SUPPLEMENTARY MATERIAL

***Scenedesmus dimorphus* (Turpin) Kützing growth with digestate from biogas plant in outdoor bag photobioreactors**

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Abstract

Digestate coming from an Anaerobic Digestion unit in a Biogas Plant, feeded on cow manure and vegetable waste from markets, has been used. As cultivation container have been employed 8-35 L polyethylene transparent bags, outdoor. Different aliquots of digestate, alone or mixed with commercial liquid fertilizer, were employed to cultivate in batch *Scenedesmus dimorphus*, a freshwater green microalga, in the ENEA facilities of Casaccia Research Center, near Rome, Italy. Cultivation period was June - July 2013. Average daily yields of dry microalgae biomass varied from 20 mg/L/d to 60 mg/L/d, mean 38.2 mg/L/d. Final dry biomass concentration varied from 0.18 to 1.29 g/L, mean 0.55 g/L. *S. dimorphus* proved to be very efficient in removing N and P from the culture medium. Another fact emerged from these trials is that *S. dimorphus* inner composition proved to be variable in response to the tested different culture conditions.

Keywords: Microalgae, *Scenedesmus dimorphus* (Turpin) Kützing, Biogas, Digestate, Photobioreactors, Yields.

3 – Experimental.

3.1 – *Microalgae cultivation and climatic parameters*

The algae (Fig. S2) were grown with different aliquots of liquid digestate (DIG), integrated or not with commercial liquid fertilizer (CLF) for plants, as from Table 3, with color code. The used strain was UTEX 1237, maintained several years in the laboratory. The culture periods of the reported experiences varied between June and July 2013 and the relative durations in days were reported in Table 4. There was one trial with 12.7 cm diameter bags (8A), the other were all with 22 cm diameter bags (Fig. S1). The digestate came from a biogas production plant, working mainly on cow manure and vegetable waste from markets as main inputs, sited some 20 km near the ENEA Casaccia Research Center. The commercial liquid fertilizer for plants had a NPK content of 7:5:6. Aeration in the PBRs was provided, at a constant flux with large bubbles of 0.18 L/sec, but no CO₂ was added. Single batch cultures were realized, with an average duration of 13.1 days.

Photosynthetic Photon Flux (PPF) data were recorded constantly, in a 100 m far weather station, such as temperatures inside the culture and in air (Fig. S3) with appropriate probe and data logger from Tinytag.

3.2 - *Details on chemical analytical apparatus.*

A muffle furnace produced by BICASA, MI (Italy), able to reach 900°C was used to obtain ashes of the samples.

A Kjeldahl apparatus produced by BICASA, MI (Italy), was used for the determination of organic nitrogen. The apparatus was constituted by three places. Each place involved a heating source with a Kjeldahl digestion and distillation flask connected with refrigerant with a glass extension inserted in a sulphuric acid solution.

A mod. ICS-3000 Dionex apparatus equipped with an anion column Ion Pack AS23 connected with a pre-column Ion Pack AG23 and a conductivity detector was used for the anions analysis.

Strong cation exchange resins Amberlite 200 C-Na were used for the determination of potassium.

3.3 - *Reagents and chemical analysis.*

Phosphosulphuric acid and CuO RPE Carlo Erba products were used without further purification. Sulphuric acid and hydrochloric acid RPE Carlo Erba reagents were diluted to have solutions with suitable concentration for analysis where they were applied. The concentration of diluted sulphuric acid were determined by titration with KHCO₃ and Ti₂CO₃ as independent standard, by using a mixed indicator constituted by metal red and bromocresol green in suitable ratio. The results of several analyses agreed within $\pm 0.1\%$.

Solution of NaOH was titrated with hydrazine sulphate as standard, using the described mixed indicator. The results of several analyses agreed within $\pm 0.1\%$.

Standard solutions of anions for the analysis of chloride, nitrate, nitrite, sulphate and phosphate were prepared from RPE Carlo Erba reagents without further purification.

3.4 - Methods of analysis.

1. The biomass was recovered from the culture water by filtration on Whatman blue paper and dried in a desiccators at 45°C until constant weight.
2. The extraction of the lipidic fraction was conducted on a Soxhlet apparatus, extracting the dried algae materials with dichloromethane for a minimum of 12 h (Martinez and Crespi, 1997). The content of total lipids was conducted by gravimetric determination after the evaporation of the solvent at reduced pressure.
3. Ashes were determined by heating at 600°C a weighed sample. The temperature was gradually increased and the analysis was carried out till to have a constant weight of the obtained ashes.
4. To determine potassium ion, cation strong exchange resins were washed with distilled water and transformed in acid form by passing a strong excess of HCl 3 M. The excess of HCl was eliminated by washing again with distilled water till neutrality. At this point the sample was introduced and the eluate was titrated with standard NaOH, using the mixed indicator. The concentration of potassium ion was obtained by applying the following formula: $V_T M_T / V_0$, where V_T and M_T are the volume and the concentration of NaOH, respectively and V_0 is the volume of sample introduced in the resin. The results of several analyses agreed within $\pm 0.3\%$.
5. The determination of organic nitrogen was performed by applying the Kjeldahl method. A weighed sample was introduced in the Kjeldahl flask. An excess of phosphosulphuric acid and a small quantity of CuO, as catalyzer, were added. The mixture was heated. The heating was carried out till to obtain a light celestial solution. To this solution a strong excess of 30% NaOH solution was added to transform ammonium ion in NH_3 , which was distilled so that it bubbled in the standard sulphuric acid solution present in an excess. The excess of acid was titrated by standard NaOH. The concentration of nitrogen was obtained by applying the following formula:

$$(V_T N_T - V_S N_S) 14 \times 100 \times 10^{-3} \times 5,70 / P,$$

where $V_T N_T$ are volume and normality of sulphuric acid, $V_S N_S$ are volume and normality of NaOH, 14 is the atomic weight of nitrogen, P is the weight of the sample and 5,70 is the conversion factor. Analysis was three times repeated obtaining results agreeing within $\pm 0.3\%$.

6. A sample was injected in the Dionex ionic chromatograph and was eluted by means of 0.25 M NaOH under He. Qualitative analysis was performed by comparing the obtained chromatogram with that of a standard solution containing several anions, such as chloride, nitrate, nitrite, sulphate, phosphate. The comparison between areas of peaks obtained from sample and standard chromatogram provided the concentration of the anions present in the sample analyzed.

Figure S1: PBRs with fresh inoculums of *S. dimorphus*

Figure S2: *S. dimorphus* , 400 x

Figure S3: Daily air temperatures (°C), max, mean, min.