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Selection of microalgae intended for valorization of digestate from agro-waste mixtures

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ABSTRACT

Digestates have been recently recognized as valuable substrates for microalgal cultivation, effectively combining wastewater remediation and biofuels production. In this regard, selection of the appropriate species for such a process is of utmost importance. In this study, the performance of seven different microalgal strains in 10% (v/v) digestate which derived from the co-digestion of several agro-waste streams was investigated. *Parachlorella kessleri*, *Acutodesmus obliquus*, *Chlorella vulgaris* and *Tetraselmis tetrahele* were able to acclimate to this new medium, resulting in biomass yields and fatty acids (FAs) content which varied between 570–1117 mg L⁻¹ and 3.9–24.5%, respectively. The main FAs detected in the four species were oleic, palmitic and linolenic acid, with significant differences in their relative abundance. Concerning nutrients removal, almost complete NH₃-N removal was observed, while % TP removal exceeded 80% for three of the four strains tested. Furthermore, induction kinetics of prompt chlorophyll fluorescence was used as a screening tool indicative of the reactions of the photosynthetic machinery of different microalgal species cultivated in digestate.

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1. Introduction

Effluents from anaerobic digesters, digestates, can represent a serious environmental threat in case of inappropriate management. Despite their beneficial use, mainly as soil amendments, direct and uncontrolled disposal of digestates can have a toxic effect on natural ecosystems, mainly due to high ammonia concentration (Tigini et al., 2016; Xia and Murphy, 2016). Additionally, several constraints of digestate handling associated with the escalating need for arable land and transportation, will lead to alternative and non-agricultural uses, if biogas technology is to prevail in global energy sector (Dahlin et al., 2015). In microalgae technology, among the numerous types of wastewater which have the potential of displacing synthetic nutrients, digestates may play a key role. Since the first demonstration of this concept in the late 1950's (Golueke and Oswald, 1959), scientific effort has focused on different aspects of the topic, including different anaerobically treated waste streams, diverse microalgal strains which are suitable for such processes and several strategies which can be adopted with view to surpassing obstacles in microalgal growth associated with digestate turbidity, ammonia toxicity, or carbon and phosphorus limitation (De la Noüe and Basseres, 1989;

Wang et al., 2010; Singh et al., 2011; Yang et al., 2011; Xia and Murphy, 2016).

The use of digestates in microalgae cultivation contributes to effluents' post-treatment, recycling of nutrients and biomass production. From an environmental point of view, significant carbon and nutrient elimination has been displayed (Xu et al., 2015), while the produced microalgal biomass can serve as a valuable feedstock for bio-products and biofuels production (Spolaore et al., 2006; Razzak et al., 2013). Concerning biofuels production, especially biodiesel, the potential of microalgae to displace fossil-based diesel stems not only from their high growth rates, but also from the ability of microalgal cells to accumulate lipids, which can reach up to 50% of their dry weight (Chisti, 2007). Therefore, it can be made clear that several criteria concerning microalgal performance can be taken into account before the appropriate species are selected for cultivation in digestate. Khanh et al., (2013) reported a high growth rate of 0.052 h⁻¹ for *Dunaliella tertiolecta* cultivated in 50% digested cattle manure. Also, in search of efficient microalgae for nutrient removal from digested mixtures of cattle slurry and cheese whey, three strains (*Neochloris oleoabundans*, *Chlorella vulgaris* and *Scenedesmus obliquus*) were found to be suitable for use in digestate remediation (Franchino et al., 2013). Furthermore, Wang et al. (2016) demonstrated the effective production of *Scenedesmus obliquus* in biogas-supplemented digestate, especially under 14 h:10 h light:dark cycle.

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Dilution of digestates with water is one of the most common pre-treatment methods followed, in order not only to prevent the toxic effect of ammonia, but also to facilitate light penetration into the culture (Cai et al., 2013). Dilution ratios of digested effluents depend on several factors, including the species which are used, the physico-chemical characteristics of digestates, as well as the desired product of the culture (Singh et al., 2011; Khanh et al., 2013). For instance, it is well known that % lipids can substantially increase during microalgal growth under nutrient-depleted conditions (Rodolfi et al., 2009). That was the case when % fatty acid content increased from 9% to 13.7% in *Chlorella* sp. cells when grown on 10% and 4% anaerobically digested dairy manure, respectively (Wang et al., 2010). However, high dilution of digestate should be avoided (Franchino et al., 2016), since large volumes of water are required to dilute small volumes of effluents, making this practice unsustainable. In a large scale system, 10% digestate concentration would be considered as relatively satisfactory for effective management of digested wastewaters (Franchino et al., 2013). In this respect, screening tests should be performed under realistic, to a certain degree, conditions.

The productivity of microalgal cultures is primarily determined by the photosynthetic performance of each strain. Of the factors that can influence the photosynthetic performance, the most important are the medium properties, such as nutrient composition, dissolved oxygen and pH, along with environmental conditions, such as light intensity, photoperiod and temperature (Raven and Geider, 2003). Each strain optimizes its photosynthetic efficiency, when certain ranges of all the above mentioned parameters meet together in a culture. Consequently, an increase in productivity by means of the best achievable acclimation of photosynthesis to the rapidly changing environment of the culture is rather difficult and becomes a challenge when algae are growing in a further unfamiliar medium as that of digestate. Induction kinetics of prompt chlorophyll fluorescence, JIP-test, is a convenient tool for rapid estimation of several parameters, related to changes of the bioenergetic state of the photosynthetic machinery (Strasser et al., 2004). For this reason, it has been proposed mainly as a screening tool for species, strains, varieties and mutants (Kalaji et al., 2016). There is also an increasing interest for using it in algal cultures (Papazi et al., 2014; Oukarroum, 2016; Touloupakis et al., 2016; Koutra et al., 2017), but to the best of our knowledge this is the first time that is used for screening the photosynthetic machinery of algal strains growing into digestates. The purpose of this study was thus to test the performance of different microalgal strains in a unique digestate derived from a mixture of agro-industrial wastes. To this end, seven strains with specific features were grown on 10% (v/v) digestate for 25 days and were evaluated, in terms of biomass production, bioenergetic state of the photosynthetic machinery, fatty acids accumulation and removal of nutrients, organic and inorganic carbon, with view to selecting the most appropriate species for a multifaceted approach including microalgal cultivation and digestate bioremediation.

2. Materials and methods

2.1. Digestate production, pretreatment and analysis

Digestate was obtained from a mesophilic (37 °C) two-stage anaerobic bioreactor, which treated mixtures of agro-industrial wastes, including end-of-life dairy products (EoL-DPs), pig manure (PM), liquid cow manure (LCM), cheese whey (CW), slaughterhouse wastes (SHW) and chicken manure (CM). More specifically, a mixture of EoL-DPs (93% milk, 5% yoghurt, 2% cheese) was fed in an acidogenic reactor operated at HRT of 3 and 6 days (HRT = 3d, 6d) and subsequently, its effluent was used as a supplementary

substrate for a methanogenic reactor, operated at HRT of 37 days (HRT = 37d). The latest was fed with a final mixture of 58% PM, 17% LCM, 11% CW, 4.1% SHW, 5.6% CM and 4.5% EoL-DPs. During methanogenic reactor's operation, an increase in organic loading rate was also performed through the addition of EoL-DPs up to 20%. The effluent produced from all the examined conditions was collected, pre-treated and analyzed as previously described (Koutra et al., 2017) and its physicochemical characteristics are shown in Table 1. In brief, digestates were mixed and separated in 1.5 L bottles. Digested mixtures were centrifuged at 4500 rpm for 15 min (x2) in order to remove solids and the liquid fraction was filtered (Whatman, GF/F) and stored at –18 °C. The pre-treated digestate was subsequently diluted with water at 10% (v/v) and was used under non-aseptic conditions, based on previous findings demonstrating the effectiveness of such a medium in total microalgal performance (Koutra et al., 2017).

2.2. Microalgal strains and culture conditions

In total, seven microalgal species were used in the present study, which were obtained from the SAG Culture Collection (University of Göttingen): *Acutodesmus obliquus* (276-6), *Parachlorococcus kessleri* (211-11g), *Chromochloris zofingiensis* (211-14), *Chlorococcum oleofaciens* (213-11), *Chlorella vulgaris* (211-11b), *Botryococcus braunii* (30.81) and *Tetraselmis tetrahele* (161-2c). The first five strains were aseptically preserved in BG-11 medium (73816, Sigma-Aldrich), after the addition of trace metal solution (Mix A5 with Co 92949, Sigma-Aldrich), at 25 ± 2 °C, under continuous illumination of 20–25 μmol photons m⁻² s⁻¹ provided by white fluorescent lamps placed above the cultures. *B. braunii* was also preserved in BG-11 medium, which was supplemented with soil extract. The brackish-strain *T. tetrahele* was preserved in BG-11, composed of 50% filtered sea water supplemented with soil extract. For the batch experiments, cultures of a total volume of 400 mL were inoculated at 10% (v/v) and incubated in Erlenmeyer flasks at 25 ± 2 °C for 25 days, using a filter-sterilized air flow rate of 0.5–1 L min⁻¹ and continuous illumination of about 200 μmol photons m⁻² s⁻¹ provided by white fluorescent lamps placed below the cultures. In *T. tetrahele* cultures, 50% of filtered sea water was used for the preparation of the digestate-based medium. Furthermore, 10% (v/v) digestate was incubated without inoculation with microalgae and was used as a control, in order to assess growth of indigenous microorganisms. No pH adjustment was performed prior to or throughout the experiments. Batch experiments were carried out in duplicate and mean values along with standard deviation values are presented in the results.

2.3. Growth determination

For microalgal growth determination, biomass production was measured as dry cell weight (DW) concentration, according to

Table 1

Physico-chemical characterization of pre-treated digestate. Data are means ± SD (n = 2).

Parameter	Mean value/concentration ± SD
pH	8.60 ± 0.11
COD (g/L)	11.34 ± 0.04
IC (g/L)	2.509 ± 0.25
Carbohydrates ^a (g/L)	0.428 ± 0.01
Total VFAs (g/L)	1.601 ± 0.14
Total Phosphorus (g/L)	0.135 ± 0.02
Orthophosphates (g/L)	0.103 ± 0.00
TKN (g/L)	4.120 ± 0.13
NH ₃ -N (g/L)	3.306 ± 0.11

^a In equivalent glucose.

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