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From piggery wastewater nutrients to biogas: Microalgae biomass revalorization through anaerobic digestion

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ABSTRACT

Microalgae grown in swine wastewater were used as a promising strategy to produce renewable energy by coupling wastewater bioremediation and biomass revalorization. The efficiency of a microalgae consortium treating swine slurry at different temperature (15 and 23 °C) and illumination periods (11 and 14 h) was assessed for biomass growth and nutrient removal at two NH_4^+ initial concentrations (80 and 250 mg L^{-1} NH_4^+). Favourable culture conditions (23 °C and 14 h of illumination) and high ammonium loads resulted in higher biomass production and greater nutrients removal rates. The initial N–NH_4^+ load determined the removal mechanism, thus ammonia stripping and nitrogen uptake accounted similarly in the case of high NH_4^+ load, while nitrogen uptake prevailed at low NH_4^+ load. Under favourable conditions, nitrogen availability in the media determined the composition of the biomass. In this context, carbohydrate-rich biomass was obtained in batch mode while semi-continuous operation resulted in protein-rich biomass. The revalorization of the resultant biomass was evaluated for biogas production. Methane yields in the range of 106–146 and 171 ml CH_4 g COD^{-1} were obtained for the biomasses grown in batch and semi-continuous mode, respectively. Biomass grown under favourable conditions resulted in higher methane yields and closer to the theoretically achievable.

1. Introduction

Third generation biofuels from photosynthetic microorganisms are currently presented as a promising renewable alternative to fossil fuels. One of the weak points of this technology to be implemented in a large scale is the supply of nutrients [1]. The use of wastewater as nutrient source could overcome this issue, contributing at the same time to wastewater bioremediation. Indeed, several studies have reported the growth of microalgae using different wastewaters as nutrient source [2]. Opposite to traditional manure management procedures where nutrients are lost, coupling swine slurry treatment with microalgae cultivation would result in sustainable nutrients recycling. However under specific circumstances, nitrogen loss by ammonia stripping and nitrification–denitrification processes has been previously reported when using microalgae-bacteria consortium to treat manure-related wastewaters [3,4]. Therefore, high nutrient removals do not always correspond to high nutrient uptakes.

Microalgae growth and nutrients removal mechanisms involved in wastewater bioremediation are highly dependent on temperature and light. Optimal growth temperatures for most microalgal species are in the range of 15–26 °C. Under low temperatures, metabolic rates of microalgae diminish and therefore, lower growth rates are attained while high temperatures may result in oxidative stress. On the other hand, light energy is stored in the cells for growth and CO_2 fixation and thus, a positive correlation between hours of illumination and microalge growth has been reported [5]. However, if the light saturation point is reached, the light receptors can be damaged resulting in photoinhibition.

Most of the studies dealing with microalgae for wastewater treatment have been focused on single microalgal species or microalgae-bacteria flocs but the use of microalgae consortia has been reported to be more efficient in terms of biomass production and nutrient recycling than individual microalgae cultivation [6]. Likewise, the use of microalgae instead of microalgae-bacteria

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consortia also provided higher nutrients uptake and thus higher nutrients recovered [7].

With regard to the microalgae biomass harvested after wastewater treatment, biogas production is probably one of the simplest applications. Anaerobic digestion (AD) is a straightforward technology for microalgae biomass valorization. AD is preferred over biodiesel or bioethanol production since it avoids the drying step of the biomass and the three macromolecules (namely carbohydrates, proteins and lipids) are converted to biogas. The production of biogas is determined by the resistance of microalgae cell wall and the macromolecular composition of the microalgal biomass. This macromolecular composition is determined by the nutrient availability in the growth media. Thus, high nitrogen and phosphorus concentrations promote protein production while a shortage of nutrients results in carbohydrate- or lipid-rich biomass, depending on the microalgae strain [8–10].

The first objective of the present work was to evaluate the ability of a microalgae consortium for biomass production using swine slurry as growth media. The different mechanisms involved in slurry bioremediation at two ammonium loads under different environmental conditions (winter and summer conditions) were studied in batch mode. The best condition (summer and high NH_4^+ load) was further studied in semicontinuous mode for comparison purposes with the batch experiment. The collected biomasses were subjected to anaerobic digestion to evaluate the effect of the cultivation parameters on the final methane yield.

2. Materials and methods

2.1. Swine manure

Swine manure was collected from a pig farm in Segovia (Spain). The chemical composition of the swine slurry is presented in Table 1. Swine slurry dilution was set in accordance to the ammonium concentration since it has been previously addressed as an inhibitory compound for microalgae [11]. The slurry was filtered and diluted approx. 7 and 22 times to achieve the desired ammonium concentrations of around 250 and 80 mg $N–NH_4^+$ L^{-1}, respectively. The carbon source for microalgae was just diluted swine manure and no external carbon dioxide supply was provided.

2.2. Microorganisms used as inoculum

Three microalgae species, i.e. *Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reindhardtii*, were used based on their robustness to grow in wastewater [12,13]. Those strains were cultivated independently in reactors of 1 L. They were grown in mineral medium containing the following components (mg L^{-1}): 240 NH_4Cl, 25 $CaCl_2 \cdot 2H_2O$, 150 $MgSO_4 \cdot 7H_2O$, 75 K_2HPO_4, 175 KH_2PO_4, 25 NaCl, 50 disodium EDTA, 31 KOH, 4.98 $FeSO_4 \cdot 7H_2O$, 11.42 H_3BO_3, 17.64 $ZnSO_4 \cdot 7H_2O$, 2.88 $MnCl_2 \cdot 4H_2O$, 1.42 MoO_3, 3.14 $CuSO_4 \cdot 5H_2O$, 0.98 $CoNO_3 \cdot 6H_2O$ and 2.42 g trisacetate in distilled water. Microalgae were grown at room temperature (22–24 °C), constant illumination supplied with four fluorescents lamps (TL-D 36W, Philips) and continuous mixing provided by magnetic stirrers. The harvest was performed by centrifugation at 5000 rpm for 10 min at 4 °C (Heraeus Multifuge, Germany) during the late-exponential growth phase (i.e. approx. after 7 days).

Table 1
Swine slurry composition.

Parameter	Unit	Average	STD
COD_t	mg L^{-1}	3750	64
COD_s	mg L^{-1}	2790	21
TS	mg L^{-1}	4188	18
VS	mg L^{-1}	2112	88
$N–NH_4^+$	mg L^{-1}	1761	41
$N–NO_2^-$	mg L^{-1}	0	0
$N–NO_3^-$	mg L^{-1}	1.1	1.0
PO_4^{3-}	mg L^{-1}	161	2

2.3. Microalgae growth on swine slurry: experimental set-up

2.3.1. Discontinuous experiments

Two different initial ammonium loads were studied, namely high (250 mg $N–NH_4^+$ L^{-1}) and low concentration (80 mg $N–NH_4^+$ L^{-1}). Based on the reported effect of temperature and photoperiod (hours of illumination) on nutrient removal efficiency and microalgae growth [14,15], those two parameters were chosen as environmental parameters. For each ammonium load, two different experiments were carried out in accordance to the light hours and average temperature of Alicante (Spain) during the months of April–October (spring–summer, favourable conditions) and for October–March (autumn–winter, non favourable conditions) (www.fomento.gob.es). Favourable conditions were set at 23 °C and 14 h of illumination while non-favourable conditions corresponded to 15 °C and 11 h of illumination. Both conditions were tested in batch mode, microalgae growth, pH and nutrients removals were monitored along approximately 10 days.

Duplicated reactors were used for each condition. Temperatures were controlled by a water bath connected to water-jacketed photobioreactors with a working volume of 1 L. To prevent oxidative damage and provide culture mixing, air was bubbled into the reactors. Light was provided by fluorescents lamps (5500 lux). The reactors were initially filled with diluted swine slurry and inoculated with 0.1 g VSS L^{-1} of each microalgae, namely *C. vulgaris*, *S. obliquus* and *C. reindhardtii*. Therefore, each reactor was inoculated with 0.3 g VSS L^{-1} of microalgae consortium. This microalgae consortium used as inoculum was characterised in terms of macromolecular distribution, presenting a carbohydrate content of 22.4 ± 3.0%, proteins of 58.1 ± 6.8% and lipid content of 19%VSS. Microalgal biomass at the end of each experimental time was concentrated by centrifugation (10 min, 5000 rpm) and protein, carbohydrate and lipid content were determined for comparison purposes.

2.3.2. Semi-continuous experiments

Based on the results obtained in batch mode, an initial ammonium concentration corresponding to approximately 300 mg $N–NH_4^+$ L^{-1}, 23 °C and 14 h of illumination were selected to set up a semi-continuous reactor treating diluted swine slurry. The experiment was carried out in duplicates. The same reactor configuration described in sub-section 2.3.1 was used. Each reactor was inoculated with 0.3 g VSS L^{-1} of microalgae consortium. The photobioreactors were operated for 25 days at hydraulic retention time (HRT) of 8 days. Slight agitation was provided by air bubbling. The reactors were fed once a day with diluted swine slurry after removal of the same volume of effluent. Samples from the photobioreactor were taken every two days in order to measure pH, soluble chemical oxygen demand (COD_s), volatile suspended solids (VSS), $N\text{-}NO_X^-$, $N–NH_4^+$ and PO_4^{3-}. Biomass at the end of the experimental time was concentrated by centrifugation (10 min, 5000 rpm). Protein, carbohydrate and lipid content were determined in the biomass grown under semicontinuous culture mode.

2.4. Biological methane potential

AD was carried out in glass bottles with 0.120 L capacity and a working volume 0.070 L. Photosynthetic microorganisms obtained