USE OF BACTERIA IN THE AWARD SELECTION ACETICA INDUSTRIAL VINEGAR FOR IMPROVING THE EFFICIENCY OF ACETIC ACID

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Abstract
This paper presents the results of the industrial experiment of obtaining acetic vinegar fermentor shavings, using Acetobacter acetic orleansis as a fermentation agent, acetic bacteria isolated and selected by the author of the paper. Acetic bacteria biomass necessary to these researches, has been easily obtained, based on laboratory experiments, the pilot station ventilation system submerged.
The selected acetic bacteria were grown in the laboratory pilot station, at the same temperature (25ºC), nutrient medium in two variants (V1 and V2). The nutrient media have in common the hidro-alcoholic liquid with 6% of refined spirit and 2% acetic acid. These media differ with the nutrient supplement of 0.5% yeast extract in V1 variant, and corn extract in V2 variant. The addition of 0.5% corn extract fluid hidroalcoolic obviously stimulates even more the process of multiplication of acetic bacteria and, therefore, under these conditions, the biomass produced increased by 28.4%, compared with the biomass produced in the variant of 0.5% yeast extract addition. Consequently, in multiplying inoculum for the industrial experiment, we used environment variant 2 (corn extract).
In the industry research we observed 5 batches of vinegar production (noted as fermentation cycles C1, C2, C3, C4 and C5) in the fermentative evolution process, in terms of cycle duration of the fermentative digestion and acetic acid yield. The duration of the control fermentation cycle (of the acetic fermentor at the start of the industrial experiment) was 8 days (192 hours), and vinegar left in the acid fermentor had an acidity of 8.1 g acetic acid/100 ml. Consequently to the use of selected culture of Acetobacter with every fermentation cycle, the batch duration was reduced from an average of 192 to 125 hours (including loading and unloading operations), with obvious results over productivity growth. Isolated, selected Acetobacter culture, previously studied and used in the industrial experiment, undoubtedly demonstrated higher fermentative qualities in vinegar obtaining biotechnology.

Keywords: fermentor acetic acid, acetic bacteria, industrial experiment

1. INTRODUCTION

Vinegar is a product of acetic fermentation known since antiquity. Since ancient times, Greeks, Indians, Babylonians, Persians obtained the vinegar from wine, beer, grapes, dates and other fruits.
Several raw materials can be used in vinegar manufacture, such as: wine, wine pique, cider; diluted alcohol (at 10-12% alcohol) made from molasses, cereals, potatoes etc.; various solutions alcoholic beverages produced by fermentation of fruit juice (pear, figs, peaches, apricots, dates, pineapples) or starchy material; honey (mead); mash of malt.
In France, Italy, Spain the wine is the main feedstock in the manufacture of vinegar whereas the cider is a common raw material used in the U.S. and malt and beer in England.
The key condition to obtain vinegar is that the raw materials must contain a certain level of ethanol; the ethanol content is adjusted between 3.5 to 17% depending on: the acetic strength to be obtained; the technology used, and the fermentation potential of the used acetic bacteria cultures. The raw materials may be used individually or in combination, with or without added water. [1].
Acetic fermentation processes may differ depending on the speed of acetic acid formation, and thus in the mode of ventilation, and can be classified into two categories: slow and fast processes.
The slow processes can be the household type and type Orleans.
Fast processes can be: a) processes that allow the contact of alcoholic solution with air by distributing the alcoholic solution into a column with inert filler; b) submerged processes. [2]

2. THE RESULTS OBTAINED IN THE PRODUCTION OF BIOMASS WITH THE SPECIES *ACETOBACTER ACETIC ORLEANSIS*

The selected acetic bacteria were grown in the laboratory pilot station [1], [2], at the same temperature (25ºC), nutrient medium in two variants (V1 and V2).
The nutrient media have in common the hidro-alcoholic liquid with 6% of refined spirit and 2% acetic acid [3], [4]. These media differ with the nutrient supplement of 0.5% yeast extract in V1 variant, and corn extract in V2 variant.
After inoculing each nutrient medium with 30% liquid *Acetobacter aceti orleansis* nutrient, cell multiplying dynamics has been observed with the following results.
In variant V1, the number of acetic bacteria number grew as follows: 1.3 times after 24 hours; 1.4 times after 48 hours; 1.4 times after 72 hours; 2.3 times after 96 hours.
The maximum concentration of cells obtained in this variant was 2.18 x 10^{8}/ml.
In variant V2 the number of cells grew even more: 1.5 times after 24 hours; 1.6 times after 48 hours; 2.1 times after 72 hours; 3.0 times after 96 hours, when maximum cells concentration was obtained, of 2.80 x 10^{8}/ ml.
The addition of 0.5% corn extract fluid hidroalcoolic obviously stimulates even more the process of multiplication of acetic bacteria and, therefore, under these conditions, the biomass produced increased by 28.4%, compared with the biomass produced in the variant of 0.5% yeast extract addition.
In conclusion, corn extract (0.5%) is more convenient in the production of biomass because, by its composition, it favors the plastic metabolism of acetic bacteria cells efficiently in the reproduction and, moreover, it is convenient and, economically, it is cheaper.
Consequently, in multiplying inoculum for the industrial experiment, we used environment variant 2 (corn extract).

2. MATERIALS AND METHODS

- Frings Acetic fermentor with shavings;
- fermentor acetic with aeration in the system submersion;
- Thoma counting chamber acetic bacteria cells;
- Total acidity - method titrimetrică;
- Ethyl alcohol by distillation and then by reading the alcoholic strength;
- pH with pH meter.

3. RESULTS AND DISCUSSION

In the industry research we observed 5 batches of vinegar production (noted as fermentation cycles C1, C2, C3, C4 and C5) in the fermentative evolution process, in terms of cycle duration of the fermentative digestion and acetic acid yield.
In all fermentation cycles we used the same white wine as raw material.
In the first batch, on top of the 2,000 litres of vinegar with 8.1% acetic acid, we added 5,600 liters of white wine with 8.6% alcohol, and 50 liters of *Acetobacter acetic orleansis* selected culture – previously grown and multiplied in the same wine.
We underline that, in each of the 5 cycles of fermentation, we added the indicated 50 liters of bacteria selected acid culture (strain T10).
In subsequent cycles, we mentained the same proportions of vinegar and wine, varying a bit the initial acidity of the vinegar left in the acetic acid fermentor, in order to ensure the initial environment in each batch fermentation.
So, after homogenization of the alcoholic environment within the acetic fermentor, the initial acidity ranged between 2.24 and 2.31% acetic acid (g/100 ml), while the alcohol of the fermentation medium was the same for all cycles of fermentation, that is 6.3% vol.
Aeration was done under the conditions prevailing in the company, setting the optimal amount of air by opening or closing the side openings of the acetic fermentor.
The wine was introduced in acetic fermentor without prior heating.
Fermentation temperature was controlled throughout the entire process, at the 3 levels of

acetic fermentor, and was within limits from 24 to 34^0 C.

The duration of the control fermentation cycle (of the acetic fermentor at the start of the industrial experiment) was 8 days, and vinegar left in the acid fermentor had an acidity of 8.1 g acetic acid/100 ml.

Dynamics of acidity of each fermentation cycle was traced in Table 1.

In Table 2 are included acetic grades obtained in the 5 cycles of fermentation, practical yields, and duration of each cycle of vinegar obtaining.

An average fermentation cycle duration realized in the industrial experiment was 125 hours.

Fermentation cycle (C4) gave the best results, i.e. a reduction in the duration of fermentation to 4 days, subsequent to increasing the fermentation speed due to adaptation and propagation of culture of acetic bacteria.

As seen in Table 3, yields obtained in fermentor with shavings, fall in the values presented in the specific literature.

In figure 1 can be observed the progress of fermentation process to the share of the growing selected culture by the speed of fermentation dynamics.

Table 1. Time evolution of acidity in acid fermentor with working capacity of 7,700 liters (Industrial Experiment)

		Fermentation cycle				
		C1	C2	C3	C4	C5
		19-24.11.2007	24.11-1.12.2007	1-6.12.2007	6-10.12 2007	10-15.12.2007
Features	Wine	Alcohol 8.6% vol Acidity 0.7 g ml acetic acid/100 ml pH 4.5				
	Fermentation mediu	Alcohol 6.3% vol Acidity 2,24-2,31 g ml acetic acid /100 ml pH 4.5				
Titrated acidity g acetic acid/100 ml	initial	2.24	2.31	2.29	2.26	2.24
	after 1 day	5.1	5.2	5.2	5.15	5.1
	2 day	6.85	6.27	7.19	8.06	6.72
	3 day	7.54	6.85	7.42	8.35	7.56
	4 day	8.23	7.48	7.54	**8.50**	7.77
	5 day	8.80	8.17	8.64		8.55
	6 day		8.70	8.70		
Alcohol residual%		0.1				
vinegar pH		3.25				

Table 2. Characteristics of vinegar and bioconversion yields on production cycles in acetic industrial fermentor

	Experimental cycle				
	C1	**C2**	**C3**	**C4**	**C5**
	19-24.11.2007	24.11-1.12.2007	1-6.12.2007	6-10.12.2007	10-15.12.2007
Composition of acetic fermentation mash in fermentor	5,600 l wine with 8.6% alcohol and 2,000 l vinegar with 8.5[a] acetic				
Fermentation cycle duration	5 days	6 days	6 days	4 days	5 days
Average duration of a fermentation cycle	125 hours (includes loading and unloading acetator operations)				
Fermentation yield,%	99.2	96.76	97.50	94.33	95.34

Table 3. Yields obtained in fermentor with shavings

Fermentation cycle	Yields		
	Practical yield ($\eta_{p),\%}$	Theoretic yield ($\eta_{t),\%}$	Fermentation yield ($\eta_{f),\%}$
C1	76.30	130	99.20
C2	74.43	130	96.76
C3	75.00	130	97.50
C4	72.56	130	94.33
C5	73.34	130	95.34
Average yields on the 5 cycles of fermentation	74.33	130	96.63

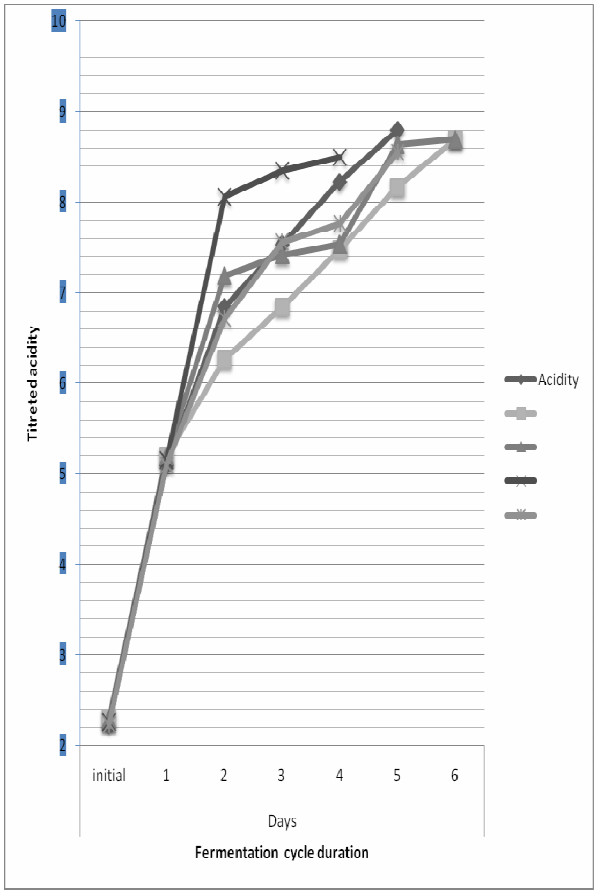

Figure 1. Dynamics of acetic fermentation for the five cycles of the industrial experiment.

4. CONCLUSION

- The vinegar obtained in the industrial experiment, had a nice color, was clear and did not require clarification with bentonite.
- Vinegar acidity of 8.5 to 8.8% acetic acid, with pH 3.2 to 3.25, corresponded to European quality standards.
- Fermentation yield of 94.33 to 99.2% showed an active culture of acetic bacteria and a correctly applied technology.
- Consequently to the use of selected culture of *Acetobacter* with every fermentation cycle, the batch duration was reduced from an average of 192 to 125 hours (including loading and unloading operations), with obvious results over productivity growth.
- Isolated, selected *Acetobacter* culture, previously studied and used in the industrial experiment, undoubtedly demonstrated higher fermentative qualities in vinegar obtaining biotechnology.

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