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A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing

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ABSTRACT

The liquid fraction of digestate produced by agricultural biogas plants is rich in macro and micronutrients that are valuable for the culturing of microalgae. Nonetheless, the high ammonium concentration may cause toxicity and the high optical density may reduce light penetration, negatively affecting the biomass production rate. Dilution with fresh water has been frequently suggested as a mean for improving the digestate characteristics in view of microalgal culturing. In this paper, the feasibility of culturing microalgae on undiluted raw digestate or on digestate after pretreatment by stripping and adsorption was investigated.

First, adsorption tests were performed using commercial activated carbon from wood in order to identify appropriate conditions for optical density (OD) reduction. Up to 88% reduction was obtained by dosing 40 g L^{-1} after 24 h of contact time. Then, culturing tests were performed on a microalgal inoculum including mainly *Chlorella* spp. and *Scenedesmus* spp. under controlled temperature and light conditions during 6–14 weeks. Raw, stripped, and stripped and adsorbed digestate samples were tested. The biomass production rate increased from 27 ± 13 mg TSS L^{-1} d^{-1} on raw digestate, to 82 ± 18 mg TSS L^{-1} d^{-1} by using stripped digestate, and to 220 ± 78 mg TSS L^{-1} d^{-1} by using the stripped and adsorbed digestate. Moreover, nitrification was constantly suppressed when using the stripped and adsorbed digestate, while relevant nitrite built-up was observed when using raw and stripped digestate. These results suggest that microalgae are able to grow on the raw digestate, provided that long hydraulic retention times are applied. A much faster growth (up to 10 times) can be obtained by pretreating the liquid fraction of digestate by stripping and adsorption, which may be an effective means of improving the areal productivity of microalgal culturing on digestates.

1. Introduction

In agricultural areas, biogas technology is extensively used for reducing organic matter load from livestock wastewater, through the transformation of organic carbon into biogas, which is in its turn a renewable biofuel [1]. Anaerobic digestion (AD) improves the environmental sustainability of animal breeding and full-scale installations (biogas plants) have spread rapidly in Europe. As a result of this rapid development, huge amounts of digestate are produced and generally separated into liquid/solid fractions at farm scale. The liquid digestate is rich in nitrogen and potassium and is normally spread into fields as fertilizer, whereas the solid phase, rich in phosphorous and stabilized carbon, is generally used as soil amendment. Nonetheless, due to restrictions imposed by to the European Nitrate directive (91/676/CEE), but also to economic and environmental issues, its conventional valorization route as soil amendment and/or fertilizer does not always fit the local context of intensive livestock areas [2]. Indeed, liquid digestate spreading may cause significant environmental problems such as water contamination and eutrophication [3]. This aspect has prompted attention toward technical solutions to reduce the nitrogen load of digestate, either by chemical-physical processes such as stripping, evaporation, membrane filtration [4] or by advanced biological processes, such as the anammox process [5]. Recent studies have focused on the possibility to use liquid digestate as a nutrient source for microalgae growth [2,6]. According to Xia and Murphy [3], the land requirement for microalgal cultivation is estimated as 3% of traditional direct land application of digestate. Furthermore, in a concept of circular economy, microalgae grown on digestate can be sent back to the anaerobic digester as co-digesting feedstock or used on soil as a slow-release fertilizer [2,7,8].

However, two major drawbacks of using the liquid fraction of digestate as a substrate for microalgae growth are related to its high nitrogen content and turbidity level [3,9]. Indeed, the liquid fraction of

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digestate is often characterized by: a high concentration of total suspended solids, causing an inherently high turbidity and reducing light transmission, and of ammonia (>100 mg N L^{-1}), which could potentially be toxic to microalgae [10]. Thus, pre-treatments have been often adopted to remove or dilute the undesirable compounds from supernatants. As concerns turbidity, the most common pre-treatments include solid/liquid separation [11], filtration [12] and dilution [13] which also helps in lowering the ammonium concentration. Another novel and promising option is the use of activated carbon (AC) for reducing the turbidity level of liquid digestate, and thus facilitating microalgae growth. The use of activated carbon has attracted attention due to its high capacity of adsorbing various kinds of components [14–16]. Furthermore, this activated carbon could be produced from renewable biomasses like lignocellulosic biomasses (i.e. wood) through a two steps process: char production by pyrolysis, followed by steam or chemical/thermal activation [17,18]. The use of AC for turbidity reduction has been largely investigated for wastewater or seawater treatment with good results [19–21]. Nonetheless, until now, no studies reported the use of AC to reduce the turbidity of liquid digestate in view of microalgae culturing.

The main objective of this study was to evaluate the feasibility of growing microalgae on digestate without addition of fresh water for dilution. To this aim, undiluted (raw) digestate was first tested as microalgal growth medium. Then, pretreatments (ammonia stripping and a combination of stripping and adsorption on AC) were applied to digestate to reduce ammonium concentration, turbidity, and optical density, thus facilitating microalgae growth. First, the effect of the AC concentration (in the range 1 to 40 g L^{-1}) and contact time (in the range 0 to 1440 min) on optical density reduction were investigated. Then, microalgae culturing tests were conducted on raw and pretreated liquid digestate.

2. Materials and methods

2.1. Liquid digestate characterization

The liquid fraction of digestate came from a piggery farm in Northern Italy, breeding up to 20,000 pigs. At the hosting farm, a full-scale wastewater treatment plant (WWTP) and a full-scale anaerobic digester followed by a solid/liquid separation were in operation. The anaerobic digester was fed on the solid fraction of piggery manure (after floatation), poultry manure, maize silage and cheese whey (more details in Scaglione et al. 2015 [5]). The liquid fraction of digestate was collected after solid/liquid separation performed by centrifugation and is here after referred to as DIG_L.

2.2. Adsorption tests

Adsorption tests were carried out by using a commercial activated carbon (AC) from wood (Norit® CA1, Sigma Aldrich, Saint Louis, USA). Tests were carried out at room temperature (20 °C), under static conditions, by varying the solid loading of AC from 1 to 40 g L^{-1}, and the adsorption time from 0 to 1440 min. After adsorption, samples were centrifuged at 12200 rpm for 2 min (MiniSpin®, Eppendorf, Hamburg, Germany) and the supernatant was separated and used for optical density analysis.

Optical density (OD) at 680 nm was used to quantify the clarifying effect of the adsorption process. Indeed, the primary pigment involved in photosynthesis is chlorophyll *a*, which has strong absorption bands in the regions 400–450 and 650–700 nm [22]. According to preliminary tests (data not shown), the mixed microalgal community used in the algal growth tests showed two maxima in the adsorption spectrum at 420 and 680 nm. A strong correlation (data not shown) was observed between OD at 680 and at 420 nm measured on samples of adsorbed DIG_L, suggesting that just one OD determination could be made and used as the reference parameter to assess the clarifying effect of the AC adsorption process. Therefore, the clarification effect of the adsorption process was assessed by comparing the OD level at 680 nm of the original DIG_L ($OD_{digestate}$) to that of the adsorbed digestate ($OD_{adsorbed\ digestate}$), as follows:

$$\text{OD reduction}\ (\%) = \left(OD_{digestate} - OD_{adsorbed\ digestate}\right)/\left(OD_{digestate}\right) \quad (1)$$

2.3. Microalgal culturing tests

Continuous algal culturing tests were performed in 150 mL glass test tubes (4.5 cm diameter, 20 cm height). Light was provided by 6 fluorescent lamps (FLUORA model, OSRAM, Munich, Germany), 18 W each one, with 12 h dark/light periods. The PAR value measured at the test tube location was 130 μmol m^{-2} s^{-1}. Air was flushed from the bottom of each tube through a fine bubble diffuser to maintain well mixed conditions. Temperature remained around 20 ± 2 °C.

2.3.1. Tests on raw liquid digestate

Each test tube was prepared by mixing a concentrated microalgal inoculum (10 mL) with 15 mL of DIG_L and 125 mL of tap water. The initial condition was selected in order to start from a non-inhibiting ammonium concentration and to ensure sufficient algal inoculum to ensure a rapid colonization. Specifically, the following conditions were set in each test tube: 160 mgN·L^{-1} of ammoniacal nitrogen concentration and an OD of 0.5. As for the algal inoculum, a mixed microalgal community dominated *by Chlorella* spp. and *Scenedesmus* spp., grown on the liquid digestate from a digester fed on waste activated sludge (Carimate (CO) wastewater Treatment Plant) was used [23]. The algal biomass was centrifuged (10 min, 3000 rpm) and a 10 mL aliquot, containing 0.255 g of dry solids, was added to each tube. The starting optical densities of the tubes were 0.50.

Every 1 or 2 weeks, a sample (10 out of 150 mL) of the algal suspension was withdrawn and substituted by an equivalent volume of DIG_L. The frequency of feeding was adjusted in order to achieve an almost complete depletion of the ammonium concentration before each feeding, thus avoiding too high ammonium concentration after each feeding event, which remained below 160 mg N L^{-1}. Tests were performed in triplicate. The concentration of N forms, chemical oxygen demand (COD), phosphorus, total and volatile suspended solids (TSS and VSS), pH, temperature, OD at 680 nm and algal counts were assessed on samples of the algal suspension withdrawn from the test tubes.

2.3.2. Tests on stripped and adsorbed liquid digestate

During these experiments, microalgae were cultured on two types of pretreated liquid digestates. The first one was pretreated by a stripping phase and will be referred to as DIG_S; the second one was prepared by further treating the stripped digestate with AC in order to reduce the OD (DIG_S&A). The stripped digestate was prepared using an air compressor providing 0.6–1.2 vvm through a fine bubble diffuser. No alkaline chemical was used since alkaline pH (around 9–9.5) was naturally achieved during air bubbling. The stripping process lasted from 5 to 7 days at 20 ± 2 °C.

The DIG_S&A was prepared by treating the stripped digestate with activated carbon. Following the results of the adsorption tests, a solid load of 40 g AC L^{-1} and an adsorption time of 10 min were chosen. The adsorption process was carried out under static conditions at ambient temperature (20 °C). Then, the activated carbon was separated by centrifuging (3000 rpm for 5 min).

Three batches of DIG_S and DIG_S&A were prepared to be used to feed the microalgal cultures. Each one was prepared from a freshly collected DIG_L which was first stripped and then adsorbed on AC. Each batch of DIG_S and DIG_S&A was then stored at 4 °C and used for microalgal feeding for no >2 weeks. Optical density at 680 nm, ammonium, nitrate, nitrite, soluble COD and phosphate concentrations were assessed for each batch of DIG_S and DIG_S&A.