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Journal of Food Engineering 63 (2004) 39–45

JOURNAL OF FOOD ENGINEERING

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# Operation in semi-continuous with a closed pilot plant scale acetifier for vinegar production

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Received 12 March 2003; accepted 21 July 2003

**Abstract**

Several semi-continuous series of acetic acid fermentations were carried out in a novel pilot plant reactor (225 l) including a gas recycling system for the recovery of volatile compounds. The behaviour of substrate and product concentrations, acetification rates, stoichometric yields and oxygen consumption were analysed. Operation with this novel reactor proved some technical advantages in order to produce high quality vinegars: stoichometric yields of 100% and minimum oxygen supply.


*Keywords:* Vinegar; Acetic acid fermentation; Acetifier; Semi-continuous

## 1. Introduction

Many technical devices have been traditionally developed for the industrial production of vinegar. Those try to increase the speed of the biological reaction: the aerobical transformation of ethanol into acetic acid in the presence of acetic acid bacteria (Tesfaye, Morales, García-Parrilla, & Troncoso, 2002). At the moment, the most common technology for this industry is based on the submerged cultures (Hromatka & Ebner, 1951) with diverse technical modifications which try to improve the general fermentation conditions (aeration, stirring, heating, etc.).

In a previous work published by the authors (de Ory, Romero, & Cantero, 1999), a novel system for the acetic acid submerged fermentation at pilot plant scale was described. This acetifier is equipped with a gas recycling system for the recovery of the volatile compounds that leave the reactor with the gas outlet stream, reducing evaporative losses to 0%. In a following work (de Ory, Romero, & Cantero, 2002) an optimum starting-up protocol for the inoculation of this acetifier was implemented, taking into account the influences of ethanol and acetic acid concentrations in the activating–inhibiting effects on the growth. This allows the reduction in start-up time to 1/3 of normal values.

The aim of the present paper is to operate with this novel pilot plant reactor in semi-continuous cycles and prove its technical advantages in order to produce high quality vinegars.

Semi-continuous is the most common operation mode in the vinegar industry at the present time: this operation mode consists of the developing of successive discontinuous cycles of acetification, each one with conversion of the ethanol that contains the medium into acetic acid. At the end of every cycle, a given volume of reactor is discharged (final product) and refilled with initial medium (fresh wine). Then, a new fermentation cycle begins.

The percentage of charge/discharge is variable in every single process but the most common in vinegar industry is the removing of the 50% of the total volume and the later charge of the same proportion of fresh wine. Producers points out to a quick discharge of produced vinegar, but the later charge of wine is recommended to be slow and progressive, trying to avoid the sudden modification of the environmental conditions for the biomass (ethanol and acetic acid concentrations, nutrients, temperature, pH, etc.), which involves a new lag phase and, consequently, a non-productive period (Bu'lock & Kristiansen, 1991).

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doi:10.1016/S0260-8774(03)00280-2

The group of assays carried out in this work has been designed for the production of high acidity vinegars by means of different semi-continuous cycles, developed in a pilot plant reactor equipped with a gas recycling system. For the beginning of every fermentation series (groups of consecutive cycles), a starting-up protocol must be applied in order to guarantee the better initial conditions for the biomass. Then, several semi-continuous series of acetic acid fermentations were carried out to study the trend of substrate and product concentrations, acetification rates, stoichometric yields and oxygen uptake rate versus time.

## 2. Materials and methods

### 2.1. Acetic acid fermenter

The pilot plant fermentation equipment was described before in previous papers (de Ory et al., 1999, 2002). Basically, it consists of a stainless steel cylinder-shaped reactor with 0.47 m internal diameter and 1.48 m height (225 l of working volume). The temperature of operation (within the optimum range for acetic acid fermentation of 30–31 °C (de Ory, Romero, & Cantero, 1998), is settled by an internal heat exchanger connected to a thermostatic bath. A closed gas recycling system has been implemented in the reactor: it consists of an air pump which re-circulate the outlet gas stream (containing the most volatile compounds) into the fermenter, after passing through an expansion and retention chamber. The re-entrance of the gas stream is carried out through the bottom of the reactor thanks to two sinterised stainless steel diffusers, also providing the necessary stirring rate (maximum air flow = 150 l/min.). The oxygen consumed by cells in their metabolic and growth functions is supplied by an industrial oxygen cylinder through an ON/OFF electrovalve connected to the expansion chamber. In such way, dissolved oxygen concentration in the liquid medium is maintained within the optimum values (≈2 mg/l), avoiding unnecessary supplies. The charge of fresh medium (wine) is made by a pump through the top of the vessel and the discharge of current percentage of final product (vinegar) is developed from the bottom with the help of another stainless steel pump. Many valves and gauges complete the described system, which is graphically shown in Fig. 1.

### 2.2. Fermentative medium and inoculum

The fermentation medium used for all the assays was a young wine from the Jerez-Xèrés-Sherry area (Spain) with an average ethanol concentration of 70–80 g/l and a low acetic acid concentration (2.5–5 g/l). This medium is introduced into the reactor at the beginning of every single discontinuous cycle, where is mixed with a given

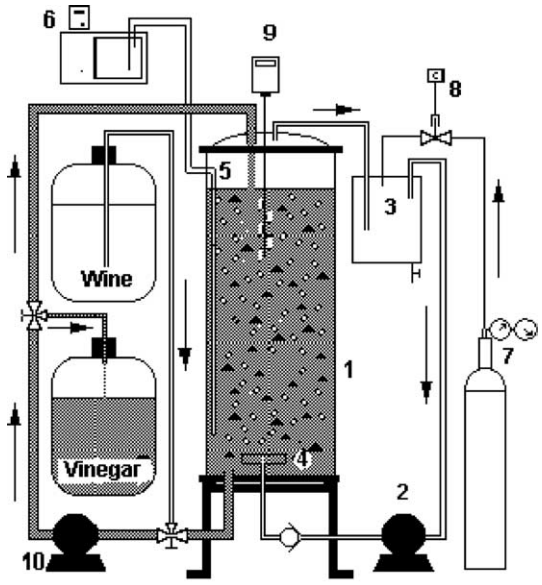


Fig. 1. Industrial acetification equipment used in the experimental work (de Ory et al., 1999).

percentage of the final product of the preceding one. In the first cycle of every series, the fresh medium is mixed with the result of the so-mentioned starting-up protocol: a medium with non-extreme concentrations of ethanol and acetic acid and a huge population of acetic bacteria in their exponential growth phase (around $500 \times 10^6$ cell/ml).

The starting inoculum was obtained from a collaborating vinegar cellar; it consists of a selected culture of acetic acid bacteria taxonomically identified as a mixed strain, prevailing *Acetobacter aceti* (Adams, 1998).

### 2.3. Starting-up protocol

An optimum starting-up protocol for industrial acetifiers has been previously established (de Ory et al., 2002) and used for the current assays. The objective is the filling-up of the reactor in several steps, beginning with 67 l–30% of total volume-constituted by an adequate mixing of starting inoculum and fresh medium, in such a way that a total acetic acid concentration of 30 g/l is established (47 g/l ethanol). In this conditions, a short lag phase is expected and in a few hours the medium has reached 45 g/l in acetic acid concentration (35.5 g/l

ethanol), reported to be the limiting conditions for a new addition. Then, 33 l more of fresh wine are introduced giving a partially filled volume of 45% and coming back to the optimum initial concentrations. Two more charges (50 l–67% and 75 l–100%) are carried out within similar ranges. The final result is the reactor completely filled with a fermentation medium with a high biomass population and the better conditions to begin a complete series of semi-continuous cycles of acetic acid fermentation.

### 2.4. Semi-continuous operation procedure

Once the reactor is completely filled, it is possible to start the semi-continuous cycles. The initial substrate is constituted by 225 l of medium with acetic acid concentration of 20–30 g/l (ethanol 40–47 g/l). In such conditions, in a few hours (a short lag phase) the medium increase its acidity by fermentation with consumption of the present ethanol. When the bulk reaches the desired acidity of the product (~80 g/l), the current cycle is considered finished.

The starting of a new cycle begins with the discharge of 50% of the total volume (112 l) and the subsequent refilling with the same volume of fresh wine. Thus, the substrate and product concentrations are settled again in adequate initial values for the starting of a new cycle (acetic acid: 40–50 g/l; ethanol: 32–40 g/l). At this point, the biomass population has been reduced to the half and new environmental conditions are established, so a short lag phase appears. In a few hours, a new fermentation occurs in similar conditions to the preceding one. So then, in every cycle a production of 112 l of wine vinegar (50%) with acidity 80 g/l is obtained.

The current protocol, schemed in Fig. 2, try to get the maximum reproducibility for the semi-continuous cycles.

### 2.5. Analytical methods

*Ethanol:* it is analysed with a gas Chromatograph HEWLETT PACKARD 5890 Series II with capillary column Carbowax 20M on Chromosorb 0.2 μm and FID detector.

*Acetic acid:* taking into account that other organic acids are present in vinegar at negligible quantities (Troncoso & Guzmán, 1988) it is possible to suppose that total acidity is a good indicator of the acetic acid concentration. This is determined by titration with NaOH and phenolphthalein as an indicator.

*Sensorial parameters:* In order to consider the quality of the vinegars obtained, samples of 750 ml were taken every 24 h, filtrated and cooled at 4 °C and analysed. Studied parameters: volatile compounds (acetaldehyde, ethyl-acetate, 2-methyl-1-buthanol, 3-methyl-1-buthanol, acetoin and ethyl lactate) by gas chromatography.

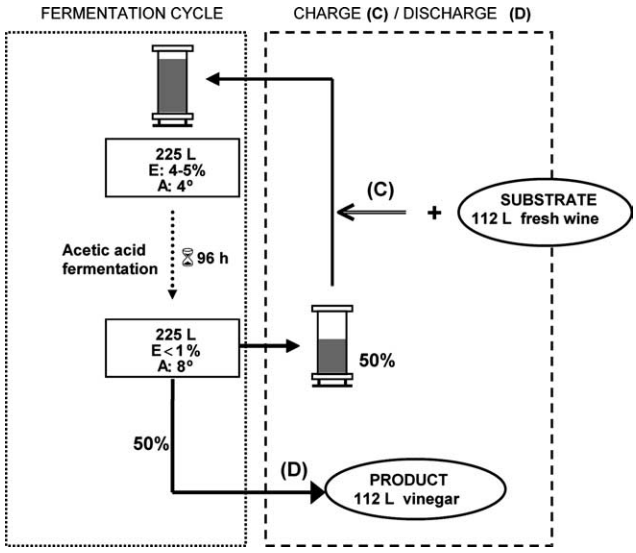


Fig. 2. Protocol for the semi-continuous operation procedure.

## 3. Results and discussion

With the aim of a correct organization of all the semi-continuous cycles carried out, the experiments were classified attending to the real operation procedure. So, all the experiments summarised in Group I were developed in a semi-continuous series; i.e., every cycle in this group follows to the preceding, after a discharge of a 50% of the reactor volume and refilled with the same quantity of fresh wine. When the last cycle of this group finished, a complete maintenance procedure was applied to the reactor (cleaning of vessel and sensors, repairing of pumps, etc.) after which, a new starting-up protocol and a new group of semi-continuous cycles (summarised in Group II) were carried out. This operation procedure tries to take into account, also, the study of the predicted adaptation effect of biomass to the current series of cycles in the reactor during the process time.

In the other hand, trying to ignore this mentioned effect, a third group of cycles (Group III) was designed. On it, every cycle is independent from the rest and it is carried out after its respective starting-up process.

In Table 1, all the developed experiments are summarised with some experimental results obtained.

Figs. 3–5 show the graphics of each cycle registered individually in the three groups, with the trends of ethanol and acetic acid concentrations versus time.

The obtained results indicate, as the first conclusion, that it is possible to operate in semi-continuous regime in the proposed reactor, producing 112 l (50% of total volume) of vinegar with a high acidity at the end of

Table 1
Experimental design for the study of semi-continuous cycles in the reactor

| Experiment | Time (days) | Total acidity (g/l) | | Medium acetification rate (g/l day) | Stoichometric yield | Total $O_2$ consumed ($L_{standard\ conditions}$) |
|---|---|---|---|---|---|---|
| | | Initial | Final | | | |
| *Group I* | | | | | | |
| Cycle 1 | 7 | 23.7 | 58.6 | 5 | 100% | 2930 |
| Cycle 2 | 6 | 33.6 | 63.3 | 4.9 | 100% | 2495 |
| Cycle 3 | 12 | 37.4 | 73.1 | 3 | 96.71% | 3000 |
| Cycle 4 | 7 | 41 | 90 | 7.1 | 100% | 4117 |
| *Group II* | | | | | | |
| Cycle 1 | 9 | 26.6 | 75.3 | 5.4 | 100% | 4093 |
| Cycle 2 | 8.1 | 39.5 | 86.8 | 5.9 | 100% | 3972 |
| Cycle 3 | 3.3 | 51.5 | 82.1 | 10.2 | 100% | 2239 |
| Cycle 4 | 3 | 42 | 75.3 | 11.1 | 100% | 2793 |
| *Group III* | | | | | | |
| Cycle 1 | 5 | 41.4 | 77.9 | 7.3 | 100% | 3066 |
| Cycle 2 | 7 | 25.6 | 73.9 | 6.9 | 100% | – |
| Cycle 3 | 4 | 49.6 | 73.8 | 6 | 100% | 2032 |

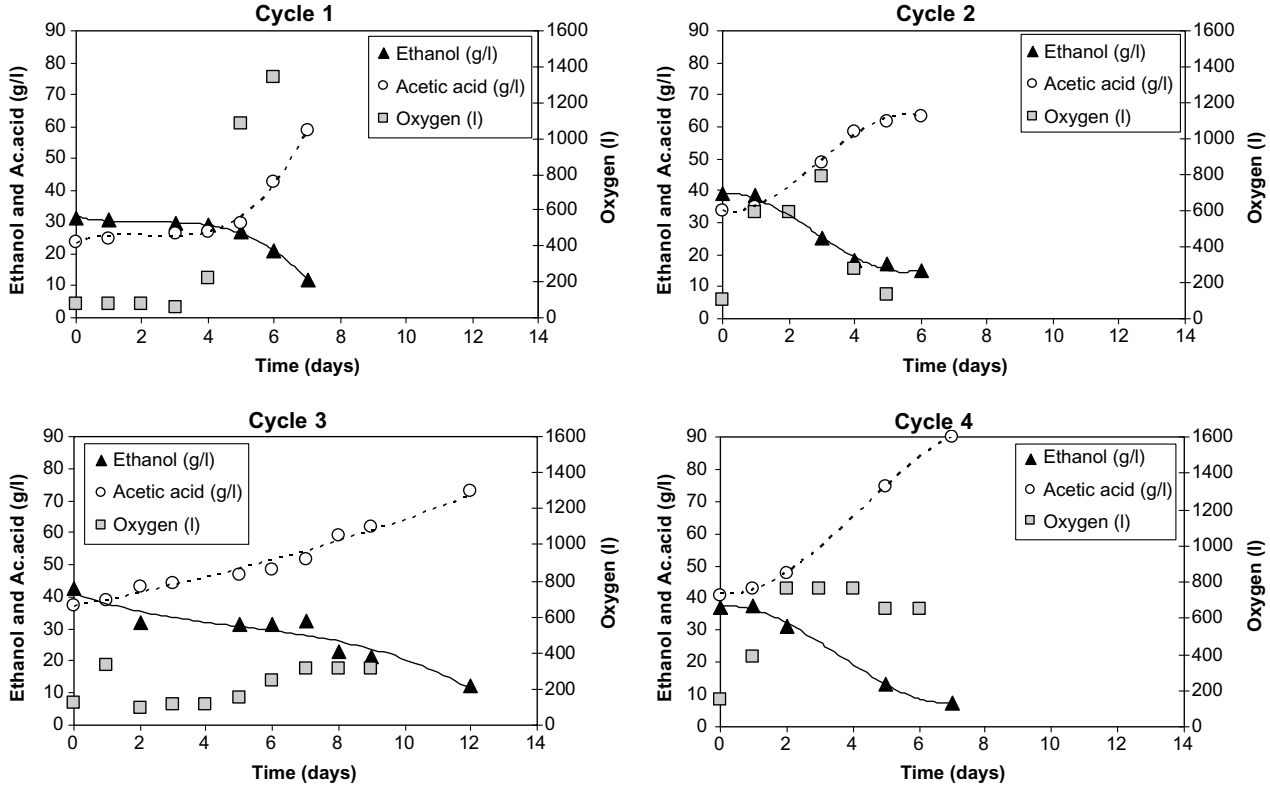


Fig. 3. Ethanol, acetic acid and oxygen concentrations versus time for the cycles of Group I.

every cycle. The closed system makes possible the obtaining of a good sensorial quality of the product due to the elimination of the losses of volatile compounds.

Taking into consideration the experimental data, several remarks could be made.

### 3.1. Acetification phases

The behaviour of ethanol and acetic acid concentrations versus process time shows the typical discontinuous trends for the microorganisms:

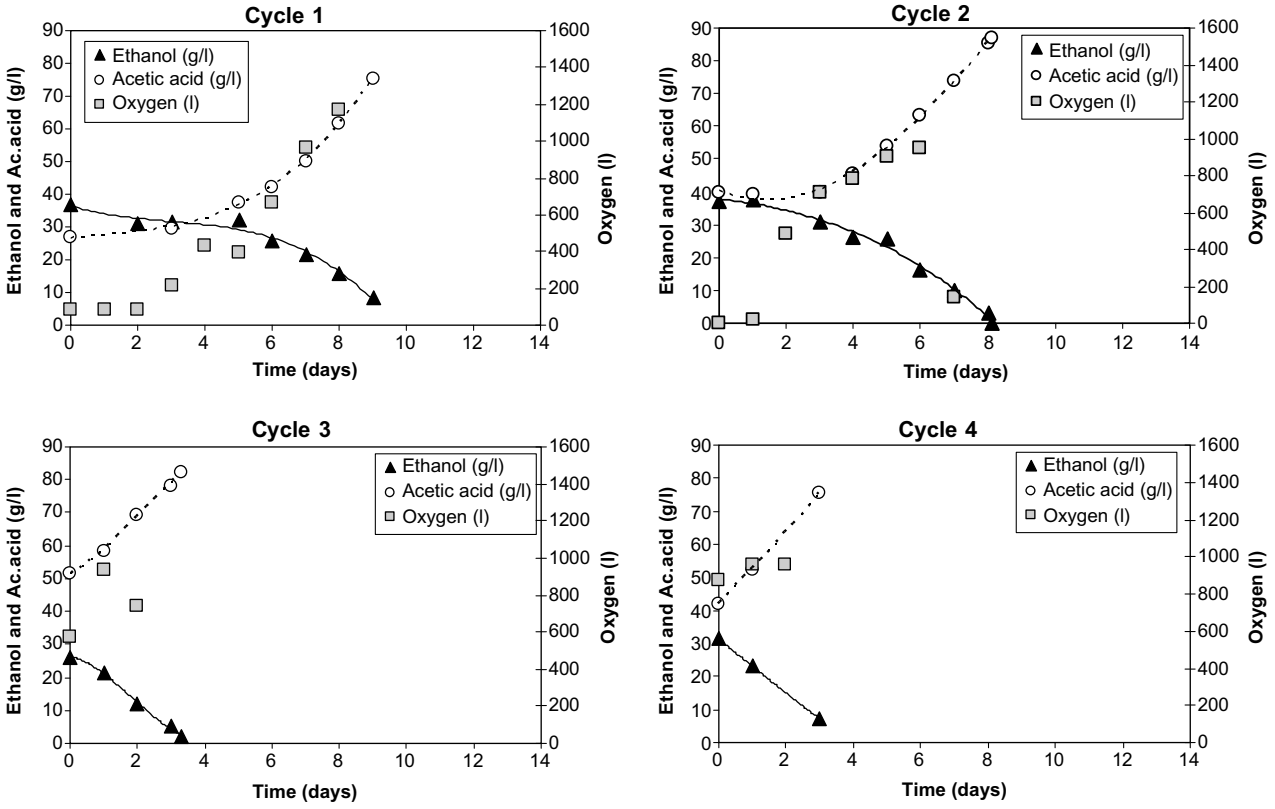


Fig. 4. Ethanol, acetic acid and oxygen concentrations versus time for the cycles of Group II.

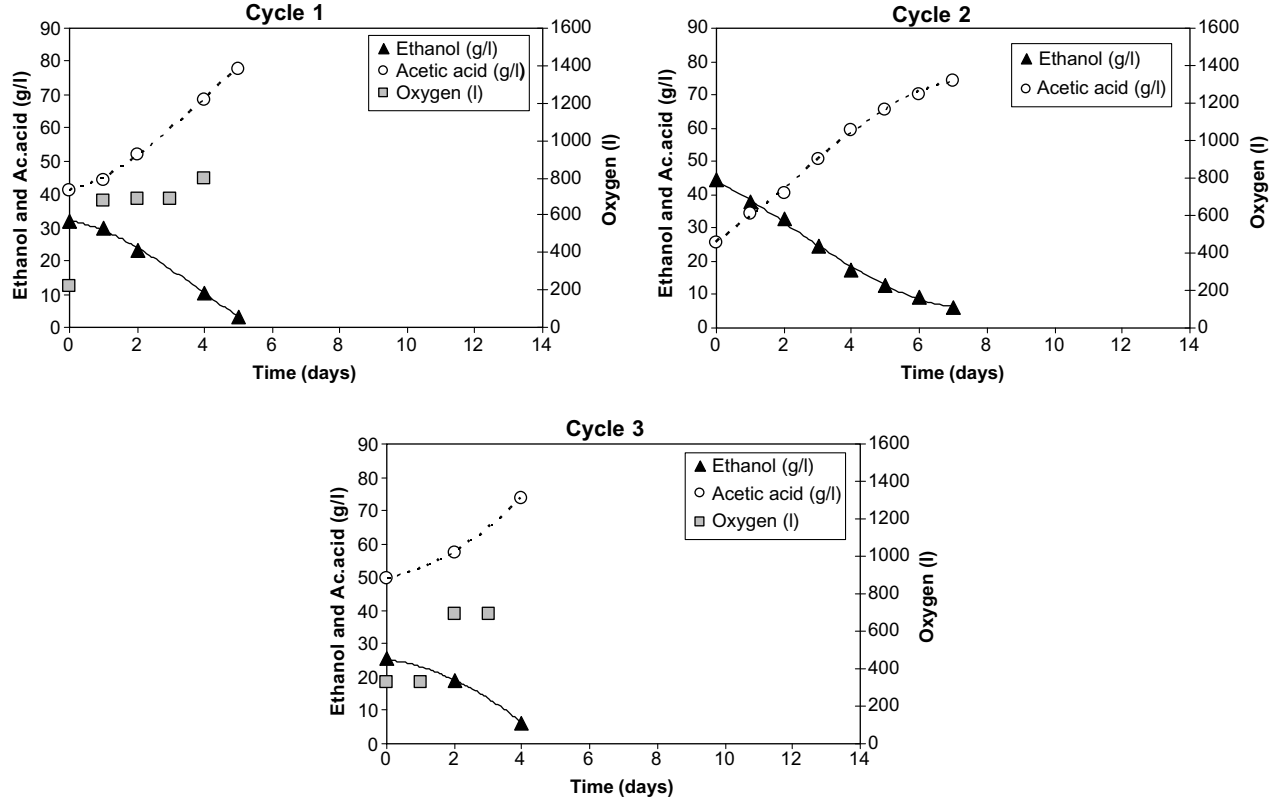


Fig. 5. Ethanol, acetic acid and oxygen concentrations versus time for the cycles of Group III.

In the first hours, and after the replacement with fresh wine, a *lag phase* is observed. On it, almost any ethanol consumption and acetic acid production is registered. Its length depends on several factors: initial concentrations of substrate and product, previous history of the culture, etc. In general terms, this could be resumed as a phase in which bacteria is spending their major energy on the metabolic adaptation to the new environmental conditions of the culture (Bailey & Ollis, 1986). In this sense, it is possible to observe that the lag phase becomes shorter in those cycles beginning with 30–45 g/l of acetic acid, versus those beginning with less than 30 g/l, because the last ones start with higher ethanol concentrations (above 47.5 g/l), considered by the bibliography as toxic for the bacteria (Nanba, Tamura, & Shiro, 1984; Soo Park, Ohtake, Fukaya, Kawamura, & Toda, 1989).

Overcoming the lag phase, non-productive from the fermentative point of view, the ethanol consumption and the acetic acid production begin. This demonstrates the start of the *exponential growth phase*.

Finally, a *stationary phase* and a *death phase* are observed. A little inevitable decrease on the production rates is registered at the end of every cycle, because of the high product concentrations obtained (80–90 g/l).

### 3.2. Acetification rates

In Table 1, the average acetification rates for every cycle have been shown. A general trend for the consecutive fermentation cycles is registered: the acetification rates increase for successive cycle in the same series (from 5 g/l day approximately in first cycles to 11 g/l day in the last ones). However, these rates are sensibly beneath those obtained by other authors using mechanical stirring, because in the proposed reactor the agitation is caused by the gas stream itself.

The progressive increasing of the rates could be explained in terms of *cellular adaptation* of the culture to the current fermentation conditions. The present biomass progressively acclimatizes to the reactor; from the initial mixed culture submerged in the medium, the best adapted strains are going to be selected. Thus, the most adequate metabolic pathways for the synthesis of new enzymes are going to stand out. The result is that the culture increases its ability for the fermentation and, then, the acetification rates.

It is possible to conclude that, with the aim of obtaining higher rates, two main aspects should be taken into account: the improvement of the volumetric oxygen transfer coefficient ($K_L a$) of the acetifier (better aeration and stirring systems) and the development of the higher number of consecutive semi-continuous cycles. However, this last point is not indefinitely possible because it is necessary to make stops for maintenance and cleaning of the system.

### 3.3. Stoichometric yields

As it is shown in Table 1, stoichometric yields of 100% are reached in most cases, with the exception of one cycle (group I, cycle 3: due to a system failure, the reactor had got to be opened to the atmosphere during some hours). These yields have been calculated as the percentage of ethanol disappeared in the liquid medium that has been converted into acetic acid. When the yield is 100%, any evaporation of substrate has been registered during the process time, but all of it has been stoichometrically converted.

These data clearly show that the closed system designed is absolutely effective for the avoiding of evaporative losses. This allows the obtaining of high grade vinegars at the end of the semi-continuous cycles (80–90 g/l), with excellent sensorial characteristics.

### 3.4. Oxygen consumption

In Figs. 3–5, the quantities of consumed oxygen in g/l day have been represented for every cycle. This consumption has been calculated taking into account the mol of ethanol converted into acetic acid in every interval of 24 h, and the stoichometry of the reaction (1:1). The total quantities (in litres of industrial $O_2$ (99.95%), standard conditions: 25 °C, 1 bar) for a complete cycle are shown in Table 1.

The consumed oxygen during the reaction is directly related to the conversion substrate/product. Then, the data represented in the figures are closely linked to the ethanol and acetic acid curves. Thus, when a lag phase is registered in those, a minimum consumption of oxygen is produced, due to the minimum conversion. When the exponential growth phase starts, maximum cell viability is expected and a sensible increase of oxygen consumption is observed. When the acetic acid production is maximum, oxygen consumption gets the top. From this point, although the acetic acid production continues, the consumption of $O_2$ falls because the cell activity decreases for the toxic effects.

## 4. Conclusions

In order to summarise the utility of the results some conclusions could be made:

- Operation in semi-continuous mode in the proposed closed pilot plant acetifier shows stoichometric yields of 100%, not detecting evaporative losses of ethanol and any other volatile compounds. Moreover, the quantity of oxygen supplied to the reactor is exactly the required for the growth of the culture, avoiding unnecessary spendings. Thus, the designed gas recirculation system is optimum for the production of

high quality vinegars, proving to be technically and economically viable.

- A general trend for the consecutive semi-continuous fermentation cycles is observed: the acetification rates increase with process time due to a progressive cellular adaptation of the culture. It is necessary the development of, at least, two cycles to obtain the higher rates.

## Acknowledgements

This work was carried out thanks to the economic support of the Spanish government (PETRI Project, 96-0110-OP) and the European Funds (CICYT-FEDER: 1FD97-0796-c02-02). Also, thanks to the invaluable collaboration with Bodegas Primitivo Collantes wine and vinegar cellar.

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