Circular zero-residue process using microalgae for efficient water decontamination, biofuel production, and carbon dioxide fixation

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Abstract

Microalgae can be easily integrated into an effective and scalable zero-carbon-emission circular process that exploits their CO_2 fixation and wastewater remediation capabilities while simultaneously using their residues as an energy and biofuel source to power global sustainable development. Herein, the use of glycogen-rich *Arthrospira platensis* microalgae for efficient water decontamination, biofuel production, and CO_2 fixation is explored. The circular process is initiated by efficient bioremediation of heavy metals (removal > 99%) during microalgal growth, followed by bioethanol production, pellet production, and mineralization of persistent organic pollutants from wastewater, and is accompanied by CO_2 fixation and/or oxygen production. Microalgal biomass generated during heavy metal bioremediation is used to

produce bioethanol via simultaneous saccharification and fermentation (0.4 L per kg of dried microalgal biomass). The residual biomass obtained after bioethanol production is dried and compressed into pellets for its use as a fuel in biomass boilers (calorific power = 20.6 ± 0.2 MJ kg^{-1}). The iron-rich ashes produced during pellet combustion are subsequently used as heterogeneous re-usable Fenton-like catalysts for the photo-Fenton degradation (mineralization > 99%) of persistent organic pollutants. Finally, the low-activity ashes are incorporated into an ash-based medium for the efficient cultivation of microalgae. For each proposed step, product characterization and evaluation were conducted to optimize and enhance the process performance while minimizing negative effects on the environment.

Graphical Abstract

Highlights

- A circular process for water decontamination, biofuel production and CO_2 fixation is proposed.
- Microalgae show heavy metal bioremediation in a wide pH range.
- Saccharification and fermentation of rich-heavy metal *Spirulina* lead to bioethanol.
- Iron-rich ashes are useful catalysts in photo-Fenton POPs mineralization.
- Pelletization of biomass residues provides effective solid biofuel.

Keywords: circular chemistry; microalgae; water decontamination; bioethanol production; pellets

1. Introduction

Microalgae are photosynthetic microorganisms with potential applications in renewable energy, water/air decontamination, and biopharmaceutical/cosmetic/nutraceutical industries, making their utilization important for developing new business models and integrating green and sustainable chemistry within a circular economy [1-5]. Microalgae offer an inexpensive opportunity to power global sustainable development as they are typically found in lakes, rivers, and oceans, but are capable of rapid growth in a variety of environments, being tolerant of broad ranges of salinity, pH, and temperature. This tolerance allows for microalgae cultivation in conditions that are unsuitable for traditional agricultural activities or directly in seas or oceans. Furthermore, the cultivation of microalgae can also make use of water that is unsuitable for human consumption or agriculture [6-8]. Therefore, microalgal cultivation does not compete with or affect traditional agriculture. Given the relatively low cost of startup for microalgal production and the fact that microalgal cultivation and posterior usage are scalable, sustainable, applicable, and suitable for developing countries, the microalgal industry can be viewed as a global job creation engine [6-8].

Microalgae can metabolize nutrients and other inorganic/organic compounds from wastewater and are well suited for the bioaccumulation and biosorption of organic compounds, which are then metabolized [9-11]. Additionally, microalgae have the capability to absorb heavy metals (e.g., Cr, Pb, Fe, and Co ions or metallic nanostructures) [12-15]. This exceptional capacity of microalgae to remove organic compounds and heavy metals, two critical wastewater pollutants, can be used for water decontamination. Since the biochemical composition (micronutrient, protein, carbohydrate, and lipid contents) of the microalgal skeleton depends on the cultivation conditions, microalgae can be used to produce biofuels, animal feeds, dietary supplements, cosmetics, bioplastics, fertilizers, lubricants, drugs, etc [5,16]. The production of biofuels such as biodiesel and bioethanol is one of the most promising applications given the high biofuel yields achieved, offering a renewable energy source with a minimal contribution to environmental pollution and global warming [5, 17-19].

Many researchers have investigated the optimization of individual microalgae-based processes such as lipid extraction or algal biomass fermentation for bioethanol production [17-21]. The development of

linear-route production processes, in which microalgal resources are consumed and wastes are generated, offers environmentally friendly alternatives to several global problems such as climate change, energy crisis or resource depletion. Still, the incorporation of circular chemistry principles to mimic the microalgal life cycle and convert wastes into new sources for other processes is necessary for total sustainability [2]. Therefore, circular thinking can reinvent business and industrial production models by integrating various processes into a single holistic, simple, green, and sustainable process with zero or quasi-zero residue production.

Figure 1 shows the proposed circular process for water decontamination and bioethanol and energy production based on the use of *Arthrospira platensis* microalgae, commonly known as *Spirulina*. The proposed circular process is replicable, simple, easily scalable, green, sustainable, and low-cost. Consequently, it is highly implementable in developing countries in terms of renewability, sustainability, and economic feasibility. The developed process features five successive stages:

1. Microalgae are cultivated using sunlight and wastewater in the presence of iron ions and wastes (i.e., iron-rich ashes) produced during the circular process, which allows the fixation of carbon dioxide and biosorption of (in)organic compounds. The excellent capability of *Spirulina* to undergo biosorption of iron ions and other (in)organic compounds; allows for the simultaneous production of clean water and oxygen in this stage.
2. The glycogen-rich microalgal biomass generated in the culture medium is used to produce bioethanol through simultaneous saccharification and fermentation. In this step, residual biomass and bioethanol are obtained, and carbon dioxide is released.
3. After glycogen extraction, the microalgal biomass is dried, pelletized, and used as a fuel that can be burned for power generation. Consequently, residual microalgal biomass is used to generate power with the simultaneous production of carbon dioxide and ashes.
4. The iron-rich ashes produced during combustion are used for the photo-Fenton mineralization of persistent organic pollutants (e.g., methylene blue) promoted by sunlight. During this process, carbon dioxide is released, while organic compounds are photodegraded.
5. To close the cycle, mineralized water obtained after the photo-Fenton process is reused to cultivate more microalgae.

This holistic process generates clean water, bioethanol, and energy while fixating carbon dioxide and releasing oxygen. The generated residues are recycled and eliminated to realize an efficient life cycle, introducing a zero-residue process based on sustainable use of resources.

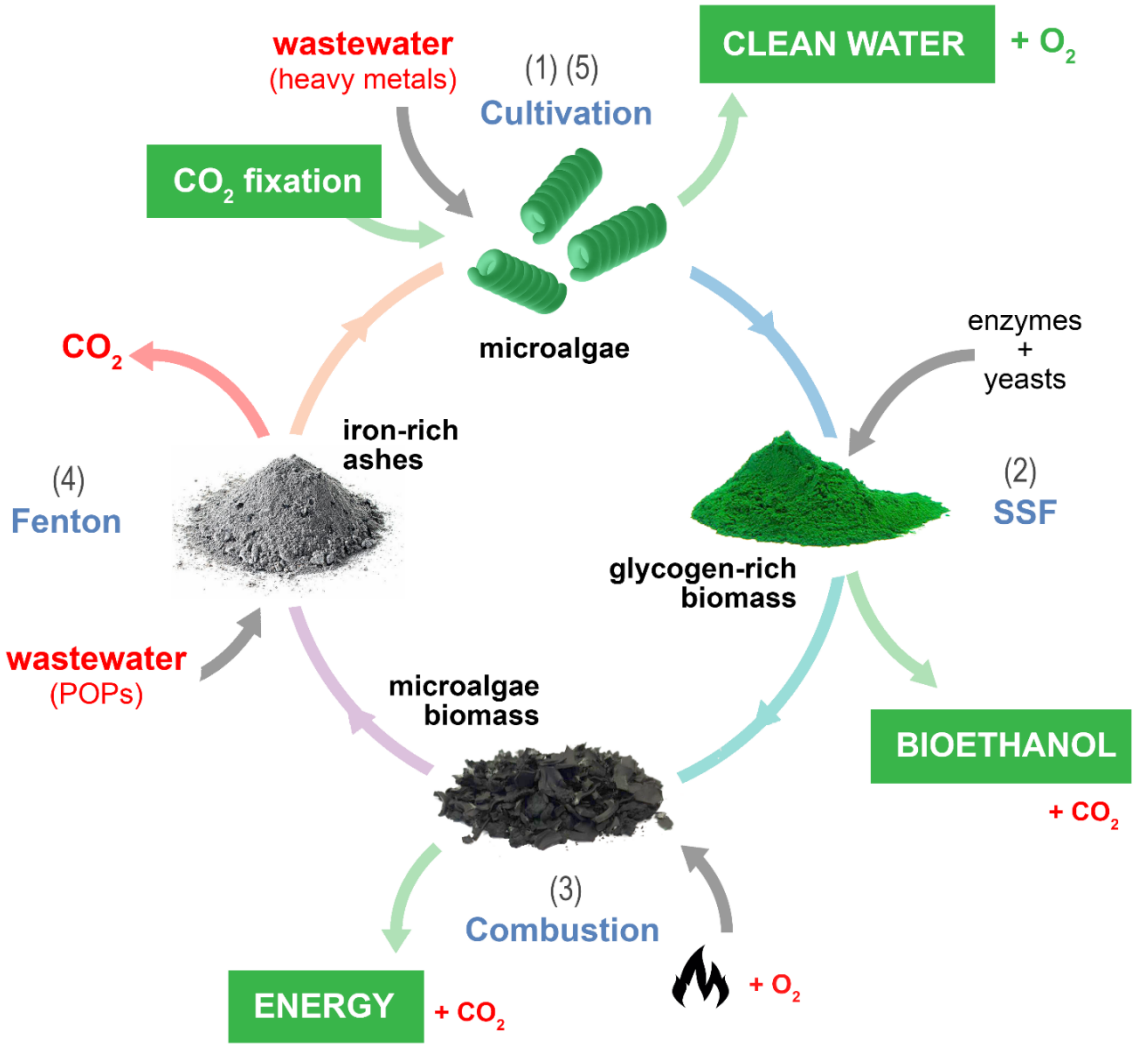

Figure 1: Schematic illustration of the circular process using *Spirulina* microalgae for water decontamination and production of bioethanol and energy through five successive stages: (1) the microalgae cultivation for carbon dioxide fixation and simultaneous production of clean water and oxygen; (2) simultaneous saccharification and fermentation of glycogen-rich microalgal biomass to produce bioethanol; (3) pelletization and combustion of microalgal residues to obtain thermal energy; (4) photo-Fenton mineralization of persistent organic pollutants using microalgal ashes (solid residue) produced during the combustion of microalgal pellets; and (5) reuse of mineralized water, containing microalgal ashes to cultivate more microalgae, closing the circular process.

2. Experimental

2.1. Microalgae cultivation and characterization

Microalgae cultivation: The microalgae *A. platensis var lonar* obtained from Espirulina Natural Company (Catalonia) were cultivated in two 30 L glass tank using either a nitrate-deficient Zarrouk's medium with 4 mM of $NaNO_3$ or a homemade ash-based medium that was 30% (v/v) Zarrouk's medium and 70% (v/v) recycled iron-rich microalgae ash solution (i.e., 1 g of ash per L of decontaminated wastewater; the elemental composition of the iron-rich microalgae ash is summarized in **Table S1**). During cultivation, the cultivation tank was set at 29.5 ± 0.4°C and subjected to intermittent mechanical agitation at 80 rpm in two 30- and 15-min on-off cycles, followed by air bubbling in two 15- and 60-min on-off cycles. The cultivation was performed under natural sunlight irradiation with an average light intensity of 1,350 ± 450 lx. The pH was controlled daily and maintained at a value of 10. Commercial *A. platensis paracas* powder, obtained from SF Superfoods (Australia), was used as a reference.

Biomass determination: Three 20-mL samples of each microalgae cultivation tank were extracted and filtered through pre-weighed Whatman glass microfiber filters (WHA1825021, Sigma-Aldrich) daily for 8 d to estimate the microalgae biomass. Filters containing the biomass were kept in an oven at 40°C, dried, and weighed. Biomass production was calculated as mg L^{-1}.

Photosynthetic pigments determination: The concentration of chlorophyll-a and carotenoids' concentration in microalgae was spectroscopically measured using Lichtenthaler and Wellburn method [22-24]. Three 5-mL samples from each microalgae cultivation tank were centrifuged at 5,000 rpm for 10 min and later washed with phosphate-buffered saline (PBS) solution 3 times. Once the supernatant was discarded, ethanol (96% v/v) was thoroughly mixed in to the microalgae residues, after which the pigments were extracted in ethanol at 65 °C for 90 min. The absorbance of the supernatant in the ethanol solution at 470, 649, and 665 nm was measured using a UV-1800 Shimadzu UV–vis spectrophotometer (Shimadzu Corporation). The concentration of chlorophyll-a and carotenoids was calculated according to Lichtenthaler and Wellburn equation's [22-24].

Glucose and glycogen determination: Glucose and glycogen were mechanically extracted from both commercial and cultivated *Spirulina* in cold PBS (i.e., 0.01 M of phosphate buffer, 0.0027 M of KCl, and 0.137 M of NaCl, pH 7.4) using a laboratory homogenizer at 20,000 rpm for 3 min. Several brief 30-s on-off cycles of mechanical extraction were performed while the sample was immersed in an ice bath. Glucose and glycogen contents were determined using a glucose assay kit (#GAGO20, Sigma-Aldrich) and a glycogen assay kit (#MAK016, Sigma-Aldrich), respectively, in analysis performed in triplicate.

Iron determination: To determine iron content, 0.2 g (dry weight) of commercial and cultivated *Spirulina* were digested in 8 mL of nitric acid in closed Teflon vessels using the microwave sample digestion system Mars 6^{TM} (CEM). After digestion, the solution was diluted to a final volume of 20 mL with Milli-Q water, and total iron content was determined using inductively coupled plasma atomic emission spectroscopy with a Optima 3200RL model ICP-AES (PerkinElmer). Each analysis was performed in triplicate.

2.2. Heavy metal bioremediation

For experiments with the heavy metal bioremediation, 0.4 g (dry weight) of commercial and ash-cultivated *Spirulina* were resuspended in both a single-pollutant laboratory sample, based on a 40-ppm iron(II) chloride (>99.0%, Sigma-Aldrich) solution prepared with Milli-Q water, and real wastewater from the main drain of a local electroplating industry. Once the real effluent was filtered and diluted to a final concentration of Cu(II) of 80 mg L^{-1}, Cu(II) was found to be the primary pollutant in the effluent water. The pH was adjusted to the desired value using 0.1 M of NaOH or HCl prior to resuspending the microalgae biomass. The bioremediation of heavy metals was performed in triplicate at 25.1 ± 0.1°C for 90 min under magnetic stirring at 200 rpm. Iron, copper, nickel, zinc and chromium contents were determined using inductively coupled plasma atomic emission spectroscopy with the same Optima 3200RL model ICP-AES. Prior to conducting the heavy metal bioremediation experiments, the functional groups of the microalgae biomass were determined using fast-Fourier infrared spectra with a Spectrum 2 IR (PerkinElmer).

2.3. Bioethanol production

A simultaneous saccharification and fermentation (SFF) process was used for the bioethanol production from commercial and ash-cultivated *Spirulina.* The following procedure was used for bioethanol production:

- Glycogen and glucose were mechanically extracted in 100 mL of YEP fermentation media (Formedium™) – 10 g L^{-1} of yeast extract + 20 g L^{-1} of Peptone - by using a laboratory homogenizer (20,000 rpm, 3 min). Multiple short on-off cycles (30s) were conducted while the sample was immersed in an ice bath. Bioethanol production was performed with the non-filtered medium using only supernatants. Supernatants were recovered through 0.2 µm syringe filters.
- At the same time, Ethanol Red^{R} (hybrid yeast) provided by Société Industrielle Lesaffre, Division Leaf (France), was prepared. Yeasts were prepared using the following procedure: (i) yeasts were first rehydrated for 30 min in five times their weight of sterile water at 31°C, and then (ii) the hydrated yeasts were grown in YPD medium (Formedium™)–10 g L^{-1} of yeast extract + 20 g L^{-1} of Peptone + 20 g L^{-1} of Glucose–at 31°C under CO_2 bubbling for 30 h, collected by centrifugation (10,000 rpm) for 3 min at 25°C, and washed twice with distilled water. The yeast concentration was controlled and stablished using a Neubauer chamber.
- The supernatants from glycogen and glucose extraction (100 mL) were mixed with 100 mL of saccharification media prepared using YEP fermentation medium to obtain a 1.5 U L^{-1} α-Glucosidase from *Saccharomyces cerevisiae* (#G5003, Sigma-Aldrich) and 3.5 U L^{-1} α-Amylase from porcine pancreas (#A3176, Sigma-Aldrich) concentration. Simultaneously, yeasts were introduced to obtain an initial concentration of ~1.55 10^7 yeast mL^{-1} of Ethanol Red^{R}.
- Next, the SFF process commenced. The SFF process was conducted under magnetic stirring (300 rpm) at 31 ± 0.3°C. The time-dependent evolution of ethanol content was determined during the 26 h period of each experiment using a GC-2010 gas chromatograph (Shimadzu) equipped with a flame ionization detector and a HP-5 column (30 m x 0.32 mm of internal diameter, 0.25 µm of film thickness).

2.4. Pelletization, combustion experiments and ash characterization

A single pelletizer machine was used to produce well-defined single cylindrical pellets. The equipment is composed by a cylinder of 6 mm of internal diameter and 10 cm in length and a piston of 6 mm in diameter and 12.5 cm in length, both placed under the crosshead of a mechanical testing machine. Temperature and force can be measured and controlled during the pelletizing process. The following procedure was used to produce microalgae pellets: (i) microalgae were dried at room temperature to attain a humidity of approximately of 10-15% (w/w); (ii) the cylinder was pre-heated to 55°C prior to introduce the microalgae biomass; (iii) the pre-heated cylinder was loaded with microalgae biomass until it was full; (iv) microalgae was compressed at a maximum force of 3500 N during 90 s; and (v) finally, the pellet was removed from the cylinder by moving the crosshead downward. The fabricated pellet was cooled at room temperature.

Based on the standard UNE-EN ISO 18134-2, humidity content of microalgae pellets was carried out by a balance (accuracy ± 0.0001 g) and an oven at 105 °C. Ashes were obtained after the microalgae pellet combustion in a Heraeus M110 Muffel oven (constant temperature of 1000 °C). Calorific power was determined using an IKA® calorimeter C 5000. Finally, thermogravimetric analysis in an inert (nitrogen) and oxidizing (air) atmosphere were performed from room temperature to 1000°C using a Mettler TGA/SDTA851^e thermobalance (gas flux: 50 mL min^{-1}; heat rate:10 °C min^{-1}).

The obtained microalgae ashes were morphologically characterized using a field emission scanning electron microscopy (FE-SEM, Hitachi S-4800) equipped with an energy-dispersive x-ray spectroscopy detector. The specific surface area of each biomimetic photocatalyst was determined based on the Brunauer–Emmett–Teller (BET) method from N_2 adsorption-desorption isotherms at 77 K using a Micrometrics Tristar-II. Ash elemental composition was carried out by X-ray photoelectron spectroscopy (XPS) at room temperature with a SPECS PHOIBOS 150 hemispherical analyzer (SPECS GmbH).

2.5. Fenton and photo-Fenton experiments

Fenton-like catalysts ($FeCl_2$ (Sigma-Aldrich, 98 %) or iron-rich ashes) were added to 100 mL beakers containing a 10 ppm methylene blue (MB) (Sigma-Aldrich, > 97%) polluted solution. The solution pH was then adjusted to the desired value (pH = 3) using a dilute sulfuric acid. Next, the Fenton or photo-Fenton process was initiated by adding H_2O_2. The initial content of MB, Fenton-like catalyst and H_2O_2 were 10 ppm, 0.1 g L^{-1}, and 10 mM, respectively. All the experiments were thermostated at $20.1 \pm 0.2°C$ and maintained under magnetic stirring (400 rpm). In order to evaluate the adsorption effect, the experiments were also done when the catalyst was kept with the pollutant solution for 30 min prior to adding H_2O_2. For photo-Fenton experiments, an 8W mercury lamp setup with the wavelength of 365 nm (light intensity of 1.4 W m^{-2}) or natural sunlight (average light intensity 1.6 W m^{-2}) were used. Iron-rich ashes were collected and reused to during five consecutive cycles for the degradation of 10 ppm MB – only under UV irradiation – to evaluate the stability, recyclability and reusability properties.

The MB degradation was tracked by UV-vis spectroscopy (UV-1800 spectrophotometer, Shimadzu) in a quartz cuvette with an optical length of 1 cm. Degradation efficiency was determined from the temporal evolution of the reduction in peak intensity at λ_m = 662 nm during a 100-min period of reaction. The mineralization of MB solutions was monitored from the abatement of their total organic carbon (TOC) after 100-min period of reaction, determined on a Shimadzu V_{CSH} analyzer.

3. Results and Discussion

The cyanobacteria *A. platensis* was selected among photosynthetic microorganisms due to its extended cultivation throughout the world and its versatility that allows for potential applications in numerous fields, including the nutritional, nutraceutical, pharmaceutical and energy industries [7, 17, 25]. There are other types of bacteria and other photosynthetic microorganisms that are also effective for heavy metal biosorption (e.g., *Cupriavidus metallidurans*) or bioethanol production (e.g., *Chlamydomonas*) [26, 27]. Consequently, the proposed effective and scalable zero-carbon-emission circular process can be extended to other algal biomass. *A. platensis* is an ideal candidate for integration in a self-sustainable circular process due to its effectiveness in water decontamination, carbon dioxide fixation, oxygen production, and biofuel production. Although the nutritional and nutraceutical applications of *A. platensis*

cultivation are not considered in this work, the extraction of pigments, lipids, or proteins can be easily achieved before the formation of microalgal pellets.

3.1. Microalgal cultivation – Beginning the circular process

Biomass production and the biochemical composition of microalgae can easily be tuned using nutrimental strategies such as varying the culture media composition (i.e., medium salinity, nitrogen and phosphorous limitations or changes in carbon sources) or by physical strategies (e.g., light intensity, irradiation dose). It is known that the cultivation of microalgae in nitrate-deficient media negatively affects biomass production but significantly enhances the production of energy-rich components like lipids and carbohydrates, as well as accessory pigments like carotenoids. This approach also reduces the yield of nitrogen-rich components such as proteins and chlorophyll [28-30].

Consequently, the selection of a proper microalgal culture medium is key to ensuring the viable industrial cultivation of *Spirulina*, since it determines the biochemical composition of the microalgal biomass, which strongly influences the subsequent bioethanol production and biomass yield [10, 15]. Among the unconventional culture media used to avoid high production costs, those based on vegetal ashes (e.g., cow dung ash) or wastewater are notable for their low cost and high performance [6, 31, 32].

Herein, *A. platensis var lonar* was cultivated in an ash-based medium comprising 30 vol. % Zarrouk's medium and 70 vol. % iron-rich recycled microalgae ash solution (1 g of ash per L of decontaminated wastewater). **Table S1** summarizes the physicochemical composition of the microalgal iron-rich ashes and the total organic content of the decontaminated water after photo-Fenton mineralization (see the photo-Fenton water decontamination section for details).

As shown in **Figure 2a** and **b** and **Table S2**, no marked differences were observed between the nitrate-deficient Zarrouk's medium (4 mM $NaNO_3$) and the ash-based medium in terms of biomass production and glycogen content of cultivated *A. platensis*. The glycogen content of the microalgae in nitrate-deficient Zarrouk's medium and the ash-based medium were approximately 70 wt. % of the dry biomass,

whereas those of commercial *A. platensis* and *ash-based A. platensis* cultured in 30 mM $NaNO_3$ Zarrouk's medium were only 7 and 21 wt. %, respectively. In addition, the content of photosynthetic pigments (chlorophyll and carotenoids) was similar between the nitrate-deficient Zarrouk's medium and the ash-based medium, where the content of chlorophyll was approximately 3.5 wt. % and the content of carotenoids was approximately 0.4 wt. %. The chlorophyll and carotenoid contents of *A. platensis* cultured in non-nitrate deficient medium were approximately 8 wt. % and <0.08 wt. %, respectively. Therefore, the biomass production was relatively low compared with production resulting from cultivation in other media, but the biochemical composition was adequate for the effective production of bioethanol.

Consequently, the recycling of the iron-rich ashes used for the photo-mineralization of persistent organic pollutants allowed for the preparation of an economical, efficient, green, and sustainable microalgal culture medium and enabled a zero-residue process. As additional benefits, carbon dioxide is fixated and oxygen is released.

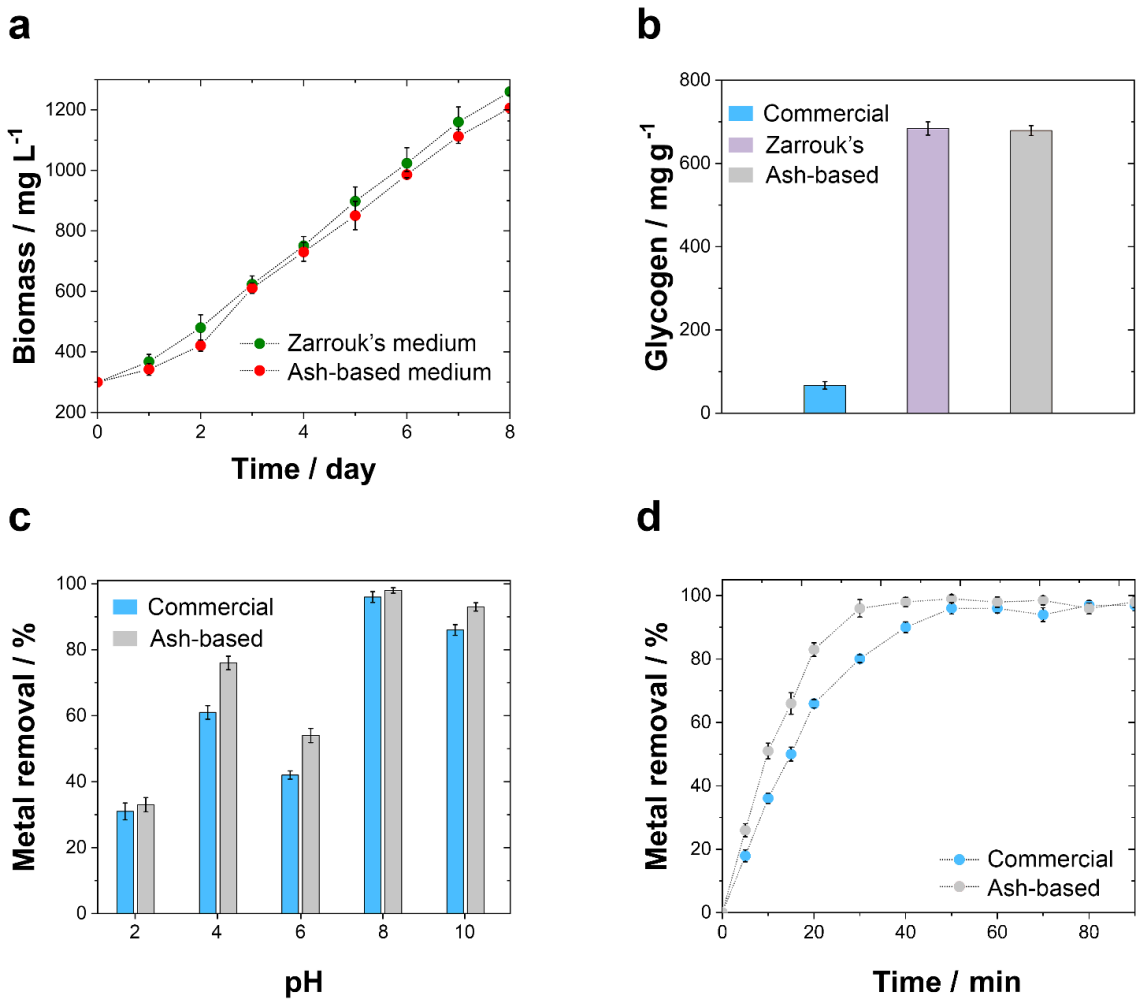

Figure 2: (a) Microalgal biomass production in nitrate-deficient Zarrouk's medium (4 mM $NaNO_3$) and an ash-based medium. (b) Glycogen content of commercial *Spirulina* powder (*A. platensis*), *A. platensis* cultivated in nitrate-deficient Zarrouk's medium, and *Spirulina* grown in the ash-based medium. (c) pH-dependent removal of iron ions (40 ppm) observed for 0.8 g L^{-1} of commercial *Spirulina* powder and *A. platensis* grown in the ash-based medium after 90 min at 25.1 ± 0.1 °C. (d) Time-dependent removal of iron ions (40 ppm) by 0.8 g L^{-1} commercial *Spirulina* powder and *A. platensis* grown in the ash-based medium at pH 8 and 25.1 ± 0.1 °C. Data points and error bars are the mean values of the three cultures and corresponding standard deviations, respectively.

3.2. Heavy metal bioremediation

A. platensis biomass has been proposed as an efficient biosorbent for heavy metal bioremediation [33-35]. For this purpose, commercial *Spirulina* powder *(A. platensis)* and *A. platensis var lonar* cultivated

in an ash-based medium (**Figure S1**) were tested for heavy metal bioremediation in a single-polluted aqueous solution of $FeCl_2$ (40 mg L^{-1}; 98%, Sigma-Aldrich) and multi-polluted real wastewater from the main drain of a local electroplating industry. In the case of the real effluent, reduction of the concentrations of Cu(II), Ni(II), Zn(II), and Cr(VI) after the bioremediation process was analyzed.

For the single pollutant solution, the bioaccumulation was evaluated as a function of pH and contact time. Several mechanisms of heavy metal bioaccumulation have been proposed, based on the activity (active or passive) of surface adsorption and intracellular bioaccumulation; normally the two processes coexist [36-38]. The pH of the medium plays a key role in bioaccumulation, as it determines the activity of functional groups on the surface of the microalgal biomass as well as the degree of ionization and speciation of heavy metals. In this study, functional groups were analyzed by Fourier-transform infrared spectroscopy (**Figure S2**), which revealed the presence of amino, carboxyl, hydroxyl, phosphate, and sulfate moieties as the main functional groups in both types of *Spirulina*. As seen in **Figure 2c**, the total iron removal primarily depended on pH, being highest at pH 8–10. At lower pH values, various negatively charged functional groups (mainly carboxyl) were protonated, which decreased the binding site availability and prevented iron ions from effectively competing with protons.

The above results highlight the importance of biosorption, which is seemingly governed by the exchange between protons and iron ions, in the uptake mechanism [39, 40]. The importance of biosorption in this bioaccumulation process is strengthened by the short contact time required for iron removal (**Figure 2d**). However, the non-depreciable removal activity under strongly acidic conditions, under which little or no uptake is expected, also adds to the low desorption of iron (<40%) under these conditions, which indicates that intracellular accumulation also plays an important role in the employed bioremediation process. Both types of *Spirulina* presented excellent biosorption capabilities, achieving removal efficiencies of >97% after 90 min of microalgae contact with the metal ion solution (**Figure 2d**).

Additionally, to prove the heavy metal bioremediation capability of *Spirulina a real* electroplating wastewater was used as a test solution. The wastewater was filtered, diluted to a final Cu(II) concentration of 80 mg L^{-1} (Cu(II) was the main pollutant of our samples), and adjusted to a pH of 4 before

bioremediation. After 90 min at 25.1 ± 0.1 °C in contact with *Spirulina*, the metal removal values for Cu(II), Ni(II), Zn(II) and Cr(VI) were 68, 75, 42, and 24 %, respectively, for ash-based *Spirulina* and 57, 70, 36, and 16 %, respectively, for commercial *Spirulina*.

Therefore, *Spirulina* is an effective, low-cost, and sustainable biosorbent for heavy metal removal from wastewater. In this circular process, the microalgal biomass used for heavy metal bioremediation is then recycled to produce bioethanol.

3.3. Bioethanol production

Over the last two decades, microalgae have attracted much attention as a source of bioethanol. However, for industrial applications, ethanol production processes require improvement, especially for pretreatment (e.g., chemical, thermal, mechanical, or biological processes, and their combination) [5, 17-19]. Pretreatment promotes cell disruption and thus facilitates the extraction of intracellular carbohydrates which determine the bioethanol production. Mechanical pretreatment methods (e.g. high-pressure homogenization, hydrodynamic cavitation, and bead milling) are the most common used in industry, as they are usually the most attractive in economic and environmental terms. However, mechanical pretreatment is both expensive and complex [41,42]. Bioethanol production from microalgae can be made more viable on an industrial scale (parallel to its optimization) by reducing raw material costs, recycling waste generated by other processes, and reusing the generated residues for other applications. Therefore, the integration of bioethanol production is expected to afford an efficient and economical circular process.

This work aimed to study the potential use of the iron- and glycogen-rich residual microalgal biomass obtained after heavy metal bioremediation for effective bioethanol production. The recycled microalgal biomass was dispersed in yeast extract peptone medium (Formedium, Hunstanton, UK) and subjected to physical lysing using a simple homogenizer to extract glycogen and glucose. The ease of *Spirulina* fragmentation showed that the mechanical process selected to extract carbohydrates was simple, economical, and industrially scalable. Next, to simplify the process, bioethanol production was carried out through simultaneous saccharification and fermentation (SSF) under two different conditions, namely

(i) the direct use of a liquid–triturated biomass mixture (i.e., without filtering) and (ii) after biomass filtering and separation of the glycogen- and glucose-containing supernatants.

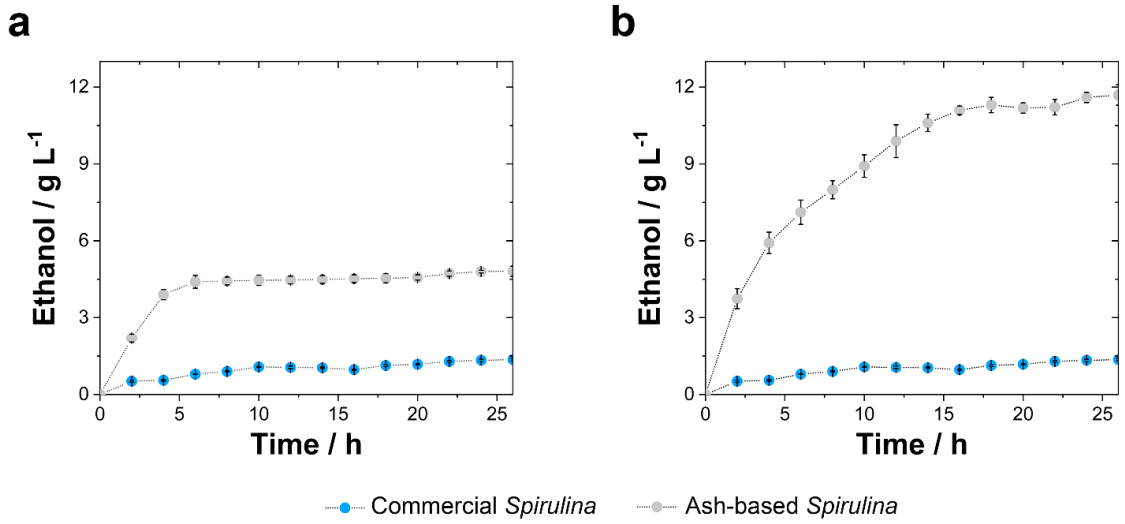

Figure 3: Ethanol production from glycogen and glucose contained in recycled commercial *Spirulina* powder and recycled *Spirulina* grown in an ash-based medium. Results were obtained in the (a) presence and (b) absence of microalgal biomass at 1.5 U L^{-1} of α-glucosidase, 3.5 U L^{-1} of α-amylase, and ~1.55x10^7 yeast mL^{-1} of Ethanol Red. Data points and error bars are the mean values of the three separate cultures and corresponding standard deviations, respectively.

The SSF step was carried out using *α-glucosidase* and *α-amylase* for saccharification (i.e., the conversion of glycogen to glucose) and a commercial industrial yeast (strains of *Saccharomyces cerevisiae*, Ethanol Red) for microbial fermentation (i.e., the conversion of glucose to ethanol), and the results are shown in **Figure 3**. In both cases, the initial glucose and glycogen concentrations were virtually identical (~1.4 g L^{-1} of glucose and ~2.5 g L^{-1} of glycogen for commercial *Spirulina* powder and ~0.8 g L^{-1} of glucose and ~26.7 g L^{-1} of glycogen for *Spirulina* grown in the ash-based medium). Without biomass filtration, bioethanol production of 1.21 ± 0.09 and 4.8 ± 0.2 g L^{-1} were observed for commercial and cultivated microalgae, respectively. However, when the biomass was separated, the respective values increased to 1.36 ± 0.05 and 11.7 ± 0.4 g L^{-1}. These differences were attributed to the reabsorption of glycogen and glucose molecules, the absorption of produced ethanol, and/or the inhibition of the SSF

process by biomass. Consequently, the biomass should be separated from supernatants before the SSF process onset.

The excellent ethanol production (~90% of the theoretical value) observed for *Spirulina* grown in the ash-based medium (~0.4 L per kg of dried microalgal biomass) was comparable to or even higher than those obtained for other carbon feedstocks (**Table S3**) [18, 42-48]. Consequently, these results reinforce the potential of the proposed circular process. The generated residual biomass was subsequently used to fabricate biomass pellets, and the concomitantly produced carbon dioxide was used to cultivate more *Spirulina*, making the ethanol production a zero-residue process.

3.4. Pelletization and pyrolysis of residual microalgal biomass

The residual microalgal biomass from bioethanol production was used to fabricate solid biofuel (non-woody) pellets. Before producing pellets, although not explored here, high value chemicals such as lipids, pigments, antioxidants, vitamins, or proteins, can be extracted. Pellet production is typically performed in multiple steps including harvesting, pretreatment, pelletization, and post-treatment, with the most expensive step being pretreatment, especially biomass drying. Herein, microalgal pellets were produced by a simple pelletizing process based on (1) the collection of fragmented residual biomass from bioethanol production, (2) sunlight irradiation to dry the biomass and reduce its moisture content to 10–15 wt. %, and (3) pellet manufacturing by biomass compression using a single pelletizer. During the last step, the temperature considerably increased because of the high pressure, and several microalgal components (such as lignin) acted as binding agents for biomass fibers to enhance the pellet quality and pelletization without the use of artificial binders. **Figure 3a** shows that well-defined microalgal pellets were easily obtained from the residues of commercial or ash-based *Spirulina*.

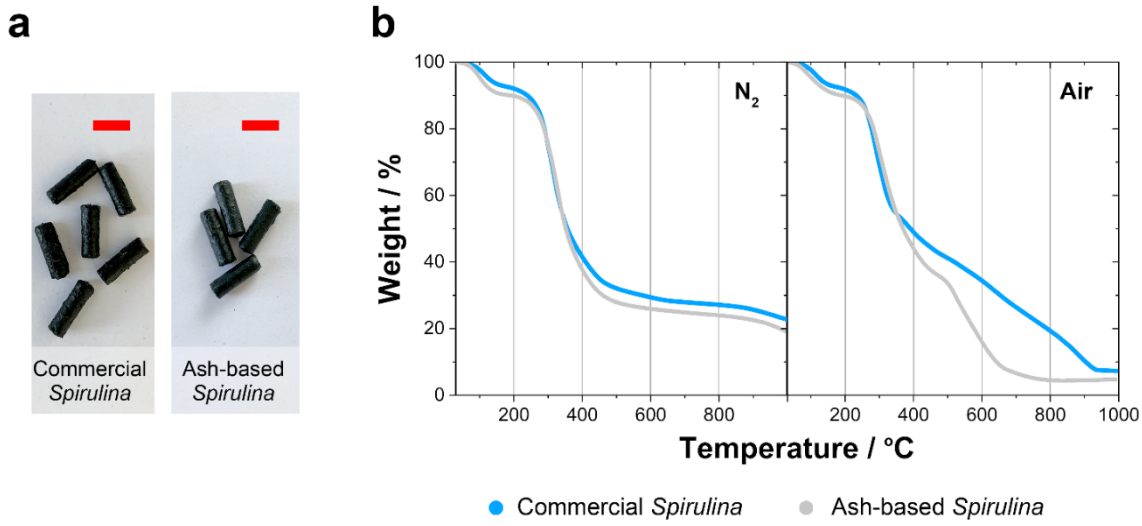

Figure 4: (a) Photographs of commercial *Spirulina* (left) and ash-based *Spirulina* (right) pellets. Scale bar: 1 cm. (b) Thermogravimetric analysis (TGA) profiles of commercial *Spirulina* and ash-based *Spirulina* pellets recorded in nitrogen and air environments

Table 1: Physicochemical properties of the manufactured *Spirulina* pellets.

Microalgal biomass	***Spirulina* species**	**Ash / wt. %**	**Humidity / wt. %**	**Calorific power / MJ kg^{-1}**
Commercial *Spirulina* powder	*A. platensis*	5.9 ± 0.1	6.5 ± 0.3	19.9 ± 0.2
Cultivated *Spirulina*	*A. platensis var lonar*	5.5 ± 0.1	5.1 ± 0.2	20.6 ± 0.2

The physicochemical properties of the manufactured pellets such as ash content, moisture content, and calorific power, were analyzed (**Table 1**) and compared with those stipulated by ISO 17225-6:2014 for non-woody pellets (ash content < 10 wt. %, moisture content < 15 wt. %, calorific power > 14.5 MJ kg^{-1}). These requirements must be fulfilled for the cultivated microalgae to be considered as a commercial biofuel and be used as a fuel in biomass boilers [49]. Our microalgal pellets complied with the

abovementioned requirements and are therefore proposed as an effective solid biofuel. The calorific power of the microalgal pellets was comparable to or even higher than that of other solid biomass fuels such as those obtained from agricultural or forestry crops [50-53].

In addition, the thermal degradation of both commercial and ash-based *Spirulina* pellets in nitrogen and air was investigated by thermogravimetric analysis (TGA) (**Figure 3b**) to confirm their viability as a solid biofuel. Three weight loss steps were observed, which were more evident in the nitrogen environment. The first step (room temperature to 175 °C) corresponding to a weight loss of ~7.5% and was attributed to the vaporization of water and volatiles. The second step (175–725 °C) corresponded to a weight loss of ~65% and 75% in nitrogen and air atmospheres, respectively, was attributed to the carbonization of carbohydrates and proteins. The biomass weight rapidly decreased for temperatures above 300 °C until it began to slow at 450 °C. Consequently, two stages attributable to the thermal decomposition of carbohydrates and sulfur/nitrogen release upon protein degradation were observed. Note that thermal degradation was higher in air (i.e., in a more oxidizing environment). The continuous weight loss (especially in air) observed above 725 °C was attributed to the slow combustion of partly carbonized residue or metal reduction, with 5.9 and 5.5 wt. % of ash (**Figure S3**) obtained for commercial and cultivated microalgae, respectively. The chemical composition of microalgal ash is summarized in **Table S1**. Thus, microalgal biomass pellets are easily fabricated and combusted as solid biofuel for energy applications to produce a small amount of residue suitable for water decontamination and microalgal cultivation. The carbon dioxide produced during combustion can also be fixated during microalgal cultivation.

3.5. Solar photo-Fenton remediation of persistent organic pollutants

The aim of the next stage of the circular process is the total mineralization of persistent organic pollutants into carbon dioxide, water, and inorganic ions to decontaminate wastewater using the Fenton process catalyzed by the recycled iron-rich ash from microalgal pellet combustion. The mineralization process was based on the classical Fenton reaction (Eq. 1) [54-57]:

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O \quad (1)$$

The hydroxyl radicals (•OH) formed during this process are powerful non-selective oxidants that can completely mineralize most organic pollutants to carbon dioxide, inorganic ions, and water. In addition, irradiation, especially with ultraviolet A light, is known to accelerate mineralization, mainly by promoting the direct photoreduction of iron (III)-hydroxo complexes (known as the photo-Fenton reaction) and consequently regenerating the Fe(II) catalyst and producing more hydroxyl radicals [54-59]. Microalgal ashes rich in iron (see **Table S1**) could thus be used to remediate persistent organic pollutants. Methylene blue (MB) was selected as a probe and iron(II) chloride was used as a reference to examine the effectiveness of microalgal ashes in organic pollutant remediation.

As shown in **Figure 5a**, the microalgal ashes comprised porous non-spherical microparticles and exhibited BET (Brunauer-Emmett-Teller) surface areas of 6.8 $m^2\ g^{-1}$ (commercial *Spirulina*) and 16.7 $m^2\ g^{-1}$ (ash-based *Spirulina*) (**Figure S4**).

MB degradation was analyzed in the dark and under irradiation with UV or sunlight, as shown in **Figure 5b** an**d c** and **Table 2**. In the absence of light, the degradation efficiencies reached 11.2, 2.8, and 25.2% after 100 min of reaction time for iron (II) chloride, commercial and ash-based *Spirulina* ashes, respectively. In contrast, degradation efficiencies of 5.6 and 30.4% were obtained for commercial and ash-based *Spirulina* ashes, respectively, under the same conditions but with introduction of heterogeneous catalyst in the pollutant solution for 30 min before H_2O_2 addition. In the dark, the removal after 100 min (**Figure S5**) was negligible in the absence of catalysts (i.e., in the presence of only H_2O_2) but reached 1.8 and 5.3% for commercial and ash-based *Spirulina* ashes, respectively, possibly as a consequence of pollutant adsorption. Moreover, the catalytic performance improved when the process was conducted under UV or solar irradiation, and the degradation exceeded 98% after 100 min of reaction time. The slower removal in the dark for the regeneration of iron(II) is ascribed to the significantly slower kinetics of the Fenton-like reaction (the reaction of hydrogen peroxide with ferric ions) compared to the photoreduction of iron(III)-hydroxo complexes and photodecarboxylation of iron(III)-carboxylate complexes. This Fenton-like reaction leads to the formation of hydroperoxyl radicals, a weak oxidant that cannot oxidize organic matter [54-59].

Mineralization was determined from the total organic content (TOC) reduction after 100 min of reaction time. The maximum mineralization was slightly lower than the MB degradation (**Table 2**), especially when the homogeneous catalyst was used.

Table 2: BET surface areas, iron contents, and photocatalytic MB (10 ppm) degradation of 0.1 g L^{-1} $FeCl_2$ and commercial and ash-based *Spirulina* ashes at 20.1 ± 0.2 °*C* in the dark and under UV light (365 nm) or solar light irradiation. Values in parentheses were obtained under the same conditions when the heterogeneous catalyst was incubated in the MB solution for 30 min before H_2O_2 addition.

	BET surface area / m^2 g^{-1}	**Iron content / ppm**	**Fenton**		**Photo-Fenton (UV irradiation)**		**Photo-Fenton (solar irradiation)**	
			MB degradation / %	**Mineralization / %**	**MB degradation / %**	**Mineralization / %**	**MB degradation / %**	**Mineralization / %**
Iron (II) chloride	–	100	11.2 ± 0.6	6.2 ± 0.3	99.9 ± 0.2	81.4 ± 0.2	99.7 ± 0.1	77.2 ± 0.2
Commercial *Spirulina* ashes	3.8	~20	2.8 ± 0.5 (5.6 ± 0.6)	1.1 ± 0.4	99.6 ± 0.1 (99.8 ± 0.1)	86.2 ± 0.4	98.9 ± 0.2 (98.9 ± 0.2)	79.4 ± 0.3
Ash-based *Spirulina* ashes	23.7	~25	25.2 ± 0.5 (30.4 ± 0.4)	14.3 ± 0.3	99.9 ± 0.1 (99.9 ± 0.2)	88.2 ± 0.3	99.9 ± 0.1 (99.9 ± 0.1)	82.1 ± 0.2

The degradation and mineralization of heterogeneous catalysts, especially for ash-based *Spirulina* ashes (high surface area), were close to or greater than those of the homogeneous catalyst (100 ppm $FeCl_2$), while the iron content of the heterogeneous systems was approximately five times lower. Depending on the heavy metal bioremediation process, microalgal ashes may contain other heavy metals. The Fenton process can be affected by other metals, for example, limited in the presence of Ni(II) ions or synergistically promoted in the presence of Co(II), Cu(II) or Mn(II) ions [60,61].

Finally, the stability and reusability of the microalgal ashes were evaluated by reusing the catalyst in five consecutive MB degradation cycles (**Figure 5d**) under UV irradiation. No significant changes in MB degradation were observed during all successive runs, and an approximately 99% degradation was

attained after five runs. The maximum mineralization was slightly reduced after cycling (from 86.2 to 77.4% for commercial *Spirulina* ashes and from 88.2 to 79.9% for ash-based *Spirulina* ashes), possibly because of the leaching of small amounts of metal ions during consecutive irradiation. Thus, we concluded that the recycled ashes produced by microalgal pellet combustion can be effectively (re)used as efficient catalysts to mineralize persistent organic pollutants.

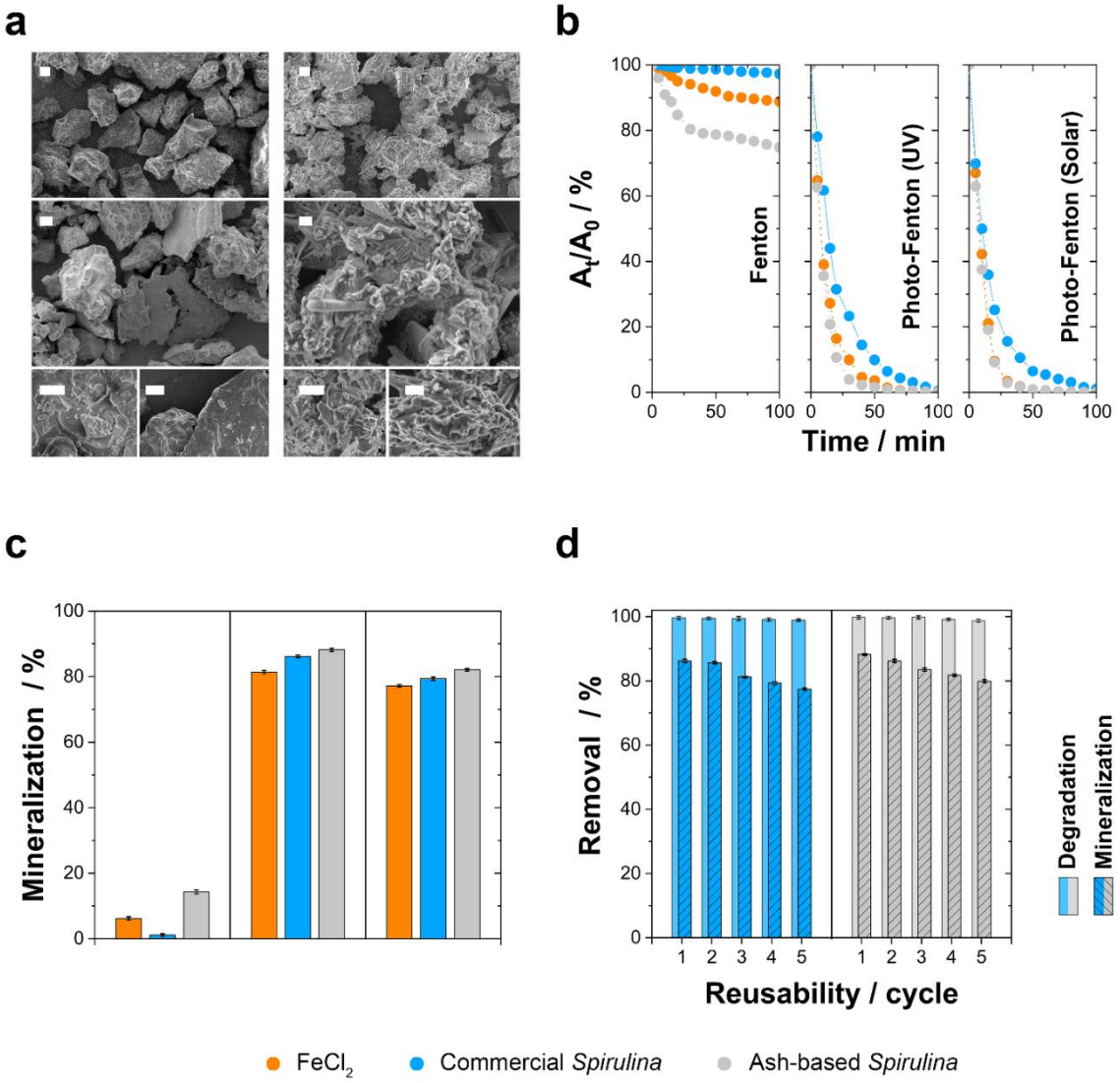

Figure 5: (a) FE-SEM micrographs of commercial *Spirulina* ashes (left) and ash-based *Spirulina* ashes (right). Scale bar: 10 µm. (b) Time-dependent methylene blue (MB) (100 ppm) photodegradation efficiencies and (c) mineralization efficiencies after 100 min of reaction time under Fenton (left), UV photo-Fenton (middle), and solar photo-Fenton (right) conditions at 20.1 ± 0.2 °*C* obtained for 0.1 g L^{-1} $FeCl_2$

and commercial/ash-based *Spirulina* ashes. (d) Reusability (degradation and mineralization efficiencies after 100 min of reaction time) of commercial and ash-based *Spirulina* ashes for the UV photo-Fenton mineralization of MB.

3.6. Closing the cycle – Microalgal cultivation

To close the cycle, the carbon dioxide produced during bioethanol production, combustion, and mineralization of organic pollutants must be effectively used, fixed, and converted to value-added products during *Spirulina* cultivation. It is well known that carbon dioxide bubbling in microalgal cultivation tanks stimulates biomass production. The microalgal ashes used to mineralize persistent organic pollutants or decontaminate water were effectively used to prepare an ash-based medium to cultivate more *Spirulina* microalgae (**Figure 1a**). During this circular process, oxygen was consumed, and carbon dioxide was generated, but the entire circular process offered a positive balance in terms of carbon dioxide fixation and oxygen production. Therefore, clean water, oxygen, bioethanol, and energy were obtained, and no residues were produced.

4. Conclusions

A holistic circular process based on the principles of green and circular chemistry was developed by integrating atmospheric oxygen production, water decontamination, carbon dioxide fixation, and bioethanol/microalgal pellet production. This process mimics the algal life cycle and does not generate any waste, as the residues obtained in each phase are the starting material of the next phase. The circular process is based on the use of *Spirulina* microalgae and sunlight, which are extremely low-cost and sustainable, demonstrating the ease of implementation even in developing countries. The cultivated microalgae and decontaminated water can be used for other applications such as cosmetics or nutraceuticals, allowing for the integration of new phases to maximize economic viability. Furthermore, the extraction of valuable chemicals before pelletization is a potential avenue for improving the profitability.

This circular process can easily be scaled up for industrial applications with minimal infrastructure investment and limited resources. Importantly, this circular process is not limited to *Spirulina* as other microalgae (or mixtures of various microalgae) can also be used.

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Appendix A. Supplementary data

Supplementary data related to this article can be found, in the online version, at doi:

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