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NITRIFICATION OF HIGH AMMONIA CONCENTRATIONS IN MEMBRANE BIOREACTORS – MICROBIOLOGICAL ASPECT

Wastewater treatment based on activated sludge is known to be one of the most effective and popular wastewater purification methods. Activated sludge, as a mixture of microorganisms, is an excellent research material for both microbiology and technology, resulting in finding a method to effectively utilize different chemicals. This research is focused on microbial community temporal changes in membrane bioreactors' flora treating wastes containing a high level of ammonia nitrogen. Knowledge about species composition and its variability over time according to the environmental factors is important from both microbial ecology and technology points of view. The aim of this study was to compare activated sludge community composition at two different sludge ages. The analysis was performed to estimate the abundance of the main species and the structure of the bacterial community pointed mainly at the ammonia and nitrite-oxidizing bacteria. The aim of the short report is mainly to familiarize the reader with problems of increasing load of nitrogen compounds resulting from different anthropogenic activities. It is obvious that the protection of our water resources is of major importance on the global scale.

Очистка сточных вод при помощи активированного ила является одним из наиболее эффективных и распространенных методов очистки загрязненных вод. Активированный ил, являющийся смесью микроорганизмов, представляет собой великолепный материал как для микробиологических, так и технологических исследований, ставящих целью нахождение способа эффективной утилизации различных химикатов. Данное исследование изучает временные изменения в микробном сообществе на мембранах биореакторов очистных заводов, чьи сточные воды содержат большое количество аммиачного азота. Знание видового состава и его изменчивости во времени в зависимости от факторов окружающей среды важно как с точки зрения экологии микроорганизмов, так и с технологической точки зрения. Задачей исследования являлось сравнение состава микробного сообщества активированного ила двух различных возрастов. Анализ проводился для оценки концентрации основных видов бактерий и структуры бактериального сообщества и сосредотачивался в основном на бактериях, окисляющих аммиак и нитриты. Основной задачей является ознакомление читателей с проблемой увеличивающейся нагрузки на окружающую среду со стороны азотсодержащих соединений в результате разнообразных видов человеческой деятельности. Очевидно, что охрана водных ресурсов является первостепенной задачей в мировом масштабе.

What is the problem with ammonia?

High ammonium content is a common feature of drainage water (or leachate). It is generated during municipal landfill and agricultural irrigation, recycling of liquid livestock slurries based on soil filtration and purification process and anaerobic sludge treatment [Hao and Martinez, 1998; Manohoran et al., 1989; Martinez, 1997; Choi et al., 2004; Fux, 2003].

Legislation has been enacted in many countries to reduce the ammonia load on receiving wastewaters. As The European Union Member State Poland are obliged to fulfil the common aims and tasks towards environmental protection. EC Directive 2000/60/EC and Urban Wastewater Treatment Directive, 91/2171/EEC imposes to the reduction of total nitrogen because nitrite and nitrate are mentioned as the factors responsible for eutrophication. For example on the territories sensitive towards biogenic components the total nitrogen contained in wastewater (for treatment plants over 10000 inhabitants) has to be diminish by 70-80 %. In a light of the stringent regulations the intensification of nitrogen removal in WWTP is required.

Ammonium-rich drainage water contained about 500-1500 g $N\text{-}NH_4/m^3$ is commonly recirculated to the main stream of municipal wastewater plants, accounting for roughly 15-20 % of the total nitrogen load to be treated in the activated sludge system [Fux, 2003]. Such injection of ammonium to a conventionally loaded stream of municipal wastewaters can lead to operational problems nitrification stage of treatment plants. So a separate treatment of such wastewater often appears more appropriate, but…

…let concentrate at first on the nitrification process

Wastewater treatment based on activated sludge is known to be one of the most effective and popular wastewater purification methods. Activated sludge, as a mixture of microorganisms, is an excellent research material both for microbiology and technology, resulting in finding a method to effectively utilize different chemicals.

Nitrification is considered as the most cost-effective, biological way to reduce ammonia from wastewater. The process, naturally appearing in the environment, covers two steps. First step is performed by ammonia-oxidizing bacteria (AOB) converting NH_4^+ to NO_2^- and then, in the second stage, nitrite-oxidizing bacteria (NOB) convert the latter to NH_3^-

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O;$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- .$$

Ammonia-oxidizing bacteria (chemoautotrophic) grow very slow comparing to nitrite oxidizers and other heterotrophs so a high biomass retention time is required to maintain the bacteria in the system and avoid their washout. The mayor players in the first step of nitrification are *Nitrosomonas sp*., *Nitrosospira sp*. while *Nitrobacter sp*. and *Nitrospira sp*. are responsible for the second step. The nitrification process are susceptible to a wide variety of factors: decrease of temperature, oxygen and/or pH concentration decrease, hydroxylamine, HNO_2, NO (which are the intermediate products), free ammonia, too short sludge age and changeable load of organic compounds and ammonia [Gernaey et al., 1997; Sundermaier and Bock, 1981; Schmidt et al., 2003; Kishino et al., 1996; Gunder and Krauth, 1999; Engelhardt et al., 1998; Cicek et al., 2001; Fan et al., 2000; Fan et al., 1996]. Lee and Werlander [Lee and Werlander, 1994] suggest that apart from chemical inhibition the nitrification process can be negatively impacted by predators. Conversely Griffiths and Rensink and Rulkens [Rensink and Rulkens, 1997] found no effect between higher organisms (protozoa/metazoa) and nitrifiers resulting from assemblation of bacteria in large agglomerates which are more resistant to grazing. Nevertheless Ghyoot and Verstrate [Ghyoot et al., 1999] report that a growth of higher organisms is greater in membrane reactors. What is the reason of such behavior – there is not many information about higher individuals in a membrane reactor.

Why membranes?

A separate treatment of wastewater much more differentiated from municipal watewaters often appears more appropriate. Various options are available for the separate treatment of high ammonia wastewaters; anaerobic ammonium oxidation and partial nitrification-denitrification are the most popular.

Operational costs of the nitrification process are related to oxygen requirements and like in every activated sludge system to an effluent suspended solids requirements.

Membrane-coupled bioreactors can resolve problem with separation of activated sludge from the treated effluent. Membrane used in presented study allows retaining the particles with diameter bigger than 0,4 μm. High efficiency and low cost are two advantages of using membrane reactors where the hydraulic residence time is independent of biocatalyst residence time. A membrane-coupled reactor is a continuously stirred tank combined with a membrane filter. Furthermore, it is possible to increase the biomass concentration considerably and thus decrease reactor volumes or sludge production rates [Rosenberger and Kraume, 2002].

The research project

The aim of the study was to observe the dynamics of activated sludge biocenosis; their consistence of nitrifiers of both stages of nitrification and a higher organisms. Among the nitrifiers Nitrosomonas oligotropha linage, Nitrosomonas eutropha/europaea (first stage of nitrification), Nitrobacter sp. and Nitrospira sp. (second stage) were investigated by using molecular methods (Fluorescence In Situ Hybridization). The group of ciliates (attached, crawling and swimming), flagellates, amebas and rotifers were considered during higher organism's analysis occurrence (classical microscopic observations). The presence and kind of filamentous bacteria is also taken into account.

Bacteria tend to aggregate in a form of flocs and than gain an advantage of the protection against predators in comparison to free living bacteria. The tendency to the floc formation can be observed by the microscopic observations of floc structure but also by it can be influenced by bacterial excretion of extracellular polymers. Polymers exposed on the microbial surface may act to absorb and bridge between cell surfaces and therefore initiate floc formation [Morgan et al., 1990; Adreadakis, 1993] and play a key role in the flocculation process. Moreover the particular bacteria can provide their competing fight by excretion of polymers. In this case extracellular matrix of activated sludge was extracted and polysaccharide, proteins, humic substances and DNA was analyzed in the extract.

If the higher organisms really have an negative effect to bacterial community the bacterial activity also should be influence by their presence. The dehydrogenase activity and respirometry activity was measured in order to obtain wide view into the state and condition of activated sludge biocenosis.

The problem with removal of ammonia is of a global importance and it is necessary to improve its effectiveness. The authors hope that the planed experiments will help with understanding of nitrification process in a membrane reactors and its better monitoring. The project is running already and the first results of the consistence of the activated sludge biocenosis will be presented during the oral presentation.

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