Advanced nitrogen elimination by encapsulated nitrifiers

M. Sievers*, K.-D. Vorlop**, J. Hahne**, M. Schlieker** and S. Schäfer*

* CUTEC-Institut GmbH (Clausthal Environment Technology Institute), Leibnizstr. 21+23, 38678 Clausthal-Zellerfeld, Germany
** Institute of Technology and Biosystems Engineering, Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany

Abstract By introducing a mixed population of nitrifiers encapsulated in gel lens beads a more selective nitrification process was found in treatment of settled sewage in lab scale at a hydraulic retention time (HRT) of about 30 to 60 minutes. The reaction rates for oxidation of soluble chemical oxygen demand (SCOD) were found to vary between 25 to 150 mg/L·h while nitrification takes place around 50 mg nitrogen per hour and litre reaction volume. However, based on this SCOD removal in the nitrification step, a consequent post-denitrification process without nitrate recycle and dosage of external carbon sources has been proven to reach substantial nitrate elimination of up to 20 mg nitrogen per litre at COD/N-ratios of approx. 6 in settled sewage. At such COD/N-ratios, suitable nitrogen elimination seems to be possible, because the bioflocs of settled sewage, produced so far by SCOD oxidation and entrapment of particulate COD, are passing through the nitrification process having a substantial contribution to the denitrification rate additionally to the remaining SCOD.

Keywords Denitrification; encapsulation; immobilisation; nitrification; nitrogen elimination

Introduction

Advanced biological nitrogen elimination is one of the most interesting treatment objectives for nitrogen loaded industrial and domestic wastewater. Several technical-scale nitrification/denitrification processes are successfully existing including novel bio-membrane reactors, bio-film reactors etc. However, optimisation of this two stage nitrogen elimination process depends on wastewater characteristics, e.g. BOD/N-ratio, level of concentrations, etc., in order to make sure first the complete nitrification and second, the utilisation of internal BOD as a hydrogen donor for denitrification as far as possible to reduce sludge production.

For complete nitrification in single sludge systems, a suitable sludge age (solid retention time, SRT) is needed with respect to the low growth-rate of autotrophics (nitrifiers), especially at temperatures around or below 10°C. This is because BOD and COD would also be oxidised during aeration in the nitrification process unit and the growth of most heterotrophics compared to autotrophics is higher. The amount of the completely oxidised BOD, which is not available as a hydrogen donor for denitrification, depends on process configuration and operation conditions.

Immobilisation

Immobilisation techniques are widely used for process intensification, especially to overcome the problem of washing-out of slow growing microorganisms. There exist two basically different immobilisation techniques. Conventionally, additional surfaces i.e. inert particles or fixed beds would be offered to mixed biological cultures during wastewater treatment achieving the immobilisation by adsorption of growing biofilms on the surfaces. The attribute of such an immobilisation technique is that the wastewater characteristics would mainly set the characteristics of bio-films within the scope of operational conditions...
(aerobic/anoxic/anaerobic, bio-film thickness by shear forces, etc.). The potential of the immobilisation technique called bio-encapsulation is much higher. It describes the immobilisation of biological catalysts by enclosing them in a stable matrix, i.e. polymer gel. Encapsulation is classified into micro-encapsulation and entrapment. For encapsulation of biological catalysts, mixed cultures including growing, resting or dead cells, purified enzymes or even enzymes from crude fermentation broth could be used. Based on the fact that encapsulation is connected to additional costs it is important to use an optimised immobilisation technique that combines lowest costs and highest efficiency for the given application. In contrast to the adsorption technique, the encapsulation method provides better protection of the biological catalysts, which is an important factor when immobilising sensitive cells. Moreover, the polymers used for the matrix should guarantee lowest toxicity against the biological catalysts while having sufficient mechanical, chemical and biological stability. An overview about encapsulation techniques and a brief description of two recent polyvinyl alcohol (PVA) based methods is given by Jahnz et al. (2001).

**Wastewater treatment using encapsulation techniques**

The use of entrapped biomass has the additional advantage that the process is less sensitive to inhibitors, pH, temperature etc. as shown for the nitrification process by Wijffels (1994). However, applications with the use of encapsulated bacteria are very few, based on the additional costs for cultivation and encapsulation. A published technical application is the PEGASUS process. Different polyethylene glycol (PEG) pre-polymers are used to immobilise nitrifying sludge (Emori et al., 1996). The cells are protected from the cross-linking reagent during immobilisation by a macromolecular coagulant. The obtained PEG pellets consist of 3 mm blocks and show good mechanical properties. The PEGASUS process is driven as a pre-denitrification activated sludge process containing PEG pellets in the aerated nitrification zone. The nitrification process has been improved and stabilised by the increased nitrification rate and its de-coupling from SRT. However, due to the large dimension of the PEG pellets, the diffusion properties are not optimal and final specific activity of immobilised biomass is limited.

Parallel to the experimental study of this paper a lab scale study on nitrification of both synthetic and municipal wastewater was carried out using two PVA pre-polymers based gels for encapsulation of autotrophic biomass (Argaman, 2000). The encapsulation technique used was developed at Federal Agricultural Research Centre (FAL), Braunschweig, Germany (Jekel et al., 1998). A volumetric nitrification rate of 20–25 mg nitrogen per litre and hour at HRT of one hour has been measured for 10% gel content. However, although the upgrading of nitrification by minimising BOD removal and improving washing-out of heterotrophics was an aim, no results concerning BOD removal have been reported.

A mathematically based example for reducing the BOD removal using entrapped autotrophic biomass has been reported with the assumption of attached heterotrophic layers on gel beads (Libman et al., 2000). Different kinetic and physical parameters (respirometric/kinetic measurements and literature-based values) were used for prediction of the heterotrophic layer effect on BOD removal. It was found that increasing heterotrophic biofilm thickness leads to increasing ammonia and decreasing BOD concentrations in the effluent. Additional measurements with different mixing intensities showed significant respiration activity of attached heterotrophic biofilms, which renders possible a post-denitrification for the assumed BOD and ammonia concentrations of 250 mg/L and 50 mgN/L respectively. However, in this study, the presence of suspended heterotrophics has not been taken into consideration. Those are substantially present even in settled sewage and subsequently it is expected that the BOD removal would practically be much higher than calculated.
Methods

For the study on advanced nitrogen removal from wastewater, PVA based gel beads were produced first by a cost effective encapsulation technique. Those beads were used for pretreatment of settled sewage in an aerated lab-scale reactor with an effective gel particle retaining system as well as a biofloc removal system.

Encapsulation

Hydrogels from polyvinyl alcohol. Polyvinyl alcohol (PVA) is a hydrophilic polymer which an aqueous solution is capable of gelling when stored for a prolonged time at low temperatures. Hydrogels from PVA by this cryogelation are mechanically very stable and show more or less no abrasion when employed in stirred reactors. In gelated form, PVA is hardly biodegradable and thus can be used when working under non-sterile conditions. The method of cryogelation of PVA often inflicts a loss in biological activity. To counteract the stress a method was developed which allows the gelation of PVA-solutions at room temperature by means of controlled partial drying (Jahnz et al., 2001).

Production of gel lens particles. A ready-to-use PVA based pre-polymer liquid solution is mixed with an externally cultivated mixed autotrophic bacteria population followed by production of small droplets on a suitable surface. These droplets are exposed to air and the water starts to evaporate, thus leading to enhanced formation of hydrogen bonds. Owing to their characteristic lens shape, the resulting gel particles are named LentiKats®. The particles formed by this procedure combine the advantages of large and small beads (see Figure 1). With a diameter of about 3 to 4 mm, retention is easily possible by sieve technology or rapid settling. As they are only 200 to 400 µm thick, diffusion limitation is decreased significantly.

The gel lens particles have the same properties as described for the PVA cryogels. Due to the mild production conditions, e.g. no harsh chemicals and the use of room temperature for gelation, even sensitive organisms show high rates of survival as it has been shown with nitrifying bacteria (Jahnz et al., 2001). The complete immobilisation process is finished in about one hour.

Nitrogen elimination process

A simplified flow diagram for the nitrogen elimination process is shown in Figure 2. The first stage contains the lens shaped particles with encapsulated nitrifiers. No heterotrophics have been encapsulated. To minimise BOD removal in the first stage by suspended heterotrophics, activated sludge would – in contrast to the PEGASUS process – not be passed through the aerated tank to this stage. Moreover, compared to post-denitrification processes, the return sludge would not be cycled back to the nitrification. Consequently, this process has been called “pre-nitrification” instead of post-denitrification (Sievers et al., 2003). Additionally, the retention of gel particles is easier and has been realised without

Figure 1 Schematic view and image of lens shaped particles with encapsulated nitrifying bacteria
any problems by an appropriate sieve plate, equipped with a back-flushing system. The process configuration has the following benefits. First, the necessity of extremely high SRT for complete nitrification in single sludge systems, especially at low temperatures, can be avoided. Second, the nitrate recycle is not necessary due to the consequent follow up of the reaction scheme. The sufficient capacity of enabling a smooth or low decrease of pH in the aerated reactor is requisite for a more cost effective application.

The second stage is operated as a conventional activated sludge system containing a stirred tank and a settling tank. To avoid the main problem of sludge flotation by further denitrification in the settling tank of post-denitrification processes, a small aeration unit between the denitrification-reactor and the settling tank was installed. This enables inhibition of denitrification in settled sludge by presence of soluble oxygen.

**Experimental**
The lab scale experiments have been carried out continuously with settled sewage, which was collected twice a week from a municipal wastewater treatment plant (WWTP). The settled sewage was fed to the system via a continuously stirred tank by different constant flow rates of settled sewage between 1.7 and 6.25 L per hour. Two reactors (nitrification and denitrification) with reaction volumes of 2.8 L (aerated) and 9 L (not aerated) were operated as stirred vessels. Additional reaction volume for a small bubble column (volume 0.4 L) after denitrification was used. The aerated reactor was filled with lens-shaped particles including encapsulated nitrifiers by volumetric content of approximately 20 to 25%. Online-measurement of pH, temperature and stirring in both reactors, flow rate of settled sewage, air, return sludge and dissolved oxygen in the nitrification reactor were realised to check correct system operation.

Additionally, batch samples were collected before and after each process unit once a day and five days per week for laboratory analysis of COD, both homogenised and filter, ammonia, nitrite, nitrate, etc.

Table 1 condenses the most relevant process parameters with their minimum, maximum and average values from day No. 200 to day No. 700 for the nitrification and from day No. 200 to day No. 400 for the denitrification.

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**Figure 2** Flow diagram for "pre-nitrification" process

**Table 1** Main process parameters

<table>
<thead>
<tr>
<th></th>
<th>Settled sewage</th>
<th>Nitrification</th>
<th>Denitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
<td>average</td>
</tr>
<tr>
<td>O₂ [mg/L]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.1</td>
<td>9.4</td>
<td>7.9</td>
</tr>
<tr>
<td>T [°C]</td>
<td>14</td>
<td>20.3</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Empty fields: not measured
Results and discussion

Nitrification and SCOD elimination

The results for nitrification and SCOD elimination are shown in Figure 3 for the periods of day No. 200 to 380 and No. 525 to 700. To eliminate variations in the measurements due to batch samples, data have been filtered with a moving-average at a period of 7 days. The nitrification rate has been increased for the first 270 days due to reduction of HRT and improved nitrogen loading. After that, a stabilised reaction rate between 40 to 60 mgN/L·h has been reached and continued for 400 days.

During nitrification a substantial SCOD elimination took place. A high variation between 25 and 150 mg/L·h was found for the SCOD reaction rate. Due to the constant operation of the reactor, the changes in settled sewage characteristics concerning different concentration of heterotrophics should be the reason. This may be caused by low efficiency of primary settling of the WWTP due to different flow rates and batch-wise water recycling from the dewatering unit.

Denitrification

For complete denitrification, a suitable COD/N-ratio with respect to a sufficient amount of carbon source is necessary. The results of the denitrification process in the period day No. 200 to 400 are shown in Figure 4 by decomposition of SCOD in relation to decomposition of total inorganic nitrogen. Additional graphs are included to compare the measured points with published sufficient COD/N-ratios for acetic acid and unspecific wastewater (Henze et al., 2000). It is obvious that the nitrate decomposition could reach 10 to 20 mgN/L while the denitrification process was driven not only by the SCOD decomposition, because too many points were below the suitable COD/N-ratio of unspecific wastewater. Therefore, the particulate COD coming from the first process contributes substantially to the denitrification rate.

By taking into consideration that the first reactor is driven as a chemostat and therefore the SRT is equal to the HRT, the concentration of heterotrophics in the reactor is relatively low. This would lead to a very high loaded sludge and therefore increased adsorption and/or biosorption effects are involved. The amount of adsorbed/biosorbed SCOD is unknown in this study, but it is possible to increase it up to 80% of the inlet depending on sludge loading, SCOD concentration in the reactor and the reactor operation mode (Morawe, 1995). Additional to adsorbed SCOD, particulate COD of heterotrophics would be produced through oxidation. Moreover, particulate COD of settled sewage is passing through the nitrification without oxidation due to the low HRT of 30 to 60 minutes. All three fractions...
of particulate COD would be present in bioflocs in the effluent of the nitrification process, which have not been measured due to variations of parameters and analytical capacities.

The particulate COD is available for denitrification only after hydrolysis to SCOD. Typical hydrolysis rates under anoxic conditions could be calculated by the Activated Sludge Model No. 2 (Henze et al., 1995). The model introduces the hydrolysis rate $r_{NO3}$ under anoxic conditions by

$$r_{NO3} = \frac{X_S/X_H}{K_X + X_S/X_H} \cdot \frac{S_{NO3}}{K_{NO3} + S_{NO3}} \cdot X_H$$

with parameters and kinetic coefficients according to Table 2.

By using typical model parameters for sewage from Table 2 above, the calculated overall hydrolysis rate would be around 20 mgCOD/L·h for an assumed concentration of heterotrophics of one third of total sludge concentration. It should be noted that the sludge concentration (sum of heterotrophics and particulate COD) was about 1 g/L or less most of the time and the portion of heterotrophics could not be measured. Additionally, changes in particulate COD in raw sewage have a high influence on the sludge characteristics. In Figure 5 the calculated SCOD production by hydrolysis has been taken into consideration additionally to the measured SCOD decomposition in the denitrification process. Most of the measurements are now more in line with the published minimum COD/N-ratios. But at low nitrate decomposition rates, the anoxic hydrolysis must be lower than calculated due to the fact that many results were below 5 mgN/L nitrate decomposition at high SCOD concentrations.

Based on the fact that the concentration of heterotrophics has a direct influence on the hydrolysis rate, the assumed concentration of heterotrophics seems to be lower at low denitrification rates and the SCOD calculated could be too high. Moreover, an adapted

**Table 2** Parameters for calculation of the hydrolysis rate under anoxic conditions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Component</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\eta_{NO3}$</td>
<td>Reduction factor</td>
<td>0.7 (ASM No.2)</td>
<td>1/h</td>
</tr>
<tr>
<td>$K_h$</td>
<td>Hydrolysis rate constant</td>
<td>0.08 (ASM No.2)</td>
<td>–</td>
</tr>
<tr>
<td>$X_S$</td>
<td>Slowly biogradable substrate</td>
<td>666 (assumed)</td>
<td>mgCOD/L</td>
</tr>
<tr>
<td>$X_H$</td>
<td>Heterotrophic biomass</td>
<td>333 (assumed)</td>
<td>mgCOD/L</td>
</tr>
<tr>
<td>$K_{NO3}$</td>
<td>Saturation coefficient for $S_{NO3}$</td>
<td>0.5 (ASM No.2)</td>
<td>mgN/L</td>
</tr>
<tr>
<td>$K_X$</td>
<td>Saturation coefficient</td>
<td>0.2 (ASM No.2)</td>
<td>mgN/L</td>
</tr>
<tr>
<td>$S_{NO3}$</td>
<td>Nitrate concentration</td>
<td>Measured</td>
<td>mgN/L</td>
</tr>
</tbody>
</table>
aerobic SCOD oxidation in the small bubble column has not taken place, thus leading to an extended SCOD decomposition.

However, the sludge mass related reaction rate of denitrification is in the range of pre-denitrification rate of sewage (Sievers et al., 2003). Additional optimisation of denitrification by higher heterotrophic concentration, a more suitable reactor volume, and better control of SCOD oxidation in a bubble column is necessary.

Conclusions
Encapsulated nitrifying microorganisms were used for advanced nitrogen elimination of settled sewage. The process to be investigated was configured as a two step process with nitrification first as an aerated chemostat and denitrification second as an activated sludge system. In the aerated reactor, a complete nitrification for approximately 700 days has been shown at a hydraulic retention time of 30 to 60 minutes and volumetric reaction rates between 50 to 60 mgN/L-h. Parallel SCOD oxidation has been found at reaction rates between 25 and 150 mgSCOD/L. The high variability of SCOD oxidation is probably based on changes in heterotrophic concentration of settled sewage. Nevertheless, suitable nitrate decomposition between 10 and 20 mgN/L at HRT of 1.5 to 3 hours has been found, because the bioflocs in the effluent of nitrification have a substantial contribution to the denitrification rate. The hydrolysis was found as the limiting factor for the denitrification rate under anoxic conditions after re-calculation based on ASM No.2. Further optimisation of the denitrification process is necessary by higher biomass concentration and suitable denitrification volume. Additionally, for practical application, the results have to be confirmed at pilot scale onsite under realistic variability of sewage characteristics.

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References

Figure 5 Calculated available SCOD in relation to measured nitrate decomposition


