Bioresource Technology 175 (2015) 224–230

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate

Cyril Marcilhac[a,b], Bruno Sialve[c], Anne-Marie Pourcher[a,b], Christine Ziebal[a], Nicolas Bernet[c], Fabrice Béline[a,b,*]

[a] Irstea, UR GERE, 17 av. de Cucillé, CS 64427, F-35044 Rennes, France
[b] Université Européenne de Bretagne, F-35044 Rennes, France
[c] INRA, UR50, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, F-11100 Narbonne, France

HIGHLIGHTS

- N:P ratio affects phosphorus removal but not microalgae growth and related N-removal.
- Nitrification was controlled by initial N:P ratio during microalgae cultivation.
- N and P assimilation up to 10.1 and 2.0 mg L^{-1} d^{-1}, respectively in coloured media.
- Microalgae perform phosphorus storage.
- *Scenedesmus* is a better competitor for phosphorus than *Chlorella*.

ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form 3 October 2014
Accepted 5 October 2014
Available online 12 October 2014

Keywords:
Phosphorus
Microalgae
Nitrification
Digestate
Community evolution

ABSTRACT

The cultivation of microalgae with digestate supernatant is a promising process for the recovery of mineralized nutrients (P, N) from anaerobic digestion. Nevertheless, the variability of phosphorus concentration in the influent could limit this process. The impact of initial N:P ratios between 3 and 76 gN gP^{-1} was studied and proved no growth limitation over 14-day batch experiments even when P was depleted. Nitrogen assimilation was not affected by phosphorus concentrations and reached 10.1 mgN L^{-1} d^{-1} whereas phosphorus removal ranged from 0.6 to 2.0 mgP L^{-1} d^{-1}. The biomass N:P ratio was found to be a function of the influent N:P ratio. Phosphorus storage by microalgae was thus confirmed. Nitrification was found to be highly dependent on the initial phosphorus concentration. The evolution of microalgae communities was also monitored and revealed the advantage of *Scenedesmus* over *Chlorella* when the media was phosphorus-depleted.

1. Introduction

Anaerobic digestion of livestock waste converts organic matter into biogas allowing the production of renewable energy. This process, which is developing fast across Europe, is now well established in Germany. In France, about 115 units of anaerobic digestion were operative at the end of 2013, their development being higher for intensive livestock regions such as Brittany. Nevertheless in these areas, nitrogen and phosphorus release into the environment through landspreading of livestock manure leads to environmental problems such as eutrophication of close-by aquatic environments (Gitton and Hurvois, 1999). Even if anaerobic digestion could allow to both increase renewable energy production and decrease greenhouse gas emissions, such a technology in itself, does not facilitate the management of nutrients contained in manure. Indeed, apart for carbon, nutrient contents of digestates are similar to raw manure while the mineralized nutrients and higher pH can favour ammonia volatilization. So, within intensive livestock areas, the large amount of mineralized nutrients (N, P) thus generated has to be treated and valorised. Phosphorus, in particular, is a non-renewable resource that is running out, while the quality of the remaining phosphorus rock is decreasing, leading to an increase in fertilizer production costs (Cordell et al., 2009). As for nitrogen, fertilizer production is highly energy-consuming. In

* Corresponding author at: Irstea, UR GERE, 17 av. de Cucillé, CS 64427, F-35044 Rennes, France. Tel.: +33 223482121; fax: +33 223482115.
E-mail address: fabrice.beline@irstea.fr (F. Béline).

http://dx.doi.org/10.1016/j.biortech.2014.10.022
0960-8524/

some areas, effluent surplus leads to environmental problems. It is therefore appropriate to better recycle nitrogen from the effluents and to export this element where it is needed. Consequently, regarding resource depletion, energetic requirements or environmental impacts, nutrient recovery from livestock effluent appears to be a good alternative. Some processes are already being applied, like reverse osmosis membrane filtration, ammonia stripping, struvite precipitation, and evapo-concentration but they involve large technical and economic constraints.

Other technologies requiring less energy and chemical additions may be a solution for nutrient recovery, in particular treatment and valorisation of N and P with microalgae (Bjornsson et al., 2013). Indeed, digestate can be an interesting culture medium for microalgae cultivation, as for higher plants. Besides, the productivity of microalgae is ten-fold higher than for conventional crops, leading to smaller cultivation surfaces (De Schamphelaire and Verstraete, 2009). After liquid–solid separation, biomass containing assimilated nutrients can be exported out of areas with a structural surplus to be used as organic amendment and fertilizer (Mulbry et al., 2005), thus limiting the locally applied amounts and reducing N losses by ammonia volatilization.

This approach essentially consists in the assimilation of the major nutrients for metabolism during the growth of microalgae, although other mechanisms such as nitrification–denitrification can also be enhanced with the oxygen production. In fact, as the process cannot be operated easily in axenic conditions, a mixture of microalgae species along with bacteria is involved, leading to complex processes, particularly for nitrogen transformations.

It has been demonstrated that microalgae are able to adapt their internal N:P ratio to the medium ratio (Lundquist et al., 2011). However, nitrogen or phosphorus still can limit the recycling of the whole N and P pools. Indeed, most N:P ratios of anaerobic digestate from livestock waste vary from 3 to 30 g $N\text{-}NH_4$ gP^{-1} in literature (Cañizares-Villanueva et al., 1994; Park et al., 2009; Levine et al., 2011; Chen et al., 2012). The heterogeneity of N:P ratios in digestate is due to AD inputs for nitrogen and phosphorus, the anaerobic digestion process and its management but also, concerning phosphate, functions of separation processes and chemical properties of digestate during anaerobic digestion. The phosphorus solubility is strongly influenced by pH, since the chemical equilibrium favours PO_4^{3-} over HPO_4^{2-} when pH rises, resulting in calcium or magnesium phosphate precipitation. Additionally, dissolved phosphorus can associate with suspended solids and other elements like iron (Möller and Müller, 2012). Moreover, struvite crystals ($NH_4MgPO_4{\cdot}6H_2O$) can be formed with mineralized N, P and Mg when the pH reaches higher values (Capdevielle et al., 2013). Furthermore, in order to grow microalgae in a digestate, an initial liquid–solid separation is required to eliminate particles, further decreasing the total phosphorus content with the removal of solid organic and mineral P, thus reducing potential crystal solubilization or desorption. All these precipitation and adsorption mechanisms lead to a wide range of N:P ratios in liquid digestate.

The internal ratio of microalgae cells issued from general formulas varies between 5.0 and 7.2 gN gP^{-1} (Grobbelaar, 2011; Watson et al., 2011). When comparing these values to digestate ratios, in most cases the quantity of phosphorus is insufficient in the digestate for enabling the removal of all the nitrogen. Hence, the aim of this study was to define the impact of the N:P ratio on microalgal growth and biochemical composition, algal diversity as well as nitrogen behaviour.

2. Methods

To study the impact of the N:P ratio on microalgae growth and nutrient removal, batch experiments were carried out at laboratory scale under controlled conditions. Analysis of nitrogen and phosphorus in liquid and solid fractions led to the estimation of nutrient removal and assimilation by the biomass.

2.1. Inoculum

A complex phytoplanktonic ecosystem, called "inoculum" was used in the experiments. This inoculum comes from an urban wastewater treatment pond located in Meze (France). The microalgae populations mainly belong to the Chlorophyta taxon and are dominated by *Scenedesmus* sp. and *Chlorella* sp. However, other algae genera are present in small amounts, along with bacteria. The inoculum also contains cyanobacteria dominated by *Synechocystis* sp. Those two green algae have frequently been observed growing on manure and digestate (Sevrin-Reyssac et al., 1996; Molinuevo-Salces et al., 2010).

2.2. Experimental set up and methods

The impact of the N:P ratio was studied in batch under controlled conditions. The experiments were performed in triplicate for all conditions. The effluent was composed of diluted anaerobic digestate and minerals to control the initial composition. The digestate originated from a mesophilic farm anaerobic digester located in Orne (France). The different N:P ratios were set with a fixed nitrogen concentration and four levels of phosphorus concentration.

The experimental set up consists of six 2.5 L tubular reactors. The temperature was maintained at 25 °C and agitation set at 230 rpm. Cultures were illuminated during daytime (12 h/12 h) with fluorescent bulbs at 240 µmolE m^{-2} s^{-1}. Pure carbon dioxide was added to control pH between 6.95 and 7.05 and was also added every 45 min during the day to provide carbon in sufficient amounts (Marcilhac et al., 2014). To avoid acidification, which had been observed during preliminary tests when microalgae growth was limited, pH was adjusted by adding 1.25 mL of NaOH 1 M every second day to each reactor. This made it possible to compensate for any differences due to night respiration and to supply a large but regulated amount of carbon dioxide.

The studied N:P ratios are 3, 9, 26 and 76 $gN\text{-}NH_4$ $gP\text{-}PO_4^{-1}$, corresponding to ratios frequently reported in the literature for digestate from livestock waste. They were respectively named HPC, IPC1, IPC2 and LPC for high, intermediate and low phosphorus content. As nitrogen is constant and equal to 190 mgN L^{-1}, the corresponding phosphorus concentrations are 67, 23, 8 and 3 mgP L^{-1}. The design of experiments includes 12 experiments; it was separated in two runs containing the triplicates of HPC and LPC, IPC1 and IPC 2.

The supernatant of centrifuged digestate was used at 0.67% v:v in the growth medium. The colour in the reactor at the beginning of the experiment was thus set at 0.51 on the basis of optical density at 680 nm (O.D.). The digestate used for these experiments was chosen for its high optical density in order to be able to control the nutrient concentration level. For this reason the digestate dilution was very high.

The composition of the growth medium was adapted from a "standard" 1/10 diluted digestate to obtain similar nutrient concentrations (except phosphorus) irrespective of the experimental conditions. Nutrients were equilibrated by adding salts based on the amount of digestate added. The characteristics of the digestate and the salts used are listed in Table 1.

Na_3PO_4, 12 H_2O was then added in solution to fulfil initial phosphate conditions. Other nutrients, which are not listed in Table 1, were added as Z8 media (Staub, 1961; Kotai, 1972; NIVA, 1976), their ratio was calculated on a nitrogen basis. Z8 trace elements were added at a rate of 1% of the total volume of the influent.