Contract nr. PO 151933 (Vito nr. 951184)

### MELISSA

# Combination of two MELISSA compartments with membrane bioreactors

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**Technical Notes 31** 

2000/MIT/R/122

Milieutechnologie

**June 2000** 

### Management summary

The objective of this project was the evaluation of the coupling of two axenic compartments in the waste water treatment system of MELISSA. Therefor the water coming from the liquefying compartment I was used for further treatment in the second compartment being composed of an photoautotrophic and a photoheterotrophic compartment with *Thiocapsa roseopersicina* and *Rhodospirillum* respectively. In the first compartment plant debris and faeces were degraded and liquifyed by an anaerobic thermophylic consortium of bacteria. The organics were degraded into volatile fatty acids. The aim of the second compartment was to remove these fatty acids and to remove eventually present H<sub>2</sub>S gas (transformation in sulfur compounds or granules by Thiocapsa).

The wastewater from the liquefying compartment I was filtrated and than added to a membrane reactor based on two chambers. The two chambers were separated from each other either by a dialysis membrane either by a Zirfon® membrane (polysulfone combined with zirconium oxide). In one compartment the Thiocapsa roseopersicina strain and in the other compartment a Rhodospirillum strain were used. The liquid, coming from compartment I, was pumped through the system (diffusion cell, flat sheet membrane reactor, bioreactor) and the volatile organic acids were measured in samples from the input, output and the *Thiocapsa* and *Rhdospirillum* compartment. It turned out that all the volatile fatty acids were completely degraded with the exception of acetic acid were always about 5 - 6 mg acetic acid/l remained as a residual concentration. The reason for this is not clear.

### **Objectives**

The objective of this research was to evaluate the combination of two axenic compartments via membrane systems in order to avoid bacterial contamination of the following compartment by the former compartment. As a test case the combination of the two parts of the phototrophic compartment were used and later on the compartments I and II were used for combination.

Compartment I is the thermophilic fermentation compartment which produces reduced sulphur compounds (H<sub>2</sub>S), volatile and non-volatile fatty acids (acetate, butyrate etc.), ethanol,  $CO_2$ , H<sub>2</sub>, urea and peptides.

Compartment II is a photoautotrophic and photoheterotrophic compartment and uses *Thiocapsa roseopersicina* and *Rhodospirillum rubrum*. The energy source is light and there is no incompatibility with the supernatant from the liquefying compartment I.

## TN 31.2. Combination of photoheterotrophic and photoautotrophic compartment

In order to test the compatibility between the *Thiocapsa roseopersicina* and *Rhodobacter capsulatus* a continuous reactor as presented in figure 1 was installed. Phennig medium, containing 0.5% acetate was pumped in the first reactor containing the *R. capsulatus*. Than the water with the dissolved components flowed through the membrane and was further treated in the second compartment *containing T. rosepersicina*. The influence of the biofilm formation on the flux through the membrane was presented in TN31.1. Several tests were done with either a dialysis membrane either organomineral membranes. Table 1 presents the characteristics of the two used organomineral membranes (Zirfon®). The organomineral membranes are composed of an organic polymer being polysulfon and an inorganic filler being  $ZrO_2$ .

Figure 1. Continuous membrane reactor.



Table 1. Characteristics of the two organomineral membranes.

| Parameter                  | Z18 | Z28  |
|----------------------------|-----|------|
| Pore diameter (µm)         | 0.1 | 3.0  |
| Thickness (µm)             | 250 | 300  |
| Flux (L/h.m <sup>2</sup> ) | 400 | 1800 |

Figure 2. SEM of a Z28 membrane.



The Zirfon® Z18 membrane did not allow any flux and no diffusion took place due to the bacterial growth in the membrane. The Zirfon® Z28 membrane could allow some flux and diffusion. The initial acetate concentration was reduced from 30 mg acetate/L to 5 mg acetate/L.

# TN 31.3. Combination of photoheterotrophic and photoautotrophic compartment with input from the liquefying compartment

Similar experiments as presented in TN31.2. were done with input from compartment I. *Thiocapsa* (9314) was added at the open side of the membrane with Phennig medium and *Rhodospirillum* (ATCC25903) was added at the skin side of the membrane with S&V medium. In the beginning a 50:50 mixture of S&V and Phennig medium was added in order to grow and adapt the bacteria. Later a mixture of 800 ml Phennig and 800 ml S&V (without Mg/NH<sub>4</sub>acetate) with 400 ml liquid from compartment I was pumped through the system. The liquid of compartment I was provided by EPAS (Gent) and centrifuged at Vito (Mol) before the introduction into the system. Several test systems were started and the volatile fatty acids determined by GC-analysis.

The first tests were done with the diffusion cell. With the membraneZ18. No flux could be obtained as it blocked nearly immediately. Than the membrane was replaced by membrane Z28. A good flux could be obtained and the results are presented in table 2.

| Fatty acid       | Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|------------------|--------|--------|-----------|----------------|--------|
|                  | (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| Acetic acid      | 15     | 302    | 8         | 7              | 30     |
|                  | 17     | 67     | 9         | 5              | 5      |
| Propionic acid   | 15     | 391    | 1         | 0              | 1      |
|                  | 17     | 27     | 1         | 0              | 1      |
| Butyric acid     | 15     | 27     | 1         | 0              | 1      |
|                  | 17     | 7      | 0         | 0              | 0      |
| Isovaleric acid  | 15     | 112    | 3         | 0              | 1      |
|                  | 17     | 25     | 0         | 0              | 1      |
| Valeric acid     | 15     | 15     | 1         | 0              | 1      |
|                  | 17     | 4      | 0         | 0              | 0      |
| Isocapronic acid | 15     | 4      | 1         | 0              | 0      |
|                  | 17     | 0      | 0         | 0              | 0      |
| Capronic acid    | 15     | 5      | 2         | 0              | 3      |
|                  | 17     | 1      | 0         | 0              | 1      |

Table 2. Volatile fatty acids concentration in the diffusion cell experiment.

It was observed that the volatile fatty acids were removed for nearly 100 % from the system. Only in the case of acetic acid some output (5 mg/l) was observed. In that way it can be said that propionic acid, present in the input in an even higher concentration than acetic acid was completely removed.

A similar experiment was done in the flat sheet reactor with membrane Z28 as a separator. Again *Thiocapsa* (9314) was added at the open side of the membrane and *Rhodospirillum* (ATCC25903) was added at the skin side of the membrane. S&V and Phennig medium were used for the growth of the bacteria. Afterwards a mixture of 800 ml Phennig (without Mg/NH4acetate) and 800 ml S&V with 400 ml liquid from compartment I was added in the *Thiocapsa* compartment. The liquid of compartment I was provided by EPAS (Gent) and centrifuged at Vito (Mol) before the introduction into the system. The volatile fatty acids determined by GC-analysis. This sytem was operated in a batch mode.

| Fatty acid       | Bacteria       | T0  | T1  | T4 | T5 | T6 | T7 | T8 |
|------------------|----------------|-----|-----|----|----|----|----|----|
| Acetic acid      | Thiocapsa      | 486 | 197 | 7  | 7  | 5  | 9  | 9  |
|                  | Rhodospirillum | 5   | 8   | 4  | 8  | 6  | 6  | 7  |
| Propionic acid   | Thiocapsa      | 139 | 73  | 0  | 0  | 1  | 1  | 1  |
|                  | Rhodospirillum | 0   | 1   | 0  | 1  | 0  | 0  | 0  |
| Isobutyric acid  | Thiocapsa      | 59  | 49  | 0  | 0  | 0  | 0  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 0  | 0  |
| Butyric acid     | Thiocapsa      | 332 | 192 | 0  | 0  | 1  | 0  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 0  | 0  |
| Isovaleric acid  | Thiocapsa      | 115 | 91  | 0  | 1  | 1  | 1  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 0  | 0  |
| Valeric acid     | Thiocapsa      | 16  | 2   | 0  | 0  | 0  | 0  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 1  | 0  |
| Isocapronic acid | Thiocapsa      | 0   | 0   | 0  | 0  | 0  | 0  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 0  | 0  |
| Capronic acid    | Thiocapsa      | 22  | 1   | 1  | 0  | 0  | 0  | 0  |
|                  | Rhodospirillum | 0   | 0   | 0  | 0  | 0  | 1  | 0  |

Table 3. Volatile fatty acids concentration in the flat sheet experiment in batch mode.

Again it can be concluded that all the volatile fatty acids are nearly comletely removed with the exception of acetic acid which was measured at the end of the system in cocentrations around 5 - 6 mg acetic acid/l. Although the removal was more than 98%.

A similar experiment was done in the flat sheet reactor with membrane Z28 as a separator. Again *Thiocapsa* (9314) was added at the open side of the membrane with Phennig medium and *Rhodospirillum* (ATCC25903) was added at the skin side of the membrane with S&V medium. In the beginning a 50:50 mixture of S&V and Phennig medium was added in order to grow and adapt the bacteria. Later a mixture of 800 ml Phennig (without Mg/NH<sub>4</sub>acetate) and 800 ml S&V with 400 ml liquid from compartment I was pumped through the system. The liquid of compartment I was provided by EPAS (Gent) and centrifuged at Vito (Mol) before the introduction into the system. Several test systems were started and the volatile fatty acids determined by GC-analysis.

The system ran until day 11 as a batch system. Afterwards a continuous input of the 5 times diluted compartment I liquid was added. The input was pumped at a speed of 10 ml/h with an hydraulic retention time of 4 days. The results are presented in table 4 till 11.



Table 4. Acetic acid concentration (mg/l) in the continuous reactor.

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 461    | 461       | 6              | Nm     |
| 4      | Nm     | 6         | 6              | Nm     |
| 5      | Nm     | 8         | 6              | Nm     |
| 6      | Nm     | 8         | 5              | Nm     |
| 11     | 923    | 5         | 3              | Nm     |
| 15     | 312    | 7         | 6              | Nm     |
| 19     | Nm     | 7         | 7              | 10     |
| 20     | Nm     | 7         | 6              | 9      |
| 22     | 10     | 9         | Мр             | Nm     |
| 25     | Мр     | 6         | 6              | 8      |

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 252    | 252       | 0              | Nm     |
| 4      | Nm     | 0         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 1         | 0              | Nm     |
| 11     | 278    | 0         | 0              | Nm     |
| 15     | 192    | 1         | 1              | Nm     |
| 19     | Nm     | 0         | 0              | 0      |
| 20     | Nm     | 0         | 0              | 1      |
| 22     | 1      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

Table 5. Propionic acid concentration (mg/l) in the continuous reactor.

Table 6. Isobutyric acid concentration (mg/l) in the continuous reactor

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 135    | 135       | 0              | Nm     |
| 4      | Nm     | 0         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 0         | 0              | Nm     |
| 11     | 103    | 0         | 0              | Nm     |
| 15     | 110    | 3         | 0              | Nm     |
| 19     | Nm     | 0         | 0              | 0      |
| 20     | Nm     | 0         | 1              | 0      |
| 22     | 0      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 342    | 342       | 1              | Nm     |
| 4      | Nm     | 1         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 1         | 0              | Nm     |
| 11     | 594    | 0         | 0              | Nm     |
| 15     | 179    | 3         | 1              | Nm     |
| 19     | Nm     | 0         | 0              | 0      |
| 20     | Nm     | 0         | 1              | 1      |
| 22     | 0      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 117    | 117       | 0              | Nm     |
| 4      | Nm     | 4         | 0              | Nm     |
| 5      | Nm     | 5         | 0              | Nm     |
| 6      | Nm     | 0         | 0              | Nm     |
| 11     | 200    | 0         | 0              | Nm     |
| 15     | 207    | 4         | 0              | Nm     |
| 19     | Nm     | 0         | 0              | 0      |
| 20     | Nm     | 0         | 1              | 1      |
| 22     | 1      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

Table 8. Isovaleric acid concentration (mg/l) in the continuous reactor.

Table 9. Valeric acid concentration (mg/l) in the continuous reactor.

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 20     | 20        | 0              | Nm     |
| 4      | Nm     | 1         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 0         | 0              | Nm     |
| 11     | 22     | 0         | 0              | Nm     |
| 15     | 4      | 0         | 1              | Nm     |
| 19     | Nm     | 0         | 0              | 1      |
| 20     | Nm     | 1         | 1              | 0      |
| 22     | 0      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

Table 10. Isocapronic acid concentration (mg/l) in the continuous reactor.

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 3      | 3         | 0              | Nm     |
| 4      | Nm     | 1         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 0         | 0              | Nm     |
| 11     | 4      | 0         | 0              | Nm     |
| 15     | 4      | 0         | 0              | Nm     |
| 19     | Nm     | 0         | 0              | 0      |
| 20     | Nm     | 0         | 0              | 0      |
| 22     | 0      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

| Time   | Input  | Thiocapsa | Rhodospirillum | Output |
|--------|--------|-----------|----------------|--------|
| (days) | (mg/l) | (mg/l)    | (mg/l)         | (mg/l) |
| 0      | 15     | 15        | 0              | Nm     |
| 4      | Nm     | 1         | 0              | Nm     |
| 5      | Nm     | 0         | 0              | Nm     |
| 6      | Nm     | 0         | 0              | Nm     |
| 11     | 19     | 0         | 0              | Nm     |
| 15     | 1      | 0         | 0              | Nm     |
| 19     | Nm     | 1         | 0              | 0      |
| 20     | Nm     | 0         | 0              | 0      |
| 22     | 0      | 0         | Мр             | Nm     |
| 25     | Мр     | 0         | 0              | 0      |

Table 11. Capronic acid concentration (mg/l) in the continuous reactor

For all the vola tile fatty acids a complete removal was observed with the esception of acetic acid were some 5 - 6 mg acetic acid/l was observed as a residual concentration.

#### Conclusions

The wastewater from the liquefying compartment I was filtrated and than added to a membrane reactor based on two chambers. The two chmabers were separated from each other either by a dialysis membrane either by a Zirfon® membrane (polysulfone combined with zirconium oxide). In one compartment the *Thiocapsa roseopersicina* strain and in the other compartment a *Rhodospirillum* strain were used. The liquid, coming from compartment I, was pumped through the system and the volatile organic acids were measured in samples from the input, output and the *Thiocapsa* and *Rhdospirillum* compartment. It turned out that all the volatile fatty acids were completely degraded with the exception of acetic acid were always about 5 - 6 mg acetic acid/l remained as a residual concentration. The reason for this is not clear.