An Air-lift Acetifier with Mash Recycling System for Corn Vinegar Production by Adsorbed Cells of *Acetobacter aceti* WK on Surface of Loofa Sponge

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Abstract—A novel air-lift acetifier with mash recycling system, called as ALMR, was designed for corn vinegar production. The filtered corn wine was supplied to ALMR using absorption of *Acetobacter aceti* WK cells on surface of recommended 1 inch thickness of sliced loofa sponge. The fresh air was supplied into the ALMR with 200 L/h of mash recycling (MR) rate. Among the 40%, 50% and 60% of charging rate of fresh filtered corn wine for semi-continuous fermentation at 30°C, the appropriate rate was 50%. Resulting in 6.8-7.2% acidity was produced within 4-5 d. The highest acetification rate (ETA) by calculation was 0.0183%/h to 0.0260%/h. The interaction between the mash recycling system in ALMR and the absorbed cells of *A. aceti* WK on loofa sponge provided the highest acetification rate when compared with our previous AL without mash recycling system and STR. Scanning electron microscope (SEM) on surface of loofa sponge strongly confirmed its appropriate structure for adsorption of *A. aceti* WK cells.

Keywords—air-lift acetifier with mash recycling system (ALMR), *Acetobacter aceti* WK, loofa sponge, adsorption, corn vinegar, semi-continuous fermentation.

I. INTRODUCTION

Design and development of acetifier for high efficiency vinegar production process have been focused during the past decades. Many types of acetifier consisting of continuous stirred tank reactor [1,2], shallow flow bioreactor [3], rotating disc reactor [4], bubble column reactor equipped with dynamic sparger [5], closed pilot plant scale acetifier with air diffuser [6], air-lift fermenter [7], and aerated-stirred fermenter [8] were applied.

According to many advantages of biological material in term of cost, safety, waste treatment resulting from its biodegradable property, it is desirable to use as eco-friendly immobilization carriers of microorganisms by passive adhesion to its surface [9]. Many studies have shown that loofa (*Luffa cylindrica*) sponge is an excellent immobilization carrier for microorganisms due to its high degree of porosity, high specific pore volume, stable physical properties [10,11]. A “Quick Process” is an alternative approach for vineger production by fixing of *Acetobacter aceti* with supporting material was developed. The differences of supporting material were recommended such as lipophilic fibrous, siran, wood chips and polyurethane foam [12]. From our previous innovative studies, loofa sponge has been proved as environmental friendly supporting material of *A. aceti* WK cells for “green vinegar production process” in air-lift fermenter [7] and stirred tank reactor [2]. Additionally, loofa sponge has been fully proved for food safety concern because it is an edible vegetable.

II. MATERIALS AND METHODS

A. Loofa sponge preparation

As mentioned in our previous reports of Krusong et al. [2,7], the dried loofa sponge was sliced into 1 inch thickness, then, washed by running tap water and dipped in vinegar containing 4% acetic acid for sterilization for 24 h (W. Krusong, Unpublished data, 2007).

B. Yeast strain and corn wine fermentation

*Saccharomyces cerevisiae* M30, a flocculate yeast, was used for corn wine fermentation. The medium used for corn wine fermentation consisting of (per 16 liter of water) corn 0.8 kg, sucrose 3.2 kg, (NH₄)₂SO₄ 8 g, MgSO₄·7H₂O 3.2 g, pH 5.5. The fermentation was conducted by using cell recycle process with 50 mL/min as recommended by our previous experiment [2, 7]. The fermentation was controlled at 32°C for 5 d.

C. Bacterial strain and corn vinegar fermentation in air-lift acetifier with mash recycling system (ALMR) at different rate of mash recycle (MR)

A screened acetic acid producer strain, *A. aceti* WK, was well adapted for vinegar production using corn wine as a substrate. The complex medium used for corn vinegar production consisted of (per liter of water) glucose 5.0 g, yeast extract 2.5 g, peptone 1.0 g. Then, the total concentration consisting of acidity of vinegar and alcohol was controlled. The 8% total concentration for starting up of the process consisted 4.5% (v/v) acidity of vinegar and 3.5% (w/v) alcohol were prepared and added into the medium in 10L ALMR. The schematic diagram of experimental apparatus was shown in Fig. 1. Based on limited efficiency of MR controller, three rate of MR consisted of 150, 175 and 200 L/h were investigated.
for vinegar production which indicated by the reduction of alcohol content to 0.5-0.8% (w/v) in the fermenting mash. The MR rate which provided the highest alcohol reduction was suitable for the subsequent experiments.

D. Semi-continuous corn vinegar fermentation in air-lift acetifier with mash recycling system (ALMR)

The corn vinegar fermentation was conducted in 10L ALMR with appropriate MR rate and type of air supply. The cycles of semi-continuous fermentation process as adapted from Krusong et al. [2,7] were studied. Each cycle was started when the alcohol content of the previous fermenting mash was diminished to 0.5-0.8% (w/v), then the fresh corn wine with 8% total concentration consisted of 1.0% (v/v) acidity of vinegar and 7% (w/v) alcohol from fresh corn wine tank was pumped into 10L ALMR. The suitable charging rate consisting of 40%, 50% and 60% of fermenting volume were investigated to maximize acidification rate (ETA).

E. Analytical procedure

The invert sugar by Lane and Eynon method and acidity expressed in term of acetic acid by titration were analyzed according to AOAC [13]. Alcohol content was analyzed by Ebulliometer compared with alcohol hydrometer while pH was examined by pH meter, model JENWAY 3510, UK. The free cell dry weight of A. aceti WK was determined from the absorbance at 660 nm with a GENESYS 10VIS spectrophotometer and converted to dry weight on the basis of a corresponding standard curve. For absorbed A. aceti WK cells, a known mass of sliced loofa sponge was dissolved in 0.05M sodium citrate. After removal of the sponge, the A. aceti WK cell concentration was determined similarly for the free cells. The microstructure of A. aceti WK on surface of loofa sponge from fermenting mash was examined by a scanning electron microscope (SEM). Sample was vacuum dried and then sputtered with gold and photographed. Image was taken on a JEOL JSM-5410LV (JEOL, Tokyo) scanning electron microscope. The acidification rate (ETA) is the increase of acetic acid concentration (AAc) during fermentation and calculated in term of %/h.

F. Statistical analysis

All experiments were carried out in triplicate and designed by using complete randomized design (CRD). Data were subjected to analysis of variance and Duncan’s new multiple range tests (DMRT), using the SPSS 10.0 for Windows pocket program, to determine if there was a significant difference (P ≤ 0.05) in the mean ETA.

III. RESULTS AND DISCUSSION

Based on our experience as reported in Krusong et al. [2,7], the corn wine containing 10.1% was produced by the flocculating yeast, S. cerevisiae M30, for 5 d at 30-32°C by using recycle cell fermenter. Filtered corn wine was used for subsequent study of vinegar fermentation in air-lift acetifier with mash recycling system (ALMR).

The adsorption of A. aceti on surface of fibrous material is an alternative vinegar production process, called “Quick Process”. Dried loofa sponge which is already proved that it could adsorb cells of A. aceti WK on its surface as mentioned by Krusong et al. [2,7]. This study aimed to prove the utilization of loofa sponge for vinegar production in a new design ALMR as experimentally demonstrated in Fig. 1. The dried loofa sponge was submerged in the fermenting broth. As mentioned in our previous study in Krusong et al. [2], the dried loofa sponges were recommended to put into ALMR for 60% of fermenting volume.

In design of ALMR, the mash recycle (MR) rate is a key factor to provide the suitable condition for oxidation of alcohol to produce acetic acid by A. aceti WK. Normally, the adaptation of A. aceti was indicated by an observation of alcohol content reduction to 0.5-0.8%(w/v) in medium. As shown in Fig. 2, it could be noticed that the adaptation of A. aceti WK was faster when cultivated in ALMR. The higher MR rate was used, the rapid decrease of alcohol occurred. However, based on limited efficiency of MR controller, the highest MR rate was 200 L/h. Therefore, the 200 L/h of MR rate was used in subsequent study.

Normally, the adsorption to surfaces of supporting material has been one of the most widely studied methods for the immobilization of acetic acid bacteria (AAB) [12]. This technique represents a particular form of cellular adhesion based on the ability of certain AAB to fix themselves to solid surfaces by means of the secretion of polysaccharides [14]. A rough surface and high degree of porosity of loofa sponge has been noticed as
The investigation of semi-continuous corn vinegar fermentation was conducted by *A. aceti* WK absorbed on surface of loofa sponge in ALMR with 200 L/h MR rate at 30°C. It could notice that *A. aceti* WK required 14 days for its first complete adaptation after inoculating in the new fermenting mash containing 8% total concentration, as shown in Fig. 4 and Table 1. The slight acid was produced while gradually reduction of alcohol in fermenting mash was investigated. Theoretically, *A. aceti* WK was successfully adapted when the alcohol content in fermenting mash was reduced to 0.5-0.8% (w/v). Then, the fresh corn wine composting 8% total concentration from fresh corn wine tank was charged at 0.3125 L/h as mentioned by Krusong et al. [2] after discharging of the fermenting mash containing acetic acid as finished product with the same rate as charging rate. The subsequent 2nd cycle, it spent shorter fermentation time. This phenomena was due to the complete adaptation of *A. aceti* WK. Then, the effect of charging rate on acidification rate (ETA) was studied. The ETA indicated by acid concentration increasing per hour by calculation. Three levels of charging rate consisting of 40%, 50% and 60% were experimented in the cycle of 3rd-5th (during day 21-38 of fermentation), 6th-8th (during day 38-52 of fermentation) and 9th-11th (during 52-79 day of fermentation), respectively. Results were shown in Fig. 4 and Table 1. The range of 0.0137%/h to 0.0217%/h of ETA was obtained from 40% charging rate while higher ETA in range of 0.0183%/h to 0.0260%/h was found in 50% charging rate. Additionally, the ETA was inversely reduced to the range of 0.0069%/h to 0.0071%/h in 60% charging rate. It is, therefore, concluded that the appropriate charging rate for semi-continuous corn vinegar fermentation by *A. aceti* WK absorbed on surface of loofa sponge in ALMR with 200 L/h MR rate was 50%.

![Figure 2](image_url)

**Figure 2.** Corn vinegar fermentation by *A. aceti* WK cells absorbed on surface of loofa sponge in 10L AFMR with different mash recycle rate at 30°C. No mash recycle as control, ♦; 150 L/h, ■; 175 L/h, ▲; 200 L/h, x. Shown in SEM (x350), Fig. 3a. It is a good evidence to indicate that loofa sponge is a good bio-resource for adsorption of AAB, *A. aceti* WK. The characteristic of adsorption of *A. aceti* WK on surfaces of loofa sponge can be seen in Fig. 3b. Additionally, the porous structure of loofa sponge helps to reduce the problems associated with oxygen diffusion, a property that becomes the most limiting factor.

Nowadays, semi-continuous fermentation is the most common operation in the vinegar industry [6]. The successive discontinuous cycles of acetification is developed. Each cycle with complete conversion of ethanol into acetic acid occurs. At the end of each cycle, a given volume of reactor is discharged as finished product and charged with new filtered fresh wine. Then, a new fermentation cycle begins. The percentage of charge/discharge is variable in every single process. As mentioned in our previous report that 40% charging rate of fresh wine was recommended for corn vinegar in air-lift fermenter [7] and 30% for STR [2]. Consequently, the the charging rate of fresh corn wine at 40%-60% was investigated to find out the optimum rate when produced in ALMR with 200 L/h MR rate instead of previous processes.

![Figure 3](image_url)

**Figure 3.** Scanning electron microscope of loofa sponge and adsorbed *A. aceti* WK cells on its surfaces: (a) surface of loofa sponge at 350x; (b) absorbed cells of *A. aceti* WK on surface of loofa sponge during corn vinegar production in ALMR.

The investigation of semi-continuous corn vinegar fermentation was conducted by *A. aceti* WK absorbed on surface of loofa sponge in ALMR with 200 L/h MR rate at 30°C. It could notice that *A. aceti* WK required 14 days for its first complete adaptation after inoculating in the new fermenting mash containing 8% total concentration, as shown in Fig. 4 and Table 1. The slight acid was produced while gradually reduction of alcohol in fermenting mash was investigated. Theoretically, *A. aceti* WK was successfully adapted when the alcohol content in fermenting mash was reduced to 0.5-0.8% (w/v). Then, the fresh corn wine composting 8% total concentration from fresh corn wine tank was charged at 0.3125 L/h as mentioned by Krusong et al. [2] after discharging of the fermenting mash containing acetic acid as finished product with the same rate as charging rate. The subsequent 2nd cycle, it spent shorter fermentation time. This phenomena was due to the complete adaptation of *A. aceti* WK. Then, the effect of charging rate on acidification rate (ETA) was studied. The ETA indicated by acid concentration increasing per hour by calculation. Three levels of charging rate consisting of 40%, 50% and 60% were experimented in the cycle of 3rd-5th (during day 21-38 of fermentation), 6th-8th (during day 38-52 of fermentation) and 9th-11th (during 52-79 day of fermentation), respectively. Results were shown in Fig. 4 and Table 1. The range of 0.0137%/h to 0.0217%/h of ETA was obtained from 40% charging rate while higher ETA in range of 0.0183%/h to 0.0260%/h was found in 50% charging rate. Additionally, the ETA was inversely reduced to the range of 0.0069%/h to 0.0071%/h in 60% charging rate. It is, therefore, concluded that the appropriate charging rate for semi-continuous corn vinegar fermentation by *A. aceti* WK absorbed on surface of loofa sponge in ALMR with 200 L/h MR rate was 50%.

![Figure 4](image_url)

**Figure 4.** Effect of charging rate on semi-continuous fermentation of corn vinegar by *A. aceti* WK cells absorbed on surface of loofa sponge in ALMR with 200 L/h MR rate at 30°C. Fermentation periods during different charging rate were day 21-38 for 40%, day 38-52 for 50% and day 52-79 for 60%. Alcohol, ■; acidity, ♦.

**Table 1. Acidification rate (ETA) by absorbed *A. aceti* WK cells on surface of loofa sponge in AFMR with 200 L/h MR rate during semi-continuous fermentation with three level of charging rate at 30°C.**
During 11 cycles of semi-continuous corn vinegar fermentation by absorbed *A. aceti* WK cells, the free and absorbed cells of *A. aceti* WK were investigated. Result in Fig. 5 showed that small amount of free cells was found during 11 cycles. On the other hand, the plenty amount of absorbed cells on loofa sponge was obtained. Moreover, it could be noticed that absorbed cells was gradually increased during adaptation period (or cycle 1). Then, an exponential growth of absorbed cells was found. The highest amount of absorbed cells was observed during the 6th-8th cycle (day 38-52 of fermentation) within the 50% charging rate. This result could support the highest ETA obtained in 50% charging rate as in Fig. 4. It was seen that higher number of cells on the surface of loofa sponge varied directly to charging rate. More cells including free and absorbed cells provided more ETA. This phenomena could also provide further information from our previous studies that if more cells of *A. aceti* WK were remained in the reactor, more cycles of semi-continuous fermentation process for corn vinegar production were conducted at the appropriate charging rate.

According to our three designs of acetifiers consisting of air-lift fermenter, STR and ALMR for semi-continuous corn vinegar fermentation by absorbed *A. aceti* WK cells, their ETA were compared as shown in Table 2. The ETA in ALMR with 200 L/h MR rate was significantly higher than that found in air-lift fermenter (without MR system) and STR which reported in our previous reports [2,7]. It was due to the efficiency of mash recycling system and the mash sprinkling plate in providing more DO content in fermenting mash. The DO is the key parameter which is necessary for the oxidation reaction of *A. aceti* WK to convert alcohol to acetic acid.

### IV. CONCLUSION

It has been widely reported in the literature that immobilization imparts a special stability to the bacteria against the negative effects of the temperature, pH, ethanol and acetic acid concentrations [4]. Regarding to loofa sponge is a natural agricultural fibrous material, it can be naturally decomposed while the plastic fibrous material could not. Additionally, loofa sponge has been fully proved for food safety concern because it is an edible vegetable. Resulting confirmed the strong recommendation in using loofa sponge as adsorption material of *A. aceti* WK cells on its surface.

**Table 2. Acidification rate (ETA) of absorbed *A. aceti* WK cells on surface of loofa sponge in AFMR, airlift fermenter (AF) and STR at 30°C.**

<table>
<thead>
<tr>
<th>Acetifier</th>
<th>Appropriate DCR/CR</th>
<th>ETA (%)</th>
<th>Average ETA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALMR</td>
<td>50</td>
<td>0.0183-0.0260</td>
<td>0.0021</td>
</tr>
<tr>
<td>AF**</td>
<td>40</td>
<td>0.0031-0.0137</td>
<td>0.0084</td>
</tr>
<tr>
<td>STR**</td>
<td>30</td>
<td>0.0028-0.0067</td>
<td>0.0047</td>
</tr>
</tbody>
</table>

DCR, discharging rate; CR, charging rate; IC, initial acidity; FA, final acidity; AP, Acidification period.

1 First fermentation cycle is adaptation period or lag phase needed in the growth cycle of *A. aceti* WK cells on surface of loofa sponge to oxidize the initial alcohol in the fermenting mash to 0.5-0.8% (w/v).
2 Discharging rate is rate of removal of fermenting mash as finished product from ALMR during semi-continuous corn vinegar fermentation. It is the same with charging rate.
3 Acidification rate (ETA) is the increase in acetic acid concentration.

The design of acetifier is one of the important factor for promoting the high ETA by *A. aceti* WK cells absorbed on surface of loofa sponge. The ALMR with 200 L/h MR rate showed the significant increase of ETA when compared with our previous two designed acetifier consisting of air-lift fermenter (without mash recycling system) and STR. Moreover, the ALMR required fresh air supply for maintaining the dissolved oxygen in fermenting mash during

### Figures

**Figure 5.** Free and absorbed cells of *A. aceti* DK on surface of loofa sponge during semi-continuous fermentation of corn vinegar with different charging rate. The fermentation was in 10L ALMR with 200 L/h MR rate at 30°C. Fermentation periods during different charging rate were day 21-38 for 40%, day 38-52 for 50% and day 52-79 for 60%. Free cells, ●; absorbed cells, ●.
adaptation period of *A. aceti* WK. The faster adaptation period, the faster cycle of semi-continuous corn vinegar fermentation occurred.

By using the semi-continuous process, the 50% charging rate of filtered fresh corn wine was an optimum for corn vinegar production in 10L ALMR with 200 L/h MR rate. Moreover, the scale up level using loofa sponge including other design of acetifiers have been further studied.

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