Microalgal post-treatment of anaerobically digested agro-industrial wastes for nutrient removal and lipids production

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HIGHLIGHTS

- *P. kessleri* outperformed *A. obliquus* in 10% ADE.
- 10% (v/v) ADE loading inhibited microalgal growth for 9–12 days.
- Non-sterilized ADE is the preferred condition examined.
- % TP removal and % NH_3-N assimilation varied between 84–94.5% and 7.87–72.7%.
- C18:0, C18:3n3 and C18:1n9t were the most abundant fatty acids detected.

ARTICLE INFO

ABSTRACT

The aim of this study was to investigate the effectiveness of cultivating *Parachlorella kessleri* and *Acutodesmus obliquus*, in anaerobic digestion effluent (ADE) derived from the co-digestion of end-of-life dairy products with mixtures of agro-industrial wastes. To this end, their performance under sterile and non-sterile conditions and different ADE loadings was evaluated, in terms of biomass and lipid production, nutrient removal efficiency and vitality of the photosynthetic apparatus. 10% (v/v) ADE loading inhibited growth over 9–12 days of cultivation, however biomass yields of 1.1 and 1 g L^{-1}, 22.7% and 19.5% (w/w) fatty acids concentration, as well as NH_3-N assimilation of 49.7 mg L^{-1} and 32.3 mg L^{-1} and TP removal of 84.2% and 84% were recorded for *P. kessleri* and *A. obliquus*, respectively. Among all the ADE-based treatments tested, *P. kessleri* outperformed *A. obliquus*, with no differences observed between sterilized and non-sterilized ADE.

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1. Introduction

Vast amounts of nutrients and toxic metals which are usually present in municipal, agricultural and industrial wastewaters may constitute a potential threat to natural ecosystems and human well-being, in case they end up in water bodies (Cai et al., 2013a). That being the case, the appropriate treatment method has to precede wastewater disposal in order to prevent pollution phenomena. To this end, the ability of microalgae to remove nitrogen, phosphorus and organic, toxic or even radioactive substances from different effluents may play a key role in wastewater bioremediation, as it has been demonstrated in several studies (Ruiz-Martinez et al., 2012; Shimura et al., 2012; Zhu et al., 2013). At the same time, depleting natural resources, climate change, industrialization and energy insecurity have shifted global interest into renewable energy sources. In this regard, microalgae-derived biofuels offer a promising alternative not only to conventional fossil fuels, but also to first and second generation biofuels, despite the fact that further research is still needed before a viable large scale production is established (Singh et al., 2011a). By cultivating microalgae for simultaneous wastewater treatment and biofuels production, process cost-effectiveness can substantially increase, constituting this process an attractive way to make microalgal biofuels sustainable (Pittman et al., 2011).

ADEs, formerly intended for land application, are nutrient-rich effluents which are increasingly becoming an environmental issue, due to the extensive application of anaerobic digestion technology. In this context, post-treatment of ADEs has been in the forefront of current scientific research due to the potential ecotoxicity of digestate, mainly attributed to its physico-chemical characteristics (Tigini et al., 2016). Microalgal ADE processing constitutes a rather advantageous option, which may not only reduce the environmental risk of the former, but could also significantly minimize the cost

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of microalgal cultivation mainly attributed to nutrients demand and organic carbon need under mixotrophic and heterotrophic microalgal growth (Xia and Murphy, 2016). Algal cultivation in digested swine manure has been proposed as a satisfactory tertiary waste treatment process (De la Noue and Basseres, 1989), while digested dairy manure was also used in order to cultivate oil-rich microalgae subsequently utilized for biodiesel production (Wang et al., 2010; Levine et al., 2011). Yang et al. (2011) investigated biomass production and oil accumulation of *Ettlia oleoabundans* grown on ADEs derived from different agricultural wastes, resulting in 65% oil content when 5% of rice hull ADE was used. Several microalgae have been tested so far in order to find the appropriate species for cultivation with digestates and many different anaerobically treated waste streams have already been used, including digested cow, swine and dairy manures, municipal and piggery wastewater (Bjornsson et al., 2013; Uggetti et al., 2014; Kim et al., 2014).

Various pre-treatment methods are usually employed prior to ADE use in order to facilitate microalgal growth. A common strategy which reduces ammonia concentration along with digestate turbidity is dilution, with the efficient ADE concentrations recorded varying between 2% and 50%, depending on the microalgae used, ADE quality and system's operation (Wang et al., 2010; Cai et al., 2013b; Khanh et al., 2013; Franchino et al., 2013). Recently, a mutant microalga, *Chlorella* PY-ZU1 was effectively cultivated in undiluted ADE and the process was optimized resulting in high biomass yield of 4.81 g L^{-1} and TP, COD and NH$_3$-N removal of 95%, 79% and 73% (Cheng et al., 2015). Furthermore, ADE sterilization can be applied with view to reducing the presence of other microorganisms in the cultures, however much too often this practice is avoided, as in a large scale microalgal production sterilization would not be feasible (Yang et al., 2011).

Due to the complexity of microalgal growth in wastewaters, the various underlying interactions and the uniqueness of each ADE produced, more research is still needed before a large-scale microalgal application linked to AD units is accomplished. The purpose of the present study was to evaluate growth, vitality of the photosynthetic apparatus, nutrient removal efficiency and lipid accumulation of two green microalgae, *Parachlorella kessleri* and *Acutodesmus obliquus*, grown on water diluted anaerobically digested mixtures of agro-industrial wastes, under different ADE loadings and sterilized or non-sterilized digestate.

2. Materials and methods

2.1. Collection and pre-treatment of ADE

The ADE used in this study derived from the co-digestion of end-of-life dairy products with a given mixture of agro-industrial wastes, a process which took place in the facilities of Laboratory of Biochemical Engineering and Environmental Technology (LBEET), in the framework of the European Project LIFE10 ENV/CY/000721 DAIRIUS "Sustainable management via energy exploitation of end-of-life dairy products in Cyprus". The raw materials used as co-substrates included end-of-life milk, yogurt and cheese, along with a mixture of other wastes including pig manure, liquid cow manure, cheese whey, slaughterhouse wastes and chicken manure. ADE was collected in 1.5 L bottles and preserved at −18 °C. Prior to use, ADE was centrifuged twice at 4500 rpm for 15 min and the liquid fraction was collected and filtered via Whatman glass microfiber filters (Grade GF/F), in order to remove large (>0.7 µm) particles. In the first run of experiments, ADE was sterilized at 121 °C for 20 min so as to eliminate the presence of other microorganisms. However, sterilization of ADE was not applied in the following experiments, aiming to investigate

the physiological performance of the two microalgae under real-scale non-aseptic conditions.

The physico-chemical analysis of the filtered effluent prior and after sterilization, which is shown in Table 1, included pH measurement with an electrode (Orion 3-Star), Inorganic Carbon (IC) measurement using a Shimadzu TOC-VCPH analyzer (Tokyo, Japan) and determination of alkalinity, COD, TKN, NH$_3$-N, Total Phosphorus (TP) and orthophosphates according to *Standard Methods* (APHA, 1995). In particular, concentration of NH$_3$-N and PO$_4$-P was determined with *phenate* and *ascorbic acid method*, respectively. Carbohydrates were measured according to Joseffson (1983) and Total Volatile Fatty Acids (TVFAs) were analyzed on a gas chromatograph (Agilent Technologies 7890A) equipped with a flame ionization detector, as described by Dareioti et al. (2010). ADE was diluted with water before use and two different ADE loadings, 2% and 10% (v/v), were applied in the experiments.

2.2. Microalgal cultures and batch-experiments

The two freshwater microalgae (Chlorophyta) used in this study, *Parachlorella kessleri* (SAG 211-11g) and *Acutodesmus obliquus* (SAG 276-6), formerly named as *Scenedesmus obliquus*, were obtained from the SAG Culture Collection (University of Göttingen). Throughout the experimentation period both strains were aseptically preserved in BG-11 medium (73816 SIGMA-ALDRICH) at 25 ± 2 °C, under continuous illumination of 20–25 µmol m^{-2} s^{-1} provided by white fluorescent lamps placed above the cultures. For cultivation in sterilized ADE, cultures of a total volume of 300 mL were inoculated at 10% (v/v) and incubated in Erlenmeyer flasks at 25 ± 2 °C, using a filter-sterilized air flow rate of 0.5–1 L min^{-1} and continuous illumination of about 200 µmol m^{-2} s^{-1} provided by white fluorescent lamps placed below the cultures, for 12 days. For cultivation in non-sterilized ADE, cultures of a total volume of 400 mL were used, which were inoculated and incubated as previously described, for 25 days. The first phase of all the conditions tested lasted 12 days, during which the effect of sterilized or non-sterilized ADE on the evaluated parameters is being compared. When non-sterilized ADE was applied, experimental time was extended to 25 days (presented as second phase) in order to fully examine this realistic scenario. No pH adjustment was performed throughout the experiments. All the treatments were carried out in duplicate and mean values along with ± SD are presented in the results.

2.3. Determination of microalgal growth

For microalgal growth determination, biomass production was measured as dry cell weight concentration, according to *Standard*

Table 1
Physico-chemical characteristics of filtered, undiluted ADE prior and after sterilization. Data are means ± SD (n = 2).

Physico-chemical characteristics	Mean value/concentration ± SD	
	Non-sterilized ADE	Sterilized ADE
pH	8.7 ± 0.11	9.7 ± 0.07
Alkalinity[a] (g L^{-1})	16.15 ± 0.01	7.775 ± 0.01
COD (g L^{-1})	10.38 ± 0.14	11.87 ± 0.12
IC (g L^{-1})	2.897 ± 0.01	1.216 ± 0.01
Carbohydrates[b] (g L^{-1})	0.436 ± 0.00	0.490 ± 0.01
Total VFAs (g L^{-1})	1.601 ± 0.25	1.363 ± 0.08
Total phosphorus (g L^{-1})	0.114 ± 0.01	0.130 ± 0.00
Orthophosphates (g L^{-1})	0.095 ± 0.00	0.108 ± 0.00
TKN (g L^{-1})	3.990 ± 0.02	1.750 ± 0.14
NH$_3$-N (g L^{-1})	3.178 ± 0.10	1.064 ± 0.00

[a] In equivalent g CaCO$_3$/L.
[b] In equivalent glucose.