Design and development of batch type acetifier for wine-vinegar production

R. Singh · S. Singh

Received: 20 March 2007 / Final revision: 5 May 2007 / Accepted: 17 May 2007

Abstract A batch type acetifier based on the principal of acetic acid fermentation was designed and tested for production of wine-vinegar from the pineapple peel waste. The pineapple peels along with starter solution was fed to the inner SS perforated peel-solid separator tank 130 mm dia having perforations of 50 mm size. The concentric perforated peel-solid separator circular tank was fitted inside the collecting tank having 255 mm dia. The pineapple peels and starter solution in perforated peel-solid separator tank was agitated and atomized by tubing agitator 200 mm long having 1 mm dia. hole to spray fermented solution at 20 rpm. The agitator was connected with stirring pump. Lift pump was fitted at the bottom of the collecting tank to lift and supply fermented solution to agitator. The capacity of the batch type Acetifier based on present working design was 3.5 liters of wine-vinegar per day for 8 hours for a quality end product at 2% acidity.

Keywords Acetifier · Submerged cultures · Fermentation · Pineapple peels

The truly large-scale aseptic fermentation vessel was firstly developed for the production of acetone by a deep liquid fermentation using *Clostridium acetobutylicum* with contaminations a serious problem, during large-scale production stage¹. The first large-scale aerobic fermentors were used in central Europe in the 1930s for the production of compressed yeast². The fermenter consisted of a large cylindrical tank with air introduction at the base. In later modifications, mechanical impellers were used to increase the rate of mixing the air bubbles. As early as 1932, in a patented system aeration tubes were provided, using water and steam for cleaning and sterilization³. Prior to 1940, the other important fermentation products produced, besides bakers yeast, were ethanol, glycerol, acetic acid, citric acid, other organic acids, enzymes and sorbose⁴. The decision to use submerged culture techniques for penicillin production where aseptic conditions, good aeration and agitation were essential, was very important factor in forcing the design and development of acetifiers. Initial agitators were studied in baffled stirred tanks to identify variables⁵. Here the geometry of the stirred vessels has been used in the design of conventional fermenters. Initially, the most important design feature of an Acetator was its self-priming aerator⁶. The most common technology in the actual vinegar industry till date is based on the submerged acetification⁷ wherein a stirred medium containing 8–12 percent alcohol (hard, cider, wine fermented malt mash or spirits) was inoculated with *Acetobacter* and held at 24 to 29°C with controlled aeration by means of finely divided air. Very little research on acetic acid bacteria has been done by the wine industry world-wide⁸. Pineapple wastes obtained during processing are broadly in form of mill juice, core, peels, stem and crown⁹. The wastes contained 7.8% solids and 89%

R. Singh · S. Singh (✉)
Agro-processing Division, Central Institute of Agricultural
Engineering, Nabibagh, Berasia Road,
Bhopal,
Madhya Pradesh - 462 038,
India
e-mail: sunitas@ciae.res.in
Tel: +91 / 755 / 2730980-Ext 297

volatile solids with 35% of total carbohydrates¹⁰. Theoretically in vinegar production, 1 g of alcohol yielded 1.3 g of acetic acid in practice, but the yield was 15–20% lower because alcohol, acetaldehyde and acetic acid tend to volatilize. The theoretical amount of air required to produce 1 L vinegar containing 6% acetic acid was about 120 L¹¹. Theoretical conversion was also reported¹² as 1g glucose to 0.51 g ethyl alcohol to 0.67 g of acetic acid. Secondary factors such as starter culture, fermenter design and working condition affected the yield and acetification rates^{13, 14}.

In view of the importance of the production of wine-vinegar from the pineapple peel waste, a batch type small capacity acetifier based on submerged acetification was designed and developed. The acetification based on operation was tested and evaluated in a designed Acetifier developed at CIAE, Bhopal.

Materials and Methods

This acetifier has been developed at CIAE, Bhopal. The main components of acetifier are mild steel stand, stainless steel parts like collecting tank, perforated solid separator (peel) tank, tubing agitator with stirring pump, and lift pump. The schematic diagram (overview) for the acetifier to be developed is shown (Fig. 1) described in results.

Keeping in view the aforesaid design consideration parameters, the acetifier was designed as detailed under:

I. Body construction

Material used: In acetifiers with strict aseptic requirements to withstand repeated steam sterilization cycles, stainless steel material (SS with 2% carbon) was used because it

Fig. 1 Schematic Design of Acetifier.

gives smooth surfaces, is non-toxic, non-corrosive and eas-
es to examine the interior of the vessel.

Base stand: Base stand of acetifier was made from 30 mm × 30 mm × 4 mm mild steel (containing 0.15–0.30% carbon) channels. Length and width of the main frame was square shaped having sides 600 mm each. The height of the frame was kept 230 mm for proper functioning of the acetifier. The main frame was covered with 2 mm thick mild steel sheet to act as a base for the acetifier. These dimensions matched with the required diameter of the stainless steel (containing 2.0% carbon) outer body of the acetifier, a place for pump and vertical mild steel square section for supporting drive motor and aseptic stainless steel pipeline.

II. Operating volume

Designed for 5 kg of pineapple peel per batch. (as per availability of peel wastes during processing pineapples). A jaggery solution of 15% TSS finally adjusted to 21% was used (28L) for ethanol production.

Volume of inner perforated tank

Average bulk density of pineapple peel = 0.704 kg/l
Therefore, Volume required for 5 kg of pineapple peel = 7.10 l

Assuming height of the tank (h_1) = 25 cm
and diameter of the inner tank (d_1) = 26 cm

$$\text{Volume of the inner SS perforated tank} = \frac{\pi d_1^2}{4} \times h_1 \quad (1)$$

Volume of the inner SS perforated tank = 13 L

Volume of outer tank

The height of the tank (h_2) = 50 cm and diameter of the outer tank (d_2) = 35 cm

$$\text{Volume of the outer SS tank} = \frac{\pi d_2^2}{4} \times h_2 \quad (2)$$

Volume of the outer SS tank = 48 L

Percent perforation on inner perforated tank:

$$\text{Surface area of inner tank} = \pi d_1 L \quad (3)$$

Where, d_1 was Diameter of inner stainless steel perforated tank.

L was Height of inner stainless steel perforated tank

$$\text{Total area of perforation} = \pi d_1 \times \text{No of perforation} \quad (4)$$

Using equations 3 and 4 percent perforation was calculated as

$$\text{Percent perforation} = \frac{\text{Total area of perforation}}{\text{Surface area of inner tank}} \times 100 \quad (5)$$

From equation 5 percent perforation provided to the inner tank of acetifier was 5.3%. The number of holes was according to area available for perforation and their size decided in such a way that the material (solid peels) may not be able to move out of the inner tank and clog the openings of the outer tank.

Liquid Height

Liquid height was kept 27 cm as per recommendation¹⁵

III The Agitation (Impellor):

The impellor functioned to:

- diminish the size of air bubbles to give a bigger interfacial area for oxygen transfer and to decrease the diffusion path and
- maintain a uniform environment throughout the vessel contents.

Impellor diameter:

Using the formula¹⁶ it was calculated as under:

$$\text{Impellor diameter} = \frac{\text{diameter of outer tank} \times 0.33}{\text{Impellor diameter} = 12 \text{ cm.}} \quad (6)$$

Length of Impellor Shaft:

Ideally the length of impellor shaft should be 1/3 to 1/2 of the outer vessel diameter above the base of the vessel¹⁷.

Diameter of outer tank = 35 cm

$$\text{Thus length of impeller shaft} = 50 - (1/3 \times 35) = 38 \text{ cm} \quad (7)$$

Baffle width: Baffle width and diameter of the outer tank ratio should be between 0.08 to 0.1¹⁸.

Baffle width = X

Diameter of outer tank = 35 cm

Therefore, baffle width was calculated the equation given below¹⁸.

$$\frac{\text{Baffle width, } X}{\text{Diameter of outer tank}} = 0.1 \quad (8)$$

Baffle width as calculated (Equation 8) was 3.50 cm. Four baffles are normally incorporated into agitated vessels of all sizes to prevent a vortex and to improve aeration efficiency¹⁸. They are metal strips roughly one tenth of the vessels diameter and allocated radially to the wall.

IV. The aeration system

Air Sparger: In the acetifier, a perforated SS pipe of 30 cm length was fitted horizontally below the lid of the SS outer tank. It was recommended¹⁹ that in sparger, holes should be at

least 6 mm in diameter as smaller holes have the tendency to block and this also minimizes pressure drop. As per the recommendation, diameter each hole on sparger was 6 mm with an appropriate distance adjusted between them (20 numbers total holes) for proper sparging from them during operation.

Stirrer glands and bearings: The satisfactory sealing of the stirrer shaft assembly has been one of the most difficult problems to overcome for long period. The most common stirrer shaft used entered the vessel from the top²⁰. Bush bearing seal as it is commonly used was used in the acetifier.

Power Requirement: Pump Power W (watt) was the energy supplied by the pump per unit of weight passing through the system. To determine this power, supplied to the system (P_s) W was multiplied by the weight flow rate (m. g).

$$\text{Then } P_s = W m g \quad (9)$$

The mass flow rate (m) may be expressed as volume flow rate by:

$$m = Q \rho \quad (10)$$

By substituting mass flow rate (m) in equation 10

$$P_s = W Q \rho g \quad (11)$$

By applying Bernoulli's equation

$$W = h_2 + V_2^2 / (2g) \quad (12)$$

The power (P_r) required by the pump, is greater than that supplied to the fluid system (P_s) due to the inefficiency of the device. This can be expressed as:

$$P_r = P_s / e \quad (13)$$

By substituting the values we have:

$$W = 0.59 \text{ J / N} \\ \text{and } P_r = 0.04 \text{ kW}$$

The total power requirement of the mono-bloc pump was 0.04 kW.

V Speed of impellor shaft

The speed of impellor shaft was calculated by using equation as below (equation 14)

$$\frac{N_2}{N_1} = \frac{d_1}{d_2} \quad (14)$$

where,

- N_2 = Speed of impellor shaft, rpm
- N_1 = Speed of driver motor, rpm
- d_1 = Diameter of driver motor pulley, cm
- d_2 = Diameter of impellor pulley, cm

The speed of the impellor shaft was calculated as 30 rpm at full load capacity by using Equation 14.

VI Length of the open belt

The length of the belt was calculated by using standard formula as below

$$\text{Length of the belt} = \frac{\pi}{2} (d_1 + d_2) + 2x + \frac{(d_1 + d_2)^2}{4x} \quad (15)$$

where, in Eqn 15

x = Distance between centre of motor pulley and impellor pulley.

Length of open belt = 123 cm (equation 15).

Cultures used: *Saccharomyces cerevisiae* NRRL 11857 and *Acetobacter rancens* NCIM 2317 were obtained from Culture Collections of NRRL Peoria, USA and NCIM, NCL Pune respectively. The cultures were subcultured regularly on Potato dextrose agar regularly.

Process for pineapple peel vinegar preparation: The method described earlier²¹ was used for vinegar (as below given).

Vinegar parameters

Titrate Acidity²²: To a diluted (known dilution) 5 ml sample was added 2 drops of phenolphthalein indicator and titrated against NaOH (0.1N). Colour change from light brown to faint pink that persisted for 30 secs was taken, as the endpoint titre of titration and titrate acidity was calculated²².

Alcohol: Rapid method of alcohol percentage was determined by using spectrophotometric method of estimation²³. Reducing sugar (RS) was estimated by Lane and Eynon titration method²². Total (Invert) sugars²² by Lane and Eynon titration method was followed after inversion of reducing sugar sample that was used for titration.

Percent T.S.S²²: For measuring the T.S.S. (°Brix) of the sample, a hand refractometer (of various ranges: 0–50 (ii) 28–62 (iii) 58–92.) was used. Sample was dropped on the prism and closed slowly. It was directed towards the light and observed through an eyepiece. While observing through an eyepiece, the scale-calibrated screw was rotated so that boundary line separating the light and the dark areas of the image was aligned.

Yield of acetification⁷: This was calculated as the change in acid during fermentation expressed as a percentage of the original alcohol content of the vinegar stock.

Operation procedure for vinegar preparation in acetifier: In the start-up cycle the reactor was filled-up of with raw material (peel wastes in jaggery-solution) (21%TSS), starter yeast (*Saccharomyces cerevisiae* NRRL 11857) as inoculum @ 0.3% (v/v) (containing 10^6 cfu/ml). When an initial scum appeared due to alcoholic fermentation the scum on surface of solution was removed. In successive stages final working volume was filled with fresh wine (as obtained in the start up process). The start of the acetification was carried out by inoculation with vinegar inoculum (*Acetobacter rancens* NCIM 2317) (@ 0.2% v/v) (containing 3×10^5 cfu/ml).

Results and Discussion

Design of the Acetifier: The main components of acetifier were fitted as detailed in Table 1.

Operation of acetifier: During operation in the first stage of a start-up cycle, the reactor contained an initial proportion of wine (after 1 d) of fermentation and vinegar inoculum was added. Ethanol of 20–25 g/l formed in 8–10 h initially. This initial alcohol produced (in the fabricated acetifier along with added pineapple wine) reached as high as 180.1 g/l (18%) if recycling without harvesting acetic acid was allowed. The ethanol concentration increased to 180–190 g/l in 15 d when it was distilled to give a pineapple flavoured edible wine.

The initial acidity of 8–9 g/l as a consequence the acetic acid fermentation reached to 17.8 g/l (Fig 2) on 3rd d, which did not increase beyond 19 g/l in 9 d. The yield expressed was in the order of 9.42%. This increased to 9.80% on 9th day after recycling on 4th day. Studies on batch scale (flasks) have shown acid levels of 3.24% starting with 2.3% alcohol in the fermented solution²⁴. The refilling of alcohol formed (with half to one third diluted concentration) was fed to the vessel for working the acetifier. The starting of a new cycle began with the discharge of 50% of the total volume (14 l of wine vinegar with acidity 14–15 g/l as obtained) and the subsequent filling with the same volume of fresh wine (on 4th day of cycle). Thus, the substrate and product concentrations were settled again, with adequate initial values for the starting of a new cycle.

Table 1 Main component of Acetifier with detail dimensions (Design).

S No	Component	Material	Dimension
1.	Base stand (l × b × h)	MS*	600 mm x 600 mm x 230 mm
2.	Outer tank	SS**	Length-50 cm Diameter-35 cm
3.	Inner perforated tank	SS	Length-25 cm Diameter-26 cm Perforation- 6mm
4.	Impellor/Agitator	SS	Impellor diameter- 12 cm
5.	Impellor shaft	SS	Length-38 cm
6.	Baffle	SS	Width- 3.5 cm
7.	Air sparger	SS	Length- 30 cm, 6 mm perforated diameter.
8.	Driver Pulley	MS	Diameter- 2 cm
9.	Impellor shaft pulley	MS	Diameter- 30 cm
10.	Pulley belts	Reinforced plastic	Length-123 cm
11.	Electric motor	-	230 V, AC, 1340 rpm
12.	Mono bloc pump		0.04 kW

* Mild steel; ** Stainless Steel.

This helped to increase the acid levels. However the intermittent conditions did not give a uniform oxygen (not calculated) distribution in the system so that it could help in increasing the acid level even higher. This can be explained by the fact that at low GK (Ger. Gesamte Konzentration) yields with longer interruptions in air supply, there was less damage to the acetification process²⁵ and that the degree of damage increased with increasing acidity. An aeration system that is to be provided in the system (a continuous slow operation of the system with agitation and recycling) may thus allow better acidity yields in the process.

The pipe diameter fixed in the monobloc pump was getting clogged and needed change/modification to work in continuous operation with refilling and in slow mode. The modifications are in progress. Therefore, in next phase of modification the pumping system for aeration of acetifier, will be taken up. This will help the acetifier to be operable in continuous slow mode (for uniform aeration in the acetous fermentation). It will then again be tested with the added inoculum levels and operation level as defined.

Vinegar production in a fabricated 'Vinegar Acetator' (Acetifier) [using Acetobacter rancens NCIM 2317 as the inoculant culture]. The titrable acidity of vinegar increased from 1.5 to approximately 2%, when observed from 1 to 9 d. The reason for low acidity was probably due to the fact that acetic acid bacteria are aerobic organisms (i.e. requiring oxygen to survive). The vinification process was a usually a fairly anaerobic process and in the past this, together with the SO₂ levels in wine, was considered sufficient reason to prevent the growth of acetic acid bacteria in wine⁸. The waste peels contained 7.8% of total sugars. The initial% TSS of raw material for vinegar was adjusted to 21% and on first day (after inoculation with *S. cerevisiae*) it decreased to 15%. It reached 7% after 9 d of the whole fermentation

Fig. 2 Changes in the fermenting solution in acetifier.

Plate 1. Fabricated acetifier

process. The pH of vinegar (4.5 on first day) decreased to 3.5 by 9 days. Percent reducing sugars of vinegar ranged from 2.54 to 2.60 and total sugars ranged from 3.18 to 3.19 from day 1 to day 4 as checked (Fig. 2). However the flavour of the vinegar was appealing and of good quality²⁶. Nutrients added in fermentation solution were necessary to establish the culture but it is reported that they have no effect on the rate of acidification²⁷. Also even if the pH reached in final vinegar was lower the percentage of acetic acid un-dissociated is still higher²⁸. Further an initial alcohol level up to 6–7% will then increase acidity levels in vinegar preparation/ fermentation process.

The system under modification as above stated, will then be operated based on designed parameters with slow aeration and agitation in a continuous operation to allow for increase in acetification yields.

Conclusion

In view of the importance to value addition of wastes, a batch type acetifier was designed and developed for production of wine-vinegar from the pineapple peel wastes. It was based on the principal of facultative anaerobic fermentation followed by aerobic fermentation. The testing of acetifier was done to produce vinegar from pineapple peel wastes and a 2% acidity level was achieved in 9 d. This acetifier was tested, however modifications are in progress. The capacity of the batch type acetifier was 3.5 L of wine-vinegar per day (in 8 h of operation with intermittent mixing/agitation) for an end product of 2% acidity. According to standards vinegar it should have a minimum of 3% acidity.

We expect the dispersion of air to all parts in the modified system to improve the acid production by *Acetobacter* in next phase of modification based on results so obtained. The influence of the time employed for the start-up step affecting the overall process is very significant and good determination of control start-up step determined the consequent productivity²⁹. So, improving the rate of this initial process –and thus decreasing the time of the non-productive stage, needs an exhaustive knowledge of the key factors that affect the viability of bacteria during the start-up are also needed.

Acknowledgements The authors wish to thank The Director, CIAE, Bhopal for necessary facilities extended and in encouraging us to design this equipment. The necessary facilities extended by the Head, Agro Processing Division and I/C Research Workshop is also placed on records here. The technical help offered by Shri K Kawalkar is gratefully acknowledged.

References

- Hasting JJH (1978) Acetone-butanol fermentation. In Economic Microbiology. Vol. 2:31–45 Academic press London
- De Becze G & Liebmann AJ (1944) Aeration in the production of compressed cheese. Ind. Eng. Chem. 36:882–890
- Strauch & Schmidt (1932) German patent 552, 241. Cited by De Becze G & Liebmann AJ (1944)
- Johnson M J (1971) Fermentation – yesterday and tomorrow. Chem. Tech. 1:338–341
- Cooper CM, Fernstorm GA and Millers SA (1944) Performance of gas liquid contactors Ind. Eng. Chem. 36:504–509
- Ebner H, Pohl K & Enekel A (1967) Self priming aerator and mechanical defoamer for microbiological processes. Biotechnol. Bioengng 9:357–364
- Hromatka, O & Ebner H (1959) Vinegar by submerged oxidative fermentation. Ind. Engng Chem. (Ind. Edn). 51: 1279–1280
- Drysdale, GS, & Fleet GH (1988) Acetic acid bacteria in winemaking: A Review. Am. J. Enol. Vitic. 39:143–154
- Anand JC & Maini SB (1997). Utilization of fruit and vegetable wastes. Indian Fd. Pkr. 51(2):45–63
- Bardiya N, Somayaji D & Khanna S (1996) Biomethanation of banana peel and pineapple waste. Bioresource Technology 58:73–76
- Macrae, et al. (1993) Encyclopedia of Food Science and Technology. Vol 7 pp 1–43
- Adams MR (1985) Vinegar. In Microbiology of fermented foods. Vol I (Wood, J.B. Brian Ed.). Elsevier Applied Science Pub. pp 1–47
- Tesfaye W, Morales, ML, Garcý' a-Parrilla MC & Troncoso AM (2002) Wine vinegar: technology, authenticity and quality evaluation. Trends in Food Science & Technology 13 (2002) 12–21
- Tesfaye W, Lourdes Morales, Garcia,-Parilla M del C, Troncoso AM (2004). Optimization of immobilization conditions for vinegar production. Siran, wood chips and polyurethane foam as carriers for *Acetobacter aceti*. FSTA Abstracts 2004 Vol 36 (5) TC 437
- Wegrich RM & Shurter RA (1953) Development of a typical aerobic fermentation. Ind. Eng. Chem. 45:1153–1160
- Blakebrough N (1967) Industrial fermentations. In Biochemical and Biological Engineering Science, Academic press London. Vol 1, pp 25–48
- Steel R & Maxon WD (1971) Power requirement of typical actinomycine fermentation. Ind. Eng. Chem. 53: 97–108
- Jackson (1958) Development of aerobic fermentation process: Penicillin. In Biochemical Engineering pp 185–221
- Walker JAH & Holdsworth H (1958) Equipment design. In Biochemical Engineering, Haywood, London. pp 223–273
- Richards JW (1968) Design and operation of aseptic fermenters. In Introduction to Industrial Sterilization, Academic press London. pp 107–122
- Fellows Peter (1997) Processing for profit. Intermediate Technology Pubs Ltd. Southampton Row, London
- Ranganna S (1986) Handbook of analysis and quality control for fruit and vegetable products (2nd Ed.). Tata McGraw-Hill Pub. Co. Ltd
- Caputi A Jr, Ueda M & Brown T (1965) Spectrophotometric determination of ethanol in wine. Am. J. Enol. And Vitic 19: 160–165
- Singh S and Pal R (2004) A comparison of pineapple peel vinegar prepared with and without yeast inoculation to ingredients in a batch process. Indian J Appl and Pure Biol. 19(3):275–282
- Allgeier RJ & Hildebrandt FM (1960) Newer developments in vinegar manufacture. Adv. Appl Microbiol 11: 163–182
- Mahindru SN (1987) Wine vinegar. Handbook of food analysis. Swan Pubs, Chief chemist Regional Agmark Laboratory, New Delhi
- Lee JW (1975) Vinegar production from papaya and pineapple by four different fermenters. Termination report, projects-566. Honolulu, hi, university of Hawaii, college of tropical Agr
- Binsted R, Devey JD & Dakin JC (1962) Pickling and Sauce Making, 2nd edn. Food Trade press, London, 274 Pp
- Ignacio de Ory, Luis E. Romero & Domingo Cantero (2004) Operation in semi-continuous with a closed pilot plant scale acetifier for vinegar production. Journal of Food Engineering 63:39–45