Upcycling microalgae side streams for biopolymers production

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Microalgae have been gaining a lot of interest due to their capacity to capture and use CO_2 as well as nutrients nitrogen and phosphorous found in liquid waste and side streams, while yielding numerous high-value compounds such as lipids, carbohydrates, proteins, pigments, among others. However, even using biorefinery concepts to valorise the various fractions of algal biomass, low value and heterogeneous residual side streams/wastewaters are produced. In the framework of a circular economy model, these side streams need to be treated and can be valorised, which enables microalgae industries to further increase their revenue, maximise resource usage efficiency, and expand sustainability. Different products can be obtained through valorisation of microalgae side streams, such as the biopolymers polyhydroxyalkanoates (PHAs). PHAs have thermoplastic properties similar to petrochemical polymers and can be a sustainable alternative to conventional plastics (Dang *et al*., 2022), being in line with European Union (EU) guidelines (European Commission, 2018). The goal of this study is to valorise an algae-based side stream into PHAs using mixed microbial cultures (MMCs) and further extract and purify the polymer. In order to produce PHAs with MMCs it is necessary to obtain a VFA-rich stream, the preferential substrate for PHA production by means of MMC by carrying out an acidogenic fermentation of microalgae streams.

The fermentation capacity of three different streams rich in microalgal biomass, namely *Nannochloropsis*, *Chlorella* and a mixed phototrophic consortium was evaluated by performing acidogenic batch tests and following production of volatile fatty acids.

The microalgae side streams provided by A4F-Algae for Future (Portugal) were firstly characterized in ash, carbohydrates, protein and lipid content (Table 1). Carbohydrate content was obtained by high performance liquid chromatography (HPLC), protein content was given by the total nitrogen content (obtained by elemental analysis) multiplying by a factor determined specifically for algae (4.78) (Laurens, 2013). The lipidic content was considered the remainder.

Table 1. Characterization of each microalgae stream.

Feedstock	% Ash	% Protein	% Carbohydrates	% Lipids
Nannochloropsis	8.7 %	26.2 %	13.7 %	51.4 %
Chlorella	10.4 %	22.8 %	25.2 %	41.6 %
Phototrophic consortium	9.9 %	25.9 %	30.3 %	33.9 %

The acidogenic batch tests were inoculated with anaerobic sludge from the wastewater treatment plant (WWTP) of Mutela (Portugal) which had TS concentration of 16.1 g/L and VS of 11.0 g/L. The batch tests were performed in triplicate for each microalgae stream (*Nannochloropsis*, *Chlorella* and phototrophic consortium) with a control for each stream (no inoculum added) and for the anaerobic sludge (no stream added). The tests were carried out at a substrate to inoculum (S/I) ratio of 2 gVS/gVS and initial pH of 5.5. The pH was controlled by the addition of a phosphate buffer solution. The batch tests were executed at 30 °C, 160 rpm, in sealed flasks with a working volume of 100 mL for 32 days. The gas phase was analysed with a gas chromatograph (GC).

Throughout the experiment no methane was detected in any of the batch tests (data not showed) which indicates that the methanogenic activity was inhibited, as desired. pH suffered no major changes across time (data not showed). *Nannochloropsis* had higher content in lipids than *Chlorella* and phototrophic consortium, 51.4 %, 41.6 % and 33.9 %, respectively (Table 1). These results are according to literature since *Nannochloropsis* usually is used to produce lipids and *Chlorella* to produce proteins and sugars (Li *et al.*, 2019). All streams were fermented into lactate, acetic acid, propionic acid, butyric acid and ethanol. *Chlorella* had the highest production of fermentation products from the three different studied streams with an average of 3000 mgCOD/L (Figure 1C). In addition, *Chlorella* fermentation produced a higher content of butyric acid (15% of the total fermentation products), which was not reached with the other two streams. *Nannochloropsis* completed its highest production of fermentation products at day 22 with around 1800 mgCOD/L (Figure 1A). From day 22 to day 32 the lactate started being converted into propionic acid. Phototrophic consortium showed the lowest fermentation capacity with only 1500 mgCOD/L in fermentation products produced (Figure 1E). The control of all the microalgae feedstocks showed the production of fermentation products (Figure 1B,D,F) indicating the presence of fermentative organisms in the microalgae side streams and thus, an intrinsic fermentative capacity. Nevertheless, the anaerobic sludge inoculum addition, enabled the production of a higher concentration and diversity of VFAs from the microalgae side streams.

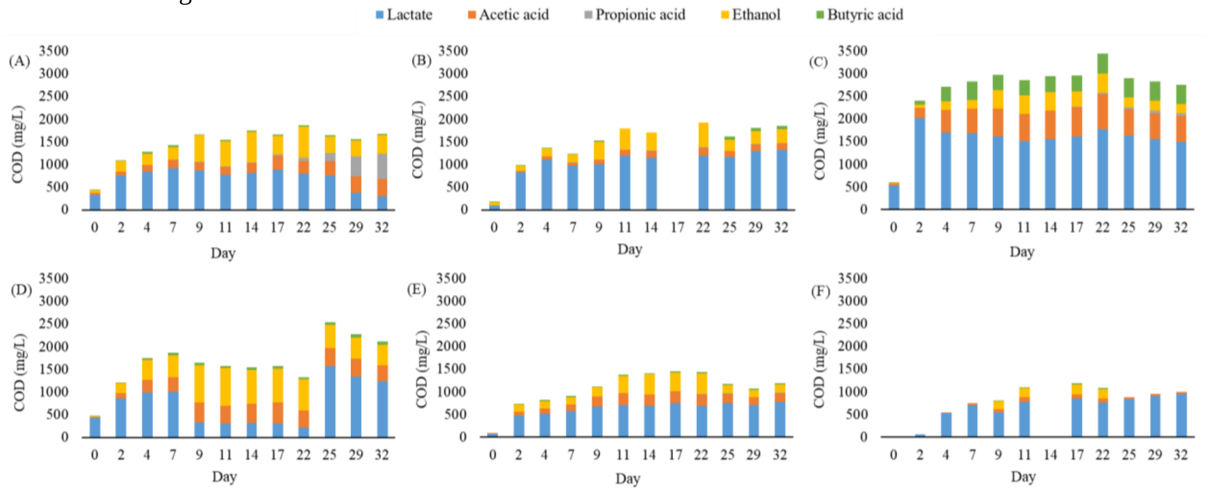

Figure 1. Results of fermentation products shown as mgCOD/L throughout the experiment. (A). Average results for triplicate experiment of *Nannochloropsis*. (B). Results of *Nannochloropsis* control experiment, results of day 17 not available. (C) Average results for triplicate experiment of *Chlorella*. (D) Results of *Chlorella* control experiment. (E) Average results for triplicate experiment of phototrophic consortium. (F) Results of phototrophic consortium control experiment, results of day 14 not available.

Chlorella feedstock showed the highest yield, on the last day, in fermentable products of 0.30 mgCOD-fermentation products/mgCODin, *Nannochloropsis* followed with 0.15 mgCOD-fermentation products/mgCODin and lastly phototrophic consortium yielding 0.12 mgCOD-fermentation products/mgCODin. The yield when only considering the production of VFAs was 0.11 mgCOD-VFAs/mgCODin, 0.09 mgCOD-VFAsmg/CODin, and 0.02 mgCOD-VFAs/mgCODin for *Chlorella*, *Nannochloropsis* and phototrophic consortium, respectively. When comparing these results with ones from literature, these are low yields (Magdalena *et al.*, 2018). However, these microalgae streams were not subjected to any pre-treatment, which impact will be assessed further studies. Current studies are focusing on an acidogenic reactor operation to optimize the VFA production from these microalgae feedstock.

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