# Carbon and Nitrogen Removal by Biomass Immobilized in Ceramic Carriers

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## **Abstract**

This experiment was conducted in a bioreactor with biomass immobilized in ceramic carriers. The influence of hydraulic retention time (HRT), carrier structure and intrinsic circulation rate on carbon and nitrogen removal from municipal wastewater were investigated. Two types of ceramic carriers were used at HRT 70, 60, 40, 30 min for carrier I, and 70, 60, 30,15 min for carrier II, and at the circulation rate of 60, 40 and 20 dm<sup>3</sup>·h<sup>-1</sup>. The highest nitrogen removal efficiency was achieved in carrier II at 30 min of reaction. The carbon removal efficiency was similar for both carriers. An increase in internal circulation rate from 20 to 60 dm<sup>3</sup>·h<sup>-1</sup> enhanced nitrogen removal efficiency from 33.0 to 47.2% and decreased in the production of surplus sludge in carrier II.

Keywords: immobilized biomass, ceramic carriers, carbon and nitrogen removal, intrinsic circulation rate

## Introduction

The advantages of biomass immobilization are: increased volumetric reaction rate [1], and reduced size of bioreactors with immobilized biomass owing to the velocities of a liquid flux through the reactor [2]. In addition, microbial solid residence time is higher than the hydraulic residence time of the reactor, which allows avoiding biomass washout problems [3, 4]. The use of immobilized biomass has a lot of advantages during nitrogen removal. Effective oxidation of ammonium to nitrates determines total nitrogen removal rate during denitrification. Limited heat diffusion in the carrier prevents the temperature fluctuating inside the carrier [5]. This causes immobilized microorganisms to be less exposed to changes in temperature. This is important particularly in the case of nitrifying bacteria, because their activity depends on temperature.

The use of porous carriers for immobilization of microorganisms has recently gained much attention [6,7]. Porous carriers are characterised by variable pore size, the possibility of high biomass loading, no diffusion limi-

tations of nutrients for the microorganisms immobilized in the carrier, inert material, high chemical and temperature resistance, mechanical strength. The carrier structure does not permit blockage by support material and ensures good distribution. Bioreactors are easy to scale up by modular assembly. The asymmetric pore size distribution is ideal for immobilization of microorganisms. During colonization they can easily enter the carrier, where they have a large surface to colonize. Besides, there is the low risk of losing the biomass due to poor separability [7].

This work presents studies on carbon and nitrogen removal in immobilized biomass. Microorganisms were immobilized in pores of ceramic carriers of different shape, diameter and number of internal channels. The carriers had different active surfaces and the size of area open for free flow. The effects of carrier structure, hydraulic retention time, and intrinsic circulation rate on carbon and nitrogen removal by immobilized activated sludge were examined.

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Table 1. Characteristics of ceramic carriers used in the investigations.

| Characteristic   | Carrier I | Carrier II |
|--|-----------|------------|
| Membrane cross-section   |           | 8          |
| External diameter [mm]   | 25        | 10         |
| Hydraulic diameter [mm]  | . 6       | 3.6        |
| Membrane length [mm]   | 1178      | 1200       |
| Number of internal channels  | 7         | 3          |
| Filtrate (internal) surface [m²]   | 0.16      | 0.04       |
| Membrane volume [dm³]  | 0.589     | 0.094      |
| Volume of liquid flux at flow velocity 1 m·s <sup>-1</sup> [dm <sup>3</sup> ·h <sup>-1</sup> ] – producer's data | 710       | 118        |

## **Experimental Procedures**

## Multi-Channel Reactor

Two types of ceramic carriers differing in internal construction were used. The characteristic of both carriers is presented in Table 1.

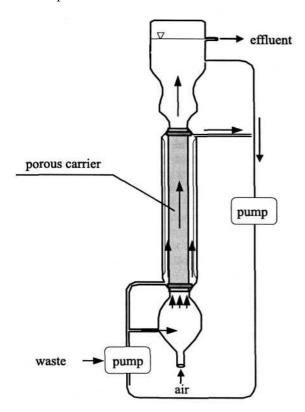


Fig. 1. Scheme of reactor.

The carriers are both made from aluminium oxide  $(Al_2O_3)$ , titanium oxide  $(TiO_2)$  and zirconium oxide  $(ZrO_2)$ . The material from which the carriers are made is porous. Pore size is 4-6  $\mu$ m.

The carriers with immobilized biomass made the stationary fillings of the reactors. The bioreactors worked under aerobic conditions, oxygen concentration in wastewater was about 2.5 g·m<sup>-3</sup>. The experiment was carried out at 20°C. The reactor design is shown in Fig. 1.

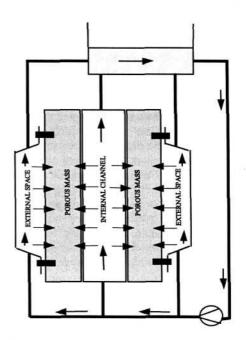


Fig. 2. Scheme of intrinsic circulation.

The carrier was fixed into the bioreactor using Orings. Two spaces were created: the internal channels and the external space. Raw wastewater flux and the circulating stream were mixed before they flew into the reactor. The influent was divided into two streams flowing parallel through the external space and internal channels. This allowed keeping the equal pressure on the internal and external surfaces. Scheme of internal circulation is shown in Fig. 2. The internal circulation has some advantages: it dilutes the wastewater, prevents excessive sludge growth and is the source of carbon needed for denitrification. The biomass does not leave the reactor and long solids residence time causes strong lysis processes. Lysis products present in the internal circulation stream can serve as an endogenous source of carbon.

#### Colonization Procedure

The immobilization of biomass in the porous carrier was made by circulating the activated sludge at an initial concentration of 20.8 g s.m.·dm<sup>-3</sup> for carrier I, and 17.3 g s.m.·dm<sup>-3</sup> for carrier II into the bioreactor for 24 h. After colonization, the concentration of biomass in the circulating effluent dropped to 11.6 g s.m.·dm<sup>-3</sup> for carrier I,

and to 15.0 g s.m.·dm<sup>-3</sup> for carrier II. The activated sludge derived from the sequencing bath reactor was the source of inoculum.

The studies were carried out for four hydraulic retention times (Table 2). In order to determine the effect of the intrinsic circulation rate on nitrogen removal by immobilized biomass, the circulation rate was varied from 60 to 20 dm<sup>3</sup>·h<sup>-1</sup> at the HRT of 40 min in carrier I, and 60 min in carrier II. In other series of investigations, the intrinsic circulation rate was stable (50 dm<sup>3</sup>·h<sup>-1</sup>). The examination period in each series was 11 to 52 days, depending on HRT. The research organization is presented in Table 2.

Average content of carbon compounds, nitrogen compounds and suspended solids in municipal wastewater used in the investigations is presented in Table 3.

## Analytical Methods

The waste was assayed for the concentration of the following: organic compounds, expressed as COD, in the influent and effluent by open reflux method; ammonium in the influent and effluent by the Nesslerization method after distillation; nitrite and nitrate in the effluent by

Table 2. Scheme of research.

| Determination | Hydraulic retention time | Intrinsic circulation rate       | Flow rate of waste | Surface loading rate | Volumetric loading<br>rate          |
|---------------|--------------------------|----------------------------------|--------------------|----------------------|-------------------------------------|
| Unit          | min                      | dm <sup>3</sup> ·h <sup>-1</sup> | dm³·h-1            | g·m-2·d-1            | g·dm <sup>-3</sup> ·d <sup>-1</sup> |
|               | 70                       | 50                               | 0.480              | 21.1                 | 5.7                                 |
|               | 60                       | 50                               | 0.510              | 29.4                 | 8.0                                 |
| Carrier I     |                          | 60                               |                    |                      |                                     |
| 40            | 40 40 0.8                | 0.840                            | 52.3               | 16.4                 |                                     |
|               |                          | 20                               | 0                  |                      |                                     |
|               | 30                       | 50                               | 1.260              | 88.3                 | 24.0                                |
|               | 70                       | 50                               | 0.078              | 15.3                 | 6.5                                 |
|               |                          | 60                               |                    |                      |                                     |
| Carrier II    | 60                       | 40                               | 0.100              | 19.2                 | 8.1                                 |
|               | 20                       |                                  |                    |                      |                                     |
|               | 30                       | 50                               | 0.165              | 50.3                 | 21.4                                |
|               | 15                       | 50                               | 0.362              | 105.2                | 44.7                                |

Table 3. Chemical characteristics of wastewater.

| Determination | COD<br>[mgO <sub>2</sub> ·dm <sup>-3</sup> ] | TKN<br>[mgTKN·dm <sup>-3</sup> ] |      |       |
|---------------|--|----------------------------------|------|-------|
| Carrier I     | 429.7  | 429.7 52.7 26. 9                 |      | 336.5 |
| Carrier II    | 376.1  | 55.1                             | 33.4 | 281.3 |

colorimetric methods; total Kjeldahl nitrogen in the influent and effluent using Kjeldahl's method and suspended solids in the influent and effluent by drying at 103-105°C [8].

### Calculation Methods

Nitrification efficiency was calculated using the following formula:

$$\%N = \frac{N_{ox}}{TKN} \cdot 100$$

where:

 $N_{ox}$  - concentration of nitrogen oxidized [mg·dm<sup>-3</sup>] TKN - concentration of total Kjelhdal nitrogen in the influent [mg·dm<sup>-3</sup>]

Concentration of nitrogen oxidized during nitrification was calculated using the following formula:

$$N_{ox} = TKN - N_{syn} - N_{NH4e} - N_{orge}$$

where:

 $N_{syn}$  - concentration of nitrogen used for biomass synthesis [mg·dm<sup>-3</sup>]

 $N_{NH4e}$  - concentration of ammonium in the effluent [mg·dm<sup>-3</sup>]

 $N_{\text{orge}}$  - concentration of organic nitrogen in the effluent [mg·dm<sup>-3</sup>]

Denitrification efficiency was calculated using the following formula:

$$\%DEN = \frac{N_{red}}{N_{ox}} \cdot 100$$

where:

N<sub>red</sub> - concentration of nitrogen reduced [mg-dm<sup>3</sup>]

$$N_{red} = N_{ox} - N_{NO2e} - N_{No3e}$$

where:

 $N_{NO2e}$  - concentration of nitrite in the effluent  $[mg \cdot dm^{^3}]$ 

 $N_{No2e}\text{-}$  concentration of nitrate in the effluent  $[mg\!\cdot\!dm^{\text{-}3}]$ 

The waste retention time and reaction time was calculated for the used liquid flow, i. e. waste flux and circu-

lation stream, assuming that the flux capacities through carrier I and carrier II at velocity of 1  $m \cdot s^{-1}$  are 710  $dm^3 \cdot h^{-1}$  and 118  $dm^3 \cdot h^{-1}$  (producer's data), respectively (Tab. 1). The efficiency of liquid flux flowing across the internal channels  $(Q_c)$  and pores  $(Q_F)$  was calculated on the basis of the following formula: Q=FA, where: Q - efficiency of liquid flux flowing through the channels and pores  $[m^3 \cdot s^{-1}],\ F$  - cross-section surface of channels and pores  $[m^2],\$ and A - flow velocity  $[m \cdot s^{-1}].$  The efficiency of liquid flux flowing through the carrier pores was the difference between the magnitudes of waste in flow to and flow through the channels. The reaction time was calculated from the ratio:

$$t = V/Q_F$$

and waste retention time was computed from the ratio:

$$t = V/(QF + Q_K)$$

where:

V - carrier volume [m<sup>3</sup>],

 $Q_F$  - liquid flux flowing through the carrier pores  $[m^3 \cdot s^{-1}]$ , and

 $Q_k$  - liquid flux flowing through internal channels  $[m^3 \cdot s^{-1}]$ .

#### Results

Two macroporous cylinder-shaped ceramic carriers were used to immobilize the activated sludge. The filtrate surface was 0.16 m<sup>2</sup> for seven-channelled carrier I, and 0.04 m<sup>2</sup> for three-channelled carrier II. The carriers differed in volume that was 0.589 dm<sup>3</sup> and 0.094 dm<sup>3</sup> for carriers I and II, respectively (Tab. 1).

Porous structure of the carriers caused the reaction time to be longer than waste retention time. The retention time of wastewater for carrier I changed from 8.17 to 9.29 s. For carrier II of above six times smaller volume, waste retention time was from 0.41 to 0.47 s. The reaction time was 3.1 times longer than waste retention time for carrier I, and 9.4 times longer for carrier II (Tab. 4).

The efficiency of nitrification in activated sludge immobilized in ceramic carriers was not dependent on the reaction time (Tab. 5). Although the reaction time for carrier II was almost 6 times shorter than for carrier I, the nitrification efficiency was higher for carrier II. An increase in volumetric loading from 5.7 to 24.0 g·dm<sup>-3</sup>·d<sup>-1</sup> for carrier I influenced nitrification effectiveness. The loading rise caused nitrification to decrease from 75.5%

Table 4. Effect of hydraulic retention time and carrier structure on retention and reaction times.

| Determination             | Unit | Carrier I |       |       |       | Carrier II |      |      |      |  |
|---------------------------|------|-----------|-------|-------|-------|------------|------|------|------|--|
| HRT                       | min  | 70        | 60    | 40*   | 30    | 70         | 60*  | 30   | 15   |  |
| Carrier volume            | dm³  |           | 0.5   | 89    |       | 0.094      |      |      |      |  |
| Calculated retention time | s    | 9.29      | 9.29  | 8.17  | 9.15  | 0.47       | 0.41 | 0.47 | 0.47 |  |
| Calculated reaction time  | s    | 28.85     | 28.85 | 25.42 | 28.44 | 4.41       | 3.90 | 4.41 | 4.39 |  |

<sup>\*</sup> values obtained for intrinsic circulation rate 60 dm<sup>3</sup>·h<sup>-1</sup>, other ones for the rate 50 dm<sup>3</sup>·h<sup>-1</sup>

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Table 5. Effect of hydraulic retention time on nitrogen conversions.

| Determination               | Unit       | Unit Carrier I |      |      |      | Carrier II |      |      |      |  |
|-----------------------------|------------|----------------|------|------|------|------------|------|------|------|--|
| HRT                         | min        | 70             | 60   | 40*  | 30   | 70         | 60*  | 30   | 15   |  |
| Volumetric loading rate     | g·dm-3·d-1 | 5.7            | 8.0  | 16.4 | 24.0 | 6.5        | 8.1  | 21.4 | 44.7 |  |
| Nitrification efficiency    | %          | 75.5           | 71.1 | 40.4 | 27.8 | 53.5       | 87.5 | 78.5 | 80.3 |  |
| Denitrification efficiency  | %          | 67.6           | 61.2 | 91.2 | 94.5 | 68.8       | 46.8 | 90.0 | 47.6 |  |
| Nitrogen used for synthesis | g·m³       | 3.3            | 4.6  | 8.2  | 3.6  | 6.5        | 3.4  | 6.0  | 4.7  |  |
| C:N in wastewater           |            | 6.3            | 7.4  | 7.0  | 10.2 | 7.5        | 5.9  | 9.0  | 7.5  |  |

<sup>\*</sup> values obtained for intrinsic circulation rate 60 dm<sup>3</sup>·h<sup>-1</sup>, other ones for the rate 50 dm<sup>3</sup>·h<sup>-1</sup>.

to 27.8%. The nitrification efficiency for carrier II was above 80%, even for the shortest hydraulic retention time of 15 min. At hydraulic residence time of 40 and 30 min in carrier I the decrease in nitrification efficiency was caused by overloading. The results of dissimilative nitrate reduction in two carriers were similar. When HRT was 30 min for both carriers, denitrification efficiency was about 90%. In these series of investigations, the respective C:N ratios in wastewater had the highest values, i.e. 10.2 and 9.0. At the loading values from 5.7 to 8.1 g·dm<sup>-3</sup>·d<sup>-1</sup> for both carriers and 44.7 g·dm<sup>-3</sup>·d<sup>-1</sup> for carrier II denitrification efficiency ranged between 46.8 and 68.8%. In the remaining series denitrification efficiency increased to above 90% at the C:N ratio in wastewater to 10.2. The use of nitrogen compounds for biomass synthesis was not dependent on technological assumptions. Nitrogen concentration used for the synthesis reached the highest value of 8.2 mg·dm<sup>-3</sup> at HRT 40 min for carrier I. In the remaining series it reached 3.3-4.6 mg·dm<sup>-3</sup>. For carrier II the maximum value of nitrogen used for biomass increase was 6.5 mg·dm<sup>-3</sup> at the lowest loading of 6.5 g·dm<sup>-3</sup>·d<sup>-1</sup>. The increase in loading did not cause the rise of nitrogen concentration used for biomass synthesis.

For carrier I, an increase in surface loading from 21.1 to 88.3 g·dm<sup>-2</sup>·d<sup>-1</sup> caused nitrogen removal to decline from 58.2 to 34.1% (Tab. 6). For carrier II a decrease in the effectiveness of nitrogen removal, as dependent on the increase in surface loading, was not observed. The highest nitrogen removal efficiency (81%) was obtained in carrier II at the loading of 50.3 g·dm<sup>-2</sup>·d<sup>-1</sup>. It is interesting that at the similar surface loadings for carrier I (52.3 g·dm<sup>-2</sup>·d<sup>-1</sup>) and carrier II (50.3 g·dm<sup>-2</sup>·d<sup>-1</sup>), but at the differ-

ent volume loadings, respectively,  $16.4~g\cdot dm^{-3}\cdot d^{-1}$  and  $21.4~g\cdot dm^{-3}\cdot d^{-1}$  (Tab. 2), nitrogen removal efficiency was different, i.e. 50.8 and 81.3%, respectively. The effect of fourfold bigger surface available for microorganisms in carrier I on carbon and nitrogen biodegradation in wastes was not proven.

The influence of intrinsic circulation rate on nitrogen conversions in immobilized biomass was investigated. At the hydraulic retention time of 40 min in carrier I, and 60 min in carrier II, the circulation of liquid through the internal channels ranged from 20 to 60  $\mathrm{dm^3 \cdot h^{-1}}$  (Tab. 7).

The change of the intrinsic circulation rate from 20 to 60 dm<sup>3</sup>h<sup>-1</sup> caused an increase in the nitrification and denitrification efficiencies in sludge immobilized in carrier II. Despite this the amount of nitrogen used for synthesis decreased, and nitrogen removal efficiency increased from 33% to 47.2%. The effect of circulation on nitrogen conversions in immobilized biomass was not observed for carrier I. A decrease in sludge production with the circulation rate was not observed for carrier I, in contrast to carrier II.

## **Discussion**

It was shown that the carrier structure determined the waste retention time and reaction time. The reaction time was 3.1 times longer than retention time for carrier I, and 9.4 times longer for carrier II. As compared with carrier I, the internal structure of carrier II more effectively enabled the reaction time to be elongated. Short diffusion way could possibly lengthen the reaction time in

Table 6. Effect of hydraulic retention time on carbon and nitrogen removal.

| Determination               | Unit                               | Carrier I<br>0.16 |      |      | Carrier II<br>0.04 |      |      |      |       |
|-----------------------------|------------------------------------|-------------------|------|------|--------------------|------|------|------|-------|
| Filtrate surface            | m²                                 |                   |      |      |                    |      |      |      |       |
| HRT                         | min                                | 70                | 60   | 40*  | 30                 | 70   | 60*  | 30   | 15    |
| Surface loading rate        | g·m <sup>-2</sup> ·d <sup>-1</sup> | 21.1              | 29.4 | 52.3 | 88.3               | 15.3 | 19.2 | 50.3 | 105.2 |
| Carbon removal efficiency   | %                                  | 74.3              | 79.8 | 78.8 | 77.8               | 79.5 | 84.0 | 86.9 | 81.6  |
| Nitrogen removal efficiency | %                                  | 58.2              | 52.3 | 50.8 | 34.1               | 51.7 | 47.2 | 81.3 | 45.5  |

<sup>\*</sup> values obtained for intrinsic circulation rate 60 dm<sup>3</sup>·h<sup>-1</sup>, other ones for the rate 50 dm<sup>3</sup>·h<sup>-1</sup>

| Determination                       | Unit                             | Carrier I |      | Carrier II |      |      |      |  |
|-------------------------------------|----------------------------------|-----------|------|------------|------|------|------|--|
| HRT                                 | min                              | min 40    |      |            | 60   |      |      |  |
| Intrinsic circulation rate          | dm <sup>3</sup> ·h <sup>-1</sup> | 60        | 40   | 20         | 60   | 40   | 20   |  |
| Nitrification efficiency            | %                                | 40.4      | 49.0 | 49.4       | 87.5 | 86.8 | 78.3 |  |
| Denitrification efficiency          | %                                | 91.2      | 95.3 | 98.4       | 46.8 | 41.8 | 24.9 |  |
| Nitrogen removal efficiency         | %                                | 50.8      | 56.4 | 53.3       | 47.2 | 42.5 | 33.0 |  |
| Nitrogen used for biomass synthesis | g·m⁻³                            | 8.2       | 5.9  | 2.4        | 3.4  | 3.6  | 7.3  |  |

Table 7. Effect of intrinsic circulation rate on nitrogen removal.

carrier II. The experiment results showed that organic biodegradation rate per time unit was higher in carrier II than in carrier I, despite that the active surface of the latter was fourfold greater. Internal channel shapes and their layout inside the porous mass had an influence on obtained results. According to Nishizawa et al. [9] the high volume reaction efficiency is caused by very short retention time (order of seconds) and high concentration of enzymes immobilized in carriers. Other authors obtained the highest volumetric nitrification rate for media with the largest surface area [7]. They showed that the increase in the surface area would hardly raise the nitrification rate, as this would require more narrow flow passages through the carrier. Optimal design of carriers requires a balance between the surface area and a space open for free flow in order to enhance mass transfer.

Our studies have shown 81% nitrogen elimination in carrier II at the hydraulic retention time of 30 min and high loading of the carrier volume (represented by COD) of 21.4 g·dm<sup>-3</sup>·d<sup>-1</sup>. By comparison, in suspended activated sludge systems volume loading during the complete biological treatment reaches the values of 1.8 g g·dm<sup>-3</sup>·d<sup>-1</sup>. The concentration of microorganisms in the carrier may be above 30 kg·mg<sup>-3</sup> [10], whereas there is about 3 to 5 kg·mg<sup>-3</sup> of biomass in suspended activated sludge systems. The critical sludge concentration for stable filtration in a reactor is 30 to 40 kg·mg<sup>-3</sup> [11]. It follows that there is some biomass concentration threshold. According to Zhao [12] diffusion resistance in cells is bigger than in the carrier, so the length of diffusion increases with biomass concentration.

Biodegradation efficiency in porous carriers is dependent on immobilization conditions, the amount of settled biomass, growth and movement conditions of microorganisms, and their distribution in the carrier structure. Pore sizes affect the amount of immobilized biomass. Cell concentration is higher in pores of bigger sizes. In carriers some part of the surface with larger pores could not be covered with cells [6]. On the other hand Nishizawa et al. [9] reported that the amount of enzymes immobilized in the carrier with pore size of 0.2 μm was about twice as high as for the carrier with 0.5 μm pore size. It has been shown that carriers with small pores size had greater immobilizing activity. The above-mentioned authors confirmed that the enzymatic activity of biomass immobilized in ceramic carriers was about ten times higher than that of enzymes immobilized in beads in a conventional column reactor.

The literature data indicate that processes held by microorganisms of long generation time can proceed at high loading because a big amount of nitrifiers is retained in the filtration bed [4]. Our research showed that the high organic loading resulted in nitrification decline in carrier I. Similar results were not really seen in carrier II. This may be a result of HRT effects on biofilm structure being different for two carriers. This needs further research. Libman et al. [13] have suggested that a slower nitrification rate in the presence of organic matter results from dissolved oxygen deficiency caused by mass-transfer limitation. Other authors have found the reaction efficiency of biomass immobilized in porous carrier mainly dependent on the mechanisms regulating substrate transport through the carrier [14].

From the present investigations it results that the use of multi-channel porous carriers ensures shortening of the oxygen and substrate diffusion ways. Oxidation of ammonium was limited in carrier I. Macroporous structure of carrier I could determine the size of air bubbles carried through the carrier pores. This seems to be an important property of porous materials, particularly in the case of high bacterial oxygen requirements. Therefore, studies showed the possibility of increasing biodegradation rates by using biomass immobilized in the carriers of suitable porosity. The number of internal channels, method of their packing and pore dimension cause different transport conditions for oxygen and nutrients. For carrier II, despite fourfold smaller filtration surface, the unfortunate effect of loading increase on nitrification efficiency was not found. At the hydraulic retention time of 15 min, the nitrification efficiency was 80.3%.

The research indicated that denitrification efficiency in immobilized biomass was not dependent on the carrier type. The nitrate reduction occured under conditions of constant oxygen supply to the reactor. The nitrogen removal performance by settled microorganisms was higher for carrier II, because the ammonium oxidation was more effective.

When oxygen was permanently supplied to the reactor with immobilized biomass, nitrification and denitrification could run simultaneously. For both carriers, the highest denitrification efficiency was observed at COD:N ratio in wastes equal to 9.0 and 10.2. Suwa et al. [4] have reported the following denitrification efficiency depending on the C:N ratio (expressed in BOD:N): when C:N was 5 to 10, the efficiency was from 30 to 50%, and at C:N above 12 it was about 75%. In our study denitrifica-

tion efficiency reached even above 90%, although C:N ratio in wastewater changed between 5.9 and 10.2 (Tab. 5). It can be concluded that additional carbon amount could originate from circulation stream. Suwa et al. [4] claimed that because of high carrier loading and in spite of aeration, denitrifiers microenvironments could be formed in the inner layers of biofilm. It takes place when the oxygen consumption rate by aerobes living outside the biofilm layers is higher than the velocity of oxygen diffusion to deeper-situated layers. Higher loading causes the biomass increase and formation of anoxic zones, even although air was supplied to the reactor constantly.

Suzuki et al. [15] obtained a high nitrogen removal rate as a result of simultaneous nitrification and denitrification. Authors have shown that a carrier with nitrifying biofilm was applied to treat carbon-containing wastewater, which resulted in the formation of denitrifying biofilm on the nitrifying biofilm, and in consequence, simultaneous nitrification and denitrification. Research conducted by Libman et al. [13] proved that the growth of the denitrifying layer on the nitrifying biofilm did not disturb the internal transport of ammonium and oxygen when the thickness of the heterotrophic layer was less than 12 µm. It can be presumed that in our experiment the anoxic zones were formed in pores, which allows simultaneous nitrification and denitrification.

In our investigations, the intrinsic circulation rate was changed from 20 to 60 dm<sup>-3</sup>·h<sup>-1</sup>. Research confirmed the effect of flow rate through the carrier with immobilized biomass on nitrogen removal efficiency in carrier II. The nitrogen removal rate by microorganisms immobilized in carrier I was not dependent on the intrinsic circulation rate. Limited oxygen diffusion through the pores made the effect of circulation rate in reactor I insignificant. Rather, the limitation of nitrification efficiency had a higher impact on nitrogen removal efficiency.

An increase in internal circulation rate decreased the production of surplus sludge in carrier II. This was concluded from the concentration of nitrogen used for biomass synthesis, which decreased from 7.3 to 3.4 mg·dm<sup>-3</sup>. It can be presumed that under conditions of high concentration of biomass in porous carriers the ratio of the available substrate to the number of microorganisms is decreased [16,17]. In our study, a drop in biomass increase depending on internal circulation was not found for carrier I. According to Casey et al. [18], one of the factors influenced the biomass increase can be biofilm thickness. Authors have called the outer layer of the biofilm - the growth layer and the layer attached to the carrier - the endogenous layer. When the thickness of growth layer was large in comparison to endogenous layer, the growth coefficient had the maximum value. Otherwise, high oxygen consumption in endogenous layer limited biomass increase and substrate reduction. It was observed in the biofilm airlift suspension reactor and three-phase fluidised bed reactor with minimal production of excess sludge. On the ground of our experiment it can also be assumed the possibility of minimizing the surplus sludge production in reactors with immobilized biomass by increasing the intrinsic circulation rate.

## **Conclusions**

- Spatial structure of carrier II made it possible to increase the reaction time 9.4-times. This was most likely caused by the shorter diffusion method than in carrier I. Despite fourfold smaller filtration surface than in carrier I, the biodegradation rate in carrier II was higher because of good transport conditions of oxygen and nu trients.
- The selection of optimal hydraulic retention time has the influence on the effective use of porous carriers in wastewater treatment; the highest nitrogen removal efficiency (81.3%) was obtained in carrier II at HRT 30 min
- An increase in intrinsic circulation rate from 20 to 60 dm<sup>-3</sup>·h<sup>-1</sup> in carrier II raised the nitrogen removal efficiency from 33% to 47.2% and decreased surplus sludge production; the amount of nitrogen used for biomass synthesis decreased by 3.9 g·m<sup>-3</sup>.

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