

MELISSA

TN 2

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Compatibility of the compartments (1), (2) and (3)
of the Melissa Cycle

1. Transformations in compartment (1)

Influent 1 :- Cellulose
(*Rat faeces*) - Carbohydrates
 - Undigestible nutrient compounds
 - Organic nitrogen
 - Ammonia
 - Minerals

- * M.o. : *Clostridium thermosaccharolyticum* and *Cl. thermocellum*
- * Carbon cycle: In compartment (1), *Cl. thermosaccharolyticum* and *Cl. thermocellum* will be used to transform organic material into compounds that can be used by the phototrophic bacteria in compartment (I). *Cl. thermoceilum* degrades cellulose in H_2 , CO_2 , lactic acid, acetic acid, butyric acid and ethanol. The same metabolites are formed by *Cl. thermosaccharolyticum* with the exception of butyric acid.
- * Nitrogen cycle: Rat faeces mainly contains ureum, NH_4^+ , amino acids and proteins as nitrogen source. The transformation of ureum and larger proteins by *Cl. thermosaccharolyticum* and *Cl. thermocellum* has to be studied. if the nitrogen recovery is insufficient, we propose to screen for a proteolytic anaerobic thermophilic bacteria. After tile first compartment, all nitrogen compounds should be converted to NH_4^+ amino acids and/or ureum.
- * Minerals: No limitation of minerals throughout the whole cycle is expected. Faeces contains 25 % of inorganic compounds, expressed on the total dry matter.

Effluent (1) :- HLac, HAc, HBut
 - Ethanol
 - CO_2/H_2
 - NH_4^+
 - Ureum
 - Amino acids
 - Minerals

Waste : - Undigestible nutrients

2. Transformations in compartment (2)

Influent 2 = *Effluent (1)*

- * M.o.: *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata*

- * Carbon cycle: *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata* both grow on lactic acid, acetic acid and butyric acid. Ethanol is one of the main transformation products of *Cl. thermosaccharolyticum* and *Cl. thermocellum* and is metabolized by *Rhodospirillum rubrum* in contrast with *Rhodopseudomonas capsulata*. However, the latter strain has the advantage to be able to grow very well on H_2 and CO_2 when no organic components are available. H_2 and CO_2 are produced in compartment (1). Especially H_2 can not be used in the other compartments. Therefore, we propose to split up compartment (2) in (2a) and (2b) and to colonize the subcompartments with the same bacteria: *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata*. Subcompartment (2a) (photoheterotrophic) would tackle with the soluble effluent (1). Subcompartment (2b) (photoautotrophic) would handle the gasphase of effluent (1) with a minimum of soluble effluent (2a). The division in 2 subcompartments is required because hydrogen consumption is inhibited by fatty acids. Otherwise, connection with regulable flow from (2a) into (2b) are easy to make and to manage. Especially *Rhodopseudomonas capsulata* will be of importance in compartment (2b) as it can grow very well on H_2 and CO_2 on condition that only NH_4^+ is present as nitrogen source and no organic compounds.
- * Nitrogen cycle: *Rhodospirillum rubrum* can grow on arginine.. This suggests that also ureum will be hydrolysed by the latter micro-organism. In compartment (2a), especially organic nitrogen compounds as peptides and ureum will be consumed and the effluent will be enriched with NH_4^+ . We propose to use partially the effluent of compartment (2a) as NH_4^+ -source of compartment (2b) (see Scheme 1).
- * Toxicity: We expect no toxicity of the metabolites of *Clostridia* for the phototrophic bacteria. In the literature, several publications are available of growth of phototrophic bacteria on the effluent of analogous anaerobic reactors in waste treatment.
- * Minerals: It has to be noticed that *Rhodopseudomonas capsulata* can, instead of H_2 , also oxidize sulfide to sulfur. This could be of importance if a considerable amount of H_2S is formed in one of the compartments.

Influent (2a):- Ethanol
 - HLac, HAc, HBut
 - Minerals
 - NH_4^+ , peptides, ureum

Influent (2b) : - CO_2/H_2
 - Minerals
 - NH_4^+

Effluent (2) : - SCP
- CO₂
- Minerals
- NH₄⁺

3. Transformations in compartment (3)

Influent (3) = Effluent (2b) + Gasphase effluent (2b)

* M.o.: *Nitrosomonas* and *Nitrobacter*

* Carbon cycle: Both, *Nitrosomonas* and *Nitrobacter*, are obligate aerobic bacteria and fix CO₂ via the Calvin cycle as source of cell carbon. Contradictory to *Nitrosomonas*, *nitrobacter* is facultative autotroph and can also oxidize organic compounds. The oxygen is supplied by the fourth compartment.

* Nitrogen cycle: *Nitrosomonas* and *Nitrobacter* use NH₄⁺ as nitrogen source. *Nitrosomonas* derive its energy and reducing power from the oxidation of ammonia to nitrite. *Nitrobacter* on the other hand oxidizes nitrite to nitrate.

Effluent (3) : - CO₂
- Minerals
- X₀₃-

* CONCLUSIONS *

Compartments (1), (2) and (3) seem to be compatible as all metabolites formed in one compartment, can be consumed by the other compartment(s). Toxicity of the metabolites produced in one compartment with respect to the following compartments, is not expected. This has already been proved for the link compartment (1) - compartment (3).

Remarks:

The transformations of the first compartment are very important with respect to the efficiency of the whole cycle. Therefore, we take into consideration some adaptations of the proposed model.

- To optimize the sulfur recuperation out of the faeces, it could be useful to supplement the microbial population of the first compartment with *Clostridium thermohydrosulfuricum*.

- It is also interesting to study if the liquification process in compartment (1) can be optimized by the addition of some strains isolated out of the rumen.

- Part of the faeces consists out of bacterial biomass, origination from the natural population of the gastro-intestinal track. The degradation of bacterial biomass by the strains proposed for compartment (1) can be studied separately to be able to evaluate the composition of the undigestible fraction of compartment (1).

We propose to study the above mentioned aspects of the transformations in compartment (1), although this is not included in the program itself. These studies could give additional information with respect to further optimisation of the Melissa cycle.

Scheme 1: The Melissa Cycle

