Improvement of Continuous Ethanol Fermentation from Sweet Sorghum Juice by *Saccharomyces cerevisiae* using Stirred Tank Bioreactor Coupling with Plug Flow Bioreactor

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**ABSTRACT**

The continuous ethanol production from sweet sorghum juice in a continuous stirred tank bioreactor (STR) and a combined bioreactor was compared. The combined bioreactor comprised an STR and three tubular (plug flow) bioreactors (TB) in series with a total working volume of 3320 ml. The fermentation was carried out at 30°C by *Saccharomyces cerevisiae* NP 01. The sweet sorghum juice containing 250 g l⁻¹ of total sugar was introduced into the systems at total dilution rates (D) of 0.007, 0.02 and 0.04 h⁻¹. Our results clearly show that the degree of ethanol production depends on the residence time of the fermentation broth in the system. In the STR system at D = 0.007 h⁻¹, the ethanol concentration (P), yield (YP/S) and productivity (QP) were 67.28 ± 0.37 g l⁻¹, 0.52 ± 0.01 g g⁻¹ and 0.47 ± 0.00 g l⁻¹ h⁻¹, respectively. Using the combined bioreactor (CSTR + TB) could improve ethanol production efficiency. The sugar consumption and ethanol production of the combined bioreactor system were markedly higher than those of the STR system. The highest P value (106.01 ± 0.47 g l⁻¹) was obtained when using the combined system at D 0.007 h⁻¹; and under this condition, the YP/S and QP values were 0.50 ± 0.01 g g⁻¹ and 0.76 ± 0.02 g l⁻¹ h⁻¹, respectively.

**Keywords:** continuous ethanol fermentation, plug flow bioreactor, stirred tank bioreactor (STR), *Saccharomyces cerevisiae*, sweet sorghum

1. **INTRODUCTION**

The use of biofuel to replace oil is one of the most viable ways to ensure a sustainable energy future. Ethanol production as an alternative fuel energy resource has been a subject of great interest since the oil crisis of the 1970s [1]. Therefore, the development of a fermentation process using economical raw materials is important for production of the biofuel ethanol on a commercial scale [1]. In Thailand, the main raw materials used for ethanol production are sugarcane molasses and cassava. Regarding the energy policy of
the Thai government, ethanol production will be increased to 9,000,000 litres/day in the year 2022 [2]. Therefore, it is possible that Thailand may face a shortage of sugarcane molasses and cassava.

Sweet sorghum [Sorghum bicolor (L.) Moench] is a high biomass- and sugar-yielding crop. It has been of particular interest as a substrate for ethanol production because the juice from its stalks contains highly fermentable sugars, primarily sucrose, fructose and glucose, as well as many essential trace elements for microbial growth and ethanol production [3-7]. The pH of the juice is also in the optimum pH range (pH 4.0 to 5.5) for yeast growth and ethanol production [8]. Moreover, after juice extraction, sweet sorghum bagasse can be hydrolysed into sugars for ethanol production [9]. The sweet sorghum can be cultivated at nearly all temperatures and tropical climate areas, and it has a growing period of 120-150 days [7,10]. In Thailand, the average yield of sweet sorghum cultivar KKU40, 90-100 days old, is 30-50 ton/ha corresponding to about 15-25 dry ton/ha [11].

Apart from the development of new sweet sorghum cultivars with high grain and sugar yields [12], fermentation process development is also important for efficient ethanol production. A very high gravity (VHG) technology is one of the methods that can enhance ethanol production efficiency. It is defined as the preparation and fermentation to completion of media containing sugar in excess of 250 g l\(^{-1}\) [13]. This technology has several advantages for industrial applications because it increases both the ethanol concentration and the rate of fermentation. In addition, it reduces capital costs, energy costs per litre of alcohol and the risk of bacterial contamination [13]. However, substrate inhibition may occur under initially high sugar levels. To increase the efficiency of ethanol production, many process improvements have been studied including the continuous fermentation system.

Continuous culture fermentation provides advantages over batch fermentation, including optimized process conditions for maximum productivity, long-term continuous productivity, reduced labour costs once it has reached steady state, reduced vessel downtime for cleaning, filling and sanitizing, and easy process control and operation [14,15]. Typical bioreactors called stirred tank bioreactors (STRs) have been used for ethanol production in both laboratory research and industry applications for a long period of time. These bioreactors are characterized by their well-mixed performance, but bioreactor engineering theories predict that strong product inhibitions can occur because of high product concentrations inside the bioreactors [16,17]. Therefore, they are not good choices for fermentation under VHG conditions, although multi-stage STRs in series (homogenous continuous culture) can lower product inhibition to some extent. It was reported that tubular bioreactors (heterogeneous continuous culture) could be used in the case of product inhibition as product concentration increases gradually along their axial directions [18].

Ethanol production from sweet sorghum juice using STRs combined with plug flow bioreactors or tubular bioreactors has not been reported. Therefore, the aim of this research was to investigate methods to increase ethanol production from sweet sorghum juice using continuous (chemostat) fermentation by Saccharomyces cerevisiae NP 01. The efficiencies of continuous ethanol production from the sweet sorghum juice by a STR and a combined bioreactor (STR and plug flow bioreactor) were compared under VHG conditions to account for differences in sugar consumption,
ethanol production and cell viability. To control the bioprocess, the effects of dilution rate (\(D\)) on ethanol production in both single and multi-stage continuous fermentation processes were also investigated.

2. MATERIALS AND METHODS

2.1 Microorganism and Inoculum Preparation

*S. cerevisiae* NP 01 isolated from Loog-pang (Chinese yeast cake) from Nakhon Phanom province, Thailand [19], was inoculated into 150 ml of yeast extract - malt extract (YM) medium and was incubated on a rotating shaker at 150 rpm, 30°C for 15 h. The YM medium contained (g l\(^{-1}\)) yeast extract 3, peptone 5, malt extract 3 and glucose 10. The yeast cells were then transferred into 350 ml of the YM medium to give an initial cell concentration of \(5 \times 10^6\) cells ml\(^{-1}\) and were further incubated under the conditions as previously mentioned. After 15 h, the cells were harvested and used as an inoculum for ethanol production.

2.2 Raw Material

Sweet sorghum juice extracted from its stalks (cv. KKU 40) was obtained from Division of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. To avoid storage problem and to prevent bacterial contamination, the juice containing total soluble solids of 18°Bx was concentrated to 75°Bx and stored at 4°C before use.

2.3 Ethanol Production Medium

The concentrated juice was diluted with distilled water to obtain a total sugar concentration of approximately 250 g l\(^{-1}\) and used as an ethanol production (EP) medium. The medium was transferred into a 2-l STR with a final working volume of 1 l and autoclaved at 110°C for 40 min, or it was transferred into a 5-l STR with a final working volume of 3320 ml and autoclaved at 110°C for 60 min. A 5-l reservoir was filled with the EP medium before sterilization. The sterile EP medium was kept at room temperature for use in continuous fermentation.

2.4 Continuous Fermentation System

Continuous fermentation was carried out into two systems, the STR system and the combined bioreactor system.

2.4.1 Stirred tank bioreactor (STR) system

All continuous fermentations throughout this work were conducted in a 5-l STR (Biostat® B, B. Braun Biotech, Germany) with a final working volume of 3320 ml. The yeast cells were inoculated into the STR to give an initial yeast cell concentration of \(1 \times 10^8\) cells ml\(^{-1}\). The STR was agitated at 100 rpm at 30°C. The sterile EP medium in the feed reservoir was fed into the top of the STR via a peristaltic pump (Biostat® B, B. Braun Biotech, Germany) when the total sugar of the fermented broth in the STR remained approximately one-third of the initial value. The dilution rates or \(D\) of the fresh EP medium into the STR were 0.007, 0.02 and 0.04 h\(^{-1}\). Samples were withdrawn at time intervals for analysis. Steady-state conditions were indicated by stable viable yeast cells, total sugar and ethanol levels in the effluent from the STR. The continuous cultures were operated for at least two times of the residence time after the system reached steady state.

2.4.2 Combined bioreactor system

The combined bioreactor system comprised a 2-l STR (Biostat® B, B. Braun Biotech, Germany) and a three-stage tubular (plug flow) bioreactor in series with a total working volume of 3320 ml. The working volume of the STR and three plug flow bioreactors were 1000, 770, 770 and 780 ml,
respectively. Figures 1A and 1B illustrate a schematic diagram of the combined bioreactor system. The yeast cells were inoculated into each bioreactor to give initial yeast cell concentrations of $1 \times 10^8$ cells ml$^{-1}$. The STR was agitated at 100 rpm and the temperature for the whole system was controlled at 30°C. The STR was connected to the 5-l medium reservoir, the three-stage plug flow bioreactor in series and a waste reservoir. The sterile EP medium in the reservoir was fed into the top of the STR at total $D$ 0.007, 0.02 and 0.04 h$^{-1}$; and the medium from the STR was fed into the bottom of the tubular bioreactor 1, 2 and 3, respectively (Figure 1B). The exhaust gas from the three-stage plug flow bioreactor was washed by bubbling it into a de-ionised water storage tank to recover the entrapped ethanol. Sterile air was supplied to the bottom of the three plug flow bioreactors at the flow rate of 0.005 vvm to prevent cell settling [18]. The continuous fermentation at each dilution rate was operated for at least two retention times at steady state.

2.5 Analytical Methods
After the system inoculation and equilibrium of continuous fermentation, samples were taken daily. The viable yeast cell numbers were determined by the direct counting method with methylene blue staining using a haemacytometer [20]. The fermentation broth was centrifuged at 13,000 rpm for 10 min. The supernatant was detected for total residual sugar by phenol sulfuric acid method [21]. Ethanol concentration ($P$, g l$^{-1}$) was analysed by gas chromatography (Shimadzu GC-14B, Japan, Solid phase: polyethylene glycol (PEG-20M), carrier gas: nitrogen, 150°C isothermal packed column, an injection temperature of 180°C, flame ionization detector temperature 250°C; C-R7 Ae plus Chromatopac Data Processor), and 2-propanol was used as an internal standard [5]. Sugar consumption ($SC$), ethanol yield ($Y_p/s$) and ethanol productivity ($Q_p$) were calculated by the following equations:

![Figure 1](image-url)
\[ SC(\%) = \frac{(ITS - RTS) \times 100}{ITS} \]
\[ Y_p/s(g\ g^{-1}) = \frac{P}{ITS - RTS} \]
\[ Q_p(g\ l^{-1}\ h^{-1}) = P \times D \]

where \( ITS \) is the initial total sugar (g l\(^{-1}\)), \( RTS \) is the residual total sugar (g l\(^{-1}\)), \( P \) is the ethanol concentration produced (g l\(^{-1}\)) and \( D \) is the dilution rate (h\(^{-1}\)) of a continuous bioreactor system based on the total working volume of the bioreactor system.

3. RESULTS AND DISCUSSION
3.1 Continuous Ethanol Production in Stirred Tank Bioreactor

The pH, viable yeast cell, total sugar and ethanol concentrations during the continuous fermentation process at the different dilution rates are illustrated in Figure 2. The amount of sugar consumption and ethanol production decreased with increasing the dilution rate. This was due to the fact that the residence time of the medium in the bioreactor decreased. The pH in the broth decreased slightly within 30 h and was constant at approximately 4.5 throughout the experiment. Similar results was observed by Narendranath and Power [8] who reported that during ethanol fermentation the pH of the medium and the ethanol concentrations were in the range of 4.0 to 4.5 and 36 to 67 g l\(^{-1}\), respectively, which was a possible way to control the bacterial contamination during the fermentation.

Figure 2 Fermentation parameters during continuous ethanol production from the sweet sorghum juice in the 5-l STR at the dilution rates of 0.007, 0.02 and 0.04 h\(^{-1}\). pH (\(\times\)), viable yeast cells (O), total sugar (\(\square\)), and ethanol (\(\bullet\)). The arrows indicate the start time of continuous mode at the different dilution rates.
The yeast cell concentrations in the broth were relatively high, ranging from log 7.38 to log 8.43 cells ml\(^{-1}\) at steady state (Figure 2). However, the yeast could not completely consume the sugar in the sweet sorghum juice at all \(D\) values (Figure 2). The total sugar utilization was approximately 49, 32 and 27% of the initial values at \(D = 0.007, 0.02\) and 0.04 h\(^{-1}\), respectively. The fermentation parameters of ethanol production in the STR are shown in Table 1. The ethanol concentration (\(P\)) decreased as the \(D\) value increased as previously mentioned, but the ethanol productivity (\(Q_p\)) increased as the \(D\) value increased. However, the productivity is in direct proportion to the dilution rate and product concentration. Productivity value will increase to a certain limit with increasing dilution rate since product concentration decreases with increasing dilution rate [22]. In this study, the ethanol yields (\(Y_{P/S}\)) at all dilution rates tested were similar with the values of 0.51 to 0.52 g g\(^{-1}\) corresponding to yield efficiencies of approximately 94 to 96% of the theoretical yield (0.54) based on sucrose (the main sugar in the sweet sorghum juice). High \(Y_{P/S}\) values indicated that most sugar in the juice was converted to ethanol. At the end of the experiments, the juice at \(D = 0.007\) h\(^{-1}\) was re-introduced to test the stability of this process. The fermentation parameters of the second feeding were not significantly different compared to those at the first feeding at the same \(D\) value (0.007 h\(^{-1}\)), indicating that the system operation could be reversed and the results obtained were reliable.

Table 1. Fermentation parameters of continuous ethanol production from sweet sorghum juice in the stirred tank bioreactor (STR) at different dilution rates.

<table>
<thead>
<tr>
<th>(D) (h(^{-1}))</th>
<th>Parameters (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(SC) (%)</td>
</tr>
<tr>
<td>0.007(^{a})</td>
<td>49.29 ± 0.85</td>
</tr>
<tr>
<td>0.02</td>
<td>31.64 ± 0.40</td>
</tr>
<tr>
<td>0.04</td>
<td>27.31 ± 0.44</td>
</tr>
<tr>
<td>0.007(^{b})</td>
<td>48.97 ± 1.06</td>
</tr>
</tbody>
</table>

\(^a\) First feeding at the beginning of the experiment.
\(^b\) Second feeding at the end of the experiment.

\(D\) = dilution rates, \(SC\) = sugar consumption, \(RTS\) = residual total sugar, \(P\) = ethanol concentration, \(Q_p\) = ethanol productivity and \(Y_{P/S}\) = ethanol yield.

3.2 Continuous Ethanol Production Using The Combined Bioreactor

In the STR system, high sugar levels remaining in the juice even at the lowest \(D\) value (0.007 h\(^{-1}\)) might have been due to substrate inhibition. This was supported by Ingledew [23] who reviewed that sugar concentrations ≥25% (w/v) affected osmotic pressure which was one of the main factors leading to the reduction of yeast viability and ethanol yield. Using the plug flow bioreactors in series, the substrate inhibition might be alleviated to some extent [24].

During the continuous ethanol fermentation in the combined bioreactor system, the pH value was relatively constant at approximately 4.0 to 4.5 at all conditions tested (Figure 3A) and no contamination was observed (data not shown). Xu et al. [25] reported that lower pH and anaerobic environments within fermenters were unfavourable for the growth of contaminated microorganisms. Therefore, contamination
could be effectively prevented, and conversion yields of ethanol to sugar could reach as high as 90 to 92% of its theoretical value.

At $D_0$ 0.007 h$^{-1}$, the steady state was evident after approximately 158 h of the continuous system as indicated by relatively stable levels of viable cell, total sugar and ethanol concentrations (Figure 3B, C and D). When $D$ value was increased to 0.02 h$^{-1}$, the sugar consumption and ethanol production by $S$. cerevisiae NP 01 was lower. The steady state was observed after 53 h of changing $D$ value. At $D$ 0.04 h$^{-1}$, the steady state was achieved faster than that at $D$ 0.02 and 0.007 h$^{-1}$, respectively. The results strongly showed that $D$ value affected sugar utilization in the fermentation broth because it related to the residence time of the broth in the system. At the end of the experiments, the juice at $D$ 0.007 h$^{-1}$ was re-introduced to the combined system. The results showed that all fermentation parameters measured were similar to those of the first feeding at the same $D$ value (0.007 h$^{-1}$), indicating that the combined system operation could be reversed. In addition, the measured ethanol loss in the exhaust gas washing tank was not detected.

**Figure 3.** Fermentation parameters during continuous ethanol production from the sweet sorghum juice in the combined bioreactor at different dilution rates. A: pH, B: log viable cell, C: total sugar and D: ethanol. •: stirred tank, △: column 1, ○: column 2, and O: column 3. The arrows indicate the start time of continuous mode at each dilution rate.
The average fermentation parameters in the combined bioreactor are summarized in Table 2. The results clearly demonstrate that the residence time of the fermentation broth in the system had a significant effect on the main fermentation parameters. At $D = 0.007$ h$^{-1}$, the average residual sugar levels in the stirred tank and the plug flow bioreactors 1, 2 and 3 were 176.10 ± 0.73, 130.17 ± 0.71, 67.21 ± 2.42 and 43.70 ± 1.13 g l$^{-1}$, respectively (Figure 3). Under these conditions, the total sugar was consumed at approximately 30, 48, 73 and 83% when the broth passed the STR and the plug flow bioreactors 1, 2 and 3, respectively. The maximum ethanol concentration of 106.01 ± 0.47 g l$^{-1}$ or 13.44% (v/v) was obtained at the last tubular bioreactor indicating that the plug flow bioreactor could improve product concentration. The value of ethanol concentration is higher than 11.5% (v/v), which is the acceptable level for industrial production [25].

When the $D$ value of the system was increased to 0.02 and 0.04 h$^{-1}$, the amount of sugar consumed and the ethanol produced increased with increasing residence time of the broth in the system as found at $D = 0.007$ h$^{-1}$ (Table 2). However, the ethanol concentrations decreased dramatically when compared to those at 0.007 h$^{-1}$. The maximum ethanol concentrations at $D = 0.02$ and 0.04 h$^{-1}$ were 67.25 ± 1.89 g l$^{-1}$ (8.52%, v/v) and 54.55 ± 1.32 g l$^{-1}$ (6.91%, v/v), respectively, in the last tubular bioreactor. However, the $Y_{p/s}$ values in each bioreactor at all $D$ values were similar, ranging from 0.50 to 0.52 g g$^{-1}$. The high $Y_{p/s}$ values implied that by-products of the fermentation were rarely occurred.

### Table 2. Continuous ethanol fermentation from sweet sorghum juice at different total dilution rates in the combined bioreactor.

<table>
<thead>
<tr>
<th>Total $D$ (h$^{-1}$)</th>
<th>Parameters</th>
<th>STR</th>
<th>STR to TB1</th>
<th>STR to TB2</th>
<th>STR to TB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.007</td>
<td>$D$ (h$^{-1}$)</td>
<td>0.023</td>
<td>0.013</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Vol. (ml)</td>
<td>1000</td>
<td>1770</td>
<td>2540</td>
<td>3320</td>
</tr>
<tr>
<td></td>
<td>$SC$ (%)</td>
<td>29.56 ± 0.73</td>
<td>47.93 ± 0.71</td>
<td>73.12 ± 1.21</td>
<td>82.52 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>$P$ (g l$^{-1}$)</td>
<td>42.62 ± 1.25</td>
<td>64.85 ± 2.36</td>
<td>100.97 ± 1.00</td>
<td>106.01 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>$Q_p$ (g l$^{-1}$ h$^{-1}$)</td>
<td>0.98 ± 0.03</td>
<td>0.86 ± 0.03</td>
<td>0.91 ± 0.01</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>$Y_{p/s}$ (g g$^{-1}$)</td>
<td>0.52 ± 0.02</td>
<td>0.52 ± 0.00</td>
<td>0.52 ± 0.01</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>0.02</td>
<td>$D$ (h$^{-1}$)</td>
<td>0.07</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Vol. (ml)</td>
<td>1000</td>
<td>1770</td>
<td>2540</td>
<td>3320</td>
</tr>
<tr>
<td></td>
<td>$SC$ (%)</td>
<td>22.69 ± 1.09</td>
<td>33.93 ± 0.26</td>
<td>40.99 ± 0.64</td>
<td>52.02 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>$P$ (g l$^{-1}$)</td>
<td>31.59 ± 1.84</td>
<td>46.76 ± 2.04</td>
<td>55.64 ± 0.95</td>
<td>67.25 ± 1.89</td>
</tr>
<tr>
<td></td>
<td>$Q_p$ (g l$^{-1}$ h$^{-1}$)</td>
<td>2.21 ± 0.13</td>
<td>1.82 ± 0.01</td>
<td>1.68 ± 0.03</td>
<td>1.34 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>$Y_{p/s}$ (g g$^{-1}$)</td>
<td>0.52 ± 0.00</td>
<td>0.50 ± 0.02</td>
<td>0.51 ± 0.01</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>0.04</td>
<td>$D$ (h$^{-1}$)</td>
<td>0.13</td>
<td>0.08</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Vol. (ml)</td>
<td>1000</td>
<td>1770</td>
<td>2540</td>
<td>3320</td>
</tr>
<tr>
<td></td>
<td>$SC$ (%)</td>
<td>8.67 ± 1.44</td>
<td>14.21 ± 3.71</td>
<td>25.97 ± 2.16</td>
<td>40.28 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>$P$ (g l$^{-1}$)</td>
<td>13.99 ± 1.24</td>
<td>22.08 ± 1.11</td>
<td>37.73 ± 0.46</td>
<td>54.55 ± 1.32</td>
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<tr>
<td></td>
<td>$Q_p$ (g l$^{-1}$ h$^{-1}$)</td>
<td>1.83 ± 0.18</td>
<td>1.77 ± 0.11</td>
<td>1.89 ± 0.02</td>
<td>2.18 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>$Y_{p/s}$ (g g$^{-1}$)</td>
<td>0.50 ± 0.02</td>
<td>0.51 ± 0.00</td>
<td>0.52 ± 0.03</td>
<td>0.50 ± 0.00</td>
</tr>
</tbody>
</table>

STR = stirred tank bioreactor; TB1, TB2 and TB3 = First, second and third tubular bioreactors, respectively.

$D$ = dilution rate, Vol. = working volume, $SC$ = sugar consumption, $P$ = ethanol concentration, $Q_p$ = ethanol productivity and $Y_{p/s}$ = ethanol yield.
3.3 Comparison of Continuous Ethanol Production Between The STR and The Combined Bioreactor Systems

The initial total sugar concentrations fed into the two systems were equal at 257 to 258 g l⁻¹ (Table 3). The results showed that the combined system promoted sugar consumption and/or reduced substrate inhibition. In addition, it also promoted cell growth of *S. cerevisiae* NP 01 (Figures 2 and 3). At the same D values, the sugar consumptions by the combined bioreactor were approximately 33, 20 and 13% higher than those by the STR at D = 0.007, 0.02 and 0.04 h⁻¹ respectively. The percentage of sugar consumption by the combined bioreactor at the lowest D value (0.007 h⁻¹) was similar to that by the batch system [26]. Therefore, the continuous operation at D lower than 0.007 h⁻¹ was not necessary. In addition, the lower D value, the lower ethanol productivity.

Table 3. Sugar consumption and fermentation parameters in batch and continuous ethanol fermentation by the STR and the combined bioreactor systems.

<table>
<thead>
<tr>
<th>D (h⁻¹)</th>
<th>System</th>
<th>Initial TS (g l⁻¹)</th>
<th>RTS (g l⁻¹)</th>
<th>SC (%)</th>
<th>P (g l⁻¹)</th>
<th>Qp (g l⁻¹ h⁻¹)</th>
<th>Yp/s (g g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.007</td>
<td>STR</td>
<td>258.21 ± 0.68</td>
<td>130.93 ± 1.90</td>
<td>49.29 ± 0.85</td>
<td>67.28 ± 0.37</td>
<td>0.47 ± 0.00</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>257.00 ± 1.34</td>
<td>43.70 ± 1.13</td>
<td>82.52 ± 0.56</td>
<td>106.01 ± 0.47</td>
<td>0.76 ± 0.02</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>0.02</td>
<td>STR</td>
<td>258.21 ± 0.68</td>
<td>176.51 ± 0.83</td>
<td>31.64 ± 0.40</td>
<td>42.81 ± 1.27</td>
<td>0.86 ± 0.03</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>257.00 ± 1.34</td>
<td>119.96 ± 0.54</td>
<td>52.02 ± 0.54</td>
<td>67.25 ± 1.89</td>
<td>1.34 ± 0.02</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>0.04</td>
<td>STR</td>
<td>258.21 ± 0.68</td>
<td>187.68 ± 0.93</td>
<td>27.31 ± 0.44</td>
<td>36.12 ± 0.24</td>
<td>1.44 ± 0.01</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>257.00 ± 1.34</td>
<td>149.29 ± 3.42</td>
<td>40.28 ± 1.71</td>
<td>54.55 ± 1.32</td>
<td>2.18 ± 0.07</td>
<td>0.50 ± 0.00</td>
</tr>
</tbody>
</table>

*D = the dilution rate, STR = stirred tank bioreactor, CR = combined bioreactor, TS = total sugar, RTS = residual total sugar, SC = sugar consumption, P = ethanol concentration, Qp = ethanol productivity and Yp/s = ethanol yield.*

The advantages of serial bioreactors have been used to reduce the residual sugar concentration at different D values. Tzeng and Fan [27] succeeded with an assimilation of 82% of glucose in cultivation of 200 g l⁻¹ of glucose, in a 8-stage fluidized-bed bioreactor with a total volume of 410 ml at D = 0.23 h⁻¹, while Bai et al. [18] achieved 92% sugar utilization in the continuous ethanol production from the enriched synthetic medium containing 280 g l⁻¹ of glucose using a cascade bioreactor at D = 0.012 h⁻¹.

At the same D values, ethanol efficiencies in terms of P and Qp values by the combined system were higher than those by the STR system (Table 3). The P and Qp values in the combined system increased approximately 51 to 58% and 51 to 61%, respectively, when compared with those in the STR system. However, the Yp/s values in the two systems were similar ranging from 0.50 to 0.52 g g⁻¹ at all conditions, indicating that most sugar in the juice was converted to ethanol. In addition, the fermentation process did not affect Yp/s or ethanol yield implying that metabolic pathway of ethanol production by *S. cerevisiae* NP 01 under all conditions were not changed.

The SC, P and Qp values in our study were lower than those (92%, 124.6 g l⁻¹ and 1.50 g l⁻¹ h⁻¹, respectively) reported by Bai et al. [18]. One of the main reasons might be due to the difference in the EP medium. The enriched synthetic medium containing 280 g l⁻¹ of glucose was used in Bai et al. [18], while the sweet sorghum juice (~260 g l⁻¹ of total sugar) without nutrient supplementation was used as the EP medium in this study.

It was reported that under no aeration in the STR system, the product inhibition was...
occurred at \( \sim 80 \text{ g l}^{-1} \) of ethanol in the fermented medium [28]. Therefore, lower \( P \) values in the STR system in our study might be mainly due to substrate inhibition as the highest ethanol concentration produced was only \( \sim 68 \text{ g l}^{-1} \). However, in the combined bioreactor, severe product inhibition was rarely observed. The viable yeast cell concentrations were still relatively high even in the plug flow bioreactor 3 which contained 106 g l\(^{-1}\) of ethanol (Figure 3). The product inhibition in the combined bioreactor in series was less because the product concentration increased gradually along the height of the plug flow reactor [18]. In contrast, well-mixed performance of the STR system caused severe product inhibition inside the bioreactor.

In this study, the sweet sorghum juice with no nutrient supplement was used as the EP medium, and the sugar consumption under the continuous system with the combined bioreactor was 83% sugar consumption. Complete sugar utilization resulting in higher ethanol production may occur if some essential nutrients for ethanol production are supplemented in the sweet sorghum juice. This was supported by Nuanpeng et al. [29] who found that 9 g l\(^{-1}\) of yeast extract was required for completion of sugar utilization in batch ethanol production from sweet sorghum juice containing 280 g l\(^{-1}\) of total sugar.

4. CONCLUSIONS

The results obtained from this study clearly demonstrated that the combined bioreactor (STR combined with three plug flow bioreactors) was successfully used for improvement of continuous ethanol production from sweet sorghum juice under VHG fermentation. The ethanol production efficiencies in terms of the ethanol concentration and its productivity increased 51 to 58% and 51 to 61%, respectively, when compared with those of the typical system (STR). Complete sugar utilization by the system may be achieved by nutrient supplementation in the sweet sorghum juice.

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REFERENCES


[19] Laopaiboon L., Nuanpeng S., Srinophakun P., Klanrit P. and Laopaiboon P., Selection of Saccharomyces cerevisiae and investigation of its performance for very high gravity ethanol fermentation, Biotechnology, 2008; 7: 493-498. ISSN 1682296X.


[26] Khongsay N., Laopaiboon L. and Laopaiboon P., Growth and batch ethanol fermentation of Saccharomyces cerevisiae on sweet sorghum stem juice under normal and very high gravity conditions, Biotechnology, 2010; 9: 9-16. ISSN 1682-296x.

