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COMPARTMENT I DESIGN : TESTED REACTOR DESIGN AND INSTRUMENTATION

prepared by/*préparé par* C.G. Dussap, H. de Baynast, B. Farges

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1 INTRODUCTION

The first compartment of MELiSSA is probably one of the most complex of MELiSSA loop having to support the first treatment of all solid and liquid wastes produced by the other compartments and principally the crew compartment, the higher plants chambers and the food preparation system. It is responsible for the biodegradation of human faecal matter and other wastes generated by the crew, *e.g.* non edible parts of plant material, paper...etc. The volatile fatty acids and ammonium produced during the anaerobic fermentation process are fed to the second phototrophic anoxygenic compartment colonized by the *Rubrum* species.

As the first step in the MELiSSA cycle, the liquefying compartment determines the fraction of organic wastes that are recycled in the loop. During the last 10 years, intensive research has been carried out so as to maximise the biodegradation efficiency. Insoluble organic matter is hydrolysed to soluble components which are further fermented into volatile fatty acids, carbon dioxide or, according to the process conditions, lactic acid or alcoholic substances. Hydrolytic acidogenic bacteria perform this fermentation step. An association of acetogenic and methanogenic bacteria may further use the fermentation products to produce biogas containing methane, carbon dioxide and traces of hydrogen. Since methane has no further use in present MELiSSA flowsheet, methanogenesis must be inhibited. The volatile fatty acids are further converted by the second compartment of the MELiSSA cycle into *Rhodospirillum* biomass and CO_2. CO_2 is reprocessed mainly in *Arthrospira* and higher plants compartments.

The hardware of the liquefying compartment has been mainly developed by EPAS. Specific developments and hardware improvements are done under the EWC contract with EPAS as prime contractor and LGCB - UBP and Sherpa as consultants, with the objective of implementation of the pilot unit at UAB. Alternative technologies are also studied under a MAP contract (Biosafe MELiSSA: a total converting and biosafe liquefying compartment for MELiSSA) with U. Ghent (LabMet) as prime contractor.

The objectives of this Technical Note are double:

- First to have an overview of the different technical choices that were made for selecting and constructing the pilot reactor at EPAS, knowing that this reactor will be implemented at UAB; the sake is here to replace these choices in the context of a bioreactor having to be interfaced in the experimental MELiSSA loop at UAB and enabling to obtain new experimental information on the liquefaction of MELiSSA waste.

- Secondly, to give the state of the art regarding on-line gas phase analysis on an anaerobic bioreactor, knowing that this kind of information is presently well documented on aerobic or photosynthetic bioreactors but much less routinely done on anaerobic gas effluents of unknown and variable compositions.

2 BACKGROUND EXPERIENCE OF TESTBED REACTORS

All this section is written considering the different Technical Notes concerning the first compartment that have been produced by EPAS and LGCB, *i.e.* TN 71.1, 71.2, 71.3, 71.4, 71.5, 74.1, 74.2....

2.1 General design of the anaerobic liquefying compartment

2.1.1 GENERAL SCHEME OF ANAEROBIC DEGRADATION

The major advantages of anaerobic biodegradation of wastewater and organic solid wastes are the high organic load that can be achieved, a low sludge production and the net energy production in the form of methane rich biogas although, in the case of the MELiSSA liquefying compartment, this last advantage falls considering that methane production is prevented rather than favoured. Anaerobic digestion of solids wastes also guarantees a significant decrease of the amount of pathogenic organisms present in the waste when operated in mesophilic and thermophilic conditions. A variety of industrial organic waste materials such as glycerine, yeast, kitchen waste, molasses, fats, fish oil, peel and pulp, stomach and intestine content can be processed in anaerobic systems, knowing that the compounds to degrade can be either dissolved in liquid stream or present as solid particles. In the last case, the first step of the degradation results in bacterial fixation on solids particles which is often the rate limiting process.

Anaerobic biodegradation is a complex biological process since several groups of bacteria are involved. The first group of bacteria is the hydrolytic-fermentative bacteria. Firstly, they hydrolyse macromolecular compounds into water-soluble polymers. Next, the polymers formed are further fermented along with the production of fermentation products such as alcohols, lactic acid and volatile fatty acids (VFA), ammonia, H_2, CO_2 and minerals. The acetogenic bacteria convert the fermentation products, such as the volatile fatty acids, into acetic acid, CO_2 and H_2. These products are then finally converted into methane by the acetoclastic and hydrogenotrophic methanogenic bacteria, knowing that the activity of these bacteria is intended to be reduced at minimal levels in the present MELiSSA liquefying compartment.

The anaerobic process is sensitive to auto-inhibition. The process is indeed inhibited by its own fermentation products. A high concentration of volatile fatty acids has an inhibitory effect on the hydrolysis of the original substrate. Also a build-up of hydrogen gas in the system will inhibit the conversion of the volatile fatty acids such as propionic acid and butyric acid into acetic acid. Apart from the auto-inhibition, sulphate reducers may successfully compete with the methanogens if sulphate is present. The production of H_2S can have an inhibitory effect on the methanogenic bacteria in case more then 200 mg/l of sulfide is formed. Finally, an ammonium concentration of more then 4 g/L at a pH of 7.1 will also inhibit the methanogenesis.

2.1.2 ANAEROBIC REACTORS CONFIGURATIONS

Several reactor configurations are possible for anaerobic processes. Different reactor technologies can be distinguished based on how the bacterial biomass is present in the reactor.

- The first group of reactors contains biomass that is fixed on a support material. The support material may be present as a fixed material but can also be fluidised. This is obviously not suitable for MELiSSA waste degradation where the support material must not compete with the solid substrate on which the attachment of bacterial flora occurs.

- The second group concerns the Upflow Anaerobic Sludge Blanket (UASB) reactors. Such reactors contain a bed of anaerobic granular sludge through which the wastewater is recycled. Basically, the interior of a UASB reactor is divided into several sections (granular sludge bed, fluidized zone, gas-liquid solid separator, settling area) in such a way that neither the solid particles into the bed (which are often biomass granules) nor the liquid phase cannot be considered as homogeneous, each zone of the reactor being responsible of a basic transformation, *i.e.* acidogenesis, methanogenesis...etc. This kind of reactor is not well adapted to MELiSSA solid waste degradation, considering that the granules in UASB reactors are mainly biomass flocks ensuring dissolved organic load degradation instead of solid waste granules. It is generally recognised that this kind of reactor is particularly well-adapted for high load of dissolved organic wastes more than for solid particles degradation. Moreover, the natural segmentation of the bed into several zones naturally drives the process towards methane production which is not the objective of MELiSSA liquefying first compartment.

-The last group is well-mixed reactors in which bacteria are both suspended in the waste stream that needs treatment and attached to the solid particles. The reactor can be a flow-through system in a completely stirred tank reactor where the hydraulic retention time is the same as the sludge retention time. The basic mixing characteristics of such reactors tend to a perfectly mixed behaviour. To keep the sludge in the reactor system, a separator can be installed to separate the liquid from the sludge. However, anaerobic sludge that is not granular has very poor settling capacities and is hard to separate. Membrane bioreactors can handle this problem. The sludge mixture is sent through a filtration system where the water phase is separated from the sludge mixture.

This last group of bioreactors has been chosen since the beginning of the studies for the degradation of MELiSSA waste for at least two reasons :

- the first reason is that it is the only way to ensure anaerobic degradation without (or minimizing) methane production ;

- the second reason is that such a configuration is well adapted to high load of solid particles to degrade.

The reactor concept used during the development phase of the liquefying compartment was a completely mixed reactor operating as a fed-batch reactor. This kind of reactor is characterised by two mean residence times : Hydraulic Residence (or retention) Time (HRT) and Solid Residence Time (SRT). In the first phase of the studies, HRT was equal to SRT ; in a later phase recycling of the solids was introduced using centrifugation and filtration to increase the SRT and also to uncouple the two process variables HRT and SRT. With this configuration, the anaerobic reactor operates in similar conditions as UASB reactor, *i.e.* with a much longer solid residence time than the liquid residence time, but in homogeneous conditions which mostly prevents methane production.

2.1.3 ANAEROBIC MEMBRANE BIOREACTOR (MBR)

Based on the promising results obtained with a well-mixed configuration with uncoupling of HRT and SRT, it has been proposed to use the same principle of reactor (*i.e.* perfectly mixed and uncoupling of HRT and SRT) with a solid liquid separation system operating continuously with a membrane separator.

The membrane acts as a selective barrier, which permits the passage of certain components out of a mixture and retains others. In order to obtain mass transport through the membrane, a driving force is applied, such as a pressure difference and / or a difference in chemical or electrical potential. Separation between components occurs because of differences in transport rates through the membrane or in particle size. As a result, the incoming stream is separated into a permeate consisting of particles small enough to pass through the pores of the membrane, and a concentrate consisting of solutes and suspended solids too large to pass through the pores.

Membrane processes can be classified according to driving force and pore size. Obviously in the case of the liquid phase of the liquefying compartment, the driving force must be the transmembrane pressure. Depending on the pore size, microfiltration, ultrafiltration, nanofiltration and reversed osmosis can be distinguished. The former two are low pressure techniques, the latter two require high pressures.

- Microfiltration is designed to retain particles sized between 0.1 and 10 µm such as bacteria or colloidal material. Generally, a overpressure of 10 to 200 kPa is applied for the mass transfer process to occur.

- Ultrafiltration is a process to fractionate and concentrate solutions containing relatively large molecules with a molecular weight (MW) larger than 10^5, such as proteins and colloids. Salts readily pass through the membrane. The operating pressures are low and remain between 200 and 500 kPa. The actual separation is based on particle size but interactions between the membrane and the solutes play an important role.

- In nanofiltration, multivalent ions such as sulphate and phosphate are retained, as well as

low-molecular-weight components with MW exceeding 200. Therefore, these membranes can be used for dissolved carbon retention and for desalting. Pressure range lies between 500 and 2000 kPa.

For the application to the liquefying compartment, the objective is to retain in the reactor higher MW molecules (polymers, proteins and of course free biomass in suspension and solid particles) in such a way that the process must function in microfiltration to ultrafiltration conditions, leading to transmembrane pressures ranging between 100 and 500 kPa. The filtrate may only contain soluble components of low MW which are the fermentation products such as volatile fatty acids, ammonium and other salts.

Depending on the type of filtration applied, two types of MBR can be distinguished :

- In the side stream MBR, the membrane module is placed at the outside of the bioreactor and the permeate (filtrate) is removed under pressure by crossflow filtration. High crossflow velocities are required to avoid excessive membrane fouling.

- Submerged MBR are a more recent development. In this case, the membrane module is immersed inside the reactor and the permeate is removed using suction. In this case, the crossflow pressure is in the order of 100 kPa (1 bar) which is the total pressure inside the reactor. Overpressurised reactors at several bars for obtaining higher transmembrane pressures require extensive developments out of the scope of this study. Gas bubbling by diffusers situated below the membranes creates a crossflow stream over the membrane surface which removes the cake layer and reduces membrane fouling. As opposed to the external MBR, no sludge recirculation is required, which substantially reduces the energy costs. On the other hand, submerged MBR operate at lower fluxes due to lower transmembrane pressure. Hence, a larger membrane surface area is needed.

In order to keep the possibility to replace the membrane system and to have a broader range of control of the permeate flow by independently manipulating a process variable (liquid flow rate into the liquid loop and / or transmembrane pressure), the first type of configuration has been chosen (external membrane module). This also prevents to use an always complex system for bubbling gas in submerged MBR.

In a dead-end filtration mode, the solution to be filtered approaches the filter medium in a perpendicular direction. The retained particles tend to build up as a filter cake, causing clogging, pressure losses and a decrease in flow. Such problems can be reduced with crossflow membrane filtration. In this case the fluid flows tangentially to the membrane and continuously sweeps away the filter cake. That is the reason why this solution has been retained.

The filtration system must separate the suspended solids and the non-biodegraded material with a high efficiency. The characterisation of the membrane remains to be done. This includes:

- The physical characterisation of the membrane, *i.e.* chemical composition, surface area, thickness, pore size...etc. ;

- The hydrodynamic characterisation, *i.e.* mainly the liquid flow in the membrane module versus the transmembrane pressure;

- The separation efficiency, *i.e.* the partition coefficient for different dissolved species such ions, VFA, carbohydrates...etc.;

- The dynamic evolution of the membrane filtration system, *i.e.* the time course change of the above properties versus the liquid liquor properties (composition, solid particles content, free biomass content) and the progressive clogging of membrane pores.

Furthermore, special attention must be paid to hygienic requirements. The filtration system needs to guarantee a pathogen free effluent. This means that the system must be expanded with a disinfection system.

2.1.4 RESULTING FLOWSHEET FOR THE FIRST COMPARTMENT

The resulting principle of the validated flowsheet of the MELiSSA liquefying compartment is pictured in Figure 1. In a first phase, a prototype fermentor reactor with a small volume (25 L) has been assembled to evaluate sensors and equipment and to collect data for model validation and controllers development. In a second step, the design is upgraded to pilot scale (100 L) and used for the demonstration tests and for final integration in the MELiSSA Pilot Plant at UAB. The pilot unit is presently assembled at EPAS. The flow diagram concerning the liquefying compartment is shown in Figure 2.

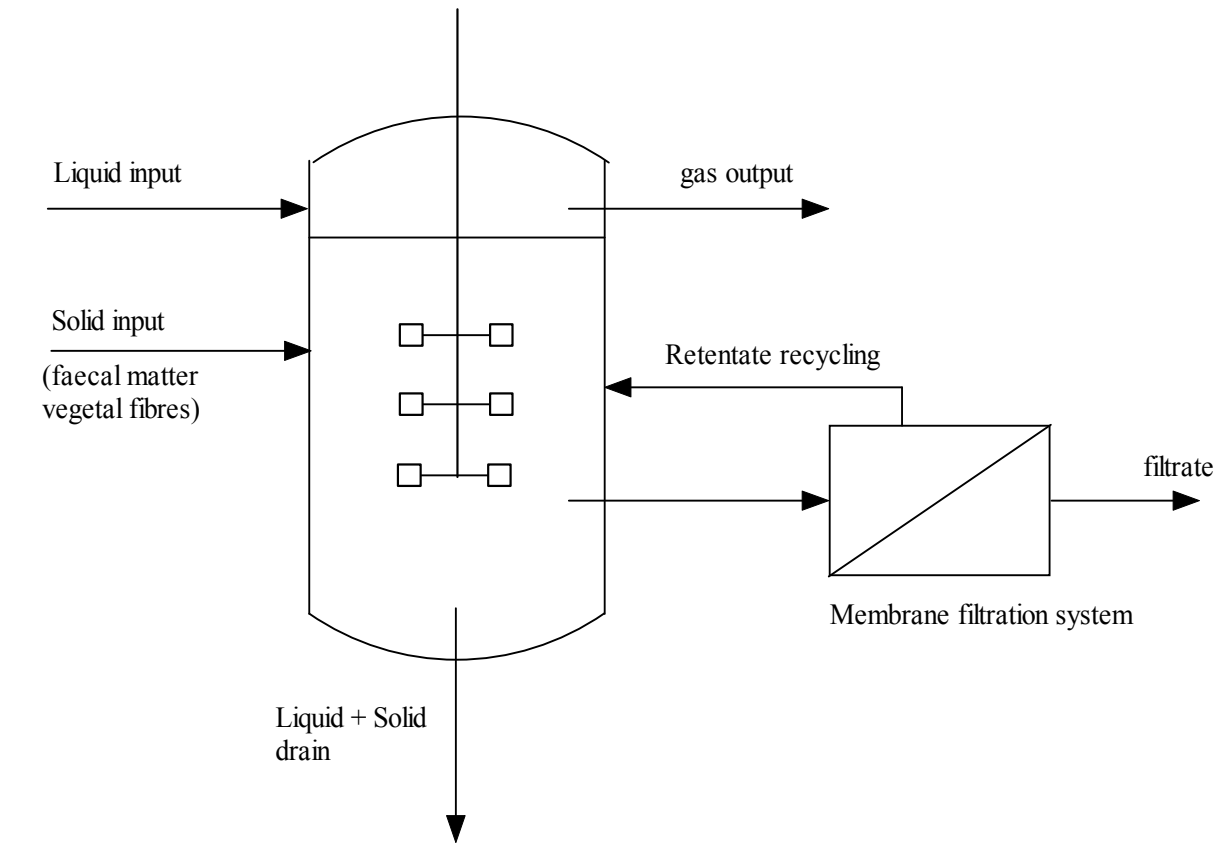


Figure 1: Principle scheme of liquefying compartment

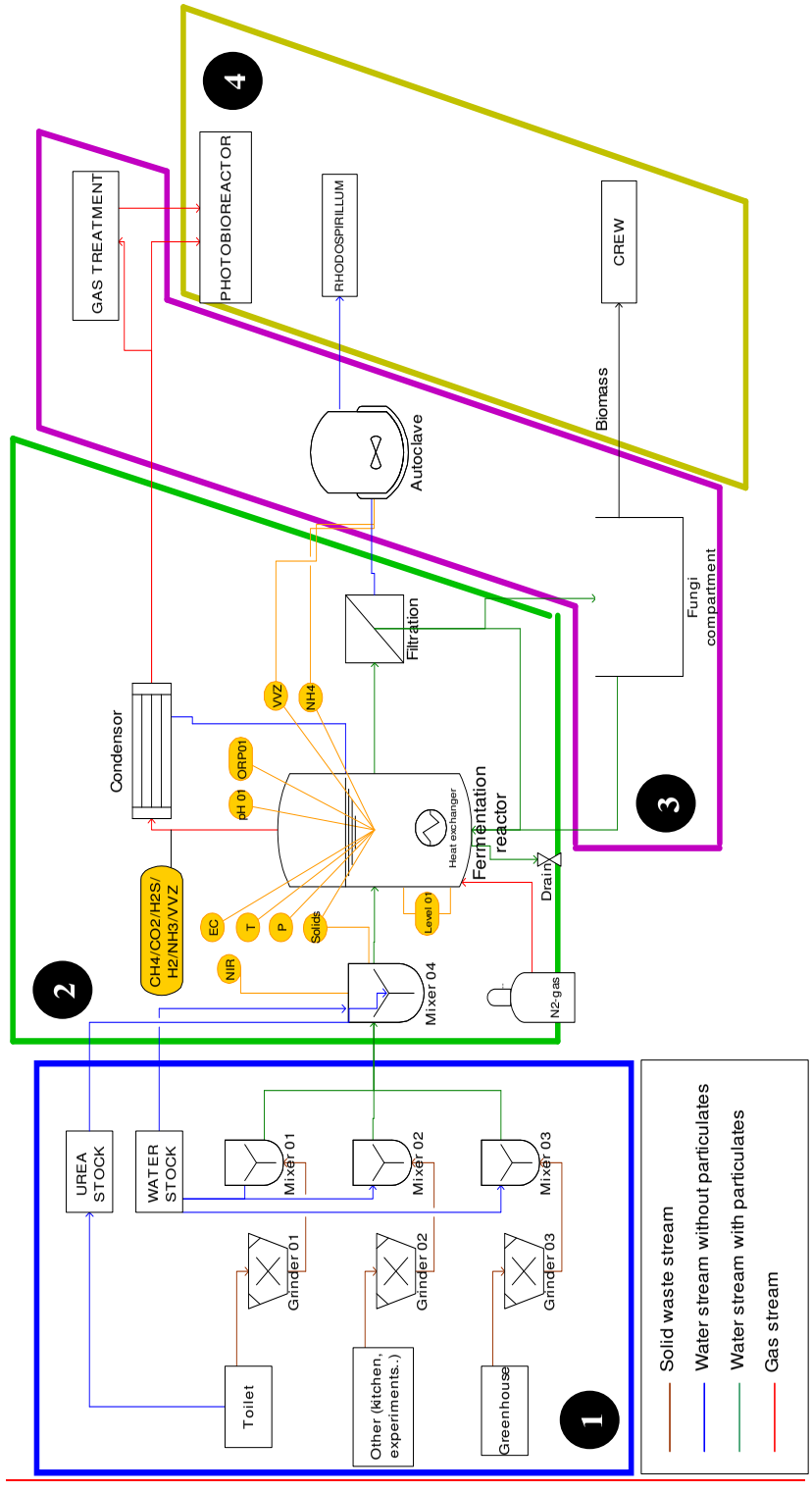


Figure 2: Schematic representation of MELiSSA waste compartment (TN 74.1, UBP)

The design enables to investigate the following ranges of the main process variables :

- Total operating volume 100L

- Nominal temperature: 55 °C (thermophilic situation in order to reduce methane production);

- Nominal pH: 6.5 (rather low pH to favour VFA production instead of methane) ;

-HRT : 5 to 15 days (calculated as the ratio of filtrate flow rate + liquid drain flow rate over liquid volume free of solid inside the bioreactor) ;

- SRT : 10 to 30 days (calculated as the dry solid drain rate over dry solid content inside the bioreactor) ;

- Organic load : 0.5 to 3 $g.L^{-1}.day^{-1}$ including faecal material, higher plant waste and toilet paper

- Transmembrane pressure : 100 to 500 kPa.

The expected performances can be summarized as follows :

- Proteins degradation : 50 to 80 % ;

- Fibre degradation : 15 to 30 % ;

- Total carbon substrate conversion to VFA and CO_2 : 60 to 70 %

- Total substrate solubilisation and degradation : 30 to 210 $g.L^{-1}.day^{-1}$

- VFA filtrate concentration (in the filtrate): 1 to 5 $g.L^{-1}$

- Ammonium concentration into the bioreactor: 0.2 to 1 $gNH_4\text{-}N.L^{-1}$

- Low CH_4 production (less than 15 %), high CO_2 content in gas phase (70 to 95 %)

- Biogas production (mainly CO_2) : 2 to 15 $sL.day^{-1}$

Although the reactor is supposed to be perfectly mixed or tends to a perfectly mixed situation, the location for liquid sampling remain to investigate as well as the estimation of imperfectly mixed situation (solid particles settling, liquid composition gradients).

The process parameters that are measured in the bioreactor liquor are dissolved volatile fatty acids, ammonium, pH, buffer capacity, conductivity, solids concentration, temperature, pressure and liquid level. The biogas production and composition must be determined (see below).

2.1.5 PRECONDITIONING OF INCIDENT WASTE STREAM

Special attention needs to be devoted to the preconditioning of the waste in the MELiSSA framework. The waste compartment needs a pre-conditioning step where it is possible to prepare the feed for the fermentation reactor, knowing that the "substrate" of the liquefying compartment needs both to be representative of a MELiSSA waste and to be standardised, for achieving valuable studies regarding the optimisation of the functioning of the bioreactor.

In MELiSSA reality, the waste will be collected from different systems, such as toilets, kitchen and greenhouse. The toilet waste includes faecal material and tissues and a separated wastewater stream containing urine. The greenhouse will produce non-edible parts of edible biomass. Additional non-edible biomass is produced in MELiSSA loop. Faeces, soiled tissues and non-edible parts of plants and biomass will be introduced into the first compartment.

A semi-automated process for grinding part of the waste stream has been built by EPAS (only wheat straw is prepared separately). This system enables to obtain a standardised MELiSSA substrate regarding the size of the solid particles, their composition and their concentration in the liquid stream.

It has been decided that the urine production may not be completely fed to the anaerobic liquefying compartment (or eventually not at all), considering that it was important to keep the possibility to prevent any possible bacteria inhibition by ammonium or urea in the compartment. Consequently urine collection system is independent of solid waste system. A system to hydrolyse urea into ammonium remains to be independently developed. This leads to uncouple ammonium and solid waste feeds to the liquefying compartment and let a degree of freedom (process variable) for controlling the fermentor. Nevertheless, at MELiSSA level, this entails that the remaining liquid can be fed directly to the other compartments, namely nitrifying, higher plant and eventually *Arthrospira* compartments, or treated by alternative methods such as physico-chemical methods.

The resulting feed of the liquefying compartment is a liquid stream containing a concentration of about 5 % particulate organic material of standardised size and composition. The stream is not viscous and can easily be transferred through classical piping and tubes. Special attention is needed for valves and pumps to prevent clogging. A wastewater stream containing urea can be used to dilute the feed for the fermentation reactor. The amount of urea (or ammonium) rich wastewater that might be used is dependent on the maximum ammonium concentration which can be acceptable in the reactor.

2.2 Main critical issues

The previous flowsheet involves a simple concept of membrane anaerobic reactor but complex technology developments, knowing that the anaerobic bioreactor of MELiSSA must function in a controlled and well-defined conditions satisfying the usual general rules of MELiSSA bioreactors, *i.e.* control by knowledge models which in turns entails complete modelling and understanding of the entire reactor and subsystems.

2.2.1 MEMBRANE MODULE

The technical limitations of MBR are related to flux deterioration and to shear stress from frequent sludge recirculation. The major hurdle for the use of membranes is caused by flux deterioration due to concentration polarization and membrane fouling. The local concentrations of the solutes at the membrane surface are higher than in the bulk. This results in a build-up of solute, which is known as concentration polarization, and may result in scaling or precipitation. Membrane fouling can be attributed to the adsorption of organic species, the precipitation of less soluble inorganic species and the adhesion of microbial cells at the membrane surface. As mentioned before, fouling can be reduced by applying sufficiently high cross flow velocities in external MBR (or by providing coarse bubble aeration in submerged MBR). Basically the shear stress prevents solutes and polymers sticking onto membrane walls. Appropriate backwash or intermittent filtration procedures also have the potential to limit the flux decline. But frequent sludge recirculation at high cross flow velocities exerts excessive shear stress on the sludge flocks and leads to an activity loss.

As mentioned above the characteristics (and the chemical composition) of the membrane remain to be investigated, considering clogging, selectivity...etc. versus the process variables *i.e.* transmembrane pressure, liquid composition, time of use and permeate flow rate.

The current knowledge of such systems is generally based on experimental (and almost empirical) trials and errors which lead to develop (in parallel) two strategies:

- Efficient on-line diagnostics of the membrane system, including transmembrane pressure, permeate flow rate, separation efficiency evaluation;

- Thorough understanding of the migration process of water and solutes through the membrane (and polarization layer) including understanding and quantification of clogging process, in order to minimize it.

2.2.2 MATERIALS SELECTION

The experience with full-scale anaerobic reactors is that corrosion can be a major problem. Corrosion is caused by to presence of H_2S and to low redox potential. The presence of CO_2 in the gas and the liquid phase can also have a corrosive effect. Special attention should thus be paid to the construction material of the reactor, the sensors, pumps, valves, connectors and seals. Corrosion resistant material like stainless steel, seawater-resistant aluminium, plastics such as polypropylene or materials with proper coatings must be applied for the construction. This includes not only the material of the reactor itself but also materials of fittings and smaller parts. The system needs to be designed to work in continuous mode. The reactor must operate on a long term without problems of clogging leading to damage or reduced capacity. Sensor failure or malfunction of actuators due to pollution must be prevented. An automated cleaning system has been implemented. The complete fermentation reactor needs to be air tight to prevent losses of odorous and hazardous components

When solid organic waste is treated, special attention must be paid to wear and tear of moving parts like pumps. The risk of clogging of ducts, filtration systems or valves must be investigated. It is therefore important to evaluate the possible formation of depositions and precipitates from organic or mineral nature.

2.2.3 STABILITY AND HOMOGENEITY

Basically, the bioreactor should be homogeneous and well-mixed with respect to the local concentrations and compositions of gas, liquid and solid phases.

In particular, this entails that gas-liquid and liquid-solid equilibria are achieved at each points in the reactor. This kind of assumption remains normally valid at time scale greater than few minutes for volumetric gas transfer coefficients greater than 10 h^{-1} (time constant lower than 5 min) which corresponds to a rather low agitation power (*ca* 1 kW/m^3) in a slightly viscous medium. Normally, these conditions will be satisfied.

The system normally will operate in continuous mode. It must be kept in mind that any representative point (concentrations, compositions, flow rates) of a stable regime (if it intrinsically exists) is only obtained after a time which is at least three times the longest residence time (HRT or SRT). Knowing that HRT is in the order of 10 days, each representative point will require a one month experiment of continuous and stable feed. In practice, this situation will be difficult to obtain due to the fact that stable solid sampling and constant behaviour of the membrane filtration system will be hardly achievable over such long periods. Therefore, it will be necessary to develop a transient knowledge of the entire system, the mean behaviour of the bioreactor being only obtained by averaging the results over long periods of time (several months). This entails that the reactor needs to be fully instrumented in order to record on-line:

- the flow rates, *i.e.* solid drain, liquid drain, permeate flow and gas production rate, inlet liquid flow rate and inlet solid flow rate ;

- the detailed compositions of the streams (solid, liquid and gas) at input and output of the reactor, including elements composition and main products characterisation, bacteria identification and concentration, knowing that a COD measurement, even useful, cannot be considered as sufficient for having information about the degradation mechanisms ;

- the composition of the sludge (liquid + solid phases) present inside the reactor, considering that, even if liquid mixing can be considered as correct at a macroscopic level, concentration gradients may occur inside the reactor (by example settling of solid particles) which may strongly affect the general behaviour of the reactor.

The quality of the analysis (reliability and accuracy) might enable to develop a thorough understanding of the bioreactor leading to predict transient behaviour in order to install a

suitable control model. It must be emphasized that the evaluation of internal heterogeneity is certainly an important point for understanding the bacterial regulatory mechanisms but add perturbations to the normal functioning of the bioreactor.

2.3 State of the art for modelling, simulation and control

2.3.1 COMMON PRACTICE OF MODELLING AND CONTROL O F ANAEROBIC DIGESTORS

Over the last fifteen years, a lot of efforts have been devoted to the development of dynamic models describing the anaerobic digestion process. These models take into account the microbial dynamics but also the gas-liquid equilibria and chemical equilibria in the liquid phase. Models have evolved from simple models putting the emphasis on a single substrate and a single population, to complex multi-pathway and multi-population models.

Anaerobic biodegradation is a complex process in which a lot of interactions and sometimes competition between bacterial groups play a role. The process is sensitive to disturbances of the process conditions like temperature and pH and to toxic components, among which also the fermentation products. Due to these aspects, anaerobic systems for processing wastewater or solids wastes are difficult to control.

The overall process control of industrial systems thus relies for a good part on the experience of the process operator. The manipulation of important process parameters, such as the load to the system is nowadays mainly controlled by experience. The process information is obtained by measuring classical parameters in the influent off-line such as Total Organic Carbon (TOC) and Organic Matter. Parameters that are measured in industrial digestors are at most volatile fatty acids, pH, alkalinity and temperature. Biogas production and its composition for methane also give important information on the process. More advanced measurements for anaerobic systems such as measurement of other biogas constituents such as hydrogen, carbon monoxide, hydrogen sulphide and ammonia are seldom done. In industrial process, automated control is mostly limited to local control such as pH and temperature. Approaches in control such as expert systems, fuzzy control and neural networks are gaining interest in the control of wastewater treatment plants. Obviously this cannot be applied, at least directly, to MELiSSA pilot reactor which requires knowledge based control models.

Up to this moment model based control for anaerobic systems is hardly reported in literature and certainly not used in practice. However, with the more detailed models developed during the last five years for MELiSSA liquefying compartment and the improved technology to estimate parameters of the model, this is subject to change.

2.3.2 CURRENT DEVELOPMENT OF MODEL APPROACHES OF THE LIQUEFYING COMPARTMENT

Globally, the same structure of modelling as for the other MELiSSA compartments has been developed for the first compartment, *i.e.* stoichiometric approach coupled to a microbial kinetic approach, the latter term eventually using physical transfer rate limiting equations.

The main originality of MELiSSA group's approach, when compared to other literature publications, certainly lies in the stoichiometric approach which is generally not considered as a prime issue for common anaerobic processes. The basic reason is that industrial anaerobic digestors are primarily used for reducing pollution; consequently the main characteristic valuable parameter for common applications is COD reduction more than CO_2, CH_4 or VFA conversion yields, knowing that in the best cases CH_4 is burned for restoring energy. Therefore, modelling efforts (and technical biodigestors developments) mostly concern the understanding and the optimisation of the reduction of incoming COD more than the prediction of the conversion yields and reactor selectivity.

Obviously, such an approach cannot be sufficient for MELiSSA purposes and understanding of the sources of variation of the conversion yields and selectivity must be considered. This has been handled through a classical (for MELiSSA group) stoichiometric approach which intrinsically considers the elements conservation as a prime issue. The behavior of the compartment is characterised by a set of stoichiometric equations, some of them involving unknown coefficients to be identified from experimental results, and each of them being associated to a kinetic rate modelling equation as a function of process parameters and liquid, gas and solid concentrations.

The structure model (toolbox AWC_MS) has been designed and completed. It is now a tool for the different MELiSSA partners to be used for:

- the study and the development of biological dynamic models for the first compartment, in order to find the most reliable model and to identify the biological parameters ;

- the study of the process itself, enabling to easily test the operating variables and improving the design (flow diagram) of the compartment (TN 74.2).

With this initial objective, the toolbox was designed in order to be flexible enough to allow a large range of scenarios. The AWC-MS toolbox is developed under MatLab ™ R13. This includes:

- the model of the process itself (reactor and membrane separation) including the hydrodynamic assumptions ;

- the biological models (set of kinetic differential equations ;

- the physical equilibria models.

The models implemented at present time in the toolbox include the bioreactor modelling (hydrodynamic modelling) with a classical chemostat approach (perfectly mixed liquid and gas phases).

The primary filtration unit was based on preliminary tests performed by EPAS based on VITO setup. Later, due to difficulties in operating the filtration unit (i.e. rapid and irreversible fouling), as well as for safety reasons (i.e. sterilisation), the membrane material, the filtration module as well as the control strategy have been changed. An Alpha ceramic membrane from Atech (50 nm pore size, Al2O3 support with TiO2 top layer) allowing sterilisation was selected, and a permeate flux control strategy was implemented.
The modelling of Filtration Unit operation is not a simple task, especially if a dynamic model is required. Usually, for preliminary studies, a simple separation model is used which represents the separating capability of the membrane in steady-state. This is the approach used at this time. This implies that transient behaviours and fouling and clogging are neglected. This would have to be improved.

Biological reactions for anaerobic degradation processes were already discussed and detailed. It was shown that often the reactions and the parameters presented in the literature are specific to the process for which they are used. The objective here was not to give a complete functional biological model for the reactor of Compartment I, but to propose the basic tools which enable to build this model. Thus, even if the equations and relations describing the models are given, the parameters of the models must be identified and adapted to the process.

As mentioned above, stable steady states of the liquefying compartment will be certainly difficult to observe, even due to intrinsic instabilities. This entails that model coefficients identification would probably be done from transient experimental observations. As a consequence, “stoichiometric” unknown coefficients will have to be determined at the same time as “kinetic” coefficients. This certainly complexifies identification work and, in turn, the global understanding of the behaviour of the compartment.

The tools currently proposed are a set of 4 different kinds of reactions, which must be combined together in order to build a complete biological model. These reactions are:

- enzymatic reactions associated to a Michaelis-Menten kinetic model with competitive and non-competitive inhibition ;

- growth reaction associated to a Monod model with the Haldane inhibition expression and the endogenous maintenance expression (Pirt model), including possible products inhibition (model Angelidaki) ;

- decay reactions;

- decomposition reactions for a compound into other ones.

Two kinds of equilibria are considered: the acid/base equilibria and the gas liquid equilibria. As for biological reactions, these equilibria are taken as dynamic phenomena (including the definition of kinetic rates).

The resulting AWS_MS MatLab toolbox is composed of about 60 scripts, most of them being related to data capture and graphical interfaces. There are 4 kinds of scripts and functions:

- Scripts for the toolbox itself (graphic interface and user interaction) ;

- Scripts and function for data capture (graphic interfaces for managing and designing the process, *i.e*. compounds, reactions and reactor parameters) ;

- Scripts and functions for simulation and computation of the process (including the rates calculations and ODE computing) ;

- Scripts for the visualisation of results.

The resulting current version of the toolbox allows performing simulations of Compartment 1, assumed to operate as a simple chemostat. The system is composed of a biphasic (gas and liquid) bioreactor with or without inputs/outputs flows, associated to an external filtration unit. The system of differential equations (ODE) representing the mathematical model of the process is integrated in the toolbox, as well as the equations required to represent the biological reactions, the gas-liquid equilibria and the acid-base equilibria. The ODE system is composed of [3 x number of compounds + 1] (*i.e.* 5 compounds involve a system of 16 ODE). It is important to keep in mind that the toolbox is not a model of the Compartment 1, but has been conceived as a tool for testing and developing various models for the process, in the limits of the core models that are implemented in the toolbox.

The toolbox was built in order to be able to :

- Define and change the set of compounds involved in the process ;

- Choose, define and fix the parameters of the set of biological reactions involved in the process, the reactions having to be chosen in the set of the 4 models currently implemented in the toolbox ;

- Design the reactor and fix all the operating variables (temperature, input flows rate and input composition….) only through a Graphic Unit Interface ;

- Save parameters of a designed process ;

- Perform simulations of the system with the choice of the ODE solver and the possibility to continue a simulation with new operating variables (typically by changing inputs flows or composition) ;

- Visualise and save the results.

The first release of the toolbox AWC_MS for the modelling and the simulation of the first compartment is currently tested by EPAS. In principle the toolbox is flexible enough to develop several kinds of models, from simple to complex ones. It would be possible to use it with most of the models already developed for anaerobic degradation processes. The architecture of the toolbox itself would permit to add new models if it is required.

The current version offers only one kind of process : a Chemostat + Filtration Unit with or without change in the volumes and the gas pressure. The modelling of the Filtration Unit is currently assumed to work in steady-state, with constant and fixed performances. The dynamic modelling of the FU would require deeper knowledge of the functioning of the membrane, and probably would also require specific studies and experiments to validate such a model. This has to be planned for further studies.

3 OUTLET GAS STREAM ANALYSIS

3.1 Important parameters for the process

Biogas is produced during the fermentation process. The composition of the biogas and the flow rate are important parameters to evaluate the process. The major component will be CO_2. However CH_4 can be produced if the system control fails and if methanogenic activity is initiated. Traces of H_2S and H_2 may be present. At pH greater than 6.5, NH_3 may be present in the gas phase. Due the equilibria between gas and liquid phases inside the reactor (depending on pH), VFA are also present in the output gas phase.

The measurement of CH_4 and H_2 partial pressures will have an important role for the functional control of the system. The production of CO_2 must be integrated in the process controller.

All these variables must be on-line recorded, on the one hand, to provide suitable information for control purposes of the bioreactor, on the other hand to obtain in real time a characterisation of the behaviour of the bioreactor and of the process itself. As for other bioprocesses, gas phase analysis of evolved biogas provides one of the most valuable clues for measuring the instantaneous kinetic rates thus enabling to enter into the understanding of the regulatory mechanisms of the complex microbial community responsible of organic matter digestion.

The important variables concerning the gas phase in the bioreactor are classified in two categories: the global variables and the composition variables.

Global variables

There are three extensive global variables (dependent of the quantity of gas) which are:

- The gas phase volume or more exactly the number of moles of the gas phase inside the bioreactor ;
- The biogas production flow rate (with conversion equations between volumic, molar and mass flow) ;
- The gas storage volume (or number of moles).

All the other global variables are intensive variables (independent of the quantity). They are :

- Temperature of gas phase inside the reactor, *i.e.* temperature of the liquid phase (namely 55 °C) assuming homogeneity of the gas phase. This is certainly not true at the reactor walls ;
- Pressure of the gas at the top of the reactor (need to be controlled) ;

- Temperature of the gas storage tank ;
- Pressure of the gas storage tank ;
- Temperature and pressure in the outlet gas circuit (see below).

Composition variables

By definition all composition variables are intensive properties. They are often reported as volumic percentages (% v or ppmv). Considering the ideal gas law applies (with high content of CO_2 this is not completely true and may introduce 1 to 3 % error in the conversion depending on temperature and pressure), the volumic percentages are equal to molar fractions. In any case, it seems preferable to work with gas partial pressures which are directly useful for understanding physical and gas liquid equilibria. The components partial pressures to assess are :

- CO_2
- CH_4
- H_2O
- H_2
- NH_3
- H_2S
- VFA
- Trace gases : organic volatiles, N_2, NO_x ...etc.

Among these components, only the water content in the gas phase is supposed to be modified between the bioreactor and the storage tank, due to mandatory condensation unit at the outlet of the reactor. Condensing the water of the gas phase may have an impact on the composition of the gas phase. Indeed, part of the water-soluble compounds is dissolved in condensed water. Nevertheless, rough calculations show that quantity of dissolved compounds may represent small part of produced metabolites. Any further condensation in the gas circuit may seriously damage the pipes and the devices due to the presence of a highly corrosive mixture of H_2S and CO_2 in combination with water.

These different variables are linked by classical thermodynamic laws. At a first approximation, ideal gas law and Dalton law (total pressure equals the sum of the partial pressures) are considered valid. More sophisticated thermodynamic models (equations of state such as Van der Waals, Redlich Kwong...etc. and non ideal gas mixing rules) can be used for further studies. These models are classically handled by commercial process simulation softwares. Presently, it can be considered that such sophistication will bring corrective terms (*ca* 1 to 3%) but will not explain the major variations which are under investigation.

3.2 Outlet gas circuit

The outlet gas circuit has the following functions :

- Storage of biogas (control of storage temperature and pressure). However, in the present design, the temperature is not controlled. It is assumed to be room temperature.
- Measurement of biogas production flow rate and of integral biogas production (redundant variables) ;
- Sanitation of gas stream before storage tank and analysers (filters) ;
- Pressure regulation inside the bioreactor (zero order control of the bioreactor pressure) ;
- Reduction of water partial pressure between bioreactor and storage tank (condensation unit) ;
- Interface with gas composition measurement units (control of pressure, temperature and humidity before analysers);

From the experience of LGCB regarding gas effluents, the flowsheet schemed in Figure 3 is proposed.

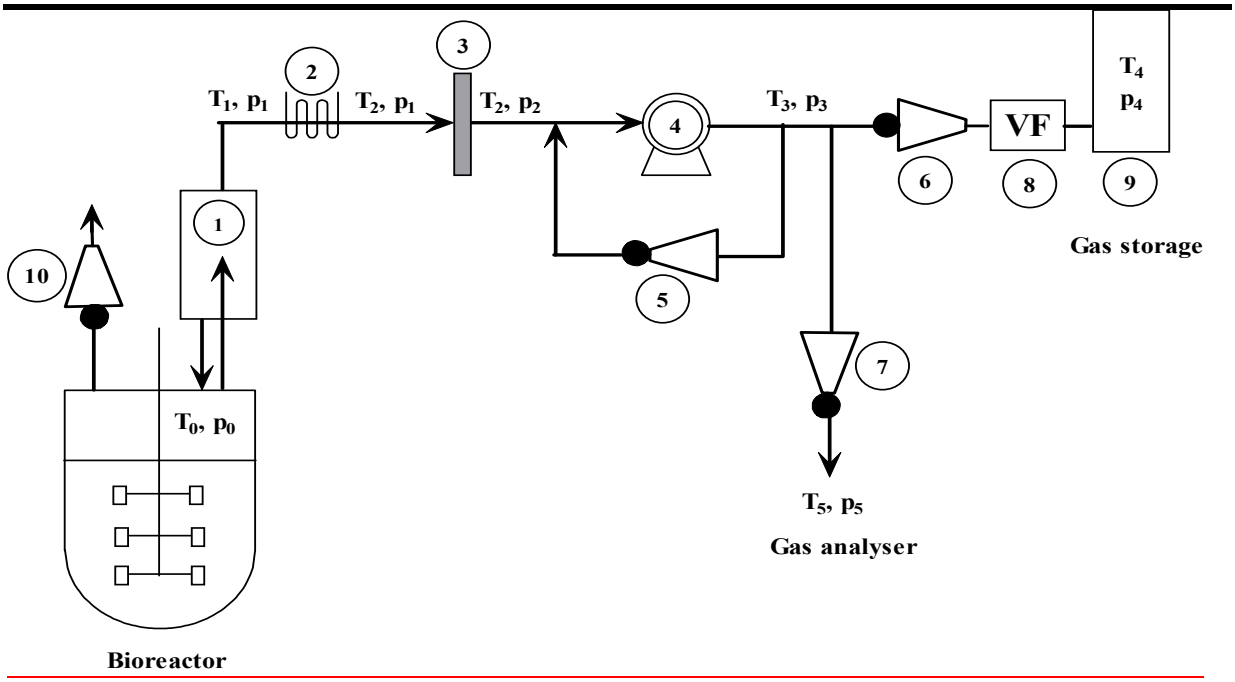


Figure 3 : Principle of outlet gas circuit of the bioreactor

1 : Condensing unit
Specifications : Inlet temperature 55°C (p_{H2O} = 15.7 kPa, 15.5 % v/v); Maximum output gas temperature 10 °C, water saturated (p_{H2O}=1.2 kPa) : condensation of 92 % of incoming vapor water.
2 : Heater
Specifications : Inlet gas temperature 10 °C ; Outlet at least 40°C (relative humidity 16 %)
3 : Filter for sanitation of gas effluent (pore size 0.2 µm) :
Remark : must function at absolutely no water condensing conditions
4 : Compressor
Specifications : Inlet pressure 100 kPa ; Outlet pressure 300 kPa ; Gas flow rate 1 to 3 $L.min^{-1}$ which is 100 to 300 times the maximum net biogas production, i.e. 15 $sL.day^{-1}$ for the 100 L pilot reactor
Remark : Corrosive gas.
5 : Downpressure regulator (Control of p_2)
Specifications : Upstream pressure 300 kPa, Downstream controlled pressure 100 kPa ; gas flow rate 1 to 3 $L.min^{-1}$
6 : Backpressure regulator (security valve) (Control of p_3)
Specifications : Upstream controlled pressure 300 kPa ; Downstream pressure 100 kPa ; Gas flow rate 15 $sL.day^{-1}$ (0.01 $L.min^{-1}$)
7 : Downpressure regulator for feed of gas analysis system. Gas flow rate TBD ; Pressure TBD
8 : Volumic flowmeter
Specifications : Gas flow rate 15 $sL.day^{-1}$ (0.01 $L.min^{-1}$), Temperature TBD ; Pressure 100 kPa.

9 : Gas storage vessel (ca. 150 L).
Remark : Possibility to follow gas volume variation by emptying liquid water.
10 : Security valve (Emergency control of bioreactor pressure) : Upflow maximum pressure 150 kPa.

The specifications reported in the figure captions are calculated considering a maximum carbon conversion of 3 $g.L^{-1}.day^{-1}$, 1/3 of the carbon being oxidised in CO_2 which leads to a maximum biogas production of *ca* 12% for the 100 L pilot bioreactor.

Importantly, the condensing unit must remove up to 90 - 95 % of the water vapour leaving the reactor with biogas. This means that this unit might not be under dimensioned. Water humidity must be kept at low value at the filter level in order to prevent any condensation on the filter and consequently a rapid clogging. In the same way, gas analysers, whatever the selected technique, will require more or less dry gas. These two reasons justify a heater device after the condensation unit.

The compressor 4 and the down-flow-pressure regulator 5 enable to control the pressure p_2 and therefore the pressure inside the bioreactor, assuming a low pressure drop between the top of the bioreactor and the outlet of the filter. In fact, it could be possible to control directly the pressure in the reactor p_0 by using an electronic down-flow-pressure regulator 5 instead of a mechanical one (basically by mechanical spring control). This would also give the advantage to have the bioreactor pressure as a process variable commanded by control software instead of a manual approximate regulation.

The measurement of biogas flow rate is much more difficult than for aerobic cultures because the composition of the gas is *a priori* not well defined. This eliminates the classical electronic mass flowmeters that use the fact that the properties of the gas are close to those of air or nitrogen. The only technical solution is to use volumetric measurement, the value of which depends on temperature and pressure at the location of the measurement. This in turns requires to perform the measurement close to atmospheric pressure and at normal temperature (0-25°C). Consequently, the gas flow rate measurement must be performed after gas sampling for analysis. This may introduce non negligible underestimation of gas production and will have to be taken into account for calculating total biogas production. The maximum net biogas production is in the order of 0.01 $L.min^{-1}$ which is in the same range as the consumption by gas analysers or gas circuit purge and washout (5 to 50 $cc.min^{-1}$). Therefore the sampling for gas composition analysis may not be considered as negligible, even for a 100 L pilot bioreactor.

The global design schemed in Figure 3 must be considered as a principle design, knowing that valves, starting circuit would remain to be add. Of course, temperature and pressure sensors must be added on the design and interfaced on the data acquisition system.

3.3 Gas composition assessment

The biogas production, even if not well defined, has a composition ranging within the following intervals :

- CO_2 : 60 to 95 %
- CH_4 : trace to 15 %
- NH_3 : trace to 1 %
- H_2 : trace to 5 %
- H_2S : trace to 1 %
- H_20 : 1 to 10 %
- Volatile fatty acids : trace to 5 %
- Inert gas (Ar, N_2, … etc)

The on-line analysis of CO_2, CH_4, H_2 and VFA partial pressures is supposed to give information on the metabolic activity of the microbial community into the reactor. The integrals of production of these compounds are important data for evaluating degradation of solid substrates and will be primarily informations for characterizing the fine kinetics of degradation. Moreover, this kind of measurement will be the clue for checking the mass balances of the compartment.

Globally speaking, it is possible to classify the gas components in 3 groups:

- Major compounds at compositions greater than 10 % v/v : CO_2 and eventually CH_4 and probably a CO_2 as the major compound (more than 90 % v/v), *i.e.* like a gas solvent ;

- Minor compounds at compositions lower than 5 % v/v : H_2O, H_2, H_2S, NH_3, VFA, aroma (organic volatiles), probably CH_4 ;

- Trace gas at compositions lower than 100 ppmv : N_2, NO_x, aroma and organic volatiles and also some VFA.

The analytical technique will mainly differ between these groups by the pretreatment procedure of the gas sample before the detector, knowing that for the last group an enrichment procedure (by passing the gas through a trap containing an adsorbent such as ceramics) may be necessary.

Regarding the gas components detection and quantification, there are basically two ways :

- compound - specific measurement techniques (CO_2 analyzer, VFA analyzer...etc.)
- generic measurement technique for all compounds.

Gas chromatography is a classical measurement technique but it must be outlined that that this technique has several drawbacks :

- frequent quantitative calibration for each gas
- no measurement of unexpected gas
- aging and clogging of separation columns
- difficulty for sequenced and automatic on-line analysis.

Quantitative Mass Spectrometry is becoming more and popular and reliable.

These two techniques are reviewed in the following sections.

4 GAS CHROMATOGRAPHY

4.1 Introduction

The advantage of the chromatography is that it is a classical measurement technique which needs a minimum experience. Gas chromatography is used to separate volatile organic and inorganic gas mixtures; the basic components are considered to be :

- a pneumatic control for the carrier gas;
- an injection device;
- a column;
- a detector (one or several techniques possible on the apparatus);
- a thermal control system.

A gas chromatographic analysis is based on the sample components being partitioned between the gas (mobile phase) and a liquid or solid adsorbent (stationary phase) by passing through a separative column. The liquid or solid adsorbent is held immobile in the column on a solid support or is coated on the walls of the column. Partition of the sample components depends on column operating parameters, including carrier gas flow rate, temperature, vapor pressure and on properties of the liquid or solid adsorbent.

Basically chromatographic analysis is a batch technique. A sample is introduced through the injection device. If it is in liquid state, it is vaporized in the injection device just before the column. It is carried through the column by an inert gas flowing at a controlled rate. Due to different retention (or adsorption properties into the column) of the gas components in the mixture, the components of the gas flow exit the column separately. The eluate from the gas chromatographic column then enters the detection system directly. An enrichment system may be used before the introduction of the gas sample into the column.

The principle is schemed in Figure 4.

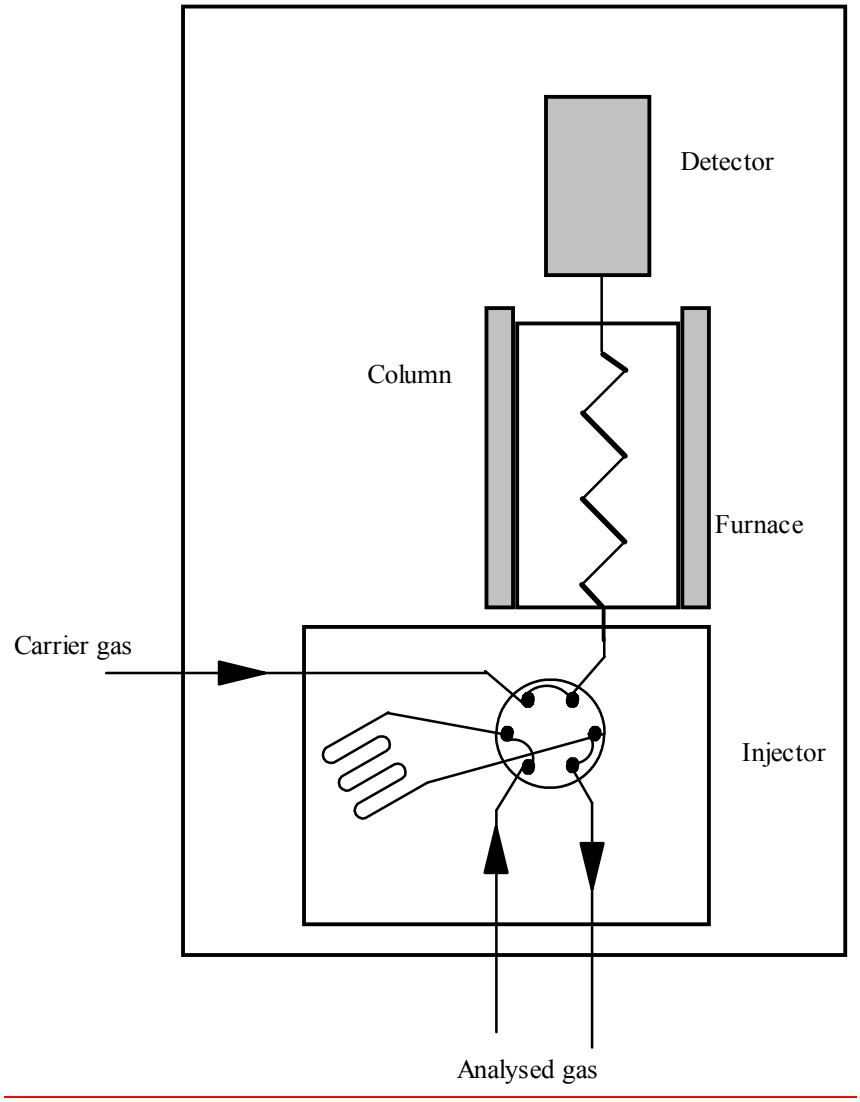


Figure 4: Gas chromatography principle

4.2 Carrier gas

The carrier gas must be sufficiently pure and inert with respect to the stationary phase. Helium is currently used. Other gases are hydrogen, nitrogen or argon. Traces of unwanted contaminants in the carrier gas must be removed by means of molecular sieves scrubbers, filters and filter-dyers. These devices must be inserted in the supply line as close as possible to the gas chromatographic column as practical. The minimum purity of any carrier gas should be 99.995 %.

The carrier gas flow rate is controlled by example over the range of 5 to 75 $mL.min^{-1}$ for a packed column. The carrier gas head pressure is controlled from at least 100 to 270 kPa (absolute pressure). The values of the flow rate and the head pressure selected must be controlled to within 1%.

It must be emphasised that the use of a carrier gas results in a consumption of external inert gas.

In the case of gas effluent from liquefying compartment, a possibility should be to use directly the biogas without any carrier gas.

4.3 Injection device

The injector is used to introduce in the column the gas mixture to analyze. In general, the injection chamber must to be at a temperature higher than the column temperature to facilitate the sample evaporation if any droplet remains in the gas sample. A heated injection device is described as having three temperatures: the injection zone, the temperature gradient to ambient and a temperature gradient to the oven. The injector must guarantee the mixture composition (reliability) and preserve the reproducibility of the injections (accuracy).

An injection device may be one of the following types: packed column, splitless capillary column, split capillary column, direct capillary column, purge-and-trap, static head space and sample enrichment injection device depending on the type of components that have to be separated on the column.

The split injection device is useful when the gas sample to analyze must be diluted in a gas solvent (carrier gas) in order to prevent detector saturation. The splitless injection is useful when all the separated compounds onto the column are sufficiently present in the gas sample to be assessed on the detector without any saturation of it. The purge-and-trap and sample enrichment device are useful when components of interest must be concentrated onto a solid support before being desorbed and released into the separation column for further analysis by the detection system.

From a general point, the basic qualities of the injection device are classified as follows :

- quality of mixing (temperature and composition) between carrier gas and gas sample;
- reproducibility and control of gas sample quantity injected into the column;
- reproducibility of gas split when required.

In general the quality of the injection makes an important part into the overall quality of the apparatus.

In the case of the biogas produced by the liquefying compartment, it can be clearly shown that the major components (CO_2 and perhaps CH_4) will be assessed with a split injection with an

inert gas (He or Ar) as carrier. The important challenge is to valuably separate these gas on the column.

The minor compounds will be assessed with a splitless device provided that the column will be able to separate H_2, NH_3 and VFA and that the major gas (CO_2 and CH_4) will not poison the detection.

The trace compounds can be assessed with an enrichment system, provided that the other major compounds (CO_2, CH_4, H_2, NH_3) will not compete to much to fixation onto the solid adsorbent with the target components (aroma, VFA...).

4.4 Columns

It is a tube or a pipe which are empty or contains an active substance called stationary phase. The column is swept by the mixture of gas sample and carrier gas. In a packed column, the constituents of the sample interact with the stationary phase. They are separated in function of their affinity with these stationary phase. In a capillary column, the mean free path of the molecules is greater than the characteristic dimension of the column (its diameter) in such a way that the flow regime is molecular. This means that small molecules travel faster than the big ones (Knudsen diffusion).

4.4.1 PACKED COLUMNS

The packed columns are pipes in which there is a solid support like grains uniformly divided.

Packed column characteristics:

- internal diameter : 3.2 mm or 6.4 mm ;
- length : 0.5 to 3 m ;
- pipe in stainless steel, glass or fused silica; the pipe must to be chemically inert and a good thermal conductor.

The efficiency of the packed columns is limited by:

- the molecular dispersion of the solute through the grains of the furnishing ;
- the difference of retention due to the non uniformity of the repartition of the stationary layer on the grains.

The packed columns are used to separate permanent gas like CO_2 - H_2 - N_2 - O_2 - CH_4…etc. These columns are often connected to an enrichment device that selectively eliminates a large portion of the major compounds and / or the carrier gas.

A schematic representation is given in Figure 5a.

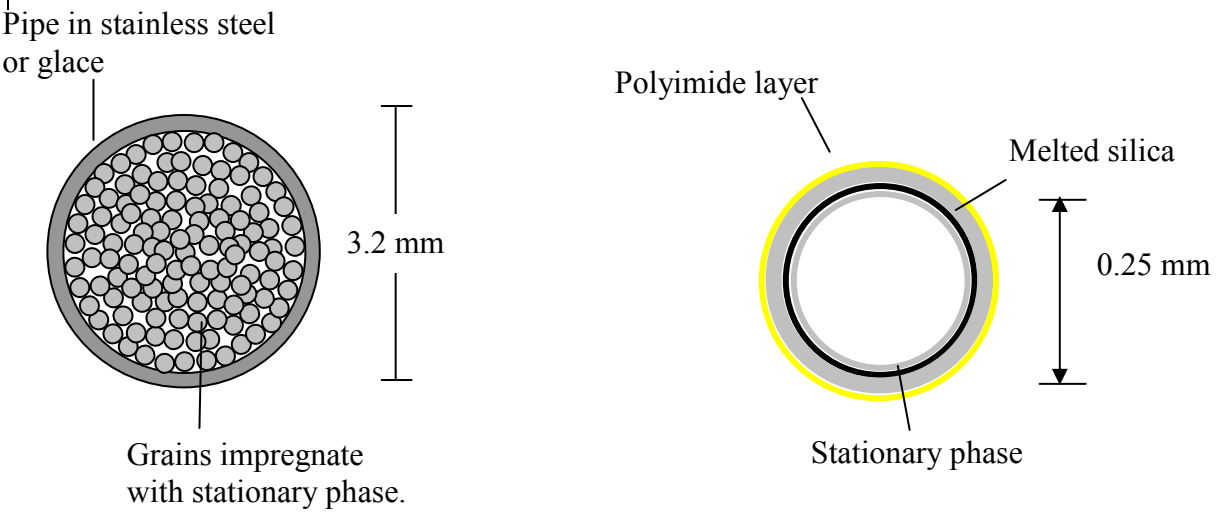


Figure 5: Packed and open tubular (capillary) columns

4.4.2 CAPILLARY (OPEN TUBULAR) COLUMNS

Usually the wall effects play a minor role for gas flow in tubes of internal diameter ranging between 0.1 and 2 mm so that the flow remains laminar instead of molecular. However wall effects become significant in tubes which are very long in comparison to their diameter and with sufficient chemical affinity between the walls and the components of the gas in such a way that molecular flow is restored.

The capillary columns have the internal wall overlayed with a stationary phase thin layer. The efficiency of these columns depends only of the rate through the stationary phase. The capillary column characteristics:

- internal diameter : 0.2 to 0.8 mm ;
- length : 15 to 100 m ;
- pipe in fused silica overlayed by a polyimide thin layer.

The capillary columns (open tubular) are used to separate polar or non polar compounds in function of the stationary phase. The system is adapted to Volatile Fatty Acids.

The stationary phases which can recommend either for packed or capillary columns are given in the following table. The carbowax stationary phase is generally a good compromise for separating classical organic polar compounds.

Compound to separate	Examples	Stationary phases
Non polar C-H and C-C	Normal hydrocarbures (n-alcans)	SPB-Octyl
		Poly(methylsiloxane), SE-30
		SPB-5, PTE-5, SE-54
Polar C-H and C-C C-Cl, -Br, -F C-N, -O, -P, -S	Alcohols, ethers, thiols, amines, carboxylic acids esters and ketones.	Poly(methylphenylsiloxane)
		Poly(cyanopropylmethylsiloxane)
		PEG, Carbowax 10, 20M
Polarizable C-H, C=C and acetylene	Aromatic alcenes	Poly(cyanopropylsiloxane)
		Poly(cyanopropylphenylsiloxane)
		TCEP

4.5 Detectors

The detector is placed behind the column to detect the different substances separated by the column. Its principal quality is to give a response that is proportional to the quantity of substance so that it measures the quantity of each sample constituents using an extensive physical property of the gaseous component which depends on the quantity.

The detector can be respectively thermal conductibility detector and flame ionisation detector depending on the nature of the column (i.e. packed or capillary column).

4.5.1 THERMAL CONDUCTIBILITY DETECTOR (TCD)

A temperature sensible resistance like tungsten or platinum is placed in the gaseous flow. A thermal equilibrium is achieved when the cooling by the vector gas flow balances the heating with an electrical current. The solute gas that is to be analysed modifies this equilibrium. It is necessary that the heat capacity, conductivity and density (therefore the thermal conductibility) of the mixture carrier + solute gas are different from the thermal conductibility of the carrier gas alone. The cooling variation leads to a resistance variation. This resistance is an element of a Wheatstone bridge. It is in opposition of other resistance where the vector gas circulates. The disequilibrium of the bridge gives a signal, indicating the presence of the solute gas and proportional to the quantity (Figure 6).

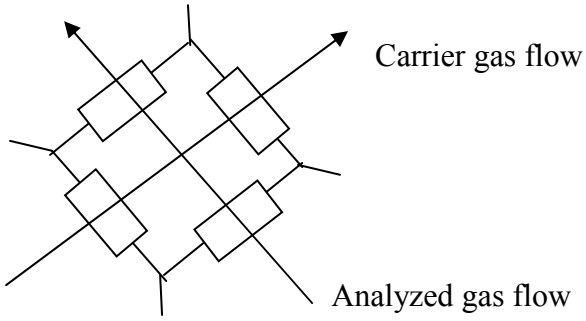


Figure 6 : Wheatstone bridge scheme for thermal conductibility detector

The characteristics of TCD are:

- moderately sensible ;
- non destructive method ;
- economic and easy maintenance ;
- sensible of temperature and flow rate changes.

The thermal conductibility detector is used to quantify permanent gases like CO_2 - H_2 - N_2 - O_2…etc. One of the drawbacks is that the thermal conductibility of solute - carrier gas mixture is not a linear function of the solute concentration because thermal conductivity is a ratio between conductivity, heat capacity and density. The calibration procedure is therefore very important except if the dilution is important or if the carrier gas is hydrogen or helium (because their conductibility is high). The following table gives an idea of the relative variations when compared to nitrogen.

Relative thermal conductibility with regard to nitrogen at 100 °C	
Hydrogen	6.95
Helium	5.54
Methane	1.72
Nitrogen	1.00
Argon	0.71

For MELiSSA liquefying compartment, the carrier gas (if any) is necessarily helium or nitrogen in order to assess hydrogen.

4.5.2 FLAME IONISATION DETECTOR (FID)

FID are generally connected to capillary columns. After the column, the gas enters the flame of a little hydrogen or air burner. The combustion produces ions that give an ionic current between two electrodes : one is the burner, the other is a collector electrode.

The FID response is proportional at the solute mass present in the burner.

The characteristics of FID are as follows:

- very sensitive ;
- linear response in function of the solute mass ;
- sensitive to temperature and flow changes ;
- not selective ;
- destructive.

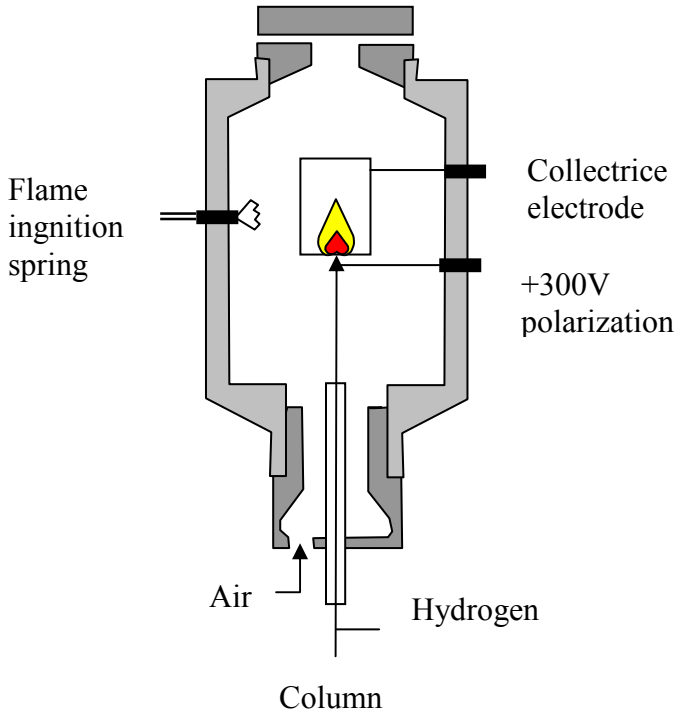


Figure 7: Scheme of flame ionisation detector

4.6 Conclusion about GC

The detectors described above are economic and universal but they don't give any information about the nature of detected substances. The retention time is not a specific characteristic of a compound. To know the composition, it is necessary to couple spectrometer with the gas chromatograph (IR or mass spectrometer)

5 MASS SPECTROMETRY (MS)

5.1 Introduction

Mass spectrometry (MS) has a long history dating back to since the mid of the previous century. The first quantitative gas analysis of volatile hydrocarbons was carried out in 1940. Breath analysis is still an important field of on-line MS application. Speed of detection and the inherent ability of MS to simultaneously analyze several compounds are the main advantages. Examples also show that MS can be used in harsh environments.

In medical areas other than breath analysis, a great deal of effort has been applied to blood gas monitoring using membrane probes. For these applications, more devoted to dissolved compounds in aqueous solutions assessment, membrane probes were designed and built.

The monitoring of isotopes in biotechnological processes (^{13}C, ^{15}N, ^{17}O, ^{32}P enriched compounds) is a field of applications which has a rapidly increasing interest due to the power and reliability improvement of the available instruments.

MS also has been applied in the monitoring of atmospheric pollution. The greatest current use of MS for process controls is submarine atmosphere control which is clearly related to Life Support applications. Recent developments also mention that a MS analyzer (MCA of Hamilton Sudstrand) has been developed for on-line monitoring of 6 major constituents (O_2, N_2, H_2, CH_4, CO_2 and H_2O partial pressure) at multiple locations within International Space Station providing the data to the environmental Control an Life Support System (ECLSS).

MS has also been applied in the direct analysis of complex gas mixtures though it is limited in this respect because of the overlapping of peak fragments from individual components (see below).

The question is here to investigate the possibility, the advantages and the drawbacks of implementing MS analysis for gas phase components assessment issued from the liquefying compartment. More generally the conclusions could be further used for other applications for on-line monitoring of gas phase analysis (and eventually liquid phase analysis see BELISSIMA project) of MELiSSA bioreactors.

5.2 Principles of MS operation

The principles of MS operation are discussed in a number of text books. Only few relevant aspects with respect to on-line analysis in fermentation are discussed briefly.

Figure 8 shows schematically the units necessary for MS use in process monitoring.

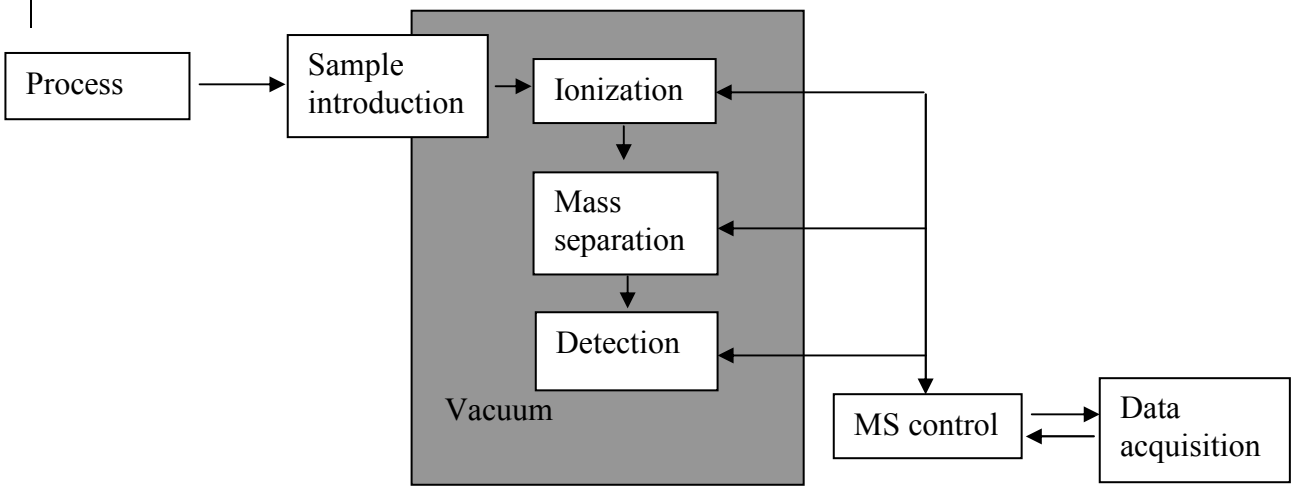


Figure 8: Basic requirements for process MS

Basically the mass spectrometer is a partial pressure analyzer of compounds carried in a gas phase. It is conceived to determine the residual gases in the secondary vacuum apparatus. It can detect the component of a gas mixture:

- qualitatively with the mass numbers ;
- quantitatively with a disconvolution procedure (see below).

A mass spectrometer is composed by three parts (Figure 9) :

- the ions source ;
- the ion separator ;
- the ion detector

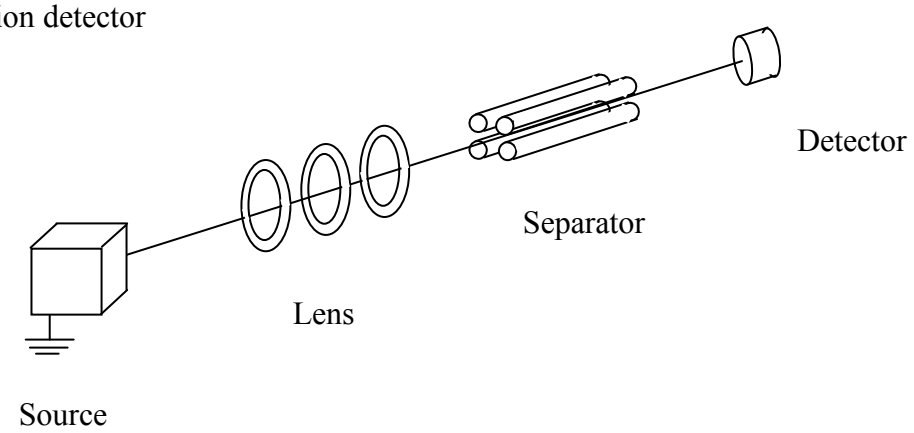


Figure 9: Different parts of the mass spectrometer

In MS, ions produced by the source will only survive sufficiently long if the vacuum is good enough. High pressure will allow collision with other molecules causing unpredictable reactions. The pressure needed in the analyzer chamber is usually < 10^{-3} Pa (10^{-8} bar).

The principle is to work a ionization cell at a very low pressure to avoid collision between the gas ions for preventing the neutral molecule is ionized by the electrons produced by the tungsten filament.

5.3 The ions source

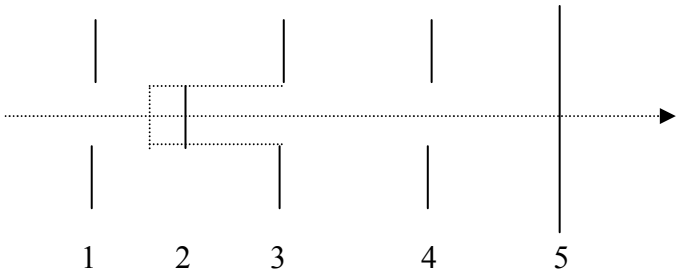
To analyze organic or mineral substances, the first step consists to produce ions of this substance in gaseous phase by electronic impact. Several kinds of ions sources are used :

- electronic bombardment source ;
- surface ionization source ;
- sparks source ;
- photo-ionization or laser source ;
- field emission source.

The electronic bombardment source (electron impact ionization) is currently used. The others are eliminated because they cannot ionize all the gas or they are less sensitive.

The electronic bombardment source (Figure 10) is composed by a heated filament which emits electrons with high energy (80 eV). The electrons bombard the gaseous molecules and give them an electric charge. The formed ions are transported toward a focal lens. So that the beam is focalized on the exit lens. The focal lens is negatively polarized like the exit lens. One part of the beam is injected in the ion separator, the other part is neutralized. It results a current flow. Its intensity is dependent on the pressure in the ions source. Total current can be used as a measure of the total pressure in the source.

Electronic bombardment source can give stable operation over long period of time. One important requirement is constant temperature. As many biological samples contain oxygen, proper selection of cathode material enhance the life time. A gas-tight ion source with cathode filament outside the ionization chamber but within the high vacuum region (< 10^{-4} Pa) improves the life time of the filament.



1- Electron repeller
2- Filament (cathode)
3- Ion cage (anode)
4- Focus lens
5- Exit lens (Total pressure plate)

Figure 10: Ions source

5.4 The ions separator: mass separation

The ion separation is the characteristic element of a gas analyzer and its performances determine the performances of the apparatus. The source provides the beam of ions which are accelerated by a extractive tension. An electrostatic optic gives the ions energy before they enter the separator. The system separates in time or in space the ions in function of the ratio mass / charge of each ion (m/z ratio).

There exist four types of ions separators:

a) Time of flight separator (TOF separator)

The time taken by the ions to go over a given distance is electrically measured. The ions are categorized in function of their speed (time of flight) during an impulsion of the ions extraction. Their major advantage of this kind of separator is unsusceptible to pollution. It is more and more widely used in protein recognition.

b) Magnetic field

The ions separation is done by a magnetic field. If a charged particle enters a magnetic field, it moves on a circle whose radius is a function of the magnetic field, the particle velocity and its mass to charge ratio (m/z). If the components are well defined and are not changing, fixed magnetic field and acceleration voltage combined with a number of simultaneously operating detectors may be used. Magnetic field based instruments, even combined with an electric field sector, can give higher resolution and has been applied in trace gas analysis. For quantitative analysis, the magnetic field separator gives better accuracy than other ions separators because they give trapezoidal peaks that are easier to interpret quantitatively.

c) Fourier transform mass separator

The ions separation is done by the measure of the ion rotation frequency which is reversely proportional of the mass.

d) Quadripole mass separator

The ions separation is done by an electrical field. The mass filter ejects all the ions excepted those which have a specific mass / charge ratio (m/z) correspondent to entire number of the oscillation period.

The quadripole analyzer consists of four rods with normally a hyperbolic section (Figure 11). In the reality, the section is cylindrical. The rods in square arrangement are connected with two at two opposed potential. A positive ion incoming between the bars is attracted by the negative potential. If the potential changes before the ion is discharged on the bar, the ion changes its direction. So, the ions are led between the four cylinders, in the parallel direction on the axis of the quadripole.

The electric field is composed of two fields :
- quadripole alternative field (radio frequency component);
- a constant field (direct current).

$\varphi_1 = U + V \cos \omega t$
$\varphi_2 = - (U + V \cos \omega t)$

ω is the pulsation of the radio frequency component
U is the direct current voltage
V is the radio frequency voltage

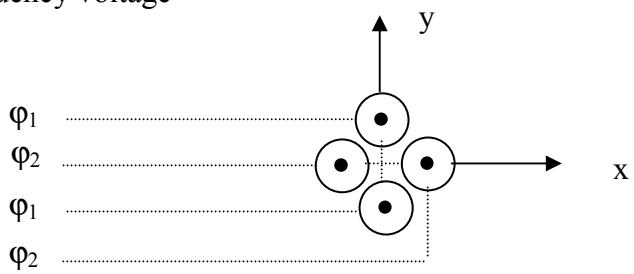


Figure 11: Voltages of the quadripole mass filter rods

The ions source produces positive and negative ions. Their selection is only function of the polarity of the quadripole. Positive ions entering the oscillating field move on oscillatory paths. Only ions with a particular mass move on stable paths, others with higher or lower masses are discharged on collisions with the rods of the quadripole. Mass selection is achieved simply by setting the DC voltage.

Quadripole instruments can be made very compact (which is of interest for space applications) and sensitive. These instruments are ideally suited for very fast selected ion monitoring and multiple ion detection.

Quadripole instruments are most frequently used in process gas analysis because they are cheaper and easier to use, though the peaks shape cannot be made trapezoidal. This separation is the most well-adapted for the analysis of residual gases in low pressure. So, this is the type of separator that must be chosen for the gas effluents analysis.

5.5 The ions detector

Ions are directly collected by a plate or Faraday cup to give an electric current which has to be amplified using an electrometer amplifier.

The Faraday cup consists only on a metallic plate where the beam is coming. The ions are neutralized on the plate and create a current. This type of detector is very accurate but the sensibility is limited.

The incoming ions are accelerated in the electron multiplicator by a potential. The collision with the surface gives the emission of secondary electrons. The ratio between ions current and secondary electrons current is the gain of the multiplicator. The electron multiplicator alone is not very accurate compared to the Faraday cup but it is very sensitive.

The association between the two previous systems for ions detection gives the best results (Figure 12). The electron multiplicator / Faraday cup system adds up the advantages of the Faraday cup and the electron multiplicator: a good sensibility and a accuracy. The gain is generally in the range 10 - 10^6.

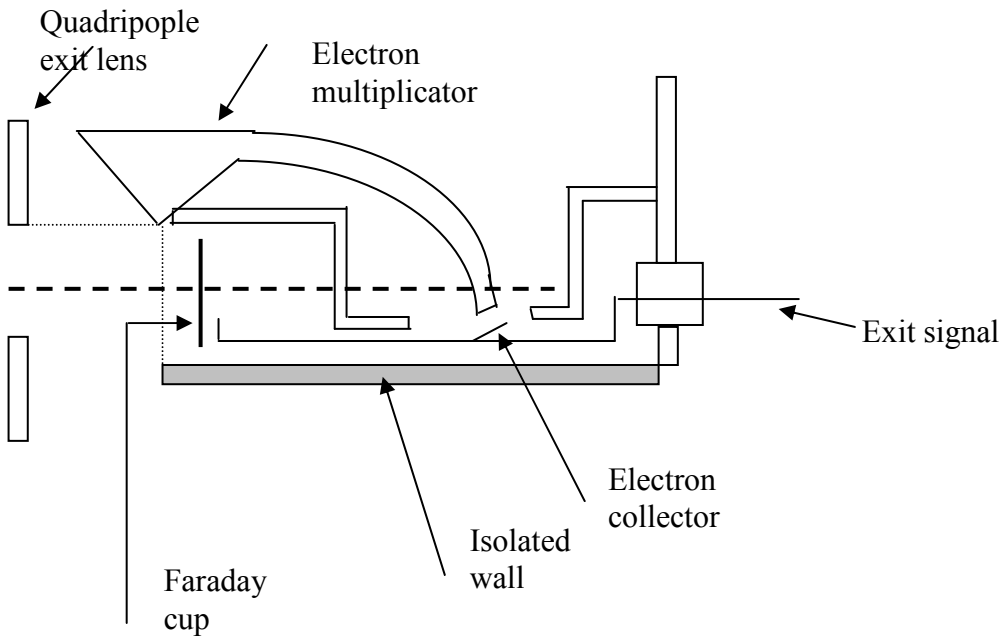


Figure 12: Electron multiplicator / Faraday cup system

5.6 Qualitative and quantitative analysis

5.6.1 QUALITATIVE ANALYSIS

The qualitative analyze consists in identifying the peaks (intensity in function of the m/z ratio). Every chemical compound being decomposed with a defined stoichiometry into a series of ions, a MS spectrum can be interpreted as a fingerprint, specific of each chemical compound. This leads to use MS to determine the chemical composition of a gas in the mass spectrometer chamber.

This application of MS is the most commonly used, especially for the new developments of MS associated with liquid chromatography and advanced studies in proteins identification and recognition (proteomics). For gas phase studies, it offers the advantages of identifying unknown compounds contained in a gas effluent. In that case, MS is also connected with gas chromatography (see below).

5.6.2 QUANTITATIVE ANALYSIS

The intensity of a peak is directly proportional to the ionic concentration in the ionization chamber for all ions having the same m/z ratio. The ionic concentration is also directly proportional to the partial pressure of the incoming gas in the source. If several ions have the

same ratio m/z but if they have a different nature, only one peak is observed, but the intensity of this peak is proportional to the sum of the partial pressure.

Dalton's law states that the total pressure in the mixture of gases is equal to the sum of its parts ($P_T = P_1 + P_2 + \ldots + P_N$). This law does not fully correlate with the output of the mass spectrometer and corrections such as :

- ionization probabilities : how easy is to make an ion ?
- fragmentation factors : how many fragments are produced when an ion is created and the amplitude of each ?
- transmission factors : how many ions actually reach the detector must be considered ?

The total pressure is taken from the total pressure plate mounted in the ion source of the sensor. This plate measures the current produced by the ions that collide with the plate on their path to the analyzer portion of the separator. A more accurate total pressure measurement can be made with an ion gauge.

The peak height is representative of the partial pressure of a component and depends on the parameters such as the ionization probability, the fragmentation factor, a transmission factor and the sensitivity of the detector. An algorithm is used to determine the partial pressure specific to each gas. The formula for determining the partial pressure of gas A based upon peak at mass B is given in equations (1) and (2):

$$PP_A = \frac{(I_{AB})x(FF_{N28})}{(FF_{AB})x(XF_A)x(TF_B)x(DF)x(S)} \qquad \text{(Eq. 1)}$$

PP_A = Partial Pressure of gas A
I_{AB} = Current of the peak at mass B from gas A (peak height in amperes)
FF_{AB} = Fragmentation Factor for gas A at mass B (from a table such as the following table)
FF_{N28} = Fragmentation Factor for Nitrogen (mass 28) (usually taken as 1,0)
XF_A = Ionization Probability of A (from a table such as the following table)
TF_B = Transmission Factor for mass B
DF = Detection Factor or relative Current per Ion (usually taken as 1,0)
S = Sensitivity for Nitrogen at mass 28 in Amperes/Torr

Equation (1) can be simplified, the Transmission Factor for Mass B being pre-programmed in software.

$$PP_A = \frac{(I_{AB})}{(FF_{AB})x(XF_A)x(S)} \qquad \text{(Eq. 2)}$$

Example of fragmentation factors are given in the following table.

Fragmentation factors and ionization probabilities for common gases			
Substance	Ionization probability	Most intense peak	Fragmentation factor
Ammonia	1.3	17	0.53
Argon	1.2	40	0.88
Carbon dioxide	1.4	44	0.85
Carbon monoxide	1.05	28	0.91
Hydrogen	0.44	2	0.98
Hydrogen sulfide	2.2	34	0.52
Methane	1.6	16	0.46
Nitrogen	1.0	28	0.94
Oxygen	1.0	32	0.95
Water	1.0	18	0.75

If there are overlapping peaks, such as the presence of both carbon monoxide and nitrogen, the calculation is more complex.

If mixtures with superimposed ion fragments intensities have to be analyzed, a system of linear equations must be solved to calculate concentrations of individual components in the mixture. The general method for quantitative analysis of mixtures with N components with contribution X_{ij} assume linear superposition of peak intensities. The measured ion currents H_J for all masses are considered to be the sum of the contributions of all components, which are proportional to the concentration of each component, and to fragment factors FF_{1J}, FF_{2J}, FF_{3J}, … FF_{NJ}.

Mass A : $FF_{1A} X_1 + \ldots + FF_{NA} X_N = H_A$

Mass B : $FF_{1B} X_1 + \ldots + FF_{NB} X_N = H_B$

Mass M : $FF_{1M} X_1 + \ldots + FF_{NM} X_N = H_M$

For the result is compatible for all peaks, it is necessary to calculate the residues.

$R_A = H_A - (FF_{1A} X_1 + \ldots + FF_{NA} X_N)$

$R_M = H_M - (FF_{1M} X_1 + \ldots + FF_{NM} X_N)$

The values of $X_1,\ldots, X_N$ are such as the sum of square of the residues is minimal. This condition is obtained only if all following equations are satisfied :

$$R_A \frac{\partial R_A}{\partial X_1} + \ldots + R_M \frac{\partial R_M}{\partial X_1} = 0$$

$$R_A \frac{\partial R_A}{\partial X_N} + \ldots + R_M \frac{\partial R_M}{\partial X_N} = 0$$

Knowing that $\frac{\partial R_J}{\partial X_I} = FF_{I\,J}$, it comes :

$$R_1\ FF_{1A} + \ldots + R_M\ FF_{1M} = 0$$

$$R_1\ FF_{1N} + \ldots + R_M\ FF_{NM} = 0$$

Which is set of N equations leading to obtain the vector X_1,..... X_N.

In matrix notation if FF is the abundance matrix, having in each column the abundance of each compound for the investigated mass (FF a matrix of M lines and N columns), the previous equation is written in a matricial form :

$$FF^T\ [H - FF\ X] = 0$$

And the composition ratio of the gas is given by :

$$X = (FF^T.FF)^{-1}\ .\ FF^T\ .\ H$$

The exact composition of the gases is given by the relation :

$$\%A = \frac{X/\sigma_A}{\sum_{gases} X/\sigma_{gzs}} \times 100$$

where σ_A is the cross section of ionization of the gas A. Further statistical analysis can be made for obtaining the error estimations.

5.7 Application to the gas effluent of the first compartment.

The previous brief presentation shows that analysis of complex mixtures of gas can be quantitatively analyzed. The main drawback is that when peaks are overlapping (that is always the case for the small m/z ratios), the linear algebra calculations may hide severe uncertainties. This point can be approached by considering the abundance matrix of a given mixture.

The abundance of the main gases supposed to be in the Compartment 1 is given in the following tables.

Hydrogen Formula : H_2 Masses and abundances*100						
2	100					

Ammonium Formula : NH_3 Masses and abundances						
14 15 16 17	3.8 4.1 90 100					

Water Formula : H_2O Masses and abundances						
16 17 18	1.8 27 100					

Methane Formula : CH_4 Masses and abundances						
2 12 13 14	2.4 1.6 4.2 10.7	15 16 17	88 100 1.2			

Nitrogen Formula : N_2 Masses and abundances						
14 28 29 30	6.2 100 0.7 0.01					

Carbon monoxide Formula : CO Masses and abundances						
12 14 16 28	1.1 0.9 0.2 100	29 30	1.1 0.2			

Carbon dioxide Formula : CO_2 Masses and abundances						
12 14 16 22 28 29	3.5 0.28 7.5 2.8 18.5 0.3	44 45	100 1.1			

Hydrogen sulfide Formula : H_2S Masses and abundances											
32 33	44.4										
34 35	42.2										
36	100										
	2.5										
	4.2										

Acetic Acid Formula : $C_2H_4O_2$ Masses and abundances											
12 13	1.4	25 26	0.8	31 32	3.7	39 40	0.1	45 46	93.8	58 60	0.1
14 15	5.1	27 28	0.7	35 36	0.4	41 42	1.1	47 55	1.1	61 62	63.6
16 24	7.4	29 30	0.4	37 38	0.2	43 44	4.5	56 57	0.4		1.4
	34.5		7.2		1.8		20.2		0.1		0.2
	5.5		12.1		0.1		99.9		0.1		
	0.3		0.5		0.5		5.2				

Propionic acid Formula : $C_3H_6O_2$ Masses and abundances										
18 26	13.1	30 31	14.1	39 41	3.6	45 46	55.7 6	57 60	30.1	
27 28	21.1	32 37	4.2	42 43	5.1	47 55	4.5	73 74	0.3	
29	61.7	38	1.4	44	5.5	56	16.8	75	48.4	
	100		2.1 1		8.4		16.4		78.5	
	83.4				7.1				2.6	

Butyric acid Formula : $C_4H_8O_2$ Masses and abundances											
17 18	0.3 1	37 38	0.9	46 47	0.5	56 57	0.3	69 70	1.3	83 84	0.1
25 26	0.2 2	39 40	1.6 8	49 50	0.5	58 59	0.5	71 72	0.5	85 86	0.1
27 28	16.4	41 42	1.3	51 52	0.1	60 61	0.1	73 74	2.3	87 88	0.1
29 30	4.4	43 44	16.8	53 54	0.1	62 67	0.2	75 81	0.2 30	89	0?2
31	9.3	45	16	55	0.1	68	100	82	1.5		1.8
	0.3 9		14.8		0.1		2.5		0.2		2.4
			0.7		0.3		0.5		0.1		0.4
			12.9		0.2		0.1		0.1		
					5.5		0.2				

Isobutyric acid Formula : $C_4H_8O_2$ Masses and abundances											
13 14	0.1	27 28	24.4 2	38 39	1.3	46 47	0.3	60 69	1.5	87 88	0.8
15 17	0.3	29 30	5 0.1	40 41	9.1	53 55	0.6	70 71	0.5	89	7.9
18 19	1.4	31 32	0.8	42 43	1.5	56 57	0.3	72 73	0.5		0.4
25 26	0.1	36 37	0.2	44 45	39.1	58 59	3.8	74 75	1.9		
	0.6		0.1		9.8		0.3		0.1		
	0.2		0.9		100		0.1		25.8		
	0.2 2				3.5		0.1		0.9		
					7.6		0.6		0.1		

Valeric acid Formula : $C_5H_{10}O_2$ Masses and abundances											
26 27	2.7	39 40	9.3	49 50	0.3 1	58 59	0.6	71 73	0.3	85 87	1.3
28 29	15.4	41 42	1.2	51 52	1 0.4	60 61	1.5	74 75	40.5	88	3.6
30 31	6.6	43 44	16.8	53 54	1.3	62 67	100 7	76 81	4.6	101	0.2
36 37	16.6	45 46	11.1	55 56	0.6 11	68 69	0.7	82 83	0.7	102	0.5
38	1.3	47	13.4	57	6.8 4	70	0.3	84	0.3		0.5
	1.7		1.6				0.3		0.4		
	0.3		11.9				0.9		0.2		
	0.5		0.8				0.2		1.2		
	1.2		0.6						0.5		

Isovaleric acid Formula : $C_5H_{10}O_2$ Masses and abundances											
25 26	0.3 3	39 40	17.9	50 51	0.8	60 61	100	74 75	0.9	88 91	0.7
27 28	25 6.1	41 42	2.7	52 53	0.9	62 64	6.9	77 79	0.3	100	0.2
29 30	11.5	43 44	37.5	54 55	0.2	67 68	0.5	82 83	0.1	101	0.9
31 36	0.2	45 46	10	56 57	1.1	69 70	0.1	84 85	0.1	102	0.4
37 38	3.4	47 49	32.9	58 59	0.6	71 73	0.2	86 87	0.5	103	0.5
	0.2		1.1		3.8 3		0.4		1.3		0.1
	0.9 2		18.1		2.8		4.2		0.2		
			0.5		0.6		0.2		1.3		
			0.2		4.2		0.2 1		0.2		
			0.2						15.3		

From the previous tables, the matricial product (FF^T . FF) can be calculated for the set of the above products and for masses ranging from 2 to 105. The statistical analysis shows that in principle all the above components can be quantified simultaneously. However some drawbacks still remain to be solved:

- H_2 quantification will be hardly possible if it is in small quantities. All the above products desintegrate in small quantities of ions of masses ranging between 2 and 12 (data not shown in the tables). Consequently, numerous interferences may pollute the H_2 signal.

- Analysis of a gas effluent where CO_2 is the major compound may render difficult the quantification of products of smaller molecular weight although CH_4 and NH_3 would in principle be determined accurately.

5.8 Mass spectrometer specifications

5.8.1 CAPILLARY INLET

For the analysis of gases, a capillary inlet must be chosen method in order to reduce the gas pressure form the sample pressure to ion chamber pressure. As can be seen in Figure 13, the pressure is firstly reduced from 300 kPa (see Figure 3) to approximately 100 Pa using a capillary. The necessary pressure drop is created by a rotary pump. Under this condition flow in the capillary will be laminar. Flow in the connection line to the rotary pump also has to be in the laminar flow region. This avoids enrichment of compounds according to their masses, which only occurs in the molecular flow region where the mean free path of molecules is larger than the inner diameter of the tube.

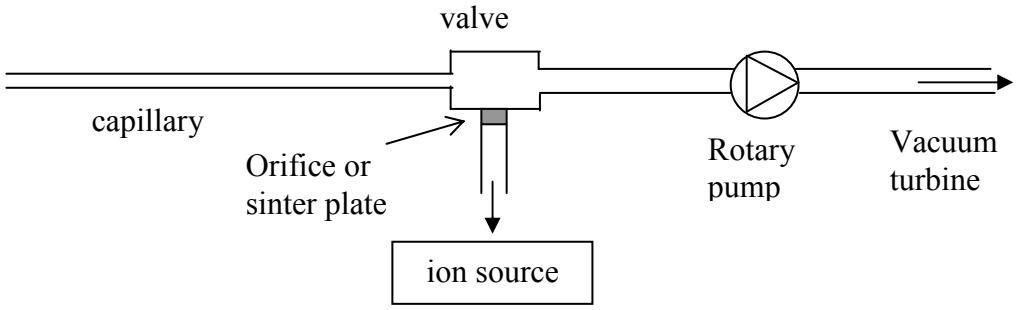


Figure 13 : Capillary inlet

At temperature below the boiling point of the volatile compounds, condensation may occur if the vapor pressure is sufficiently low. It is necessary to heat the capillary and to use inert materials.

5.8.2 TECHNICAL SPECIFICATIONS.

The specifications for the mass spectrometer, coupled with the Compartment 1 are summarized in the following table.

Mass range	1 to 200 uma
Detector type	High performance combination electron multiplier Faraday cup.
Base pressure Performance	$< 6.6. 10^{-7}$ Pa
Ambient temperature	20°C to 50°C
Relative humidity	≤ 80%
Utility requirements	No rack space required Compressed air located near the compound pump 220/240 VAC, 2 amp outlet located at the power module 220/240 VAC, 0.75 amp outlet located at the foreline pump

6 GC / MS ANALYSIS

From the above, it clearly appears that neither GC nor MS alone can solve the problem of on-line analysis of the gas phase effluent of the first compartment.

The best solution is to use, from one hand the separation capacities of a chromatograph and the general capacities of MS for identifying and quantifying gas compounds, even if they are present in gas mixtures. Gas chromatography coupled to mass spectrometry is a method that combines the features of gas chromatography and mass spectrometry to identify different substances. The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph uses chemical differences to separate the molecules within the compound sample by controlling the time required for different kinds of molecules to arrive at the mass spectrometer. The mass spectrometer then breaks each molecule into ionized fragments and identifies each molecule from the charge and mass of the ionized fragments

For compartment I, the exhaust gas contains permanent gases (CO_2, CH_4, NH_3, H_2, H_2S, H_2O,…) and Volatile Fatty Acids. Considering that the simultaneous quantification of VFA and permanent gas will be hardly achievable simultaneously because of the difference in concentrations, the analyzer has to be composed of two gas chromatographic columns : one with a packed column for permanent gases separation and one with a capillary column for VFA.

As the outlet gas flow of the fermentor, the circuit to the Mass Spectrometer analyzer must be heated to at least 40 - 65°C for avoiding any condensation. Water remains a problem either for the detection system or for the chromatographic columns. The choice of water trap depends of the gaseous phase. For example, silica gel adsorbs CO_2 and others can adsorb VFA. But to protect the analyzer, it is necessary to have a dew-point of - 40°C at the entrance of the gas analysis system.

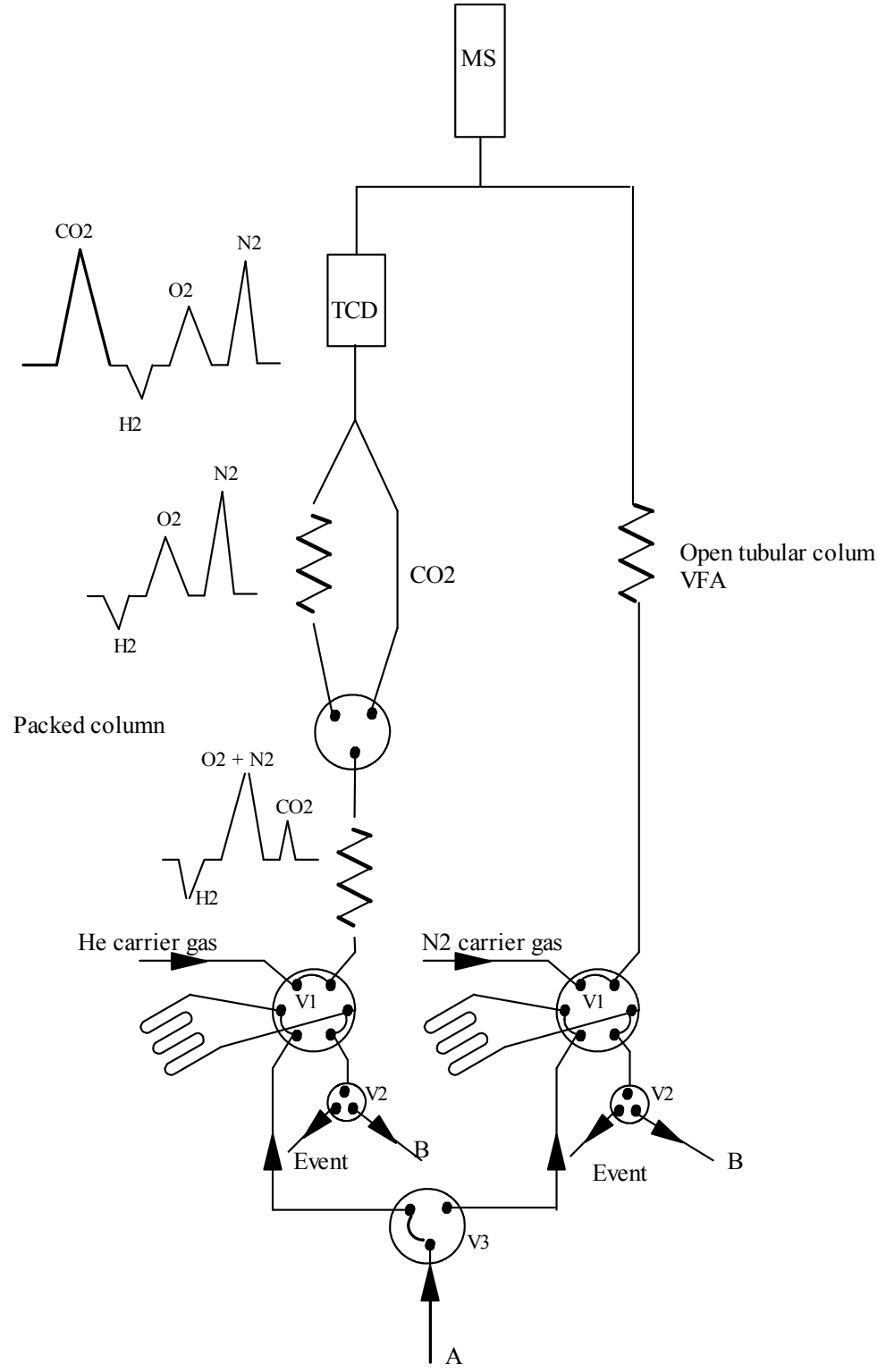


Figure 14: Two ways gas chromatograph circuit

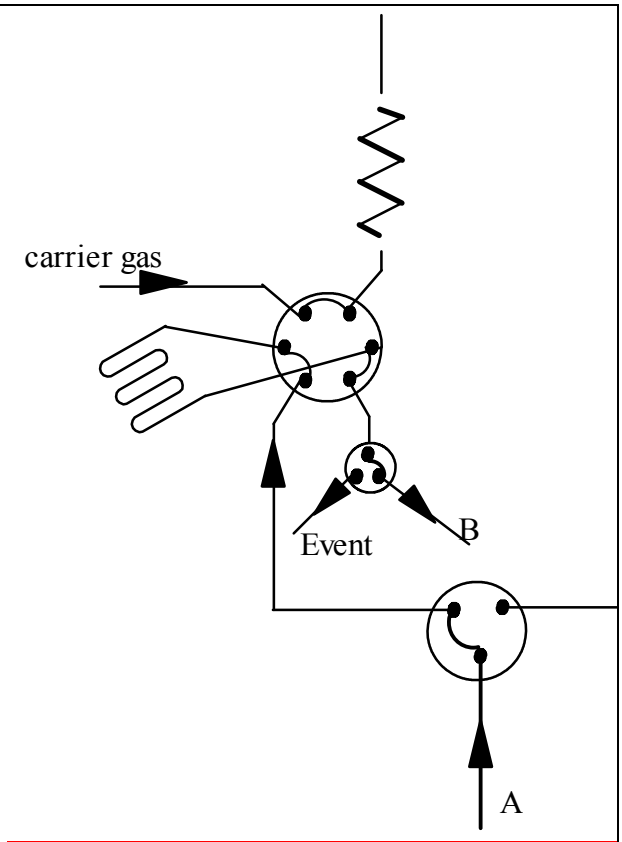
The requirements can be summarized as follows:

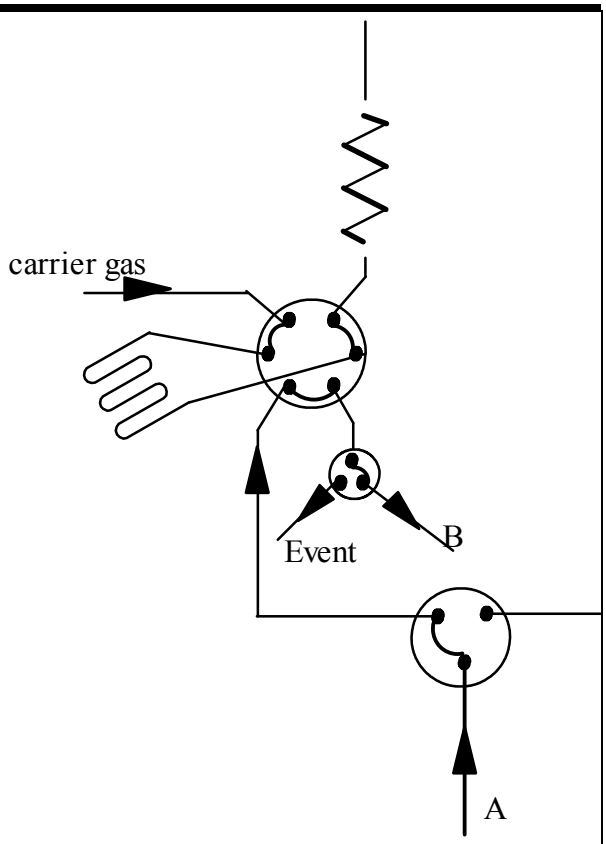
- separate permanent gas and enable identification and quantification knowing that some of them can be present at low concentration, *i.e.* 1 % H_2 in gas containing 95 % CO_2.

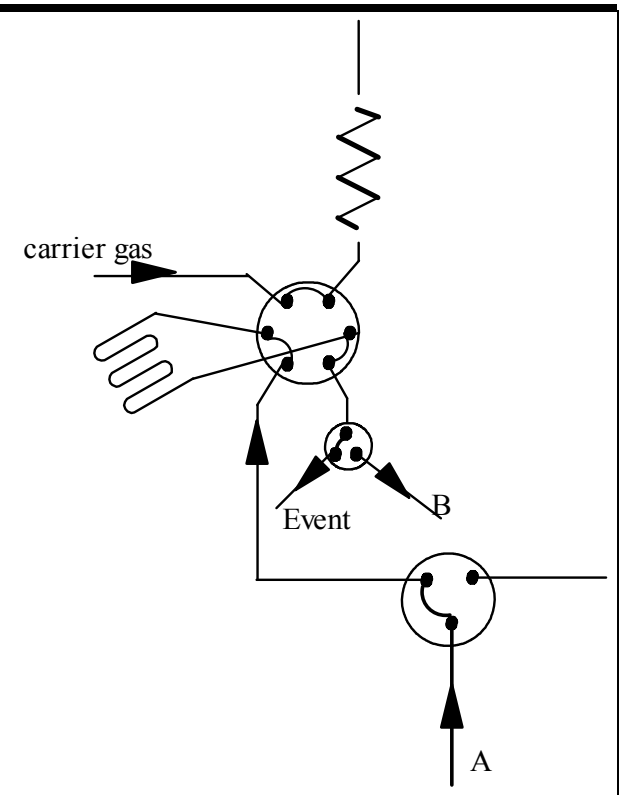
- separate VFA and enable identification and quantification in terms of partial pressure into the incident gas.

The solution that is proposed consists in using packed column chromatography for separating permanent gas and capillary column for VFA and other diluted organics.

The basic principle of such an operation is schemed in Figure 14. The gas is alternatively distributed in the packed column and the capillary column with the V3 valve. It is important not to inject carrier gas (He or N_2) in the outlet gas circuit, so design the two ways gas chromatograph circuit with two events. Analysis is performed in 3 steps to avoid pollution with vector gas. The three steps are described in the table hereafter.

Step 1 Compartment I gas is introduced in the loop to rinse the loop. Gas return in the outlet gas circuit in B. In general, the loop have a volume of 0.25 ml.	

Step 2: Carrier gas is introduced during 12 sec. the vector gas pushes the compartment I gas present in the loop toward the separation column. Analyze can be possible.	

Step 3: Compartment I gas is introduced in the loop to purge vector gas. To no pollute gas circuit, this gas is ejected by event. The purge time is estimated at time necessary to pass 5 loop volumes.	

The interface between the gas chromatograph and mass spectrometer is a separator that preferentially removes the gas chromatograph carrier and permits a flow of enriched gas sample to enter the spectrometer. For the permanent gas, a TCD detector detects the peaks in order to switch the gas sample (after column separation) for entering the spectrometer. Generally, there is a molecular jet separator between GC and MS. Direct coupling to the mass spectrometer is usually used for capillary columns.

The specifications for the two ways mass spectrometer gas chromatograph, coupled with the Compartment 1 are summarized in the following table.

Permanent gases	Packed column
Volatile Fatty Acids	Capillary column
Detection	Quadripole mass spectrometer
Transfers lines	Sample and injection with thermal regulation until 150°C
Sampling	Internal pump with heating valve
Cycle time	Complete cycle : 6 minutes : adsorption, - disorbtion - injection - cleaning - cooling down
Alimentation	110/220V or 12V with batteries Max 80 W

Of course, this experimental scheme needs to be further improved, but it must be outlined that on-line analysis of gas effluent composition, including trace compounds and major compounds such CO_2, H_2 and CH_4 can be performed. The main advantage of such a technique is that in the case of presence of unexpected compounds, the analytical system will record a signal and propose identification and determine a concentration.

Hereafter, a non exhaustive list of companies that propose equipments, some of them being already miniaturized for embarked application or specially designed for functioning in harsh or industrial environments.

Gas chromatograph companies

- Agilent technologies
- Perkin Elmer instruments
- SRA Instruments
- Varian Analytical Instruments

.Mass spectrometer companies

- Balzers AG
- Bruker Instruments Inc.
- Edwards High Vacuum International
- Hewlett-Packard Co
- JEOL Inc
- Kurt J. Lesker Co
- Leybold Inficon Inc
- Micromass UK Ltd
- MKS Instruments Inc
- Perkin-Elmer Corp
- Seiko Instruments USA Inc
- Varian Analytical Instruments

7 CONCLUSION

The above study was intended to predict the general behavior of the pilot facility of Compartment I of MELiSSA considering the results obtained from previous experiments on a smaller volume bench scale reactor. The values of substrate degradation and of biogas production which are given must be considered as orders of magnitude considering global stoichiometric conservation with a steady state assumption based on preliminary results already obtained.

Some critical issues remain to be faced. One of the most difficult point for evaluating the preformances of this kind of reactor lies in the fact that it will hardly be at steady state, the time constants of potentially rate controlling processes ranging from few hours to several months. However it must be stressed that the general methodology adopted for other MELiSSA compartments must still be applied. This includes stoichiometric constraints evaluation and on-line analysis of gas phase composition which will provide in real time information of the physiological state of the reactor.

The review from literature data and other experimental works performed in the lab. of on-line gas phase analysis methods show that the GC/MS technique has to be seriously investigated for anaerobic reactor characterisation. This is a new challenge for this kind of fermentation culture. From a technical point, the main conclusion is that the analyzer has to be composed of two gas chromatographic columns : one with a packed column for permanent gases separation and one with a capillary column for VFA. The quantification of gas composition can be performed by MS, eventually in parallel with other detection systems.

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