

Wastewater from Biodiesel Production as a Carbon Source for Denitrification of Sludge Liquor in SBR

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Sludge liquor from an anaerobic sludge digester with an average N–NH₄ concentration of $\gamma = 1185 \text{ mg L}^{-1}$ was treated in a pilot-scale SBR (sequencing batch reactor) system. The returned activated sludge of a WWTP was used as inoculum. The average efficiency of N–NH₄ removal was over $\bar{\eta} = 90 \%$. Concentrations of N–NH₄ in the effluent were typically below 10 mg L^{-1} . The maximal achieved nitrification rate was $r_N = 9.1 \text{ mg g}^{-1} \text{ h}^{-1}$ (relative to MLVSS). Wastewater of methyl ester wash arising during biodiesel production was used as an external carbon source for denitrification. A dosage of 3.5 – 4.5 g of COD per 1 g of nitrogen available for denitrification was found optimal. Typical effluent N–NO₃ concentration was about $\gamma = 25 \text{ mg L}^{-1}$ and maximal achieved denitrification rate was $r_D = 14.5 \text{ mg g}^{-1} \text{ h}^{-1}$. Operation of the SBR was stable at a HRT of $\tau = 4 - 5$ days.

Key words:

Sequencing batch reactor, nitrogen, denitrification, biodiesel

Introduction

Supernatant from anaerobic sludge digesters is an element of the internal liquid flow of a wastewater treatment plant (WWTP), having high mass concentrations of N–NH₄ and organic non-biodegradable compounds. It is usually recirculated to the plant inlet. Even though it represents a small part of the overall wastewater flow, because of its high ammonia concentration it could make it difficult to reach the legal limit of total nitrogen (N_{total}) concentration in the WWTP effluent.¹ Therefore, separate treatment of this stream is recommended. Many processes have been developed including physico-chemical and biological ones. Biological nitrification/denitrification is usually preferred because of the lack of consumed chemicals, the low amount of sludge produced and its usability in agriculture.² Due to the small flow rate of the sludge water, a sequencing batch reactor (SBR) seems to be an optimal reactor for carrying out the biological processes.³ Biological nitrification/denitrification requires the interchanging of sequential aerobic and anoxic phases. The process has been described in the literature and consists of two reactions: aerobic nitrification, which takes place in two steps (nitritation and nitrification), and anoxic denitrification.^{4,5,6} Denitrification requires the presence of an electron donor, such as organic carbon.⁷ As the sludge liquor is poor in biodegradable organic compounds, an external carbon source (methanol, acetic acid) is to be used. The external carbon source must fulfill requirements such as

high biodegradability, high COD, low mass concentrations of N, P, and compounds inhibiting the biological treatment process, simple handling, low price, good availability, etc. The suitability of some industrial waste products containing easily biodegradable organic compounds like yeast, corn, whey or spent sulfite liquor has been tested and it has been proved that they yield denitrification efficiencies comparable to that of methanol.^{8,9,10,11}

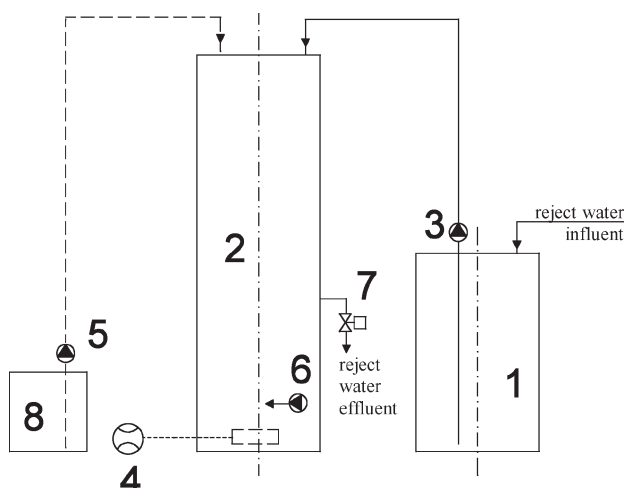
Most of the biodiesel produced in the Czech Republic is done via the base catalyzed transesterification of plant oil. Methyl ester as the main product is washed with diluted acid. The amount of arising wastewater is about 10 % of the produced methyl ester. The wastewater has an extremely high COD ($\gamma = 300$ to 500 g L^{-1}), with a volume fraction of methanol in the range of $\varphi = 6$ to 10% , and it contains traces of glycerin and soaps. Its treatment or further use is difficult. Sometimes it is used for biogas production. Use of the methyl ester wash wastewater as a substrate for the denitrification phase of the biological treatment of nitrogen-rich wastewaters such as sludge liquor from anaerobic sludge digestion in an SBR could be an interesting option.

Experimental

The reactor and its operation

This study was performed on a pilot-scale SBR system located at the Brno WWTP in the Czech Republic. The SBR system was constructed by

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(1) sludge liquor storage vessel 0.317 m³; (2) reaction vessel 0.634 m³, outlet pipe 900 mm from the bottom of the tank, i.e. 285 L of mixed liquor always remained in the tank after each cycle; (3) sludge liquor pump; (4) aerator; (5) external carbon source pump; (6) stirrer – submersible pump; (7) servo valve; (8) external carbon source storage vessel

Fig. 1 – Process flow sheet for the SBR system

EVH s.r.o. of Brno. Its process flow sheet is shown in Fig. 1. The reactor worked in a 12-hour cycle. Each cycle included the phases presented in Table 1.

The reactor operation was divided into two- or three-day periods determined by sampling times (Monday, Wednesday, and Friday). Each period was characterized by constant parameters such as hydraulic retention time (*HRT*), sludge load, influent quality, external carbon source dosage, etc.

During the start-up, sludge liquor was fed into the reactor at the beginning of the oxic phase. Feeding took several minutes according to the filled amount.

At higher hydraulic loads, the concentration of N–NH₄, i.e. (N–NH₃ + N–NH₄⁺) in the reactor increased considerably after feeding in the sludge liquor. The N–NH₃ form is toxic to microorganisms and thus it could affect the process negatively.⁷ At a temperature of 25 °C and a pH of 8.0, fraction of N–NH₃ comprises about $w = 5.4\%$ of the total N–NH₄.¹² To avoid the potential negative effect of N–NH₃, feeding was divided into 3 time intervals and the reactor was fed stepwise: at the beginning of the oxic phase, after 60 min and 120 min.

Feed, inoculum, and the external carbon source

The sludge liquor used in the experiments originated from the centrifuges dewatering sludge from the anaerobic sludge digesters of the Brno WWTP.

Table 1 – Cycle phases and their durations

Cycle phase	Time, <i>t</i> /min
oxic phase – nitrification (aeration)	400
anoxic phase – denitrification (mixing)	270
settling	30
discharge	15
pause	5
total	720

Table 2 – Characteristics of sludge liquor from an anaerobic sludge digester

Component	γ	Average	Median	Minimum	Maximum
N–NH ₄	mg L ⁻¹	1023	1029	783	1419
N _{Kj}	mg L ⁻¹	1144	1111	814	1443
N _{org}	mg L ⁻¹	121	70	20	515
alkalinity (4.5)	mmol L ⁻¹	74	77	30	87
COD	mg L ⁻¹	1026	975	847	1548
pH	–	8.06	8.06	7.82	8.22
SS	mg L ⁻¹	428	396	36	896
VSS	mg L ⁻¹	388	328	28	876
P _{total}	mg L ⁻¹	16.9	18.4	2.9	21.9

Table 2 shows its main characteristics. Besides N–NH₄ and organic non-biodegradable compounds, another problem was the large concentration of suspended solids, particularly in the first periods of the experiment.

The start-up inoculum was taken from the returned activated sludge stream of Brno's WWTP, the activated sludge process of which included nitrification-denitrification.

During the start-up of the reactor, methanol was used as a time-tested external carbon source. Afterwards, a wastewater of methyl ester wash (BIO) was tested. This BIO was obtained from a biodiesel manufacturer, who produced the biodiesel via KOH-catalyzed transesterification of rape oil with methanol. The methyl ester product is washed with a mixture of water and sulfuric acid. The wastewater from this process (BIO) is a clear yellowish liquid without suspended solids characterized by the following values: $\gamma(\text{COD}) = 400 \text{ g L}^{-1}$, $\gamma(\text{N–NH}_4) = 12.9 \text{ mg L}^{-1}$, $\gamma(\text{N–NO}_2) = 0.29 \text{ mg L}^{-1}$, $\gamma(\text{N–NO}_3) = 16.1 \text{ mg L}^{-1}$, $\gamma(\text{N}_{\text{org}}) \leq 1 \text{ mg L}^{-1}$, $c_{\text{acidity}(4.5)} = 25 \text{ mmol L}^{-1}$, pH = 2.3. The BIO was diluted with tap water ($\psi = 510 \text{ mL L}^{-1}$) for accurate dosing.

Sampling and analyses

The following samples were taken:

- influent sludge liquor,
- influent organic substrate (BIO),
- effluent from the last operation cycle, corresponding with the water quality in the reactor,
- a mixed effluent sample from all operation cycles constituting the assessed period, and
- mixed liquor from the reactor.

The following parameters were analyzed (dependent on the sample type): pH (Radiometer PHM210), alkalinity (titration method), ammonia nitrogen N-NH₄ (Nessler's method), nitrite nitrogen N-NO₂ (spectrophotometric method with sulphanic acid and 1-naphthylamine), nitrate nitrogen N-NO₃ (spectrophotometric method with salicylic acid), Kjeldahl nitrogen N_{Kj} (Hach-Lange Digesdahl), P_{total} (decomposition with Oxisolv – Merck – and spectrophotometric determination as a molybdophosphate complex), COD in the filtered sample (spectrophotometric semi-micromethod), suspended solids (SS), and volatile suspended solids (VSS) (both gravimetric methods).

Evaluation of the data

As the biomass production was low, the part of nitrogen incorporated into the biomass was neglected. The material balance of nitrogen related to each operation period (*B*, g/period) was calculated according to the following scheme:

- nitrogen available for nitrification:

$$B(N_{\text{nitr}}) = B(N_{\text{Kj}})_i - B(N_{\text{org}})_e$$

- nitrified nitrogen:

$$\Delta B(N_{\text{nitr}}) = B(N_{\text{Kj}})_i - B(N_{\text{Kj}})_e = \Delta B(N_{\text{Kj}})$$

- nitrogen available for denitrification:

$$B(N_{\text{den}}) = B(N_{\text{total}})_i - B(N_{\text{Kj}})_e$$

- denitrified nitrogen:

$$\Delta B(N_{\text{den}}) = B(N_{\text{total}})_i - B(N_{\text{total}})_e = \Delta B(N_{\text{total}})$$

The *i*- and *e* indexes corresponded to the influent- and effluent parameters. The main parameters used for evaluation of the process were as follows:

Specific nitrification rate r_N (mg g⁻¹ h⁻¹) is defined as the quantity of nitrified nitrogen related to MLVSS and aeration time. It depends on the oxic phase loading rate of nitrogen available for nitrification $B_{\text{oxo}}(N_{\text{nitr}})$, and the temperature.

The specific denitrification rate r_D (mg g⁻¹ h⁻¹) is evaluated from the quantity of denitrified nitrogen related to MLVSS and the anoxic phase time. The main factors controlling the denitrification rate are the nitrogen available for denitrification loading rate $B_{\text{oxo}}(N_{\text{den}})$ and the dosage of external carbon source related to the nitrogen available for denitrification.

The relative dosage of the external carbon source (*RD*, g g⁻¹) was calculated as the dosed amount (per period) expressed in oxygen equivalents *B*(COD) related to the amount of nitrogen available for denitrification: $RD = B(\text{COD})/B(N_{\text{den}})$.

Stoichiometric consumption of the carbon source depends on the initial nitrogenous compound (N-NO₃, N-NO₂) and the final denitrification product, which can be N₂ or N₂O. The external carbon source is decomposed by many bacterial processes during denitrification. That is why its recovery is usually not complete, particularly when it is added excessively in relation to N_{den}. The substrate recovery (*SR*, g g⁻¹) was calculated as a ratio of the amount of denitrified nitrogen (g) to the dosage of the external carbon source: $SR = \Delta B(N_{\text{den}})/B(\text{COD})$.

Results and discussion

Start-up and operation of the reactor

The reactor was filled with returned activated sludge, the MLSS concentration of which was approx. 4 g L⁻¹. At start-up, an initial feed of the sludge liquor into the reactor was used, with methanol as the external carbon source. Acclimation of the microorganisms was fast. Its course is evident from Fig. 2.

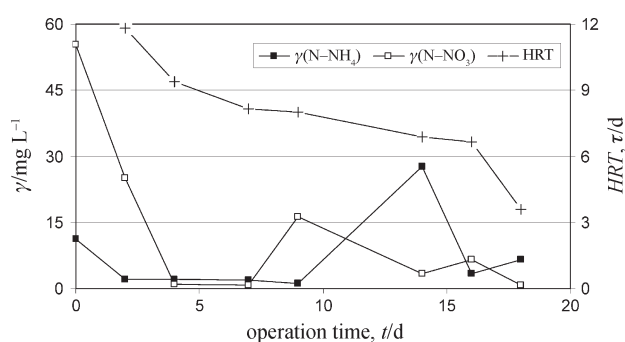


Fig. 2 – Variations in HRT and N-NH₄ and N-NO₃ concentrations in the effluent during SBR start-up

After start-up, the reactor was operated for 61 days with BIO as the external carbon source. The operation time was divided into 25 experimental periods (with a length of 2 or 3 days). At the beginning, the hydraulic retention time (*HRT*) was 20 – 40 days. The feed flow rate was gradually increased and the BIO additions were adjusted according to the obtained results, usually in an over-stoichiometric amount. In some periods the *RD* was below this level and in period No. 10 the reactor was operated with no addition of external carbon source. In the last experimental periods *HRT* was reduced to about 5 days (Fig. 3).

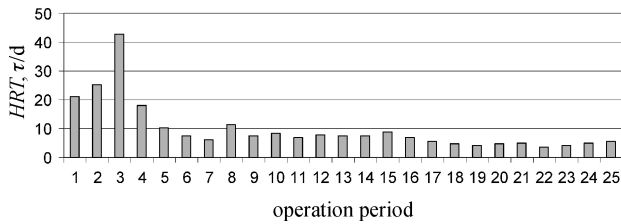


Fig. 3 – Variations in HRT

Besides HRT, MLVSS concentration also played an important role. The variation over time of the MLSS and MLVSS related to the minimal reactor volume is illustrated in Fig. 4. The average MLSS was 3400 mg L^{-1} . MLVSS represented 77 % of MLSS on average. Production of biomass was low and there was no need to withdraw the sludge from the reactor during the experiments. The settleability of the sludge was good. Yet, a small part of the sludge flocs floated and occasionally caused high concentrations of suspended solids in the reactor effluent, especially in periods of high nitrate concentration (670 mg L^{-1} on average).

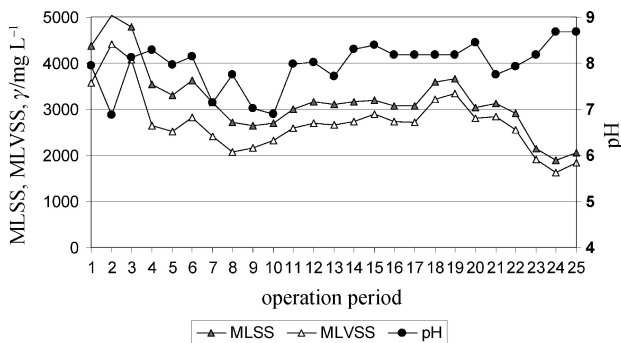


Fig. 4 – Variations in pH of the effluent and MLSS and MLVSS in the reactor

Acid-base conditions in the reactor changed during the cycles according to whether processes of nitrification or denitrification were running. Fig. 4 shows the time behavior of pH measured in the effluent. The pH was usually between 7 and 8 with extremes of 6.9 and 8.7. Due to the high buffering capacity of the sludge liquor, the acidity of the BIO usually caused no significant drop in pH.

The reactor was located in the building where the digested sludge was dewatered and dried. Thus, temperatures measured in the reactor were relatively high and varied from 23 to 26 °C.

Nitrification phase

Fig. 5 shows concentrations of N-NH_4 in the effluent. The mass concentrations varied from $\gamma = 2.3$ to 153 mg L^{-1} and were typically below $\gamma = 10 \text{ mg L}^{-1}$ (median 5.1 mg L^{-1}).

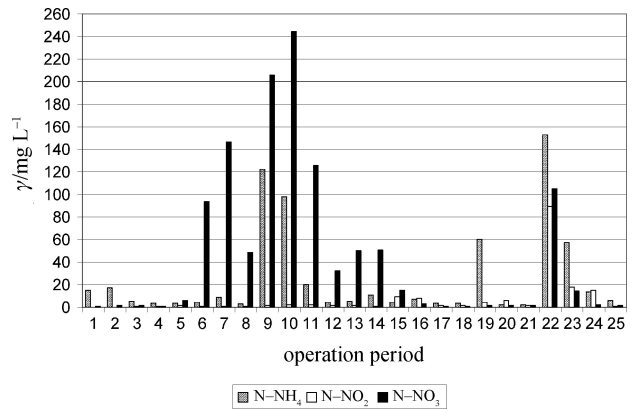


Fig. 5 – Concentrations of nitrogenous compounds in the effluent

Relatively high concentrations in periods 9 and 10 were probably caused by a low dosage of external carbon source accompanied by a decrease in pH to values below 7.0. In the oxic phase, the pH dropped to even lower values (6.0 to 6.5). Optimal pH ranges from 7.9 to 8.2 for *Nitrosomonas* and from 7.2 to 7.6 for *Nitrobacter*.⁴ In period 22 the effluent concentration of N-NH_4 reached an extreme – 153 mg L^{-1} – because besides low dosage of the BIO, the hydraulic load was suddenly increased to $B_{xoo}(\text{N}_{\text{nitr}}) > 10.5 \text{ mg g}^{-1} \text{ h}^{-1}$. Also, at these conditions the efficiency of N-NH_4 removal was about 80 %. After 5 days, the nitrifying bacteria became acclimated to the new operating conditions and the effluent concentration of N-NH_4 dropped to $\gamma = 13.5 \text{ mg L}^{-1}$. Nitrified nitrogen was predominantly N-NO_3 . Raised N-NO_2 concentrations were also temporarily recorded, especially when accompanied by a sudden increase in sludge loading. The average influent N_{org} concentration of $\gamma = 45 \text{ mg L}^{-1}$ decreased to the effluent concentration of $\gamma = 32 \text{ mg L}^{-1}$. This fact indicated that this form of nitrogen was not important for nitrogen balance.

The specific nitrification rate r_N depended primarily on the oxic phase loading rate of nitrogen available for nitrification $B_{xoo}(\text{N}_{\text{nitr}})$. The $B_{xoo}(\text{N}_{\text{nitr}})$ vs. r_N dependence was linear to the tested $B_{xoo}(\text{N}_{\text{nitr}})$ of $10 \text{ mg g}^{-1} \text{ h}^{-1}$ (Fig. 6). The slope of this dependence multiplied by 100 indicates the efficiency of Kjeldahl nitrogen removal, which was 96.4 % on average. The efficiency was lowered in 4 operation periods with extremely low RD , which disturbed the reactor operation. These periods were excluded from the evaluation (filled squares in Fig. 6). Maximal achieved r_N was $9.1 \text{ mg g}^{-1} \text{ h}^{-1}$.

Nitrification reactions require a DO (dissolved oxygen) concentration greater than 1 mg L^{-1} ; activated sludge systems designed for carbon oxidation and nitrification typically require DO levels greater

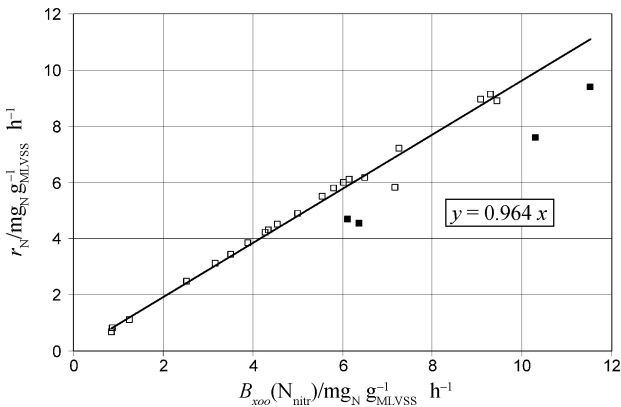


Fig. 6 – Nitrification rate as a function of the oxyc phase loading rate of nitrogen available for nitrification

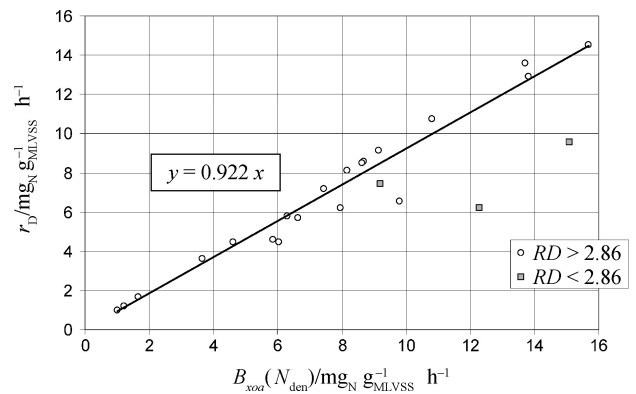


Fig. 7 – Denitrification rate as a function of the anoxic phase loading rate of nitrogen available for denitrification

than 2 mg L^{-1} .¹³ The DO concentration in the reactor during the oxyc phase at the highest loading of about $B_{xoo}(N_{\text{nitr}}) = 10 \text{ mg g}^{-1} \text{ h}^{-1}$ was between $\gamma = 0.2$ and 2 mg L^{-1} . The DO concentration of 1 mg L^{-1} in this case was reached only after 4 hours of aeration. Thus, the oxygenation capacity of the aerator seems to be a limiting factor of the nitrification phase. A more efficient aerator would probably enable parameters approaching those reported in the literature from a full-scale SBR: $\tau = 1.3 \text{ d}$, $r_N = 14 \text{ mg g}^{-1} \text{ h}^{-1}$.¹⁴

Denitrification phase

Denitrification of 1 g of N-NO_3 to N_2 requires a stoichiometric amount of 2.86 g COD , which represents 7.13 mL of BIO. The dosage of BIO relative to the nitrogen available for denitrification (RD) was higher than the theoretical amount in all testing periods except periods 6, 7, 9, and 22. In period 10, no external carbon source was dosed. The lack of organic substrate resulted in a sudden increase in N-NO_x concentrations.

Similarly as during the nitrification phase, the specific denitrification rate r_D depended on the anoxic phase loading rate of nitrogen available for denitrification $B_{xoo}(N_{\text{den}})$. Fig. 7 shows two sets of the measured data. Set 1 represents periods with $RD \geq 2.86 \text{ g g}^{-1}$; set 2 represents periods with $RD < 2.86 \text{ g g}^{-1}$. Set 1 was used for construction of a dependence, which was linear over the whole tested range, i.e. up to $15 \text{ mg g}^{-1} \text{ h}^{-1}$. Its slope multiplied by 100 demonstrates the efficiency of N_{total} removal and equals 92.2 %. In these periods, the RD ranged from 3.22 to 9.91 g g^{-1} with an average of 4.83 g g^{-1} . It follows from the data in Fig. 8 that over-stoichiometric amounts of COD had no effect on the r_D . With under-stoichiometric amounts of COD, the denitrification rate and the denitrified amount of nitrogen decreased with the decreasing

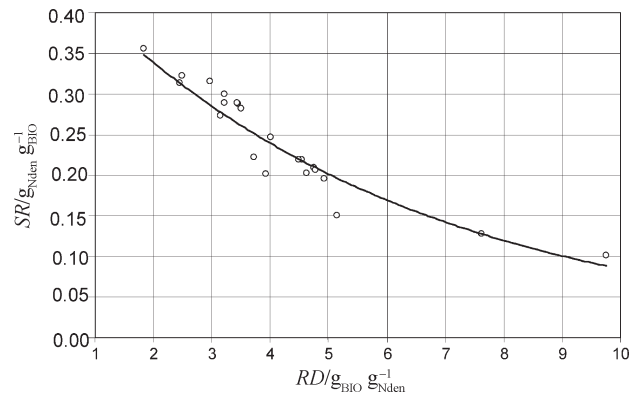


Fig. 8 – Recovery of BIO as a function of its relative dosage

amount of COD. In these periods, elevated concentrations of N-NO_3 were measured in the effluent (Fig. 5). After increasing the amount of BIO, N-NO_3 concentrations in the effluent soon decreased. Concentrations of N-NO_2 as intermediate products of denitrification reactions in the effluent were typically below 10 mg L^{-1} , even in period No. 10 without a dosage of BIO. A significant increase in N-NO_2 effluent concentrations (89.5 mg L^{-1}) accompanied by a drop in pH value was observed only in period No. 22, characterized by a low COD dosage (0.38 g g^{-1}) and a low HRT (3.7 days). In the following periods, both RD and HRT were increased and the N-NO_2 effluent concentration dropped to 0.7 mg L^{-1} in period No. 25.

Fig. 8 shows the dependence of the recovery of the external carbon source (SR) on its relative dosage. COD recovery decreased with increasing RD . A relative dosage of $3.5 - 4.5 \text{ g g}^{-1}$ (i.e. slightly over-stoichiometric) is recommended. Higher COD amounts do not bring better results. Furthermore, the non-consumed organic matters have to be decomposed in the oxyc phase, which requires increased oxygen supply. Otherwise, they remain in the treated water and cause elevated COD concentrations.

Removal of organic matter

Sludge liquor contains high concentrations of organic matter. During the time of operation, the input of COD in the sludge liquor was 2 896 g and in the BIO it was 11 510 g. 2 556 g of COD was released. Under the presumption of total decomposition of the COD from the BIO, the efficiency of COD removal from the sludge liquor was only 11.7 %. It means that organic matter in the sludge liquor has high biological resistance and is not very suitable as a substrate for denitrification.

Material balance of nitrogen compounds

Table 3 presents the material balance of nitrogen compounds and COD in periods with a *HRT* of about 5 days and with a *RD* of about 4 g g⁻¹. Excellent efficiencies of ammonia- and total nitrogen removal were achieved in these periods (total operation time of 19 days). The predominant nitrogen compound in the effluent was N_{org}, whereas other analyzed N-compounds occurred only in concentrations below 10 mg L⁻¹. Technological parameters relative to these periods are presented in Table 4.

Conclusions

Wastewater of methyl ester wash arising during biodiesel production has been demonstrated to be an appropriate external carbon source for the denitrification phase of biological treatment of nitrogen rich wastewaters such as sludge liquor from anaerobic sludge digestion in an SBR.

Adaptation of microorganisms to the influent is fast if hydraulic and nitrogen load is increased gradually.

The nitrification rate relative to MLVSS depended primarily on the loading of MLVSS with nitrogen available for nitrification. The dependence was linear up to $B_{xoo}(N_{nitr}) = 9.5 \text{ mg g}^{-1} \text{ h}^{-1}$. The maximal achieved nitrification rate was $r_N = 9.1 \text{ mg g}^{-1} \text{ h}^{-1}$. Concentrations of N-NH₄ in the effluent were typically below $\gamma = 10 \text{ mg L}^{-1}$.

The denitrification rate relative to MLVSS depended primarily on the load of MLVSS with nitrogen available for denitrification and external carbon source (BIO) dosage. The dependence $B_{xoa}(N_{den})$ vs. r_D was linear up to $B_{xoa}(N_{den}) = 15.7 \text{ mg g}^{-1} \text{ h}^{-1}$. The typical effluent N-NO₃ mass concentration was about 25 mg L⁻¹ and the maximal achieved denitrification rate was $r_D = 14.5 \text{ mg g}^{-1} \text{ h}^{-1}$. The dosage of BIO (expressed as the COD relative to the nitrogen available for denitrification) of 3.5 to 4.5 g g⁻¹ was found optimal.

Table 3 – Balance of nitrogen compounds and COD (experimental periods 15 – 18, 20 – 21, 26 – 27)

	Balance values			Average values	
	inlet m/g	outlet m/g	removal eff. η/%	inlet γ/mg L ⁻¹	outlet γ/mg L ⁻¹
N-NH ₄	1025	6.5	99.4	962	6.0
N _{Kj}	1193	42.3	96.5	1120	38.8
N-NO ₂	0	6.2		0	5.7
N-NO ₃	0	2.8		0	2.6
N-NO _x	0	9.0		0	8.3
N _{org}	168	35.8	78.7	157.9	32.8
N _{total}	1193	51.3	95.7	1120	47.1
COD (sludge liquor)	1033	914			
COD (BIO)	5071				

Table 4 – Technological parameters (experimental periods 15 – 18, 20 – 21, 26 – 27)

		Total	Average
duration	t/d	19	
sludge liquor flow		1065 L	56.1 L d ⁻¹
total flow		1090 L	57.4 L d ⁻¹
<i>HRT</i> (oxic phase)	τ/h		72.9
<i>HRT</i> (anoxic phase)	τ/h		49.2
<i>HRT</i>	τ/d		5.47
<i>RD</i>	g g ⁻¹		4.41
<i>SR</i>	g g ⁻¹		0.225
temperature	θ/°C		26.7
VSS	γ/mg L ⁻¹		2618
$B_{xoo}(N_{nitr})$	mg g ⁻¹ h ⁻¹		6.12
$B_{xoa}(N_{den})$	mg g ⁻¹ h ⁻¹		8.23

The average efficiency of N-NH₄ removal during SBR operation (except days with effluent pH below 7.0) was $\bar{\eta} = 96.4 \%$.

List of symbols

- $B_{xoo}(N_{nitr})$ – oxic phase loading rate of nitrogen available for nitrification, mg_N g⁻¹_{MLVSS} h⁻¹
 $B_{xoa}(N_{den})$ – anoxic phase loading rate of nitrogen available for denitrification, mg_N g⁻¹_{MLVSS} h⁻¹
 RD – dosage of BIO relative to the nitrogen available for denitrification, g_{BIO} g⁻¹_{Nden}

HRT – hydraulic retention time
 r_D – specific denitrification rate, $\text{mg}_N \text{g}^{-1}_{MLVSS} \text{h}^{-1}$
 r_N – specific nitrification rate, $\text{mg}_N \text{g}^{-1}_{MLVSS} \text{h}^{-1}$
 SR – substrate recovery, $\text{g}_N \text{g}^{-1}_{BIO}$
 t – time, h, d
 w – mass fraction, %
 γ – mass concentration, mg L^{-1} , g L^{-1}
 η – efficiency, %
 ϑ – temperature, °C
 τ – hydraulic retention time, d
 φ – volume fraction, %
 ψ – volume ratio

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