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MELISSA

COMBINATION OF TWO MELISSA COMPARTMENTS WITH MEMBRANE BIOREACTORS

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I. INTRODUCTION

The objective of this project was the study of the second compartment of the MELISSA concept. In this phototrophic compartment different bacteria will be used together. These bacteria are *Thiocapsa roseopersicina*, *Rhodobacter capsulatus* and *Rhodospririllum rubrum*. The second compartment must further degrade the compounds formed in the anaerobic liquefying compartment I. These compounds are volatile and non-volatile fatty acids, carbon dioxide, peptides, aminoacids, ammonium ions, hydrogen and hydrogensulfide. In order to fulfil this task a combination was necessary between photo-autotrophic and photo-heterotrophic bacteria. Therefore the compatibility between *Thiocapsa* and *Rhodobacter* or *Rhodospirillum* is an essential factor for the design of the second compartment.
The compatibility of the growth media was studied followed by the determination of possible toxic effects of compound produced by one of the strains. In order to distinguish *Thiocapsa* from the *Rhodobacter* or *Rhodospirillum* strains a specific growth medium was tested. In order to be able to make mass balances the chemical composition of the strains was analysed. Finally it was decided to construct a lab scale reactor in which the *Thiocapsa* was separated from the *Rhodobacter* by a dialysis membrane in order to prevent the mixing of the bacteria and to allow free transport of the compounds in the solution. Finally this reactor was transformed in a continuous reactor system.

It could be shown in TN16.3 that the membrane bioreactor concept (Diels et al., 1995a, 1995b) was very useful for the linking of two Melissa compartments. Some preliminary results showed that the photoheterotrophic and the photoautotrophic compartment could be linked together via a transfer cell separating the two subcompartments with a Zirfon® or a dialysis membrane. However in order to avoid clogging on one or both sides of the membrane some extra research will be necessary.
In the following period some special attention will be paid to the kind of membrane supports and the membrane type, being either Zirfon® or a dialysis membrane. Also special attention will be paid to the design of the membrane reactor cells. This can be based on the former used Flat Sheet Reactor (FSR) or on a new kind of diffusion-cell reactor. Further different combinations of compartments will be evaluated : photoheterotrophic - photoautotrophic ; photoheterotrophic - photoautotrophic combined with imput from the liquefying compartment ; liquefying photoheterotrophic compartment.

II. THEORY

The need for the incorporation of *T. roseopersicina*, which is a sulfide oxidizer, is caused by hydrogen sulfide itself produced by the fermentative thermophilic Clostridia of the liquefying compartment (Mortimer, 1981). This hydrogen sulfide has a toxic effect on the microorganisms of the other compartments (chemical reactions and pH changes) and on humans (10 ppm = threshold value). As a matter of fact there is a real need for a sulfur cycle.
T. roseopersicina is a purple non sulfur bacterium (= Chromatiaceae) which belongs to the anoxygenic phototrophic bacteria. This means that *T. roseopersicina* is a bacterium growing under anaerobic conditions with light as energy source and without production of oxygen. The electrons which are activated by the light to produce energy are available from inorganic compounds (minerals). *T. roseopersicina* uses sulfides as inorganic compound.

The sulfides are oxidized to sulfur, which is stored in the bacterium as granules and then further oxidized to sulfates.
H. Van Gemerden made the observation that as long as there are some sulfides left in the medium, the hydrocarbons will be stored and only used when the sulfides will be disappeared (Van Gemerden, 1968). At that moment the sulfides will oxidize to sulfur, 42% and to sulfate, 58%. The quantity of synthetised cell material is dependent of the amount of reducing power used.

The environment where *T. roseopersicina* can grow is not rigorously defined. Some experiments showed that the bacterium is able to grow in light and dark anaerobic conditions and in light and dark aerobic conditions. In these conditions, the two possible systems for energy gain are photosynthesis and respiration, which are in competition for their electrons, because both systems use the same redox couples. The situations with the best growth are in the light under anaerobic conditions and in the dark under aerobic conditions with regular addition of thiosulfate for example.

In the nature the bacterium is found in fresh and salt stationary water. In fresh water, a concentration of about 5 ppm hydrogen sulfide is enough for the growth of the bacterium. In salt water the sulfate concentration is much higher than in fresh water (28 mM against 150 μM). The presence of high sulfate concentration results in a higher amount of sulfate reducing bacteria. These bacteria produce a lot of hydrogen sulfide (± 500 μM = 16 ppm S^{2-}). This hydrogen sulfide results in the growth of phototrophic sulfur bacteria, such as *T. roseopersicina*. At the upper layer an algae population will grow. This structural consortium of algae, sulfide oxidizing and sulfate reducing bacteria is called a sulfureticum or microbial mats and are mostly present in estuaries.

The reason of using *T. roseopersicina* is its ability to oxidize reduced sulfur compounds to sulfate, through and internal formation of S-granules together with light as energy source. The bacteria is also able to assimilate different organic compounds and can grow in different conditions (versatility).

A batch reactor consisted of two reaction vessels from which liquid was pumped in and out. The liquid was tangentially filtred over a dialysis membrane. The soluble compounds could pass the membrane and the bacteria were stopped by the same membrane. A flow sheet of the reactor is presented in figure 3. The system allowed *Thiocapsa* and *Rhodobacter* to grow without inhibition by each other.

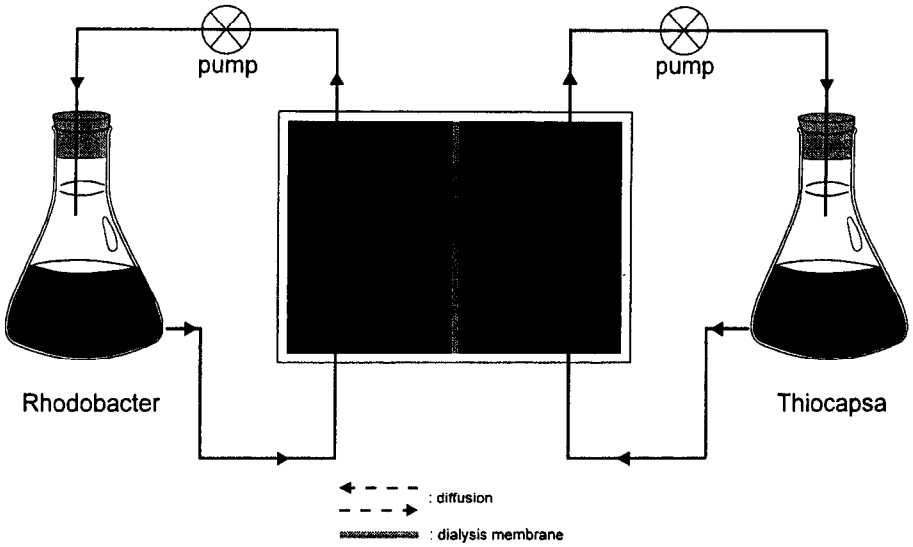

Figure 3. Flow sheet of batch reactor system.

Efforts were made to find a good membrane which allows the diffusion of chemical compounds and no transfer of bacteria. Dialysis membranes and organo-mineral membranes (Zirfon®) were tested. It could be concluded that Zirfon® membranes allowed a better diffusion and an easy biofilm formation. A problem arose by the supporting polyester layer. This layer seemed to limit the diffusion although the porosity and pore diameter were very high. In a next project supporting layers with different air permeability characteristics will be tested. Figure 4 shows a photograph of the lab scale batch reactor system. Until now the experiments were done to show that such a combined reactor system could work. Data will be presented in the next report.

Figure 4. Photo of the lab scale batch reactor system.
The combination of a *Thiocapsa* and *Rhodobacter* culture via a membrane reactor system.

III. TN31.1 MEMBRANE BIOREACTOR

III.3. DIFFUSION EXPERIMENTS THROUGH THE MEMBRANE OF THE TWO CHAMBER REACTOR

In order to model the reactions occurring in both reactor chambers it was important to investigate the diffusion of specific compounds of the influent through the membrane separating both chambers containing *Thiocapsa* and *Rhodobacter* respectively. The determination of the organo-mineral membrane diffusion characteristics was done in a simple diffusion cell. The diffusion cell is composed of two plexiglass chambers separated by an organo-mineral membrane (Z1890:10). The diffusion can be described by the first law of Fick.

$$J = - D.\frac{dSm}{dx}\ (x = 0) = \frac{-K.(Se - Sn)}{A} \qquad (1)$$

$$\text{with } K = \frac{A}{\frac{L1}{D} + \frac{L2}{De} + \frac{L3}{D}} \qquad (2)$$

J = flux through the membrane (mol/m² s)
D = diffusion coefficient in water (m²/s)
De = diffusion coefficient in the membrane (m²/s)
Sm = concentration in the membrane (mol/m³)
Sn = concentration at the open side of the membrane (mol/m³)
Se = concentration at the skin side of the membrane (mol/m³)
x = coordinate in the membrane (m)
K = mass transport coefficient (m³/s)
A = membrane surface (m²)
L1 = thickness of the stagnant layer at the skin side (m)
L2 = membrane thickness (m)
L3 = thickness of the stagnant layer at the open side (m)
Ve = volume of the skin side chamber (m³)
Vn = volume of the open side chamber (m³)

The mass balans at the skin side is

$$\frac{\delta\, Se\,(t)}{\delta t}.\ Ve = - K.[Se(t)-Sn(t)] \qquad (3)$$

The residence time in the membrane is neglected which makes

$$Sn\,(t) = \frac{Ve(0).Se(0) - Ve(t).Se(t) + Vn(0).Sn(0)}{Vn(t)} \qquad (4)$$

or

$$Se((t) = \frac{Se(0) + Sn(0)}{2} + \frac{Se(0) - Sn(0)}{2} . \exp\left(\frac{-2.K.t}{Ve}\right) \qquad (5)$$

The diffusion can than be calculated with the following input :
D (nitrate) = 1.902e-009 m^2/s (in water)
D (sulfate) = 1.065e-009 m^2/s (in water)
D (phosphate) = 6.9e - 010 m^2/s (in water)
porosity = $\varepsilon = 0.58$
tortuosity = $\tau = 2$
A = 45e-004 m^2
V = 0.0006 m^3
L2 = 250e-006m

The figures 6, 7 and 8 present the diffusion of nitrates, sulfates and phosphates respectively through the organo-mineral membrane and compare experimental and model values.

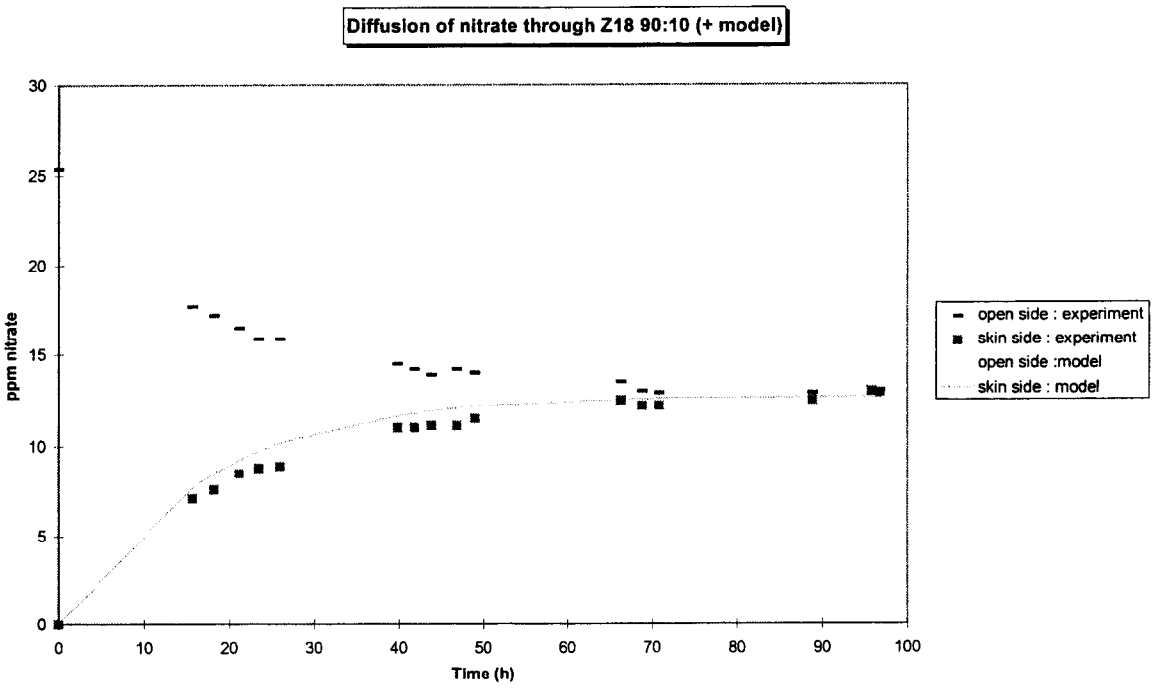

Figure 6. Diffusion of nitrates through an organo mineral membrane.

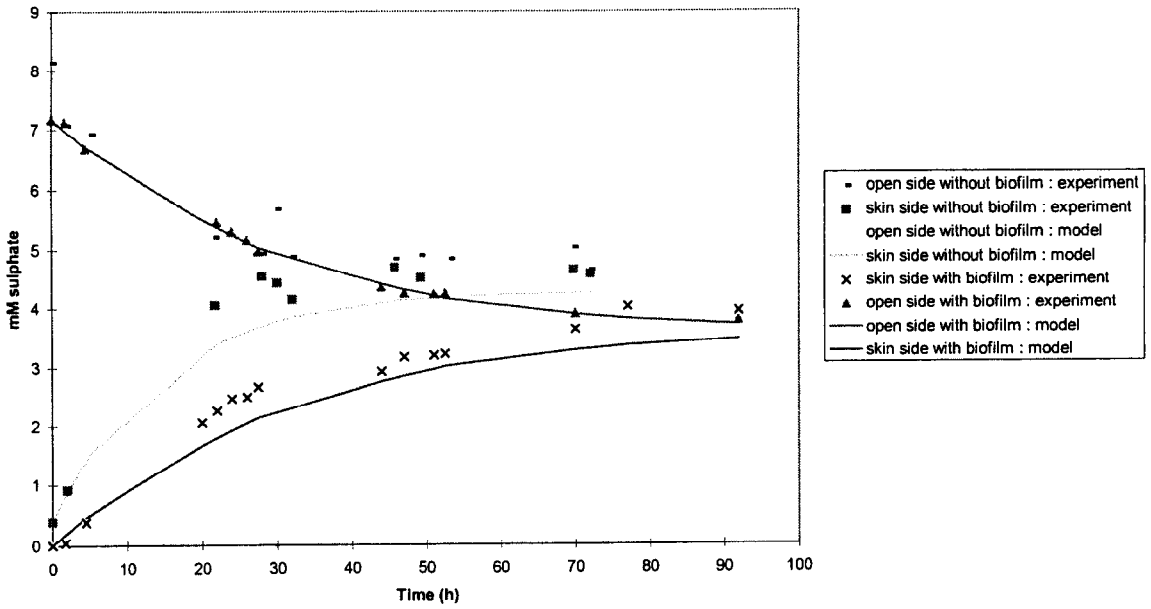

Figure 7. Diffusion of sulfates through an organo mineral membrane.

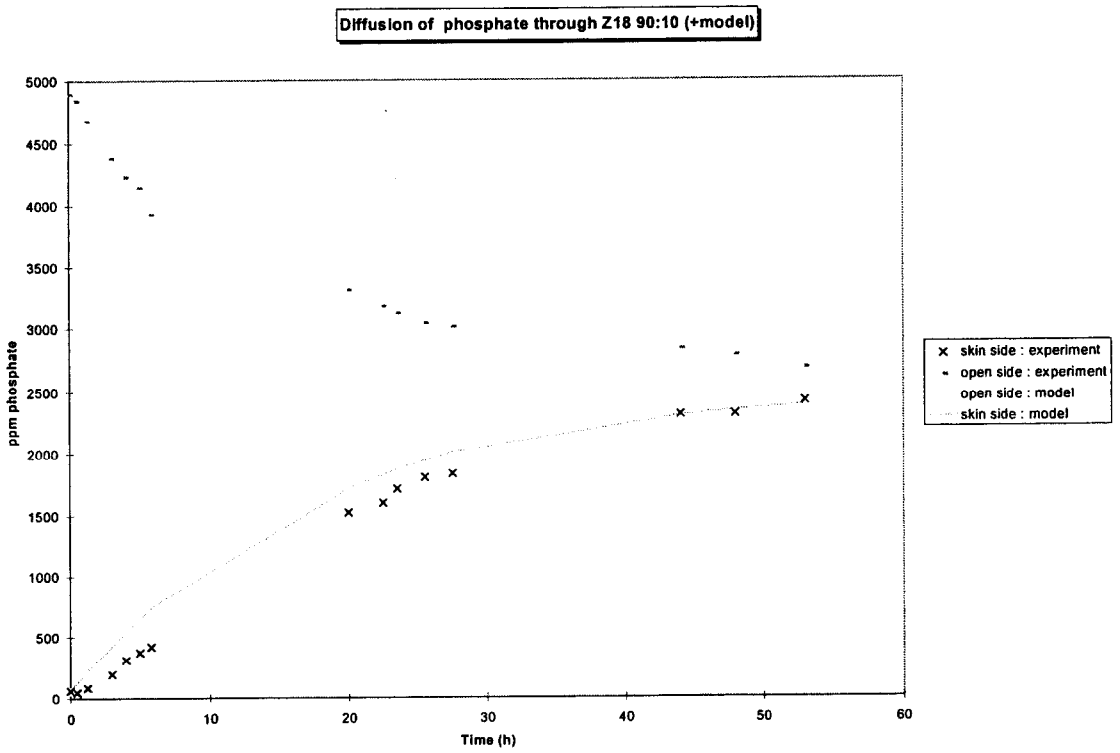

Figure 8. Diffusion of phosphates through an organo mineral membrane.

During the cell growth in both chambers also a biofilm develops on the membrane and will reduce the diffusion. This was measured in a diffusion cell with a biofilm on one side of the membrane. A comparison was made between a membrane with and one without biofilm for the diffusion of a carbon source like 3-chlorobenzoate. Figure 9 shows the diffusion curves for both situations.

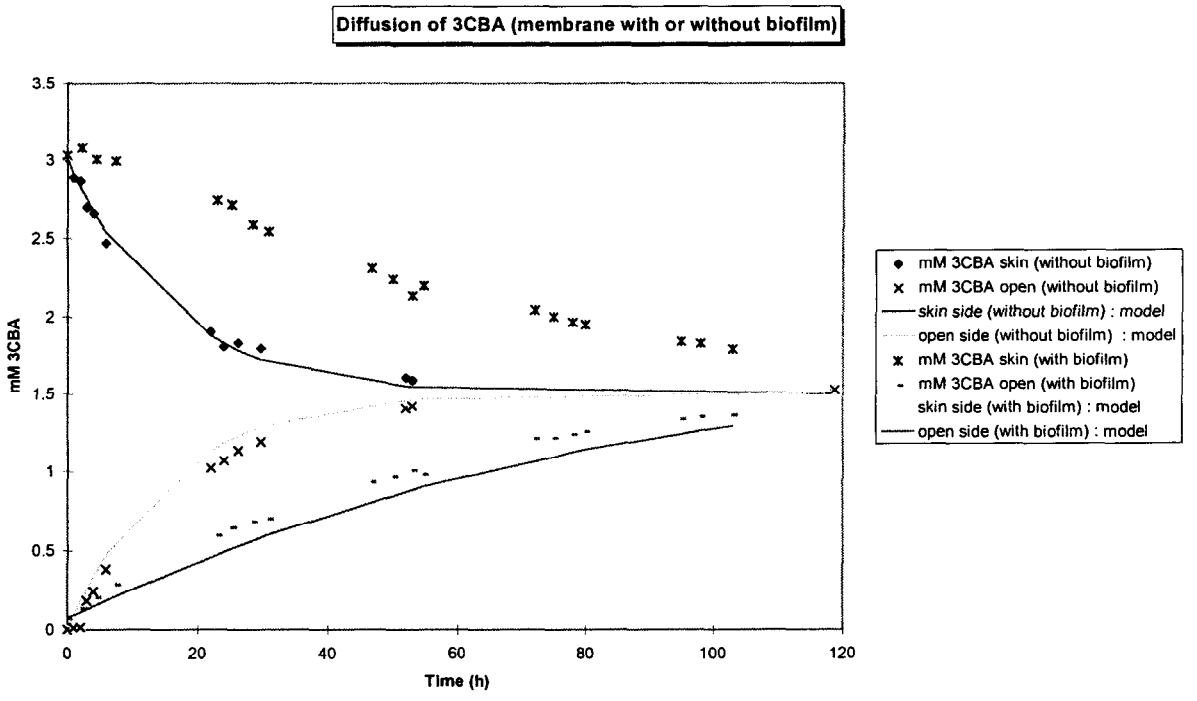

Figure 9. Diffusion curves for an organo-mineral membrane with and without a biofilm.

The mean porosity of the biofilm is unknown. In the used model the system biofilm membrane is considered as one unit with a mean porosity ε and tortuosity τ. The model is fitted with the experimental results with an unknown parameter ε/τ. The optimal ε/τ was 0.11 for a biofilm-membrane system. The ε/τ of the membrane alone as about 0.29.

Further diffusion experiments are going on and will later be combined with degradation characteristics in one model.

IV. CONCLUSIONS

Modelling of diffusion is done for nitrates, sulfates and phosphates and will be extended for acetate, lactate and butyrates. Further the model shows the influence of biofilm formation on diffusion rates. The diffusion data will be combined with degradation kinetics in a final model. This model will describe the sulfate removal and biodegradation of volatile organic acids in a two chamber phototrophic compartment II.

V. REFERENCES

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