Process Biochemistry 41 (2006) 1338–1342

Process Biochemistry

www.elsevier.com/locate/procbio

Development of denitrifying and methanogenic activities in USB reactors for the treatment of wastewater: Effect of COD/N ratio

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Received 8 November 2005; received in revised form 10 January 2006; accepted 15 January 2006

Abstract

Nitrification–denitrification is the traditional biological process for nitrogen removal from wastewaters. During its second step, denitrification, nitrate formed during nitrification is reduced to gaseous nitrogen under anoxic conditions. Under the presence of organic matter and nitrogen, also methanogenesis and dissimilatory nitrate reduction to ammonia (DRNA) may also take place. COD/N has been referred to be a key factor in the expression of these metabolic pathways. During this research, five upflow sludge bled (USB) reactors were operated at different COD/N ratios, in order to study the evolution of the methanogenic and denitrifying activities in the sludge. The use of nitrogen and organic matter through denitrification, DNRA and methanization was also studied through mass balances, as well as its granule structure. COD/N ratio showed a strong influence on biomass activity, and therefore on the metabolic pathways of nitrate and organic matter utilization. Low values generated high denitrifying activities, and high value, elevated methanogenic activities. Even though it was possible to perform methanization and denitrification in one single reactor, feasible loading rates will be limited by the available activities, so in many cases separated reactors will be more suitable. Granular structure could not be maintained in denitrifying reactors at low COD/N ratio (COD/N 5 and lower): granules disappeared and were replaced by flocculent sludge, with low settling velocities.

Keywords: Denitrification; COD/N ratio; Methanogenic activity; Denitrifying activity; USB; Granule

1. Introduction

Biological nitrification–denitrification is the most common process for nitrogen removal from wastewater, especially for sewage. Extensively research has been conducted in the application of this process for industrial wastewater with high ammonia concentration [1–6]. Due to the high oxygen demand for ammonia oxidation, aeration represents one of the main costs involved in this treatment technology. Nevertheless, important advances have been made related with the development of new operation strategies, oriented to reduce operational costs [7–10].

Biological nitrogen removal is performed through two individual sequential processes: nitrification and denitrification. During nitrification, ammonia is oxidized to nitrate with nitrite as an intermediary compound, by the action of autotrophic nitrifying bacteria that use ammonia (and nitrite) as energy source [11]. During denitrification, nitrate is reduced to gaseous nitrogen (with nitrite, nitrous oxide and nitric oxide as intermediaries) by the action of anoxic bacteria that use NO_x as final electron acceptor. Organic matter is the electron donor for this process.

If organic matter and nitrate are present in a wastewater treatment bioreactor, three main processes may occur (beside nitrogen assimilation) in the absence of oxygen: denitrification, methanization and dissimilatory nitrate reduction to ammonia (DNRA). Fig. 1 presents a schematic representation of the metabolic flows of organic matter and nitrogen proposed by Akunna et al. [12], for a system where denitrification, methanization and DNRA are feasible. DNRA represents an undesired pathway since ammonia is produced, which represents a step back in the treatment procedure. Methanization could be a positive factor in a denitrifying reactor, since excess of organic matter can be removed through this route. Nevertheless, its widely accepted that nitrate has an inhibitory effect over methane production, so methanogenesis is only possible once denitrification has finished [12–14]. This could be the result of an inhibitory effect of nitrate, nitrite or other

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1359-5113/$ – see front matter
doi:10.1016/j.procbio.2006.01.007

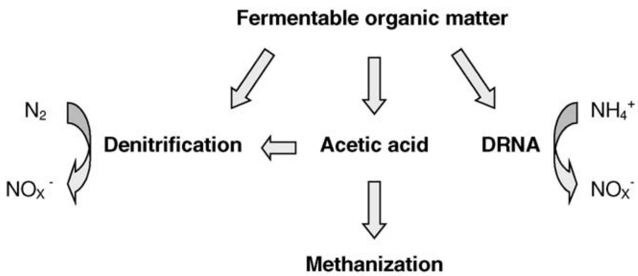

Fig. 1. Metabolic flows for nitrogen and organic matter when denitrification, methanization and DNRA processes are present [12].

intermediary compounds in denitrification pathways [15]. Another option is a negative effect of the increase in the redox potential by the presence of oxidized nitrogen compounds. Nevertheless, this inhibition seems to be reversible and fixed biomass reactors have shown some capability to develop both processes (denitrification and methanization) simultaneously at laboratory scale [12,14,16–18]. Then, nitrate and organic matter could be potentially removed simultaneously in one reactor. Anyway, research is still needed in order to clarify basics aspects related to the interaction of the microorganisms involved and the effect of operational conditions like the COD/N ratio.

This work was focused on the study of COD/N ratio on the performance of upflow sludge bed (USB) reactors inoculated with methanogenic granular sludge. The organic matter and nitrate utilization through denitrification, methanization and DNRA was studied, along with the sludge activity evolution.

2. Material and methods

2.1. Experimental set-up

Experiments were conducted in five parallel laboratory USB digesters. Reactors were assembled with a 0.4 L body, and a 0.4 L head to improve biomass retention. For calculation purposes a useful volume of 0.4 L was used, since head is considered only to enhance biomass retention. Reactors were inoculated with anaerobic granular sludge from a pilot UASB reactor treating brewery wastewater, never exposed to nitrate before. Anaerobic granule had a specific methanogenic activity (SMA) and denitrifying activity (SDA) of 1.2 g CH_4/g VSS day and 0.1 g N-NO_x/gVSS day, respectively. Biomass ash content was 60% and its sludge volumetric index (SVI) was 22 mL/g. Reactor temperatures were maintained in the range of 28–30 °C. Fig. 2 presents a schematic representation of each reactor's set-up. A synthetic wastewater was used to feed the reactors, prepared through the dilution of a concentrated media (Table 1) and the addition of the required amount of $NaNO_3$ to achieve the desired nitrate concentration.

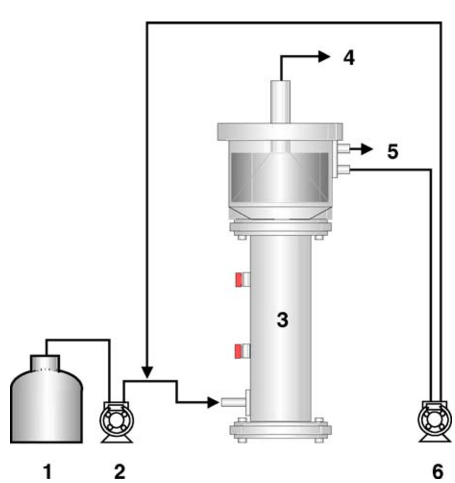

Fig. 2. Experimental set-up of the USB reactors: (1) feed tank, (2) feed pump, (3) USB reactor, (4) biogas outlet, (5) effluent outlet and (6) effluent recycling pump.

Table 1
Composition of the concentrated synthetic wastewater [13]

Compound	Concentration (g/L)
$NaCH_3COO{\cdot}3H_2O$	82.92
Peptone	4.8
Yeast extract	2
$NaHCO_3$	8
K_2HPO_4	70
KH_2PO_4	54
$MgSO_4{\cdot}7H_2O$	0.135
$FeCl_2$	0.014
$CaCl_2$	0.014
KCl	0.014
$CoCl_2$	0.003

2.2. Analytical methods

Ammonia was analyzed by an ion selective electrode (Orion 95-12). Nitrate was determined by UV absorption at 220 and 275 nm and nitrite by the sulphanilamide acid reaction [19]. Biomass was determined by volatile solid suspended concentration and COD was measured by closed reflux dichromate oxidation, both based on standard methods [19]. Specific methanogenic and denitrifying activities were determined as described by Field et al. [20] and Akunna et al. [21], respectively.

2.3. Reactors operation

In order to activate the biomass, a 40 days start-up procedure was performed before conducting experiments, increasing in steps the applied organic loading rate (ORL) up to 7.5 kg COD/m^3 day, reaching a stable operation with high COD removal at this operational condition (data not shown). No nitrate was added to the influent during this period. Once start-up was finished, reactors were operated at different COD/N ratios, as shown in Table 2. A control experiment without nitrate was considered (experiment B) for comparison purposes. Experiment 1 was stopped after 55 days of operation. Hydraulic retention time was fixed thought the whole operation period. Organic and nitrogen loading rates (OLR and NLR) were increased during the operation, by increasing COD and nitrate concentrations. Consumption of substrates (organic matter and nitrate) by denitrification, DNRA and methanization were calculated based in mass balances. Nitrate removal was computed including complete denitrification to N_2 plus nitrite accumulation and ammonification, i.e., nitrate depletion.

3. Results and discussion

Fig. 3 presents COD and nitrate removal for all the experiments. Values at the end of reactors operation are presented. High COD removals were obtained for all the experiments, except for COD/N of 10 (close to 50%). Under the latter condition, organic matter consumption was low, since COD concentration is widely higher than stoichiometric requirements for nitrate denitrification (2.9 g COD/g N-NO_3^-). In