EFFECT OF ACIDIC PH ON THE GROWING OF SACCHAROMYCES CEREVISIAE CS1-1 ON HSS BASED MEDIA.
Effect of Acidic pH on the growing of *Saccharomyces cerevisiae* CSI-1 on HSS based media.

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A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science with Honours Resource Biotechnology

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DECLARATION

I, Dehanjey Julse Mosinoh, Matric no 29993, from Faculty of Resource Science and Technology hereby declare that the work entitled Effect of acidic pH on the growth of *Saccharomyces cerevisiae* CSI-1 on HSS based media is my original work. I have not copied from any other students work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

I had contributed my effort about 80% on this project, 10% from my supervisor Associate Professor Dr. Cirilo Nolasco Hipolito, and another 10% from my friend and colleague Jorim anak Ujang.

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TABLE OF CONTENTS

Acknowledgement 1
Declaration 11
Table of Contents III - IV
List of Abbreviations V
List of Tables and Figures VI
Abstract 1
1. Introduction 2-3
2. Literature Review 4-6
3. Materials and Methods
   3.1 Preparation of Yeast 7
   3.2 Preparation of fermentation medium and bioreactor 7
   3.3 Controlling the pH 8
4. Results
   4.1 Grow rate of S. cerevisiae CSI-1 at different pH media. 9-10
   4.2 Dry cell weight of S. cerevisiae CSI-1 at different pH media. 11
   4.3 Glucose consumption of S. cerevisiae CSI-1 at different pH media. 12
   4.4 Viability of cells of S. cerevisiae CSI-1 at different pH media. 13
   4.5 Ethanol production of S. cerevisiae CSI-1 at different pH media. 14-15

III
4.6 Lactic Acid Production of S. cerevisiae CSI-1 at different pH media. 16

4.7 Cell morphology of S. cerevisiae CSI-1. 17

5. Discussion 18-22

6. Conclusion 23

References 24
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>mililiter</td>
</tr>
<tr>
<td>g/L</td>
<td>Grams per litre</td>
</tr>
<tr>
<td>g/L/h</td>
<td>Grams per litre per hour</td>
</tr>
<tr>
<td>CSI-I</td>
<td>Cirilo Shimazaki Ishizaki - 1</td>
</tr>
<tr>
<td>Rpm</td>
<td>Rotation per minute</td>
</tr>
<tr>
<td>HSS</td>
<td>Hydrolysed sago starch</td>
</tr>
<tr>
<td>DCW</td>
<td>Dry cell weight</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
</tbody>
</table>
LIST OF TABLES AND FIGURES

Figure 1: Absorbance of S. cerevisiae CSI-1 in 565 nm 9

Figure 2: Absorbance of S. cerevisiae CSI-1 in 780 nm 10

Figure 3: The dry cell weight of yeast (S. cerevisiae CSI-1) in anaerobic fermentation between different pH 11

Figure 4: Glucose consumed by yeast (S. cerevisiae CSI-1) in anaerobic fermentation between different pH 12

Figure 5: The difference of viability of yeast cell (S. cerevisiae CSI-1) under anaerobic fermentation between different pH 13

Figure 6: Ethanol volumetric production of S. cerevisiae CSI-1 in anaerobic fermentation in different pH 14

Figure 7: Ethanol production of S. cerevisiae CSI-1 in anaerobic fermentation in different pH 15

Figure 8: The amount of lactic acid (g/L) produced by S. cerevisiae CSI-1 in different pH 16

Figure 9: Picture of yeast cells (S. cerevisiae CSI-1) 17
ABSTRACT

The control for contamination and decent ethanol production in fermentation using the yeast cell *S. cerevisiae* CSI-1 can be found very useful to be able to gain the utmost product in the fermentation industry. Recent studies found that the optimum pH for *S. cerevisiae* is around pH 6 – pH 5. *S. cerevisiae* CSI-1 produced the highest ethanol production rate (4.74 g/L/h) in anaerobic fermentation at pH 5.5 followed by pH 4.5 (3.98 g/L/h) and pH 3.5 (3.38 g/L/h). The cell growth measured by using the dry cell weight, pH 5.5 is the highest at 7.17 g/L followed by pH 4.5 (4.93 g/L) and pH 3.5 (4.26 g/L). Interpreting all the results obtained, pH 5.5 is the optimum pH for the yeast *S. cerevisiae* CSI-1 strain.

**Keywords:** *S. cerevisiae* CSI-1, low pH, ethanol production, dry cell weight.

ABSTRAK

Kawalan terhadap kontaminasi disamping menghasilkan sejumlah ethanol yang berpatutan menggunakan sel yis *S. cerevisiae* adalah antara faktor penting dalam industri penapai. Parameter pH merupakan salah satu faktor utama yang mempengaruhi pertumbuhan *S. Cerevisiae*. Oleh itu, kajian ini bertujuan untuk membandingkan kesan pH berbeza ke atas pertumbuhan *S. cerevisiae* CSI-1 menggunakan media daripada hidrolisis kanji sagu. Kajian terbaru menunjukkan bahawa pH yang optima bagi *S. cerevisiae* adalah di antara pH 6 – pH 5. Menggunakan yis *S. cerevisiae* CSI-1 dalam proses penapaian anarobik, proses fermentasi yang di jalankan dalam pH 5.5 menunjukkan penghasilan ethanol yang tertinggi iaitu 4.74 g/L/h dan diikuti dengan pH 4.5 (3.98 g/L/h) dan seterusnya pH 3.5 (3.38 g/L/h). Tumbesaran sel juga di ambil dengan mengukur berat sel dalam keadaan kering, dan fermentasi di bawah pH 5.5 adalah yg tertinggi iaitu 7.17 g/L diikuti dengan pH 4.5 (4.93 g/L) dan seterusnya pH 3.5 (4.26 g/L). Analisis kesemua hasil data menunjukkan pH 5.5 adalah pH yang optimum bagi sel yis *S. cerevisiae* CSI-1.

**Kata kunci:** *S. cerevisiae* CSI-1, pH rendah, hasil ethanol, berat sel dalam keadaan kering.
1. INTRODUCTION

Using ethanol as a clean fossil fuel has been a promising plan for an alternative and may also be unlimited fuel supply for the future if there is enough research and development. Utilization of yeast has made a remarkable breakthrough and pushing the development of ethanol to another level. *S. cerevisiae* is yeast that has been widely used in multiple industries. What remains as a question is how to utilize its ability to convert carbon source into ethanol to its fullest. In ethanol fermentation, several kinds of raw materials can be used (Lin et al., 2012). In this experiment, the raw material that used is glucose obtained from sago starch.

The ability of the CSI-1 strain of *S. cerevisiae* that assessed is to grow and produce ethanol in acidic pH under optimum temperature and substrate concentration. The acidic pH makes the *S. cerevisiae* CSI-1 to be infused with undissociated weak acids into its cytoplasm that has an alkaline environment. An alkaline environment causes the accumulation of H\(^+\) ion in the cytoplasm hence, disturbance of the cell metabolism (Pampulha & Loureiro-Dias, 2000). The adaptation of the *S. cerevisiae* CSI-1 towards the acidity is the key point and also to be able to evaluate the extent of this strain of *S. cerevisiae* to grow and perform fermentation under low pH. As the fermentation proceeds, the *S. cerevisiae* CSI-1 grows under anaerobic condition and produces ethanol in which also lowers down the pH in the bioreactor.
Batch fermentation was used to access the S. cerevisiae CSI-1 growth and to produce ethanol. In batch fermentation, the process is only one cycle and at the beginning of the process, all the carbon source, media and the yeast are mix in one batch to a fixed volume. The fermentation finalised when the substrate is exhausted. Batch fermentation mode is used because of its low maintenance cost and low risk of contamination hence, compared to another method of fermentation it is easier to set up (Goh, 2012).

The main metabolic pathway that the yeast undergo is normally glycolysis where one molecule of glucose is metabolize to two molecules of pyruvate, then the pyruvate was reduced to ethanol and produced CO$_2$ and theoretically 0.511 for ethanol and 0.489 for CO$_2$ produced for each glucose molecule (Bai et al., 2008).
2. LITERATURE REVIEW

*S. cerevisiae* is a unicellular fungi or, yeast that reproduced through budding and divide unevenly (Tortora et al., 2010). *S. cerevisiae* can reproduce at high specific growth rate and this enable to the culture of this yeast and reproduce a new strain to be possible. Researcher had found, created or mutated many of the *S. cerevisiae* strain and had found that this new strain can adapt to a specific environment and grow exponentially. Some of the new strains are being research on to produce yeast that is beneficial to the industry specifically on fermentation and also biofuel production.

The *S. cerevisiae* BY4742 is a strain that had been studied by Lin et al. (2012) which found to be tolerant for high temperature under fermentation process. When conducting simultaneous saccharification and fermentation (SSF), the problem faced is the different optimal temperature for the saccharification and the fermentation where the optimal temperature for saccharification is between 40-50°C and for the fermentation is between 20-35°C (Lin et al., 2012). Using the BY4742 strain, the fermentation can be carried under high temperature and have almost the same optimal temperature as the saccharification process (40-50°C).

Vriesekoop and Pamment (2002) reported the effect of acetaldehyde over the growth of different strains of *S. cerevisiae*. The idea was to induce the flexibility of the *S. cerevisiae* strain at the early stage or the lag phase so it can grow and continue the fermentation process under variety of environmental stresses. In industrial fermentation process, there is always environmental stress encountered by the microorganism be such as contamination, pH, temperature or the presence of toxic compounds. Inducing adaptability
at lag phase of the *S. cerevisiae* is a new research to shorten the lag phase, then shortening the fermentation process.

According to Pampulha and Loureiro-Dias (2000), when *S. cerevisiae* is under condition of low pH, the undissociated weak acid diffused through the plasma membrane and interrupts the cell’s metabolism. An increasing concentration of organic acids may inhibit the fermentation process of *S. cerevisiae*. Thus, assessing the effect of acetic acid towards the growth of the *S. cerevisiae* is important to study the extent of the adaptation of the *S. cerevisiae*.

Besides producing ethanol, *S. cerevisiae* also produce β-Carotene. B-Carotene is an antioxidant and acts as a colouring agent for food products (Luo et al., 2013). Using different pH values to utilize the ability of *S. cerevisiae* to produce the β-Carotene is another great breakthrough where modification and control of the stress conditions towards the *S. cerevisiae* enable the synthesis of desired product.
*S. cerevisiae* is a microorganism or specifically yeast can promote industrial growth and also building of the economics of the biofuel industry. Biomass such as sugarcane bagasse, sago, and other cellulose compounds can be degraded to produce ethanol releasing as well carbon dioxide. (Kasavi et al., 2012). This research on the effect of pH towards the growth of CSI-1 strain of *S. cerevisiae* could be one of that researches that help to bloom the biofuel production. Moreover, this research can aid towards future research on the capabilities of the *S. cerevisiae* to produce ethanol and also to the research foundation of other strain of *S. cerevisiae*. 
3. MATERIALS & METHODS

3.1 Preparation of yeast.

A vial containing *S. cerevisiae* CSI-1 was obtained from the Laboratory of Biofuel, R&D of the Department of Molecular Biology in the Faculty of Resource Science and Technology of UNIMAS. The strain was grown in glucose based media at 30±5 g/L and was cultured at 36°C for 12–18 hours. Sub-culturing was conducted in order to keep the original strain survive and to prevent it from contamination. Inoculation was carried to activate culture to start the batch fermentation.

3.2 Preparation of fermentation medium and bioreactor

Fermentation medium was prepared by using glucose from sago starch and also yeast extract. The media was poured inside a 3L bioreactor and the fermentation started at this point. A 15 ml sample was taken out at the start of the fermentation and after each next 3 hours, until the substrate inside the fermenter jar was consumed by the yeast. The working volume used in the fermenter was 2 litres and the agitation fixed at 100 rpm. The samples were withdrawn every 3 hours until the glucose was exhausted. The growth of the yeast was measured by optical density as well as cell counting by counting cells using a haemocytometer.

After the exhausting of the glucose, and just before continuing to the next fermentation a sample was taken around 1 ml and observed under microscope. If no or minimal (1 foreign cell: 1000 yeast cells) contamination is seen the cell on the previous batch was recycled.
The process used was withdrawing out the fermentation broth and centrifuging approximately 200 – 300 ml of the broth under 3000 rpm and 5 minutes. The cell was collected by rinsing with distilled water and poured into the bioreactor until the target OD was achieved. All of these processes were performed in laminar chamber for sterile purposes.

3.3 Controlling the pH

The pH of the fermentations was controlled at 5.5, 4.5 and 3.5. All the fermentation was done by duplicate.
4. RESULTS

4.1 Grow rate of *S. cerevisiae* CSI-1 at different pH media.

Figure 1 and Figure 2 shows the grow rate of the *S. cerevisiae* CSI-1 by using two different absorbance's that is 565 nm and 780 nm. Spectrophotometer was used to measure the absorbance at 565 nm and laser turbidometer that was attached to the fermenter was used to measure the absorbance at 780 nm.

![Absorbance of S. cerevisiae CSI-1 in 565 nm](image)

**Figure 1:** Absorbance of *S. cerevisiae* CSI-1 in 565 nm
Figure 2: Absorbance of *S. cerevisiae* CSI-1 in 780 nm
4.2 Dry cell weight of *S. cerevisiae* CSI-1 at different pH media.

The growth of yeast (*S. cerevisiae* CSI-1) was measured by taking the absorbance reading from spectrophotometer (565 nm) and converting it into dry cell weight by using the standard linear equation between absorbance and dry cell weight as reference (Appendix A).

From figure 1, the yeast that was fermented under pH 5.5 shows the highest growth followed by yeast fermented under pH 4.5, and the least was yeast fermented under pH 3.5.

![Figure 3: The dry cell weight of yeast (*S. cerevisiae* CSI-1) in anaerobic fermentation between different pH.](image-url)
4.3 Glucose consumption of *S. cerevisiae* CSI-1 at different pH media.

The glucose consumption was measured by analyzing the glucose concentration in each sampling time by using the OSITM BF-5 Glucose Analyzer. According to figure 2, the yeast fermented under pH 3.5 consumed the glucose faster at the lag phase followed by pH 4.5 and then yeast fermented under pH 5.5 is the slowest at the lag phase. At the end of the log phase, yeast fermented under pH 3.5 was the highest, following pH 5.5 and then pH 4.5 as the lowest. The trend changes as the graph continues at the end.

![Graph showing glucose consumption](image-url)

**Figure 4:** Glucose consumed by yeast (*S. cerevisiae* CSI-1) in anaerobic fermentation between different pH
4.4 Viability of cells of *S. cerevisiae* CSI-1 at different pH media.

Figure 3 shows the viability of the yeast cell (*S. cerevisiae* CSI-1) under different pH and it shows that in pH 5.5 the yeast cell had the highest viability of 99.17% followed by pH 4.5 (97.34%) and the lowest was pH 3.5 (93.92%). The trend of the bar chart is increasing as the pH increases.

![Viability Chart](chart.png)

**Figure 5:** The difference of viability of yeast cell (*S. cerevisiae* CSI-1) under anaerobic fermentation between different pH.
4.5 Ethanol production of *S. cerevisiae* CSI-1 at different pH media.

The ethanol volumetric production of yeast had been calculated by using the Ideal Gas Law equation,

\[ PV = nRT \]

This equation used the raw data taken from FLC and converted it into ethanol volumetric productivity in grams/liter/hour. From the graph shown above, fermentation under pH 5.5 produced the highest amount of ethanol volumetric production that was 4.74 g/L/h and followed by pH 4.5 (3.98 g/L/h) and the lowest ethanol volumetric production compared was fermentation under pH 3.5 (3.38 g/L/h).

![Graph showing ethanol production over time at different pH levels](image)

Figure 6: Ethanol volumetric production of *S. cerevisiae* CSI-1 in anaerobic fermentation in different pH
The ethanol volumetric productivity in g/L/h can be derived into ethanol production of the fermentation that is in g/L as shown in Figure 7.

**Figure 7:** Ethanol production of *S. cerevisiae* CSI-1 in anaerobic fermentation in different pH.
4.6 Lactic Acid Production of *S. cerevisiae* CSI-1 at different pH media.

The lactic acid production of the yeast was measured by monitoring the NaOH consumption. Using the titration method the amount of lactic acid present in the fermenter jar was measured. The data for pH 3.5 was not recorded due to lack of instrument.

![Graph showing lactic acid production at pH 4.5 and pH 5.5](image)

Figure 8: The amount of lactic acid (g/L) produced by *S. cerevisiae* CSI-1 in different pH