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Production of the Freshwater Microalgae *Scenedesmus Dimorphus* and *Arthrospira Platensis* by Using Cattle Digestate

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Microalgae are considered one of the most promising feedstocks for biofuels; these microorganisms are also able to enhance the nutritional content of conventional food preparations, or can be converted into other fuel products, such as hydrogen, ethanol, long-chain hydrocarbons resembling crude oil, or biogas. *Scendesmus dimorphus 1237* is an oleaginous eukaryotic microalga, able to produce and accumulate lipids up to a fraction around 43%. In condition of nitrogen starvation this percent grow sup to 50% of dry weight. Therefore this microalga is considered a promising feedstocks for biofuels. *Arthrospira platensis* is a cyanobacterium with a considerable potential as a source of high biologic value proteins ("superfood"), pigments (phycocyanin and beta-carotene) and poly-unsaturated fatty acids (PUFA) which have been shown to have therapeutic effects on humans.
Anaerobic digestion liquid effluents feature the presence of nutrients, such as nitrogen and phosphorous, which makes them interesting for a potential application in microalgal biomass production.
Aim of this work is investigating the use of liquid anaerobic cattle manure digestate for the photosynthetic growth of these microalgae.

1. Introduction

Economics is considered a key barrier to full-scale algal biodiesel production as a drop-in fuel, energy source, and commodity. There is a need to couple micro-algal oil production with other revenue streams and/or with other forms of energy production, such as anaerobic digestion (Chisti, 2013). This latter creates, however, a nutrient-rich waste stream, called digestate, which can be spread as crop fertilizer but has the potential to be used as a nutrient source for micro-algal growth. Recently, Ras et al. (2011) and Wang et al. (2010) investigated culturing of *Chlorella vulgaris* and *Chlorella* sp. as a means for fixing nitrogen and phosphorous contained in anaerobic manure digestates while Bjornsson et al. (2013) investigated culturing of *Scenedesmus* sp. for the same purpose. *Scenedesmus dimorphus* 1237, on the other hand, was investigated by Cicci et al. (2013) also to remediate olive oil mill wastewater.
Aim of the present work is characterising biomass production and nutrient removal from an anaerobic cattle-based digestate.
Scenedesmus dimorphus 1237 and *Arthrospira platensis*, in aseptic culture, were adopted as target photosynthetic microorganisms. The former is an oleaginous microalga that has been shown to grow also on some organic substrates (glycerol, i.e. exhibiting mixotropy), potentially usable for producing bio-oil. The latter is a fast-growing, high-protein-containing, intrinsically mixotrophic cyanobacterium, therefore capable of both metabolising dissolved organic carbon and accumulate a significant amount of nutrients per unit weight of outgrown biomass.
In this work a potential use, as nutrient sources, of liquid anaerobic digestate for microalgal growth has been investigated. A series of treatments was adopted to eliminate solids and biological contaminants,

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and two set of experiments were carried out: cultures insufflated with only air and cultures insuflated with air 5% CO_2 at different dilution ratios of synthetic medium and treated digestate.

2. Materials and Methods

2.1 Cattle digestate pre-treatment

The feedstock used is the liquid fraction obtained from the mechanical separation of the product of an anaerobic co-digestion of cow manure and agricultural products carried out in a 1-MW plant located in Cicerale (Southern Italy). The digestate was subsequently sieved at a 710 µm aperture to get rid of suspended solids and micro-filtered with a poliammide (type JX) (pore size 0.3 µm). Finally, the digestate was thermally sterilized (20 minutes at 120 °C).

2.2 Cultivation set up

Scenedesmus dimorphus (UTEX 1237) was obtained from the Culture Collection of Algae at the University of Texas at Austin, USA. The strain on agar was inoculated into a Modified Basal (MB) medium at the following composition (in mM/L): $CaCl_2$: 0.17, $NaNO_3$: 2.21, $MgSO_4 \cdot 7H_2O$: 0.3, K_2HPO_4: 0.43, KH_2PO_4: 1.29, NaCl: 0.43, $Na_2EDTA \cdot 2H_2O$: 2, $FeCl_3 \cdot 6H_2O$ 0.36, $MnCl_2 \cdot 4H_2O$: 0.21, $ZnCl_2$: 0.037, $CoCl_2 \cdot 6H_2O$: 0.0084, $Na_2MoO_4 \cdot 2H_2O$: 0.017. *Arthrospira platensis* was obtained from the CNR-ISE (Sesto Fiorentino, Italy) culture collection. The *A. platensis* strain on agar was inoculated into Zarrouk medium (Zarrouk 1966). Three set of experiments were performed for the digestate feedstock. The sterilised digestate was diluted 1:1, 1:5, 1:10 and 1:20 by volume, for each species, with the species-relevant media and with tap water, and then used for algal growth experiments. The cultures were bubbled with air and air supplemented with 5% CO_2; pH was adjusted to a value of 9.0 for *A. platensis* and 6.7 for *S. dimorphus* 1237. Cultures were grown in 400-mL cylindrical glass tubes, with diameter 6.5 cm, fed with filtered and humidified air; flow rate was 130 NL/h. The batch cultures were carried on for 5 days, with daily switching between lighting (16 h), provided by cold white fluorescence lamps (400 - 700 nm, 32 W, 80 µmol photons m^2 s^{-1}), and darkness (8 h). The temperature was maintained constant at 24 ± 1 °C. At the end of the batch, the biomass was collected, centrifuged and washed twice with distilled water. Afterwards, the collected microalgal biomass was transferred in a dry glass flask, dried in a vacuum oven for 4 hours, and weighed.

2.3 Biomass measurements

Absorbance, measured in correspondence to the chlorophyll absorption peak, was correlated to cellular density, determined by Burker chamber count and dry weight measurements. At the end of the experimental run the culture was collected, centrifuged and washed twice with distilled water. Afterwards, the recovered biomass was transferred in a pre-dried glass flask and dried in a vacuum oven for 4 hours. Dry weight was calculated subtracting the glass flask weight from the total weight. Absorbance data were determinated by spectrophotometry (UV-1800PC by Shanghai Mapada Instruments Co., Ltd). Specific growth rate was calculated from absorbance at 690nm.

2.4. Chromatographic analysis

A portion of culture was collected at 0 and 96 hours and centrifugated at 15000g for 15 minutes. Supernantants were collected and filtered 0.22 µm; ionic species concentration was determined by means of ionic chromatography using Dionex DX 120 equipment.

3. Results and discussion

The potential use of cattle digestate as medium for microalgal cultivation *(Scenedesmus dimorphus 1237* and *Arthrospira platensis*) was investigated; two sets of growth tests were carried out by using mixtures of cattle digestate with tap water (first set) and digestate with synthetic medium (second set). The first set of tests was performed to characterize the nutritional supply of cattle digestate; the second set was performed to study the digestate effects on growth in non-limited nutrient supply.

In figures 1 the growth rates of *S. dimorphus* 1237 in cattle digestate-based media at different concentrations are shown. Analysing the first four samples (cattle digestate diluted with synthetic medium), a growth increment corresponding to the dilution increment can be noticed; in the 1:20 digestate/synthetic medium case, a marked change of growth rate between “air” and “air+CO_2 5%” insufflations can be observed. Comparing with the control culture data, it is possible to find a similar value for the case with air only but not for the case where the insufflation was done with air enriched with CO_2. This allows to hypothesize that the limiting growth factor is represented by the available light intensity rather than nutrients limitation. Observing the growth rates in cattle digestate diluted with tap water it is

possible to deduce a strong nutrient limitation; in the case where the digestate was diluted 1:20 in tap water, growth rate decreased by 53% with respect to the 1:20 digestate/zarrouk sample.
In figure 2 (A), (B) and (C) the nutrients removal efficiencies by *S. dimorphus* in digestate/synthetic medium and digestate/tap water are shown; in (A) and (B) the cultures were insufflated only with air; (C) and (D) cultures were insufflated with air enriched with CO_2 (5% V/V).

Analysing the samples grown in digestate diluted with synthetic medium, (B) and (D), is possible to observe a greater removal efficiency when the cultures were insufflated with CO_2-supplemented air. These results are consistent with growth data where the presence of CO_2 (inasmuch a photosynthetic microalga) promotes growth and the concomitant nutrients uptake.

At dilutions 1:1, 1:5 and 1:10 of cattle digestate with synthetic medium, similar results in terms of growth and removal efficiency were obtained; in these samples, where the presence of digestate in terms of volume is high, the color of the cultures is dark and, considering the tubular geometry of the reactors, only a small fraction of the biomass received a sufficient quantity of light to use for maintenance and replication.

In (B) and (D), biomass had grown in digestate diluted with tap water insufflated by air and by air + CO_2 5% v/v; in addition to the physiological stress due to limitation and/or absence of substrates, light starvation is also present, due to the low penetration depth of light in the medium; the combination of these two factors gave an irregular response of the cultures.

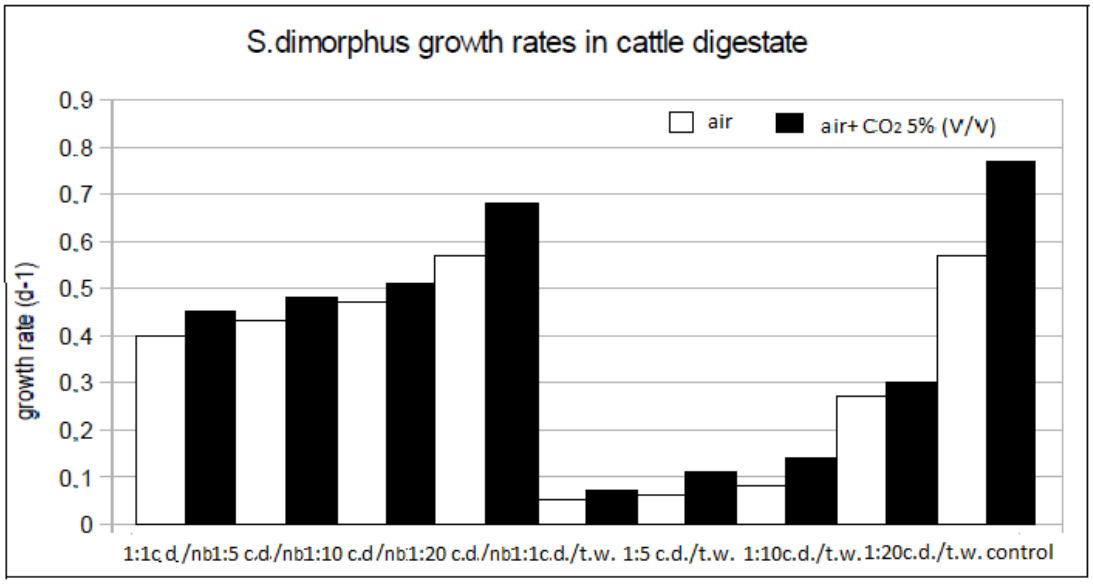


Figure1: S. dimorphus growth rates on different dilution ratio of cattle digestate (c.d.) and 3NB medium (nb) and tap water (t. w.) insufflated with air.

A. platensis was found to be more sensitive to the combined effect of nutrient starvation and toxic effects than *S. dimorphus*. Furthermore, *A. platensis* was found to be more sensitive toxic effects than to nutrient starvation. As possible to see in figure 3 (A), this results very clearly from the fact that a modest growth rate was only exhibited by those diluted cultures which are most nutrient-limited, but also less concentrated in toxic compounds and less light limited. It should be reminded, here, that *A. platensis* is a diazotropic species, which means that it can internally produce ammonium nitrogen from dissolved molecular nitrogen, when both ammonium and nitrates are absent in the medium. However, this occurs at an energy expense which determines a lower uptake preference with respect to the other nitrogen forms (when available) on the one hand, and a higher uptake energy cost which ultimately results in a reduced growth rate on the other hand.

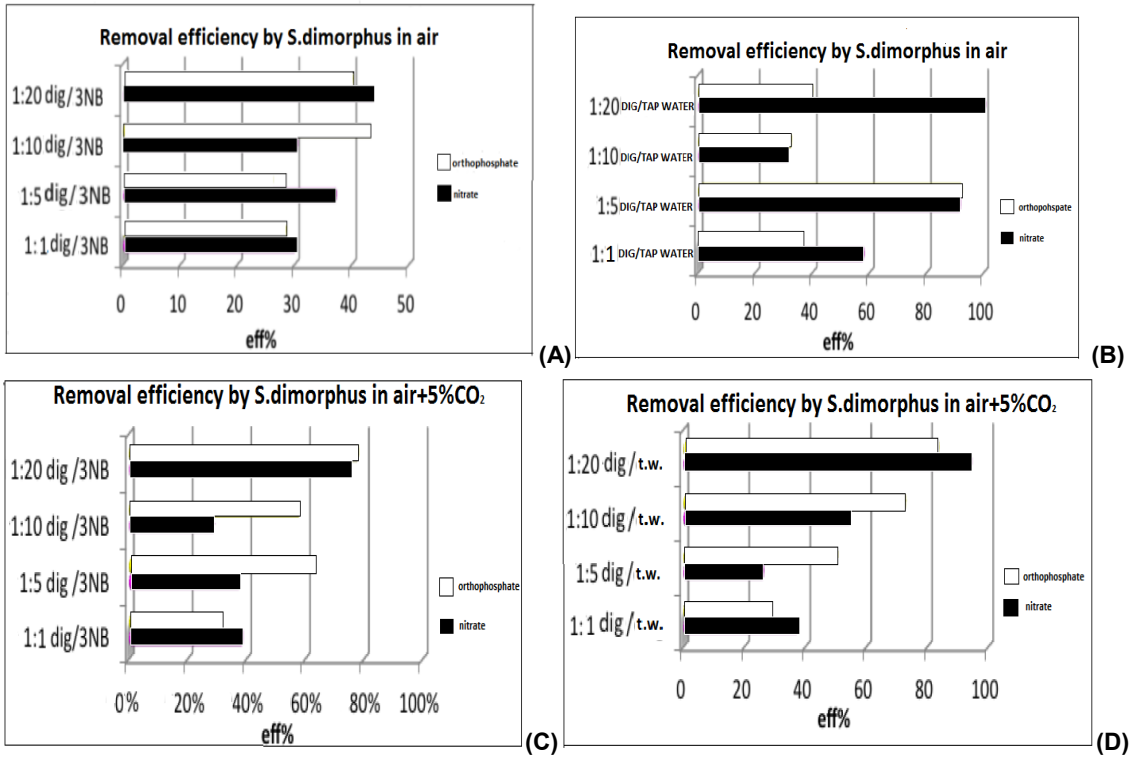
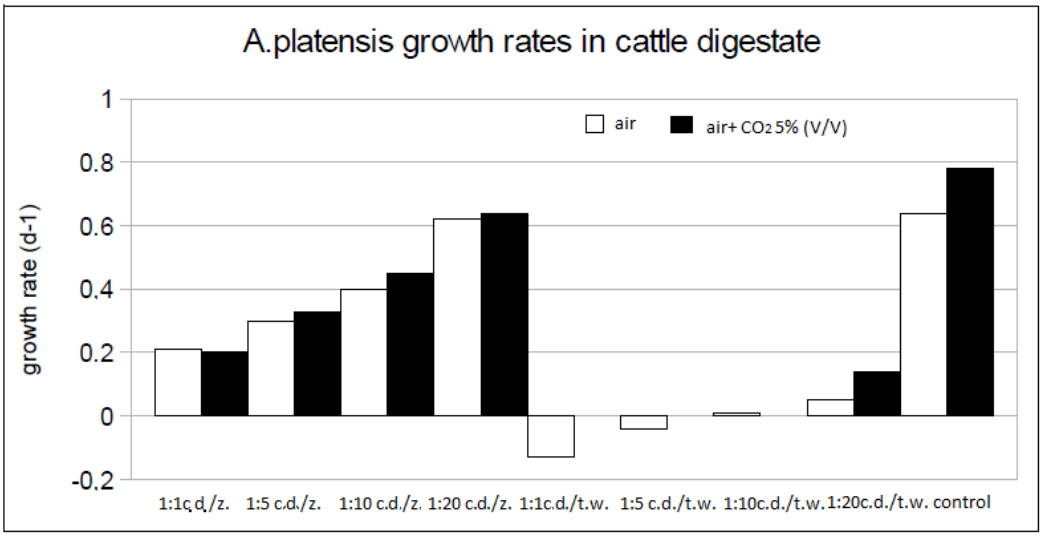


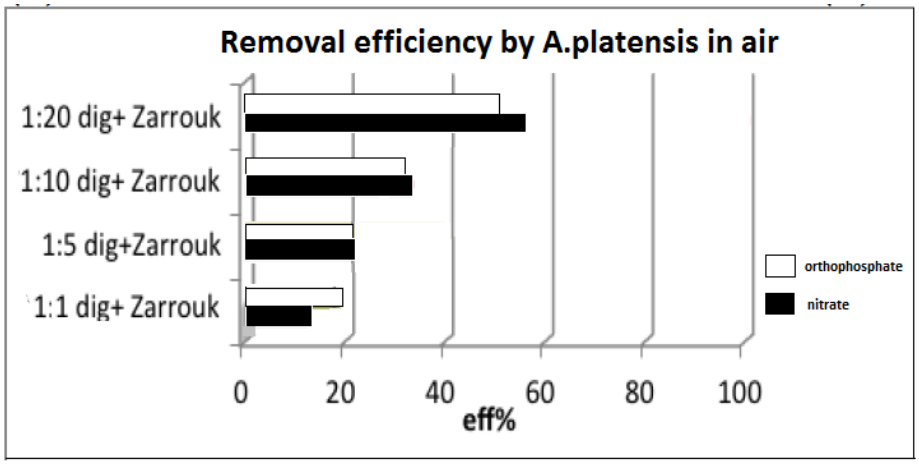
Figure 2: (A): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with synthetic medium insufflated with air. (B): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with tap water insufflated with air. (C): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with synthetic medium insufflated with air enriched in CO_2 5% v/v. (D): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with tap water insufflated with air enriched in CO_2 5% v/v.

In figure 3 (B) and (C) the removal efficiencies of nutrients by *A. platensis* in digestate and synthetic medium are shown. It can be checked that the removal efficiency data between cultures in air (B) and air + CO_2 (C) are similar, and that the relevant growth rates are also similar.

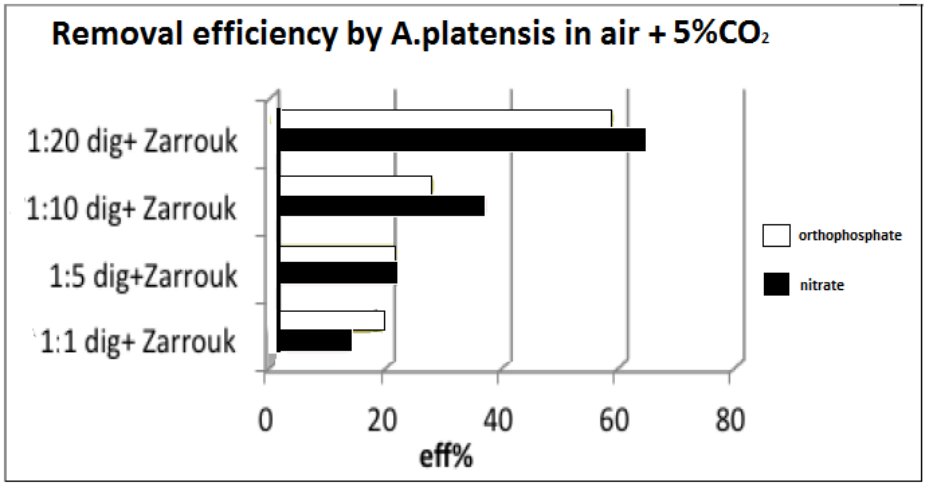


(A)

Figure 3: (A): A. platensis growth rates on different diluition ratio of cattle digesate (c.d.) and zarrouk medium (z.) and tap water (t. w.).



(B)



(C)

Figure 3: (B): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with synthetic medium insufflated with air. (C): removal efficiency of orthophosphate and nitrate ions in cattle digestate diluted with synthetic medium insufflated with air enriched in CO_2 5% v/v.

4. Conclusions

The liquid fraction of anaerobic cattle digestate was used as the base of a semi-synthetic media for culturing *S. dimorphus* (an oleaginous microalga) and *A. platensis* (a high-protein cyanobacterium) obtained by diluting it at various degrees with tap water and the respective, species-relevant synthetic medium. The performed culturing runs, carried out with air (with and without CO_2 supplementation) showed that *S. dimorphus* is more resistant to physiological stress consequent to nutrient starvation and light limitation. Specific growth rates in the presence of major proportions of cattle digestate are low, due to limiting nutrients, and exhibit minor variations in the presence of CO_2 , due to a light limitation effect.
A. platensis shows greater sensitivity than *S. dimorphus* both in terms of nutrients supply and of light supply; in tap water-diluted digestate as the only nutritional source it grows only at high dilution ratios, corresponding to a significant medium transparency. In synthetic medium-diluted digestate, *A. platensis* grows also at low dilutions and, under CO_2 supplementation, growth can be improved at high dilutions. It can be concluded therefore that *A. platensis* growth is light supply-limited at low dilutions and CO_2 supply-limited at high dilutions.

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