USE OF *GALDIERIA SULPHURARIA* (CYANIDIOPHYCEAE, RHODOPHYTA) FOR WASTEWATER PHYTODEPURATION

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ABSTRACT: The use of natural resources is facing significant challenges due to current global environmental issues. The provision of clean water to the human population is becoming a worldwide problem. Municipal, industrial, and agricultural waters contain various organic and inorganic impurities, such as microplastics, high nutrient loads, and heavy metals, which pose a threat to our nutrition and health. The increasing importance of efficient wastewater treatment technologies and circular economic approaches has led to the exploration of alternative and/or supplementary methods to traditional wastewater treatment. The increasing importance of efficient wastewater treatment technologies and circular economic approaches has led to the exploration of alternative and/or supplementary methods to traditional wastewater treatment. The increasing importance of efficient wastewater treatment technologies and circular economic approaches has led to the exploration of alternative and/or supplementary methods to traditional wastewater treatment. Microalgae have emerged as a potential solution for wastewater treatment plants. *Galdieria sulphuraria*, a single-celled red algae belonging to the class Cyanidiophyceae, is known for its high tolerance to extreme conditions, including high salinity, acidic pH and high temperatures. This article summarises current research on the use of *Galdieria sulphuraria* for phytoremediation of wastewater, discussing the phytoremediation mechanisms of this alga, the types of pollutants it can remove, and potential applications in wastewater treatment systems.

Keywords: extremophilic microalgae, Galdieria sulphuraria, wastewater, and phytoremediation

1. INTRODUCTION

Water is a crucial natural resource on our planet. However, water quality has reached an alarming state in many industrialised countries, in addition to the inadequate supply of clean water in many developing countries (Sousa et al., 2018). Municipal, agricultural, and industrial wastewater is polluted with a large number of organic and inorganic contaminants, including microplastics and xenobiotics. The presence of pathogens, heavy metals, and high concentrations of nitrates, phosphates, and carbon (C) compounds strains the food chain and, consequently, the basis of human life (Eerkes-Medrano et al., 2019; Farmer et al., 2018; Sousa et al., 2018).

Conventional wastewater treatment plants (WWTs) focus on mechanically removing suspended solids and reducing biological oxygen demand through activated sludge (Wang et al., 2017). Biodegradation involves the breakdown of organic molecules and inorganic constituents, including nitrogen (N) and phosphorus (P) compounds. This process is crucial in preventing downstream waters, such as rivers and lakes, from becoming eutrophic. The capacity of conventional technologies to degrade pollutants is limited, particularly in the case of heavy metals, extremely high nutrient loads, and xenobiotics. This leads to an accumulation of these substances in groundwater (Sousa et al., 2018; Eerkes-Medrano et al., 2019; Farmer et al., 2018).

Microalgae have the potential to treat various wastewater sources due to their metabolic flexibility. They can perform photoautotrophic, mixotrophic or heterotrophic metabolism (Hu et al., 2018; Subashchandrabose et al., 2013). The use of microalgae in wastewater treatment (WWT) systems has two main objectives: (1) the direct adsorption or transformation of water contaminants and (2) the improvement of the purification performance of bacterial systems through the provision of additional oxygen from photosynthesis (symbiotic cocultures), thereby reducing the total energy costs of direct oxygen supply (gassing performance) or indirect oxygen supply (agitation performance) (Quijano et al., 2017). Research on wastewater treatment using algae has primarily concentrated on conventional microalgae and cyanobacteria (Zhai et al., 2017; Ansari et al., 2019) due to their ability to accumulate high levels of lipids and starch. This review specifically examines the use of extremophilic microalgae, *Galdieria sulphuraria*, for phytodepuration of wastewater.

2. *GALDIERIA SULPHURARIA*-ACUF427

Galdieria, a red polyestrematophile microalga belonging to the class Cyanidiophyceae, is a new and interesting approach in the field of algal wastewater treatment. It grows in volcanic soils with temperatures up to 50 °C and high concentrations of sulphur. The presence of a large number of membrane transporters and carbohydrate metabolism enzymes gives Galdieria exceptional metabolic versatility. These microalgae are able to switch from photoautotrophic to mixo-heterotrophic growth using over 50 different carbon sources, including glucose, glycerol and organic food waste. It is highly flexible and can adapt to pH levels as low as 5.5, even though it naturally exists in conditions with a pH below 2. Due to its ability to cope with and neutralise biohazardous heavy metals commonly found in volcanic areas and acid mines, it has great potential for use in biotechnological applications and as a reliable medium for bio-based remediation processes. This is thanks to its wide variety of carriers. In addition, this microalga can tolerate temperatures up to 56°C, making it suitable for cultivation in closed photobioreactors exposed to full sunlight and high daytime temperatures without the need for energy-intensive cooling technologies. This results in a reduction in system energy costs. Moreover, these organisms are of great interest due to their high levels of phycocyanin, which are approximately 1-2 orders of magnitude higher than other species. Additionally, they are capable of accumulating up to 50% glycogen under appropriate conditions. The glycogen produced by these organisms has a lower molecular weight and a more branched chain than that produced by other organisms (di Cicco et al., 2021a, b).

The efficacy of *Galdieria sulphuraria* in removing pollutants from wastewater has been evaluated in several studies in recent years. Under optimal experimental conditions, *Galdieria sulphuraria* can remove almost 100% of heavy metals from aqueous solutions (Kharel et al., 2023), and bioabsorb precious metals even at very low concentrations (Palmieri et al., 2022; Ciniglia et al., 2017; Iovinella et al., 2022). In addition, it can reduce the microbial load of various pathogens, including Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Aeromonas sp., Clostridium sp., Legionella sp., and Streptococcus sp. As a result, disinfection measures may not be necessary (Pleissner and Händel, 2023). Selvaratnam et al. (2022) reported a reduction in NH_4-N concentrations from 71.4% to 99.8% and almost complete removal of PO_4-P concentrations. Hejna et al. (2022) found the method to be effective in the removal of pharmaceuticals.

3. PHYTOREMEDIATION MECHANISMS

Galdieria sulphuraria uses several mechanisms to phytoremediate wastewater, including bioabsorption, bioaccumulation and biodegradation. Bioabsorption is a passive process in which a biological material binds and concentrates contaminants in the water. Biosorption is a mass transfer process. A material is transferred from the liquid phase and bound to the surface of a solid. Biosorption involves physical, chemical, and metabolic independent processes supported by various mechanisms including precipitation, ion exchange, surface complexation, electrostatic interactions and adsorption (Chia et al., 2020). The cell wall of microalgae is directly responsible for bioabsorption. Its chemical composition plays an important role in the process and determines the mechanism by which this phenomenon occurs. In addition, the material's surface contains pores and surface charges that promote bioabsorption. They act as binding sites for metal ions and as effective ion exchangers (Soto-Ramírez et al., 2021). In contrast to bioabsorption, bioaccumulation is an active metabolic process involving the utilisation of various substrates in the cell lumen. It requires energy and is slower than bioabsorption. Galdieria is used for waste detoxification by bioaccumulation, where it absorbs substances and then accumulates or metabolizes them. This process is essential for removing organic and inorganic pollutants such as sulphates, nitrates, phosphates, heavy metals, and pesticides. These substances can be transferred into cells (Mustafa et al., 2021). Bioaccumulation and bioabsorption are fundamentally different processes, and it is difficult to quantify the pollutants that are bioabsorbed and bioaccumulated because the two mechanisms interchange dynamically. Galdieria has the ability to accumulate various pollutants, as well as nutrients and trace elements (Rempel et al., 2021). Its ability to adapt to the environment allows it to resist pollutants at low concentrations. Additionally, it has a high tolerance for a wide range of pollutants from domestic, agricultural, and industrial sectors, which enhances its bioremediation capacity (Mojiri et al., 2020). Biodegradation, which involves the breakdown of complex compounds into simple and safe chemical elements, is one of the most effective processes for removing pollutants from wastewater. Unlike bioaccumulation and bioabsorption, where Galdieria act as biological filters to concentrate and separate contaminants from the surrounding water, bioremediation degrades target contaminants by complete mineralisation of the original molecules into CO_2 and H_2O or by biotransformation, which involves a series of enzymatic reactions to produce various metabolic intermediates (Sutherland et al., 2019). Basic biodegradation mechanisms can be divided into two categories: (i) metabolic degradation, where pollutants act as electron donors/acceptors and carbon sources for microalgae, and (ii) cometabolism, where pollutants act as both electron donors and carbon sources for abiotic matter (Leng et al., 2020; Tiwari et al., 2017).

4. WATER TREATMENT APPLICATIONS

Galdieria sulphuraria is considered an unrivaled microorganism candidate for various biotechnological applications in water treatment systems due to its unique extremophilic features. Based on its characteristics, table 1 summarises the potential applications of *Galdieria sulphuraria* in wastewater treatment systems.

Table 1. Potential applications of *Galdieria sulphuraria* in water treatment considering its characteristics (adapted from Maamoun et al., 2020).

No.	Feature	Designated Application
1	Enormous metabolic versatility in the utilization of carbon sources (including a large range of sugars and alcohols) for heterotrophic growth.	Perfect choice for the removal of nutrients and dissolved organic carbon from wastewater (WW).

2	Spherical shape with thick-walled cells.	Higher specific surface area towards selectivemetal precipitation and for metal bio-sorption from wastewater.
3	Remarkable acidophilic ability.	Biological Oxygen Demand (BOD) and nutrients (phosphorus, nitrogen-forms) removal from municipal wastewater.
4	High resistance to high metal concentrations in aqueous solutions (even at low pH < 2.5).	Recovery of rare metals from industrial wastewater (even if present at low concentrations) orfrom solid waste materials.

As mentioned, unlike current energy-intensive technologies, algal processes can be powered by sunlight or wastewater. Algal systems are classified as photoautotrophic, heterotrophic or mixotrophic according to their metabolic carbon/energy choices. Many applications, including WWT, have adopted photoautotrophic and chemoheterotrophic systems. Mixotrophic systems have the potential to meet their carbon and energy needs from either organic or inorganic chemicals (Fig. 1). Each process can occur independently, resulting in either CO_2 or O_2 accumulation or depletion (Maamoun et al., 2020).

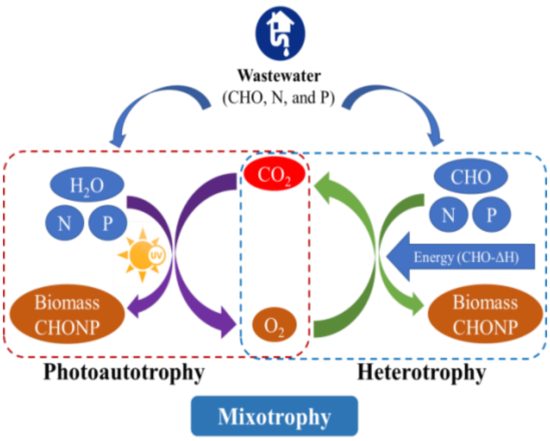

Figure 1. Schematic of mixotrophic process for utilizing nutrient in wastewater and energy from light (in photoautotrophy) or from organics (in heterotrophy) (from Maamoun et al., 2020).

The pilot-scale version of this mixotrophic system (700 L) produced discharge-ready effluent in less than three days of fed-batch processing, while reducing BOD and nutrients in primary settled effluent in a single step. These results demonstrate the system's efficacy in treating effluent. The mean volumetric removal rates of BOD5 and ammonium nitrogen in this mixotrophic system were found to be similar to those reported for the photoautotrophic high-rate algae pond (significance level 0.05), with values of 16.5 ± 3.6 mg L^{-1} d^{-1} and 6.09 ± 0.92 mg L^{-1} d^{-1}, respectively. The removal rate of phosphorus (1.40 ± 0.57 mg L^{-1} d^{-1}) was found to be greater than that reported for the photoautotrophic high-rate algae pond (significance level 0.05). In this specific mixotrophic system, the extreme culture conditions allowed a simultaneous reduction of E. coli (> 5 log) and other pathogens. It is important to note that this statement is objective and not subjective (Nirmalakhandan et al., 2019).

5. CHALLENGES

Despite its potential in phytodepuration, the use of *Galdieria sulphuraria* poses some challenges that need to be addressed in order to optimise the effectiveness and scalability of this technology:

- Optimising growth conditions: The growth and bioremediation effectiveness of *Galdieria sulphuraria* can be influenced by factors such as temperature, light, pH and nutrient concentration. It is important to optimise these conditions for best results.
- Scalability: Scalability of *Galdieria sulphuraria* phytodepuration to large wastewater treatment plant

CONCLUSIONS

Galdieria sulphuraria has been shown to be a promising alga for the removal of various pollutants from wastewater and has several advantages over other methods of wastewater treatment. Although there are still challenges to overcome to optimise its use for large-scale phyto-purification, it represents a potential and viable alternative to traditional wastewater treatment due to its lower operational and energy costs, as well as the ability to harvest a resource such as biomass. The ability to use CO_2 emitted by other industrial plants for microalgae cultivation, thereby reducing atmospheric emissions, is an advantage of this approach.

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