ABSTRACT OF THE PH.D. THESIS

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INVESTIGATION OF THE APPLICATION OF *PSEUDOMONAS BUTANOVORA* IN BIOTECHNOLOGICAL PROCESSES

BY

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INTRODUCTION

One of the great challenges of the mankind is the recognition at the beginning of the new century that protection of our environment is one of the most important problem to be solved.

By now the intensive industrial and agricultural production of the past 50 years has caused ever-increasing problems both in the soil and in the groundwater, which is used as drinking water in certain areas. In many areas nitrate concentration in the groundwater and the amount of the aromatic hydrocarbons exceeds the limit set up by the World Health Organisation and the European Community.

Due to the nitrate contamination of the groundwater the water of the wells in many little Hungarian settlements is unfit not only for drinking but for watering as well. Although the toxicity of nitrate is low, the consumption of drinking water contaminated with nitrate causes serious health risks because of the secondary and tertiary effects, i.e. the reduction to nitrite can cause anaemia and even death in the case of children under 2 years. Aromatic hydrocarbons have also adverse health effects inducing the development of several types of cancer.

Despite the fact that the treatment of the contaminated water and soil is a very expensive and time consuming processes a lot of chemical and physical technologies have been developed. In spite of their effectiveness these methods do not always guarantee a final solution.

Applying the special bacterial metabolisms with the ability to utilise organic pollutants as growth substrate, the environmental biotechnology has allowed the development of several efficient treatments.

The nitrate content of the groundwater can be reduced with the help of denitrifying bacteria, the final product of which is inert nitrogen gas.

Bioremedation of soil contaminated with aromatic hydrocarbons is one of the most cost effective methods applying bacteria able to degrade pollutants. The

effectiveness of the bioremedation processes depends on a lot of chemical and physical parameters from which the oxygen supply of the contaminated area was thought to be the most important factor. The bacterial degradation of aromatic compounds via ring cleavage is catalysed by the oxygenase enzymes. It has been recently proved that the oxygen originated from the nitrate reduction covered the oxygen requirement of the oxygenase reactions under anaerobic circumstances. Some bacteria are able to cometabolise nitrate and different aromatic hydrocarbons.

The potential of the application of an environmental biotechnology process depends on the behaviour and properties of the applied bacteria. Therefore both the investigation of the applicability of an uncharacterised bacterium in different remedation technologies as well as the deeper understanding of its behaviour promote the improvement of environmental biotechnology.

AIM

Our aim was to investigate the applicability of the *Pseudomonas butanovora* cells in water purification and soil remedation technologies.

- Besides the detailed characterisation of the bacterium nitrate, nitrite reduction and denitrification at the presence of different organic electron donors, high salt concentration and some heavy metal ions as well as the comparison of its denitrification activity to other bacteria denitrification activity were the basis of our study.
- Development and operation of different types of bioreactors.
- Investigation of the nitrate and a simple aromatic substrate cometabolism under anaerobic condition. The exact determination the nitrate requirement of a consecutive monooxygenase and dioxygenase enzyme reaction showing the right amount of nitrate ions will be applied in situ bioremedation process to avoid the additional environmental contamination by the usage of abundant nitrate ions.
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METHOD

In order to determine the denitrification activity of *Pseudomonas butanovora* the cells were immobilized into composite gel matrix and the resulted beads were filled into different reactor systems. The characterisation of the effluent of the bioreactors was carried out on HPLC and spectrophotometer instruments, furthermore classical microbiological methods were also used.

The anaerobic nitrate reduction and the ability of the bacterium to degrade a simple aromatic substrate were investigated in New Brunswick fermentor systems. The oxidative degradation of salicylic acid as a simple aromatic substrate was studied, since salicylic acid is a very common intermediate of the biodegradation of many 2-3-ring aromatic hydrocarbons.

SUMMARY OF THE SCIENTIFIC RESULTS

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 bacterium. The denitrification ability of the bacterium provided the connection between the two different environmental biotechnological processes and our experiments allowed the detailed characterisation of the denitrification process of the *P. butanovora*.

- 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 ratio of the inhibition of the nitrite reduction in the cell turned 50% as soon as the pH of the medium had attained 8.8.
- *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 ones.

- 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 amount of 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.
- 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 stimulant of 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 increasing pH.
- The results of the bioreactors loaded with media containing nitrate and sodium salt of high concentration showed that the biotreatment of such media can be solved effectively and a similar technology can be run in the long run where 100% nitrate removal efficiency is obtainable during 8-10 day retention time.
- 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 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 allowing the enumeration of the amount of the nitrate ions will be applied during an in situ bioremedation process; being aware of the type and the concentration of the contaminants the additional environmental contamination can be avoided by the usage of nitrate ion in excess.

LIST OF PUBLICATIONS

Kesserű P., Kiss I. Bihari Z. Polyák B. Investigation of the denitrification activity of immobilized Pseudomonas butanovora cells in the presence of different organic substrates Water Research 2002. 36: 1565-1571.
Impact factor (2000) 1.285

Kesserű P., Kiss I. Bihari Z. Polyák B. The effects of NaCl and some heavy metals on the denitrification activity of Ochrobactrum anthropi Journal of Basic Microbiology. 2002. 42:268-276.
Impact factor (2000) 0.753

Kesserű P., Kiss I. Bihari Z. Polyák B. Biological denitrification in a continious-flow pilot bioreactor containing immobilized Pseudomonas butanovora cells. Bioresource Technology 2002. Elfogadva
Impact factor (2000) 0.881

PRESENTATIONS, POSTERS

Hansel M., Kesserű P., Tatar-Kis T., Szemes M., Kiss I., Polyak B.: New prospects in aerobic water denitrification. Third International Symposium and Exhibition on Enviromental Contamination in Central and Eastern Europe, Warsaw, Poland 1996.

Tatár-K.T., Kesserű P. Mató T., Hansel M., Kiss I., Dr. Polyák B.,: Biológiai nitrátmentesítés alkalmazása az ivóvizek tisztításában Magyar Hidrológiai Társaság XV. Országos Vándorgyűlés Kaposvár 1997. (előadás)

Kesserű P. Ivóvizek nitrátmentesítése. Bay Zoltán Alkalmazott Kutatási Alapítvány Biotechnológiai Intézet, Szakmai Nap, 1998. Szeged

Perei K., Bodrossy L., Rákhely G., Kovács L. K. Bihari Z., Kesserű P., Kiss I., Polyák B., Bioremedációs eljárások alkalmazása jelentős környezetszennyezést okozó vegyületek eltávolítására ivó- és szennyvizekből, Mezőgazdaság - Környezetvédelem - Biotechnológia 1999 szimpózium, Veszprém 1999.

Kesserű P., Kiss I. Bakteriális Víztisztító rendszerek, Mesterséges szimbiózisok Budapesti BEST Bizottság, Budapesti Műszaki Egyetem Nemzetközi Tanfolyama 1999.

Kesserű P. Anaerobic biodegradation of aromatic compounds by denitrifying bacteria Hungarian Science Days, Minisymposium on Hungarian, Finnish Estonian Biotechnology, 2001. Helsinki

OTHER PUBLICATIONS

Tatár-K.T., Kesserű P. Mató T., Hansel M., Kiss I., Dr. Polyák B.,: Biológiai nitrátmentesítés alkalmazása az ivóvizek tisztításában Magyar Hidrológiai Társaság XV. Országos Vándorgyűlés Kaposvár Magyar Hidrológiai Társaság XV. Országos Vándorgyűlésének Kiadványa 1997 I Kötet 91-102 oldal

Dr. Polyák B, Perei K., Bihari Z, Eördegh G., Gárgyán A., Hansel M., Kesserű P., Kiss I., Tatár-K. T., Tóth M.: "Sárkány ellen sárkányfű" Technika Műszaki Szemle, XL. évf. 1997. 4, 19-21,

Perei K., Bihari Z., **Kesserű P.**,Dr. Polyák Béla, Dr. Bodrossy L., Dr. Rákhely G., Dr. Kovács K.: A környezetszennyező veszélyes hulladékok biológiai lebontása *Biokémia* XXII.évf. 1998. 3:61-65,

K. Perei, L. Bodrossy, G. Rákhely, Cs. Bagyinka, Z. Bihari, **P. Kesserű,** I. Kiss, P. Polyák, K. L. Kovács: Biodegradation of selected hazardous waste. Proc. COST Action 831 1999.

Kesserű P., Kiss I. Bihari Z. Polyák B. Nitrátionok kettős szerepben, az ártalmas néha hasznos lehet? Technika Műszaki szemle 44. Évfolyam, 9. Szám, 37-39. 2001.

Kiss I., **Kesserű P.,** Fehér B., Bihari Z. és Polyák B. Co-immobilization of symbiotic green algae and *Saccharomyces unispora. Symbiosis* 2002. 32:247-256. Impact factor (2000) 0.895

Hatvani N., **Kesserű P.,** Mécs I. Effects o f different inorganic salts and organic nutrient components on the growth of *Lentinula edodes* (Shiitake) mycelium on solid medium. *Journal of the Science of Food and Agriculture* (elbírálás alatt)

Impact factors (2000) : 3.814