Dark fermentation potential in increasing circularity of biotechnological wastes

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Introduction

Within the last 20 years, dark fermentation gained considerable interest between the technologies to produce green H_2. Although promising, its application as a “stand-alone” process is currently hampered by several factors. The main constraints are related to high reaction volumes, the need for renewable and alternative substrates to sugars, the difficulty to obtain robust biocatalysts, the reduction of pretreatments and further valorisation of its by-products (e.g., CO_2, short chain fatty acids-scVFAs, residual solids) (Ren et al. 2011; Łukajtis et al. 2018; Jain et al. 2024).

Along with dark fermentation, cultivation of both microalgae and cyanobacteria gained increasing interest in the development of a green and sustainable economy. Such interest lays in their capacity of growing in phototrophic or mixotrophic conditions, allowing use and valorisation of anthropogenic CO_2 streams and different industrial effluents, whilst generating added value products of interest for the chemical, nutraceutical and pharmaceutical industries coupled with bioremediation applications (Olabi et al. 2023; Saravanan et al. 2023; Prabha et al. 2022). Despite their properties, high cultivation costs often limit microalgae and cyanobacteria production and commercialization. Main actions reported in the literature to increase process feasibility are directed towards the exploitation of waste gas stream and liquid effluents from other processes. Although use of low-cost resources like wastewaters for their growth would not allow use of the biomass in the nutraceutical and food industry, valuable chemicals like pigments (i.e. phycobilins, chlorophylls and carotenoids) could still be recovered and further ways to achieve biomass full and efficient utilization could be deployed. (Slade and Bauen 2013; Mutale-Joan, Sbabou, and Hicham 2023). Given the requirements of these two technologies a possible solution could consider their integration. Cyanobacterial/microalgal biomass grown in wastewater could be used as feedstock as is, or after denaturation and extraction of valuable chemicals, in a dark fermentation process generating renewable H_2, CO_2 and scVFAs. The latter two could then be used as substrates for further growth and production of algal or cyanobacterial biomass/products, resulting in a cyclical process that would valorise the waste products of both processes (Figure 1).

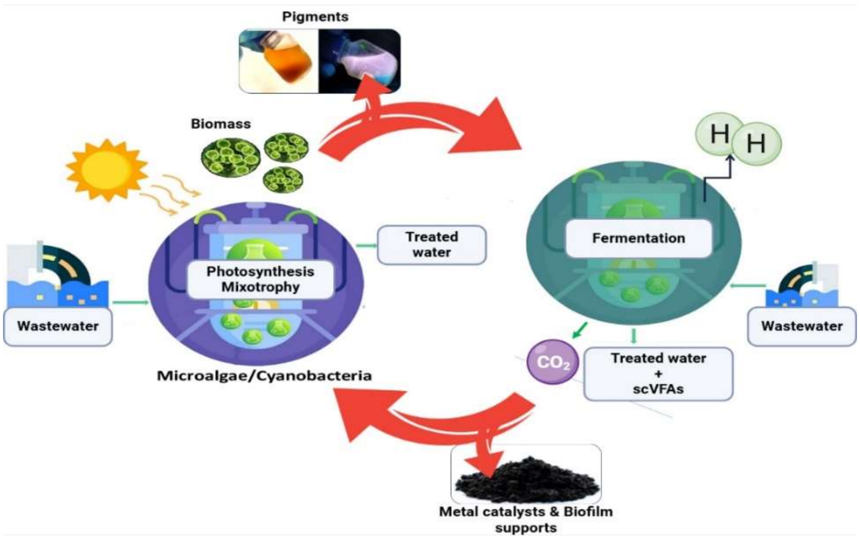

Figure 1: Integration of dark fermentation and micro-algae/cyanobacteria driven photosynthetic/mixotrophic processes with potential flows of substrate interchanges

Materials and methods

In this work a microbial consortium obtained through adaptative laboratory evolution was characterized at physiological level displaying potential for application in a coupled biotechnological process where dark fermentation utilises waste algal biomass grown on effluent aiming at the full valorisation of all products (i.e. gases, liquid and solids). The performances of the biocatalyst were recorded during repeated batch cultivation in mesophilic conditions (37°C) with cyanobacteria biomass (*Spirulina* spp.) used for bioremediation representing the main substrate for the process. Fermentation of lyophilized biomass (Spyro) was tested at substrate concentrations of 100 and 50 g/L. Furthermore, residues obtained after phycobilin extraction were also tested as potential substrates. Residues were obtained with two high-pressure denaturation protocols running for 2 hours at 100°C (Treat 1) and for 5 hours at 80°C (Treat 2), respectively. Fermentation of denatured residues was tested at a concentration of 50 g/L. All performances were screened against positive controls provided with 100 and 50 g/L of glucose (Ctrl). Liquid from the organic fraction of municipal solid waste digestate was diluted 1:3 with tap water and used as source of minerals. To assess the robustness of the biocatalyst, sterilization procedures of liquid and solid substrates were not performed.

Results, discussion and conclusions

Production of H_2 was reported in all tested conditions. Generally, it was observed that higher substrate input (100 g/L) led to higher H_2 productivity and increased quality of output gas stream characterized by higher H_2 concentrations when compared to tests provided with 50 g/L substrate (Figure 2).

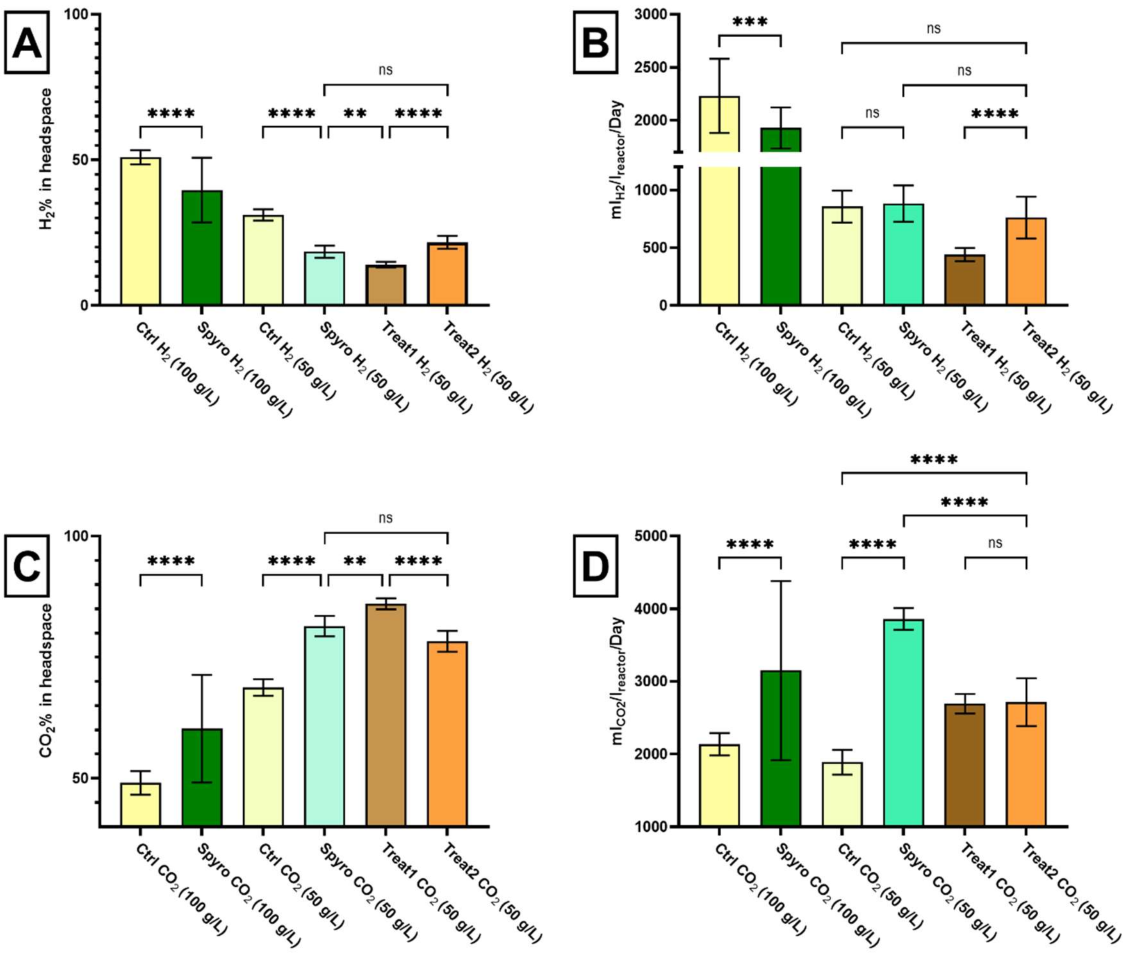

Figure 2: H_2 and CO_2 concentrations in output gas (A;C) and average productivity (B;D) measured during dark fermentation of Spirulina biomass and residues of its denaturation for phycobilin extraction. Comparisons were performed through a two-way ANOVA test.

Fermentation of denatured residues revealed that milder extraction methodologies (Treat 2) would allow for their possible valorisation through dark fermentation. Residues treated at lower temperature displayed H_2 production rates similar those of control and *Spirulina* provided samples, whilst considerably lower amounts were obtained from residues treated at higher temperature (Treat 1) (Figure 2B). Given such results, the utilization of fine-tuned extraction methodologies should be considered to maximise the production of H_2 in the fermentative process.

Along with H_2, scVFAs were also produced. Cultures fed with *Spirulina* biomass displayed in general a wider panel and higher production of acid species in comparison to control samples, with acetic and butyric acid being the most represented (Figure 3).

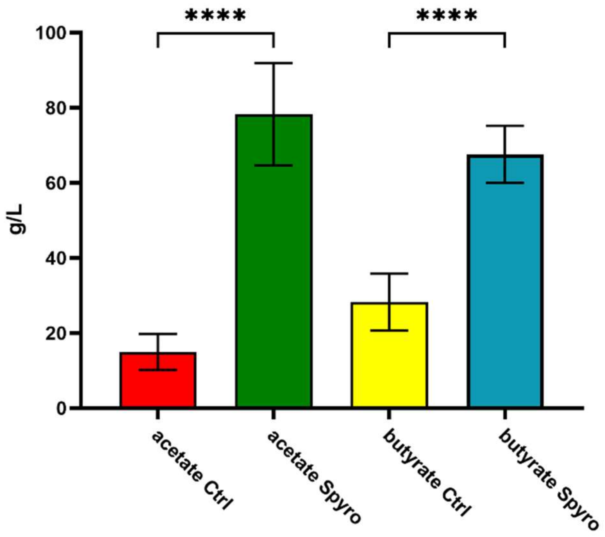

Figure 3: Levels of acetate and butyrate produced during dark fermentation of Spirulina biomass and control samples (substrate concentration 100g/L). Comparisons were performed through a two-way ANOVA test.

Despite carbohydrate-rich wastes are regarded as the best feedstocks for biological H_2 production, results reported in this study shows the potential of using cyanobacteria biomass derived from biotechnological and chemical processes as a possible substrate for dark fermentation processes.

Along with H_2, fermentation of cyanobacteria biomass allowed for high production of both CO_2 and scVFA (i.e. acetic and butyric acid) which could be re-deployed as feedstocks for cyanobacterial phototrophic and mixotrophic processes respectively. Furthermore, residual fermentation solids could represent a precursor for biomaterial production allowing for an efficient valorisation of all fermentation products. Fermented biomass and residues were used as templating agent to produce metal-based catalysts, which could exploit the activation of oxygenated water for catalytic processes. Furthermore, finely tuned pyrolysis conditions in absence of metals would produce sponge-like materials that could promote microbial adhesion and biofilm formation. A feasibility study was also carried out to define the possibility to deploy the technology.

Further research should focus on process scalability and the development of continuous operations. Moreover, optimization of feedstock provision (i.e., possible co-feeding with wastes rich in carbohydrates) and the utilization of other cyanobacteria or algal strains representing possible substrate for such process should be considered.

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