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ORIGINAL PAPER

Removal of nitrates from high-salinity wastewaters from desulphurization process with denitrifying bacteria encapsulated in Lentikats Biocatalyst

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Abstract Successful elimination of high concentrations of $N–NO_x^-$ (up to 250 mg/L) from high salinity wastewaters (up to 35 g/L Cl^- + 17 g/L SO_4^{2-}) originating from desulphurization process within coal power stations was achieved using pure cultures of denitrifying bacteria encapsulated in porous polyvinyl alcohol lenses (so called Lentikats Biocatalyst, LB). Laboratory batch tests revealed inhibitory influence of the raw wastewater on the denitrification activity, which was partially mitigated by the addition of $P–PO_4^{3-}$. In following continuous tests, the denitrification activities reached the range 150–450 mg N/h/kg LB, i.e., values suitable for industrial scale applications. The higher activities were achieved under a lower salinity, higher $N–NO_x^-$ influent concentrations and a prolonged retention time. The effluent $N–NO_x^-$ concentrations were below the determination limit of 5 mg/L. After a period of 3 months, a significant decrease of denitrification activity of Lentikats Biocatalyst was observed. Addition of nutrients into the wastewater enabled fast regeneration of the initial activity. The overall results proved the applicability of Lentikats Biocatalysts for the removal of nitrates from high-salinity desulphurization water and other industrial wastewaters of similar character.

Keywords Brines · Denitrification · Immobilization · Industrial wastewaters · *Paracoccus pantotrophus* · Polyvinyl alcohol · *Pseudomonas fluorescens*

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Introduction

Biological treatment of industrial wastewaters is often a complicated task due to their unfavorable conditions for common treatment methods, such as high toxicity, high salinity, specific pollution or insufficient nutrient content. Common low-cost biological methods utilizing activated sludge often fail when applied to industrial wastewaters and special pretreatment is often necessary to remove biological inhibitors (Beliavski et al. 2010; Cyplik et al. 2007; Ersever et al. 2007; Lefebvre and Moletta 2006; McAdam and Judd 2008; McAdam et al. 2010; Wisniewski et al. 2002). Salinities higher than ~1 % cause inhibition of nitrification and denitrification of the activated sludge (Dincer and Kargi 1999; Lefebvre and Moletta 2006; Mariangel et al. 2008). The use of immobilized microorganisms presents a possible alternative for biological treatment and potential reuse of industrial brines. The immobilization enables intensification of the treatment process due to high biomass retention within the system, low biomass washout and elimination of undesired microbial activities by use of pure cultures (Sievers et al. 2002). It also provides protection for the immobilized microorganisms (Hanaki et al. 1994; Bouskova et al. 2011). Several works report application of immobilized microorganisms as a possible solution for overcoming of loss of

biological activity when treating of high-salinity wastewaters (Foglar et al. 2007; Park et al. 2001; Trogl et al. 2011a; Yang et al. 1995). Among the most beneficial immobilization methods currently available is the so called Lentikats Biotechnology according to a patented method of Vorlop and Jekel (Jekel et al. 1998a, b; Vorlop and Jekel 1999) and its product—Lentikats Biocatalyst (further LB), i.e., biological material encapsulated in polyvinyl alcohol (PVA) gel.

The resulting PVA lenses are durable, non-toxic, non-biodegradable and of adequate elasticity and density for biotechnological applications. Unlike other encapsulation methods utilizing PVA gel (using boric acid, freezing etc.), the encapsulation into Lentikats Biocatalysts is highly biocompatible and the loss of activity during the encapsulation process is negligible. Bacteria, yeast and enzymes encapsulated in Lentikats Biocatalyst to date have found applications in food industry (Grosova et al. 2008, 2009; Hronska et al. 2009), biotransformations (Bruss et al. 2002; Kubac et al. 2006; Vejvoda et al. 2006), bioethanol production (Rebros et al. 2005a, b, 2009) and wastewater treatment, including saline waters (Bouskova et al. 2011; Trogl et al. 2011a, b). For the removal of inorganic forms of nitrogen from wastewaters, Lentikat's Inc. has developed two different Lentikats Biocatalysts, nitrification LB and denitrification LB, containing high concentrations of pure cultures of nitrification and denitrification bacteria, respectively. The technology has got a proven lab-scale track record as well as industrial scale applications.

Increased robustness of the biomass immobilized in Lentikats Biocatalysts observed in previous works (Bouskova et al. 2011) was recently (May–October 2010) challenged when applied for the elimination of high nitrate concentrations (up to 250 mg/L N–NO_x^-) from desulphurization wastewaters characterized by a high salinity (up to 35 g/L Cl^-, 17 g/L SO_4^{2-}, up to 2 g/L Ca^{2+} and 3 g/L Mg^{2+}), low phosphorus (<0.075 mg/L), high COD (up to 1,400 mg/L) and low BOD_5 concentrations (up to 260 mg/L). The aim of this work was to verify the efficiency and stability of denitrifiers encapsulated in LB under these extreme conditions.

Materials and methods

Wastewaters

Real wastewater samples from running coal power stations in Poland were used in the tests (see Table 1 for detail composition). Due to the variability in composition, the wastewaters were partly standardized by the addition of NaCl and $CaCl_2$ to achieve a final chloride concentration of 20 g/L or 35 g/L. The nitrate concentration was adjusted by the addition of KNO_3 to a standard level of 200 mg/L N–NO_3^-.

Table 1 Composition of the real wastewaters (assembly from three consequent samples)

Parameter (Unit)	Value
N-NO_x^- (mg/L)	Up to 250
Cl^- (mg/L)	4,000–35,000
SO_4^{2-} (mg/L)	4,000–17,600
P-PO_4^{3-} (mg/L)	<0.075
COD (mg/L)	400–1,400
BOD_5 (mg/L)	40–260
TSS (mg/L)	5–24
TDS (mg/L)	16,000–80,000
N-NH_4^+ (mg/L)	<2.5
Ca^{2+} (mg/L)	750–2,000
Mg^{2+} (mg/L)	440–3,140
pH	7.0–7.5
T (°C)	<35

Immobilization of microorganisms into Lentikats Biocatalyst and their cultivation

The encapsulation of denitrification bacteria (*Pseudomonas fluorescens* or *Paracoccus pantotrophus*) into Lentikats Biocatalysts was carried out by LentiKat's Inc. using an industrial automatic manufacturing line according to the method of Vorlop and Jekel (1998, 1999). After the encapsulation, LB were cultivated in the production plant using a mineral denitrification medium (MDM, Trogl et al. 2011a) to a final denitrification activity of ~500 mg N–NO_x^-/h/kg LB.

Experimental setup

The tests were carried out in 5-L glass reactors (working volume 3L) equipped with a slow-rotating magnetic stirrer (Heidolph). Continuous set-up was achieved by a set of peristaltic pumps (Watson-Marlow) and a sieve separator withholding the LB inside the reactor. The pH, concentration of dissolved oxygen and temperature values were monitored by on-line probes (Gryf) placed inside the reactor. The temperature was maintained at 30 °C. Quantity of 209 or 300 g (wet weight) of LB with encapsulated *P. pantotrophus* and *P. fluorescens*, respectively, was used. A commercial mixture of predominantly biodegradable hydrocarbons Brenntaplus VP1 (BRENNTAG CEE Group, http://www.brenntag-cee.com) was dosed as necessary carbon source for denitrification (5:1 COD:N).

Batch tests description

Initial batch tests were focused on the inhibition of denitrification activities in a set of six consecutive batch tests to compare two encapsulated microorganisms—*P. fluorescens* and *P. pantotrophus*. Two tests were first carried out with MDM serving as non-inhibiting starting reference environment, followed by two tests with the real wastewater (adjusted to 20,000 mg/L chlorides) and concluded by two more control tests with MDM. The same sequence was applied to determine the effect of phosphorus addition (KH_2PO_4, P:N 0.07:1) on the denitrification activity of *P. fluorescens*. Initial $N–NO_x^-$ concentration of all batch tests was 200 mg/L. The initial pH 7.0 was not further adjusted during the course of the batch experiments.

Continuous test description

A long-term denitrification continuous test was run for the period of 102 days. A single portion of 209 g of LB with *P. pantotrophus* was used during the whole period. The influent flow rate of real wastewater supplemented with NaCl (to maintain unified Cl^- concentrations in wastewater) was set to 500 mL h^{-1} (±5 %) giving the retention time of ~6 h. A solution of organic substrate (COD:N 5:1) amended with phosphorus (H_3PO_4 P:N 0.07:1) was dosed in parallel. The pH values between 7.4 and 7.8 were maintained automatically by the addition of 2 M NaOH. The influent salinity and concentration of $N–NO_x^-$ were varied throughout the test to determine the denitrification activities under different conditions (Table 3).

Analytical methods and calculations

Sampling was performed at appropriate intervals. The nitrate concentration was determined by ion chromatography (DIONEX ICS 1000, IonPac® column AS22 4 × 250 mm, guard column AG22 4 × 50 mm, mobile phase 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$, flow-rate 1.2 mL/min). Merck kits 1.14776, 1.14752, 1.14848, and 1.14560 were used for the determination of $N–NO_2^-$, $N–NH_4^+$, $P–PO_4^{3-}$ and COD, respectively. These determinations were tested for interferences with high salinity, high nitrate and nitrites concentrations to ensure correct results. These interferences resulted in the need of sample dilutions and increased determination limits (in mg/L) 5, 0.5, 1, 250, and 1 for $N–NO_3^-$, $N–NO_2^-$, $N–NH_4^+$, COD, and $P–PO_4^{3-}$, respectively (Pilarova et al. 2011; Trogl et al. 2011a).

System efficiency determination

The systems based on Lentikats Biotechnology are assessed using the efficiency of nitrogen elimination (i.e., percentage of removed input nitrogen) and the activity of LB expressed as the amount of $N–NO_x^-$ or $N–NO_3^-$ removed per 1 h by 1 kg of wet LB. In the batch experiments it was calculated from the time needed to reduce the initial $N–NO_x^-$ or $N–NO_3^-$ concentration below the determination limits. In the continuous experiments it was calculated from the difference between the $N–NO_x^-$ influent and $N–NO_x^-$ effluent concentrations in steady state. The activity reflects the mass of bacteria inside the carrier and their vitality. This correlation can be demonstrated on the batch cultivation of freshly produced LB, when the activity exponentially increases with the number of repeated batches (Rebros et al. 2005b).

Microscopy

Microscopy analysis was performed on a sample of LB withdrawn from the reactor. The comparative analysis was carried out using optical microscope Olympus BX51. QuickPHOTO MICRO 2.3 software fitted with Deep Focus 3.1 module was used for photos processing. Each deeply-focused final photo was software-assembled from a series of shots with successive focus (Kriklavova and Lederer 2010).

Results and discussion

Batch tests: inhibition of denitrification

The kinetics of the batch denitrification tests (Fig. 1) followed zero-order kinetics with obvious nitrite peaks as described previously (Glass and Silverstein 1998, 1999; Trogl et al. 2011a; Vackova et al. 2011). The Biocatalyst's denitrification activities obtained in the preliminary batch tests (Table 2, lines 1 and 2) revealed obvious inhibitory effect of the real wastewater. Achieved denitrification activities in the second control tests with MDM were slightly lower than the initial ones. This suggests that obtained reduction of the denitrification activity of LB in the real wastewater was caused both by inhibition of denitrification pathways and by partial depletion of the encapsulated denitrifies. The range of denitrification inhibition was comparable for both tested microorganisms *P. fluorescens* and *P. pantotrophus*.

Since the real wastewaters contained minimum (<0.075 mg/L) concentrations of phosphorus necessary for the growth and activity of denitrification bacteria (Mohseni-Bandpi and Elliott 1998), a hypothesis of a positive

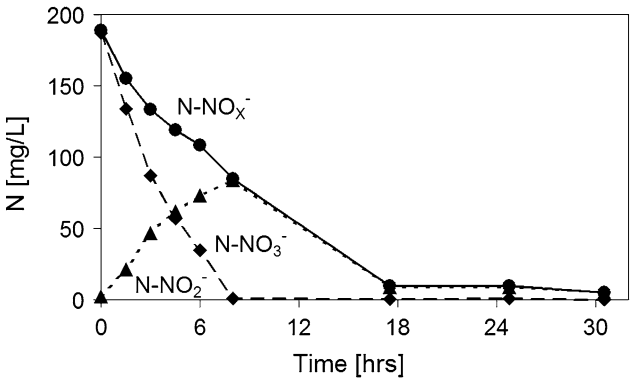

Fig. 1 Kinetics of batch denitrification (Lentikats Biocatalyst with encapsulated *Pseudomonas fluorescens* applied to real wastewater adjusted to 20 g/L Cl^- without phosphorus addition). Depicted are concentrations of nitrate nitrogen (*diamonds*), nitrite nitrogen (*triangles*), and their sum (*circles*)

effect of phosphorus addition was tested. Line 3 of Table 2 indeed confirms this hypothesis. The addition of phosphorus completely eliminated the inhibition of nitrate reduction and substantially suppressed the inhibition of nitrite reduction. The activities achieved in the second control tests were even higher than the initial values, which indicated a successful proliferation of the encapsulated bacteria.

Continuous test

The main results of the continuous test under various conditions are chronologically listed in Table 3. The main goal was to achieve effluent $N–NO_x^-$ concentrations bellow the determination limit (5 mg/L) together with maximal denitrification activities of LB.

Table 2 Denitrification activities measured in the batch tests

Line		Activity of LB in elimination of **$N–NO_x^-$** (in bold) and $N–NO_3^-$ [mg N/hr/kg LB)] (percentage of initial activity %)		
	Microorganism in LB	Initial activity in MDM	Activity in the wastewater	End activity in MDM
1	*P. pantotrophus*	**467 ± 16 (100 %)**	**79 ± 8 (17 %)**	**293 ± 8 (63 %)**
		805 ± 209 (100 %)	192 ± 15 (24 %)	363 ± 92 (45 %)
2	*P. fluorescens*	**322 ± 50 (100 %)**	**55 ± 6 (17 %)**	**236 ± 82 (73 %)**
		420 ± 149 (100 %)	202 ± 74 (48 %)	229 ± 63 (54 %)
3	*P. fluorescens*, addition of phosphorus	**310 ± 104 (100 %)**	**201 ± 62 (65 %)**	**386 ± 60 (125 %)**
		365 ± 28 (100 %)	384 ± 119 (105 %)	409 ± 60 (112 %)

Table 3 Achieved parameters during 102 days continuous experiment under various conditions (retention time in reactor 5.7–6.0 h, pH values 7.4–7.8)

Line	Time (days)	Set parameters			Achieved parameters		
		Cl^- (g/L)	SO_4^{2-} (g/L)	$N–NO_x^-$ Influent concentration (mg/L)	$N–NO_x^-$ Effluent concentration (mg/L)	Maximum denitrification activity (mg $N–NO_x^-$/hr/kg LB)	
1	16.83	20	10.5	200	<5	456	a,b
2	43.99	35	17	248	157	201	b
3	47.32	35	17	132	42	192	b
4	47.91	35	17	80	<5	167	b
5	51.80	35	17	114	21	212	b,c
6	76.12	20	3.5	203	23	407	b
7	88.08	20	3.5	97	19	187	b
8	90.06	5	3.5	76	5	171	b
9	98.82	5	3.5	157	<5	479	d
10	101.82	5	3.5	206	<5	477	b

[a] Without pH regulation (pH ~7)

[b] Without nutrients

[c] Increased working volume (4 L) and retention time (~8 h) due to a partly suspended effluent

[d] Added nutrients (MDM without $MgCl_2$ and $CaCl_2$, P:N 0.07)

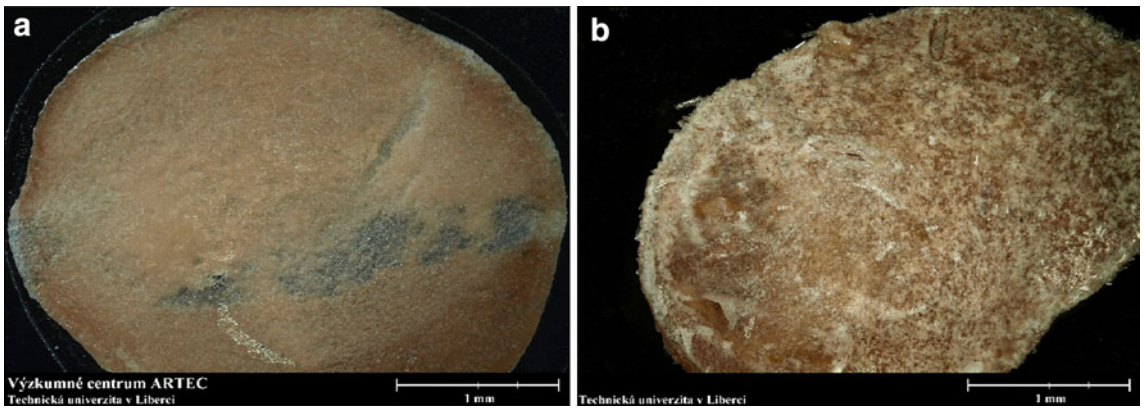

Fig. 2 An example of deeply-focused microscopy photo of Lentikats Biocatalyst in the continuous experiment: **a** before cultivation (90 days), **b** after cultivation (99 days). Increase of encapsulated bacteria is indicated by increase in brown pigmentation

At the beginning, the system was capable to eliminate all nitrogen to a level below the analytical determination limit and to achieve a high denitrification activity of 456 mg $N–NO_x^-$ h^{-1} kg^{-1} LB at the concentrations of 20 g/L Cl^- (Table 3, line 1). The following tests focused on the effect of maximum expected salinity (35 g/L Cl^- and 17 g/L SO_4^{2-}) on the denitrification activity. An increase in the salinity resulted in a decrease in the denitrification activity (Table 3, line 2) and a consequent high $N–NO_3^-$ concentration in the effluent (158 mg/L). To simultaneously achieve an effluent $N–NO_3^-$ concentration below the determination limit and a high denitrification activity, the influent $N–NO_3^-$ concentration was varied (lines 3 and 4). A comparison between the lines 2–4 suggests that these two demands are contradictory. Under the same conditions, higher $N–NO_x^-$ influent concentrations led to a higher denitrification activity. An increased LB denitrification activity (Table 3, line 5) was also achieved after an unintentional increase in the working volume and associated increase in the retention time from $\sim$6 to 8 h. These observations suggest that the capacity of LB is not fully used at low retention times and low influent $N–NO_x^-$ concentrations, likely due to the inhibition of denitrification caused by the high salinity.

Passing 71 days from the beginning, the initial test (line 1) was repeated (line 6). The LB activities achieved were lower and also the effluent $N–NO_x^-$ concentrations were higher. To achieve effluent $N–NO_x^-$ concentrations bellow the desired 5 mg/L, the influent $N–NO_3^-$ concentration was decreased (line 7). Nevertheless, the LB denitrification activity decreased predominantly as opposed to the expected decrease in the effluent concentration. Even a decrease of salinity did not improve the activity (line 8), which suggested a partial loss of viability of the encapsulated bacteria. Hence, the following tests were run with an addition of mineral nutrients and indeed resulted in a restoration of the original denitrification activity (lines 9 and 10) as well as low effluent $N–NO_x^-$ concentrations. Successful proliferation of encapsulated bacteria was indicated by increased COD:N consumption ratios (4.2 ± 2.7 during the cultivation opposed to 2.3 ± 1.3 before and after nutrients addition). The decrease and restoration of viability of the encapsulated bacteria was also confirmed by deep-focus-microscopy (Fig. 2). The bacteria are visible as brown areas; higher color intensity corresponds to thicker layers of bacteria (Kriklavova and Lederer 2010). Before the cultivation (Fig. 2a) the bacteria were spread, their quantity was low (indicated by light-brown pigmentation), and significant part of the LB lacked bacteria. After cultivation (Fig. 2b) the increase in bacterial population (indicated by darker brown pigmentation) is obvious.

LB can be applied for as long as 2 years without a need for re-cultivation or loss of activity in nutrient rich municipal wastewaters. The obtained $\sim$3-month period of high activity with a consequent deterioration in tested high-salinity wastewater is a significantly lower value and reflects the extreme conditions the Biocatalyst was exposed to. This comparison suggests that a lack of nutrients (including phosphorus) in the given wastewater was the rate-limiting step. However, a regular dosage of nutrients seems to facilitate a long-term high denitrification activity of Lentikats Biocatalyst.

In general, the continuous operation confirmed the previously observed inhibition of denitrification activity, especially the reduction of nitrites. At all tested levels of salinity, the effluent nitrites generally dominated over nitrates. Also, an accidental decrease of COD:N dosage to

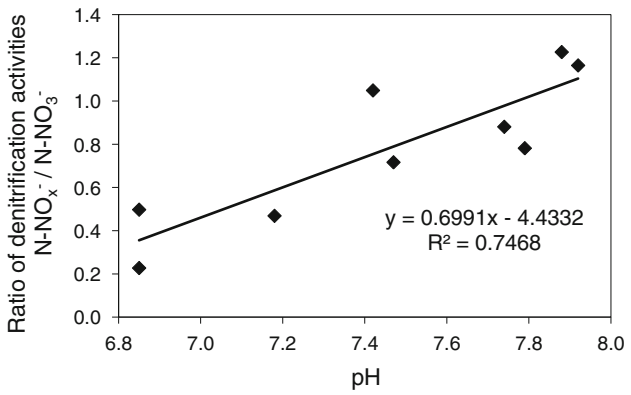

Fig. 3 Effect of pH on the ratio of activities of reduction of $N\text{-}NO_x^-$ and $N\text{-}NO_3^-$ in continuous set-up

2:1 resulted in a complete conversion of the influent nitrates to nitrites, while the activity of nitrite reduction was zero (not shown).

The pH in this experiment was maintained at values 7.4–7.8, corresponding to a previously published optimum for denitrification (Estuardo et al. 2008). However, pH values affect also the metabolic preferences between reduction of nitrates and nitrites. Since reduction of nitrites is the second step of overall denitrification, denitritation activities alone are inaccessible. Instead, ratios of activities of reduction $N\text{–}NO_x^-$ and $N\text{–}NO_3^-$ were calculated and they exhibit linear increase with pH (Fig. 3). This implicates that at pH above ~7.8, reduction of nitrites should be faster than reduction of nitrates repressing nitrites accumulation. This was indeed confirmed later using synthetic brine and new LB (not shown). Nevertheless Glass and Silverstein (1998, 1999) observed higher accumulation of nitrites in batch cultures of activated sludge just opposite at higher pH (8–9). This contradiction might have many reasons and is currently under investigation.

Deformation of LB

During the initial batch experiments, extensive irregular scrolling and clustering of PVA lenses were observed, but without notable effect on the denitrification activity and separation of LB from the bulk liquid. The clusters disappeared again in the later continual experiments, however, the lens scrolling persisted. Complementary tests with fresh LB revealed that an addition of phosphorus (P:N 0.07:1) slowed down the clustering process. Neither clustering nor scrolling occurred in pure solutions of NaCl and $CaCl_2$ (up to concentration of 35 g/L/Cl^-) in a 3-month period, which disregards the salinity as a sole cause. The problem remains further under investigation.

Application aspects

The technology was capable of removing high $N\text{–}NO_x^-$ concentrations (tested up to 250 mg/L) to the levels below the determination limit (<5 mg/L) in the continuous setup, i.e., high nitrogen removal efficiency >98% was achieved. Obtained denitrification activities (~450 and 150 mg $N\text{–}NO_x^-$/h/kg LB for ~4 and 8 % salinity, respectively) were lower than maximal achievable activities in non-inhibiting media (~1,000 mg $N\text{–}NO_x^-$/h/kg LB, Trogl et al. 2011a, b), nevertheless they are still high enough to enable industrial full-scale applications. Previous experiences with denitrification of high-salinity waters (Bouskova et al. 2011; Trogl et al. 2011a) indicate that much higher concentrations of $N\text{–}NO_3^-$ (up to 2,300 mg/L) can be effectively reduced to as low as 0.3 mg/L $N\text{–}NO_x^-$ using LB. These results together demonstrate the applicability of LB for the treatment of industrial high-salinity wastewaters.

Conclusion

Successful elimination of high concentrations of nitrates and nitrites (up to 250 mg/L $N\text{–}NO_x^-$) from high-salinity wastewaters from desulphurization process using denitrifying bacteria (*P. pantotrophus*, *P. fluorescens*) encapsulated in Lentikats Biocatalyst (polyvinylalcohol) was demonstrated. Inhibition of denitrification was markedly overcome by the addition of limiting phosphorus. Obtained denitrification activities (150–450 mg $N\text{-}NO_x^-$/h/kg LB) were suitable for industrial-scale applications even at the highest tested salinity (35 g/L Cl^- + 17 g/L SO_4^{2-}, i.e., ~8%). The technology was capable of removing high influent concentrations (in this study up to 250 mg/L $N\text{–}NO_x^-$) with >98% removal efficiency (<5 mg/L $N\text{–}NO_x$ in the effluent). Accumulation of undesired nitrites was significantly reduced by operating at pH > 7.8. These results demonstrate promising applicability of Lentikats Biotechnology for treatment of other similar (high salinity, nutrient-limiting) industrial wastewaters.

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References

Beliavski M, Meerovich I, Tarre S, Green M (2010) Biological denitrification of brines from membrane treatment processes using an upflow sludge blanket (USB) reactor. Water Sci Technol 61(4):911–917

Bouskova A, Mrakota J, Stloukal R, Trogl J, Pilarova V, Kriklavova L, Lederer T (2011) Three examples of nitrogen removal from industrial wastewater using Lentikats Biotechnology. Desalination 280(1–3):191–196

Bruss T, Mika D, Westermann B, Warnecke HJ (2002) Enantioselective hydrolysis of racemic epoxides with LentiKats((R))-encapsulated microorganisms. In: Landbaufor. Volkenrode, SH241, pp 127–129

Cyplik P, Grajek W, Marecik R, Kroliczak P, Dembczynski R (2007) Application of a membrane bioreactor to denitrification of brine. Desalination 207(1–3):134–143

Dincer AR, Kargi F (1999) Salt inhibition of nitrification and denitrification in saline wastewater. Environ Technol 20(11): 1147–1153

Ersever I, Ravindran V, Pirbazari M (2007) Biological denitrification of reverse osmosis brine concentrates: I. batch reactor and chemostat studies. J Environ Eng Sci 6(5):503–518

Estuardo C, Marti MC, Huilinir C, Lillo EA, Von Bennewitz MR (2008) Improvement of nitrate and nitrite reduction rates prediction. Electron J Biotechnol 11(3). doi:10.2225/vol11-issue3-fulltext-6

Foglar L, Sipos L, Bolf N (2007) Nitrate removal with bacterial cells attached to quartz sand and zeolite from salty wastewaters. World J Microbiol Biotechnol 23(11):1595–1603

Glass C, Silverstein J (1998) Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. Water Res 32(3):831–839

Glass C, Silverstein J (1999) Denitrification of high-nitrate, high-salinity wastewater. Water Res 33(1):223–229

Grosova Z, Rosenberg M, Rebros M, Sipocz M, Sedlackova B (2008) Entrapment of beta-galactosidase in polyvinylalcohol hydrogel. Biotechnol Lett 30(4):763–767

Grosova Z, Rosenberg M, Gdovin M, Slavikova L, Rebros M (2009) Production of D-galactose using beta-galactosidase and Saccharomyces cerevisiae entrapped in poly(vinylalcohol) hydrogel. Food Chem 116(1):96–100

Hanaki K, Hirunmasuwan S, Matsuo T (1994) Protection of methanogenic bacteria from low pH and toxic materials by immobilization using polyvinyl-alcohol. Water Res 28(4):877–885

Hronska H, Grosova Z, Rosenberg M (2009) Hydrolysis of lactose in milk by Kluyveromyces lactis beta-galactosidase immobilized in polyvinylalcohol gel. J Food Nutr Res 48(2):87–91

Jekel M, Buhr A, Willke T, Vorlop KD (1998a) Immobilization of biocatalysts in LentiKats. Chem Eng Technol 21(3):275–278

Jekel M, Buhr A, Willke T, Vorlop KD (1998b) Novel gel inclusion immobilisation systems (LentiKats) in biotechnology. Chem Ing Tech 70(4):438–441

Kriklavova L, Lederer T (2010). The use of nanofiber carriers in biofilm reactor for the treatment of industrial wastewaters. In: Nanocon 2010, 2nd International Conference, pp 165–170

Kubac D, Cejkova A, Masak J, Jirku V, Lemaire M, Gallienne E, Bolte J, Stloukal R, Martinkova L (2006) Biotransformation of nitriles by Rhodococcus equi A4 immobilized in LentiKats (R). J Mol Catal B Enzym 39(1–4):59–61

Lefebvre O, Moletta R (2006) Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res 40(20):3671–3682

Mariangel L, Aspe E, Marti MC, Roeckel M (2008) The effect of sodium chloride on the denitrification of saline fishery wastewaters. Environ Technol 29(8):871–879

Mcadam EJ, Judd SJ (2008) Biological treatment of ion-exchange brine regenerant for re-use: a review. Sep Purif Technol 62(2):264–272

Mcadam EJ, Pawlett M, Judd SJ (2010) Fate and impact of organics in an immersed membrane bioreactor applied to brine denitrification and ion exchange regeneration. Water Res 44(1):69–76

Mohseni-Bandpi A, Elliott DJ (1998) Groundwater denitrification with alternative carbon sources. Water Sci Technol 38(6):237–243

Park EJ, Seo JK, Kim MR, Jung IH, Kim JY, Kim SK (2001) Salinity acclimation of immobilized freshwater denitrifier. Aquacult Eng 24(3):169–180

Pilarova V, Trogl J, Synek V (2011) Validation of photometric determinations of nitrites and COD_{Cr} using Spectroquant® sets in saline waters. Chem Listy 105(13):s55–s57

Rebros M, Rosenberg M, Kristofikova L, Stloukal R (2005a) Microbial production of fuel ethanol: Bacteria or yeasts? Chem Listy 99(6):402–409

Rebros M, Rosenberg M, Stloukal R, Kristofikova L (2005b) High efficiency ethanol fermentation by entrapment of Zymomonas mobilis into LentiKats((R)). Lett Appl Microbiol 41(5):412–416

Rebros M, Rosenberg M, Grosova Z, Kristofikova L, Paluch M, Sipocz M (2009) 170 Ethanol Production from Starch Hydrolyzates using Zymomonas mobilis and Glucoamylase Entrapped in Polyvinylalcohol Hydrogel. Appl Biochem Biotechnol 158(3):561–570

Sievers M, Schafer S, Jahnz U, Schlieker M, Vorlop KD (2002) Significant reduction of energy consumption for sewage treatment by using LentiKat (R) encapsulated nitrifying bacteria. In: Landbaufor. Volkenrode SH241, pp 81–86

Trogl J, Bouskova A, Mrakota J, Pilarova V, Krudencova J, Mechurova J, Krizenecka S, Stloukal R (2011a) Removal of nitrates from simulated ion-exchange brines with Paracoccus denitrificans encapsulated in Lentikats Biocatalyst. Desalination 275(1–3):82–86

Trogl J, Bouskova A, Pilarova V, Mrakota J, Stloukal R (2011b) Application of Lentikats Biotechnology for removal of nitrates from ion-exchange brines: Implications for adaptation of encapsulated denitrifiers. Afr J Biotechnol 10(79):18304–18310

Vackova L, Srb M, Stloukal R, Wanner J (2011) Comparison of denitrification at low temperature using encapsulated Paracoccus denitrificans, Pseudomonas fluorescens and mixed culture. Bioresour Technol 102(7):4661–4666

Vejvoda V, Kaplan O, Klozova J, Masak J, Cejkova A, Jirku V, Stloukal R, Martinkova L (2006) Mild hydrolysis of nitriles by Fusarium solani strain O1. Folia Microbiol 51(4): 251–256

Vorlop KD, Jekel M (1999) Process for preparing a biocatalyst with a polyvinyl alcohol gel and biocatalyst produced by this process. German patent DE 198(27):552

Wisniewski C, Persin F, Cherif T, Sandeaux R, Grasmick A, Gavach C, Lutin F (2002) Use of a membrane bioreactor for denitrification of brine from an electrodialysis process. Desalination 149(1–3):331–336

Yang PY, Nitisoravut S, Wu JS (1995) Nitrate removal using a mixed-culture entrapped microbial cell immobilization process under high-salt conditions. Water Res 29(6):1525–1532