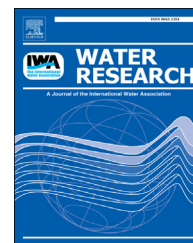


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Digestate color and light intensity affect nutrient removal and competition phenomena in a microalgal-bacterial ecosystem

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ABSTRACT

During anaerobic digestion, nutrients are mineralized and may require post-treatment for optimum valorization. The cultivation of autotrophic microalgae using the digestate supernatant is a promising solution; however the dark color of the influent poses a serious problem. First, the color of the digestates was studied and the results obtained using three different digestates demonstrated a strong heterogeneity although their color remained rather constant over time. The digestates absorbed light over the whole visible spectrum and remained colored even after a ten-fold dilution. Secondly, the impact of light and of substrate color on the growth of *Scenedesmus* sp. and on nitrogen removal were assessed. These experiments led to the construction of a model for predicting the impact of influent color and light intensity on N removal. Maximum N removal ($8.5 \text{ mgN-NH}_4^+ \text{ L}^{-1} \text{ d}^{-1}$) was observed with an initial optical density of 0.221 and $244 \mu\text{molE m}^{-2} \text{ s}^{-1}$ light and the model allows to determine N removal between 15.9 and $22.7 \text{ mgN-NH}_4^+ \text{ L}^{-1} \text{ d}^{-1}$ in real conditions according to the dilution level of the influent and related color. Changes in the microalgae community were monitored and revealed the advantage of *Chlorella* over *Scenedesmus* under light-limitation. Additionally microalgae outcompeted nitrifying bacteria and experiments showed how microalgae become better competitors for nutrients when phosphorus is limiting. Furthermore, nitrification was limited by microalgae growth, even when P was not limiting.

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1. Introduction

Within intensive livestock farming systems such as found in Brittany (France), the over-application of manure on agricultural soils leads to nitrogen and phosphorus discharge into the

aquatic environment, resulting in serious eutrophication problems and decreasing water quality (Jongbloed and Lenis, 1998). Manures are heterogeneous complex substrates that contain volatile compounds like ammonia. Such substrates are difficult to handle in an environmentally-friendly way and

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bad management can lead to losses into the atmosphere, increasing environmental impacts and decreasing recycling efficiency. For example, ammonia emissions from pig slurry used as crop fertilizer without prior treatment represent up to 50% loss of total excreted nitrogen (Portejoie et al., 2003). Simultaneously, anaerobic digestion of livestock waste is becoming increasingly common in Europe, since it can produce renewable energy and reduce direct and indirect greenhouse gas emissions. However, the nitrogen and phosphorus contents of the waste are not modified by anaerobic digestion, and the increase in pH and ammonia content may further increase potential losses into the atmosphere (Massé et al., 2011). Consequently, additional post-treatment is required to improve nutrient recovery through better management either to reduce environmental impacts and/or to allow them to be exported from the intensive livestock breeding region. Accumulating mineralized nutrients in autotrophic microalgal biomass by cultivating microalgae, using the digestate as substrate, was identified as an efficient way to achieve this objective (Lens et al., 2001). In addition, the combination of the two treatments allows better use of the energy that is produced in cogeneration by using excess heat for microalgae growth. If the flue gas produced by cogeneration is used as a carbon source for the cultivation of microalgae, thereby reducing the amount of carbon dioxide released into the atmosphere, the global carbon print will also be reduced. After the nutrients have been accumulated in the biomass and harvested, the microalgae could then be exported and used as organic “slow release fertilizer” (Mulbry et al., 2005; Patel et al., 2012) thus reducing short term ammonia emissions and losses.

Consequently, such a process allowing recycling nutrients from manure or digestate or quite similar effluents by means of cultivating microalgae has previously been studied (de la Noüe and Bassères, 1989; Tam and Wong, 1994; Sevrin-Reyssac et al., 1996; Molinuevo-Salces et al., 2010; Park et al., 2010; Levine et al., 2011). However, the biomass productivity observed in these studies varied greatly from 31 to 332 mg DW L⁻¹ d⁻¹, with nitrogen removal rate varying from 3.4 to 36.5 mgN L⁻¹ d⁻¹ considering an N content of 11% in the microalgae biomass. Additionally, the data based on DW productivity included the heterotrophic biomass growth with the microalgae growth. The heterotrophic biomass could be significant, with such concentrated effluents and the exact microalgae productivity rate is consequently not well-known. In the same way, when data are based on N removal rate, the processes involved including microalgae growth but also NH₃ stripping and nitrification/denitrification are often not well quantified whereas these processes other than microalgae growth could represent more than 50% of N removal in some cases (de Godos et al., 2009). Moreover, the impact of the effluent characteristics and the related effect of the cultivating conditions are little documented while one of the main difficulties involved in the use of digestate as substrate for the cultivation of microalgae is the dark color of the influent (Gonzalez-Fernandez et al., 2011). The dark color reduces light penetration and hence the amount of light available for the autotrophic growth of microalgae. The growth of microalgae in such a medium requires its dilution, mainly to promote light penetration, but also to reduce the high nutrient content,

which could inhibit growth (Azov and Goldman, 1982). Light penetration depends not only on the color of the influent but can also be reduced by too high concentrations of microalgae (Fallowfield and Garrett, 1985), which in turn, depend on the culture conditions and the initial concentration of microalgae. The amount of water used to dilute the digestate during the process has to be as small as possible not only to ensure the process is economical and ecological but also that it is feasible. In another connection, the intensity of the color not only reduces light penetration, but could also be responsible for a change in species composition (Bartosh and Banks, 2007). However, despite the important role of the color of the influent in the efficiency and consequently the feasibility of the recycling process, data on the characteristics of the digestate and their impact on the growth of microalgae are rare.

The aim of this work was thus to characterize the color of digestates and to quantify the effect of their color and light intensity on microalgae growth (quantity and species) in order to be able to predict microalgae growth and nutrient assimilation yields.

2. Materials and methods

The color of digestates from three industrial sites was characterized over the period of a year to observe differences between the three sites as well as any changes the digestates may have undergone with time. The ability of light to penetrate different colored digestates and microalgae concentrations was measured to compare their influence.

To study the effect of the intensity of the light and the color of the digestate on microalgae growth, batch experiments were carried out at laboratory scale according to an experimental design. Different colored influents and light intensity conditions were tested to measure microalgae growth and nutrient removal over a period of 14 days. At the beginning of each experiment, the reactors were seeded with an inoculum mainly composed of microalgae. Microalgae growth and nutrient removal yields were monitored as a function of the light and color conditions, and changes in the microalgae species were measured by comparing initial and final states.

2.1. Characterization of digestates

Three digestates originating from farm anaerobic digesters (D1 and D2) and collective anaerobic digester (D3) facilities were monitored over a period of one year. Anaerobic digestion inputs were cattle manure, cereal waste and cattle slurry (D1), cattle manure, vegetable waste and paper paste (D2), and pig slurry and activated and greasy sludge from a slaughterhouse and wastewater treatment plant (D3). The three digestates studied corresponded to a supernatant resulting from decanter centrifuge separation with polymer addition (D3) and screw press (D1 & D2).

Digestates were sampled five times over a period of one year (9/2011, 11/2011, 2/2012, 4/2012, 6/2012). The ammonium content of each sample was measured each time, whereas the phosphate content was measured the two first times. The color of the digestates was also determined.

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