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BIOFILMS GROWING ON GAS PERMEABLE MEMBRANES

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ABSTRACT

In a Membrane Biofilm Reactor (MBR) gas permeable membranes are used as a substratum for bacteria to grow in. The membrane separates an oxygen containing gas space from a compartment through which wastewater is pumped. Biofilms attached to the membrane are supported with oxygen from the adhesion site, and with substrates from the bulk liquid.

The MBR is a promising tool for the aerobic treatment of industrial wastewaters. Volatile organics are kept from getting stripped into the atmosphere. Bacteria with special metabolic properties can be immobilized and exploited under controlled process conditions. By using porous membranes the bacteria are allowed to colonize not only the membrane surface but also its pores. By that, a starter culture is maintained and used as an inoculum in case biofilm gets lost through erosion, abrasion, grazing or sloughing.

Experiments have been conducted to study structure and functions of membrane bound biofilms. Visualisation of biofilms by scanning and transmission electron microscopy revealed that the pores of the membranes get densely colonized. *In situ* identification of microorganisms by genetic probes in cross sections of a biofilm could successfully be carried out. Oxygen concentration profiles measured by means of microelectrodes demonstrated the efficiency of the oxygen partial pressure as a parameter to control oxygen supply of membrane bound biofilms.

KEYWORDS

Gas permeable membranes, Membrane Biofilm Reactor, membrane bound biofilms, microelectrodes, oxygen concentration profiles, genetic probes

INTRODUCTION

Aerobic treatment of industrial process wastewaters or contaminated groundwaters is often difficult for a number of reasons. Bacterial strains with special metabolic capabilities may be required to eliminate the pollutants. Often, those strains grow very slowly, and may be easy prey of grazing organisms. For enrichment of slow growing specialists under non-sterile conditions, biofilm reactors packed with porous support media are used in practice. A reasonable biomass residence time can be achieved, and the bacteria - at least those growing inside the micropores - are protected against grazing and abrasion.

Porous membranes separating oxygen containing gas from the wastewater to be treated provide additional

advantages. Oxygen that diffuses through the membrane supports preferentially bacterial cells growing close to and inside the membrane (Fig. 1). Unlimited oxygen supply gives those cells a competitive advantage over cells growing in the distance of the membrane surface.

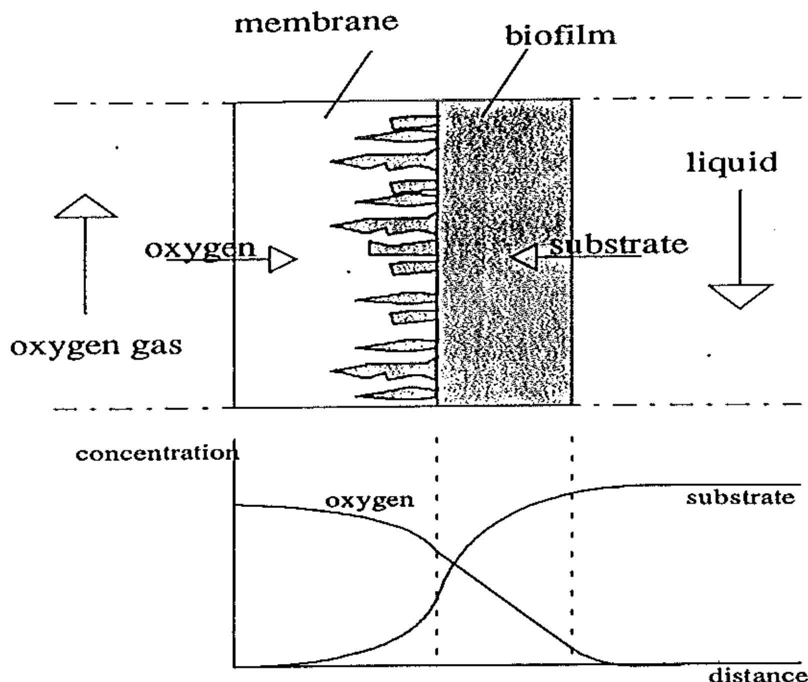

Fig. 1 Schematic representation of a biofilm system associated with a porous gas permeable membrane

Kniebusch et al. (1990) provided a detailed description of membrane biofilm systems, and discussed potential applications. Wilderer and Märkl (1989) introduced first thoughts on the design of Membrane Biofilm Reactors (MBR).

A MBR is of particular interest when the wastewater to be treated contains volatile organic substances such as benzene, toluene and xylenes (BTX compounds). As Debus and Wanner (1992) demonstrated, BTX compounds can be effectively degraded in MBRs. Stripping losses to the atmosphere are effectively minimized. MBRs can also be of advantage when surfactants are to be degraded. Since no gas bubbles are formed foaming is no problem at all.

Sufficient supply of dissolved oxygen (DO) to the bacterial cells is a prerequisite when aerobic degradation of pollutants is to be achieved. In contrast to other bioreactors, the performance of an MBR does not depend on an enhanced concentration of DO in the bulk liquid. It is sufficient when the bacteria in the biofilm are adequatly supported with oxygen. The oxygen partial pressure maintained in the gas compartment of the MBR allows easy control of the penetration depth of oxygen into the biofilm. By keeping the DO in the bulk liquid close to zero the membrane is the only location in the reactor where aerobic growth can be accomplished. Thus, the DO gradient across membrane and biofilm appears to be a strong attraction factor for aerobic strains. Strains with special degradation capacities inoculated into the MBR during start-up can be lured to the membrane and subsequently immobilized at distinct locations in the reactor.

With respect to process stability over time it is important that the specialized strains inoculated into the reactor during start up remain active despite the invasion of all kinds of other microorganisms including predators. The question to be answered is: Do the specialists mix with other bacteria once the reactor is exposed to the open biosphere, or will they stay in separate layers? In case of sloughing, abrasion or loss of the biofilm through grazing, will the specialists remain in the pores of the membrane, and provide an efficient inoculum for regeneration of the biofilm?

For modelling and design of MBRs information about colonization patterns and spatial distribution of cells in the biofilm are needed (Task Group Report, 1989). The continuum approach mostly used to describe

development and performance of biofilm reactors is certainly not applicable for MBRs, when started up with a distinct starter culture.
Species distribution in mixed cultures can be monitored by genetic probes, in particular by fluorescently labelled, rRNA targeted oligonucleotides (DeLong et al., 1989; Amann et al., 1990). This technique has been successfully applied to biofilms by Amann et al. (1992) and Manz et al., (1993). In the following, first results of microscopic investigations on species distribution of membrane bound biofilms are presented. Biofilms growing on gas permeable membranes were investigated using light and electron microscopy. Genetic probes were used to identify at least groups of bacteria in the biofilm matrix.

MATERIAL AND METHODS

Membranes of polyetherimid with an assymetric distribution of pores were used for the experiments. The membrane material is described by Kniebusch et al. (1990). The pore size was up to 10 µm at the side of the membrane exposed to the bulk liquid of the reactor. The overall thickness of the membrane was 200 µm. A cross section of the membrane is presented in Fig. 2.

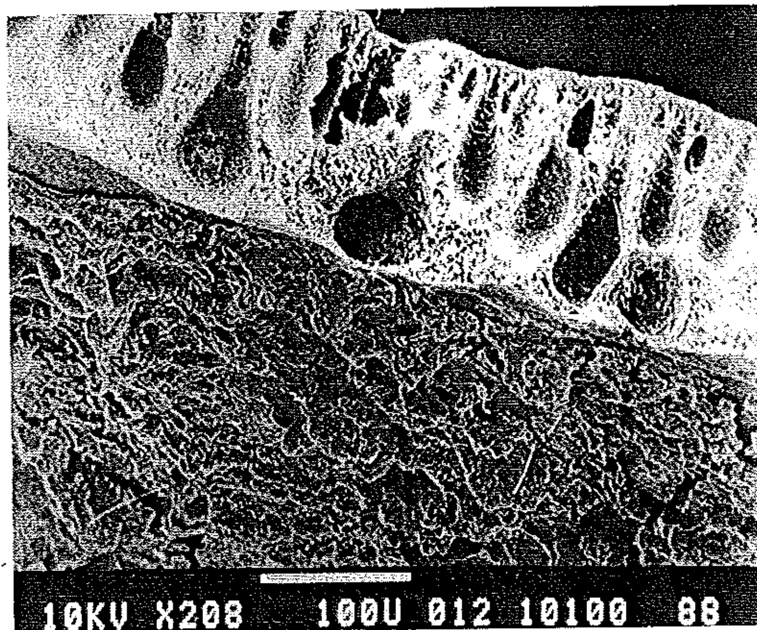

Fig. 2 Cross section of the polyetherimid membrane used for the experiments described herein. (SEM micrograph)

The membrane was placed in the centre of a flow chamber (Fig. 3). A nutrient broth was passed across the top surface of the membrane, whereas oxygen containing gas was blown through the compartment underneath the membrane. The Reynolds number in the liquid chamber was set to about 2,000. Thus, turbulent conditions in the bulk liquid can be assumed. The DO in the bulk liquid was artificially kept close to zero by purging the dissolved oxygen with nitrogen gas from the reservoir. By continuously feeding the system with a fresh nutrient solution the substrate concentration was kept constant, and suspended cells were washed out. In general, the dilution rate was kept in the range of 0.7 h^{-1}.
The MBR was inoculated with a pure culture of *Pseudomonas aeruginosa*, *Alcaligenes eutrophus* JMP 134, and with a mixed population derived from freshwater respectively.
Two types of feed compositions were applied, a complex and a mineral medium. The complex medium consisted mainly of 0.05 g/l yeast extract, 0.1 g/l casein and 0.05 g/l glucose. The pH was set to 7.0. Components of the mineral medium were 0.02 g/l glucose, 0.02 g/l acetate, 0.02 g/l NH_4Cl and various trace elements.

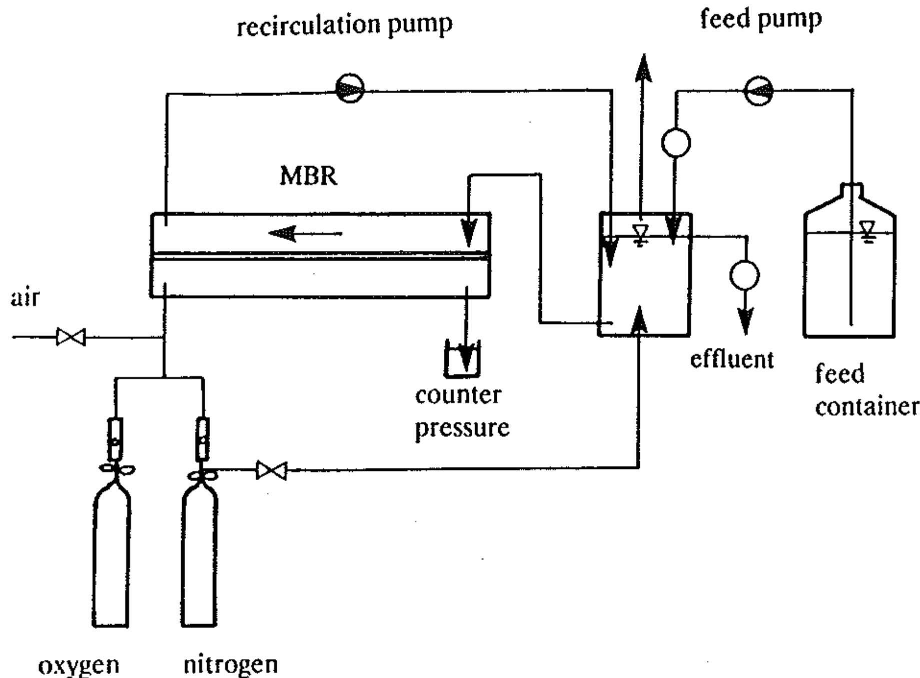

Fig. 3 Experimental set up.

After inoculation the reactor system was operated under non-sterile conditions. A wide variety of microorganisms were allowed to establish themselves at the membrane. Once the biofilm had reached a reasonable thickness (roughly after 3 weeks) the reactor was opened, and the membrane was removed for further investigation. For scanning electron microscopy the samples were critical point dried.

In situ identification of the microorganisms was performed with genetic probes. The samples were embedded in paraffin, sliced and hybridized with specific probes. During hybridization fluorescently labelled oligonucleotides bind to ribosomal ribonucleic acid (rRNA) as target molecules in the cells. When the sequences of the probe and the target molecule match, binding takes place and the fluorescent signal can be detected under the microscope (Delong *et al.* 1989).

Samples for transmission electron microscopy were doublefixed in osmiumtetroxide and embedded in Spurr`s resin (1969). Counterstaining was done after Reynolds (1963).

Oxygen profiles perpendicular to the membrane surface were taken during normal operation conditions in the MBR. For that purpose microelectrodes were employed. The tip diameter of the electrodes was 2 µm. Dr. Z. Lewandowski of the Center for Biofilm Engineering, Montana State University at Bozeman, USA conducted these experiments.

RESULTS

DO profiles were taken under true operation conditions. In figure 4 profiles are presented which were taken perpendicular to a fully developed biofilm of *Pseudomonas aeruginosa.* The biofilm was grown on mineral medium. Biofilm thickness was measured to 97 µm. The biofilm system was developed under natural oxygen partial pressure in the gas compartment, and - due to purging - at almost zero DO in the bulk liquid (profile at the top of Figure 4). Increase in the oxygen partial pressure by a factor of 5 (pure oxygen instead of air) increased the steepness of the DO profile but the DO in the bulk liquid remained almost unchanged (at the bottom of Figure 4). Obviously, the flux of oxygen, and by that the respiration activity of the bacteria

was enhanced. The increase of the steepness of the DO profile suggests that the bacteria had lived under oxygen supply limitation as long as the oxygen partial pressure was low. Increase of the oxygen partial pressure in the gas compartment caused the respiration rate to increase. From this result it should be realized that oxygen supply management is of major importance with respect to the metabolic activity of biofilms.

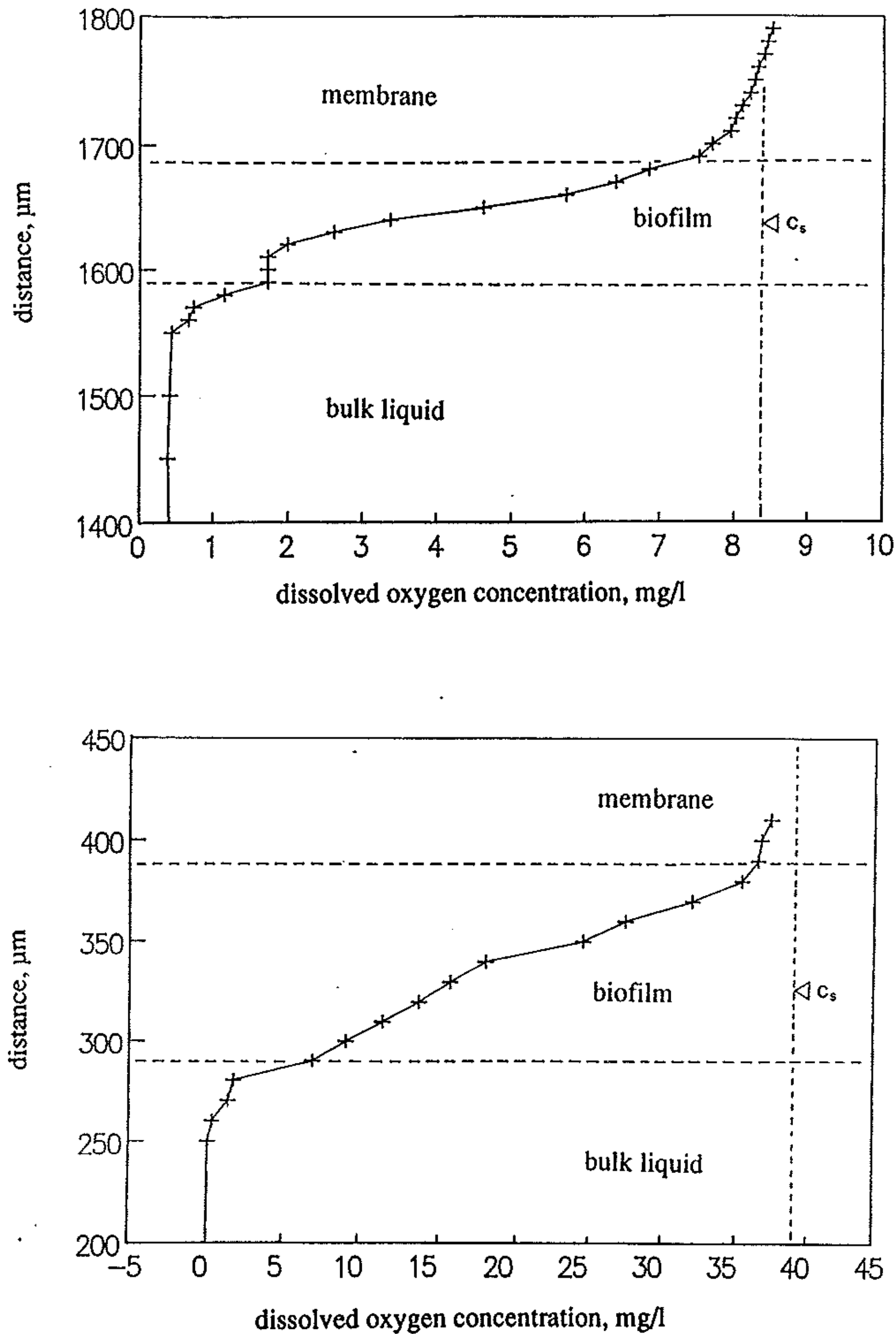

Fig. 4 DO profile with air blown through the gas compartment of the MBR (top) in comparison with the effects of the application of pure oxygen (bottom). Temperature 25 °C, Reynolds number 1,970

Figure 5 shows a cross section of the membrane and the biofilm attached to it. In the TEM micrograph it can be seen that most of the pores of the membrane were colonized by bacterial cells (presumably *Pseudomonas aeruginosa*). Some of the cells inside the pores appear empty. Starvation conditions and the resulting low growth rate may have caused lysis of cells. It is one of the goals of further investigations to study the survival rate of bacterial cells in the pores of the membrane. Only when the metabolic capacity of the cells living inside the membrane can be maintained, a potential starter culture will be available for biological regeneration of the MBR after system break down.

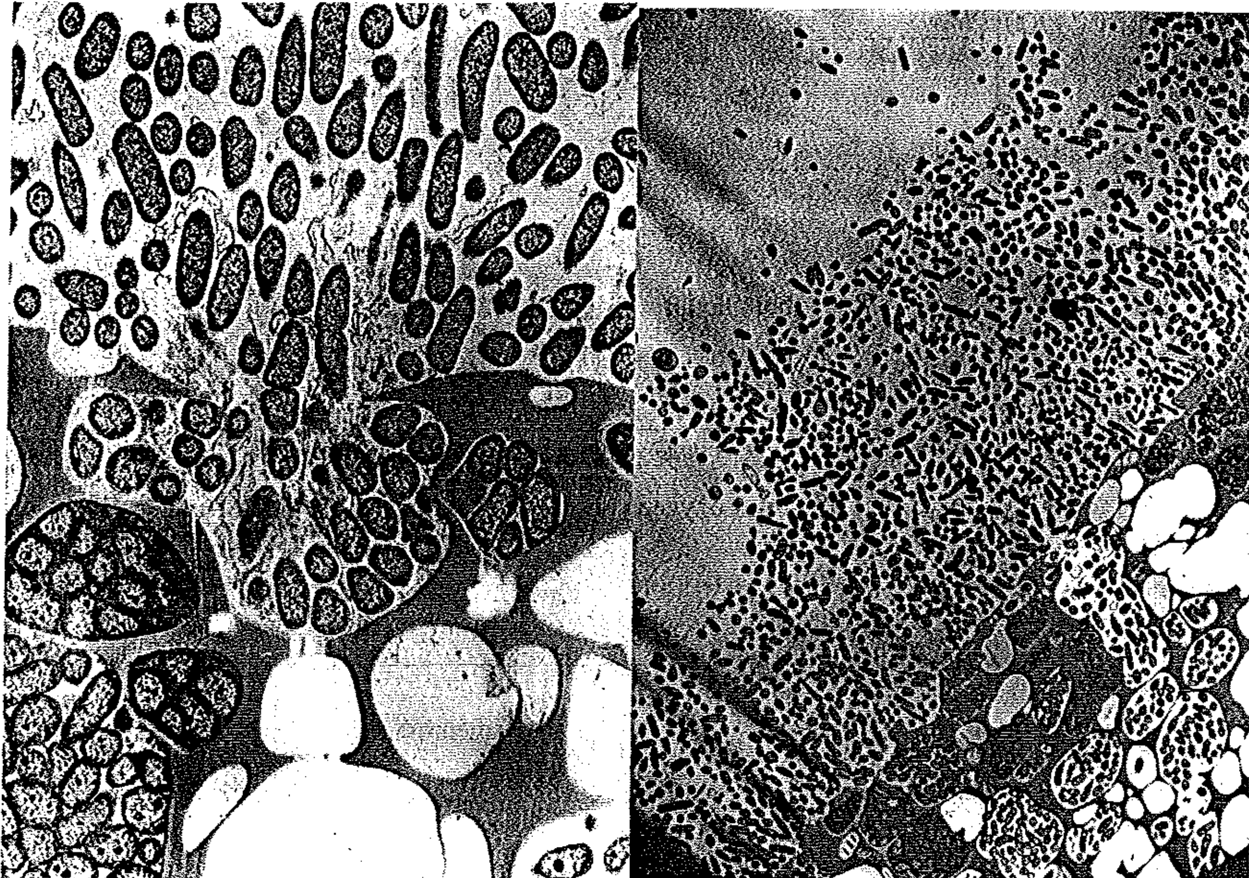

Fig. 5 TEM micrograph showing a cross section of the membrane and the membrane bound biofilm. The biofilm was started up with a pure culture of *Pseudomonas aeruginosa*.
Left: Biofilm growing out of the pores of the membrane (14850 x).
Right: Colonization of the pores 21 days after inoculation (4500 x).

A biofilm with *Alcaligenes eutrophus* JMP 134 as inoculum was grown over several weeks. The nutrient broth was a complex medium containing 10 mg/l of 2,4-dichloro-phenoxy-acetic acid (2,4-D). Biofilm morphology and 2,4-D degradation were monitored. After 5 days of growth cell clusters were observed (figure 6, left hand side). The cell distribution in the biofilm became inhomogeneous, by that. The effects of the spatial distribution on biofilm activity is yet to be studied.
A totally different picture appeared after 20 days . Figure 6, right hand side shows a cross section of the membrane (lower part of the micrograph) and the biofilm attached to it (upper part). The *Alcaligenes* cells had been overgrown by a variety of other strains. Eukariotic organisms dominated the top layer of the biofilm. A thin layer of bacterial cells (presumably *Alcaligenes eutrophus*) remained close to the membrane

surface. That layer was covered by a thick layer of testaceae. On top a layer of filamentous organisms can be seen. Despite of the dramatic change in the biofilm morphology, the 2,4 D degradation rate remained stable over the whole period of observation.

We intend to correlate these results with *in situ* localisation of *Alcaligenes eutrophus* in the biofilm. While specific probes are still under development, it could already be demonstrated that genetic probes can be effectively applied to membrane bound biofilms.

With specific probes for the three main phylogenetic groups of the proteobacteria (alpha, beta, gamma), it could be demonstrated that *in situ* identification of the microorganisms in the biofilm is possible. In a biofilm with freshwater inoculum only bacteria belonging to the beta and gamma group could be detected. The biofilm was dominated by organisms of the beta group. Only relatively few organisms belonging to the gamma group were found (the micrographs taken are not presented herein. They are informative only in color; reproduction in black and white is not appropriate, unfortunately). The distribution of both groups of organisms was fairly uniform. But that does not automatically mean that the individual strains were homogeneously distributed.

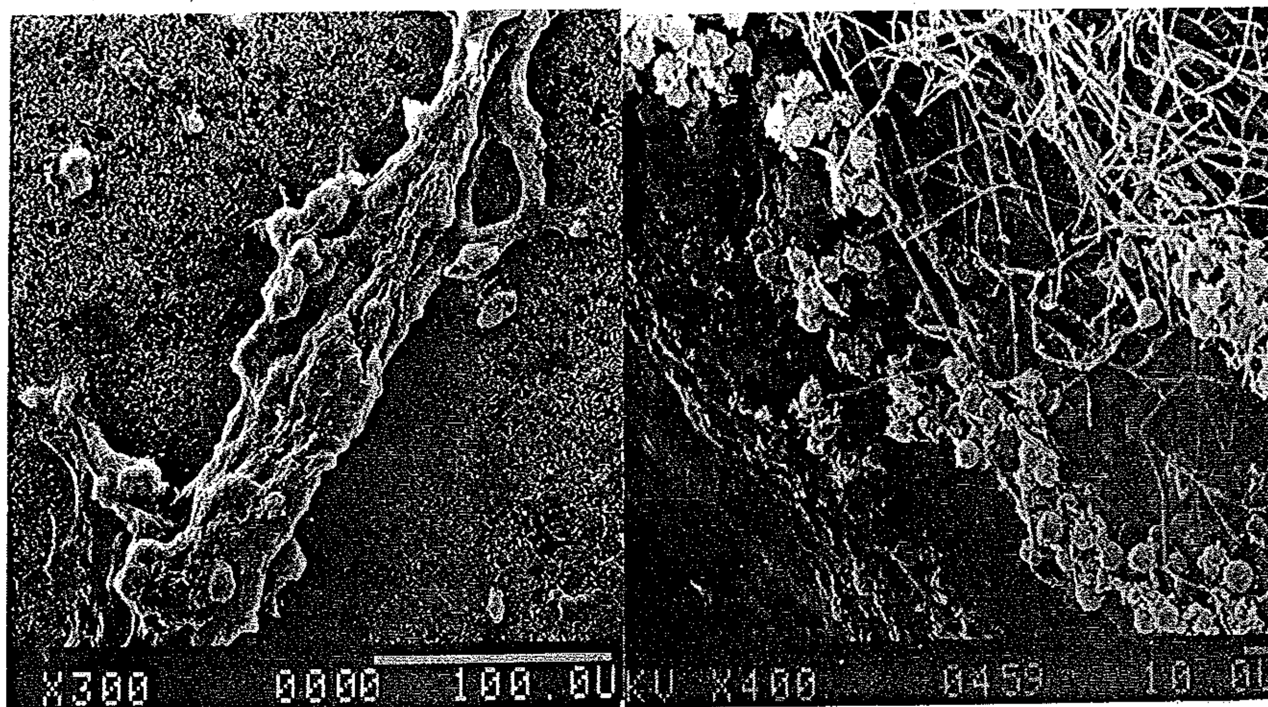

Figure 6: *Alcaliges eutrophus* biofilm 5 days and 20 days after inoculation.

CONCLUSIONS

Membrane biofilm reactors are an interesting tool to study development and performance of biofilms. DO profiles measured by means of microelectrodes revealed the importance of the flux of electron acceptors, of substrates and metabolites in and out of biofilms. Increase of the DO flux enhanced the respiration rate significantly, and by that the rate of metabolic conversion. DO flux can be controlled in a MBR by manipulation of the oxygen partial pressure in the gas phase. Problematic is - due to diffusion limitations - the accumulation of metabolites and waste products such as hydrogen and chlorine ions in the deeper regions of the biofilm. A local drop of the pH may effect the metabolic activity of the organisms, despite of the pH in the bulk liquid remaining unchanged.

The pores of the membrane were colonized by bacteria. It was observed, however, that certain strains including *Alcaligenes eutrophus* had difficulties to attach themselves to the polyetherimid surface. Once the surface was conditioned by other bacteria, further adhesion and growth of *Alcaligenes eutrophus* was relieved. It is concluded, therefore, that single species experiments are of limited value with respect to the development of bioreactors. However, detailed information is required about the interactions between various species to control development of bioreactors, and maintenance of biofilms. The continuum approach used for modelling of biofilm systems must be put in question. In the MBR used for the experiments, the inhomogeneity of the biofilm did not affect the overall performance of the reactor. Further investigations are to be conducted to quantify that observation. Genetic probes for single species of microorganisms will help to get a better understanding of the development and metabolic behaviour of biofilms.

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