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# Anaerobic digestion of lipid-extracted microalgae: Enhancing nutrient recovery towards a closed loop recycling

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ABSTRACT

Nutrient recycling is essential to make microalgae cultivation sustainable at industrial scale. To this aim, lab-scale anaerobic digestion experiments of *Chlorella vulgaris* after lipid extraction were carried out to evaluate biogas yield and nutrients recovery in the liquid digestate. Then, this liquid fraction was used as source of nutrients for cultivation of a closely related *C. vulgaris* species, in order to assess the possibility of re-cultivating microalgae towards a closed-loop nutrient recycling process. The biological methane potential tests resulted in biogas production of about 347 NmL $gVS^{-1}$ with 43% v/v methane content. In re-growth experiments, the liquid digestate showed an insufficient amount of soluble sulfate and phosphorus. However, by amending these two nutrients, the specific growth rate and final biomass concentration increased to about 2 $d^{-1}$ and 2 g $L^{-1}$, respectively, which were comparable to those obtained in a defined control medium. The low content of soluble phosphorus in liquid digestate was mainly due to precipitation and removal with the solid phase. Several techniques were hence tested to enhance phosphorus solubilisation, and highest recoveries of up to 41% were obtained when using $NaHCO_3$. Finally, *C. vulgaris* was grown in such treated digestate, obtaining a final biomass production comparable to that of the control, without the need of external phosphorus supply.



## 1. Introduction

The increasing world energy demand, together with the concern raised by $CO_2$ emissions related to fossil fuels utilization, has led to the development of several alternative and renewable energy sources. Among these, microalgae have received wide attention as a prospective biomass feedstock for biofuels and bioenergy production, especially related to liquid fuels for transportation, as they are characterized by fast growth rates and high lipid contents compared to other crops [1,2].

Nonetheless, in order to quantitatively replace fossil fuels with third-generation biofuels derived from microalgal biomass, number of issues have to be properly addressed and solved yet. In terms of both economic and environmental sustainability, the problem of nutrients (particularly N and P) supply required by microalgae to grow is of greater concern, as a simple mass conservation balance suggests that huge amounts of fertilizers would be needed in view of a quantitative replacement of transportation fossil fuels by biofuels. These amounts are in competition with food crops cultivation, in the case of nitrogen, and well beyond the available natural resources for phosphorus [3]. On the other hand, biofuels do not need to be based on components other than mixtures of hydrocarbons, which do not contain either N or P, whereas microalgae, as well as any other biomass, need them to grow and to produce hydrocarbon precursors. Therefore, the only possibility and goal are pursuing maximum nutrient recovery from the spent biomass and subsequently recycle them for further cultivation.

Among the possible techniques available to this aim, anaerobic digestion (AD) appears to be a viable and promising solution, as it allows obtaining a liquid phase in which nutrients are re-mineralized, while at the same time producing biogas as an additional energy output [4–7]. The literature is quite rich in papers about AD of different microalgal species, both on whole-cells [8–10] and after lipids extraction [11–13], proving that this type of biomass can be a good substrate for biogas production. The feasibility of using microalgal biomass for AD was proved also in semi-continuous system [12]. In addition, the possibility of growing microalgae on the liquid digestate effluent originated from AD of various feedstocks (e.g., cow or swine manure, municipal wastewaters, etc.), rich in nutrients such as ammonium nitrogen

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and phosphorus, has also been investigated and proved to be feasible for different algal species [14–16].

However, the composition of the biomass fed to the anaerobic digester clearly influences the final composition of the digestate. So that, if a given nutrients ratio is required by algae growth, and the same biomass is digested, some unbalanced recovery of nutrients may occur. This fact is likely to affect the suitability of algal digestate as nutrients source. In the perspective of an industrial-scale microalgal process development, a closed-loop should be considered, where the algae-based digestate is recycled to the culture, but the availability of nutrients in the correct ratio must be checked, when the same biomass is used for AD. Prajapati et al. [10] investigated the possibility of a closed-loop cultivation/AD process using the cyanobacteria *Chroococcus* sp. In their work, the biomass produced after cultivation is sent directly to the anaerobic digester, so that such a process is clearly aimed at the production of biogas as the main and only fuel. When instead the main goal is obtaining algal liquid fuels for transportation, the lipid fraction has to be extracted from microalgae, then the residual biomass (which has a somehow different composition) is sent to AD for additional energy production and nutrients recycling. In a recent paper [17] assessed the possibility to re-grow algae of a different species (*C. sorokiniana*) on LEA (filamentous algae) digestate,

In this work, a lipid-extracted microalgal biomass (*Chlorella vulgaris*) was used for AD, and the liquid digestate was assessed as cultivation medium to re-grow the same species and evaluate the availability of macro and micro nutrients, in order to highlight and quantify the need of nutrients make-up. In particular, the lack of some micro or macro nutrients can affect the exploitability of this nutrient recycling technique, and it has to be accounted for when assessing the process feasibility. In fact, even if the same elemental composition of the one fed to the digester should result in the digestate, some different digestion yields or precipitation phenomena may result in a loss of nutrients.

## 2. Materials and methods

### 2.1. Lipid extraction and BMP test

Pre-dried *Chlorella vulgaris* (provided by NEOALGAE™) was used for AD experiments. Lipids were extracted using a Soxhlet apparatus and a mixture of ethanol-hexane (2.5:1 volumetric ratio) as extraction solvent. Such a mixture was chosen as, even though chlorinated solvents are more efficient in extracting lipids from the biomass, their residuals cause inhibition of the digestion process [18]. Laboratory scale tests were then performed to evaluate the Biochemical Methane Potential (BMP) of the lipid-extracted algal biomass (LEA). Batch tests were carried out using six 500 mL glass bottles, with a working volume of 250 mL. In addition, two bottles were added containing only inoculum (no algae), as a control. These were subsequently sealed with silicon plugs. Anaerobic sludge collected from an anaerobic digester of sewage sludge from a municipal wastewater treatment plant located in Padova, Italy, was used as inoculum. Microalgal concentration and microalgae/sludge ratio (F/M, i.e. food to microorganism) were 2.76 gVS $L^{-1}$ and 0.5 $gVS_{algae}$ $gVS^{-1}{}_{sludge}$ [18], respectively.

The bottles were flushed with $N_2$ gas for 3 min to ensure anaerobic conditions and incubated at a temperature of 35 ± 1 °C. Total solids (TS) and volatile solids (VS) of the inoculum and LEA were analyzed in triplicate according to standard methods [19].

The volume of biogas produced during the AD process was measured by means of the water displacement method, using an acidified (pH < 3) and saline (NaCl 25%) solution in order to avoid the dissolution of methane and carbon dioxide into the liquid. The produced gas composition in terms of $CH_4$ and $CO_2$ was analyzed using a portable gas analyzer (LFG 20-ADC, Gas Analysis Ltd).

Methane and carbon dioxide volumes produced between two subsequent measurements were calculated according to Ginkel et al. [20]:

$$V_{c,t} = C_{c,t}V_{b,t} + V_H\left(C_{c,t} - C_{c,t-1}\right) \quad (1)$$

where:

$V_{c,t}$ = Volume of $CH_4$ or $CO_2$ produced at intervals between t and t-1

$C_{c,t}$ = Concentrations of $CH_4$ or $CO_2$ in headspace at time t

$V_{b,t}$ = Volume of total biogas produced at intervals between t and t-1

$V_H$ = Volume of bottles headspace

$C_{c,t-1}$ = Concentrations of $CH_4$ or $CO_2$ in headspace at time t-1.

The net production of biogas was calculated by subtracting the amount of biogas produced by control experiments (bottles without algal biomass).

At the end of BMP tests, which lasted 41 days, the products were collected and homogenized: part of it was immediately centrifuged and filtered (using qualitative filter paper, declared pore size 15–20 μm) to separate the liquid fraction from the solids. The remaining products were kept for subsequent phosphorus solubilisation treatments, carried out prior to the solid-liquid separation (Section 2.4). All the products were stored at −20 °C until use.

### 2.2. Algae strain and culture media

*Chlorella vulgaris* Emerson/3 was used for growth experiments. The culture was maintained in sterile BG11 medium with 1.5 g $L^{-1}$ $NaNO_3$ (247 mg $L^{-1}$ N) and 30.5 mg $L^{-1}$ $K_2HPO_4$ (5.4 mg $L^{-1}$ P), buffered with 10 mM HEPES pH 8, in 250 mL Drechsel bottles, as a pre-inoculum. Since in the anaerobic digestate nitrogen is mainly present as ammonium, for the control experiment BG11 was modified so that this nutrient was supplied as $NH_4Cl$, keeping an equivalent concentration of 247 mg $L^{-1}$ N. All other nutrients were provided in the same amount and form as standard BG11.

For experiments carried out in the digestate, the medium was diluted with distilled water in order to have the same N concentration of control BG11. When necessary, additional P and S were added as $K_2HPO_4$ and $MgSO_4{\cdot}7H_2O$ salts, respectively, at the concentration reported in subsequent sections. The pH was measured daily and kept in the range of 7.3–7.7 by addition of NaOH or HCl solutions. No sterilization was carried out on the digestate prior to algae inoculation, to effectively measure the growth capability in conditions similar to industrial ones.

### 2.3. Cultivation set-up and analytical procedures

Growth experiments were performed in Drechsel bottles having 5 cm diameter, with a culture volume of 100 mL. A mixture of $CO_2$-enriched air (5% v/v, regulated by two flow-meters) was continuously bubbled through the microalgal suspension at approximately 1 L $h^{-1}$ total flow, to ensure non limiting carbon supply. To avoid sedimentation, the culture was continuously mixed by a stirring magnet, placed at the bottom of the reactor. Light was provided by fluorescent lamps, placed in front of the cultivation bottles. The light intensity used for the experiments was equal to 120 μmol $m^{-2}$ $s^{-1}$ of PAR (Photosynthetically Active Radiation, 400–700 nm), measured with a photoradiometer (Delta OHM HD 2102.1). The cultures were placed in a refrigerated incubator, at a constant temperature of 28 ± 1 °C. Each experiment started with a microalgae inoculation of $OD_{750}$ = 0.2, which corresponds to a cell concentration of about $2{\cdot}10^6$ cells $mL^{-1}$, and was carried out at least in duplicate.