Summary

The objective of this work was to investigate the application of *Pseudomonas butanovora* cells in nitrate-elimination technologies and in anaerobic bioremedation technology based on the nitrate and aromatic substrate cometabolism of the bacteria. The denitrification ability of the bacterium provided the connection between the two different environmental biotechnology processes and our experiments allowed the detailed characterisation of the denitrification process of the *P. butanovora*.

The results of the *P. butanovora* denitrification:

- The nitrite accumulation of the cells in batch and reactor experiments revealed that *P. butanovora* is a group C denitrifying bacteria according to the classification of Martienssen and Schöps.
- The nitrite reduction of the bacterium was followed by the increase of the pH independently from the applied organic electron donor that negatively influenced the nitrite reduction. The nitrite reduction of the cell was inhibited by 50% as soon as the pH of the medium had attained 8.8.
- *Succinic acid, ethanol and acetic acid served as sufficient electron donor for the bacterium denitrification, but the measured denitrification activity was always higher in the presence of ethanol and acetic acid. The determined optimum C:N ratios for the different substrates were in great accordance with the other authors results.*
- *P. butanovora* proved to be a moderately halophilic bacterium capable of denitrifying under high salt concentration.
- The growth of the *P. butanovora* was seriously inhibited by most of the examined heavy metal ions; the cells tolerated the presence of Pb^{2+} and Cu^{2+} ions up to the tolerance level determined by Nieto et al. The heavy metal ions had severe effects on the denitrification of *P. butanovora*. Complete

denitrification at the maximum concentration of the tolerance was achieved in media containing Pb (1 mM).

- *P. butanovora* proved to be a more effective denitrifying bacterium than the other investigated bacteria.
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Results of the nitrate elimination (I):

- *P. butanovora developed biofilm on the surface of both carriers (zeolite and the ion exchange resin) but reactor filled with the carriers had not effective denitrification because of the instability of the biofilm (loss of cells due to the shear stress).*
- The bacterium immobilized into composite gel matrix exhibited high denitrification activities independently from the bioreactors set up and the operation parameters. The composite beads showed great mechanical resistance against shear stress and contributed to the concentrated denitrifying biomass available in the reactor despite the high flow rate and the appearance of other strains.
- The increase in the size of the bioreactor did not affect dramatically the denitrification activity, which was close to the activity measured in smaller bioreactor.
- At the end of the scaling up process the function of a pilot scale denitrification bioreactor with loading rate of 40-50 l^{-1} influent day^{-1} was tested. The bioreactor had 100-90% nitrate removal efficiency and the average concentration of the nitrite in the effluent was always near the prescribed level throughout the experiment in spite of the high oxygen concentration and excessive flow rate of the influent.

Results of the nitrate elimination (II):

- *The denitrification activity of the P. butanovora as a moderately halophilic bacterium was dependent on the type of the applied organic electron donor in the presence of high salt concentration. In this systems acetic acid proved to be the best substrate to drive the bacterial denitrification. The nitrite reduction of the cells was affected by the applied sodium salt as well. $NaHCO_3$ inhibited the nitrite reduction by the increasing pH. .*
- *The results of the bioreactors loaded with media containing nitrate and sodium salt in high concentration showed that the biotreatment of such media can be solved effectively and a similar technology can be run in long time where 100% nitrate removal efficiency is obtainable during 8-10 day retention time.*

Results of the bioremedation experiments:

- *In an anaerobic chemostat system we showed that the P. butanovora was able to utilise a simple aromatic substrate, salicylic acid as a growth substrate and reduce nitrate simultaneously. Under the anaerobic circumstances the oxygen necessary for the enzymatic ring cleavage was originated from the nitrate ions. During the experiments we determined the nitrate requirement of a consecutive monooxygenase and dioxygenase enzyme reaction and it was in great accordance with the amount of the theoretical ratio of 1:1. This ratio makes countable the amount of the nitrate ion will be applied during in an in situ bioremedation process being aware of the type and the concentration of the contaminants avoiding the additional en*

The high denitrification capacity, degradation ability of short chain hydrocarbons, chlorinated aromatic compounds, aromatic compounds under

anaerobic circumstance, tolerance of some heavy metal ions and being moderately halophilic bacteria all promote the application of the Pseudomonas butanovora in further environmental biotechnology processes.