., Halioui, M., Seyedsalehi, M., and Sánchez, S. (2019). Integrated process for olive oil mill wastewater treatment and its revalorization through the generation of high added value algal biomass. *Water Res.* *151*, 332–342.
 46. Zhu, C.J., and Lee, Y.K. (1997). Determination of biomass dry weight of marine microalgae. *J. Appl. Phycol.* *9*, 189–194.
 47. Giordano, M., Kansiz, M., Heraud, P., Beardall, J., Wood, B., and McNaughton, D. (2001). Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (Bacillariophyceae). *J. Phycol.* *37*, 271–279.
 48. Friedl, T., and Lorenz, M. (2012). The Culture Collection of Algae at Göttingen University (SAG): A Biological Resource for Biotechnological and Biodiversity Research. *Procedia Environ. Sci.* *15*, 110–117.
 49. Zhu, C.J., and Lee, Y.K. (1997). *Determination of Biomass Dry Weight of Marine Microalgae* (Kluwer Academic Publishers).
 50. European Commission. Commission Regulation (EU) 915/2023 of 25 April 2023 on maximum levels for certain contaminants in food. <https://www.fao.org/faolex/results/details/en/c/LEX-FAOC217510/#:~:text=This%20Regulation%20prohibits%20the%20food,set%20out%20in%20Annex%20I>.
 51. European Commission. (2005). Commission Regulation (EC) 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics.
 52. FAO. (2006). Legislative Decree 152/2006 - Norme in materia ambientale. <https://www.fao.org/faolex/results/details/en/c/LEX-FAOC064213/#:~:text=Italy-,Legislative%20Decree%20No.,is%20composed%20of%20six%20Parts>.