MICROBIOLOGICAL ASPECTS OF THE AEROBIC PROCESSING WINERIES WASTEWATER

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SUMMARY

The aerobic devices of processing of the effluents of cellar can be comparable with organic-engines which comprise a heterogeneous biomass. Yeasts, then the bacteria and finally of the protozoa, the parameciums, the vorticelles ones, the amoebas and the rotifères take part successively in the degradation of the organic matter by ways anabolic and catabolic leading to a purification by formation of biomass and CO2 and water elimination.

From an industrial point of view, various devices are implemented. The operating processes are variable: batch, semi-continuous, continuous. The tweaking of the boot and the operation of the engine relates at the same time to microbiological sowing, and the conditions of medium (pH, nutritive factors, temperature). The current ways of research relate to mainly the fastenings of the biomass and the implementation of an active biomass ensuring of the optimal performances in the middle of the point of rejections grape harvest (biological activity choke).

In the future, the microbiological aspects and genetic knowledge, could allow significant progresses in particular in the following fields: facility of sedimentation and dehydration of muds, adaptation to the toxic compounds related to hygiene, speed of degradation of the macromolecules, reduction of smell pollutions and the foam formation.

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ABSTRACT

Devices for winery wastewater aerobics purification edge Be assimilated to bioreactors comprising has heterogeneous biomass. Yeast, then bacteria and eventually protozoa, paramecium, vorticella, amibium, rotaria are successively involved in the degradation of organic to subdue by anabolic and catabolic leaflets, resulting in toilets purification by forming biomass, carbon dioxide and toilets.

From year industrial not of view, different devices are At work. There are several running modes: batch, half-continuous, continuous. Microcomputer-biological seeding and media conditions control (pH, nutritive factors, temperature) could lead to the tweaking of the reactor' S starting and running. Current research topics are mainly concerned with biomass fixing devices and with the uses of optimal

year activated biomass allowing results At the highest pollution level, during harvest bunches.
In future, microbial gold genetic knowledge may allow significant progress in the following fields: sludge sedimentation ability and dehydration, adaptation to toxic compounds linked to hygiene, macromolecules degradation quickness, reduction of olfactory pollution and foam formation.

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INTRODUCTION

Since Pasteur, enology gradually integrated microbiological knowledge in order to understand the stages of the vinification and to improve the development of the wines.

The use of yeast stocks selected in the form of active dry yeasts and more recently the control by sowing of malolactic fermentation, are part of the common practices of modern enology.

Beyond the development of the wines, the environmental constraints justify the study of the microbiological aspects in a new field for the cellars: the processing liquid waste viticultural, set of themes which is integrated more and more in the course of formation and the practice of the oenologists.

With the image of a vinification, the purification of the effluents of cellar utilizes a true biological factory in which the micro-organisms are the actors of the metabolic transformations, which lead by anabolism and catabolism to the degradation of complex organic compounds.

This talk aims to draw up an inventory of current knowledge in this field and to consider the tracks of search which can be explored in the future.

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I - GENERAL INTRODUCTION

1 - History

The natural degradation of the organic matters in the waterways is known for a very long time, however this self-purification, progressive, proved to be sufficient more to face the increase in the rejections resulting at the same time from the development of the urban centres and the industrialization. Thus, the biological processing of urban waste waters then industrial effluents gradually developed after the second world war.

In the agro-alimentary sector, after the very polluting considered branches of industry (sugar refineries, distillings), the cellars were gradually concerned with the processing of their effluents, under the action in particular of lawful and financial pressures.

Put except for the rustic techniques of standard spreading or evaporation, the viticultural liquid waste processing is generally carried out by aerobic biological way.

2 - Principle of the aerobic processing

This mode of processing which takes as a starting point the natural self-purification of a river can be summarized in the following way: by oxygen contribution (mixing, dynamic contribution), the organic matter is eliminated by ways anabolic (cellular multiplication) and catabolic (supply of energy) (Fig. 1).

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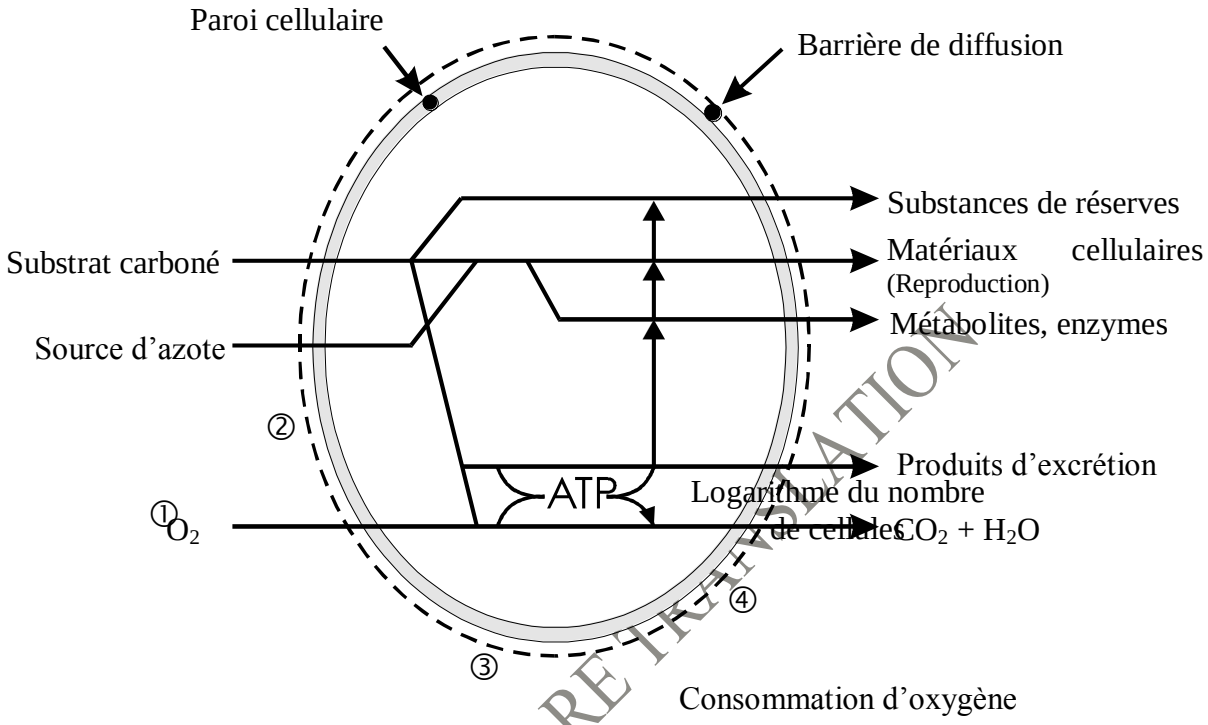

Figure 1: Representation of the cellular metabolism according to LEVEAU and BOUIX (1993)

The rollout of the organic matters towards the biomass intervenes according to the following process (DAIGGER and GRADY, 1982):

- the fine particles and the large molecules are adsorbed on the biomass of the device of purification. Fixed way exocellulaire, the compounds resulting from the hydrolysis by the exoenzymes, as well as the low-weight compounds molecular, can penetrate in the cells of the micro-organisms.
- once penetrated in the cell, the organic compounds are transformed into intracellular reserve, new expanded material, or oxidized thus providing energy for the cell.

Time

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This process leads to the development of biomass (Fig. 2) and thereafter to the mud formation made up of biomass and not hydrolized organic matters.

Figure 2: Development of the purifying biomass according to SCHLEGEL (1976)

①Launching Phase② exponential Phase
③Stationary Phase④ Phase of deterioration

According To the residence time, a more or less significant part of the micro-organisms autolysis, thus providing to the medium a new biodegradable substrate. The organic matter is thus recycled by died and successive regeneration, with in parallel an elimination in the form of CO2 and H2O.

This microbiological process complexes which intervenes particularly during the extensive processing, at the same time makes it possible to reduce the quantity of muds to be eliminated, to improve their aptitude for the decantation, and to stabilize them biologically, thus limiting smell pollutions. In practice, the clear biomass produced is generally understood in a fork from 0.3 to 0.45 kg of dry matters per kg of eliminated DCO what represents a volume of about 3 to 5% when muds are separated by decantation.

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3 - Characteristics of the biomass

It is generally composed of a complex population associating procaryotic and eucaryotes for which the bacteria constitute the purifying base.

The nature of the organic compounds to degrade and the conditions of medium will support the development of a biomass determined at a given moment of the processing. The evolution of the medium conditions the characteristics of the biomass for which a succession of species can be noted (FANTEI *and Al*, 1992).

Starting from studies conducted out of Champagne on devices led in batch (ventilated storage) on effluents of pressing, (ROCHARD *and Al*, 1996 and WISSER, 1997) a cycle of purification can be broken up schematically into four phases according to the evolution of the purifying biomass and the evolution of the physicochemical characteristics of the effluent in processing (Fig. 3).

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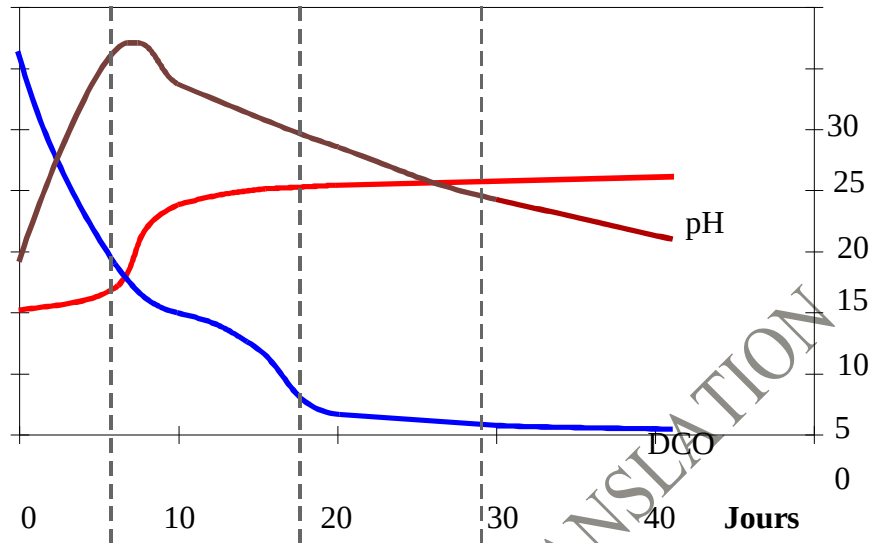

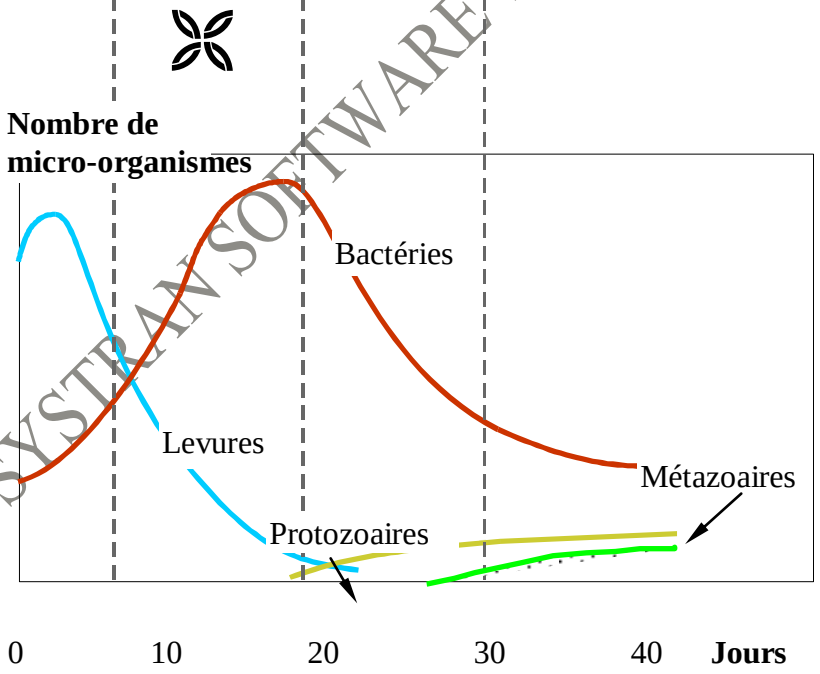

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Figure 3: Schematic Evolutions of the characteristics of the effluents treated by ventilated storage and the purifying biomass

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Launching Phase

This phase is characterized by a preponderance of yeasts because of a share of the effect of sowing of musts and favorable conditions of development (pH, nutritive sugars, factors).

The development levurien leads to a lowering of the fast DCO with for consequence a very important demand for oxygen, and a rise in significant temperature.

This stage, the separation of the biomass by decantation is difficult.

Phase of bacterial growth

Gradually, the activity of yeasts decreases with the profit of that of the bacteria. This significant evolution is related on the one hand to the increase in the pH (oxidation of the organic acids) and on the other hand to the rise in temperature during the launching phase.

This stage of purification, the biomass develops in free form in the liquid and of this fact the decantation is very difficult.

Stationary Phase

This phase corresponds to a stabilization even a reduction in the bacterial population and to the formation of the first flocs because in particular of exhaustion of the medium in easily biodegradable substrate and the fall of temperature related to the attenuation of the biological activity.

The appearance of protozoa generally bactérivores corresponds to the successions of species of the ecological chain classically met in the purification of the domestic effluents. One notes however a prevalence

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of the parameciums which is characteristic of the mediums deprived of nitrogen, which is the case of the viticultural effluents.

This stage, the sedimentation of muds becomes more effective.

The level of the DCO reached at the end of this phase, generally makes it possible to consider a networked rejection, but the level of purification is generally insufficient when the discharge system is a natural environment (ditch, river).

☙ *Phase of deterioration*

The bacterial population continues to decrease because of the low level of biodegradable carbonaceous substrate and a consumption by the higher species. This stage, the bacteria are mainly in the form of flocs easily decantable.

The evolution of the higher species is characterized on the level of the protozoa by a proliferation of the parameciums and the more occasional appearance the vorticelles ones, amoebas,…. , in certain cases, the presence of métazoaires is then observed: rotifères, nematodes (VEDRY, 1994).

This stage also corresponds to a very progressive degradation of fraction classically called “DCO hard”. Although few studies was carried out on this fraction effluents cellar, one can suppose that it corresponds to compounds with high molecular weight for which complex phases of hydrolysis are necessary before their microbial assimilation.

This phase finishes the liquid waste processing which can generally be rejected in the natural environment after a simple decantation.

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It is also at the end of this phase that the microphytes in the open basins appear (PLUCHART, 1997) their development and the contribution of pure oxygen that they produce often make it possible to exceed saturation and to undoubtedly accelerate the process of degradation of the hard DCO. On the other hand, they present the disadvantage of increasing the concentration in My with the rejections.

II INDUSTRIAL DEVELOPMENT

1 - Presentation

The devices of processing can be comparable with organic-engines for which the biomass can be developed under different conditions.

In the viticultural field, there does not exist universal solution of processing, each cellar is a typical case what justifies a diversity of the processes of purification to be implemented.

The homogeneity or the heterogeneity of the rejections throughout the year, the organic load of the effluents, the constraints related to the establishment, the technical skills of the staff of the cellar is as many factors which will direct the persons in charge of the cellars towards extensive systems or on the contrary very intensive.

2 - Mode of control of the devices

The development of the purifying biomass can be considered in various modes of control of the devices of purification.

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- *Discontinuous Processing (batch)*

With the image of a traditional alcoholic fermentation, the effluent is stored in one or more tanks corresponding to the volume rejected during pressing and/or the vinifications, even the whole of the year. This process developed out of Champagne under the name of "ventilated storage" is well adapted to the cellars which present a strong point of pollution during the grape harvest.

The technical follow-up of this extensive operating process is limited enough, on the other hand this device imposes a volume of important storage with sometimes of the difficulties of establishment in urban site. This mode of control also justifies a great vigilance with respect to the risks of nuisance (anaerobic metabolism if ventilation is insufficient).

- Discontinuous Processing

In this case of figure, the tank of processing preserves a foot of tank permanently corresponding to elutriated muds of the preceding cycle. The feeding of the device of purification intervenes is by contribution in only once of the totality of the effluents of a cycle (*Sequential Reactor Batch*: SBR), is by progressive feeding (*EDF batch*).

This mode of control makes it possible to ensure a purification with an important biomass and activates (exponential phase). The difficulty lies in the management of the cycles of purification (feeding, residence time) in particular when the effluents have a great variability in their composition.

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- *Continuous Processing*

These processes aim at maintaining a state of balance in the tanks of processing supplied and tapped uninterrupted. Generally purification intervenes in only one basin with into final a separation of muds in a decanter. This operating process corresponds to the principle classically used for the urban effluents (sewage treatment plant with activated sludge). For the peak period, the station can be supplemented in first stage by another basin functioning for example on the principle of the activated sludge in strong load. An alternative of this process consists in splitting in several tanks the phase of purification with for consequence of the gradients of concentration in DCO associated with a biomass specialized in each engine.

Generally, these processes are well adapted to the cellars rejecting of the effluents whose characteristics are rather constant (volume and composition). The technical follow-up of these intensive processes must be carried out by a staff of cellar formed to biological purification or by a company specialized service provider.

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3 - Influence physicochemical factors

These factors were the object of a Champagne study within the framework of microcomputer-purifications in batch of 100 liters (VIAUD *and Al*, 1994)

The principal conclusions are summarized below:

- A complementation of the nitrogen effluents and cogitates has a significant effect.

- Neutralization presents way a less positive effect.
- The combination of the neutralization and the phosphorus and nitrogen addition does not make additional improvement compared to the only nitrogen complementation and cogitates.
- The heating of the effluents at a temperature of 35°C contributes to accelerate the process of purification significantly

Within the framework of the technological applications, the tweaking of the conditions of medium is especially effective for the intensive systems. This tweaking relates in particular to the following aspects: regulation of the pH with 7, contribution of nutritive factors on the basis of a contribution DBO5/N/P of 100/5/1 and possibly, control partial of the temperature by insulation or underground establishment of the engine.

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4 - Sowing with selected micro-organisms

Beyond the purifying performances, sowing with micro-organisms can be required in order to obtain an active biomass at the beginning of the point of pollution related to the grape harvest (biological effect choke). Indeed, in most cellars, it is difficult to maintain a good viability of the biomass because of the lack of carbonaceous substrate during the period which precedes the grape harvest. Within this framework, interesting results were got with activated sludge of sewage treatment plant (VIAUD *and Al*, 1994)

This study also related to tests of commercial stocks, however, the results are not very significant compared to the witness.

The way of research relating to sowing currently is the specific object of study opposite in particular of the adaptation and the conservation of the biomass within the framework of a Community project CLEAN ENOLOGY associating various industrialists and European research centres.

An original project (Vinipur®) based on the principle of a first stage of processing where sowing is carried out with yeasts and a second stage with activated sludge functioning in prolonged ventilation is currently developed industrially in a cellar of Bordeaux. The purifying performances of yeasts (EHLINGER *and Al*, 1994), associated with an important level of oxygenation, makes it possible to obtain an output of purification raised for a very short residence time (3 to 4 days). The effect of competition yeast-bacteria, justifies the regulation of the pH with 4 in the first engine.

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This particularly compact process can be easily integrated in urban site. This thorough intensification imposes a very pointed technical follow-up. In addition, the separation of yeasts, not easily decantable, justifies the installation of a horizontal centrifugal separator. In addition, in order to avoid the dissemination of yeasts in the wine storehouses, a system of sterilization of the air of the effluents, as well as a liming of muds were set up.

5 - Fastenings of the micro-organisms

Classically, the purifying biomass is put in work in free culture. This technique is limited by the acceptable concentration in micro-organisms in the basins, leading to volumes of important work. In order to stage with this disadvantage, of the processes with fixed culture were developed. They are based on the property which have the bacteria to produce of the exopolymères (with the image of the disease of the grease of the wines) allowing their fixing on a support in the form of a film.

Many supports being different by their specific surface (m2/m3), their form, the type of material used, were already tested or are under development.

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Conclusion

The devices of processing liquid waste of cellar, can be comparable to bioréacteurs, for whom the biomass is at the origin of the complex metabolism épuratoire which associates anabolism and catabolism. If enology already largely integrated the use of specific micro-organisms, the field of purification is inspired still largely by empirical processes based on the use of an indigenous biomass. However, this way deserves to be thorough in order to answer in particular the need for obtaining a biological effect choke in the initial phase of the points of linked activities to pressing and the vinifications.

Beyond the performances épuratoires, microbiological search, possibly integrating the techniques of genetic recombination, can also relate to the implementation of the processing, in particular in the following fields:

- reduction of smell pollutions and the production of foam;
- facility of sedimentation and dehydration of muds
- speed of the degradation of the “DCO lasts” (macromolecules);
- adaptation to the toxicity of the products of cleaning;

It is also important to note that whatever the level of tweaking of the device of purification, possibly controlled by modeling, this one will be able to function satisfactorily only on condition that integrating the constraints of control of water consumption and the polluting load during the development of the wines. These constraints open a vast

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field of investigations which associates, at the same time formation, sensitizing and evolutions of the practices and technologies of vinification.

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