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

G. Ruiz [a,*], D. Jeison [b], R. Chamy [a]

[a] *Biochemical Engineering School, Universidad Católica de Valparaíso, General Cruz 34, Valparaíso, Chile*
[b] *Department of Chemical Engineering, Universidad de La Frontera, Casilla 54-D, Temuco, Chile*

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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

* Corresponding author. Tel.: +56 23 273651; fax: +56 32 273803.
E-mail address: gonzalo.ruiz@ucv.cl (G. Ruiz).

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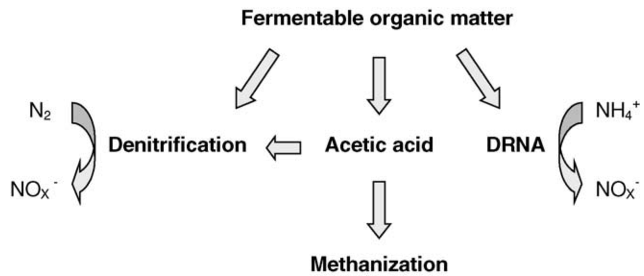

Fig. 1. Metabolic flows for nitrogen and organic matter when denitrification, methanization and DNRA processes are present [12].

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

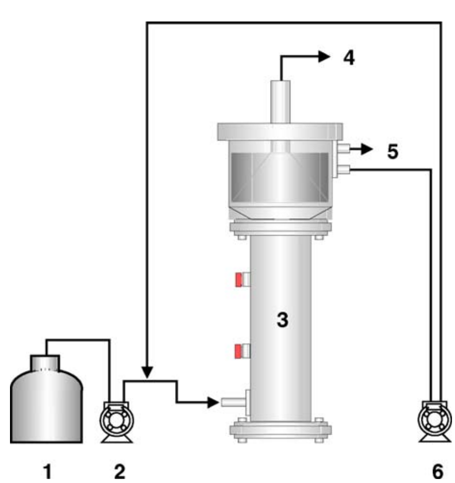

Fig. 2. Experimental set-up of the USB reactors: (1) feed tank, (2) feed pump, (3) USB reactor, (4) biogas outlet, (5) effluent outlet and (6) effluent recycling pump.

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

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.

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

Table 2
Operational conditions of conducted reactor operations

Parameter	Experiment B	Experiment 1	Experiment 2	Experiment 3	Experiment 4
COD/N	–	1	5	10	100
Initial COD (mg COD/L)	2000	2000	2000	2000	2000
Final COD (mg COD/L)	6000	2000	6000	6000	6000
Initial NO_3 (mg N/L)	0	2000	400	200	20
Final NO_3 (mg N/L)	0	2000	1200	600	60
Initial OLR (kg COD/m^3 day)	7.5	7.5	7.5	7.5	7.5
Final OLR (kg COD/m^3 day)	22.5	7.5	22.5	22.5	22.5
Initial NLR (kg N/m^3 day)	0	7.5	1.5	0.75	0.075
Final NLR (kg N/m^3 day)	0	7.5	4.5	2.25	0.225
HRT (h)	6.4	6.4	6.4	6.4	6.4
Operation time (days)	190	55	102	166	166

addition, no other routes of COD consumption are present under that condition, as can be seen in Fig. 4: methanization only accounts for 10% of the removed organic matter. COD/N had a strong effect on the organic matter utilization pathways as can be clearly seen in Fig. 4: for a ratio equal or lower than 10, denitrification represents by far the main route of organic matter consumption. At a ratio of 100, methanization is over 97%.

On the other hand, nitrate is practically 100% eliminated for COD/N over 5 (Fig. 3). At COD/N of 1, nitrate removal is poor, since the organic matter requirement for denitrification is not fulfilled. This also caused nitrite accumulation (Fig. 5).

COD/N influence in the nitrogen utilization route is presented in Fig. 5. Denitrification is the main route of nitrogen utilization. Nitrite accumulation takes place at a COD/N ratio of 1 due to insufficient organic matter availability as mentioned earlier.

Ammonia generation was detected at higher values of COD/N. Effluent ammonium on experiments 3 and 4 (COD/N 10 and 100) was about 60–80 mg N-NH_4^+/L. Probably this is the result of the degradation of peptone and yeast extract present in the macro and micronutrients (Table 1). Stoichiometric calculations confirm this hypothesis. Therefore, no significant occurrence of DRNA was detected.

Specific methanogenic and denitrifying activities were highly influenced by the operation at different COD/N ratios, as can be seen in Figs. 6 and 7. The methanogenic activity remained practically unchanged if no nitrate is present in the feed (experiment B), and decreased faster, and reached lower levels, as nitrate concentration increased. This reduction would be the result of the presence of nitrate, nitrite or another intermediary compound in the denitrification process, like nitrous oxide [15]. Another alternative could be a toxic effect of some free radical generated during nitrogen metabolization, which could affect methanogenic bacteria [22], since they do not have a free-radical defense system due to the absence of the

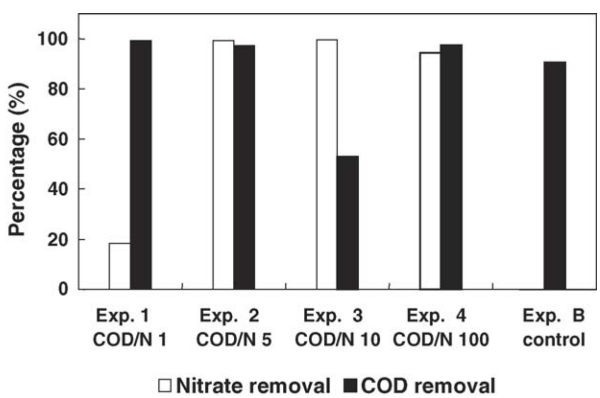

Fig. 3. Influence of COD/N ratio on the COD and nitrate removal: values computed at the end of reactors operation (standard deviation was less than 5%).

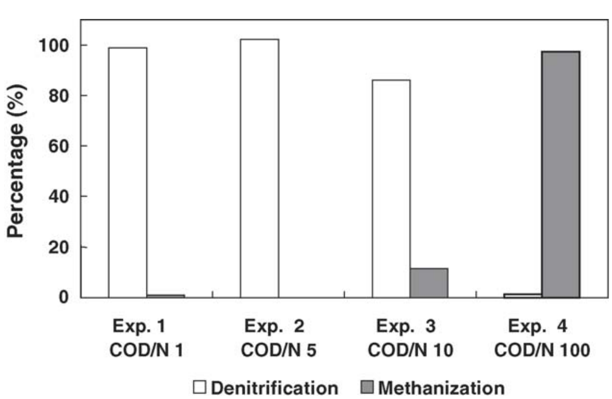

Fig. 4. Effect of COD/N ratio over organic matter utilization route: percentage of COD used for denitrification, ammonification and methanization, at the end of reactors operation (standard deviation was less than 2%).

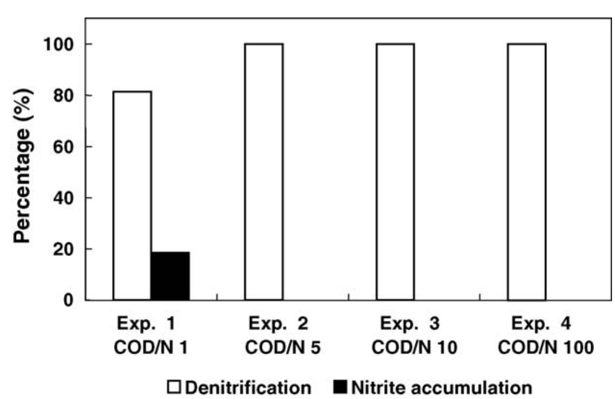

Fig. 5. Effect of COD/N ratio over nitrate utilization route: percentage of N-NO_3^- used for denitrification, ammonification and nitrite accumulation, at the end of reactors operation (standard deviation was less than 5%).

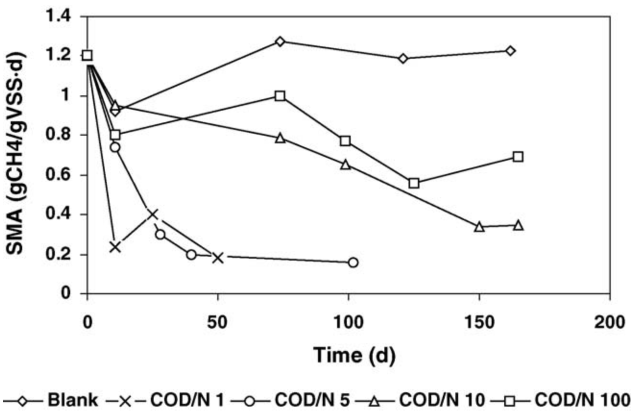

Fig. 6. Evolution of the specific methanogenic activity (SMA) of sludge from the reactors.

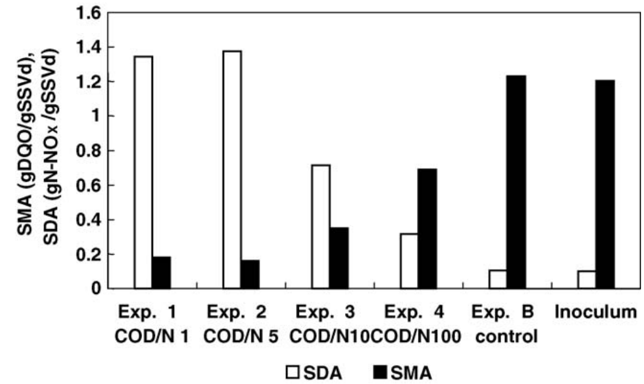

Fig. 8. Specific methanogenic and denitrifying activity (SMA, SDA) of sludge from the reactors at the end of its operation. Inoculum activity is also presented for comparison porpoises.

superoxide dismutase enzyme [23]. The denitrifying activity showed the opposite tendency, as can be seen in Fig. 7. Activity increased as COD/N decreased, up to the ratio of 5. The denitrifying activity increased quickly during the first 30 days of operation, and remains fairly constant from that point onwards. This implies that anaerobic granular sludge is a useful source of inoculum for denitrifying processes, since high levels of activity are reached in short periods of time. Fig. 8 presents both activity levels at the end of the operation of the reactors. The behavior of the metabolic activities is in close relation, as expected, to the metabolic pathways observed during the reactors' operation (Figs. 4 and 5).

The obtained results confirm the difficulties found when methanization and denitrification want to be performed in a single reactor. Conditions that improve the activity of denitrifying microorganisms (low COD/N) seriously affect the activity of methanogenic bacteria. Even though both activities can be found at COD/N ratios around 10, their values are much lower than those that would be found in fully methanogenic or denitrifying reactors. This means that if both processes are going to be performed in a single stage, low loading rates should be applied. Indeed, low organic and nitrogen loads are in general a common characteristic of those researches that have successfully performed both pathways in one single reactor [24,25]. This situation explains the low COD removal of experiment 3 (COD/N 10): low methanogenic activity was not enough to remove the excess organic matter for denitrification, due to the high organic loading applied.

Notorious differences were observed in sludge physical properties. Sludge volumetric index increased quickly for low COD/N ratios, as can be seen in Fig. 9. For experiments 1 and 2

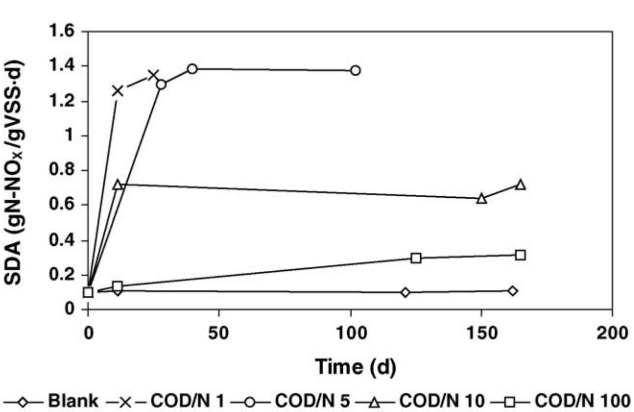

Fig. 7. Evolution of the specific denitrifying activity (SDA) of sludge from the reactors.

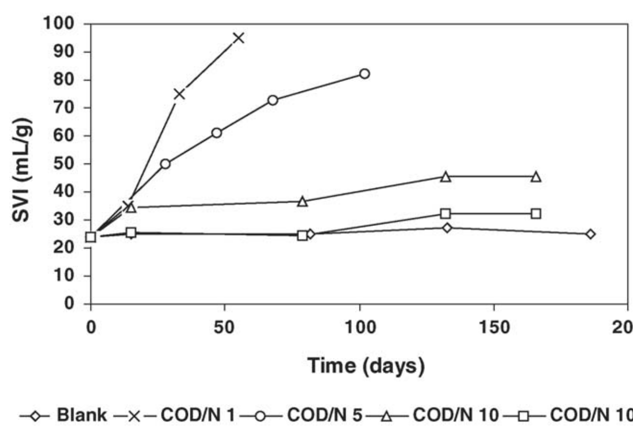

Fig. 9. Evolution of the sludge volumetric index (SVI) of reactors biomass throughout their operation.

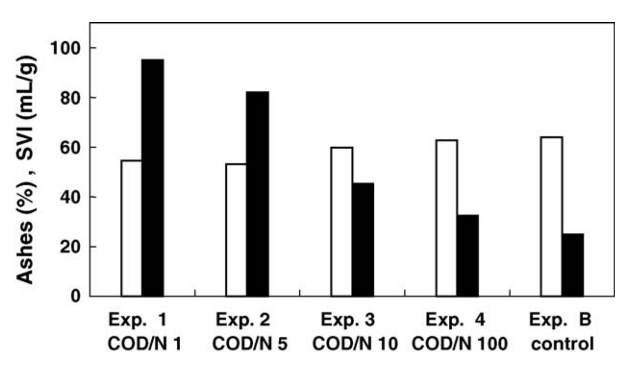

Fig. 10. Effect of COD/N ratio on sludge ash content and volumetric index (SVI), values computed at the end of reactors operation.

(COD/N ratio 1 and 5), SVI reached values close to of 100 mL/g, amount that is characteristic of flocculent sludge. On those experiments, granules tended to disappear, inducing the formation of smaller flocs that presented poor settling-ability and a tendency to float (data not shown). Experiment 3 presented clear grey granules with a ''hairy'' like surface. Experiments B and 4 presented granules with the typical characteristics of anaerobic granular sludge bed reactors. Differences in sludge characteristics can be more clearly seen in Fig. 10, which presents the SVI at the end of the reactors operation. These differences influenced the height of the sludge bed inside the reactors (VSS were kept constant), and therefore their operation, by increasing the possibility of sludge washout. Fig. 10 also presents the ash content. No effect is apparent in this parameter, since values from all experiments were similar.

4. Conclusion

The COD/N ratio has a strong influence on biomass activity. Low values generate the development of high denitrifying activities, and high values produce elevated methanogenic activities. Even though it is possible to maintain both processes in one reactor, feasible loading rates are limited by the available activity. This means that in many cases low loaded reactors are necessary to achieve good levels of removal. Therefore, economical convenience could be doubtful and separated processes may be more suitable. Conditions and requirements of each treatment situation would determine the most convenient alternative, and no generalizations are possible.

Granular structure could not be maintained in denitrifying reactors (COD/N 5 and lower). The development of flocculent sludge, with low settling velocities, made the overall reactors operation difficult, by increasing sludge washout. On the other hand, during the operation with a COD/N ratio over 10, granules properties remained stable with good settling-ability properties.

The experiments shown that it is not possible to maintain at a long term both, methanogenic and denitrifying activities in the same reactor, limiting the application of both process at the same reactor.

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