

## Denitrification of Spent Regenerated Brine Using Molasses

Brigita Tepuš,<sup>a</sup> Marjana Simonič,<sup>b,\*</sup> Aleksandra Petrovič,<sup>b</sup> and Jasmina Filipič<sup>a</sup>

<sup>a</sup>Municipal Enterprice Ptuj, Puhova 10, SI–2250 Ptuj, Slovenia

<sup>b</sup>Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, SI–2000 Maribor, Slovenia

RECEIVED SEPTEMBER 10, 2013; REVISED JANUARY 22, 2014; ACCEPTED JANUARY 30, 2014

**Abstract.** Spent BRINE from the regeneration of exhausted resins has to be properly treated before its disposal. The heterotrophic denitrification of regenerated brine was studied in present work. Molasses which served as a carbon source has until now not been applied during brine denitrification. The nitrate and nitrite consumptions were observed according to different ratios between total organic carbon and nitrate (TOC / NO<sub>3</sub> ratios) and the influence of NaCl was studied during batch experiments. This work ascertained that molasses is a very good source of carbon, and no addition of other nutrients is necessary. The complete nitrate removal from regenerated brine could be achieved using molasses.

**Keywords:** nitrate, denitrification, regeneration brine, molasses, kinetics

### INTRODUCTION

In drinking water treatment systems, nitrate is usually removed using ion exchange. After the regeneration of ion exchange resin, the spent brine solution has to be properly treated before its disposal. Denitrification of spent regeneration brine relies on an external carbon source as an electron donor and microorganisms which use nitrate rather than oxygen as an electron acceptor.<sup>1,2</sup> If there is insufficient carbon in the wastewater, an external source is necessary. Various carbon sources in different amounts could be used for denitrification processes, however, discrepancies have been reported regarding process efficiency.<sup>3</sup> Some authors claim that better efficiencies could be achieved using acetic acid as a carbon source when compared to glucose, methanol or ethanol.<sup>4</sup> Other have shown comparable results using acetic acid and methanol.<sup>5,6</sup> Another group of researchers gained the best results using ethanol and methanol.<sup>7,8</sup> Some authors have reported that ethanol has many advantages over methanol, acetic acid, and glucose.<sup>9</sup>

A strong salt (NaCl) solution is used to regenerate the resin resulting in the production of concentrated waste brine. Only a few species of bacteria are suitable for nitrate removal from a high salt brine solution. A haloalkaliphilic species, *Halomonas campisalis*,<sup>10–12</sup> or *H. denitrificans*<sup>13</sup> were isolated in order to perform denitrification using different sources of carbon, such as

glycerole, lactate, acetate, while metanol and ethanol were not utilised under denitrification conditions. This research indicated that a readily biodegradable substrate is an important factor effecting denitrification rate. In the first rapid denitrification phase, a readily biodegradable substrate such as reduced sugar would be used as a carbon source, and in the second slow denitrification phase, slowly biodegradable substrates such as unhydrolyzed polysaccharide.<sup>14</sup> Some authors claim that activated sludge from wastewater treatment plants could be used for denitification of regenerated brine with a high salt content.<sup>15</sup>

Research based on utilizing molasses as a carbon source during treating waste brine has not been described in literature so far. However, its application and effectiveness have been examined in few studies relating to the denitrification of wastewater.<sup>13,16</sup> Molasses is potentially a good carbon source because it contains invert sugars (saccharose, glucose and fructose), raffinose, chetose, starch, dextrane (total sugar content 47–48 %), proteins and other substances (30 %), growth stimulants, and inhibitors, vitamins, and water (26 %). It contains cations, such as K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and anions such as Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>. Several metals have been determined in molasses, such as Fe, Zn, Mn, Cu, Pb, Se, Al, and others.<sup>17</sup> Accordingly, it contains all those nutrients necessary for the development of microorganisms, without requiring external sources and consequently, the denitrification becomes

\* Author to whom correspondence should be addressed. (E-mail: marjana.simonic@um.si)

**Table 1.** The composition of the regenerated brine and molasses

Parameter	Molasses	Regenerated brine
pH	5.66	9.08
$\gamma\text{NO}_3^-/\text{mg L}^{-1}$	69	1996
$\gamma\text{SO}_4^{2-}/\text{mg L}^{-1}$	26	1220
$\gamma\text{Cl}^-/\text{mg L}^{-1}$	17	16800
$\gamma\text{TOC}/\text{mg L}^{-1}$	3007	460
$\gamma\text{C}/\text{mg L}^{-1}$	13	610
$\gamma\text{TN}/\text{mg L}^{-1}$	131	425

more cost effective. The optimal pH reported for denitrification was within the range from 7 to 9, whilst the pH-value of the regenerated brine was normally alkaline, because the hydrogencarbonate concentration increased during the denitrification.<sup>18</sup> Lower or higher pH values resulted in the accumulation of  $\text{NO}_2$ ,  $\text{NO}_2^-$  and  $\text{N}_2\text{O}$ .<sup>19</sup> Most investigators agree that denitrification of nitrate to nitrite follows zero order kinetics.<sup>20</sup> The varieties of carbon substrate as well as TOC/ $\text{NO}_3$  ratio affect the denitrification rate.<sup>21</sup>

The goal of this study was to assess the stability of the denitrification process when using simultaneous heterotrophic denitrification of waste brine, and the determination of kinetic denitrification parameters. Molasses which until now has not been applied during brine denitrification, was used as the carbon source. The anoxic process was applied using acclimated municipal wastewater treatment activated sludge. The nitrogen removal was calculated using a nitrogen mass balance methodology.

## EXPERIMENTAL

### Materials

Lewatit mono plus M 600 nitrate selective resin is a strongly alkaline, gelular anion-exchange beads of styrene-divinylbenzene copolymer. It contains a crosslinked polystyrene matrix and quaternary amine functional group. A breakthrough point was determined at the effluent concentration of nitrate of  $50 \text{ mg L}^{-1}$ . The regeneration was done using NaCl. Molasses was obtained as a by-product from sugar beet processing.

Molasses was dark brown colored with density of  $1.4 \text{ g cm}^{-3}$ . The chemical composition of molasses was: dry matter 78–82 %, sugar content 45–48 %, proteins 7–9 % and small quantities of Ca, Mg, K, P and Cu. The composition of the regenerated brine and molasses ( $\gamma = 10 \text{ g L}^{-1}$ ) is shown in Table 1.

Activated sludge was taken from a municipal wastewater treatment plant. It was wintertime, and salt was needed to melt the snow on the roads, resulting in increased inflow of salted water to the municipal wastewater treatment plant. Therefore, it was assumed that microorganisms from the sludge would have to be adapted for denitrification at higher salt concentrations.

### Batch Process

12 batch experiments were carried out under anoxic conditions, whereby dark reactors were used in order to determine the suitability of molasses application during heterotrophic denitrification. The feed data for the first 8 reactors are presented in Table 2. In each reactor 0.1 L of activated sludge, 0.1 L of regenerated brine, and nitrate solutions in different appropriate volumes were added (Table 2). All the reactors were filled with Millipore water to the mark of 1 L. They were kept at a constant temperature of  $20 \pm 1 \text{ }^\circ\text{C}$  and the solutions stirred using magnetic stirrers.  $\zeta(\text{TOC}/\text{NO}_3)$  ratios were set at 5.1, 4.8, 4.4, 4.7 in reactors 1, 2, 3 and 4, respectively. 20 g of molasses was added into each reactor except for reactors 3 and 4. In reactor 3 only 15.0 g of glucose was added, and in reactor 4 both molasses and glucose were added in concentrations of 10 g and 7.5 g, respectively. The influence of  $\zeta(\text{TOC}/\text{NO}_3)$  on the nitrate and nitrite removal was studied. In reactors 5, 6, 7 and 8, the  $\zeta(\text{TOC}/\text{NO}_3)$  were set at 3.1, 2.2, 1.6, and 1.3 respectively, for this purpose. The initial TOCs were set in all the reactors at approximately  $6300 \text{ mg L}^{-1}$ , the mass concentrations of nitrate in reactors 5 to 8 were  $2064 \text{ mg L}^{-1}$ ,  $2911 \text{ mg L}^{-1}$ ,  $3928 \text{ mg L}^{-1}$ , and  $4909 \text{ mg L}^{-1}$ , respectively. Additionally, 20 g of molasses was added into reactors 6, 7 and 8 after 5 days. The pH value was adjusted to 7.5 in reactors 6 and 7, and to 8.5 in reactor 8, respectively, after one week, using  $2 \text{ mol L}^{-1}$  NaOH.

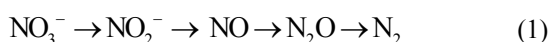
Last four experiments were performed in order to study the influence of NaCl on nitrogen removal. NaCl in concentrations 5, 10, 15, and  $20 \text{ g L}^{-1}$ , respectively,

**Table 2.** The reactors feed data for batch experiments

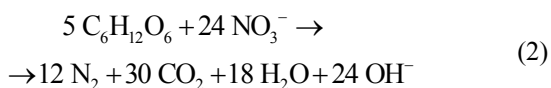
Reactor No.	1	2	3	4	5	6	7	8
$m_{\text{molasses}}/\text{g L}^{-1}$	20	20	–	10	20	20	20	20
$m_{\text{glucose}}/\text{g L}^{-1}$	–	–	15	7.5	–	–	–	–
$\gamma\text{NO}_3/\text{mg L}^{-1}$	1200	1284	1260	1150	2064	2911	3928	4909
$\zeta(\text{TOC}/\text{NO}_3)$	5.1	4.8	4.4	4.7	3.1	2.2	1.6	1.3

were added into reactors from 9 to 12. Other reactors feed date were the same as in reactor 2 (Table 2). Samples were taken daily. Analyses of the contents of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{SO}_4^{2-}$  ions, total organic carbon (TOC), total nitrogen (TN), the concentration of suspended solids (SS), and pH were performed according to ISO standards. The samples for analyses of TOC and TN were not taken from reactors from 9 to 12 due to higher salt concentrations, which could affect the determinations of these two parameters.

The nitrate reduction reactions involve the following reduction steps from nitrate to nitrogen gas (Equation 1):<sup>21</sup>



In biological nitrogen removal processes nitrate is used as electron acceptor and external source such as glucose or molasses served as electron donor. If glucose is available, following chemical reaction occur:<sup>22</sup>



The denitrification rate can be expressed by Equation 3:<sup>21</sup>

$$\frac{d\gamma_{\text{N}}}{dt} = q_{\text{m}}\gamma_{\text{SS}} \quad (3)$$

where  $d\gamma_{\text{N}}/dt$  is the denitrification rate ( $\text{mg L}^{-1} \text{h}^{-1}$ ) with regard to the sum of nitrate and nitrite ions,  $q_{\text{m}}$  the specific denitrification rate ( $\text{mg g}^{-1} \text{h}^{-1}$ ) and  $\gamma_{\text{SS}}$  the suspended solid's concentration ( $\text{g L}^{-1} \text{h}^{-1}$ ). The denitrification rate is independent of nitrate ion concentration while it is a function of the suspended solids.

## RESULTS AND DISCUSSION

### Effect of Carbon Source on Denitrification

To test the efficiency of nitrate removal with molasses and glucose, chosen physico-chemical parameters were monitored in reactors from 1 to 4. The results are presented in Table 3. When molasses was used as a carbon source the total nitrate and nitrite removals were achieved within 48 hours as seen from Table 3 in reactors 1 and 2. If only glucose was used as a carbon source (reactor 3), the nitrate ions were removed within 3 days and the nitrite within 5 days which meant the slowest denitrification rate compared with other reactors. In experiment where molasses and glucose together were used as a carbon source (in the ratio of 1:1), the nitrate ions were removed within 48 hours, and

the nitrite ions within 4 days, respectively. This was attributed to the fact that molasses is easily biodegradable and readily usable for denitrification. These results were in agreement with another study where applied hydrolyzed molasses was found to be more effective and economical than methanol.<sup>14</sup> Ge *et al.*<sup>19</sup> also reported that better denitrification was achieved by a combination of glucose and molasses compared with that using glucose alone. Decreasing trends of total organic carbon (TOC) and total nitrogen (TN) concentrations were observed throughout the experiments. The share of carbon uptakes during denitification were 14 % and 15.8 % in reactors 1 and 2, respectively. In reactor 4 it was 7 % , which is half of the shares in reactor 1. This fact was in agreement with the mass of molasses in reactor 4 which was half of that in reactor 1. It seemed that molasses was more freely available than glucose. If molasses was used as a carbon source beside total nitrate removal, TOC decreased, therefore, again the molasses seemed to be more readily biodegradable substrate than glucose. The highest amount of nitrite formed was observed in reactor with glucose. The nitrite concentration had not decreased even after 7 days. Similarly, it was reported that the maximum nitrite accumulation was obtained if glucose was used as an external carbon source.<sup>19</sup> It could be explained by the differences in the metabolism and electron-flow velocities amongst the different carbon sources. Different types of carbon sources could show various accumulation characteristics. The nitrite accumulation during denitrification with glucose could have been caused by *Alcaligenes faecalis* and it was suspected that the biomass was probably not fully acclimated to the glucose.<sup>19</sup> Molasses proved to be the more effective than glucose, because the denitrification process was completed in less than 2 days and nitrite formation was not observed.

The initial concentrations of suspended solids (SS) in the reactors varied from  $2.7 \text{ g L}^{-1}$  at the beginning of the process upwards up to  $4 \text{ g L}^{-1}$  during the 3<sup>rd</sup> day. The suspended solids' concentrations oscillated in all four reactors. Only slight variations in sulphate ion concentrations were observed. Both parameters did not show any effects on denitrification efficiency.

### The Effect of $\zeta(\text{TOC}/\text{NO}_3)$ on Denitrification

Figure 1a shows nitrate consumption at different  $\zeta(\text{TOC}/\text{NO}_3)$  for reactors 2, 5, 6, 7 and 8. It can be seen that at a  $\zeta(\text{TOC}/\text{NO}_3)$  of 4.8, denitrification was completed within 24 h, and at a lower ratio of 3.1 this time increased to within 48 h. The majority of nitrate ions were removed within 72 h at a  $\zeta(\text{TOC}/\text{NO}_3)$  of 2.2, but concentration of  $\text{NO}_3$  still remained at  $137 \text{ mg L}^{-1}$ . The nitrate ions were not removed totally at lower ratios of 1.6 and 1.3. The concentration significantly decreased

**Table 3.** Results obtained in first four batch reactors

Reactor No.	1			2			3					4		
<i>t</i> /d	0	1	2	0	1	2	0	1	2	3	7	0	1	2
$\gamma\text{NO}_3/\text{mg L}^{-1}$	1199	1.3	0	1284	0.8	0	1259	674	9.7	0	0	1152	0.8	0
$\gamma\text{NO}_2/\text{mg L}^{-1}$	63	285	0	40	147	0	21	198	358	154	33	77	215	0
$\gamma\text{SO}_4/\text{mg L}^{-1}$	235	222	229	223	233	196	180	141	120	102	51	193	174	189
$\gamma\text{TOC}/\text{mg L}^{-1}$	6101	5127	5244	6180	5450	5206	5482	5195	5134	4937	4654	5465	5064	4986
$\gamma\text{TN}/\text{mg L}^{-1}$	540	342	294	533	320	288	233	171	104	56	17	347	178	139
$\gamma\text{SS}/\text{g L}^{-1}$	3.0	3.6	3.3	2.8	3.1	3.3	2.7	3	2.6	/	/	2.8	4.3	3.2
$\zeta(\text{TOC}/\text{NO}_3)$	5.1	/	/	4.8	/	/	4.4	/	/	/	/	4.7	/	/

over the 72 h but over the next 48 h it remained unchanged. The reason could have been an insufficient carbon source, therefore, 20 g of molasses was added into reactors 6, 7 and 8. This is denoted as a vertical line at day 5 in Figure 1a. The pH values for all reactors slowly decreased. Denitrification could be incomplete at effluent pHs below 6, therefore on day 5 the pH values were adjusted to 7.5 in reactors 6 and 7, and to 8.5 in reactor 8 (denoted as a vertical line at day 7), respectively. However, improved conditions by adding molasses and the adjusting of pH values did not enhance the denitrification process, although higher efficiencies have been reported for halophilic communities at higher pH values.<sup>10</sup>

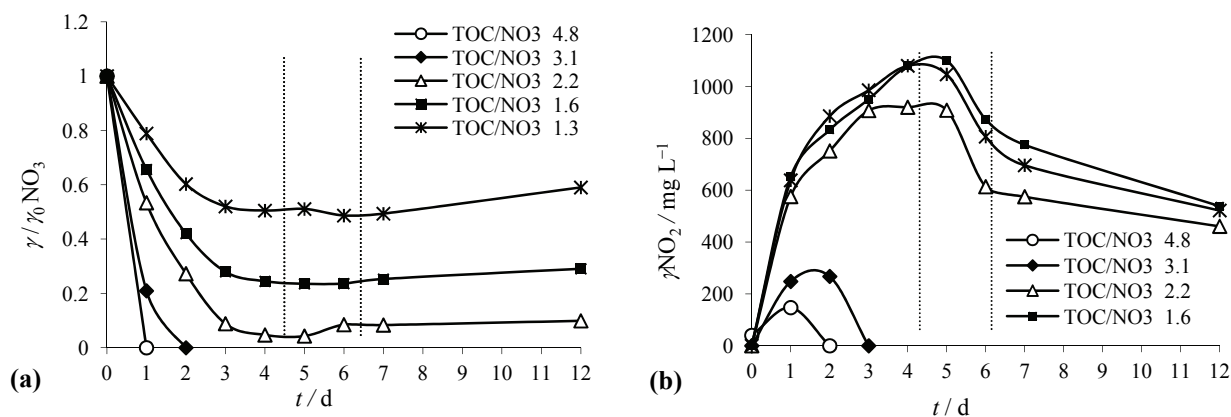
Figure 1b shows the nitrite formations and consumptions for reactors 2, and from 5 to 8. Initially the concentrations of nitrite ions increased at all  $\zeta(\text{TOC} / \text{NO}_3)$ , whilst the pH values decreased. The nitrites were completely removed only in reactors 2 and 5. The highest nitrite concentrations at approximately 1000 mg L<sup>-1</sup> were formed in reactors 6, 7 and 8 with low  $\zeta(\text{TOC}/\text{NO}_3)$ . In these reactors molasses was added to the solutions on the 5th day, and the pHs were adjusted on the 7th day. However, the nitrite and nitrate consumptions could not be enhanced which could be explained by low  $\zeta(\text{TOC} / \text{NO}_3)$ . Due to insufficient

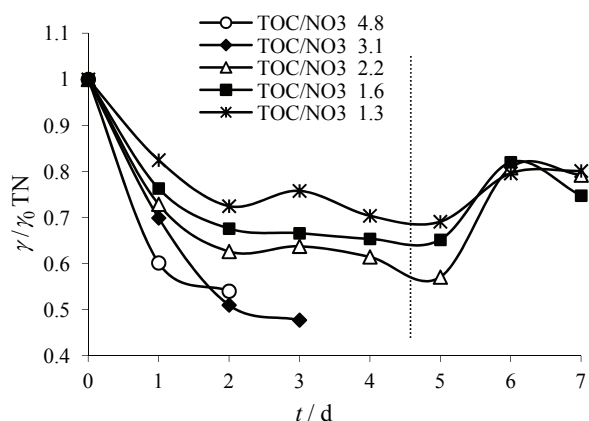
carbon supply the nitrite accumulated. The results showed that the  $\zeta(\text{TOC} / \text{NO}_3)$  should be around 3.1 which is in accordance with the reported<sup>1</sup>  $\zeta(\text{TOC}/\text{NO}_3)$  value equal to 2.7. Lower  $\zeta(\text{TOC} / \text{NO}_3)$  had negative impact on denitrification and nitrate could not be removed completely.

The initial TOC concentration (total, not filtered) was approximately 6300 mg L<sup>-1</sup> in all the reactors and it slowly decreased throughout the experiment. The consumption of TOC was lower at lower  $\zeta(\text{TOC} / \text{NO}_3)$ . Figure 2 presents the decrease in total nitrogen. The concentration of total nitrogen decreased in accordance with decreased nitrate concentration, whilst maximum reduction was observed in those reactors with higher  $\zeta(\text{TOC}/\text{NO}_3)$ .

### The Effect of Salt on Denitrification

Tables 4 and 5 shows the NaCl effect on denitrification process. No inhibitory influence of NaCl was observed at 5 g L<sup>-1</sup>. Nitrates and nitrites were found in the reactors, even after 7 days if the concentration of NaCl was above 10 g L<sup>-1</sup>. The concentration of nitrate at the end of experiment increased due to salt inhibition and partly due to biomass starvation. The experiments also showed the inhibitive effects of NaCl on sulphate consumption.

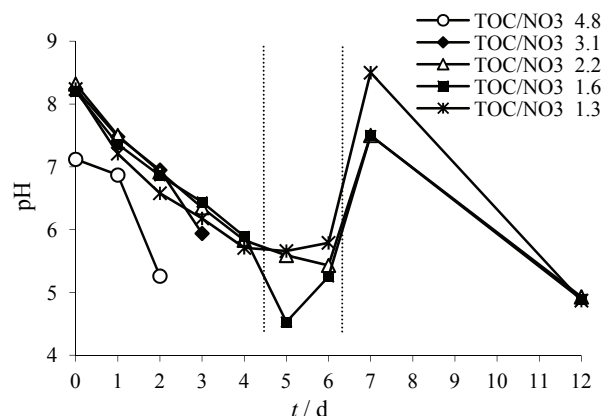
**Figure 1.** (a) Nitrate and (b) nitrite removal in batch experiments.



**Figure 2.** The content of total nitrogen in batch experiments at different TOC/NO<sub>3</sub> ratios.

The values of pH in all the reactors slowly decreased during the experiment, similar as in reactors from 5 to 8 (Figure 3). The results showed that denitrification in the batch experiments was successful at initial nitrate concentrations below 2000 mg L<sup>-1</sup>, and at the concentrations of NaCl up to 5 g L<sup>-1</sup>. At 10 g L<sup>-1</sup> of NaCl significant amounts of nitrite were observed in the solutions and at the end of experiments some of nitrite still remained (52 mg L<sup>-1</sup>).

The results also showed that NaCl inhibits both denitrification steps, the reduction of nitrate to nitrite, and to a greater extent nitrite to nitrogen. The reason could be high osmotic pressure of the brines that could lead to cell death as a consequence of dehydration.<sup>13</sup>



**Figure 3.** pH values in batch experiments at different TOC/NO<sub>3</sub> ratios.

### The Specific Denitrification Rate Calculation

The specific denitrification rate  $q_m$  (denitrification constant) was calculated according to Equation (3). The calculations for batch experiments are collected in Table 6. The denitrification rates varied between 0.16 and  $25.4 \times 10^{-2}$  (mg g<sup>-1</sup> h<sup>-1</sup>). The highest denitrification rate (0.254 mg g<sup>-1</sup> h<sup>-1</sup>) was achieved during batch trials with molasses used as a carbon source at  $\zeta(\text{TOC}/\text{NO}_3)$  of 3.1 in reactor 5. Glucose, in comparison with the molasses, provided lower specific denitrification rates (reactors 2 and 3). In general, with any decrease in  $\zeta(\text{TOC}/\text{NO}_3)$  the concentration of nitrate ions increased, and therefore the specific denitrification rate subsequently decreased. The specific denitrification rates for reactors 9 to 12

**Table 4.** Nitrate consumption in reactors 9 and 10

Reactor No.	9 ( $\gamma(\text{NaCl}) = 5 \text{ g L}^{-1}$ )			10 ( $\gamma(\text{NaCl}) = 10 \text{ g L}^{-1}$ )					
	t/d	0	1	2	0	1	2	3	7
pH		7.12	7.07	5.77	7.12	6.78	5.17	4.9	4.87
$\gamma\text{NO}_3/\text{mg L}^{-1}$		1000	323	0	1000	430	4.3	7.6	48
$\gamma\text{NO}_2/\text{mg L}^{-1}$		0	249	0	0	225	150	121	52
$\gamma\text{SO}_4/\text{mg L}^{-1}$		94	92	/	94	95	85	84	94
$\gamma\text{SS}/\text{g L}^{-1}$		2.8	2.9	5.6	2.8	3.8	5.7	9.7	/

**Table 5.** Nitrate consumption in reactors 11 and 12

Reactor No.	11 ( $\gamma(\text{NaCl}) = 15 \text{ g L}^{-1}$ )					12 ( $\gamma(\text{NaCl}) = 20 \text{ g L}^{-1}$ )					
	t/d	0	1	2	3	7	0	1	2	3	7
pH		7.12	6.88	5.61	5.04	4.79	7.12	6.71	5.99	5.36	4.79
$\gamma\text{NO}_3/\text{mg L}^{-1}$		1000	517	6.1	5.6	57	1000	641	116	67	113
$\gamma\text{NO}_2/\text{mg L}^{-1}$		0	211	309	267	199	0	158	386	436	374
$\gamma\text{SO}_4/\text{mg L}^{-1}$		94	94	86	83	99	94	96	100	91	102
$\gamma\text{SS}/\text{g L}^{-1}$		2.8	4.6	8.6	6.8	/	2.8	4.3	8.0	7.5	/

**Table 6.** The denitrification constants ( $q_m$ ) at batch trials

$t/d$	1	2	3
$\zeta(\text{TOC}/\text{NO}_3)$	$q_m/10^{-2}(\text{mg g}^{-1} \text{h}^{-1})$		
5.1	16.7	/	/
4.8	24.7	/	/
4.4	7.8	11.6	6.2
4.7	15.0	/	/
3.1	25.4	5.1	3.5
2.2	11.7	6.4	2.8
1.6	7.8	9.2	3.4
1.3	3.5	6.1	2.5
4.8	8.0	7.9	/
4.8	4.7	6.2	0.25
4.8	2.9	3.0	0.56
4.8	2.3	1.8	0.16

decreased if the concentration of NaCl increased. Even an amount of  $5 \text{ g L}^{-1}$  NaCl could significantly inhibit the process of the denitrification and thus the specific denitrification rate decreased. The denitrification rate of approximately  $0.87 \text{ mg g}^{-1} \text{ h}^{-1}$  was reported in sequential batch reactor treating sewage using molasses as the carbon source.<sup>16</sup> Stated value of denitrification rate is fairly comparable with the results from present research (Table 6).

## CONCLUSION

The results achieved demonstrated that the denitrification process using molasses as carbon source was efficient. The kinetic parameters data obtained can be considered as a useful tool for process design, and the operation of regeneration brine treatment. The  $\text{TOC}/\text{NO}_3$  ratio must be around 3.1 to allow heterotrophic denitrifiers to perform denitrification of regeneration brine. The sulphate and suspended solids' concentrations did not influence the denitrification rate. Total nitrate removal was achieved in 24 hours at initial concentrations of  $\text{NO}_3$  and NaCl up to  $1 \text{ g L}^{-1}$  and  $5 \text{ g L}^{-1}$ , respectively. At  $10 \text{ g L}^{-1}$  NaCl only partial

denitrification accomplished in seven days is feasible, whilst the concentration of nitrite accumulates.

## REFERENCES

1. D. A. Clifford and X. Liu, *Water Res.* **27** (9) (1993) 1477–1484.
2. E. J. McAdam and S. J. Judd, *Sep. Purif. Technol.* **62** (2008) 264–272.
3. H. Chonstatin and M. Fick, *Water Res.* **31** (1997) 583–590.
4. M. Henze, P. Harremoës, J. C. Jansen, and E. Arvin, *Wastewater treatment: biological and chemical processes*, 3<sup>rd</sup> ed., Springer, Berlin, Germany, 2002.
5. U. Nyger, H. Aspergren, B. Andersson, J. Jansen, and I. S. Villadsen, *Water Sci. Technol.* **26** (1992) 1077–1086.
6. S. Lee, B. Koopman, S. Park, and K. Cadee, *Water Environ. Res.* **67** (1995) 1119–1122.
7. M. Christensson, E. Lie, and T. Welander, *Water Sci. Technol.* **30** (1994) 83–90.
8. B. Andersson, H. Aspergren, U. Nyberg, and H. Odegaard, *Water Sci. Technol.* **37** (1998) 175–183.
9. G. Cao, O. Zhao, X. Sun, and T. Zhang, *Enzyme Microb. Tech.* **30** (2002) 49–55.
10. B. M. Peyton, M. R. Mormile, and J. N. Petersen, *Water Res.* **35** (17) (2001) 4237–4242.
11. A. A. Shapovalova, T. V. Khijniak, T. P. Tourova, G. Muyzer, and D. Y. Sorokin, *Extremophiles* **12**(5) (2008) 619–625.
12. E. J. McAdam, M. Pawlett, and S. J. Judd, *Water Res.* **44** (2010) 69–76.
13. P. Cyplik, W. Grajek, R. Marecik, P. Króliczak, and R. Dembczyński, *Desalination*. **207** (2007) 134–143.
14. Z. X. Quan, Y. S. Jin, C. R. Yin, J. J. Lee, and S. T. Lee, *Bioresour. Technol.* **96** (2005) 1690–1695.
15. B. U. Bae, C. H. Kim, and Y. I. Kim, *Water Sci. Technol.* **49** (2004) 413–419.
16. M. F. Rodriguez, F.G. de Giner, A. A. Rodriguez, and E. J. Lomas, *Bioresour. Technol.* **88** (2003) 215–219.
17. P. W. Van der Poel, H. Schiweck, and T. Schwartz, *Sugar Technology, Beet and cane sugar manufacture*, Verlag Dr. Albert Bartens KG, Berlin, Germany, 1998.
18. Y. Tang, C. Zhou, E. M. Ziv, and B. E. Rittmann, *Water Res.* **45** (2011) 232–240.
19. S. Ge, Y. Peng, S. Wang, C. Lu, X. Cao, and Y. Zhu, *Bioresour. Technol.* **114** (2012) 137–143.
20. A. Carruci, R. Ramadori, S. Rossetti, and M. C. Tomei, *Water Res.* **30** (1996) 51–56.
21. G. Tchobanoglous, L. B. Franklin, and H. D. Stensel, *Wastewater engineering treatment and reuse*, Metcalf & Eddy, 4<sup>th</sup> Ed., McGraw-Hill Higher education, 2003, Chapter 7 and 11.
22. S. Saggarr, N. Jha, J. Deslippe, N. S. Bolan, J. Luo, D. L. Giltrap, D. - G. Kim, M. Zaman, and R. W. Tillman, *Sci. Total Environ.* **465** (2013) 173–195.