

Comparative Production of Sugarcane Vinegar by Different Immobilization Techniques

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ABSTRACT

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Sugarcane juice was converted to ethanol by *Saccharomyces cerevisiae* producing 8% (v/v) ethanol. This ethanol was used for vinegar production using adsorbed (bagasse, corn cobs and wood shavings) and entrapped (calcium alginate) cells of *Acetobacter aceti* NRRL 746. All three adsorbed carrier materials were statistically similar for acetic acid production and produced acidity from 5.9 to 6.7% after 28 days of submerged fermentation. By recycling bagasse adsorbed cells, the time of acetic acid fermentation was reduced to 13 days. Semi-continuous fermentation of bagasse adsorbed cells using a packed bed column further reduced the fermentation time to 80 h.

Key words: *Acetobacter aceti*, adsorption, entrapment, semi-continuous fermentation, vinegar.

INTRODUCTION

Natural vinegar is a superior food additive over synthetic vinegar as it carries essential amino acids from its fruit source and is reported to act as a medicine for aches and gastric troubles¹. However, it is generally ignored by both the consumer (due to the higher price) and the producer (due to the long fermentation time of 5–6 weeks). In rural areas the population resorts to traditional fermentation methods without the use of proper cultures/cultural conditions. Entrepreneurs hesitate to fund vinegar production ventures due to the low efficiency of the process and the long fermentation times. Moreover, there is also a lack of awareness of the properties of natural vinegar, besides the problem of the high cost of investment.

Sugarcane is a prime crop in Northern India and its juice is a substrate of choice for natural vinegar because of its high sugar content and availability¹⁰. Therefore, in order to make this process suitable for a low cost cottage industry, it is essential to produce natural vinegar at low cost and in as short a time as possible.

Cell immobilization provides a means to improve upon the fermentation process by increasing biomass, option of reusability, protection of cells from toxic effects of low pH, temperature, inhibitors etc. (as ionic/hydrophobic interactions of the immobilization matrix induces in-

creased stability and a buffered zone is provided by the immobilization materials^{3,6}). Cell immobilization also helps in early clarification of the product. Further, the choice of immobilization material in the form of inexpensive easily available inert biological materials can help reduce the cost of the process.

The present study was undertaken to develop an efficient process using free cells of *Saccharomyces cerevisiae* and immobilized cells of *Acetobacter aceti* and comparing different inert materials for immobilization of the *A. aceti* cells for vinegar production from cane juice.

MATERIALS AND METHODS

The *Saccharomyces* yeast strain employed was *S. cerevisiae* strain G (an isolate from local brewery waste) and an *Acetobacter aceti* bacterial strain, (*A. aceti* NRRL 746 sourced from the USDA). These organisms were used for the dual fermentation.

Sugarcane juice was boiled for 10 min, cooled to room temperature, and the dextrins allowed to settle. The supernatant (clarified juice) for the ethanol fermentation was placed in 2 L flasks with 1.25 L juice per flask at 14°C. The fermentation medium was inoculated with an overnight culture (grown for ~16–20 h) of *S. cerevisiae* G at 10⁶ cell mL⁻¹. Samples were withdrawn every 12 h and °B determined using a hand refractometer. Ethanol was determined by the method of Caputi et al.⁴ The fermented sugar cane was allowed to settle for 2 days at 4°C and the supernatant was siphoned off. The ethanol content was adjusted to 7% (v/v) using distilled water and was inoculated with the adsorbed/entrapped cells of *A. aceti* cells.

For entrapment, a cell paste (36 h old cells of *A. aceti* NRRL 746) and a 4% (w/v) sodium alginate solution were mixed and extruded through a syringe into a 0.2 M CaCl₂ (anhydrous) solution to form the calcium alginate beads. A total of 200 beads (3.1 × 10⁶ cells/bead) per 400 mL of fermentation medium (cane alcohol) were inoculated. For adsorption, 20 mL cell paste (36 h old cells of *A. aceti* NRRL 746) and 20 g dried and sterile crushed corn cobs (40 mesh size), sugarcane bagasse (1–2 cm pieces) or soft wood shavings (1–2 cm pieces) were mixed, shaken for 6 h and inoculated in 400 mL of 7% (v/v) cane alcohol.

In all sets of experiments, mother vinegar @ 5% (v/v) was added at the start of fermentation before passing the fermented ethanol through the carrier materials adsorbed with cells of *A. aceti* NRRL 746. Alongside, fermentation by free cells (as control) was also carried out whereby 7% (v/v) cane alcohol was inoculated with a 5% (v/v)

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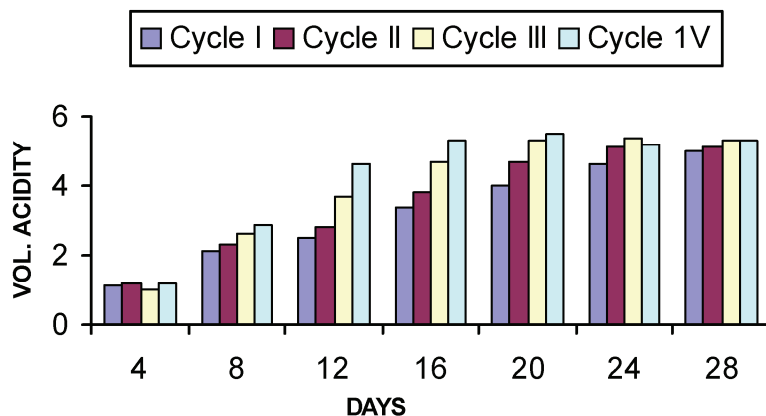


Fig. 1. Effect of recycling of bagasse adsorbed cells of *A. aceti* on volatile acidity production during vinegar production.

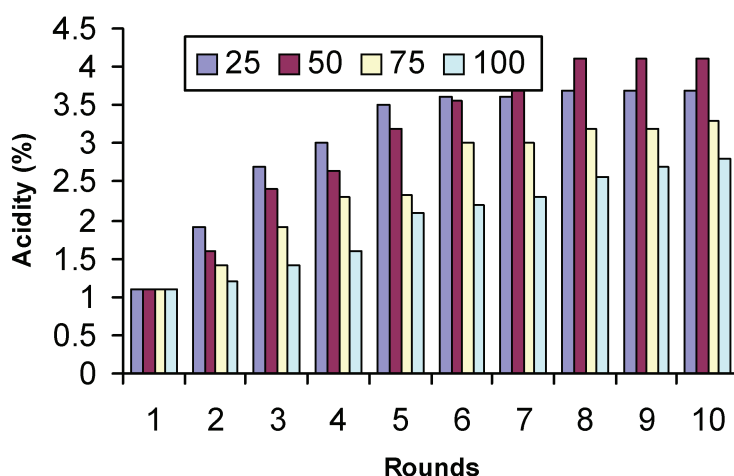


Fig. 2. Effect of flow rate of 25, 50, 75 and 100 mL/h on the semi-continuous fermentation of cane alcohol for vinegar production.

36 h old culture of *A. aceti* NRRL 746 and a 5% (v/v) of mother vinegar in 400 mL cane alcohol at 30°C.

Samples were withdrawn every week and analyzed for residual ethanol⁴ and acetic acid by the distillation method of Amerine et al.¹ The latter were correlated with acetic acid analysis using a gas liquid chromatograph (Nucon, India make) with a PoraPack-N column (mesh range 80–100, internal diameter 2 mm and column length of 2 m) and under conditions of injector, oven and detector temperatures of 200, 75–200 and 200°C respectively. For gas chromatography, samples were clarified through charcoal and 10 µL samples were injected.

A semi-continuous process for vinegar production was studied using a packed bed column (45 cm × 5 cm). The column was filled with 100 g of sugarcane bagasse containing adsorbed cells of *A. aceti* NRRL 746 at a cell load of 10⁶ g⁻¹.

The fermentation efficiency of acetic acid production was calculated as:

$$\text{Fermentation efficiency} = \frac{\text{Acetic acid (g/100 mL)} \times 0.64}{\text{Ethanol consumed (v/v)} \times 0.51} \times \frac{100}{1.304}$$

Where 0.51/0.64 is the factor used to convert ethanol consumed (v/v) into (w/v). All experiments were conducted in triplicate and the data was analyzed statistically by Analysis of Variance (Random Block Design).

Vinegar produced was stored at 4°C, for 3–4 days, and the settled bacterial cells and sediment were separated. This partially clarified vinegar was bottled and pasteurized (using a water bath at 65°C for 30 min) and stored at room temperature.

RESULTS AND DISCUSSION

The fermentation of the clarified juice by *S. cerevisiae* G produced 8.0% (v/v) ethanol in 48 h and the total soluble sugar (TSS) of the juice was reduced from 14°B to 2°B as reported in earlier studies⁷.

The cane alcohol was fermented using the alginate entrapped cells and the *A. aceti* NRRL 746 cells adsorbed on corn cobs, sugarcane bagasse and wood shavings. The maximum acetic acid was produced (6.7 g/100 mL of acetic acid) with the corn cobs at a fermentation efficiency of 99% after 28 days of submerged fermentation.

Table I. Production of acetic acid using immobilized cells.

Carrier material	Acetic acid (g/100 mL) in days (d)							Mean (carrier materials)
	0 d	7 d	14 d	21 d	28 d	35 d	42 d	
Calcium alginate	0.7	1.9	2.8	4.1	4.6 (77%) ²	4.4	4.1	3.229
Corn cobs ¹	0.12	0.7	1.6	2.8	6.7 (99.1%) ²	6.6	6.4	3.560
Sugarcane bagasse ¹	0.08	0.3	0.76	3.1	5.9 (87.3%) ²	6.0	6.1	3.177
Wood shavings ¹	0.26	1.2	2.8	4.5	5.9 (87.3%) ²	5.6	5.2	3.637
Free cells (control)	0.14	1.4	3.3	4.1	4.5 (76%) ²	4.3	3.8	3.077
Mean (time)	.26	1.10	2.25	3.7	5.52	5.38	5.12	

ANOVA table

Source	d.f.	M.S.	C.D. 5%
Time	6	23.262010	1.179
Carrier	4	.42811580	—
Error	24	.81539850	

¹ 20 mL cell paste (36 h old culture of *A. aceti* NRRL 746) and 20 g sterile adsorption material were mixed, shaken for 6 h and inoculated in 400 mL fermentation medium.

² Figures in parenthesis indicate fermentation efficiency.

The residual ethanol ranged from 0.5 to 0.8% (v/v) in different experiments. Moreover, all the adsorbed materials were statistically similar in their acetic acid production potential and were significantly superior to the alginate entrapped and free cells (Table I). Similar observations have previously been made by Berraud² and Ory et al.⁹ Further, adsorbed cells showed better fermentation efficiency than entrapped cells because of the increased surface area and the direct contact between the substrate and air spaces within the adsorbed materials. On the other hand, fermentation efficiency with the alginate entrapped cells was low due to limited transport of substrate and oxygen across the beads and hence the diffusion gradient between gel matrix and the cells⁵.

However, a fermentation time of 28 days was still too long and thus the adsorbed cells were recycled. The four time recycling of the bagasse adsorbed cells, under the same set of fermentation conditions, reduced the acetous fermentation time from 28 days to 13 days and a volatile acidity of more than 4 g/100 mL was obtained (Fig. 1). The volatile acidities of the different cycle means were 3.24, 3.57, 4.00 and 4.30 g/100 mL respectively, with a CD 5% of 0.43, suggesting that the 3rd and 4th cycles were statistically similar.

The batch process was compared with a semi-continuous process, whereby the packed bed column was fed with a batch size of 500 mL, containing 7% (v/v) alcohol and an initial acidity of 1% (w/v). The effect of flow rate (25–100 mL/h) was observed on volatile acidity production for 10 rounds (each round was 10 h). The data is presented in Fig. 2 and reveals that 50 mL/h was the optimum flow rate and 4.1% (w/v) acetic acid was produced in 8 rounds. Although the mean values of 3.05 with the 25 and 50 flow rates with CD 5% of 0.24 were statistically similar, the required value of above 4% (w/v) was only obtained with the latter. Therefore, the semi-continuous process produced vinegar in 80 h and this was four times faster than the batch process. Earlier, Mehaia and Cheryan⁸ reported a value of 20 times more vinegar productivity using a membrane bioreactor, rather than free cells and Ory et al.⁹ reported a vinegar productivity of 4.74 g/l/day using polyurethane adsorbed cells of *A. aceti*.

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REFERENCES

- Amerine, M.A., Kunkee, R.E., Ough, C.S., Singleton, V.L. and Webb, A.D., *The Technology of Wine Making*. 4th ed. The AVI Publishing Co: Westport, CT, 1980.
- Berraud, C., Production of highly concentrated vinegar in fed-batch cultures. *Biotech. Lett.*, 2000, **22**, 451–454.
- Brodellius, P. and Vndamme, E.J., Immobilized cell systems. In: *Biotechnology*. Vol. 7A, H.J. Rehm and G. Reed, Eds., Verlag Chemie: Germany, 1987, pp. 405–464.
- Caputi Jr. A. and Wright, D., Collaborative study of the determination of ethanol in wine by chemical oxidation. *J. Assoc. Off. Anal. Chem.*, 1969, **52**, 85–88.
- D'Souza, S.F., Immobilized cell techniques and applications. *Ind. J. Microbiol.*, 1989, **29**, 83–117.
- Durham, D.R., Marshall, L.C., Miller, J.G. and Chmurny, A.B., New composite biocarriers engineered to contain adsorptive and ion exchange properties improve immobilized cell bioreactor process dependability. *Appl. Environ. Microbiol.*, 1994, **60**, 4178–4181.
- Kocher, G.S., Kalra, K.L. and Tewari, H.K., Production of vinegar from cane juice. *Electronic Proceedings of Symposium on Food and Nutritional Security: Technological Interventions and Genetical options*, Sept 18–19, 2003, HPKV, Palampur, India. Contribution, In Late arrivals.
- Mehaia, M.A. and Cheryan, M., Fermentation of date extracts to ethanol and vinegar in batch and continuous membrane reactors. *Enz. Microbial Technol.*, 1991, **13**(3), 257–261.
- Ory, I. De., Romero, L.E. and Cantero, D., Optimization of immobilization conditions for vinegar production. Siran, wood chips and polyurethane foam as carriers for *Acetobacter aceti*. *Process Biochemistry*, 2004, **39**(5), 547–555.
- Tewari, H.K., Marwaha, S.S., Gupta, A. and Khanna, P., Quality vinegar production from juice of sugarcane variety CoJ-64. *J. Res. PAU*, 1991, **28**, 77–84.
- Yamagishi, K., Kimura, T., Kameyama, M., Nagta, T. and Kikuchi, Y., Purification and identification of blood fluidity improvement factor in brewed rice vinegar. *J. Japan Soc. Fd. Sci. Technol.*, 1998, **45**, 545–549.

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