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# Maximum yield acetic acid fermenter.

## Comparative fed-batch and continuous operation studies at pilot plant scales

I. de Ory, L.E. Romero, D. Cantero



**Abstract** Acetic acid fermenters in submerged culture are most currently used in vinegarmaking industry. However, they have the disadvantage of being open to the atmosphere with the subsequent evaporation losses of volatile compounds. This paper studies an acetic acid fermentation reactor equipped with a closed gas recycling system which prevents any loss of volatile compounds due to evaporation. Experiments have been conducted in two different operation regimes: fed-batch and continuous. Operation protocols are detailed for both cases, paying particular attention to optimum performance conditions, and comparison of yields and acetification rates.

## 1 Introduction

Vinegar, as a food by-product from wine, has lately acquired an important role in salad dressings, ketchup and other sauces, etc. This fact demands industrial fermentation systems capable of affording great production volumes, reliably controlled, and operational in optimum conditions for acetic acid bacteria fermentations.

As film culture systems are slow and expensive [1], they are not used nowadays, and submerged culture fermenters [2] have become widely used at industrial scale. In these fermenters, the biomass is suspended in the medium, and it is stirred and aerated [3] by means of fans or compressors. Likewise, these fermenters are usually fitted with a thermal jacket for the maintenance of the optimum temperature in the fermentation process.

A very widespread patent for submerged culture is the Frings acetifier [4]. This patent allows for the acetification of large volumes with a low air uptake. Another advantage of these systems is the increase of fermentative yields and the increase in acetification rates, often due to highly effective oxygen transfer. Other popular acetification devices are the tower fermenters or bubble column fermenters, the cavitator, and the Jet fermenter.

Such systems, however, have an important disadvantage which, still today, remains unsolved: they are systems that work open to the atmosphere. As a consequence, high evaporation losses of volatile compounds, such as ethanol, acetic acid or ethyl acetate occur with the subsequent reduction of yields about 5–10%, and an increase in operational costs [5].

Our research group has obtained good results with the use of similar fermenters at laboratory scale [6] and has studied the proposed pilot scale reactor in discontinuous operation [7]. Following this experience, this paper describes the starting-up and behaviour of this pilot scale reactor for wine acetification which aims to avoid volatile compound losses (100% fermentative yields) without increasing operational costs. At the same time, two operation regimes are studied and compared: fed-batch and continuous.

## 2 Materials and methods

### 2.1 Equipment and optimum operation conditions

The before-mentioned equipment is located in the pilot plant belonging to the Department of Chemical Engineering, Food Technology and Environmental Technologies of the Spanish University of Cadiz. It is a stainless steel cylinder-shaped acetic acid fermenter, 47 cm internal diameter and 148 cm height. By using an internal heat exchanger connected to a thermostatic bath, the temperature of operation is controlled at 30 °C, since this has been considered the optimum value [8]. As a technical novelty the equipment has been fitted with a closed gas recycling system. Thanks to this system, the volatile compounds in the gas stream are impelled by a 0.6 KW air pump to the bottom of the fermenter after passing through a 60 l expansion chamber. Hereafter, they are admitted again into the medium through two sinterized stainless steel diffusers, also providing the necessary stirring rate (air flow = 150 l/min). At this stage, the reactor is fully closed to the atmosphere. The oxygen required for cellular metabolic and fermentative functions is supplied from an industrial oxygen cylinder (N50) using an ON/OFF electrovalve controlled by times. The optimum dissolved oxygen concentration (2 mg/l) can be assured by using this device [9]; thus, avoiding non-necessary oxygen supplies. This working protocol makes the operation at high acetification rates with no yield losses possible. The equipment

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is also fitted with safety valves and various gauges for liquid level control. A diagram of the maximum yield fermenter is shown in Fig. 1.

## 2.2 Protocols of operation

### 2.2.1 Inoculation

The first step in the fermentation process is inoculation, developed with 40 l of a selected culture in exponential growth phase. This inoculum is mixed with 40 l of wine (9% ethanol and less than 5 g acetic acid/l). When this medium has 30–40 g acetic acid/l, 70 l of wine are added, and, in the final stage, when similar concentrations have been reached, the last 75 l of wine are added. Resulting total volume is 225 l containing 30–40 g acetic acid/l with biomass growing in exponential phase and a high fermentative potential.



### 2.2.2 Fermentation

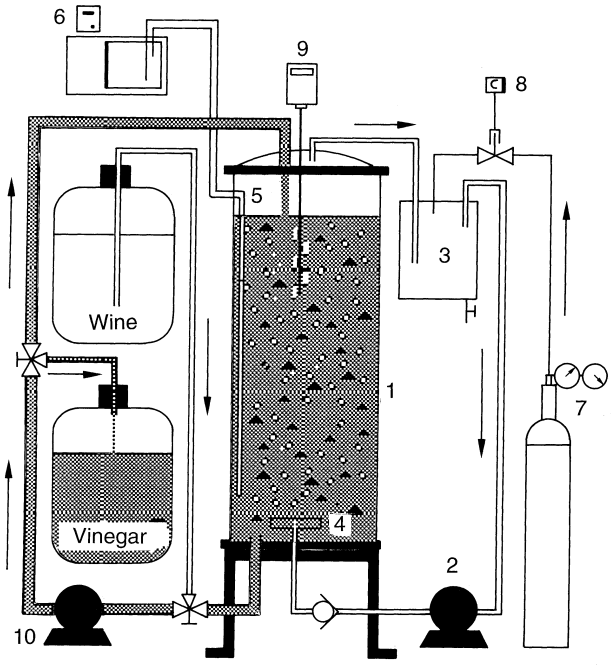
*1. Fed-batch fermentations*

Several acetic acid fermentations have been developed until acetic acid concentration was 80 g/l, with an 8 g/l residual ethanol concentration. When these conditions were achieved, 125 l (50% of the fermenter) were discharged and this volume refilled with the original wine. The resulting medium had an acetic acid concentration of 40 g/l and an ethanol concentration of 48 g/l, thus avoiding possible microbial proliferation and reducing the negative effects of a high concentration of ethanol and acetic acid over Acetobacter aceti [10][11]. This procedure operates between 40 and 80 g acetic acid/l, this being the interval in which the specific growth rate of Acetobacter aceti best suits high acetification rates.

*2. Continuous fermentation with submerged culture*

Once the inoculation has taken place, the medium in the vessel is fermented to 70 g acetic acid/l (16 g ethanol/l), which are considered suitable conditions for standard continuous operation. The continuous feed flow rate is provided by a membrane pump, which is able to supply feed flow rates between 0.2 and 5 l/h. The outlet effluent is discharged by means of overflowing through a pipe fitted at the required liquid level.

In order to avoid the so-called effect "microorganisms washing" (i.e. decrease in bacterial population due to short residence times), experiments were developed by gradually increasing the feed flow rates until the stationary states were reached. Other experimental conditions such as aeration, stirring, temperature, or maintenance of dissolved oxygen concentration remained at the optimum values.




1 Reactor
2 Gas recycling pump
3 Expansion tank
4 Air diffusers
5 Heat exchanger
6 Thermostatic bath
7 Oxygen cylinder N-50
8 Electrovalve ON/OFF
9 Dissolved oxygen sensor
10 Feed inlet and effluent outlet pump

**Fig. 1.** Maximum yield acetic acid fermenter

## 2.3 Methods of analysis

Several analytical determinations were developed:

- Ethanol; by gas chromatography (Hewlett Packard 5890 Series II) with a flame ionisation detector and a Capillary column Carbowax × 20 M.
- Acetic acid by titration with a 0.3 M NaOH solution, and phenolphtaleine indicator. Organic acids different to acetic acid were considered non-relevant for titration.
- Dissolved oxygen; by a polarographic electrode OXI-92 (Crison).
- Total Biomass; by counting in an optical microscope with a Neubauer Chamber.

## 3 Results and discusion

### 3.1 Fed-batch experiments

A large group of fermentation cycles were developed between 40 and 80 g acetic acid/l following the protocols previously described. When experiments were finished vinegar was 80 g acetic acid/l, 125 l of vinegar were discharged and replaced with 125 l of wine.

Figure 2 shows total acidity data versus time for seven representative fermentation cycles.

As it can be observed, it is possible to develop short fermentation cycles in short periods of time. The first cycle corresponds to lag-phase of the acetic acid bacteria to the medium. This cycle is not very representative for the estimation of acetification rates and, in this sense, it is only shown to illustrate the whole process. The average acetification rate for fermentation cycles was 8.4 g acetic acid/l·d

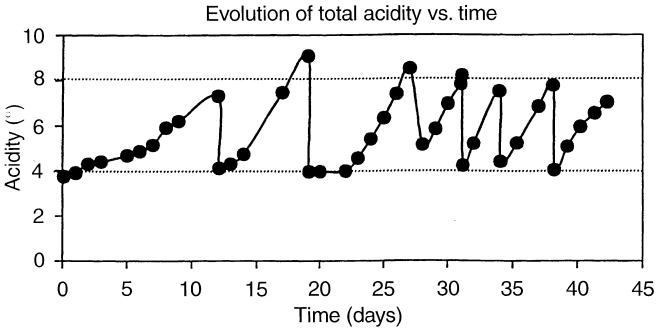



**Fig. 2.** Fed-batch fermentation experiments

with half of the fermenter volume produced in 4.75 days as average cycle duration. The maximum value of acetification rate in a fermentation cycle was about 12 g/l·d.

Stoichiometric yields were in all cases 100% thanks to the operation with the volatile compounds recovery system which allowed evaporative losses to be established at 0%.

## 3.2 Continuous experiments

The experiments were developed following the protocols previously described in materials and methods. An increasing series of feed inlet rates was essayed and, for each experiment, the steady state (i.e. biomass, acetic acid and ethanol concentrations were constant) was reached. After this, some new increases in the feed inlet rates were provoked in order to measure and record a decrease in biomass concentration. This state corresponds to the continuous operation shutting down.

Figures 3 to 6 show the evolution of total acidity versus time of process for four different feed inlet rates: 0.2, 0.9, 1.1 and 1.7 l/h.

Experiments were started at a very low feed inlet rate: 0.2 l/h. The specific growth rate of acetic acid bacteria was high enough to prevent the shutting down of the reactor at this hydraulic residence time (HRT). After a short period of lag time, a steady state was reached. Here, dilution and specific growth rates were equalled and, consequently, concentrations of acetic acid, ethanol and biomass remained at constant values.

When this experiment was finished, those 0.9 and 1.1 l/h were essayed as feed inlet rates with very similar results. A steady state was achieved in both cases. If feed inlet rate was fixed at 1.7 l/h, a shutting down of the reactor (i.e. gradual decrease in acetic acid and biomass concentrations) could be observed. This fact was due to the impossibility of microorganisms to grow at a specific rate high enough to compensate the dilution rate of the reactor. As a consequence, a decrease in the microbial population caused the shutting down of the fermenter in few hours.

Other essays with higher feed flow rates made possible to assure that 1.7 l/h is a critical feed flow rate for the equipment. This fact demands the use of a maximum feed flow rate about 1.1 l/h in the equipment as well as the operation of such equipment with suspended biomass in the fermentation medium. By using this feed flow rate, it is possible to produce 125 l of a vinegar containing 70 g acetic acid/l in 4.75 days.

## 4 Conclusions

– The proposed pilot plant scale fermenter is equipped with a gas recycling gas system. Experimental data lead to



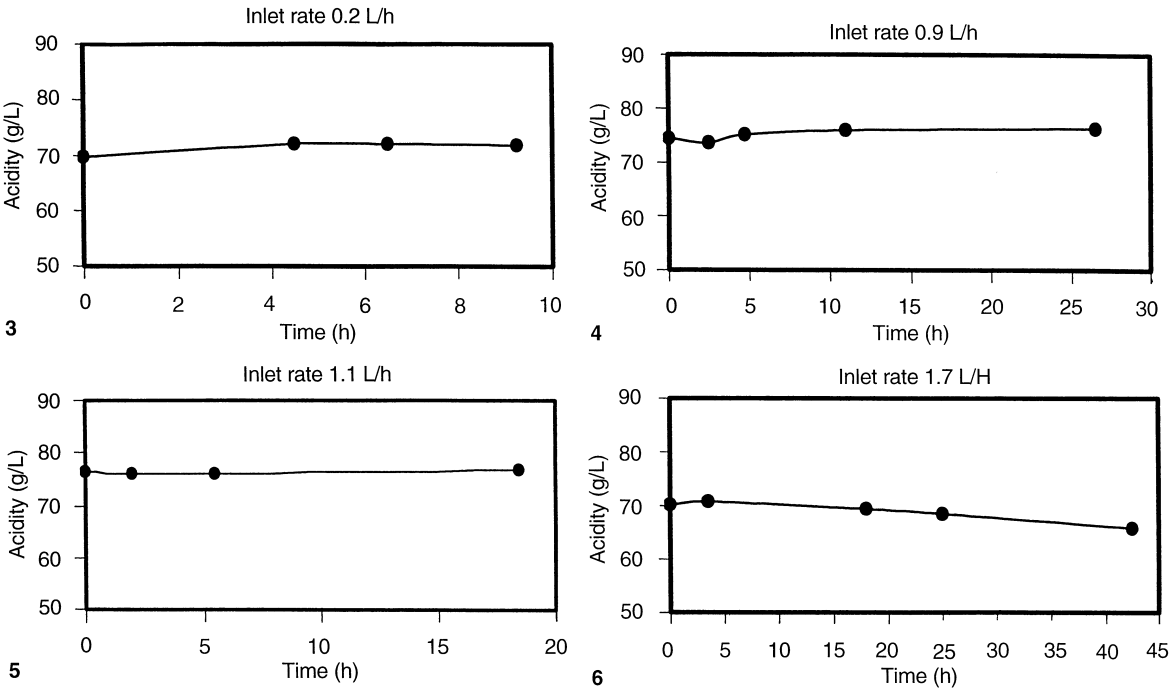



**Fig. 3–6.** Total acidity data versus time for different feed inlet rates: 0.2, 0.9, 1.1 and 1.7 l/h

the conclusion that evaporative losses are reduced to 0% during acetic acid fermentation processes.

– Proposed protocols for inoculation and fed-batch or continuous operation described are suitable for both purposes: reduction of time of lag phase, and increase of acetification rates.

– Better yields are obtained when working in fed-batch operation than when working in continuous operation with suspended biomass. In fed-batch operation it is possible to obtain vinegars with higher acetic acid concentrations in similar times of process. For this reason the commercial value increases.



– The best method to boost the productivity of the fermenter in continuous operation and avoid the limits that the shutting down exerts upon the dilution rate, would be to apply those traditional methods of biomass immobilisation described in the literature to the equipment designed.

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