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Kinetics of denitrification with crude glycerine as a carbon source in landfill leachate treatment

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ABSTRACT

The effect of COD/N ratio (3–15) on the kinetics and efficiency of nitrogen removal from stabilized landfill leachate with crude glycerine as a carbon source was investigated. Then, using the optimal COD/N ratio, the dependence between kinetic parameters of denitrification and the volumetric exchange ratio (*n*) (0.1–0.6 cycle^{-1}) was determined. At or above the optimal COD/N ratio of 7, the effectiveness of denitrification was almost 100%. Increasing COD/N ratios above 7 not only did not improve the denitrification rate (ca. 14 mg N-NO_3/L·h and ca. 8 mg N-NO_x/L·h), but also led to higher COD in the effluent. At chosen COD/N ratio of 7, only at *n* in the range of 0.1–0.3 cycle^{-1}, the denitrification rates depended on *n*, and increased from 6.4 to 13.6 mg N-NO_3/L·h and from 2.7 to 7.4 mg N-NO_x/L·h. Moreover, increase in each 1 mg N-NO_3/L in initial concentration of N-NO_3 increased the denitrification rate by 0.18 mg N-NO_3/L·h and 0.09 mg N-NO_x/L·h. Further increases in the *n* from 0.4 to 0.6 cycle^{-1} did not substantially increase the rate of denitrification. However, because the denitrification rate remained the same despite the increase in nitrate concentration at the beginning of the SBR cycle, the time needed for complete nitrogen removal increased to 21 h, which means that a 24 h SBR cycle may be too short to ensure process stability.

Keywords: Kinetics of denitrification; Crude glycerine; Landfill leachate; Nitrogen removal; COD/N ratio

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1. Introduction

Biological nitrogen removal is still one of the main problems during wastewater treatment, particularly nitrogen removal from wastewater with a low COD/N ratio, because of the lack of available carbon for denitrification. Landfill leachates are considered difficult to treat because they undergo continuous qualitative and quantitative changes with landfill aging. Leachate from stabilized landfill contains high levels of ammonium, often reaching several thousands of mg/L [1–3] and a low concentration of biodegradable organics. Thus, it is considered wastewater with an unfavorable COD/N ratio, making it necessary to supply carbon from external sources to improve the effectiveness of nitrogen removal. As an external carbon source, waste products offer many advantages in comparison to commercial carbon sources. For example, although commercially available low molecular weight alcohols or volatile fatty acids are most often used because they are easily biodegraded and enable high denitrification rates, the use of these carbon sources generates additional treatment costs. Recently, alternative sources of organic compounds, i.e. waste products, have been the focus of research. Waste products from the agro-food industry seem to be the most useful [4]. So far, molasses [5,6], residues from distillery (spent wash, fusel oils) [7,8] or crude glycerine [9–11] have been successfully used as carbon sources for nitrogen removal, both in denitrification and denitritation. In many countries nowadays, diesel is supplemented with biodiesel, so biodiesel production has been increasing [12]. Glycerine is a by-product of biodiesel production, so glycerine supply exceeds demand. However, when choosing an organic car-

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bon source, some aspects should be considered: low cost, high effectiveness of denitrification/denitritation and a high process rate.

Although the effect of waste carbon sources on process effectiveness has been shown, most of those studies were conducted with synthetic or municipal wastewater, but not with real landfill leachate. Moreover, studies that investigate the dependence between the technological parameters and denitrification kinetics and organics removal are rather scarce.

In the present study, the effect of COD/N ratio on the efficiency and rate of denitrification with crude glycerine as a carbon source was determined. Next, using the optimal COD/N ratio that was indicated in the first part of the study, the effect of volumetric exchange ratio on the efficiency and the rate of denitrification and organics removal was examined.

2. Materials and methods

2.1. Landfill leachate

Leachate used in the experiment originated from a landfill located in the Warmia and Mazury, Poland, that has been operating for 20 years. Animal and vegetable organic waste (25.3% of the waste mass), glass (14.0%), paper and cardboard (12.2%), plastics (4.4%), metals (3.1%), textiles (2.7%), and the fine fraction (38.3%) are collected at the landfill side. The landfill does not accept fecal material, liquid waste, toxic and radioactive substances, or other hazardous substances. Leachate is collected by a system of drains and stored in a reservoir. The physico-chemical composition of the landfill leachate is presented in Table 1.

2.2. Process configuration and system design

2.2.1. Nitrification

Nitrification of landfill leachate was conducted in two parallel SBRs with a working capacity of 5 dm^3 each. The reactors were inoculated with activated sludge from the nitrification chamber of municipal wastewater treatment plant (MWTP). The reactors operated with 24 h cycles with the following phases: filling (5 min), stirring-aeration (23 h) and sedimentation and decantation (55 min). The volumetric exchange ratio (n) was 0.3 d^{-1}. The system was operated at room temperature (20–22°C) for 90 d. The biomass concentration was ca. 3.5 g MLSS/L and SRT 20 d. The reactors were equipped with a stirrer with adjustable speed (36 rpm), and a fine bubble aeration system at the bottom of the reactor. DO concentration in the aeration phase was ca. 2.5 mg O_2/L. Nitrifying SBRs were fed by the landfill leachate which characteristic was given in the section *Landfill leachate*.

2.2.2. Denitrification

For denitrification, leachate after nitrification was directed to six parallel SBRs with a working capacity of 3 dm^3 each. These reactors were inoculated with activated sludge from the denitrification chamber of the MWTP. The biomass concentration was maintained at ca. 3.5 g MLSS/L; SRT 15 d. Because of the lack of organic compounds susceptible to biodegradation (BOD_5/COD in the leachate after nitrification was 0.004), crude glycerine, a waste product of biodiesel production, was added as an external carbon source. The chemical composition of the crude glycerine was as follows: glycerol 80–85%, ash (NaCl) < 7%, M.O.N.G. (non-glycerol organic matter) < 2%, methanol < 0.5%, with the remaining portion consisting of water (product specification from Biodiesel Manufacturing Plant, Poland). The solution of crude glycerine was prepared in the following way: 113 g of crude glycerine were dissolved in 1 L of distilled water, yielding ca. 100 mg COD/ml.

Investigations of the kinetics and effectiveness of denitrification were performed in separately. First, the effect of COD/N ratios on the efficiency and rate of denitrification was determined. The volumetric exchange rate in the six parallel SBRs was 0.3 d^{-1}. The following COD/N ratios were tested: 3, 5, 7, 9, 12, and 15. Second, the effect of volumetric exchange ratios on the efficiency and the rate of denitrification was examined (using the COD/N ratio selected in part one). The n were 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 cycle^{-1}. To enable the microorganisms to adapt to the COD/N ratios (part one) and to n (part two), the six denitrifying SBRs were operated at room temperature (20–22°C) for 60 days in each part. Then, under steady state conditions, the kinetics of denitrification and COD removal were determined. The kinetics of denitrification was determined with regard to both N-NO_3 removal and the removal of the sum of nitrite and nitrate (N-NOx removal).

2.2.3. Analytical methods

Measurements of pollutant concentration in the leachate, effluent from nitrifying and denitrifying reactors, and during cycles, included: chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen, total phosphorus, total solids, volatile solids nitrites and nitrates. The activated sludge was analyzed for mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). All analyses were performed according to APHA [13].

3. Results and discussion

3.1. Nitrification step

The ammonium concentration in the landfill leachate was 890±18 mg/L, but taking into account the volumetric exchange ratio (0.3 d^{-1}), the initial concentration of ammonium at the beginning of the cycle in the nitrifying SBR was ca. 260 mg/L. During leachate nitrification with a 24 h operational reactor cycle, the adaptation period in both nitrifying SBRs lasted about 40 days. At the beginning of this time nitrite was formed as an intermediate product of nitrification. Afterwards, the ammonium concentration in the effluent dropped below 1 mg N-NH_4/L and complete nitrification to nitrate took place. In the following days of the experiment (40–90 d), stable nitrification took place, and the ammonia concentration in the treated leachate did not exceed 0.56 N-NH_4/L or 0.45 N-NH_4/L, with a nitrate concentration of ca. 250 N-NO_3/L. The effectiveness of nitrification in both SBRs was 99.88%.

On the basis of changes in the concentrations of ammonium, nitrite and nitrate during the SBR cycle, the rate constants of ammonium oxidation (removal) and of the formation of the sum of nitrite and nitrate (nitrification) were determined. Both ammonium removal and nitrification proceeded according to zero order kinetics. The ammonium removal rate was 31 mg N-NH_4/L·h. The rate of formation of both nitrite and nitrate was similar. During the first two hours of the cycle only the first phase of nitrification (the oxidation of ammonia nitrogen to nitrite) took place. After this time nitrite was oxidized to nitrate and finally the effluent contained nitrate only. The time needed for complete ammonium removal was 8 h, which constituted ca. 35% of the aeration phase in the SBR cycle. This means that it would have been possible to shorten the SBR cycle to 12 h or to increase the volumetric exchange ratio, but this was not done because the main goal of the present study was to investigate denitrification of the effluent prepared in the nitrifying reactors.

3.2. Denitrification

To allow the microorganisms to adapt to new carbon source, denitrification was carried out in six parallel SBRs. In the leachate after nitrification, the organics (COD) concentration was 902 mg O_2/L, but these organic compounds were difficult to biodegrade, as shown by the low BOD_5 and BOD_{20} of 3.73 and 6.21 mg O_2/L, respectively, and by the low value of the rate constant of oxidation of easy-to-degrade organic compounds (BOD_5) in the leachate (k_{BOD5} 0.09 d^{-1}). Even though the COD concentration in leachate is high, the ratio of biodegradable organics (BOD_5) to total organics (COD) is very low, so that even at a high COD/N ratio, a supply of external carbon may be necessary. Thus, in the present study to assure effective nitrogen removal, crude glycerine was added. Crude glycerine is an attractive alternative as a carbon source because of its cost and availability. The potential of a carbon source to support denitrification also depends on its biodegradability, and various industrial wastes have been found to have high biodegradability, thus giving favorable process kinetics. For example, dairy waste is useful for denitrification because it has a high value of readily biodegradable COD such as lactose and lactate [14]. Corn syrup or high-fructose corn syrup are widely used in the food industry and have been suggested for nitrogen removal because of their high content of glucose or fructose [15,16]. The crude glycerine used in this study was characterized by high BOD_5/COD ratio of 0.68, and high rate constant for oxidation of easy-to-degrade organic compounds (k_{BOD5} 0.5 d^{-1}).

Both when COD/N ratios were being tested (part one), and when volumetric ratios were being tested (part two), denitrification in the six parallel SBRs was considered stable when the concentration of COD, N-NO_2, and N-NO_3 did not change by more than 5–10% within 7 d.

3.3. Effect of COD/N ratios on kinetics of denitrification and COD concentration in the effluent

It known that effective denitrification requires a COD/N ratio of 5–10 in wastewater, bearing in mind a BOD_5/COD ratio of 0.5–0.6, and that some of the organic compounds are removed in the settlement stage, and used also by other microorganisms like phosphorus accumulating bacteria. However, the COD/N ratio can be lower when a highly biodegradable compounds, like acetic acid or methanol, are added directly to the denitrification chamber to serve as carbon sources. When using waste products as a carbon source, such as crude glycerine, the COD/N ratio may need to be higher. This is due to the fact that only part of the organic compounds in waste products are highly biodegradable. For this reason, the present study tested the effect of six different COD/N ratios (from 3 to 15) on the efficiency and rate of denitrification (Fig. 1). At a COD/N ratio of 3, the rate of denitrification was lowest: 2.29 mg N-NO_3/L·h and 1.2 mg N-NO_x/L·h. Denitrification effectiveness was 41.25%; this low level of effectiveness led to a high nitrite concentration in the effluent (ca. 35 mg N-NO_2/L). Nitrite, not nitrate, was present in the effluent, indicating that the second phase of denitrification (nitrite to nitrogen gas) was inhibited. At a COD/N ratio of 5, the rate of denitrification was almost two times higher (6.3 mg N-NO_3/L·h and 2.2 mg N-NO_x/L h), which resulted in higher process effectiveness (81.78%), although nitrite was still present in the effluent (15 mg N-NO_2/L). At both COD/N ratios, the COD concentration did not exceed 40 mg COD/L after denitrification (Fig. 1).

When the COD/N ratio was 7 or higher, the effectiveness of denitrification was almost 100%. Increasing COD/N ratios above 7 not only did not improve the denitrification

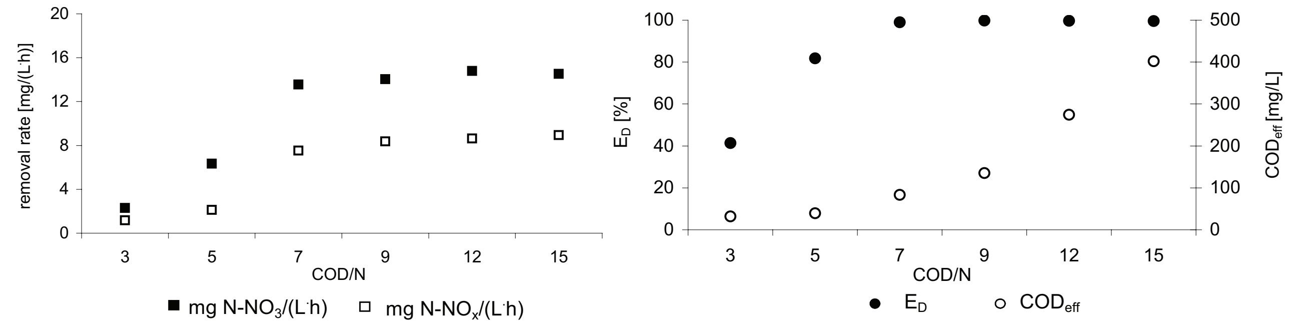


Fig. 1. Relationships between COD/N ratios and the removal rates of N-NO_3 and N-NOx (a), and effectiveness of denitrification (E_D) and the concentration of COD in the effluent (COD_{eff}) (b).

rate, but also led to higher organics concentrations, as indicated by COD, in the effluent (Fig. 1b).

Taking into account the rate of denitrification, the efficiency of the process and the concentration of organic compounds in the effluent, a COD/N ratio of 7 was chosen to investigate the effect of volumetric exchange ratio on the denitrification rate in the second part of the study.

3.4. Effect of the volumetric exchange ratio on kinetics of denitrification and COD removal

Volumetric exchange ratios from 0.1 to 0.6 cycle^{-1} resulted in initial concentrations of nitrate at the beginning of the SBR cycle of 25, 50, 75, 100, 125, 150 mg N-NO$_3$/dm^3 (taking into account that leachate after nitrification contained nitrate at a concentration of ca. 260 mg/L).

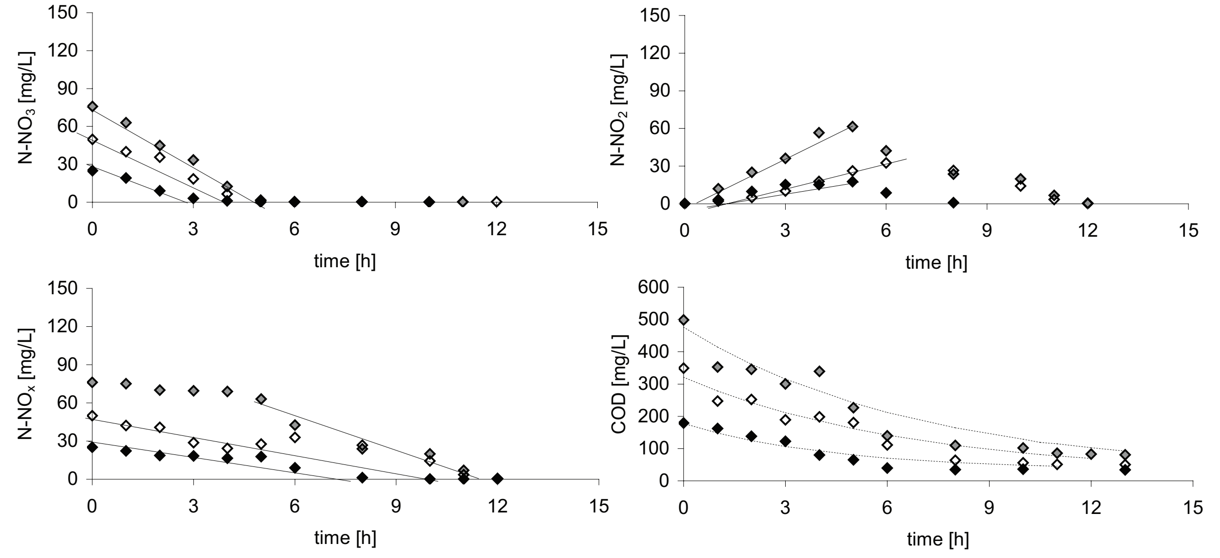
Figs. 2 and 3 present changes in concentrations of nitrite, nitrate, and COD in the SBR cycles. When the initial nitrate concentration at the beginning of the cycle ranged from 25 to 75 mg N-NO$_3$/L, the time needed for nitrate removal did not exceed 5 h. However, it should be emphasized that nitrite accumulated. This caused the time for removal of both oxidized forms of nitrogen (N-NO$_x$) to last ca. 12 h. Similar tendencies in denitrification (nitrite accumulation) were observed at the three higher initial concentrations of nitrate (100, 125 and 150 mg/L). These higher nitrate concentrations at the beginning of the cycle caused the time needed for nitrate removal (6–9 h) and N-NO$_x$ removal (14–21 h) to be almost two times longer than at the lower initial concentrations.

On the basis of the changes in concentrations of nitrite, nitrate, and organic compounds as COD, the following rates and the rate constants were determined (Figs. 2, 3): i) nitrate removal (r_{N-NO3}, k_{N-NO3}) ii) nitrite increase (r_{N-NO2}, k_{N-NO2}), iii) N-NO$_x$ removal (r_{N-NOx}, k_{N-NOx}), and iv) the removal of organic compounds (r_{COD}, k_{COD}).

Nitrate removal, nitrite increase and N-NO$_x$ removal proceeded according to zero-order kinetics (the rates of these processes were equal to the rate constants); however, COD removal followed first-order kinetics.

When the initial concentration of nitrate in leachate at the beginning of the cycle increased from 25 to 75 mg N-NO$_3$/L, the nitrate removal rate increased from 6.4 mg N-NO$_3$/L·h to 13.6 mg N-NO$_3$/L·h (Fig. 2). At initial nitrate concentrations of 100–150 mg N-NO$_3$/L, the rate of nitrate removal was almost stable and was ca. 15.5 mg N-NO$_3$/L·h. The rate of nitrate removal exceeded the rate of nitrite increase (Fig. 3), indicating that part of the nitrate was completely reduced to nitrogen gas (complete denitrification), while at the same time, the other part was reduced to nitrate only (the first step of denitrification). During the first hours of the cycles, at nitrate concentrations in the leachate up to 75 mg N-NO$_3$/L, nitrate removal to nitrite prevailed for a period of 4-6 h. The rate of N-NO$_x$ removal was much lower than the rate of nitrate removal alone. The nitrite concentration increased, so that the overall rate of nitrogen removal (as N-NOx removal) in denitrification slowed down.

At the same COD/N ratio of 7, the rate constants of COD removal (k_{COD}) at all volumetric exchange ratios were almost the same, 0.18–-0.19 h^{-1}. However, because COD



Initial N-NO$_3$ at the beginning of the cycle [mg/L]		25	50	75
N-NO$_3$ removal	r_{N-NO3}; k_{N-NO3} [mg/L·h]	6.37	10.27	13.62
N-NO$_2$ increase	r_{N-NO2}; k_{N-NO2} [mg/L·h]	3.26	4.94	11.61
N-NO$_x$ removal	r_{N-NOx}; k_{N-NOx} [mg/L·h]	2.67	3.78	7.41
COD removal	r_{COD} [mg/L·h]	31.51	54.12	99.70
	k_{COD} [h^{-1}]	0.18	0.18	0.19

Fig. 2. Changes in N-NO$_2$, N-NO$_3$, N-NO$_x$ and COD concentration during SBR cycle at *n* 0.1–0.3 cycle^{-1} (initial nitrate concentrations of 25–75 mg N-NO$_3$/L) at COD/N ratio of 7.0; table presents kinetics of denitrification and COD removal.

removal followed first-order kinetics, the organics removal rate (r_{COD}) increased from 31.51 mg COD/L·h to 159.32 mg COD/L·h, as a result of the higher content of organics at the beginning of the SBR cycle (Figs. 2, 3).

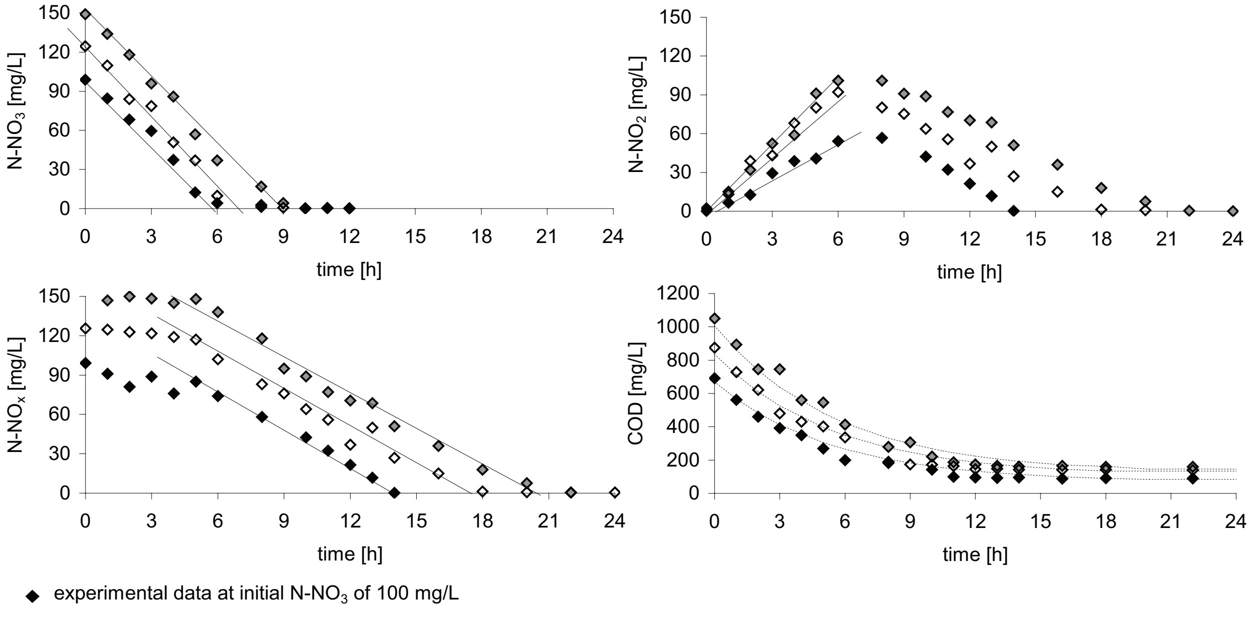
It should be emphasized that at all volumetric exchange ratios (*n*), COD in the effluent averaged 15% of the COD introduced with the crude glycerine. Thus, at higher volumetric exchange ratios, and the correspondingly greater amounts of organic compounds added, COD in the effluent was higher. This COD that remained in the effluent was probably composed of difficult-to-degrade compounds.

In Fig. 4, it can be seen that, up to an initial nitrate concentration of 75 mg N-NO_3/L, each 1 mg N-NO_3/L increase in this initial concentration caused the rate of N-NO_3 removal (r_{N-NO3}) to increase by 0.18 mg N-NO_3/L·h. In contrast, at initial concentrations of nitrate of 100–150 mg N-NO_3/L, the N-NO_3 removal rate only increased by about one-sixth this amount for every additional 1 mg N-NO_3/L increase in the initial concentration (by 0.03 mg N-NO_3/L·h). A similar tendency was observed for the rate of N-NO_x removal (0.09 mg N-NO_x/L·h at initial nitrate concentrations up to 75 mg N-NO_3/L, then 0.02 mg N-NO_x/L·h).

The influence of the COD/N ratio on the effectiveness and rate of denitrification/denitritation has been reported in different studies when alternative carbon sources were used. However, most of these studies were carried out with the use of synthetic or municipal wastewater not with landfill leachate. Alternative sources of carbon (wastewater from a sweets factory, the residue from a soft drinks factory with high-sugar content and the residue from a dairy plant with high content of lactic acid) were tested for the denitrification of synthetic wastewater containing 2500 mg N-NO_3/dm^3 in an SBR [17]. The optimum COD/N ratios varied between 4.6 for the lactic-acid-rich carbon source and 5.5–6.5 for the sugar-rich carbon sources. The authors obtained specific denitrification rates of 42–48 mg N-NO_3/g VSS·h, nitrate-free effluents, and very low COD concentrations in the effluent with 4–6 h of SBR reaction time, especially with the sugar-rich carbon sources.

Torá et al. [18] tested different carbon sources (ethanol, acid-fermented primary sludge centrate, acid-fermented secondary sludge centrate, glycerol and landfill leachate) in heterotrophic denitrification from nitrite (denitritation) in an SBR. Efficient denitritation of a synthetic high-strength nitrite wastewater was achieved using these carbon sources, with the exception of the fermented secondary sludge centrate, with COD/N ratios of 3.0 for ethanol, 3.8 for glycerol, 5.5 for primary sludge centrate and 8.8 for landfill leachate. The maximum specific nitrite removal rate of 0.25 g N/gVSS·d was achieved with glycerol, while values of 0.13–0.17 g N/g VSS·d were obtained with ethanol, landfill leachate and fermented primary sludge centrate.

Prentice [15] conducted batch experiments as well as a full-scale investigation and found that the addition of corn syrup improved the nitrogen removal process, with an empirical dosing of about 7.9 g COD/g N, and a denitri-



Initial N-NO_3 at the beginning of the cycle [mg/L]		100	125	150
N-NO_3 removal	r_{N-NO3}; k_{N-NO3} [mg/L·h]	15.43	15.81	16.22
N-NO_2 increase	r_{N-NO2}; k_{N-NO2} [mg/L·h]	7.89	13.31	14.79
N-NO_x removal	r_{N-NOx}; k_{N-NOx} [mg/L·h]	7.81	8.62	8.92
COD removal	r_{COD} [mg/L·h]	108.21	134.49	159.32
	k_{COD} [h^{-1}]	0.18	0.19	0.18

Fig. 3. Changes in N-NO_2, N-NO_3, N-NO_x and COD concentration during SBR cycle at *n* 0.4–0.6 $cycle^{-1}$ (initial nitrate concentrations of 100–150 mg N-NO_3/L) at COD/N ratio of 7.0; table presents kinetics of denitrification and COD removal.

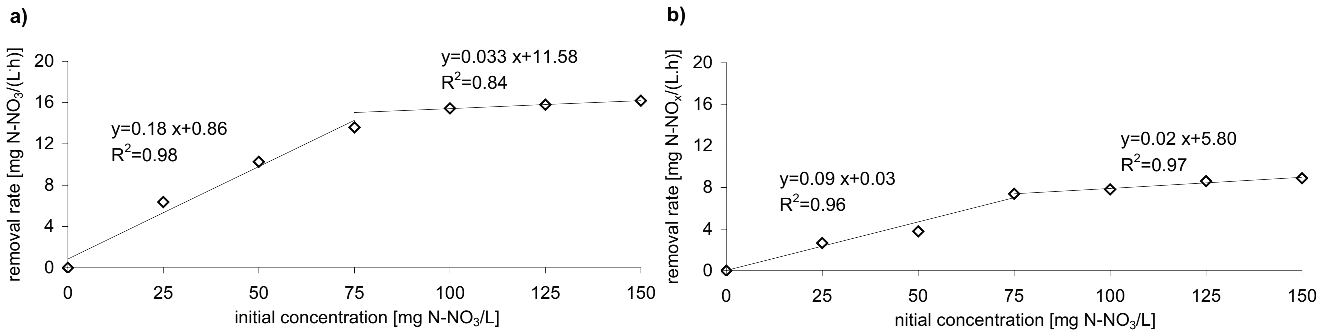


Fig. 4. Relationships between removal rates of N-NO_3 and the nitrate concentration at the beginning of the cycle (a), removal rates of N-NO_x and the nitrate concentration at the beginning of the cycle (b).

Table 1
Characteristics of municipal landfill leachate

Characteristic	Value
pH	8.45 ± 0.12
COD, mg O_2/L	978 ± 31
BOD_5, mg O_2/L	32 ± 4
k_{BOD5}, d^{-1}	0.13
BOD_5/COD	0.033
BOD_5/TKN	0.035
Total Kjeldahl nitrogen, mg TKN/L	917 ± 17
Ammonium nitrogen, mg N-NH_4/L	890 ± 18
Total phosphorus, mg P/L	76.6 ± 4.8
Total solids, mg/L	7324 ± 272
Volatile solids, mg/L	1640 ±110

k_{BOD5} – the rate constant of oxidation of easy-to-degrade organic compounds as BOD; this reflects the rate of oxidation, and together with the BOD concentration, this value indicates the concentration of easy-to-degrade compounds and the their susceptibility to biodegradation

fication rate of about 4.4 mg N/g VSS·h. Similar results, in terms of dosing and increased efficiency, were obtained with high-fructose corn syrup [16]. Effective denitrification at a lower C/N ratio (4.5) was obtained by Mokhayeri et al. [19].

Although studies have investigated the use of waste products from biodiesel production for nitrogen removal, they have not done so in the context of landfill leachate treatment. For example, glycerine was investigated as an organic carbon source for the removal of nitrate from municipal wastewater with a low BOD_5/N ratio (1.7:1) [20]. Biodiesel waste, at an amount of 500 kg COD/d, was dosed to obtain a concentration of N_{total} in the effluent that was below 10 mg/L. Denitrification efficiency increased by 2-5 mg N-NO_3/L per 100 dm^3 of glycerol phase added to the denitrification tank. Glycerol has been used as an external carbon source for denitrification in a pilot study [21]. The average denitrification rate was 1.8 mg N/gVSS·h; the authors also highlighted the need for acclimatization of the biomass in order to efficiently remove the nitrate.

Bernat et al. [10] previously showed that crude glycerine was useful as the sole carbon source; however, this study concerned nitrite removal (100 mg N-NO_2/L) from synthetic wastewater. Those results indicated a high denitritation rate of 18.85 mg N-NO_2/g VSS·h with a 12 h cycle length at a COD/N ratio of 3.5. In the present study, the denitrification rates were lower (ca. 15.5 mg N-NO_3/L·h which correspond to ca. 4.4 mg N-NO_3/g VSS·h) and demanded COD/N ratio higher, of 7; however, it should be emphasized that the process proceeded from nitrate, not nitrite, and in landfill leachate, not synthetic wastewater.

4. Conclusion

During nitrogen removal from landfill leachate with crude glycerine as a carbon source, at COD/N ratio was 7 and above this value, the effectiveness and the rate of denitrification did not increase, and the quality of the effluent deteriorated, as shown by an increase in organics (as COD) concentration.

At a COD/N ratio of 7, the rate of denitrification (the rates of N-NO_3, and N-NO_x removal) increased with increases at the volumetric exchange ratio in the range only from 0.1 to 0.3 $cycle^{-1}$. Further increases from 0.4 to 0.6 $cycle^{-1}$, did not lead to further improvements in the rate of denitrification. However, as a result of the increases in nitrate concentration at the beginning of the SBR cycle, resulted from the increase of *n*, the time needed for complete denitrification increased to 21 h, which means that a 24 h SBR cycle may be too short to support stable denitrification in landfill leachate treatment.

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