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Aeration Effects on Vinegar Production from Coffee Pulp: Laboratory Fermentation and 100-L Scale-Up

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ABSTRACT: Aeration was tested as an operating variable in vinegar fermentation from coffee pulp. *Acetobacter pasteurianus* TISTR 102 was grown in a 10-L stirred-tank bioreactor supplied with air at 0.5, 1.0, or 1.5 vvm. The 1.0-vvm treatment reached 5.12% acetic acid after 144 h and an estimated viable cell concentration of 7.1467 log CFU/mL. It gave the highest acid concentration and shortest fermentation time among the three treatments. The logistic fit at this rate had $R^2 = 0.9975$, $\chi^2 = 0.3587$, RMSE = 0.5821, and MBE = 0.3388. Oxygen limitation and adverse effects of greater aeration could explain the lower performance at the other rates, but their contributions were not established experimentally. When the 1.0-vvm condition was used in a 100-L fermenter, acetic acid reached 4.48%. Thus, this air supply rate performed best in the laboratory comparison and also supported fermentation in the larger vessel. Its transfer to another scale needs to account for vessel mixing, oxygen transfer, and temperature conditions.

1. INTRODUCTION

Coffee processing leaves a substantial pulp fraction, equivalent to 40–50% of berry weight [1]. Discarding this material can cause odors and contaminate soil or water, while also attracting insects and pathogens. Its composition offers a reason to examine fermentation as an alternative use. Comparable agro-industrial residues, including furniture-industry bamboo waste, cinnamon leaves, and low-grade longan, have been directed to fermentation-based valorization routes such as biohydrogen, bioethanol, and biogas [32, 33, 34]. Along with fiber and protein, the pulp contains potassium, calcium, magnesium, and phenolic compounds such as chlorogenic acids [2]. Antioxidant, antidiabetic, and antiobesity properties have been reported for these compounds [2]. Aqueous pulp extracts reduced cholesterol absorption and showed lipid-lowering effects in cell and animal experiments [3]. Those findings concern the extracts and experimental systems studied, rather than health effects of a fermented vinegar.

Fruit and cereal substrates, including grapes, apples, and rice, are used for vinegar, and residues such as rice bran have also been studied [9]. Coffee pulp provides another substrate to test. The final product needs sufficient acidity because acetic acid is its principal acid, with additional quality requirements set by the relevant standards [4, 5, 6]. The Thai notification, for example, specifies at least 4 g of acetic acid per 100 mL for fermented vinegar [6]. Reviews describe antioxidant and antimicrobial activity and possible metabolic effects across different vinegars [7, 8], but these responses depend on the product and the evidence available.

The conversion proceeds through an anaerobic stage, in which Saccharomyces cerevisiae produces ethanol, and an aerobic stage, in which Acetobacter spp. oxidize that ethanol to acetic acid [10]. The second stage depends on aeration, agitation,

inoculum, and dissolved oxygen [11, 12]. Providing more air can relieve restricted oxygen supply, yet the response may change as gas flow increases. Acid over-oxidation and hydrodynamic stress have been discussed as possible adverse effects [14, 15, 16]. These relationships make oxygen supply a practical variable to examine before transferring a fermentation to a larger vessel [13]. Co-digestion and effluent-fermentation studies likewise show that operating variables identified at bench scale govern product yield and gas quality in biogas and biomethane systems [35, 36].

A logistic growth model gives a compact description of the cell concentration profile and can be used to compare aeration treatments [17]. Oxygen availability affects Acetobacter respiration [18, 19], and changes in air supply have been used in high-acidity fermentation strategies [20]. Responses differ across organisms and production conditions [21, 22]. Fitting a growth curve therefore helps describe a treatment response, while explaining that response requires information about oxygen transfer and cell stress.

In this study, three aeration rates were compared for coffee pulp vinegar production in a laboratory bioreactor. Acid production and cell growth were followed during fermentation, and logistic parameters were estimated from the growth profiles. The laboratory treatment with the best acid-production result was then tested in a 100-L fermenter to examine its performance at that scale.

2. MATERIALS AND METHODS

2.1 Alcoholic fermentation

Hillkoff Co., Ltd., Chiang Mai, Thailand, supplied the Arabica coffee pulp (*Coffea arabica* L.). The alcoholic fermentation used *Saccharomyces cerevisiae* Lalvin EC-1118 according to [23]. A mixture of 30 g dried pulp and 2.5 L water was boiled, cooled, and strained through filter cloth. White sugar brought the soluble solids to 20 °Brix. The medium was transferred to a fermentation tank after cooling to 50 °C, and yeast was added at 25 g/100 L with 0.5 g/L diammonium phosphate. An airlock allowed fermentation under anaerobic conditions (Fig. 1). Ethanol was checked every 12 h with a Per Vinum J. Salleron Dujardin ebulliometer (France). At 8% (v/v) ethanol, potassium metabisulfite was added to inhibit further fermentation and microbial activity that could affect flavor. The antimicrobial use of this compound in fermentation has been described in [24].

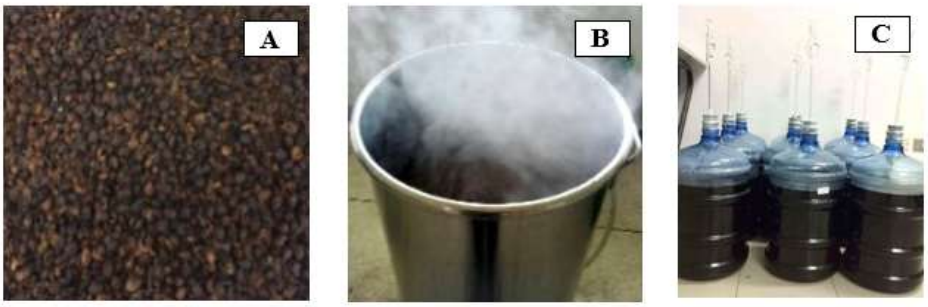


Figure 1. Preparation of the alcoholic substrate. (A) Dried coffee pulp, (B) boiling, and (C) fermentation tank fitted with an airlock.

2.2 Acetic acid fermentation

Batch acetic acid fermentation was carried out in a 10-L stirred-tank bioreactor (Bioneer-neo-10L, B.E. Marubishi, Thailand) at an 8-L working volume. Aeration rates of 0.5, 1.0, and 1.5 vvm were compared. These treatments bracketed 1.0 vvm, a reference rate selected from earlier work on oxygen supply and vinegar fermentation [9, 13, 20, 25]. Agitation was fixed at 300 rpm in all treatments. Dissolved oxygen was monitored continuously with the reactor's built-in probe, but no dissolved oxygen setpoint was imposed. Aeration was the operating variable changed in the laboratory comparison, with the other fermentation conditions held constant. The reactor is shown in (Figure 2.)

Acetobacter pasteurianus TISTR 102 was introduced aseptically at 10% (v/v) into the reactor containing the alcoholic substrate from Section 2.1 and sterile medium. Seed cultures were grown in 500-mL Erlenmeyer flasks with 10% (w/v) glucose and 1% (w/v) yeast extract, the medium selected in our earlier study of coffee pulp fermentation [23]. The flasks were incubated for 48

h at 30 °C and 150 rpm. Acetic acid fermentation was conducted at 30 °C. Samples were collected every 12 h for measurements of cell concentration, acetic acid, residual ethanol, and pH. The endpoint was a plateau in acetic acid concentration.



Figure 2. Stirred-tank bioreactor used for the laboratory fermentation experiments.

2.3 Analytical methods

2.3.1 Ethanol measurement

The ebulliometer was calibrated against the boiling point of distilled water. For each sample, the boiling point was recorded after the temperature had remained stable for approximately 20 s. Its difference from the water reference was converted to ethanol concentration in % (v/v) on the instrument scale. The chamber was cleaned and the cooling water changed between samples. The instrument was a Per Vinum J. Salleron Dujardin unit (France), shown in Figure. 3(A).

2.3.2 Acetic acid titration

For titration, 0.1 mL of sample was combined with 20 mL distilled water and three drops of phenolphthalein in an Erlenmeyer flask. The mixture was titrated with 0.1 N NaOH, and the consumed volume was used in Eq. 1 to estimate acetic acid concentration. Triplicate measurements were made for each sample. (Figure. 3B) shows the titration setup.

$$\%\ acetic\ acid = \frac{NaOH\ concentration\ (0.1N) \times Used\ NaOH\ volume\ (ml.) \times 0.064}{volume\ of\ vinegar\ (ml.)} \times 100 \quad (1)$$

2.3.3 pH measurement

Sample pH was measured with a FiveEasy F20 meter (Mettler Toledo, Switzerland). For each measurement, 20 mL of vinegar was placed in a container, and the reading was recorded after stabilization. Measurements were made in triplicate. The setup is shown in Figure. 3(C).

2.3.4 Estimation of viable cell concentration

Cell concentration was estimated from optical density at 600 nm, measured with a GENESYS 30 VIS spectrophotometer (Thermo Scientific, USA). An OD600 calibration against viable counts for *Acetobacter pasteurianus* TISTR 102 was used to express the estimates as log CFU/mL. Figure. 3(D) shows the measurement setup.

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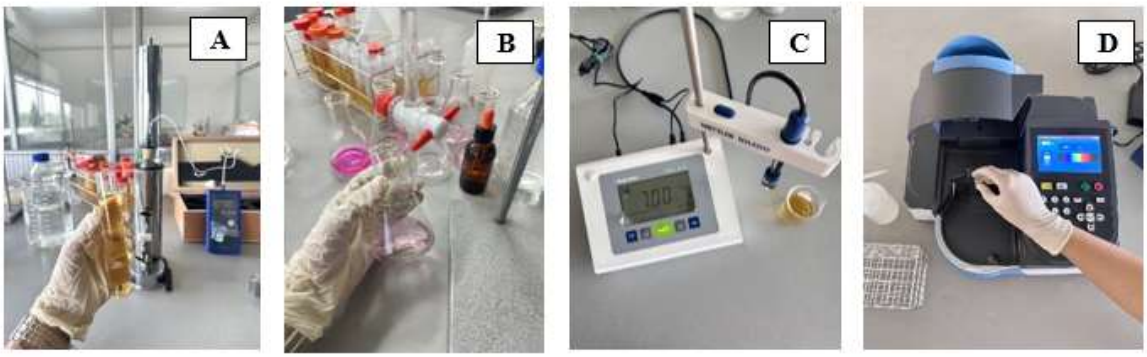


Figure 3. Analytical measurements. (A) Ethanol by ebulliometry, (B) acetic acid by titration, (C) pH, and (D) optical density for estimating cell concentration.

2.4 Kinetic study of microbial growth

The logistic model was used to describe the sigmoidal cell concentration profiles during batch fermentation [17]. It provides estimates of the growth rate parameter μ_m and maximum cell concentration X_m without using substrate concentration as an input. Kinetic models have also been applied to acetic acid and brewing fermentations [26, 28]. Here, the differential logistic equation was fitted to the growth of *Acetobacter pasteurianus* TISTR 102 using Eq. 2.

$$\frac{dX}{dt} = \mu_m \left(1 - \frac{X}{X_m}\right) X \tag{2}$$

In Eq. 2, X is the estimated viable cell concentration (log CFU/mL), X_m is its maximum value (log CFU/mL), and μ_m is the growth-rate parameter (h^{-1}). Time t is expressed in hours.

Integrating Eq. 2 with the initial condition $X = X_0$ at t = 0 gives the logistic expression in Eq. 3. This form was used to fit the cell concentration profiles without a substrate term.

$$X = \frac{X_m}{1+(\frac{X_m}{X_0}-1)e^{-\mu_m t}} \tag{3}$$

Model fit was assessed using R^2, χ^2, root mean square error (RMSE), and mean bias error (MBE), calculated with Eqs. 4–7.

$$R^2 = \left[\frac{\sum_{i=1}^{n} y_{experimental} \times y_{predicted}}{\sqrt{\left(\sum_{i=1}^{n} y^2{}_{experimental}\right)\left(\sum_{i=0}^{n} y^2{}_{predicted}\right)}}\right]^2 \tag{4}$$

$$\chi^2 = \frac{\sum_{i=1}^{n}\left(y_{experimental} - y_{predicted}\right)^2}{N - n_p} \tag{5}$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{n}\left(y_{experimental} - y_{predicted}\right)^2} \tag{6}$$

$$MBE = \frac{1}{N}\sum_{i=1}^{n}\left(y_{experimental} - y_{predicted}\right) \tag{7}$$

In Eqs. 4–7, N is the number of observations and n_p the number of model parameters. The statistics denoted R^2 and χ^2 are calculated with Eqs. 4 and 5, respectively. RMSE describes the root mean square error and MBE the mean bias error. The terms $y_{experinmental}$ and $y_{predicted}$ represent the observed and model predicted values.

2.5 Fermentation in a 100-L vessel

The best performing laboratory aeration rate was tested at Hillkoff Co., Ltd. in a custom-designed 100-L fermenter with an 80-L working volume (Figure 4).



Figure 4. The 100-L fermenter was used for the scale-up trial.

2.6 Statistical analysis

Experiments were performed in triplicate, and results are reported as mean ± standard deviation (n = 3). Analysis of variance was conducted in IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). Means were compared with Duncan's multiple range test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of aeration on acetic acid production

The highest acetic acid concentration was obtained at 1.0 vvm, reaching 5.12% after 144 h (Figure 5). At 0.5 vvm, fermentation took 192 h and produced 3.2% acetic acid. Increasing aeration to 1.5 vvm gave an intermediate fermentation time of 168 h, but the final acid concentration was lower, at 3.84%. Thus, increasing air supply from 0.5 to 1.0 vvm improved the outcome, whereas a further increase to 1.5 vvm did not.

Ethanol declined from the initial 8% during acidification and was no longer detected after 192, 144, and 168 h at 0.5, 1.0, and 1.5 vvm, respectively (Figure 6). This decline is consistent with ethanol oxidation by acetic acid bacteria [29]. Acid accumulation slowed as the ethanol supply was depleted. The initial ethanol level was also within the 5–8% range described as supporting Acetobacter growth [30].

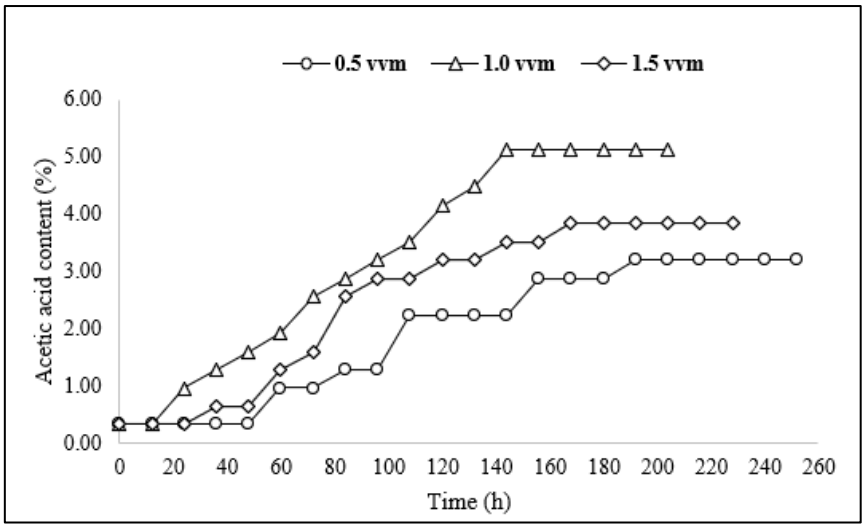


Figure 5. Acetic acid concentration during coffee pulp vinegar fermentation at the three aeration rates.

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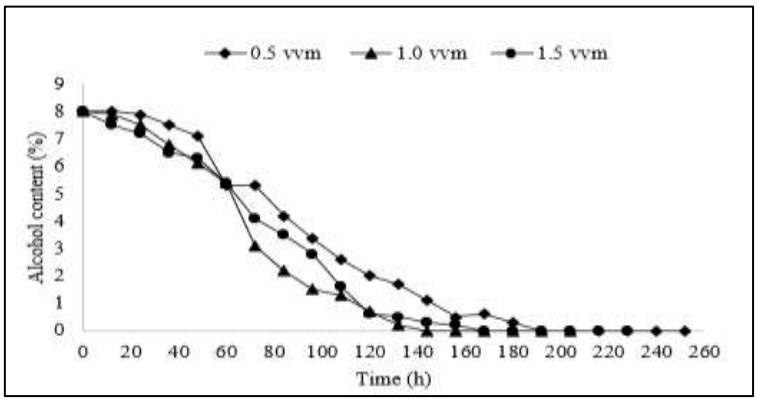


Figure 6. Ethanol concentration during coffee pulp vinegar fermentation at the three aeration rates.

Restricted oxygen supply could account for the low acetic acid concentration at 0.5 vvm [13], while acid over-oxidation is a possible explanation for the reduction at 1.5 vvm [14]. The measurements do not separate these mechanisms. The result at 1.0 vvm agrees with the rice bran vinegar study in [9]. Multistage fermentation with oxygen enrichment has also been described for continuous acetic acid production [25]. In contrast, onion vinegar fermented with *Acetobacter orientalis* MAK88 showed less acid at 1.0 vvm than at lower aeration. That experiment compared 0.2, 0.5, and 1.0 vvm, found a slight acid increase at 0.2 vvm, and reported pH values of 2.63–2.80 [31]. The differing outcomes place the laboratory result in the context of its particular strain and substrate.

3.2 Effect of aeration on cell growth

Estimated viable cell concentration peaked at 7.1467 log CFU/mL after 144 h with 1.0 vvm (Figure 7). At 0.5 vvm, the maximum was 5.3797 log CFU/mL at 192 h. The 1.5 vvm treatment reached 5.6946 log CFU/mL at 156 h. The intermediate air supply rate therefore supported the greatest cell concentration, and the highest rate gave an intermediate result. Figure 7 also shows the logistic fits used to compare these growth profiles.

Aerobic respiration provides a reason for expecting oxygen supply to affect *Acetobacter pasteurianus* growth [18, 19]. Increased gas flow could also impose hydrodynamic stress [15, 16], although the agitation rate stayed at 300 rpm and stress was not established by the reported measurements. Other fermentation systems show different responses to air supply. *Aspergillus niger* SS7 reached its highest xylanase activity and biomass at 1.00 vvm in a 3-L stirred tank [12]. For *Azospirillum brasilense*, reducing aeration from 1.0 to 0.1 vvm increased biomass tenfold [22]. Work on aerobic enzyme production likewise treats aeration as a process variable [21]. These comparisons show why the rate selected for coffee pulp needs to be evaluated within its own fermentation conditions.

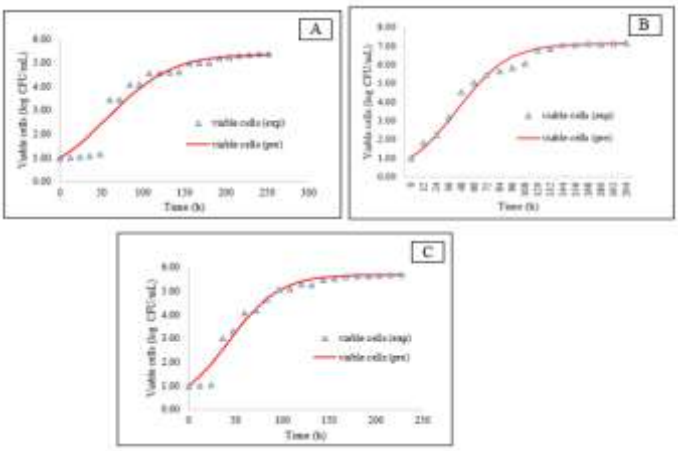


Figure 7. Measured cell concentration profiles and logistic fits for *Acetobacter pasteurianus* TISTR 102 in the 10-L bioreactor. (A) 0.5 vvm, (B) 1.0 vvm, and (C) 1.5 vvm.

The growth rate parameter μ_m was estimated by minimizing the difference between measured cell concentrations and the predictions from Eq. 3. The profiles in Fig. 7 show an increase after 24 h at 1.0 vvm, while the other treatments showed little apparent growth at that point. The largest fitted μ_m was 0.0414 h^{-1} at 1.0 vvm, compared with 0.0262 h^{-1} at 0.5 vvm and 0.0370 h^{-1} at 1.5 vvm (Table 1). These values are of a similar order to the 0.042–0.091 h^{-1} reported for *Acetobacter pasteurianus* CICIM B7003-02 in high-acidity vinegar fermentation [20]. Differences between studies may reflect the strain, substrate, aeration, temperature, and pH, as well as the basis used to estimate growth.

Table 1. Logistic parameters and fit statistics for coffee pulp vinegar fermentation at the three laboratory aeration rates.

Parameter	Aeration Rate (vvm)		
	0.5 vvm	**1.0 vvm**	**1.5 vvm**
X_0 (log CFU/mL)	0.9760	1.0035	0.9760
X_m (log CFU/mL)	5.3797	7.1467	5.6946
μ_m (h^{-1})	0.0262	0.0414	0.0370
R^2	0.9908	0.9975	0.9969
χ^2	0.6871	0.3587	0.2969
RMSE	0.8098	0.5821	0.5310
MBE	0.6558	0.3388	0.2820

Across the laboratory treatments, the reported R^2 values ranged from 0.9908 to 0.9975 (Table 1). The corresponding χ^2 values were 0.2969–0.6871, and RMSE was 0.5310–0.8098. MBE remained positive at all three rates, ranging from 0.2820 to 0.6558, so the fitted curves were not unbiased under this measure. Together, these statistics describe the fit to the available data. They do not establish predictive performance in a different fermentation system.

3.3 Performance in the 100-L fermenter

At 1.0 vvm in the 100-L fermenter, acetic acid reached 4.48% and the maximum estimated cell concentration was 6.8830 log CFU/mL (Figure 8). The fitted μ_m was 0.0257 h^{-1}. Fit statistics were R^2 = 0.9907, χ^2 = 0.7893, RMSE = 0.8536, and MBE = 0.7286. The larger vessel trial therefore showed a growth profile that could be described by the logistic model, but its fitted growth rate was lower than that of the 1.0 vvm laboratory treatment.

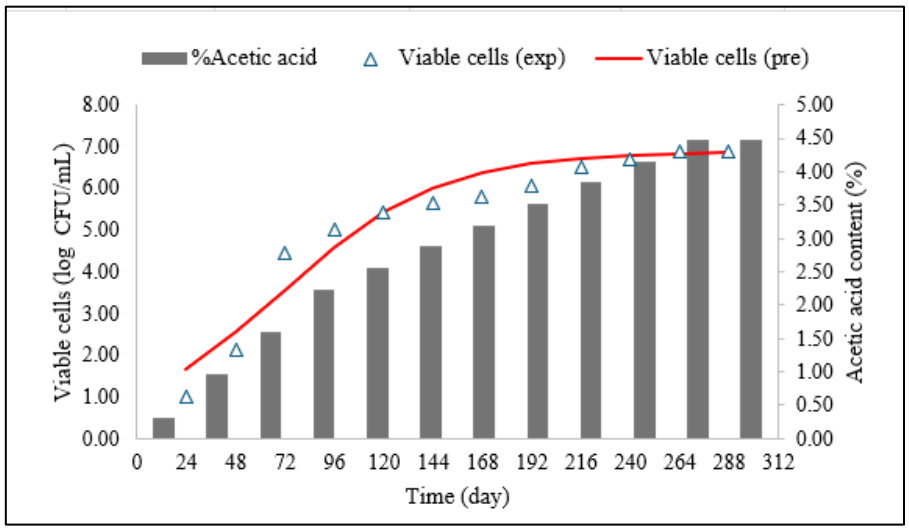


Figure 8. Measured cell concentration, logistic fit, and acetic acid concentration for *Acetobacter pasteurianus* TISTR 102 in the 100-L fermenter at 1.0 vvm.

The 100-L trial produced vinegar with a reported acetic acid concentration of 4.48% and no detectable residual ethanol. The Thai notification cited in [6] requires at least 4 g of acetic acid per 100 mL at 27 °C for fermented vinegar. Direct comparison with the reported percentage requires the same acidity reporting basis. Acidity and residual ethanol alone do not demonstrate compliance with all of the product's quality and safety requirements.

The 100-L fermentation produced less acid than the 10-L treatment, at 4.48% compared with 5.12%, and its fitted μ_m was lower, at 0.0257 compared with 0.0414 h^{-1}. Both used 1.0 vvm. Mixing and oxygen transfer may have changed with vessel size, and factory temperature was less closely controlled than laboratory temperature. Because the volumetric oxygen transfer coefficient, k_La, was not measured, the scale comparison cannot quantify the oxygen transfer contribution. These differences provide specific reasons to evaluate temperature, mixing, and oxygen transfer together when selecting conditions for further scale-up. Multi-criteria decision and techno-economic frameworks developed for other biomass conversion routes offer a structured way to weigh such operating, siting, and deployment factors before committing to a larger vessel [34, 37].

4. CONCLUSION

For the laboratory system, 1.0 vvm gave the best result among the three air supply rates tested. At this rate, acid reached 5.12% in 144 h and estimated cell concentration reached 7.1467 log CFU/mL. Logistic fits supplied growth parameters for comparing the treatments. Fermentation at the same air supply rate in a 100-L vessel produced 4.48% acid, with lower growth and acid production than at 10 L. Coffee pulp supported vinegar production in both systems, but the scale comparison shows that a common vvm value does not by itself reproduce the laboratory outcome. Further development needs to relate air supply to temperature control and the mixing and oxygen transfer conditions of the production vessel.

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AUTHOR CONTRIBUTIONS

Naruemon Boonmee: Investigation, Data Curation, Formal Analysis, Visualization, Writing – Original Draft.

Somkiat Jaturonglumlert: Methodology, Validation, Writing – Review & Editing.

Nukrob Narkprasom: Methodology, Validation, Writing – Review & Editing.

Kanokwan Tandee: Validation, Writing – Review & Editing.

Kanjana Narkprasom: Conceptualization, Methodology, Supervision, Writing – Review & Editing.

All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study because it did not involve human participants or animals.

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