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Microalgae Cultivation: Nutrient Recovery from Digestate for Producing Algae Biomass

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The aim of this work was to compare the growth rate of algae in mineral medium and in ultrafiltrate medium. The ultrafiltrate allowed us to recover nutrients from a stream of digestate from agroindustrial sources. Two microalgae strains (*Phaeodactylum tricornutum* and *Pavlova lutheri*) were cultivated in both of the media. The stream used as growth medium was obtained from an anaerobic digestion plant used to digest mixtures of agro-zootechnical material. The digestate was treated through an ultrafiltration process and then diluted in order to meet the needs of the algae strains. The algae growth on standard medium versus ultrafiltrate medium was similar for both species. *Phaeodactylum tricornutum* grew in almost 12 days with a similar average productivity on standard medium and ultrafiltrate (respectively of 25 and 24 mg L^{-1} d^{-1}) while *Pavlova lutheri* grew in 24 days with an average productivity of 15 mg L^{-1} d^{-1} on standard medium and 17 mg L^{-1} d^{-1} on ultrafiltrate. The results show that microalgal biomass production offers real opportunities for addressing issues such nutrient recovery from wastewater streams and CO_2 sequestration, and the resulting biomass could be employed as biofertilizer or to produce added-value organic chemicals as new raw materials.

1.Introduction

The world is currently facing a severe energy crisis due to the incessant increase of energy demands and gradual depletion of fossil fuels (Maity, 2015). The use of biomass as a sustainable renewable source is the only way to replace carbon from fossil sources for the production of carbon-based products such as chemicals, raw materials and liquid fuels, with a remarkable reduction of CO_2 releases into the atmosphere (Fava et al., 2013). In this context, biogas production represents one of the most well developed bioenergy sources in the European Union. The biogas industry grew up thanks to financial incentives and the support of specific legislative tools aimed at increasing the production of biogas in different economic sectors. Nowadays these industries need to self-sustain and to reduce costs. Anaerobic digestion (AD) is an anaerobic biological process by which, in the absence of oxygen, organic matter is transformed into biogas, which principally consists of methane (50–80 vol.%) and carbon dioxide, the former used to produce energy and heat (Tani et al., 2006). Anaerobic digestion also produces a final biologically stable and partially hygienic organic product: the digestate (Tambone et al., 2009). Moreover, it is characterized by a high mineral load, mainly nitrogen (N) and phosphorus (P) and it is usually used as a fertilizer in agriculture. Alternatively, recovery of downstream wastes (digestate) could become a resource in term of nutrients, heat and CO_2 for producing third generation biomass such as microalgae. Microalgae are autotrophic microorganisms which utilize light energy and inorganic nutrients (carbon dioxide, nitrogen, phosphorus etc.) and synthesize valuable biomass compounds, such as lipids, proteins, carbohydrate and pigments (Markou et al., 2013). These compounds could be extracted for several applications in animal feed, agriculture, green chemistry and the bioenergy sectors (Pulz and Gross, 2004). Different studies have tested algal strains for the treatment of the digestate. The results are still preliminary but promising (Ras et al., 2011). Digestate from AD has been used as the substrate to support the growth of microalgae, since it is rich in macro and microelements. Furthermore, CO_2 and heat, by-products of the bio-methane production, could be exploited to support microalgal growth in order to contribute in reducing the price of the whole process. Following these concepts we can introduce the term "Biorefinery", to

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describes a nexus of integrated bio-based industries using a variety of technologies to make products such as chemicals, biofuels, food and feed ingredients, biomaterials, fibers, heat and power, aiming at maximizing the added value (Fava et al., 2013). A biorefinery is an integrated facility which combines various processes and equipment to produce biofuels, power, and high-value chemicals from biomass (Markou et al., 2013). The multiple commodity production of a biorefinery improves the utilization of biomass feedstock, maximizing its value (Demirbas, 2009; Nobre et al., 2013).

2. Materials and methods

2.1. Microorganism and culture conditions

For these experiments *P.lutheri* strain 926-1 and *P.tricornutum* strain SAG1090-1a were used, acquired from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut (Universität Göttingen, Germany).
The inoculum was prepared batch-wise using sterilized f/2 medium (Guillard, 1975).The inoculum was maintained in Erlenmeyer flasks, 0.5 L, the operational conditions were constant aeration and mixing with filtered air (0.2 µm), under continuous light of 120 µmol m^{-2} s^{-1} provided by fluorescent tubes, and incubated at a controlled temperature of 22 ± 1°C.

2.2 Experimental set-up

All the strains were cultivated in batches in 3 L borosilicate flasks: the cultivations were performed in triplicate. Airflow was continuously provided, with a CO_2 flow supplied on demand according to pH value. The cultures were maintained under continuous illumination of 120 µmol m^{-2} s^{-1} provided by fluorescent tubes, and at a constant temperature of 22 ± 1°C. The experiments were set up as follow: microalgae were first placed in enriched nutrient replete medium during the growing phase, in particular f/2 medium for both strains. When the stationary phase was reached, the cultures were collected. The same procedure was implemented for the growth on digestate. Both steps were carried in order to compare the kinetics of growth and therefore the performance of microalgae in a standard medium versus digestate.

2.3 Analytical methods

Microalgal growth was monitored by optical density (OD560nm) and dry weight determination by filtering the culture with Whatman GFC filter 1.2 µm pre-weighed, and by desiccation at 80°C overnight: the filtrate was then placed in a vacuum desiccator over silica gel and weighed. Determination of specific growth rate (day^{-1}) was calculated from Eq(1) as reported by Richmond (2008). N1 and N2 are the concentrations of cells at the beginning (t1) and at the end (t2) of the exponential growth phase, respectively.

$$\mu = (\ln N_2 - N_1)/(t_2 - t_1) \quad (1)$$

Biomass productivity (g L^{-1} d^{-1}) during the culture period was calculated from Eq(2) (Richmond, 2008), where Xt is the biomass concentration (g L^{-1}) at the end of the exponential growth phase (tx) and X0 the initial biomass concentration (g L^{-1}) at t0 (day):

$$\text{Productivity} = (\text{Xt} - \text{X0})/(\text{tx} - \text{t0}) \quad (2)$$

The average irradiance (in the range of photosynthetically active radiation, PAR) at which cells are exposed inside a culture (Iav) is a function of irradiance in the absence of cells (Io), the biomass extinction coefficient (Ka), the biomass concentration (Cb) and the light path inside the reactor (p). It can be approximated by using Eq(3) (Molina Grima et al., 1997).

$$\text{Iav} = (\text{Io}/(\text{Ka} * \text{p} * \text{Cb})) * (1 - \exp(-\text{Ka} * \text{p} * \text{Cb})) \quad (3)$$

The extinction coefficient (Ka) is calculated by dividing optical density at 560 nm by the biomass concentration (Cb) and cuvette light path (p) Eq(4) (Molina Grima et al., 1997).

$$\text{K}\alpha = \text{OD560}/(\text{Cb} * \text{p}) \quad (4)$$

Further parameters to take into account were pH and temperature (°C) that were recorded daily. Biomass was recovered by centrifugation (4300 rpm) followed by freeze-drying. The Ultrafiltrate (UF) derived from a digestate that was undergoing treatment with a solid liquid separation, later liquid fractions were [obtained by adding?] added by polyamide flocculants and sent to a decanter centrifuge (MAMMOTH 570/3, Pieralisi, Jesi-Italy), allowing the separation of another solid fraction vs. liquid stream. The liquid entered an ultrafiltration unit equipped with a 40 kDa grafted Poly-acrylonitrile membrane, resulting in a brown liquid as shown in Figure1a.

The UF effluent was sampled from (Fiolini and Savani s.r.l) stored in 10 L tank at 4°C overnight and later analyzed, taking into account pH, amount of Total Solids (TS), and then measured according to standard procedures (APHA, 1992) the Total Kjeldhal Nitrogen (TKN) and ammonium nitrogen ($N\text{-}NH_4^+$), which were determined using fresh material according to the analytical methods for wastewater sludges (IRSA CNR, 1994). Total phosphorus was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-MS, Varian, Fort Collins,USA).

3. Results and discussion

3.1 Characterization of ultrafiltrate stream

UF chemical characterization compared with the synthetic medium is shown in Table 1. All concentrations are reported on a wet weight (w.w.) basis. Most of the nitrogen in the digestate was in the form of ammonium (1435 ± 10 mg kg^{-1}) and therefore was readily available to algae (Wang et al., 2014). Although this is the preferred form of nitrogen used by algae, an high concentration of ammonium is toxic for the microalgae because it is lipid-soluble and so it easily diffuses through membranes (Collos & Harrison 2014).
To avoid the toxic effect caused by a high concentration of ammonium, and also to reduce the shading effect due to the brown color of the effluent, UF was diluted ten times (UF 1:10) with deionized water, resulting in a clarified liquid as shown in Figure 1b.

Table 1: Composition of f/2 medium and ultrafiltrate medium

Parameters	f/2	UF	UF 1:10
pH	8.21	8.68	8.68
TS (g kg^{-1})	0	8.9 ± 0.5	0.89 ± 0.4
TKN (mg kg^{-1})	12	1488 ± 15	148.9 ± 12
$N\text{-}NH_4^+$ (mg kg^{-1})	0	1435 ± 10	146 ± 10
N-org (mg kg^{-1})	0	27 ± 5	2.2 ± 6
P (mg kg^{-1})	1	31.3 ± 13.5	3 ± 10

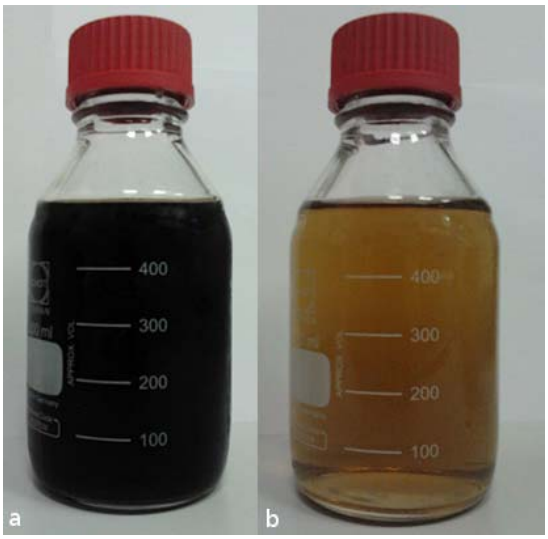

Figure 1: a) Pure UF b) UF diluted 1:10

3.2 Microalgae growth

In order to compare the performance of the strains, growth curves are plotted, with the graphs showing the values of biomass as dry weight vs time in days (Figure 2).

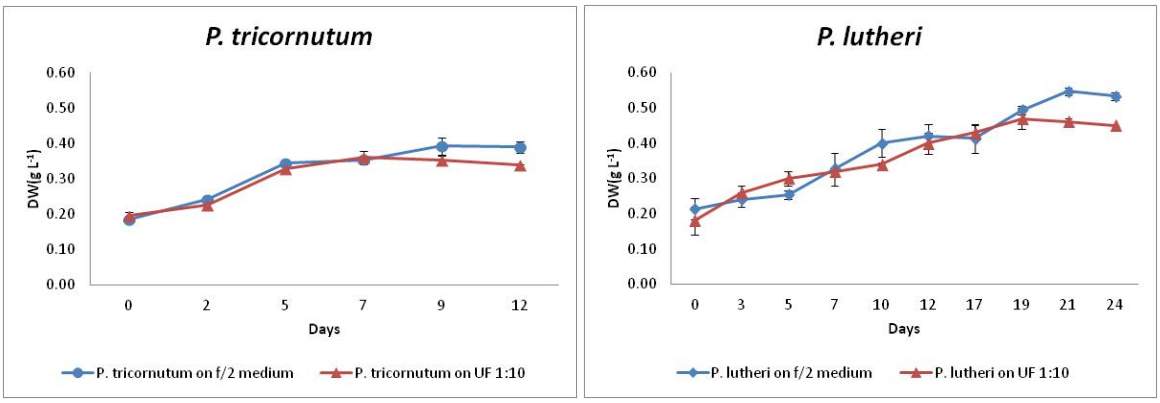

Figure 2: P.lutheri and P.tricornutum growth

In all treatments, microalgae showed a typical batch growth: *P.tricornutum* has an exponential phase of about 5–8 days during the 12 days of incubations; while for *P.lutheri* the exponential phase was much longer at 17-19 days. In both case it was assumed that the results do not show a significant lag phase for any of the trials because the inocula were carefully prepared before each experiment starting with a 0.2 g L^{-1} (Figure 2). The curves show slight differences in both species, related to standard medium versus the UF 1:10. In the late phase of the curve, the variation is more evident, probably caused by the higher self-shading effect in the UF 1:10 due to reduction of light penetration, from 120 to 70 µE m^{-2} s^{-1} in f/2 medium, whereas in UF 1:10 the light was reduced from 120 to 47 µE m^{-2} s^{-1}, determined by the Molina Grima method (Molina Grima et al., 1997).
This factor could explain the reduction of the maximum dry weight produced (Table 2), although the range of productivity in the two different media remained comparable,(i.e. 18-32 mg L^{-1} d^{-1} and 15-41 mg L^{-1} d^{-1}) for *P.tricornutum* on f/2 medium and UF 1:10, respectively. The productivity value for *P.lutheri* was substantially lower in both of the media used, and ranged from 8-17 mg L^{-1} d^{-1} to 11-26 mg L^{-1} d^{-1} for f/2 and UF 1:10, respectively. In the final phase of the experiment, *P.lutheri* recorded the highest biomass production, after 20 days it reached 56 mg L^{-1} in the standard medium vs 47 mg L^{-1} in the UF 1:10, whereas *P.tricornutum* reached 40 mg L^{-1} for f/2 and 34 mg L^{-1} UF in only 12 days.

Table 2: Microalgae growth parameters

Parameters	*Phaeodactylum tricornutum*		*Pavlova lutheri*	
	f/2	UF	f/2	UF
µ (d^{-1})	0.1	0.24	0.05	0.08
Productivity (g L^{-1} d^{-1})	0.024	0.025	0.015	0.017
Max DW (g L^{-1})	0.40	0.34	0.56	0.47

3.2 Nutrient recycling

Previously published (Norsker et al., 2011) total costs for producing microalgae at an industrial scale, estimated for 100 hectares of operative plant, are around 5 € per kg of dry weight. The estimate included all the operative costs like the investment, cultivation process and downstream processing, including the different

costs of the operational processes, within which fertilization, i.e. the medium and carbon dioxide, represented high costs ranging from 1 to 1.2 € per kg^{-1} dry mass (Norsker et al., 2011).
Utilization of effluents (flue gases and wastewaters) as raw materials, will allow the competitive production of microalgal biomass. Roughly, algal growth requires, per kg of dry weight produced, 1.8 kg of CO_2, 0.3 kg of N and 0.03 kg of P, as reported by Richmond (2008). According to the results we obtained, UF supported algae growth so that nutrients can be usefully recycled from the digestate stream, resulting in a money saving of about one € per kg of biomass produced, due to CO_2 and nutrient recycling. The biorefinery flow chart (Figure 3) summarizes a production scheme in which anaerobic digestion produces biogas and digestate, this latter could be used to sustain and produce algal biomasses, reducing total production costs. In particular, using the algal species studied in this work and based on literature data, potential productivity of high added-value products could be : Eicosapentaenoic acid (EPA) ranging from 20 to 30% of the total fatty acids with a total lipids productivity of 44.8 mg g^{-1} l^{-1} d^{-1} in the biomass for *P.tricornutum* (Singh & Gu 2010); Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) that account for 29% to 35% respectively of the total fatty acids, with a production of 39.5 mg g^{-1} l^{-1} d^{-1} for *P.lutheri* (Guihéneuf & Stengel 2013). In addition, these algal strains also produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feed or fertilizer (Singh & Gu 2010).

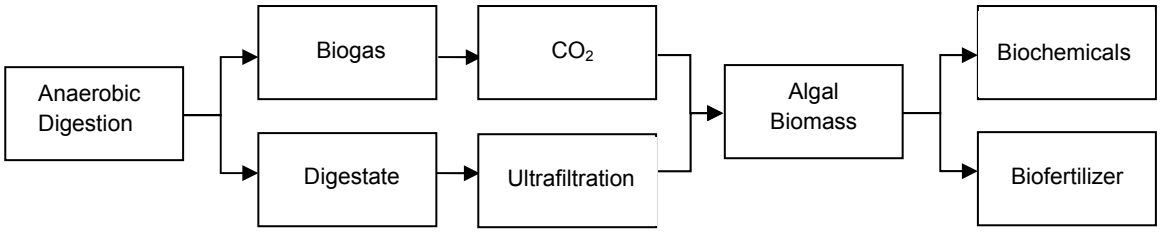

Figure 3: Biorefinery flow chart

4.Conclusion

Based on current technologies, algal cultivation and production on its own is unlikely to be economically viable. Dual-use microalgae cultivation coupled with an existing technology such as biogas production is therefore an attractive option in terms of reducing the cost, GHG emissions, and the nutrient (fertilizer) and freshwater resource costs of third generation algal biomass. The biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for obtaining biochemical products and is likely to be one of many approaches suitable for the production of high added-value chemicals, thus profitably integrating traditional agriculture and bioenergy production.

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