EXPLORING SUSTAINABLE CULTIVATION OF GALDIERIA PHLEGREA USING URBAN WASTEWATER: INTEGRATION OFIOT-ENABLED PHOTOBIOREACTOR FOR BIOMASS PRODUCTION AND SILICON-RECOVERY APPLICATIONS

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ABSTRACT: Microalgae, renowned for their sustainable biomass production and the synthesis of valuable compounds, hold promise for various applications, including biotechnology and environmental remediation. Extremophilic strains like *Galdieria* within *Cyanidiaceae* exhibit potential in biometallurgical methodologies. However, scaling up cultivation methods, particularly with the cost-effective urban effluents, presents challenges. Innovative technologies like Twin Layers photobioreactors coupled with IoT integrated systems offer scalable and energy-efficient solutions. This study investigates *Galdieria phlegrea* cultivation using urban wastewater, focusing on growth dynamics, biomass characterization, and metal recovery potential, particularly silicon from photovoltaic panels. Experimental results indicate the effectiveness of selected cultivation conditions in promoting biomass growth, while revealing the presence of Silicon aggregates on algal biomass that was treated with HF acid leachate from dismantled solar panels, suggesting a viable route for silicon recovery. These findings underscore the significance of microalgae in sustainable resource management and circular economy paradigms.

Keywords: Microalgae cultivation; Galdieria phlegrea; Urban wastewater treatment; Biomass production; Resource recovery; Silicon bio-recovery; Photovoltaic panel waste; IoT-based monitoring systems

1. INTRODUCTION

Microalgae are highly valued for their role in sustainable plant biomass production and the valorizationof industrial and biomedical products (Malavasi et al., 2020). Their photosynthetic abilities, CO_2 fixation, and production of valuable compounds like fatty acids, proteins, and carbohydrates make them incrediblyversatile. Widely studied species such as *Chlorella*, *Spirulina*, and *Nannochloropsis* are known for their rapid growth, high productivity, and nutritional composition (Fernández et al., 2021). In recent years, extremophilic strains capable of thriving in extreme environmental conditions have gained attention for their biotechnological potential (Varshney et al., 2015). For instance, Cyanidiaceae species flourish in acidic, high-temperature, and contaminant-rich environments, offering opportunities for the recovery of metals and rare earth elements (Ciniglia et al., 2004).

Among these extremophiles, *Galdieria*, a genus within Cyanidiaceae, stands out due to its potential in innovative biometallurgical methodologies aimed at replacing traditional metallurgical treatments (Iovinella et al., 2023; Minoda et al., 2015). However, challenges persist in scaling up cultivation methods,especially when utilizing urban effluents as nutrient sources (di Cicco, Iovinella, et al., 2021). Innovative technologies, such as Twin Layers cultivation systems integrated with IoT-based monitoring, offer scalable and energy-efficient solutions to address these challenges (Carbone et al., 2020; Naumann et al., 2013; Podola et al., 2017). Furthermore, understanding the metabolic processes of microalgae through isotopic analysis enhances the efficiency and sustainability of cultivation methods.

Additionally, raw tests have been conducted with the biomass cultivated using these innovative photobioreactors to attempt to recover silicon from dismantled photovoltaic panels (PV-WEEE). Silicon, indeed, a crucial component of photovoltaic cells, is a finite resource with high demand in various industries, including electronics and solar energy. With the burgeoning utilization of solar panels worldwide, the efficient recovery of silicon becomes paramount for sustainability and resource conservation (Sapra et al., 2021; Xu et al., 2018). Given the imperative need to recover valuable resources such as silicon (Si) from discarded photovoltaic panels, our study assumes significance. Hence, in our experimental design, we also investigated the possibility of utilizing dry biomass for bio-recovery preliminary tests. This approach aligns with the growing interest in employing biomass, particularly microalgae, for resources recovery processes (Finkel, 2016). Microalgae, with their high surface area and metal-binding capabilities, present a promising avenue for extracting critical elements from complex matrices suchas photovoltaic panel waste. Moreover, exploring the efficacy of utilizing biomasses in such processes not only offers a sustainable alternative to conventional chemical methods but also contributes to the circular economy paradigm by valorizing organic waste streams (Ansanelli et al., 2021; Azeumo et al., 2019; Chakankar et al., 2017; Chowdhury et al., 2020). By integrating biomasses into metal recovery processes, we move towards a more environmentally friendly and resource-efficient approach to waste management and resource extraction.

Therefore, our study not only addresses the immediate need to find an innovative system for the sustainable cultivation of biomasses, but it also explores innovative strategies for resource recovery from photovoltaic panel waste, thereby fostering sustainability and circularity in the solar energy sector.

2. MATERIALS AND METHODS

2.1 Design of the Cultivation system

The study employs Twin Layers photobioreactor technology for sustainable cultivation of *Galdieria phlegrea* using urban wastewater. This system features two layers: a source layer for culture medium flow and a substrate layer for biomass adhesion. Advantages include reduced energy consumption compared to liquid-phase systems and efficient collection of dense, low-moisture algal biomass. *G. phlegrea*'s natural growth pattern on mineral substrates enhances nutrient uptake and gas exchange. Thetechnology isolates algae from impurities in urban wastewater, resulting in clean biomass with high marketvalue (Carbone et al., 2017; Carbone et al., 2020; Shi et al., 2014). Notably, using untreated primary wastewater poses challenges due to higher turbidity levels. However, the system enables clean biomass production with low energy demand, avoiding trade-offs between biomass purity and process performance common in liquid-phase cultivation with urban wastewater (González et al., 2020).

The photobioreactor development process involved designing 3D models in collaboration with external companies for prototype construction. Multiple versions of structural components were produced to assess usability and durability. The prototypes consist of four main parts: a supporting structure, base, lid for liquid circulation, growth chamber, and a peristaltic pump. The supporting structure ensures stability and accessibility, constructed with aluminum profiles and steel bolts. The base and lid direct liquid growthmedium flow, and they are made from ABS polymer via 3D printing for chemical resistance. The growth chamber, made of tempered glass, houses the algal cultivation substrate. A customized sensor system monitors environmental parameters in real-time, facilitating remote process management. The system transmits data to an online platform for analysis and control, enabling optimization of operations and maintenance in large-scale applications.

Figure 1. Laboratory-scale Twin Layers photobioreactor prototypes with sensors, polycarbonate discs andattached biomass.

2.2 The cultivation tests

2.2.1 Experimental Setup Summary and Preparation of culture media

The experiments aimed to monitor biomass growth under varied environmental conditions at the laboratory scale, anticipating future scale-up. Key process variables affecting microalgal growth performance include pH, nutrient availability, lighting conditions, and temperature. Five cultivation tests were conducted over 15 days, with the experimental setup summarized in Table 1.

Table 1 – Experimental setup of the 5 cultivation tests

ID Experiment	Duration (days)	pH	Temperature	Light/Dark hours	Illumination (lux)	Control	Test	Frequency of fresh feeding
Exp 1	15	2.5	37°C	16:8	~1000	Allen medium	Wastewater	never
Exp 2	15	2.5	28-35°C	16:8	~1000	Allen medium	Wastewater	never
Exp 3	15	2.5	28-35°C	16:8	~32000	Allen medium	Wastewater	never
Exp 4	15	2.5	28-35°C	16:8	~32000	Allen medium	Wastewater	2-3 days
Exp 5	15	2.5	28-35°C	16:8	~32000	Allen medium	Wastewater	continuously

Culture media were prepared using urban primary effluents (W), sourced from a Conventional Activated Sludge wastewater treatment plant, and Allen medium (A), the latter being prepared as in previous studies (di Cicco, Palmieri, et al., 2021). Effluents were filtered, pH-adjusted, and sterilized for experiments. A continuous flow of fresh culture medium was supplied for one experiment, requiring large volumes stored under controlled conditions.

Temperature was controlled at 37°C or varied between 28-35°C, through the use of appropriate air-conditioning systems. Light intensity ranged from 1000 to 32000 lux, and lighting cycles were adjusted accordingly. Medium refresh rates varied from continuous to periodic replenishment, depending on the experiment.

2.2.2 Growth monitoring, Biomass Characterization, and Analysis of NH_4^+, NO_3^-, and PO_4^{3-} in W and A

In order to start the cultivation tests, a thermophilic algal strain (*Galdieria phlegrea* ACUF 784.3) was cultured under controlled conditions and inoculated for experiments, using polycarbonate microfilters membranes and customized cylindrical-shaped moulds. Biomass concentration was calibrated prior to inoculation. Biomass growth was monitored gravimetrically, while other samples were harvested every 2- 3 days in order to perform physical biomass characterization analyses throughout the experiments.

In particular, alongside gravimetric measurements, compositional and morphological analyses were conducted. Elemental analysis with isotopic ratio mass spectrometry (EA-IRMS) and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) provided insights into biomass compositionand morphology. The references for the protocols involved in these analyses can be found in (di Cicco, Palmieri, et al., 2021) and (Germinario et al., 2019).

Finally, ionic concentrations in culture media were analyzed over time using colorimetric assays to track variations in ammonium, nitrate, and phosphate levels. To perform these investigations, we used a portable spectrophotometer Hach DR1900 coupled with proper test-in-cuvette kit ready to use and from the same company.

2.3 Raw tests for Silicon recovery from PV-WEEE

As anticipated in the Introduction, biomass cultivated with urban wastewater has been employed to investigate its potential use as a substrate for the bio-recovery of silicon from discarded photovoltaic panels (PV-WEEE). Therefore, in these raw tests, the aim was to investigate the possibility of solubilizing and bonding Silicon and other potential elements of interest from photovoltaic panels onto the biomass. To accomplish this, a preliminary qualitative test was conducted, which consisted of two phases:

- In the first phase, manually shredded coarse pieces of discarded photovoltaic panels were placed in four different acidic solutions at equimolar concentration (5M). The acids selected for the investigation were HF, HCl, H_2SO_4, and H_3PO_4. At the end of the reaction period, the obtained liquid was filtered and properly stored.
- In the second phase, a certain amount of biomass were brought into contact with the acidic solutions for a short period (30 minutes). Afterward, the resulting material was transferred onto the same polycarbonate membrane filters used for cultivating the microalgae, aiming to obtain dense biomass disks for lyophilization and analysis.

The obtained disks underwent qualitative evaluation using SEM-EDS methodology (Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy), following the working parameters presented in (Germinario et al., 2019).

3. RESULTS AND DISCUSSION

The experimental setup in this study allowed for the comparison of biomass response in terms of growth under five different environmental conditions. These conditions varied in ambient temperature, quality of lighting, or the supply of fresh culture medium, using a Twin Layers biomass cultivation system equipped with smart sensor technology for real-time and continuous process parameter monitoring.

Upon initial observation, operational conditions in Exp3 proved to be the most effective, yielding significantly higher growth after 15 days of observation. Specifically, with W, a final average biomass surface density of 25.4 g/m^2 was recorded in Exp3, representing a 91% increase compared to the average of 13.3 g/m^2 obtained from the other four experiments (Fig. 2). With culture medium A, Exp3 recorded a surface density 56% higher than the average obtained in the other four tests (19.6 vs. 12.6 g/m^2).

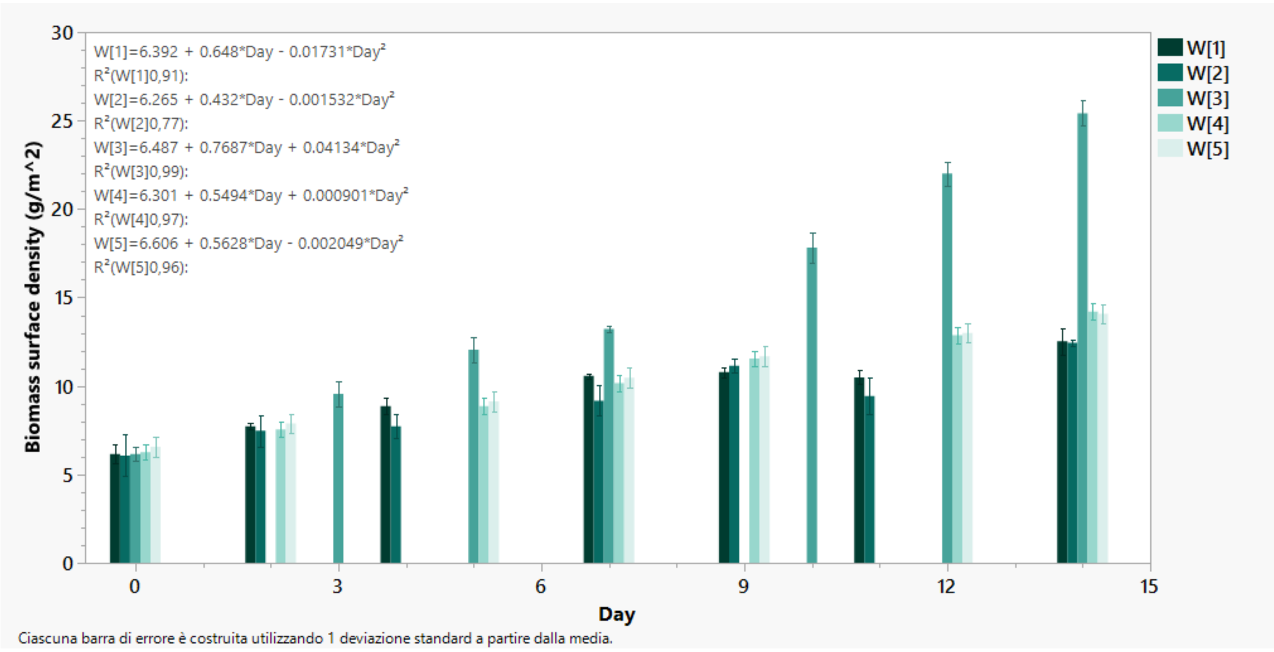

Figure 2 - Time trend of biomass grown with culture medium W in Exp 1 to 5, expressed as surface density.

Further analytical detail involved the use of mathematical models to describe the temporal trends observed in the five experimental conditions, providing additional insights into growth dynamics. Three mathematical models were applied separately to treatments A and W. Despite the usual sigmoidal growthcurve exhibited by biological organisms, quadratic polynomial regression emerged as the best fit for both A and W conditions, indicating a parabolic trend. Zooming into Exp3, the models revealed long-term biomass yields, suggesting the potential for significant biomass production over time. The exponential model, in particular, forecasted a biomass yield of 1 kg/m^2 in approximately 2 months for both culture mediums (54 days with W, 64 days with A). Energy performance estimations suggested substantial potential for energy recovery from biomass production. Calculations indicated the recovery of approximately 5 kWh of energy per kg/m^2 of dry biomass produced using a single liter of culture medium,with further energy gains expected in large-scale cultivation systems.

Apart from energy production, microalgal biomass of the Galdieriaceae family harbors biocompounds of high biotechnological interest. Protein, carbohydrate, and lipid content make it a valuable resource for various applications, including biofuel production and extraction of biocompounds. Ensuring biomass purity and structural integrity is crucial for biofuel production and biocompound extraction processes, andSEM analyses confirmed the homogeneous structure and lack of contamination in the algal biofilm.

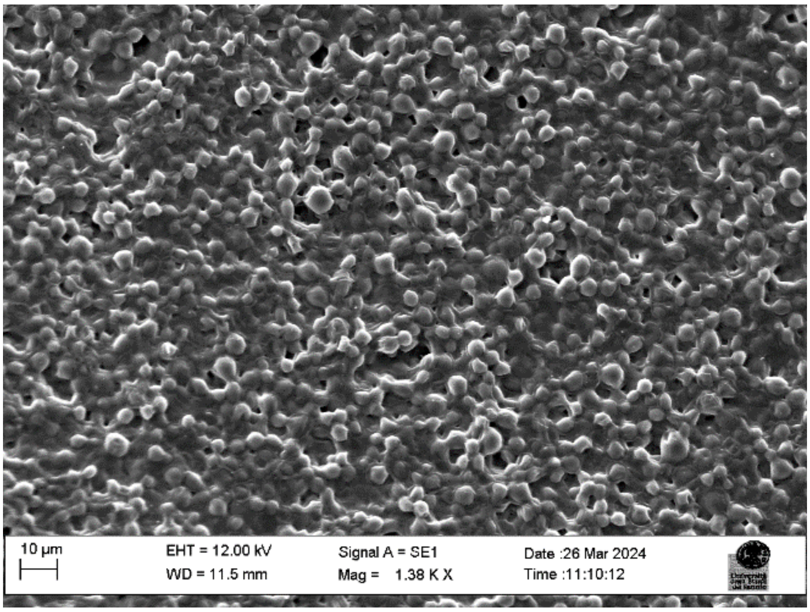

Figure 3 – SEM observations on microalgal biomass grown with urban wastewater.

Isotopic analyses provided insights into nutrient assimilation dynamics, revealing temporal growth trends in carbon and nitrogen content. These analyses, combined with elemental composition measurements, highlighted the uptake of nutrients from the culture medium, with implications for metabolic processes and environmental interactions, as previously envisaged in (di Cicco, Palmieri, et al., 2021). Sensor technology implementation facilitated real-time monitoring of pH levels and environmental parameters, ensuring stable chemical equilibrium and providing valuable insights into experimental conditions. Future investigations should focus on long-term growth dynamics, efficient biomass harvesting, and the development of environmentally friendly materials for cultivation systems. Additionally, ongoing research can explore the diverse applications of microalgal biomass in biotechnology and bioenergy sectors, contributing to sustainable development goals.

With regard to the preliminary tests for the potential recovery of silicon from photovoltaic panels, SEM-EDS analyses positively revealed the presence of aggregates in contact with the surface of the microalgae, whereas microanalysis revealed the presence of Silicon and Aluminium in the spectra observed for the biomass that was placed in the presence of hydrofluoric acid. This is a positive finding that anticipates the possibility of actually solubilizing silicon via acid leaching and using algae as a substrate for the recovery of this important metalloid, Silicon, from awaste material with a high social, economic and environmental impact.

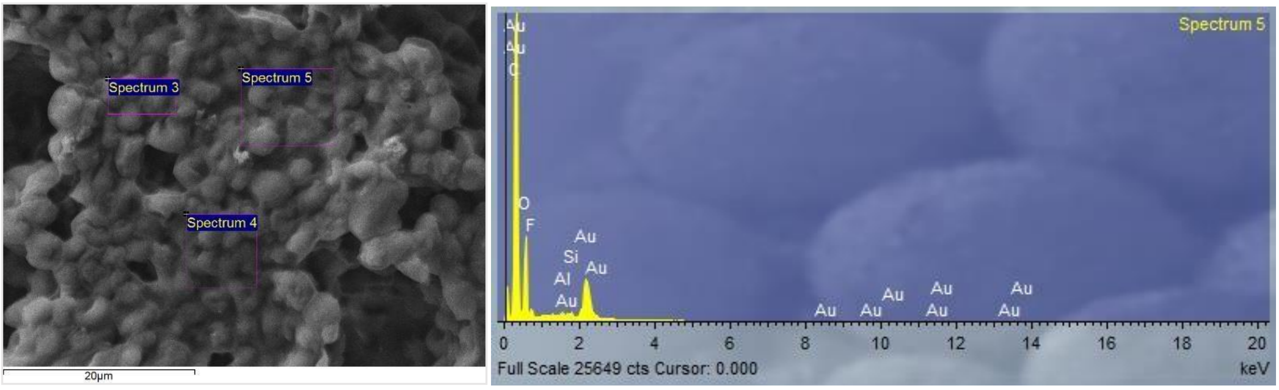

Figure 4 - An example of an SEM image with its corresponding EDS spectrum of biomass that has been incontact with a solution of HF and residues from photovoltaic panels.

4. CONCLUSIONS

This study underscores the potential of microalgae, particularly *Galdieria phlegrea*, in sustainable biomass production and resource recovery. The investigation employed Twin Layers photobioreactors forcultivating *G. phlegrea* using urban wastewater, demonstrating efficient biomass production under varied environmental conditions. Mathematical modeling revealed long-term biomass yield forecasts, suggesting significant energy recovery potential. Biomass characterization analyses confirmed the purity and structural integrity of algal biomass, essential for various biotechnological applications. Moreover, isotopic analyses provided insights into nutrient assimilation dynamics, contributing to our understanding of metabolic processes.

Preliminary tests for silicon recovery from photovoltaic panels showed promising results, with SEM-EDS analyses indicating the presence of silicon aggregates on algal biomass surfaces. These findings highlight the multifaceted potential of microalgae in sustainable development initiatives, emphasizing the importance of further research in biomass cultivation, resource recovery, and environmental applications.

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