

Optimization of The Bioconversion of *Spirogyra Hyalina* Hydrolysates to Become Ethanol Using *Zymomonas Mobilis*

Sulfahri*, Ni'matuzahroh and Yosephine Sri Wulan Manuhara

Department of Biological Science, Airlangga University, Surabaya, 60115

ABSTRACT

This study aims to determine the effect of different types of gases, initial pH, and the fermentation duration to cell biomass, pH, reducing sugar concentration, and ethanol concentration that was produced from fermentation of the *Spirogyra hyalina* hydrolysates to become ethanol using *Zymomonas mobilis*. Fermentation performed under anaerobic conditions with the variations of nitrogen gas and hydrogen gas in the space fermenter. The results showed that different types of gases, pH, and the fermentation duration give an effect on the cell biomass, pH, reducing sugar concentration, and ethanol concentration that was produced from fermentation of *Spirogyra hyalina* hydrolysates using *Zymomonas mobilis*. The highest levels of ethanol and biomass achieved by *Zymomonas mobilis* in the presence of hydrogen gas. It means that the hydrogen gas that was injected into the fermenter space can act as a reducing agent for the formation of NADH. NADH in the cell metabolism of *Zymomonas mobilis* functioning for the formation of ethanol.

Keywords: *Spirogyra hyalina*, Fermentation, Ethanol, *Zymomonas mobilis*, nitrogen, hydrogen

INTRODUCTION

One of the algae with the potential to be developed as the raw material of ethanol is the algae *Spirogyra*. Fermentation of algae *Spirogyra* by using bacterium *Zymomonas mobilis* more effective in anaerobic conditions with the addition of nitrogen gas [15]. The addition of nitrogen gas is meant by passing nitrogen into the jar fermentor. It is intended to remove all the Oxygen in the bottle and replace it with nitrogen. Meanwhile, anaerobic conditioning using the Hungate technique with the addition of nitrogen gas, can also be done by using carbon dioxide and hydrogen [4].

The addition of nitrogen gas in the fermentation of *Spirogyra* can produce ethanol at 11.36% (v/v) [15]. Whereas nitrogen in the process can not act as a reducing agent. Meanwhile, the addition of hydrogen gas having a function for anaerobic conditioning, also has a function as a reducing agent for the formation of NADH. This is because reduction potential of hydrogen is at -0.4 volts vs. NHE, while the reduction potential of NADH is at -0.32 volts vs. NHE. Reduction potential difference allows the hydrogen acts as a reducing agent for the formation of NADH [10]. NADH in the cell metabolism of *Zymomonas mobilis* function in the formation of pyruvic acid and ethanol [16].

The other factors that influence to the effectiveness of the fermentation are pH and fermentation duration. The pH of the fermentation is important for microbial growth, because only certain enzymes will break down the substrate in accordance with a specific pH [6]. Therefore, pH regulation is very important in the fermentation process [7].

This study aims to determine the effect of different types of gases, initial pH, and the duration of fermentation to cell biomass, pH, reducing sugar concentration, and ethanol concentration produced from fermentation of the algae *Spirogyra hyalina* which has been hydrolyzed using *Zymomonas mobilis*.

MATERIALS AND METHODS

Pretreatment and Hydrolysis Process of *Spirogyra hyalina*

Spirogyra hyalina was collected from a pond located within the campus of Sepuluh Nopember Institute of Technology, Surabaya, Indonesia. *Spirogyra hyalina* obtained and identified under a microscope using a *Sedgewick Rafter Cell* to ensure that the algae are *Spirogyra hyalina*. *Spirogyra hyalina* which has been identified then dried in oven with a temperature of 80°C for 24 hours. *Spirogyra hyalina* which has been dried then blender until crushed and sieved to 40 mesh size sieve. *Spirogyra hyalina* which passes 40 mesh sieve was weighed as much as 62.5 grams of distilled water and added as much as 1 liter, in order to obtain water and *Spirogyra hyalina* ratio is 15 to 1, then stirred [19].

Spirogyra hyalina which has been through a pretreatment process put in Erlenmeyer and heated on a hot plate. Heating process lasts for ± 2 hours with a heating temperature of $\pm 100^\circ\text{C}$ and then cooled until the temperature reaches $\pm 45^\circ\text{C}$ [19], and α -amylase enzyme is added (Liquozyme Supra, Novozymes, Denmark) as

*Corresponding Author: Sulfahri, Department of Biological Science, Airlangga University, Surabaya, 60115, Indonesia. Phone, +6282140839288, e-mail: mynameisfahri@gmail.com