# Kinetic Parameters for Bioethanol Production from Oil Palm Trunk Juice

A. H. Norhazimah, C. K. M. Faizal

**Abstract**—Abundant and cheap agricultural waste of oil palm trunk (OPT) juice was used to produce bioethanol. Two strains of *Saccharomyces cerevisiae* and a strain of *Pichia stipitis* were used to produce bioethanol from the OPT juice. Fermentation was conducted at previously optimized condition at 30°C and without shaking. The kinetic parameters were estimated and calculated. Monod equation and Hinshelwood model is used to relate the specific growth to the concentration of the limiting substrate and also to simulate bioethanol production rate. Among the three strains, single *S. cerevisiae* Kyokai no. 7 produce the highest ethanol yield of 0.477 g/l.h within the shortest time (12 h). This yeast also produces more than 20 g/l ethanol concentration within 10 h of fermentation.

**Keywords**—Oil palm trunk, *Pichia stipitis, Saccharomyces cerevisiae*.

## I. INTRODUCTION

BIOETHANOL production has been dynamically investigated since the increase of oil price. Different types of raw material have been explored using different approaches, especially using agricultural waste. There are several research have been done by researchers to produce biofuel from agricultural waste such as using grape stalks and sweet sorghum juice as raw material [1], [2]. Oil palm trunk (OPT) is an agricultural waste that generated from replantation of oil palm tree. It constitutes large amount of liquid in the form of juice waste. Previous studies found that OPT juice contain good amount of sugar that can be used directly for biofuel production such as bioethanol and biobutanol [3], [5].

During the microbial selection for bioethanol production, it is essential to explore the kinetic parameter involved during fermentation. Fermentation in a relatively similar and equitable environment should produce almost similar and constant rate multiplication of microbes. Therefore, kinetic models can be used to describe microbial behaviour and mechanism of the process. Several phases of cell growth can be observed during batch fermentation including lag phase, acceleration phase, exponential phase, stationary and death phase. Maximum specific growth rates, half saturation constant and biomass yield are calculated from the end of exponential phase until decline phases. The performance of different strains was carefully analyzed and compared.

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The specific growth rate is described by the Monod equation. The *Monod equation* (1) derived from the basis that a single enzyme system with Michaelis-Menten kinetics are responsible for S uptake and catalytic activity is low enough from to be growth-rate limiting.

$$\mu(s, e) = \mu\, max\, \frac{s}{s+Ks} \qquad (1)$$

The Hinshelwood model is described by (2).

$$\mu = \mu\, max\, \frac{s}{s+Ks} \left(1 - \frac{P}{Pm}\right) \qquad (2)$$

where $\mu$ is the specific growth rate, $\mu$ max is the maximum specific cell biomass growth rate, $P_m$ is the maximum ethanol concentration above which cell growth ceases. In order to make accurate assessments of ethanol production from OPT juice for use as an energy sources, it is necessary to access the amount of sugar conversion and ethanol yield.

*S. cerevisiae* Kyokai no. 7 grows exponentially under anaerobic conditions for 12 h. Therefore, the time period of below 18 h will be used throughout the simulation experiment. The growth yield measurement can provide useful data concerning the relationship among substrate utilization and bioethanol production

## II. METHODS

### A. Microorganisms and Inoculum

Two strains of *Saccharomyces cerevisiae* and a strain of *Pichia stipitis* were used throughout this research (*S. cerevisiae* JCM2220, *S. cerevisiae* Kyokai no.7, *P. stipitis*). Co-culture is combination of *S. cerevisiae* Kyokai no. 7 and *P. stipitis*. Inoculums was prepared by transferring 3 loopful of pure culture into 250 ml conical flask containing 100 ml OPT juice.

### B. Extraction of Oil Palm Trunk Juice

Extraction of juice from OPT was carried out using trunks obtained from replantation area in Negeri Sembilan, Malaysia. Extractions were carried out using press machine. No dilution and pH adjustment was done to the OPT juice. All juice was sterilized by autoclaving at 121°C for 15 minutes

### C. Fermentation

Fermentations were carried out in 250 ml conical flask with 100 ml working volume. The fermentation was carried out under anaerobic conditions for 72 h. The temperature was kept constant at 30°C and static condition. Samples were withdrawn periodically at predetermined time intervals for analysis.

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### D. Analytical Method

Bioethanol concentration was measured by using Gas Chromatography with flame ionized detector (HP-InnoWAX column, carrier gas helium, initial oven temperature 80 °C, with temperature rate of 15 °C per minute until 120 °C and hold for 2 min). The optical density (OD) was measured spectrophotometrically at 620 nm and compared with standard curve of yeast's cell dried weight. For sugar concentration, it was determined using High Performance Liquid Chromatography (Agilent 1200, Agilent Carbohydrate Analysis Column, mobile phase ACN: pure water at 75:25 at 1.4 ml/min). All reading was compared with standard curve to obtain the actual value.

### III. RESULT AND DISCUSSION

#### A. Effect of Different Strains on Bioethanol Production Inoculum

During this experiment, three independent cultivations were carried out with OPT juice as fermentation medium. The result represents an average reading obtained during all experiments. The composition of OPT juice used in this work was: 13-34 g/l fructose, 22-40 g/l glucose and 6-41 g/l sucrose which formed total sugar of 67-86 g/l. Fig. 1 illustrated the fermentation course of microbes in 72 h of fermentation, while Figs. 2 and 3 show the effect of residual limiting substrate concentration on the specific growth of microorganism and comparison between actual and predicted data by using the Hinshelwood model.

From Fig. 1 (a), it can be seen that the concentration of bioethanol increased with the increase of fermentation time until 12-24 h of fermentation, and then it decreased a little bit. *S. cerevisiae* Kyokai no. 7 showed highest specific growth rate of 1.1169 g/l.h, followed by *S. cerevisiae* JCM2220 (0.7026 g/l.h) and co-culture (0.6223 g/l.h). Under 30°C fermentation condition, *S. cerevisiae* Kyokai no. 7 demonstrated superior kinetics and produce faster ethanol production which exceeding 20 g/l within 10 h. This is supported by Fig. 2 which showed that *S. cerevisiae* Kyokai no. 7 has a greater growth rate. Since bioethanol production is a primary growth-associated product, where the product is produced simultaneously with microbial growth, greater cell growth rate is beneficial for higher production. Even though co-culture gained the highest concentration of bioethanol (39.38 g/l) at the end of fermentation, it has a slower bioethanol specific rate and consumes more time compared to a single strain of *S. cerevisiae* Kyokai no.7. In industry, faster production should be considered as one advantage for good production.

The maximum specific growth rate, $\mu_{net}$ and substrate saturation constant, $K_S$ were found to be; 1.871 and 0.205 for *S. cerevisiae* Kyokai no. 7; 1.550 and 0.085 for co-culture; 1.443 and 0.204 for *S. cerevisiae* JCM 2220.

Comparison between actual and predicted data obtained using the Hinshelwood model in Fig. 3 show the model does not have the adequate fitting to suit the data. Further study on nonlinear equation should be done to ensure better fitting.
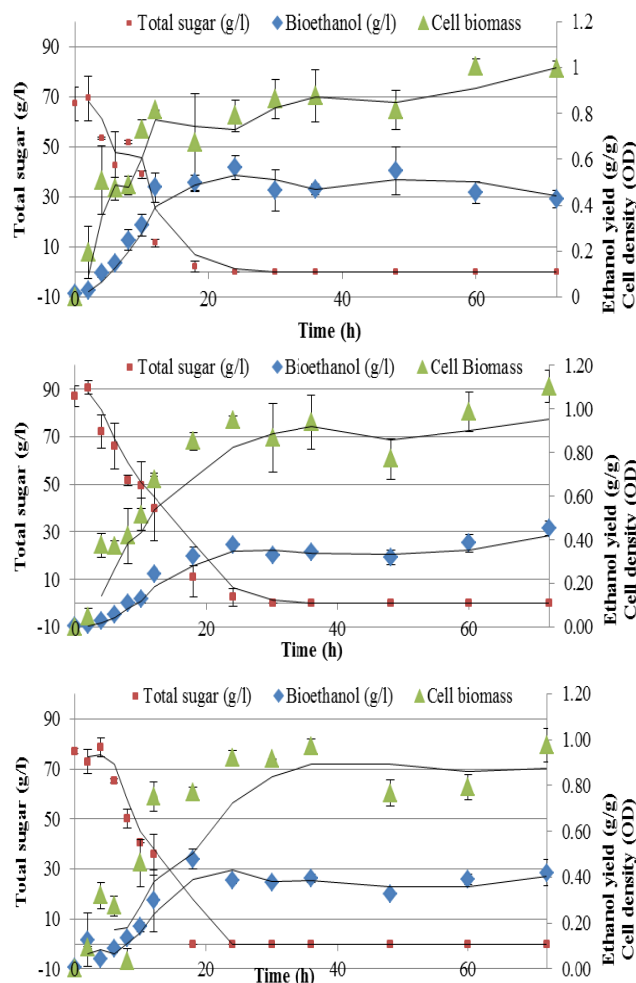


Fig. 1 The fermentation course of microbes in 48 h of fermentation (a) *S .cerevisiae* Kyokai no. 7 (b) Co-culture, (c) *S. cerevisiae* JCM2220
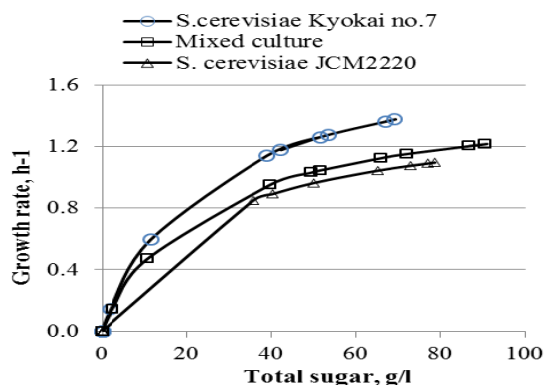


Fig. 2 The effect of residual limiting substrate concentration on the specific growth of microorganism
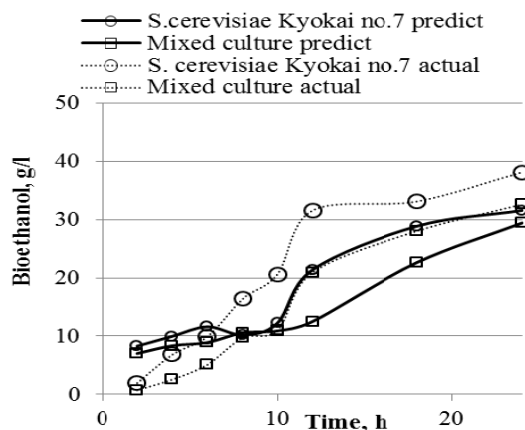
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Fig 3 Comparison between actual and predicted data obtained using the Hinshelwood model.

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Table I summarizes the kinetic parameter involved in the experiment.

TABLE I
PARAMETERS OF DIFFERENT STRAINS PERFORMANCE IN OPT JUICE

| Parameter | *S. cerevisiae* Kyokai no.7 | Co-culture | *S. cerevisiae* JCM2220 |
|---|---|---|---|
| Ethanol production specific rate, $r_e$ ($g_e$/l.h) | 2.7972 | 1.9178 | 2.0920 |
| Maximum concentration of ethanol produced, $E_{max}$ | 38.12 | 39.38 | 36.91 |
| End of ethanol production, $\varepsilon$ | 28.31 | 39.38 | 32.19 |
| End of exponential growth phase, $\Phi$ (h) | 12 | 24 | 24 |
| Maximum concentration of biomass $X_{max}$ | 39.98 | 44.64 | 21.30 |
| Net increase of biomass $\Delta X$ | 31.18 | 18.27 | 17.59 |
| Apparent cell growth, cells/ g substrate | 0.2622 | 0.2099 | 0.2280 |
| Biomass specific growth rate (g/ l.h) | 1.1169 | 0.6223 | 0.7036 |
| Net specific growth rate, $\mu net$ | 0.9538 | 0.1837 | 0.2819 |
| Sugar consumption specific rate, $r_s$ | 4.3667 | 3.9645 | 4.921 |
| Time of complete sugar consumption, $\alpha$ | 30 | 36 | 24 |

Table I shows that *S. cerevisiae* Kyokai no. 7 gain largest net increase of biomass (31.18 g/l) followed by co-culture (18.27 g/l) and *S. cerevisiae* JCM 2220. The result also indicates that single *S. cerevisiae* Kyokai no. 7 has good characteristics compared to other strain where this is reflected by the apparent cell growth (cells/ substrate) of 0.2622 cells per gram substrate

## IV. CONCLUSION

In this work, the effect of different selected strains to the bioethanol production from OPT juice has been investigated. In view of these results, the fermentation of OPT juice conducted by *S. cerevisiae* Kyokai no. 7 at 30°C was selected as the most suitable strains for bioethanol production from OPT juice and it could serve as a reference for future large scale study. Many more explorations should be conducted using nonlinear models and this will be done as future study.