MELISSA

TN 1

MELISSA - 1989

(Microbial Ecological Life Support System Alternative)

Technical Note N° 1 : TN1 (WP1)

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MELISSA WP 1.000

A. BIBLIOGRAPHIC REVIEW OF MICROBIAL GROWTH AND SURVIVAL IN SPACE CONDITIONS

Basic bibliography is included in the Biorack report (1) in the compilation and made by Gmünder and Cogoli (2).

Most of the available data come from the experiments carried out on

- Biosatellite II (NASA published in 1971)
- Saliout 7 (Cytos 2) (published in 1984)
- Spacelab (Biorack) (published in 1988).

Growth of procaryotes as Bacillus subtilis, Staphylococcus aureus, Proteus, Salmonella typhimurium, Escherichia coli and some microeucaryotes (Chlamydomonas reinhardtii, Saccharomyces cerevisiae ...) seem not to be impaired in space microgravity conditions.

The tested microorganisms were mainly grown in heterotrophic (broth media for B. subtilis, E. coli, Saccharomyces) or in photoautotrophic conditions apparently without damage. Growth rate and speed seem to be improved (Chlamydomonas, Chlorella) in some cases.

These results seem to authorize a reasonable extrapolation to most of the microorganisms as able to survive or to thrive in space conditions. Nevertheless, there is a need for substantial information about the growth of microorganisms more relevant for a project as MELISSA as i.e. obligate and facultative chemolithotrophs for anaerobic microbes (saprophytes, anoxygenic photoautotrophs, etc.).

More information is also required about long term survival, mutation rates, role of selection pressure to maintain the desirable phenotypes and appropriate modelization.

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1. Biorack on spacelab D1. ESA SP-1091. February 1988. An overview of the first flight of Biorack, an ESA facility for life science research in microgravity.

2. F.K. Gmünder & A. Cogoli. Cultivation of single cells in space. Appl. Microgravity tech I (1988) 3. Hanser publishers, Munich 1988.

B. LIST OF STRAINS FOR POSSIBLE USE IN MELISSA

1. THERMOPHILIC CLOSTRIDIA

AVAILABLE STRAINS : (RUG : DeLey ; Kersters)

Clostridium thermaceticum
Clostritium thermocellum
Clostridium thermosaccharolyticum
Clostridium thermohydrosulfuricum

1. Special attention will be given to isolates growing optimally at 60-65°C and totally unable to grow at temperatures lower than 50°C.

2. Selection procedures should be developed to isolate thermophilic clostridia with higher capabilities of proteolysis.

2. PHOTOAUTOTROPHS

AVAILABLE STRAINS IN SCK (*)
IN RUG (Verstraete)

Rhodobacter sphaeroides ATCC17023 (*)
Rhodobacter capsulatus ATCC23782
Rhodobacter capsulatus ST407
Rhodobacter gelatinosus ATCC17011 (*)
Rhodospirillum rubrum ATCC19613 (*)
Rhodospirillum rubrum ATCC25903 (*)
Rhodomicrobium vannielli

Strains available in RUG (De Ley ; Kerstens)

Rhodobacter sulfidophilus (Hansen & Veldkamp, 1973)
Imhoff, Truper & Pfennig, 1984

Rhodocyclus gelatinosus
Rhodocyclus purpureus
Rhodocyclus tenuis
Rhodomicrobium vannielli
Rhodopila globiformis
Rhodopseudomonas acidophila
Rhodopseudomonas blastica
Rhodopseudomonas palustris
Rhodopseudomonas viridis
Rhodospirillum fulvum
Rhodospirillum molischianum
Rhodospirillum photometricum
Rhodospirillum rubrum

3. NITRIFYING BACTERIA :

Available strains :

Nitrobacter sp. ATCC25381
Nitrobacter winogradskyi ATCC25391
Nitrosomonas europaea ATCC19718
Nitrobacter agilis ATCC e 14123

4. STRAINS INVOLVED IN SULPHUR (H_2S) RECYCLING

Available strains RUG, SCK(*)

Thiocapsa roseopersicina
Thiobacillus A2 (*)
Thiobacillus novellus (*)

Comments :

Thiocapsa roseopersicina would come in the phototropic compartment ; Thiobacilli in the nitrifying compartment.

5. SPIRULINES

a. Strains received from Göttingen (Germany)

- Spirulina maxima (Setchell et Gardner) Geitler — n.ax.
  B8479
  Mr. Lefevre, nr M132/1, 1963
  Chad, Natron lake, gas vesicles - Medium 2

- S. platensis (Nordstedt) Geitler
  B85.79 G. Lajorte nr M132/2b, 1963 — n.ax.
  gas vesicles, Natron lake - Medium 2

- S. platensis
  B. 86.79 P. Compère
  Lake Chad-Natron lake, gas vesicles — n.ax.
  filaments no more spiral - Medium 2

- S. platensis
  B.257.80 E. Hegewald nr 1977/229-1977 — n.ax.
  Peru, Laguna Huacachira, Dpts. Ica
  gas vesicles, filaments partly not spiral - Medium2

b. Strain received from Institut Pasteur

Spirulina platensis nr 8005 — axenic

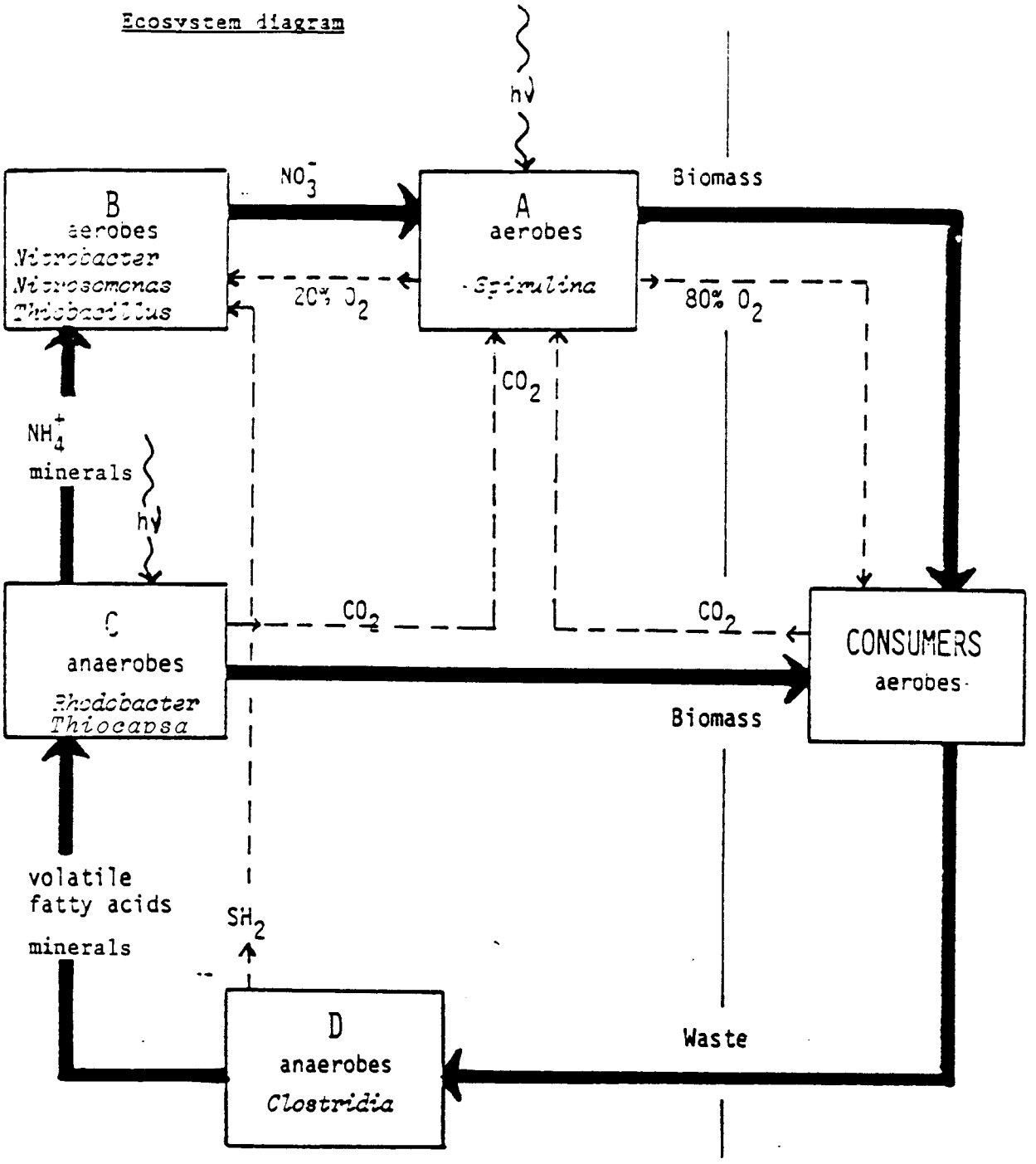
Comments :

Axeny of german strains now in progress
Strain 8005 : possible mixture of right and spiral filaments

WP 1.100 :

DESCRIPTION OF THE MELISSA COMPARTMENTS

Ecosystem diagram



A : Photosynthesis compartment

B : Nitrification compartment

C : Photoheterotrophic compartment

D : Wastes liquefying compartment

a. LIQUEFACTION COMPARTMENT

I. DESCRIPTION

1. Organisms

For the liquefaction compartment special attention is drawn towards thermophilic clostridia. Indeed, their metabolic characteristics are adequate for the anaerobic degradation of polymers, the main components of faecal matter. Moreover, their thermophilic characteristics allow anaerobic fermentation to occur under thermophilic conditions, with higher conversion rates as compared to mesophilic temperatures and a lower susceptibility for contaminations. More specifically, Clostridium thermocellum and Clostridium thermosaccharolyticum deserve further examination for their complementary metabolism concerning degradation of polymers in faecal matter. Clostridium thermocellum is a predominant species in anaerobic digestion processes. These bacteria readily degrade cellulosic and hemicellulosic substrates, converting them into ethanol, acetic acid, lactic acid, CO_2 and H_2 (Ng et al., 1977). Clostridium thermosaccharolyticum degrades dextrins, pectins and starch to the following endproducts : acetic acid, butyric acid, lactic acid, H_2 and succinic acid. Another item of considerable importance for extensive liquefaction of organic matter is the proteolysis. Siebert and Toerien (1969) pointed out that species of the genus Clostridium form an important group under the protein degrading bacteria in anaerobic digestion. However, Ng et al. (1979) observed a lack of proteolytic activity in cultures of Clostridium thermocellum grown on a defined medium with cellobiose as a carbon source.

2. Process

2.1. Growth conditions

* For its growth on cellulosic substrates Clostridium thermocellum is equipped with a set of various complementary cellulases, endo- and exoglucanases, organised in a defined supramolecular fashion, the latter being a critical factor for an efficient biodegradation (Lamed et al., 1983). According to Johnson et al. (1982), these enzymes require Ca^{2+} and a thiol-reducing agent for an extensive cellulose solubilization.

* Certain physiological features of Clostridium thermocellum have been a source of controversial reports. Growth of this bacterium has been demonstrated by Patni and Alexander (1971) on glucose, fructose and mannose. On the other hand, Ng et al. (1977) and Shinmayo et al. (1979) observed lack of growth with several strains on any carbon source except cellulose and cellobiose. It appears that the ability to ferment the above mentioned carbon sources depends upon the yeast extract concentration (must be above 0.5 %). A defined medium with cellobiose as a carbon source and urea as a nitrogen source has been composed by Johnson et al. (1981). Besides minerals, Clostridium thermocellum also requires the growth factors biotin, pyridoxamine, vitamine B12 and p-aminobenzoic acid. Growth of Clostridium thermocellum was evaluated on several synthetic media by Saddler and Chan (1982). The optimum temperature for growth is 60-64 °C and no growth occurs below 37 °C. The optimal pH is around 7.0 and drops to 5.6-5.8 after 4 days fermentation in a yeastextract-peptone cellobiose medium. Clostridium thermosaccharolyticum shows an optimal growth at 55-62 °C and reduced growth at 37 °C. This bacterium appears to have a higher acid tolerance level since after a fermentation of 5 days a pH of 4.6 is reached.

2.2. Processefficiency

The main factors determining the degradation rates of cellulosic substrates are the recalcitrance of the substrates, the growthconditions (pH and temperature), the type of microorganisms and microbial interactions and reactortype.

Weimer and Zeikus (1977) reported that the degradation of cellulose by Clostridium thermocellum amounted up to 50 %, 3 days after the onset of fermentation with a final pH of 5.5. Cooney et al. (1978) observed the specific rates of product formation by Clostridium thermocellum on corn residue, cellulose and cellobiose at a 1 % substrate concentration (Table 1). The main reaction products were reducing sugars, ethanol anb acetic acid. The ratio of ethanol/acetate depends on the type of strain, stirring and partial pressure of H_2. Stirring decreases the ratio ethanol/acetate by a factor 2 to 3 while pH_2 has an inverse effect (Lamed et al., 1988).

It should be noticed that pure culture fermentations are often confronted with accumulation of end products which become inhibitory

for microbial activity at certain concentrations (Table 2). De Baere et al. (1985) have shown that in different fermentation processes, the maximum concentration of organic acids attained is 20-30 $g.l^{-1}$ while Cooney et al. (1978) demonstrated a growth reduction of Clostridium thermocellum of 30 % at 0.5 % ethanol and of 50 % at 1 % ethanol concentration.

Furthermore, it must be emphasized that when compared to axenic fermentations, the performance of natural or artificial anaerobic reactors with mixed cultures is at a much higher level. Naturally, the microbial interactions with a continuous endproduct removal, for instance by methanogens, highly contribute to this more rapid and extensive degradation of cellulosic materials. In Table 3, some data are presented about the conversion rates and efficiences at which some natural and artificial reactors operate.

Table 1. Product formation of Clostridium thermocellum on different substrates (after Cooney et al., 1978)

Substrate	Time of fermentation (h)	Specific rate of product formation ($g.g\ cell^{-1}.h^{-1}$)		
		reducing sugars	ethanol	acetic acid
Corn residue	4	1.03	0.20	0.32
	10	0.52	0.23	0.16
Cellulose	4	0.35	0.11	0.07
	10	-	0.16	0.06
Cellobiose	4	-	0.26	0.16
	10	-	0.12	0.10

Table 2. Inhibitory concentration of organic acids on anaerobic bacterial activity (after De Baere et al., 1985)

Product	Concentration ($g.l^{-1}$)
Formic acid	0.1 - 1
Acetic acid	5 - 10
Propionic acid	1
Lactic acid	5 - 10

Table 3. Performance data of some natural and artificial anaerobic microbial ecosystemns (after Gijzen, 1987)

Reactortype	Substrate	Loading rate g.VS.l^{-1}.d^{-1}	Retention time (d)	Conversion (%)	Reference
Stirred reactor	domestic refuse	1 - 2	20 - 40	50	Van der Vlugt & Rulkens (1984)
Stirred reactor	pig manure	3 - 6	10 - 20	30 - 40	Von Velsen (1981)
Two fase reactor	tomato plants	-	14 - 21	40	Goffenk (1983)
Upflow anaerobic sludge blanket	waste water	15 - 18	0.13-0.33	95	Lettinga et al (1980)
luidized bed	waste water	20 - 60	0.04-0.08	90	Heijnen (1983)
SSF reactor	straw	12 - 24	10 - 20	30 - 40	Vandevoorde & Verstraete (1987)
SSF reactor	solid waste	10 - 20	21	30 - 40	De Baere et al (1985)
Rumen	grass	50 - 100	1 - 2	40 - 70	-

II. COMPARTMENT STABILITY

Thermophilic growth conditions not only increase conversion rates but also exclude or at least minimalize microbial contaminations. Thermophilic methanogenic bacteria, often associated with hydrolytic organisms e.g. clostridia in non-axenic fermentations, can possibly be repressed by monitoring the pH between 5.6 and 6.0.

III. SAFETY OF THE ORGANISMS

Considering Clostridium thermocellum does not show any growth at 37 °C, one might expect them to be harmless for human health.
The culture supernatans of Clostridium thermosaccharolyticum was proven to be non-toxic for mice. Moreover, broth cultures injected intravenously in rabbits or fed to rats did not show any pathogenic effect.

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b. THE COMPARTMENT OF PHOTOTROPHS

I. COMPARTMENT DESCRIPTION

1. Organisms

Purple non-sulfur bacteria are characterized by their photoheterotrophic growth on an array of organic carbon sources, based on a large set of inducible pathways. Besides, a number of these bacteria among which Rhodobacter capsulatus, Rhodobacter palustris, Rhodobacter gelatinosus and Rhodospirillum rubrum have been reported for their ability to grow photoautotrophically with H_2 as an electron donor and CO_2 reduction (Yoch, 1978).

Purple sulfur bacteria carry out an anoxygenic photosynthesis in which sulfide is oxidized to sulfate via elemental sulfur. The energy source of photobacteria when growing anaerobically, is light, which permits a complete separation of nutrient acquisition and ATP-synthesis. Therefore, the conversion of organic carbon is carried out with a considerably higher efficiency than by aerobic non-photosynthetic organisms (Gibson, 1984).

2. Process

2.1. Growth conditions :

* Purple non-sulfur bacteria assimilate a number of fatty acids and derivates, most of which are final products of the fermentative metabolism. Under conditions of non-limitation for both nitrogen and carbon, the amount of nitrogen assimilated depends on the amount of carbon available (Schick, 1971). Ammonium nitrogen limitation activates the "nitrogenase" enzyme and energy losses via H_2 production can occur.
* Concerning the C- and N-level, Kobayashi and Kurata (1978) reported optimal growth of pure culture photobacteria at a substrate concentration of 3 $g.l^{-1}$ of fatty acids.
  In experiments by Suhaimi et al. (1987), complete recovery of ammonium nitrogen and lactate carbon was obtainable in cultures with C/N ratios of 5, while maximum biomass-density was achieved at a nitrogen and carbon level of 0.8 $g.l^{-1}$ and 4.0 $g.l^{-1}$, respectively.
* For optimal growth, these photobacteria require besides carbon and nitrogen also minerals, trace elements and vitamins : thiamine,

biotine and nicotinic acid. Balloni et al. (1982) reported that for cultivation of photobacteria on a sugar-refinery waste water, the amount of nutrients required for photoanaerobic elimination of 1 g COD was (mg) : N 60; P 12; S 5; Mg 2. As a synthetic medium the basal medium of Segers and Verstraete (1983) can be used. The optimum pH is around 7 (6,0 - 8,5) and the optimum temperature between 30 °C and 35 °C.

2.1. Process efficiency and reactor design

The process efficiency depends upon several factors : the phototrophic organisms, the light intensity and penetration, carbon and nitrogen source, carbon and nitrogen levels, retention time, contaminations, reactor design.

* Seven photosynthetic bacteria were examined by Vrati (1984) for their ability to grow and produce SCP on clarified effluents of a biogas plant. The organisms tested were : Rhodobacter capsulatus, Rhodobacter palustris, Rhodobacter sphaeroides, Rhodobacter gelatinosus, Rhodobacter acidophilus, Rhodospirillum rubrum and Rhodospirillum tenue. Rhodobacter capsulatus was found to show the highest cell yield i.e. 0.08 $g.l^{-1}$ over a period of 6 days and also the highest protein content. Moreover, analysis of the SCP derived from Rhodobacter capsulatus revealed a high content of essential amino acids with special reference to the amounts of methionine, usually scarce in photobacterial SCP and lysine, comparable to the FAO reference protein (WHO 1973).

  Since light energy is the sole energy source, the growth rate of photobacteria largely depends upon the light intensity and penetration through the reactor. Suhaimi et al. (1987) obtained improved ammonium nitrogen assimilation rates and amounts of Rhodobacter capsulatus by increasing the light intensity from 12 to 120 $\mu E.m^{-2}.s^{-1}$ measured at the surface of the reactor. Dark and turbid effluents readily reduce the penetration of light in the reactor and seriously hampers the applicability of photobacteria for SCP production. Therefore, effluents slurry of the anaerobic fermentation process should be clarified prior to the phototrophic process.

* In Table 1, a short overview is presented of ammonium assimilation rates in photoanaerobic processes on wastes and defined media.

Table 1. Assimilation of NH_4^+-N from several substrates containing different C/N ratios

Substrate	NH_4^+-N ($g.l^{-1}$)	C/N	Assimilation (%)	Rate of assimilation ($g.l^{-1}.d^{-1}$)	Organism	Reference
Clarified sugar refinery wastewater	0.05	9.8	70.0	0.013	Rhodospirillaceae	Balloni et al. (1983)
Acetate and NH_4^+-N	0.30	2.7	36.7	0.030	Rhodospirillaceae	Balloni et al. (1983)
Malate and NH_4^+-N	0.40	5.0	87.1	0.230	R. gelatinosus	Shipman et al. (1975)
Lactate and NH_4^+-N	0.07	12.7	71.4	0.250	R. capsulatus	Jouanneau et al. (1984)
Lactate and NH_4^+-N	0.40	5.0	99.1	0.170	R. capsulatus	Suhaimi et al. (1988)
Silage filtrate and NH_4^+-N	0.40	5.0	99.1	0.170	R. capsulatus	Suhaimi et al. (1988)

Besides batch processes, the SCP production rate of Rhodobacter capsulatus was also studied in a continuous flow-though reactor by Driessens et al. (1987). The photobioreactor operating at upflow velocities of 1 $m.h^{-1}$ and a hydraulic residence time of 2.4 hours yielded biomass production rates 5 to 10 times higher compared to growth in batch cultures. Indeed, with Ca-lactate as a carbon source and a C/N ratio of 5, up to 10.4 g biomass l^{-1} d^{-1} could be attained. Moreover, at short residence times the photothrophs grew in dense flocs with favourable sedimentation characteristics. The reactor design is outlined in Figure 1.

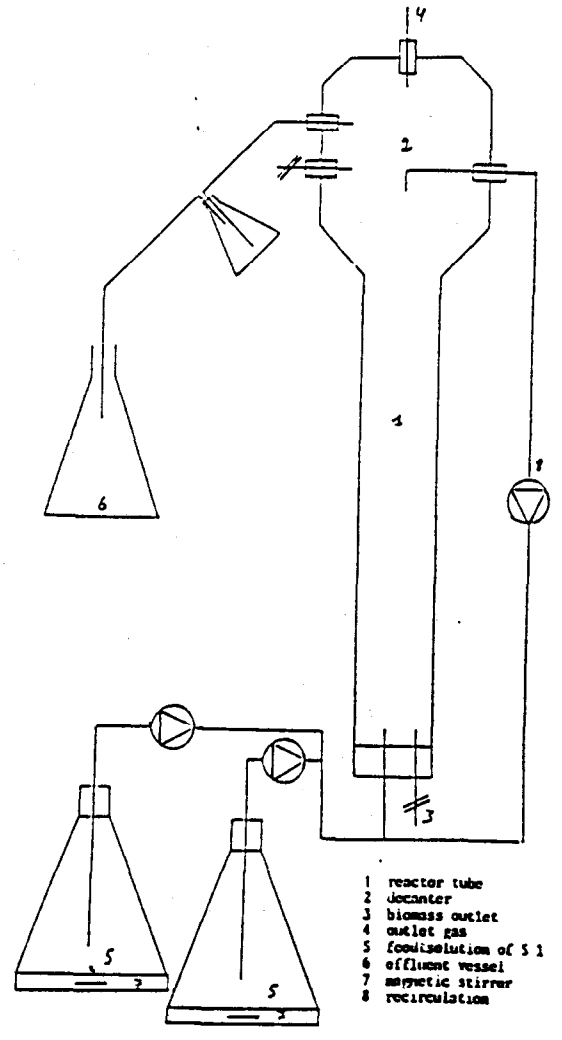

Figure 1. Arrangement of the photo-reactor system

II. Compartment stability

In illuminated non-sterile anaerobic reacotrs, photosynthetic bacteria are rather poor competitors. Algal development and the concomittant oxygen production suppress their photobiochemistry.

In addition, in the presence of sulfate, they can also be outgrown by sulfate reducing bacteria. Nevertheless, Balloni et al. (1983) reported a successfull cultivation of photobacteria grown non-axenically on sugar-refinery wastewater. The risk of contamination by oxygen evolving microalgae was reduced by maintaining a BOD-level of about 500 in the effluent.

Increasing the retention time of the wastewater in the photoreactors enhanced the possibility for algae contamination. In an experiment by Suhaimi et al. (1987), the non-axenic growth of Rhodobacter capsulatus on lactate was studied by a batch-fed mode during 45 days. As bacterial

contaminants coliforms, streptococci and clostridia were detected in small numbers (< 10^5/ml) compared to the numbers of phototrophs (10^8-10^9/ml). Selective growth inhibitors for algae were not really necessary to assure dominance by the photobacterium unless the C-level and N-level dropped below respectively 0.25 $g.l^{-1}$ and 0.05 $g.l^{-1}$.

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c. The nitrifying compartment

1. Theoretical study of the compartment process schematic

Spirulina requires NO_3^- as a source of nitrogen for growth in optimal conditions. Because the anoxygenic phototrophs compartment (Rhodobacter)mainly produc NH_4^+, a nitrifying compartment should transform ammonium to nitrate. Nitrificatio is a respiration from mineral products (chemolithotrophic bacteria). The biological conversion of ammonium to nitrate is collectively referred to as nitrification but is carried out by two gram-negative chemolithotrophic bacteria of the family Nitrobacteraceae (1) : the ammonium oxidizers and the nitrite oxidize Both groups fix carbon dioxide via the Calvin cycle for their major source of cell carbon and derive their energy and reducing power either from the oxidation of ammonia (Nitrosomonas) or nitrite (Nitrobacter)

$$\text{Nitritation} : NH_4^+ + 1.5\ O_2 \xrightarrow[\text{Nitrosomonas}]{} 2\ H^+ + H_2O + NO_2^- + 58\text{-}81\ \text{kcal}$$

$$\text{Nitratation} : NO_2^- + 0.5\ O_2 \xrightarrow[\text{Nitrobacter}]{} NO_3^- + 15\text{-}21\ \text{kcal}$$

Nitrobacteria are slow growers and produce limited amounts of biomass. Ther is a main difference between two species : Nitrobacter is a facultative autotroph (it can also oxidize organic products) while Nitrosomonas is a strict autotroph.

For pure culture of Nitrosomonas spp. the pH optimum ranges from 7.5 to 9.0 (2, 3) while optima for Nitrobacter have been given to range from 7.0 to 8.6 (4). The optimum pH for overall nitrification activity by activated sludge was 7.5 to 8.5 (5). The temperature optimum for nitrification lies between 30°C and 35°C (a little bit higher for Nitrobacter than for Nitrosomonas in axenic conditions). Oxygen is an obligate requirement for all species concerned, making adequate aeration essential. The required oxygen will come from the photosyntheti compartment (Spirulina).

Inhibition of nitrification by light has been reported by several authors. A photoinhibition of 50 % of Nitrosomonas europaea has been observed (6) after light exposure with even higher sensitivity under iron limitation. Moreover,

photoinhibition can occur after one 12 h dark-light cycle while maintaining the cycle for one week, prolonged the recovery period for several months (7). Nitrification processes had been extensively studied for waste water treatment and are well understood. Fixed culture processes should be developped in order to increase the microorganisms concentration in limited volume and to reduce plants (8). Moreover, cell fixation induces a reorganisation of membrane structure which increases its permeability (9), thus resulting in accelerated exchanges between broth and cytoplasm and cell metabolism (e.g. for Nitrobacter, cellular activity may be multiplied by 2 to 10 (10)) and in higher potential activity for fixed bacteria than for free cells (11).

Fixation offers another main advantage for nitrification processes. In fact, growth rate of nitrifying bacteria is relatively low, and washing out of plants can occur. This can be avoided by fixation which contributes to maintain biomass into the reactor. This compartment is of special interest to study immobilization of bacteria in various carriers in order to optimize their catalytic properties. Development of immobilization techniques and materials will be of prime importance for CELSS.

The nitrifying compartment could also be the place to aerobically reprocess the sulphides still remaining in the effluents coming from the upper compartments. Thiobaccilli growing autotrophically at the expense of sulphides can oxidize them to sulphate, thus allowing the completion of the sulphur-cycle.

2. Theoretical study of the material balance through the compartment

The following formula, $C_5H_7NO_2$ is generally admitted for nitrifying bacteria. Thus the stoechiometric equations for each species are as follows : (12)

Nitrosomonas : $55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \longrightarrow C_5H_7NO_2 + 54\ NO_2^- + 52\ H_2O + 109\ H^+$

Nitrobacter : $400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 \longrightarrow C_5H_7NO_2 + 400\ NO_3^- + H^+ + 2\ H_2O$

or by summation (13) :

$NH_4^+ + 1.83\ O_2 + 1.98\ H\ CO_3^- \longrightarrow 0.021\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.041\ H_2O + 1.88\ H_2CO_3$

From these equations, it is possible to find that oxidation of 25 mg ammonium produces only 3 mg of Nitrosomas and 0.5 mg of Nitrobacter. These yields (lower than 10 per cent of those observed for heterotroph bacteria) account for the low

formation of biomass in this compartment. During these reactions, nitrites can never be observed because the growth rate or Nitrosomonas is lower than growth rate of Nitrobacter under optimal conditions. It will be necessary to derive about 25 per cent of photosynthetic oxygen from Spirulina compartment, in order to get no limitating nitrification.

3. Theoretical compartment process efficiency

The maximal growth rate for nitrifying bacteria is between 0.06 and 0.09 h^{-1}.

These slow values are primarily due to the energy-demanding fixation for CO_2. In Table 1, an overview is presented of maximum nitrification activities of some nitrifying species. CO_2 does not appear to be a rate limiting substrate in sewage since no increase in growth rates in enriched cultures was noticed over the range 0.03 - 20 % (v/v) (14).

Table 1. Maximum activities per cell determined during exponential growth of nitrifiers in pure culture (after BELSER, 1979)

Culture	Activity per cell ($\mu mol.cell^{-1}.h^{-1}$)		Reference
Ammonium oxidisers			
Nitrosomonas europaea	0.020	(24)	ENGEL and ALEXANDER (1958)
Nitrosomonas europaea ATCC	0.011		BELSER and SCHMIDT*
Nitrosomonas sp.	0.023		BELSER and SCHMIDT*
Nitrosomonas briensis	0.004		BELSER and SCHMIDT*
Nitrosomonas multiformis	0.023		BELSER and SCHMIDT*
Nitrite oxidisers			
Nitrobacter strain	0.011	(23)	CHIANG (1969)
Nitrobacter "Engel"	0.018	(22)	BELSER (1977)
Nitrobacter agilis	0.042	(22)	BELSER (1977)
Nitrobacter agilis	0.009	(25)	RENNIE and SCHMIDT (1977)
Nitrobacter winogradskyi	0.012	(25)	RENNIE and SCHMIDT (1977)

* = unpublished data

Nevertheless, the efficiency of nitrifying compartment is limited by a technological parameter which is the volumetric coefficient of oxygen transfer.

In fact, as mentioned above, nitrification is a very aerobic process and the oxidation of one mg ammonium requires theoretically 4.57 mg O_2. Although the processus can present a variable stoechiometry, nitrification is limited by oxygen transfer into the reactor. The rate of O_2 consumption is given by :

$$r\,O_2 = k_e.a\ (C^* - C)$$

$r\,O_2$ rate of respiration by microorganisms ($kg.m^{-3}.^{-1}$)
$k_e.a$ volumetric transfer coefficient of O_2 (h^{-1})
C^* saturation concentration in dissolved oxygen ($kg\ m^{-3}$)
C phase " " " " "

Thus, higher results will be obtained with higher volumetric transfer coefficient of oxygen wich depends on technological parameters. During nitrification in the activated sludge process, the transfer coefficients are relatively low and rates of 0.75 kg $N.m^{-3}.d^{-1}$ are obtained. However, a rate of 3.2 kg $N.m^{-3}.d^{-1}$ had been reached with a three phases fluidised bed and a transfer coefficient into this reactor of about 80 h^{-1} (with a flow rate of 20 air volume per volume of reactor and per hour) (15).

Finally, the part of photosynthetic oxygen available to be derived to the nitrifying compartment will constitute a good regulation of the rate of nitrates production. The pH may be a key factor especially as ammonium and nitrite oxidation leads to an acid environment (pH < 6), which markedly decreases the nitrification rate. If the sewage has a pH value of 7 - 8, the oxidation should proceed quite normally, at least if the buffering capacity is adequate.

At higher pH values (> 8.5) nitrite starts to accumulate which is attributed to the sensitivity of the Nitrobacter group to ammonium salts under alkaline conditions.

It has been demonstrated that the presence of organic material stimulates cell growth and culture filtrates of heterotrophic bacteria increase nitrite oxidation (16). However, the presence of pyruvate, acetate or glycerol may induce heterotrophic growth of Nitrobacter and repress the nitrite-oxidizing system (17)

4. Intrinsic stability of the compartment and microbiological safety of selected organisms

The nitrification/denitrification is a well-known and currently used process for waste water treatment (18, 19, 20) in order to :

- limit oxygen concumption in environment,
- limit eutrophisation of ponds and rivers,
- facilitate surface water employment for industrial or domestic applications.

Thus, in the waste water treament plants, these processes work in steady state to respect norms on nitrogen rejections in environment for each country. Stability and microbiological safety of such processes are no more to be demonstrated.

Since the growth media of autotrophic nitrifying bacteria do not contain any or only small amounts of organic material, cultures can be grown without too much risk for contamination. Algal development can be repressed by protecting the nitrification reactor from light exposure.

5. Theoretical trade off between proposed microorganisms and alternative microorgani

Nitrification can theoretically be performed by a lot of microorganisms.
Nitritation : Nitrosomonas, Nitrocystis, Nitrospira, Nitrosolobus, Nitrosoglea...
Nitratation : Nitrobacter, Nitrococcus, Nitrospina, Nitrocystis, Bactoderma, Microderma.....

But, Nitrosomonas and Nitrobacter usually grow alone. Moreover, these 2 microorganisms have the higher specific rates for nitrates production. Nitrosomor is believed to be the dominant genus of the ammonia-oxidising bacteria in all habitats except for some soils. According to WALKER (21) this genus is most commonly associated with sewage or manured agricultural land, while BELSER and SCHMIDT (22) found it to be a major genus in a sewage effluent. On the other hand, Nitrobacter appears to be the dominant, if not the only, genus of nitrite oxidisers in terrestrial and freshwater habitats.

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d. Photosynthetic compartment

1 - Theoretical study of the compartment process schematic

The photosynthetic compartment will have to ensure the major tasks of the ecosystem since it will have to regenerate the atmosphere and to produce consumable biomass.

The photosynthetic filamentous Cyanobacteria, Spirulina are good candidates for this compartment on account of their high photosynthetic efficiency, their reasonable biomass production and ease of harvesting by simple filtration.

They are easily cultivated at 35°C at optimal pH 9.5. Beside limited needs for minerals as phosphate and sulfates, Spirulina mainly requires light, CO_2 and nitrates for growth.

- Light will be provided by sun or any artificial source. The main limitation of the process is light limitation of photosynthetic reactions by shadowing. One crucial problem to be solved therefore will be efficient light conduction inside the bioreactor.
- CO_2 will originate from the consumers and from the Rhodobacter compartment. Its high solubility at pH 9.5 should maintain non limiting concentrations in the culture medium. However, microgravity conditions will imply a gas exchange process (O_2/CO_2) between the culture and the surrounding atmosphere through gas permeable membranes.

2 - Theoretical study of the material balance through the compartment

Biomass of Spirulina platensis can be expressed by the chemical formula $C_5H_{8.3}O_{2.2}N$(1). The photosynthetic capacity and growth of this organism therefore can be expressed by this overall stoechiometric equation :

$$5\ CO_2 + 3.7\ H_2O + HNO_3 \longrightarrow C_5H_{8.34}O_{2.2}N + 7.25\ O_2$$

The production of 1 g biomass therefore requires 1.86g CO_2 and 0.53g nitrates and releases 2g O_2.

On the other hand, the production of 1 mole nitrate by the nitrifying compartment requires 1.87 moles O2 (see stoechiometric equations for this compartment). About 25 % of the evolved photosynthetic oxygen therefore will have to be derived to the nitrifying compartment.

3 - Theoretical compartment process efficiency

The growth rate of Spirulina platensis under non limiting light conditions has been estimated to approximately 0.028 h^{-1} [2]. Slightly slower values should be considered if cells are cultivated in continuous cultures in the ecosystem. Then, with 1 g to 1.5 g of dry biomass per litre of the culture efflux, a minimal productivity of dry biomass of 0.5 Kg/m^3/day (330-400 g proteins/m^3/day) can be expected. Considering the above steochiometric equation, this biomass production corresponds to the evolution of 1 Kg/m^3/day of oxygen, which corresponds to the needs of one person per day.

The efficiency of the photosynthetic compartment is mainly limited by light and should significantly be improved by increasing available light intensity inside the bioreactor.

4 - Intrinsic stability of the compartment

Spirulina have been cultivated in laboratory conditions for many years without significant changes. However, intrinsic genetic stability of the cells should be checked in space radiative conditions.

The stability of the photosynthetic compartment could be disturbed by some competition with a contaminant, which should not occur with axenic cultures, or by some imperfect recycling resulting in changes in the composition of the culture medium. Such possibility will have to be checked in the complete system.

5 - Microbiological safety of selected organisms

Spirulina are not pathogen to humans and not toxic. They have been and still are traditionally consumed by Aztecs and by populations around lake Chad. Moreover, they are industrially cultivated and sold as dietetic food. The theoretical possibility that they would be associated with some pathogens has never been observed and is ruled out in our case, since we will use axenic strains.

6 - Theoretical trade-off between proposed microorganisms and alternative microorganisms

Biomass production in long space flights or in planetary stations has to obey to various constraints : the limited space available i.e. directs the choice of the photosynthetic source of food towards those microalgae known for their high rate of O_2 evolution, CO_2 fixation and volumic ratio.

Eucaryotic green algae as Chlorella and Scenedesmus respond mostly to such preliminary criteria. Moreover, such an organism should easily be cultivated in liquid conditions and harvested. Resistance to pathogenes and unsensitivity to parasites is also an asset and additional criteria as digestibility and absence of toxicity should be decisive to make a final choice. Cyanobacteria belonging to the Spirulina genes respond to these major criterions. These helicoidal, multicellular filamentous microorganisms (up to 0.2 mm) have a reasonable short generation time and a good biomass production. Their decisive advantage upon green algae as Chlorella or Scenedesmus lies in 1) their digestibility, their mild flavoured taste and their dietary features (high content of proteins with a well balanded aminogram, high content in vitamines and in essential unsaturated fatty acids, digestible mucoproteic cell wall), 2) their absence of toxicity, 3) their culture medium which limits the susceptibility to pathogenes or parasites, 4) the simple harvesting methods or filtration.

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WP 1.200 :

WASTE DESCRIPTION

WP 1200 - WASTE DESCRIPTION

List of contents

WP 1200 - WASTE DESCRIPTION

1. *PREAMBLE*

Being based upon a purely biological processing concept, the MELISSA system does not pretend it could cover the processing of all waste generated onboard a manned spacecraft. In that respect, specific research is required to ensure that MELISSA would cope with human biological waste.
The following pages underline the main features of in space waste classification as they stay now and help to prepare the next steps of the introduction of MELISSA into an operational system.

2. *WASTE PROCESSING POLICY GUIDELINES*

Wastes generated during space missions are dealt with for the following reasons :

1. *to stop the build up of unpleasant or toxic smells and gases,*
2. *to eliminate health risks from primary infections or subsidiary microbial growth,*
3. *to facilitate storage in a stable and compact form,*
4. *to collect similar materials together so as to facilitate recycle or recovery of key elements.*

Wastes are categorised as gases (metabolic products and leakage), structural materials (metals, ceramics and rigid plastics) packaging flexible plastics and natural polymers), inorganic reagents (acids, based and salts), and organic material (complex mixtures from life support systems, metabolic activity of the crew and scientific experiments involving organic, chemical and biological material). In considering the total treatment of these wastes it has been considered useful to categorise them on the basis of their phase (gas, liquid and solid and whether they must be considered as regenerable or non-regenerable.

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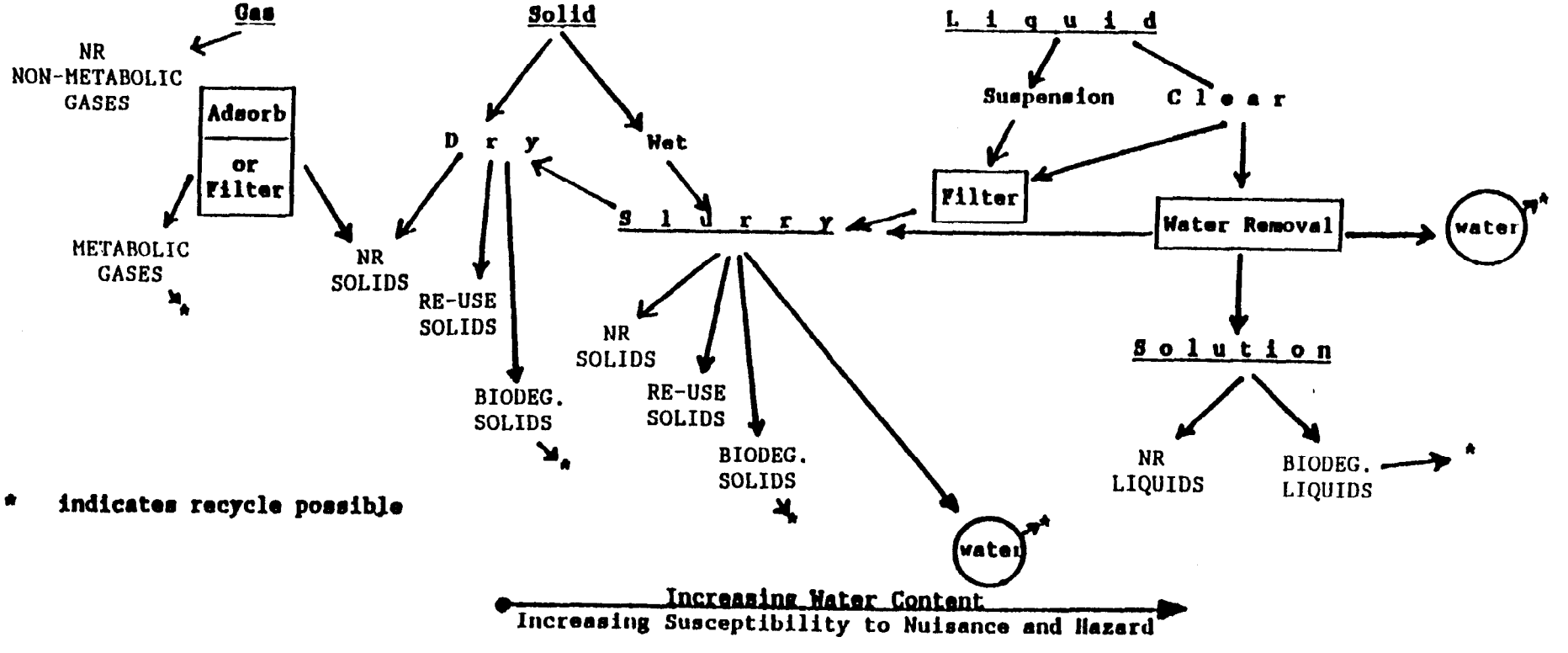
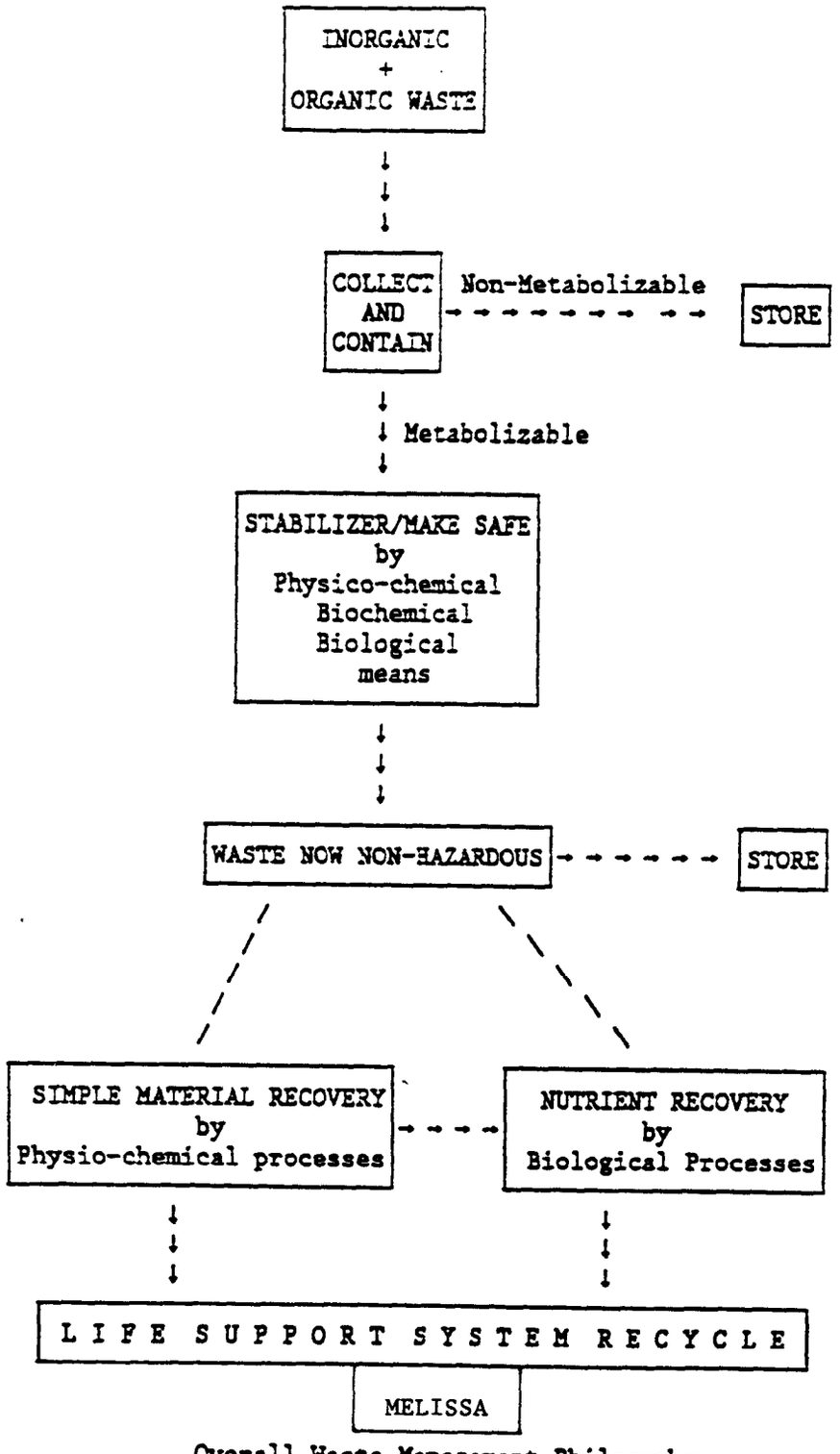
* *Most of the information contained in paragraphs 2 and 3 is taken from Waste Processing "Note to accompany Final Presentation" ESTEC 7499/87/NL/MA.*



* indicates recycle possible

GENERAL POLICY: Prevent contamination of wastes to right by wastes on the left

Relationship and Flow of Waste Types



Overall Waste Management Philosophy

3. TYPES OF WASTE

Tables 1 and 2 hereafter list best estimated quantities of wastes normally generated on board maned stations :

Gases present a unique set of problems, since they must be selectively adsorbed from the spacecraft atmosphere and concentrated before being treated. Liquid and solid wastes require detailed consideration to meet the objectives listed above. The situation is simplified by the fact that the largest proportion of the liquid wastes will be aqueous solutions. Hence one is principally concerned with treating hydrated solutes and suspended solids, in order to facilitate the recycle of the water.

Solids such as metal, ceramic or rigid polymer materials will have been selected and used because of their stability and so unlikely to pose anything other than a mechanical problem on the waste treatment side. Reactive, inorganic chemicals are unlikely to be produced in significant amounts in the open areas of a spacecraft. Any wastes produced within an experiment will have been dealt with in the experimental plan and will be contained. Hence, segregation of these contained wastes if of importance but the stabilization of organic waste does not require consideration, except where it can assist in treating other wastes.

In contrast to these generally stable inorganic wastes, a substantial proportion of the organic wastes will be biodegradable and hence require stabilization if the objectives noted above are to be satisfied. The flight crew will be a major source of organic waste (wee Tables 2 and 3 for composition of the main components : feces and urine).

Human waste is mainly characterized by human excretes, i.e. feces and urine (Tables 3 and 4), and water from washing. The excreta being mainly exhausted food have a high ratio of nitrogen to carbon as carbon skeletons have been dissimilated. Generally, all inorganic compounds are enriched. Washing procedures consume much water and enrich the waste-water with the constituents of the detergents. According to a recent paper from Japan (SHIGETA et al., 1984a) the pollutant load from use of surfactants contributed 40.9, 3.7 and 9.8% to the total pollution of domestic sewage as COD, total-N and total-P, respectively. The total load of the domestic sewage per day and person was 173 L water, 12.4 g COD, 22.1 g BOD, 13.0 g TOC, 3.91 g anionic detergents (as methylene-blue-active substances). 3.40 g n-hexane extractable matter, 1.16 g total-N, and 0.47 g total-P (SHIGETA et al., 1984b).

Origin - Types	Quantity	
HUMAN METABOLISM		
Metabolic carbon dioxide	kg/person-day	1
Metabolic produced water	kg/person-day	0,35)
Perspiration/respiration H_2O	kg/person-day	1,8)
Fecal water	kg/person-day	0,09)
Urine (3,3) plus flush (1.1)	kg/person-day	2)
Urine solids	kg/person-day	0,06)
Fecal solids	kg/person-day	0,03)
Sweat solids	kg/person-day	0,02)
EVA waste water	kg/8-hr EVA	0,9
EVA carbon dioxide	kg/8-hr EVA	0,7
CLEANING AND RECYCLING SYSTEMS (IF ANY)		
Hand wash water	kg/person-day	3,2)
Shower water	kg/person-day	2,3)
Clothes wash water	kg/person-day	12,5)
Dish wash water	kg/(8) crew-day	7,3)
Trash solids	kg/person-day	0,06)
Trash water	kg/person-day	0,14)
Hygiene latent water	kg/person-day	0,4
Laundry latent water	kg/person-day	0,06
Hygiene water solids	% of H_2O usage	0,06
Waste wash water solids	% of H_2O usage	0,2
Charcoal (odour control)	/person-day	0,06
Lithium hydroxide cartridges		?
Unspecified secondary wastes		?
Methane secondary waste		?
Used air filters	l/person/day	
Used water membranes	l/person/day	
Vacuum cleaner filters and bags	l/person/day	
Sponges	l/person/day	
Containers of products	l/person/day	
Used velcro strips	l/person/day	

ORIGIN AND QUANTITY OF WASTES PRODUCED WITHIN A SPACECRAFT.

Origin - Type	Quantity
FOOD CONDITIONING	
Containers metallic or plastic	0,4 kg/person/day
TECHNICAL MAINTENANCE	
Used light tubes (glass and metal)	1/person/day
Other components (electronics, plugs, wires and plumbing, o.rings)	1/person/day
OTHER LIVING ACCESSORIES	
Books, papers, pens	1/person/day
MEDICAL WASTE	
Medical products	
Medical containers	
HYGIENE ESTHETICS AND COSMETICS	
Towels	4/person/day
Brushes	1/person/day
Sponge	1/person/day
Wipes	2/person/day
Dentifrice tube	1/person/day
Tooth picks	2/person/day
Feminine tampons	
Containment bags	2/person/day
Cut hair, nails	
Product containers (soap, lotion, cream, etc.)	1/person/day
Used instruments	1/person/day
Clippers	1/person/day
Applicators	1/person/day
Headbands	1/person/day
Combs	1/person/day
Clothing	1.13 kg/person/day
SCIENTIFIC EXPERIMENTS*	
Bio samples	
Products (chemical, biological, medium, substrates)	
Instruments-Tools	
Water	

ORIGIN AND QUANTITY OF WASTE PRODUCED WITHIN A SPACECRAFT (continued)

TABLE 2

WASTE CHARACTERISTICS	*WASTE*
Biodegradable Liquid Waste	*Hand wash water* *Shower water* *Clothes wash water* *Dish wash water* *Metabolically produced water* *Perspiration/Respiration* *Fecal water* *Urine and flush* *EVA waste water* *Hygiene latent water* *Laundry latent water* *Biosamples* *Scientific expt. products*
Biodegradable Solid Waste	*Trash solids* *Trash water* *Urine solids* *Fecal solids* *Sweat solids* *Sponges* *Some clothings?* *Hygiene water solids* *Waste wash water solids* *Towels, brushes, sponges* *Clothing* *Product containers* *Used instruments* *Clippers, Applicators,* *Headbands, Combs* *Carbon dioxide*

TABLE 3
Composition of Human Feces (ROCHE LEXIKON MEDIZIN, 1984)

Amount per person per day	*60-250 g (about 30% dry matter)*
pH-value	*about neutral*
Organic components	*Lime soaps, cholesterine, urine compounds, proteinaceous putrefaction products, decomposed bile components*
Inorganics (about 25% of dry matter)	K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, PO_4^{3-}, Na^+, Cl^-, S^{2-}
Undigestible nutrient components	*Cellulose, hair, horn, plant components*
Not digested nutrient residue	*Fibers of muscle and connective tissue, starch, fat*
Microorganisms (about 25% of dry matter)	*Pathogenic and saprophytic bacteria, yeasts and parasitic organisms*

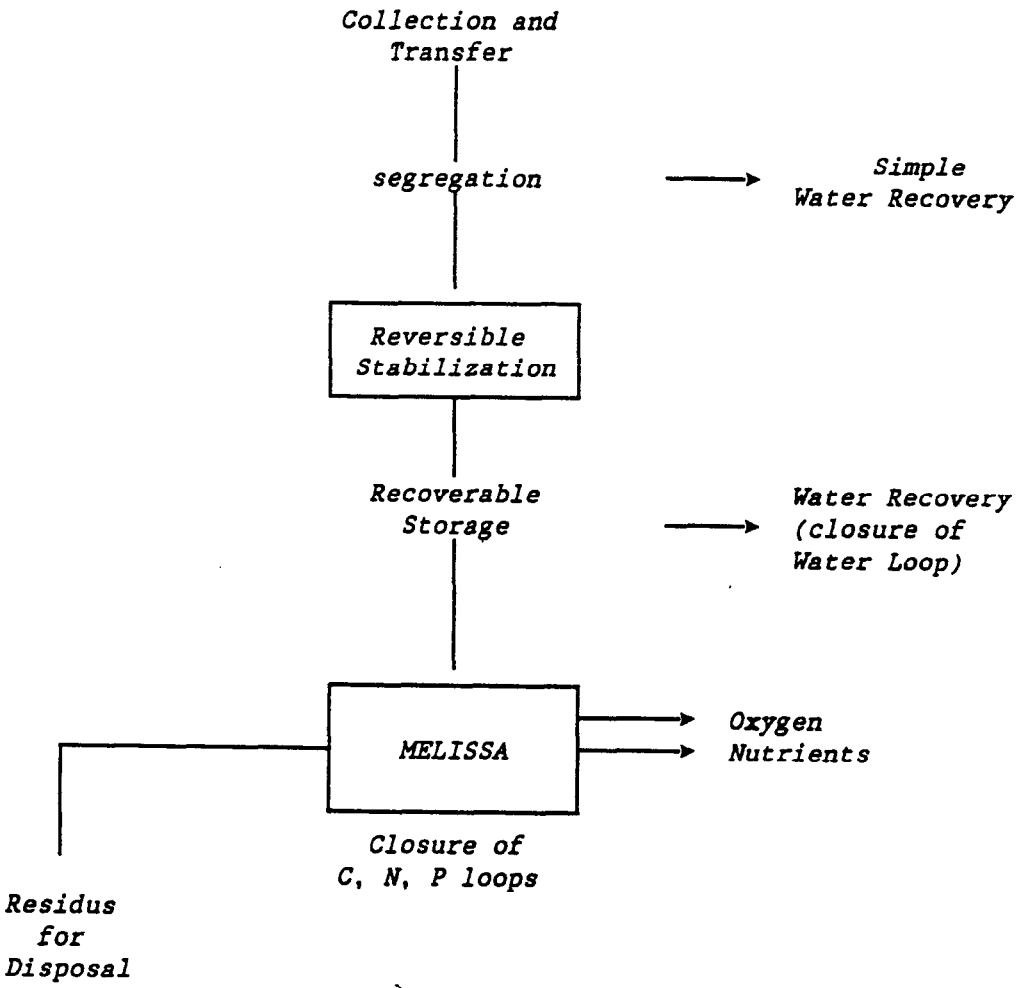
TABLE 4

Composition of Human Urine per person per day
(ROCHE LEXIKON MEDIZIN, 1984)

Amount	*500.0 - 2000.0 mL*
Dry matter	*40.0 - 60.0 g*
pH-value	*4.8 - 7.5*
Total nitrogen (N) as % of total N	*7.0 - 17.0 g*
Amino acid-N	*< 2.0*
NH_4-N	*4.6*
Uric acid-N	*1.6*
Urea-N	*82.7*
Creatinine-N	*3.7*
Potassium	*1.4 - 3.1 g*
Sodium	*2.8 - 5.0 g*
Chloride	*4.3 - 8.5 g*
Phosphorus, total	*0,8 - 2.0 g*
Sulfur, total	*1.24 - 1.50 g*
Other inorganic substances (mg) : Calcium, 130-330; magnesium, 60-200; zinc, 0.14-0.70; iron, 0.04-0.15; copper, 0.03-0.07; iodine, 0.02-0.5	
Amino acids, total	*1.3 - 3.2 g*
Bile acids	*5.0 - 10.0 g*
Urea	*12.0 - 30.0 g*
Hippuric acid	*1.0 - 2.5 g*
Creatinine	*0.5 - 2.5 g*
Uric acid	*0.08 - 1.0 g*
Purines	*0.2 - 0.5 g*
Citric acid	*0.15 - 1.2 g*
Sugar (reducing substances)	*0.5 - 1.5 g*
Other organic compounds (mg) : Fatty acids, 8-50; glucuronic acid, 200-600; lastic acid, 100-600; oxalic acid, 10-25; proteins, 10-100; creatinine, 10-190|270; ketones, 10-100; hydroxyindole acetic acid, 1-14.7; indicane, 4-20; indoxyl sulfuric acid, 15-100; bile pigments, 0.07-4.4; porphyrines, 2-10	

On the other hand, some types of paperwaste (tissues, toilet paper, kitchenpaper, not printed sheets,...) should be considered for recycling in MELISSA : indeed, they are an excellent source of cellulose to be processed in the liquefaction compartment.

The natural microbial flora in the human waste and in the environment of the spacecraft will act upon the wastes and must be taken in account for the retreatment of waste before introduction in the liquefaction compartment (cfr. the description of suitable stabilization methods as reported in the Waste Processing Contract Report (ESTEC, 1989).


Collection and Transfer
segregation
Simple Water Recovery
Reversible Stabilization
Recoverable Storage
Water Recovery (closure of Water Loop)
MELISSA
Oxygen
Nutrients
Closure of C, N, P loops
Residus for Disposal

WP 1.300 :

BIOMASS QUALITY OF SPIRULINES

WORK PACKAGE 1300

BIOMASS QUALITY

The developing interest for Spirulina as source of food lies on the discovery that they were and still are consumated by Atzecs in Mexico, by people around the lake Chad and in several other places in the world. Moreover, they are industrially produced, mainly in Mexico and sold as dietetic food. Analyses confirm their high dietetic value.

I - Overall composition of Spirulina (1)

	CEE standard for yeast	Candida utilis	Spirulina
Crude proteins (%)	> 45	51	72
Nucleic acids (%)		6	4
Lipids (%)		5	7.3
Carbohydrates (%)		35	13
Ashes (%)	< 10	8,5	4,7
Energy (K cal/100 g)		370	406
DNp cal		25	43
Calcium (mg/100 g)		550	98
Phosphore (mg/100 g)		1800	870
Ca/P		0.3	0.11
Iron (mg/100 g)		19	53
Vitamin C (mg/100 g)		-	10
Vitamin B1 (mg/100 g)	> 1	2.5	5.5
Vitamin B2 (mg/100 g)	> 3	5	4
Vitamin PP (mg/100 g)	> 30	42	11.8
Vitamin B6 (mg/100 g)		3.5	0.3
Folic Acid		2	0.05
Ergosterol		3	-

Compared to the composition of yeasts which are the main source of SCP and to CEE standards, Spirulina appears as a good alternative.

Remarks :

- The calcium concentration depends on the culture medium.
- The low value of the Ca/P ratio is characteristic of both organisms.
- Iron requirements of men can be covered by Spirulina.

II - Nitrogenous compounds

1) Protein nitrogen

a) Men requirements for essential amino acids (2)

	mg/day/70 Kg adult
Isoleucine	700
Leucine	980
Lysine	840
Methionine + cystine	910
Tyrosine + phenylalanine	980
Threonine	490
Tryptophane	210
Valine	700

The protein requirements of an adult are coreved with 100 g Spirulina

b) Aminogram stability (3)

The aminogram of Spirulina platensis was followed during a two years long continuous culture in 5 m^2 pools in the Institut Français du Pétrole. Essential amino acids composition appeared very stable.

	g/100 g Spirulina	
	first measurement	second measurement two years later
Isoleucine	4.37	4.55
Leucine	6.24	6.44
Lysine	3.21	3.50
Methionine	1.86	1.82
Cystine	0.66	0.66
Phenylalanine	3.17	3.22
Tyrosine	3.39	3.43
Threonine	3.66	4.03
Tryptophane	1.12	1.12
Valine	4.72	4.90

c) Deficiency for lysine

Spirulina presents a monodeficiency for lysine, which can constitute a limiting factor for Spirulina protein efficiency. They should therefore be supplemented either with lysine or with proteins from other origin.

	Spirulina		egg	wheat	
	g/100 g protein	deficit	ref. protein g/100 g prot.	g/100 g prot.	deficit
Lysine	4.93	- 26	6.70	2.80	- 58
Methionine	3.05	+ 2	3.00	1.45	- 52
Threonine	5.30	0	5.30	2.85	- 46
Isoleucine	6.15	+ 6	5.80	3.60	- 38
Tryptophane	1.59	+ 7	1.50	0.95	- 37

d) Protein efficiency (4)

	Real nitrogen digestibility %	real nitrogen retention %	net protein utilisation
Beef muscle	99	76	76
Spirulina	84	72	61
Wheat flour	86	46	41
Yeasts	83	63	53

The effects of the monodeficiency for lysine in Spirulina proteins are decreased by their good nitrogen retention.

2) Nucleic acids nitrogen (5)

	% nucleic acids
Higher plants	1 - 2
Spirulina	4
Eukaryotic algae	5 - 6
Yeasts	10 - 15

Although the nucleic acids concentration of Spirulina is higher than that of higher plants, it remeains largely below that found in yeasts which are the major source of SCP.

III - Fatty acids (6)

	mg/100 g Spirulina minimum	maximum
Total lipids	6000	7000
Total fatty acids	4900	5700
Saturated : lauric (C12)	18	22.9
myristic (C14)	52	64.4
palmitic (C16)	1650	2114
stearic (C18)	traces	35.3
unsaturated : palmitoleic (C16)	149	203
palmitolinolenic (C16)	175	256.5
heptadecenoic (C17)	9	14.2
oleic (C18)	197	301
linoleic (C18)	1092	1378.4
alpha linolenic (C18)	69.9	700
gamma linolenic (C18)	875	1197

Two essential fatty acids, the linoleic and gamma linolenic acids represent 40 - 45 % of total fatty acids.

IV - Carbohydrates (7)

	g/100 g Spirulina
Total carbohydrates	15
Rhamnose	9
Glucan	1.5
Cyclitols- phosphates	2
Glucosamines and muramic acid	1.5
Glycogen	0.5
Sialic acid and others	0.5

Spirulina cells present an undigestible rhamnosan and glycosan mucilage and traces of simple sugars and sucrose.

V - Vitamins (9)

	Recommended daily allowance	Composition for 100 g Spirulina	% of daily allowance covered by 100g Spirulina
Vitamin A	3000	-	-
Provitamin A (Beta carotene)	-	170 mg	144
Vitamin E	15 mg	19 mg	127
Vitamin C	80 mg	10 mg	13
Vitamin PP	16 mg	11.8 mg	74
Vitamin B1	1.4 mg	5.5 mg	393
Vitamin B2	1.6 mg	4 mg	250
Ca panthothetate (8)	5 mg	1.1 mg	22
Vitamin B6	2.1 mg	0.3 mg	14
Vitamin B12	3 µg	200 µg	6667
Folic acid	400 µg	50 µg	13
Biotin	300 µg	40 µg	13

Spirulina cells contain high amounts of vitamins B12, B1, B2 but a level for vitamin B6 two low, specially for a product of high protein content.

VI - Conclusion

The biomass production yields of Spirulina are high (10) .

	Yield in tons/ha/year	
	dry matter	Proteins
Wheat	4	0.1
Maize	7	1
Soja	6	2.4
Spirulina	50	35

Spirulina cells as food present a high protein value with a well balanced aminogram (however with a deficit for lysine). It can be used as a correcting factor for energetic nutriments rich in lipids and carbohydrates.

Moreover, Spirulina present some interesting dietetary characteristics:

- a lipid composition devoid of sterols and rich in two essential fatty acids, the linoleic and gamma linolenic acids which are typical of animals.
- a high level of organic phosphorus, mainly associated to cyclitols.
- a high level of vitamin B12, usually found in animal tissues (liver) and of vitamins E and beta carotene which protect lipids against oxidization and rancidness.

Diets where 100 % of the proteins of the dietetary allowance were furnished to animals as Spirulina provided growth rates similar to those obtained with usual diets. Moreover, anomalies or pathological effects were never observed (11, 12, 13).

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WP1400 :

BIOMASS QUALITY OF PHOTOTROPHS

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1. Biomass production

The protein from microorganisms, known as single cell protein, offers the best hope for being a new source of major protein, independent of agriculture. Both photosynthetic and non-photosynthetic microorganisms, grown on various carbon and energy sources, are used in fermentation processes for the production of biomass. A high production rate is obtained with the photosynthetic bacterium Rhodobacter capsulatus. About 10 g VSS/l.d is produced in a continuous flow through reactor (Driessens et al., 1987). The highest yield obtained for a batch reactor was about 2,97 g VSS/l.d (Shipman et al., 1975). Also the flocculation of the cells grown in the continuous reactor, is of iotechnological significance because it permits easy harvesting of the SCP.

2. Biomass content

In Table 1, the amount of crude protein in several microorganisms is presented (Kobayashi and Kurata, 1978).

Table 1. Crude protein content (g/100 g DW)

Photosynthetic bacteria :	60,95
Chlorella :	55,52
Yeasts :	50,50
Fungi :	45

The photosynthetic bacteria contain more crude protein than other microorganisms.
Vrati (1984) reported that Rhodobacter capsulatus, when grown on clarified effluents, contained ca. 69 % crude protein.

Also the quality of the protein is very high. The amino-acid composition is given in Table 2.

Table 2. Amino-acid composition of different types of single cell protei

Protein source	Amino Acid (a)							
	Histi-dine	Isoleu-cine	Leu-cine	Lysine	Methi-onine	Phenyl-alanine	Threo-nine	Vali
Rh. capsulatus	2.82	5.24	8.02	5.41	3.23	5.23	5.12	7.2
Rh. palustris	2.02	4.32	7.23	5.20	3.33	4.22	4.86	6.5
Rh. acidophilus	2.75	4.43	6.88	4.82	3.41	4.43	4.82	6.8
Rh. gelatinosus	3.02	3.98	7.01	4.66	2.88	4.80	4.75	6.4
Rh. sphae-roides	2.90	3.85	7.14	5.60	3.00	4.75	5.05	6.5
Rsp. rubrum	3.82	4.10	6.56	4.93	3.05	5.12	5.40	7.0
Rsp. tenue	2.80	4.30	7.72	5.05	3.41	5.20	4.80	7.3
FAO reference protein (b)	1.90	4.00	7.00	5.50	3.50	6.00	4.00	5.0
Chlorella protein (c)	1.90	4.39	8.03	4.88	0.48	4.77	4.10	5.4
Yeast protein (e)	1.73	5.20	7.00	7.44	1.00	4.35	5.24	6.3
Meat protein (d)	1.80	3.40	6.40	5.00	1.30	3.60	3.40	5.0
Egg protein (e)	2.40	6.60	8.80	6.40	3.10	5.80	5.00	7.4
Soybean protein (e)	2.40	5.40	7.70	6.30	1.30	4.90	3.90	5.2
Wheat flour (f)		4.20	7.00	1.90	1.50	5.50	2.70	4.1

(a) Amounts of amino acids are given as percent protein
(b) WHO (1973)
(c) Kobayashi and Kurata (1978)
(d) Cited by Shuler et al. (1979)
(e) Shipman et al. (1975)
(f) Erdman et al. (1977)

Table 2 shows that the phototrophs have a high content of essential amino-acids. The amino-acid composition compares favourably with that of Chlorella and yeasts. According to Vrati and Shipman et al. (1975), phototrophs can contain till 3 % methionine in their proteins.

The problem with most single cell proteins is that they are poor in methionine content. However, methionine contents of SCP obtained from photosynthetic bacteria are comparable with that in the FAO reference protein and are superior to soybean and meat proteins. The cereals are poor in their lysine contents, but higher amounts of lysine are present in the proteins of animal origin. SCP from photosynthetic bacteria have comparable amounts of lysine to FAO reference protein but lower compared to the proteins of animal origin.

Besides, results in Table 2 show that there are substantial differences in amino-acid composition of SCP obtained from different photosynthetic bacteria. Moreover, the protein content and amino acid composition also depends on the way of cultivation, in batch or in a continuous flow through reactor (Table 3).

Table 3. Crude protein and amino-acid composition of Rhodobacter capsulatus ATCC 23782

Protein source	Crude protein (2)	Amino Acid (1)						
		Isoleu-cine	Leu-cine	Lysine	Methi-onine	Phenyl-alanine	Threo-nine	Valine
Rh. capsulatus ATCC 23782 grown in Batch Culture	615	3.78	7.45	5.58	1.75	-	2.62	4.52
Rh. capsulatus ATCC23782 grown in Upflow Reactor	605	5.13	9.89	4.52	1.61	4.95	7.13	7.66

(1) amounts of amino acids as % of the protein

(2) expressed as g kg^{-1} volatile suspended solids

Analyses of SCP from different photosynthetic bacteria, and in particular Rhodobacter capsulatus, shows that it has a high content of essential and sulphur amino-acids. Besides, photosynthetic bacteria contain also a lot of vitamins (B group) (Kobayashi and Kurata, 1978). The use of photosynthetic bacteria has been found of significant importance in pisciculture, the poultry industry and horticulture (Kobayashi, 1978).

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