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Impact of temperature and photoperiod on anaerobic biodegradability of microalgae grown in urban wastewater

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ABSTRACT

This study was designed to elucidate how temperature and photoperiod, two of the principal parameters affecting microalgae culture conditions influenced the anaerobic digestion of harvested biomass when grown in wastewater under different scenarios (I: 23°C/14 h illumination, II: 15 °C/14 h and III: 15 °C, 11 h). With respect to biomass cultivation, temperature affected biomass productivity but not final biomass concentration. Scenario I mediated faster ammonium and phosphate removal (100% for all the evaluated scenarios) and greater organic matter removal (80.5% compared to 56.5% and 70.8% obtained for Scenario II and III, respectively). Biomass grown under unfavorable conditions of light and temperature (Scenario III) evidenced the highest nitrogen assimilation due to the lowest ammonia stripping (6%). Different cultivation scenarios resulted in a different macromolecular profile of the harvested biomass. Carbohydrates accumulation prevailed under Scenario I while low temperature (Scenario II) and short photoperiod (Scenario III) increased lipid and protein content. Harvested biomass was subjected to anaerobic digestion. Anaerobic biodegradability of the three types of biomass remained in the narrow range of 36–42%, however different hydrolysis constant rates were calculated. Comparison between the theoretically calculated and experimentally obtained methane yield values showed that biomass collected at Scenario III only reached 36.1% of the theoretical methane yield achievable compared to 46.5% attained with the biomass collected at Scenario I. Further research on microalgae communities and cell wall composition is required to understand the methane yield mismatch.

1. Introduction

Although microalgae are considered to be a promising feedstock for biofuel production, this application is limited due to the consumption of nutrients and water resources for microalgae cultivation (Lardon et al., 2009). One alternative is to grow microalgae biomass in wastewater media. This strategy entails a more sustainable practice while providing additional benefits. Contrary to expensive conventional aeration systems, the application of microalgae based systems can be envisaged as a cost-effective *in situ* oxygenation via photosynthesis. Additionally, this biotechnological process is preferable since it removes water pollutants while recovering nutrients via biomass uptake. Conventional activated sludge processes transform wastewater contaminants into non-valuable products such as N_2 and CO_2 while in the case of microalgae based systems, these nutrients can be recovered. Assuming an average carbon content of 47% in the dry biomass (González-Fernández et al., 2010), to obtain each g of biomass 1.72 g of CO_2 is stoichiometrically required, and thus, no CO_2 is released into the atmosphere.

The synergistic relationship between microalgae and aerobic bacteria for wastewater remediation has been previously described. Algal–bacterial consortia are able to establish an O_2/CO_2 cycle production and usage thereof (Munoz and Guieysse, 2006). Different driven forces governing algal-bacterial systems have been described in the literature. Nitrification and denitrification are the main nitrogen transformations accounting for nitrogen removal in those types of systems (González-Fernández et al., 2011a; Posadas et al., 2015; Aguiar do Couto et al., 2015). Wastewater chemical composition and operational conditions

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applied to photobioreactors strongly influence nitrogen transformation in reactors inoculated with alga–bacteria consortium (Risgaard-Petersen et al., 2004; Marcilhac et al., 2014). For instance, nutrients recovery (biomass uptake) accounted for approximately 40% when the microalgae-bacteria consortium was cultivated at 31 °C and 16 h of photoperiod, which diminished to 10% when operated at 24 °C and 9 h of photoperiod when using fresh swine slurry (González-Fernández et al., 2011a). Likewise, nitrogen abiotic losses are also identified as a principal mechanism for nitrogen removal. Due to the uptake of inorganic carbon by autotrophic microorganisms, pH of the medium is increased. In principle, high microalgae activity raises the pH, however, the pH can also be close to neutrality by simultaneous processes of nitrification and carbon dioxide consumption. This rise in pH also affects phosphate removal. Given the high content of other elements such as magnesium and calcium in wastewater, high pH achieved in the culture broth may also cause phosphate precipitates hydroxyapatite and struvite (Pratt et al., 2012), which ultimately results in microalgae deprivation of this nutrient.

As an alternative to avoid nitrogen loss through simultaneous nitrification-denitrification, wastewater treatment can be conducted by microalgae consortium (no aerobic bacteria addition). Until now several studies have reported the growth of individual microalgae strains but few studies have focused on microalgae co-culture. Indeed, co-culture has been reported to provide higher biomass growth and nutrients removal efficiencies than individual microalgae cultivation (Asmare et al., 2014). This is probably because different metabolic abilities of diverse microalgae strains result in a more robust operating system. Moreover, when dealing with wastewater, microalgae monoculture presents a possible risk since the stability of microalgae productivity may be hampered by the development of native algae (Fouilland, 2012). Likewise, it should be considered that during wastewater treatment, microbial population dynamics will take place. This will also compromise the use of the harvested microalgae biomass.

With regard to biomass grown in wastewater, the most common and simplest application is the production of biogas through anaerobic digestion. With respect to this, the ability of microalgae for wastewater nutrients recovery will affect not only the prevailing microalgae strain but also the macromolecular composition of the harvested biomass that will be anaerobically digested subsequently. This study was designed to elucidate how temperature and photoperiod, two of the principal parameters affecting microalgae culture conditions, influenced the anaerobic digestion of harvested biomass when grown in wastewater. Nitrogen and phosphorous removals were assessed in batch culture under three different scenarios. Finally, harvested biomass was chemically characterized and subjected to anaerobic digestion to further evaluate the effect of those cultivation parameters on the methane yield achievable by biomass harvested under the different scenarios.

2. Materials and methods

2.1. Microorganisms

Microalgae strains (namely *Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reindhardtii*) were selected based on their robustness to grow in wastewater and therefore for wastewater bioremediation (González-Fernández et al., 2011b; Passos et al., 2013; Amengual-Morro et al., 2012). *C. vulgaris* was collected at the wastewater treatment plant of Valladolid (Spain) while *C. reinhardtii* and *S. obliquus* was obtained from the bank SAG Culture Collection of the University of Göttingen (Germany). Those strains were cultivated in independent reactors of 1 L. These microalgae inocula were grown in mineral medium containing the following components (mg L^{-1}): 1680 NH_4Cl, 25 $CaCl_2.2H_2O$, 150 $MgSO_4.7H_2O$, 75 K_2HPO_4, 175 KH_2PO_4, 25 NaCl, 50 disodium EDTA, 31 KOH, 4.98 $FeSO_4.7H_2O$, 11.42 H_3BO_3, 17.64 $ZnSO_4.7H_2O$, 2.88 $MnCl_2.4H_2O$, 1.42 MoO_3, 3.14 $CuSO_4.5H_2O$, 0.98 $CoNO_3.6H_2O$ and 2.42 g trisacetate in distilled water. Microalgae inocula were cultured at room temperature (22–24 °C) and constant illumination supplied with four fluorescents lamps (TL-D 36W, Philips). Magnetic stirrers were used to improve culture broth mixing. Microalgae were harvested during the late-exponential growth phase (approximately after 7 days). To harvest the culture, microalgae were centrifuged at 5000 rpm for 10 min at 4 °C (Heraeus Multifuge, Germany).

2.2. Wastewater

Fresh urban wastewater was collected from the wastewater treatment plant of Valladolid (Spain). Raw wastewater was centrifuged (4000 rpm, Centrifuge 5810R, Heraeus Multifuge, Thermofisher, Germany) and the supernatants directly used as cultivation media for microalgae. At this point it should be stressed that the sole feeding source was the wastewater and no external carbon dioxide supply was provided. More specifically, the chemical characterization of this wastewater displayed a total COD concentration of 259.3 ± 5.7 mg L^{-1} out of which 61% was in soluble form. For the nitrogen and phosphorus, ammonium concentration was 80.4 ± 0.9 and phosphate was 14.5 ± 0.2 mg L^{-1}. Nitrate and nitrite was not detected.

2.3. Microalgae culture conditions: experimental set-up

Among the possible operational conditions that may be changed, temperature and photoperiod (hours of illumination) were chosen. These two operational parameters have been described by other researchers as responsible for efficient nutrients removal and biomass growth in outdoor open ponds (Lan et al., 2015; Béchet et al., 2015). In summary, three scenarios were studied. Scenario I was set at 23 °C and 14 h of illumination and Scenario III was set at 15 °C and 11 h of illumination. These two scenarios reproduced the light hours and average temperature of Alicante (Spain) during the months of April–October (Scenario I) and October–March (Scenario III) (www.fomento.gob.es). In order to be able to attribute the observed differences to any of the two parameters, Scenario II was established, corresponding to the cultivation of microalgae at 15 °C with photoperiod of 14 h.

Those conditions were run in water-jacketed photobioreactors to appropriately work at the selected temperature. A water thermostat was connected to the photobioreactors which presented a working volume of 1 L. To prevent oxidative damage, air was sparged into the reactors. The reactors were illuminated with fluorescents lamps (5500 lux). Each condition was tested in three reactors; therefore each scenario was conducted in triplicate. The reactors were initially filled with wastewater and inoculated with 300 mg VSS L^{-1} of microalgae consortium. For the inoculation of each reactor, 100 mg VSS L^{-1} was used of each microalga, namely *C. vulgaris*, *S. obliquus* and *C. reindhardtii*. Once mixed, the microalgae biomass used as inoculum was characterized in terms of macromolecular distribution. This biomass contained 22.4 ± 3.0% carbohydrates, 58.1 ± 6.8 proteins and 19% lipids.

2.4. Biomethane potential assays

Anaerobic sludge employed was collected at the wastewater treatment plant of Valladolid (Spain). Anaerobic biomass presented total solids (TS) concentration of 16.2 g L^{-1} and volatile solids (VS)/TS of around 70%. Anaerobic digestion was conducted in batch