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Short communication

# Bioremediation and biomass harvesting of anaerobic digested cheese whey in microalgal-based systems for lipid production

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ABSTRACT

Agro-industrial wastewaters are potential resources for production of microalgae biofuels. The aim of the present work was to determine the feasibility of a semi-continuously fed microalgal-based system for the treatment of anaerobic digested cheese whey (AD) and to evaluate biomass productivity and lipid accumulation for a period of 77 d. The effect of increasing ammonium loading rate (ALR) and decreasing hydraulic retention time (HRT) was evaluated. Maximum biomass productivity and lipid content were 12.0 g $m^{-2}$ $d^{-1}$ and 12.3%, respectively, achieved when operating at an ALR of 12.9 mg $L^{-1}$ $d^{-1}$ and at a HRT of 5 d. Under these conditions, soluble chemical oxygen demand (SCOD), ammonium and soluble phosphorous (SP) removal accounted for 94%, 92% and 20%, respectively. Additionally, the effectiveness of flocculation induced by increase pH to harvest produced biomass was investigated. Flocculation efficiencies up to 90% were obtained for a pH of 13.5 regardless culture broth characteristics, and therefore, this process can be used as a pre-concentrated step of microalgal-bacterial suspensions.



## 1. Introduction

Microalgae will play an important role as feedstock for biodiesel production in a near future (Salim et al., 2012). However, their cultivation require considerable amount of nutrients, raising questions about the environmental and economic impact of microalgal production systems. For this reason, the use of wastewaters, especially those derived from agro-industrial facilities which usually present high nutrient concentration, seems an interesting route that deserves deeper investigation to reduce the total production cost of microalgal biodiesel (Malla et al., 2015; Pittman et al., 2011).

Cheese industry is of major importance in Castilla y León (Spain), producing 34% of cheese made in Spain (more than 104,000 t per year) (JCYL, 2016). Cheese whey (CW), the liquid resulting from the precipitation and removal of milk casein in cheese making process, constitutes a serious environmental problem of dairy industries due to its high organic load. Anaerobic digestion of CW has achieved a great development facing management difficulties in small and medium factories (Prazeres et al., 2012). However, anaerobic effluents still present a high nutrient concentration (N and P) that requires a further treatment (Wang et al., 2010). In this context, microalgal-based processes present several advantages over conventional technologies, mainly faster uptake of nutrients, mitigation of $CO_2$ emissions due to their autotrophic metabolism and potential cost saving (Molinuevo-Salces et al., 2010; Pittman et al., 2011), meanwhile producing a high-added value biomass for biofuel applications. There have been some recent studies of microalgal cultivation on anaerobic effluents, most of them focused on the use of anaerobic digested manure (Chen et al., 2012; González-Fernández et al., 2011; Olguín et al., 2015; Wang et al., 2010). The experience gained for the treatment of anaerobic digested cheese factory effluents in microalgal-based systems is limited to few works (Blier et al., 1995). These studies mainly focused on algae productivity and nutrient removal efficiency, however, the lipid content and, more importantly, the lipid productivity of microalgal-based system fed with this type of substrates has seldom been reported. Additionally, the search of cost-effective harvesting methods with emphasis on preconcentration of microalgal biomass prior to centrifugation should be faced (Salim et al., 2012). Flocculation is considered to be an efficient process, which allows rapid treatment of large culture volumes and not consume much energy (Vandamme et al., 2013; Wu et al., 2012). Flocculation of microalgal biomass is particularly sensitive to pH of the culture broth. pH increase may influence the cell charge and change the existing forms of metal cations in culture

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suspension due to their hydrolysis (Wu et al., 2012). Flocculation simply by pH increase could be an attractive alternative because it is low-cost, non-toxic to microalgal cells and it avoids the use of flocculants, allowing recycling the separated medium after biomass flocculation (Wu et al., 2012). Most works on flocculation induced by pH increase carried out so far have focused on single microalgae species cultivated under particular conditions. The evaluation of the efficiency of this process applied to microalgal-based systems requires a deeper research.

This work aims to test the potential of microalgal-based systems for bioremediation of the anaerobic digested cheese whey (AD) and simultaneously to evaluate biomass growth and its lipid content. The effectiveness of flocculation induced by pH increase for pre-concentrating the produced biomass was also evaluated.

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

*Chlorella sorokiniana* was obtained from the Culture Collection of Algae of the University of Goettingen (SAG) (Goettingen, Germany). Microalgae inoculum was prepared according to Guieysse et al. (2002). This specie was selected for this study for its high tolerance to polluted environments (De Godos et al., 2009). The aerobic sludge was obtained from an activated sludge reactor of the municipal wastewater treatment plant of Valladolid (Spain). Prior to inoculation, microalgae and bacteria were centrifuged (5 min; 3000 rpm) and resuspended in distilled water.

### 2.2. Substrate source

Cheese whey was collected from a cheese factory located in Valladolid (Spain). Anaerobic digestion was carried out in a continuously stirred tank reactor with a working volume of 5 L under mesophilic conditions, at an organic loading rate (OLR) of 1.7 g total COD (TCOD) $L^{-1}$ $d^{-1}$ and at a hydraulic retention time (HRT) of 28 d. Anaerobic effluent was centrifuged (5 min, 3000 rpm) and the supernatant diluted to the desired ammonium loading rate (ALR) prior feeding the photobioreactor. Centrifuged anaerobic effluent (AD) presented a soluble COD (SCOD) of 16.7 g $L^{-1}$. Ammonium ($NH_4^+$-N) and soluble phosphorus (SP) concentrations were 735 mg $L^{-1}$ and 87 mg $L^{-1}$, respectively. Noteworthy that the sole feeding source was AD and that no external carbon dioxide was provided to the microalgal-based system.

### 2.3. Experimental set-up

The experimental set-up consisted of an open to the atmosphere photobioreactor with a total working volume of 3 L (14 cm wide, 27 cm long, 11 cm high). Photobioreactor was continuously illuminated using four fluorescent lamps at 54 μE $m^{-2}$ $s^{-1}$ (Sylvania Grolux F36W/GRO, Germany). The average temperature was 27.0 ± 1.9 °C. The culture broth was gently suspended by means of magnetic stirrers. The volume of the photobioreactor was checked daily and any water lost due to evaporation was corrected (lower than 3% of culture broth volume).

Photobioreactor was initially filled with tap water and inoculated with 18 and 20 mg volatile suspended solids (VSS) $L^{-1}$ of aerobic sludge and microalgae, respectively. Right after inoculation, photobioreactor was fed with diluted AD in three different periods for 77 days, varying ALR and/or HRT (Table 1). Operational conditions were changed when steady-state conditions were assumed. In this manner, the change took place after a period of time equivalent of three times the corresponding HRT.

Feeding was carried out sequentially once a day (300 mL $d^{-1}$ for periods I and II and 600 mL $d^{-1}$ for period III). Influent and samples from culture broth were withdrawn twice a week to monitor TCOD, SCOD, VSS, total kjeldahl nitrogen (TKN), $NH_4^+$-N, nitrite ($NO_2^-$-N), nitrate ($NO_3^-$-N) and SP. Total VFA concentration in influent and effluent samples was weekly measured. The culture broth collected for a time period equal to each HRT during the steady period was accumulated for flocculation assays described in Section 2.4. This biomass was lyophilized (Lyoquest 85 Plus Eco, Spain) to determine lipid content.

### 2.4. Flocculation induced by pH increase

Flocculation experiments were performed in 250 mL glass beakers with 100 mL of microalgal-bacterial broth. Firstly, the pH of the sample was adjusted to the desired value by dropwise addition of 6 N NaOH. After that, stirring was maintained for 5 min at 130 rpm and allowed to settle at room temperature for 10 min. A liquid sample of 25 mL was then withdrawn for the analysis of absorbance at 550 nm ($OD_{550}$) at 1 cm below the surface of the treated culture broth. Flocculation efficiency was calculated based on $OD_{550}$ of culture broth before flocculation. Additionally, $Mg^{2+}$ content and microalgal population in culture broths before and after flocculation were determined. Two tests were also run without pH adjustment to determine the natural settling of the culture broth. All tests were conducted in duplicate.

### 2.5. Analytical procedures and statistical analysis

Analyses of VSS, TCOD, SCOD, TKN, $NH_4^+$-N and SP were performed in accordance with APHA Standard Methods (2005). $NO_2^-$-N and $NO_3^-$-N were determined using continuous flow analysis equipment (Braud and Luebbe, Analytical AA3, Norderstedt, Germany). Lipids were determined following the method proposed by Kochert (1978). pH and temperature were determined using a multi-probe system model, YSI 556 MPS (YSI Incorporated, USA). Total volatile fatty acids (VFA) were analyzed using a gas chromatograph (Varian CP3900) equipped with a CP-Was 58 Varian capillary column (25 m × 0.53 mm × 1 μm) and a flame ionization detector. The identification and quantification of microalgae were carried out by microscopical examination (OLYMPUS IX70, USA) of culture broth samples according to Sournia (1978).

$OD_{550}$ and pH in flocculation assays were measured in a Spectronic Helios Gamma UV–vis Spectrophotometer (Thermo Fisher Scientific, USA) and a Crison Basic 20 pH-meter (Crison Instruments, SA, Barcelona, Spain), respectively. Magnesium was analyzed using an atomic absorption spectrometer (AA 240 FS, Varian).

Results obtained were analyzed using one-way ANOVA with significance at $p < 0.05$.

## 3. Results and discussion

### 3.1. Biomass productivity

Biomass concentration in culture broth significantly increased from period I to period II (Table 1) concomitantly with increasing organic carbon and nitrogen availability, as reported in previous works (Choudhary et al., 2016; De Godos et al., 2009). Lowering HRT (from 10 d in period II to 5 d in period III) led to a significant decrease in biomass concentration in the culture broth. However, volumetric biomass productivity considerably increased from period II to period III, reaching a value of 0.15 g VSS L $d^{-1}$ (Table 1). These productivities proved higher in comparison to those obtained by other authors operating open systems fed with anaerobic digested effluents, mainly coming from livestock manure (Table 2). These differences could be attributed to the fact that diluted AD used in present study still present high VFA concentrations (ranging