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# Integration of microalgae production with anaerobic digestion of dairy cattle manure: an overall mass and energy balance of the process

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### ABSTRACT

This work aimed to investigate the possibility of integrating microalgae production with anaerobic digestion of dairy cattle manure and subsequent digestate treatment, thus helping to reduce the cost of slurry treatment and improving the energy balance of the process. Real biogas and digestate-treatment units were monitored for energy, mass and nutrient balances. The existing system produces electrical energy (2182 ekWh d<sup>-1</sup>) and organic fertilizers/amendments (11,655 t y<sup>-1</sup>), among others. Microalgae production was integrated with this system by using untreated ultra-filtered digestate as the growth medium for the production of *Scenedesmus* sp. The tolerance of this strain to digestate was evaluated and results demonstrated that a percentage of digestate of over 10% inhibited the growth of this microalga, but below this value productivity of up to 124 mg L<sup>-1</sup> d<sup>-1</sup> could be obtained. The composition of the culture medium also influenced the biomass composition, with protein, carbohydrate and lipid content being direct functions of ammonia concentrations. Integrating microalgae production with anaerobic digestion, it is possible to produce 166–190 t y<sup>-1</sup> of valuable microalgal biomass. Overall energy and mass balances of the process allow us to show that the integration of both anaerobic digestion and microalgae production steps are positive and technically feasible.

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### 1. Introduction

Water resources management has become an important issue due to anthropogenic effects caused by population increasing (i.e. urbanization) and agricultural and industrial activities, which affect both quantity and quality of shallow and deep waters (Peters and Meybeck, 2000). As consequence of that there is a need to improve the sustainable use of water resources by considering innovative productive processes in which a product-to-waste-toproduct scheme has to be pursued.

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The development of livestock production has gained attention for its environmental impact because of the production of massive volumes of polluted slurries characterized by high organic and mineral load, this latter mainly represented by nitrogen (N) and phosphorus (P) (Petersen et al., 2007). Nitrogen from livestock slurries contributes largely to environmental pollution through ammonia and nitrogen oxides emission to the atmosphere and nitrate leaching to ground and surface water bodies (Brandjes et al., 1996). In order to reduce these impacts a wide range of treatment techniques has been proposed, although most of them are considered too expensive for livestock farming (Burton, 2007) and also difficult to apply. Livestock slurries are frequently treated by anaerobic digestion to produce renewable energy. By this process the organic matter is transformed into biogas (50-80% v/v methane), used to produce energy and heat. Anaerobic digestion modifies the composition of the slurry, particularly regarding N. Proteins are degraded and ammonia is

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Abbreviations: ekWh, electric kWh; TKN, Total Kjieldahl Nitrogen; GHG, Greenhouse Gases; GMO, Genetically Modified Organism.

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produced so that the N–NH<sub>4</sub><sup>+</sup>/TKN ratio increases (Schievano et al., 2009). Moreover ammonia accumulation increases the fertilizing properties of digestate (Schievano et al., 2009), but, also, can facilitate N removal from digestate when stripping technologies are used.

The recovery of nutrients from wastes (especially N, P and K), is becoming a research priority as they are finite resources concentrated in a few countries (Cordell et al., 2009), or they require high energy input to be produced and/or transported, causing high GHG emissions (Wood and Cowie, 2004). Therefore the possibility of recovering nutrients to be employed in new productive processes is becoming fascinating and important, above all for farmers who need to find new possibilities of improving agricultural incomes because of reductions in subsidies from central governments (EC MEMO/13/937). In a recent work Ledda et al. (2013) showed an innovative process for livestock manure treatment, coupled with anaerobic digestion, that reduced treatment costs jointly with both water and nutrient recovery, so that slurry became a resource rather than a waste.

Concerning recovery of nutrients, microalgae are photosynthetic microorganisms capable of taking up inorganic N and P and transforming them into valuable organic compounds using solar energy. Microalgae have been exploited for this purpose due to their high productivities and the possibility to use them as single-cell bio-factories for the production of nutraceuticals, pharmaceuticals, pigments, biopolymers, chemicals and animal feed products (Pulz and Gross, 2004). Moreover, algae have been reported to grow efficiently on wastewater (Oswald and Gootas, 1957) because of their tolerance to high concentrations of nutrients and ability to produce large amounts of biomass (De la Noüe et al., 1992).

Among the various different species studied, the green microalga *Scenedesmus* sp. has shown extraordinary vitality in urban wastewaters, with growth rates similar to those reported on complete synthetic media, so that it has commonly been used for the phytoremediation of industrial, urban and agricultural wastewaters (Xiao et al., 2011; Ji et al., 2013; Franchino et al., 2013).

Recently a model was proposed to develop a farm level integrated system focused on the anaerobic digestion process (Manenti and Adani, 2014). In that paper, particular attention was paid to the re-use of digestate as the nutrient medium to produce microalgal biomass. Nevertheless no experimental data were reported and the proposal remained academic.

When using the digestate to cultivate microalgae, another key point could be the negative effect of bacteria on microalgal biomass survival and quality. To solve this problem, slurry should be sterilized (Cai et al., 2013), adding costs and energy to the process and leading to an important constraint in the scale-up of microalgae cultivation using animal slurries (Zhu et al., 2013).

This issue could be solved by finding full-scale technologies, applicable at farm scale and at a low cost, to ensure the sterilization of the slurry. It would then be possible to achieve the remediation of the waste stream by recovering the nutrients into a high quality biomass.

The work which we report here represents a first attempt to develop a farm-level integrated process that considers the production of food from farming and of energy from anaerobic digestion, the recycling of nutrients and water from digestate treatment, and the production of algal biomass (*Scenedesmus* sp.) using the nutrients contained in the liquid streams (ultrafiltration permeate). Results from this work are of interest for the integration of livestock slurry treatment with biogas production and microalgae production, enabling farm product diversification and an increase in agricultural incomes.

#### 2. Methods

#### 2.1. Biogas and digestate-treatment units

A real anaerobic digester  $(1500 \text{ m}^3)$  serves a GMO-free dairy farm with 1200 Italian Friesian cattle, 400 of which are in lactation. The annual production of milk is about 12 t which are entirely delivered to the "Grana Padano Consortium". Farm land availability is about 200 ha, used for cattle and biogas plant feed. The biogas plant has an installed electrical power of 180 kW and it reutilizes the cattle manure as the liquid substrate, but enriches it by codigesting various biomasses such as cattle manure solid fraction among others. The digestate from the anaerobic digester is further treated by a fractionation process to separate the liquid from the solid fraction and to produce ammonium sulfate usable as fertilizer (Ledda et al., 2013). The treatment unit operates in batch mode. The first separation is achieved with a screw press separator after which the liquid fraction is treated by the addition of a polyamide flocculant and sent to a decanter centrifuge. Then, the centrifuged liquid enters the ultrafiltration unit, equipped with a 40 kDa grafted polyacrylonitrile membrane.

The ultra-filtered permeate (UFP) can potentially be used to produce microalgae, but under real conditions it is subjected to a reverse osmosis step. Finally, the permeate from reverse osmosis is refined in a zeolites bed and then discharged to surface-water bodies. The concentrate from reverse osmosis also enters a cold ammonia stripping unit where lime is added, raising pH up to 12-12.5. Under these conditions, NH<sub>3</sub> is stripped in gaseous form by using a controlled air flow, which is then scrubbed with sulfuric acid, producing liquid ammonium sulfate.

#### 2.2. Characterization of anaerobic digestion step

All the streams coming from the biogas and the digestatetreatment units were sampled within three different cycles of the treatment process and chemically characterized. Representative samples of wastes were used to carry out all the analyses. Total solids (TS) and volatile solids (VS) were determined according to standard procedures (APHA, 1998). The values of biochemical methane potential (BMP) of the samples were determined by using a standardized method reported in Schievano et al. (2008). BMP values detected for both input and output material were used jointly with mass balance data to determine the bio-methane yield (BMY) of the biogas plant, by using the Equation (1) (Schievano et al., 2011) where BMP<sub>in</sub> is the bio-methane potential in the fed mixture ( $m^3 kg^{-1} TS$ ), BMP<sub>out</sub> is the bio-methane potential in the output digestate ( $m^3 kg^{-1} TS$ ),  $TS_{in}$  are the total solids fed during the observed period (kg) and  $\mathrm{TS}_{\mathrm{out}}$  are the total solids output with digestate during observed period (kg).

$$BMY(\%) = \frac{(BMP_{in} \times TS_{in} - BMP_{out} \times TS_{out})}{BMP_{in} \times TS_{in}} \times 100$$
(1)

TKN, ammonia nitrogen  $(N-NH_4^+)$  and nitric nitrogen  $(N-NO_3^-)$  were determined using fresh material according to the analytical methods for wastewater sludge (IRSA CNR, 1994). Elemental analysis was performed according to the 3051A method (EPA, 1998). Total phosphorus (P) content was determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian, Fort Collins, USA). A certified standard reference material (2782 Industrial Sludge) from the National Institute of Standards and Technology (Gaithersburg, US) was used in the digestion and analysis. To ensure the accuracy and precision in the analyses, reagent blanks were run with samples. All analyses were performed in triplicate.

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