Lab Work Textbook of Fermentation Engineering Technology

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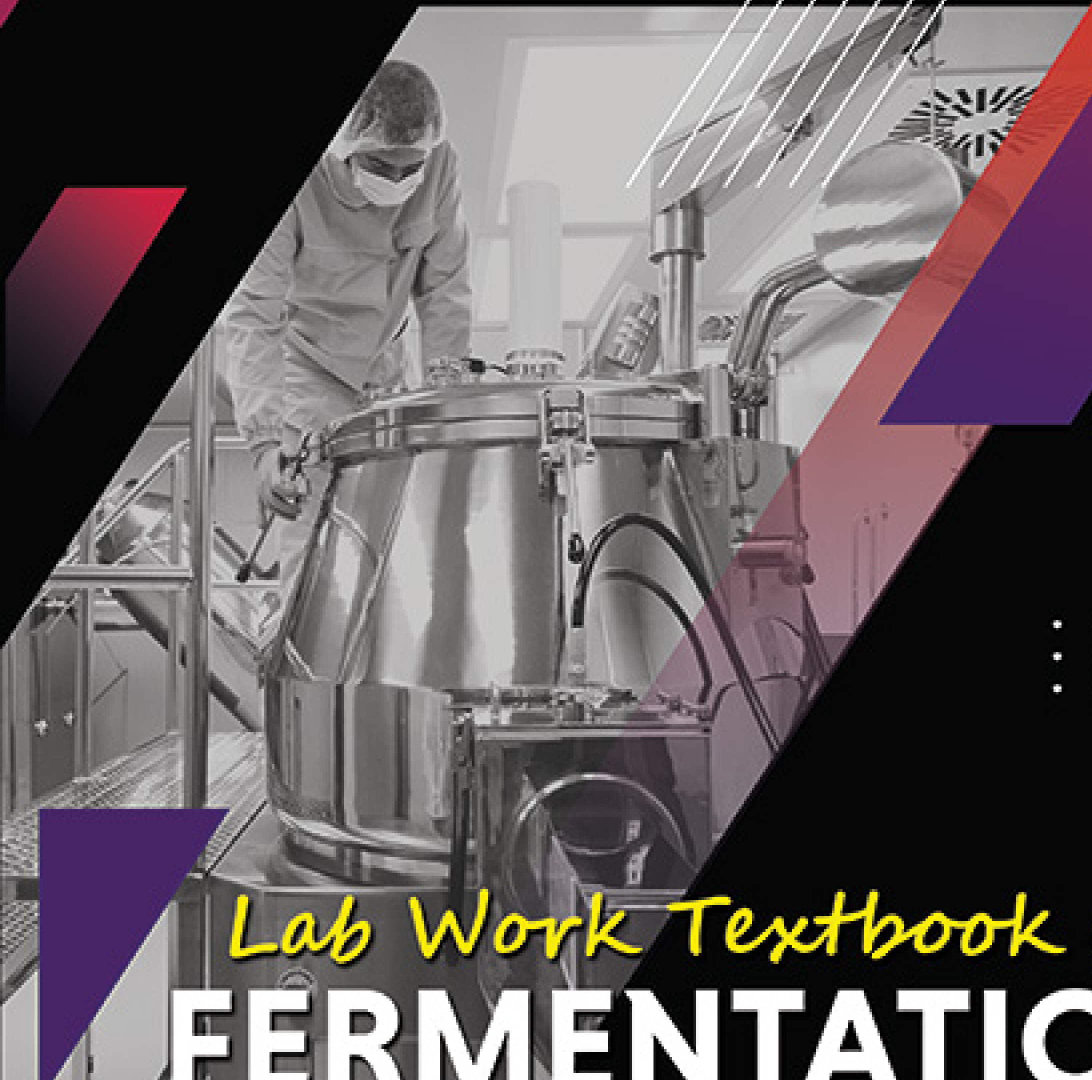
Abstract:
It gives us great pleasure to provide this book to our esteemed readers. This book is intended to serve as a resource for students enrolled in the Fermentation Engineering Technology course given by the Faculty of Engineering Technology, UTHM.

This book has a number of unique elements, including chapters that are organized precisely to follow the modified Fermentation Engineering Technology (BNN30304) syllabus, point-by-point explanations of each chapter's topic and a lengthly exercise at the end of each chapter.

If the book proves helpful to the course's students and instructors, we will feel that our efforts have been well rewarded.

Keywords: bioreactor, colorimetric, exponential phase, impeller, oxygen transfer rate



Lab Work Textbook of

FERMENTATION ENGINEERING TECHNOLOGY

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Penerbit UTHM

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Content

PREFACE

It gives us great pleasure to provide this book to our esteemed readers. This book is intended to serve as a resource for students enrolled in the Fermentation Engineering Technology course given by the Faculty of Engineering Technology, University of Tun Hussein Onn Malaysia.

The book has a number of unique elements, including chapters that are organized precisely to follow the modified Fermentation Engineering Technology (BNN30304) syllabus, point-by-point explanations of each chapter's topics and a lengthy exercise at the end of each chapter.

If the book proves helpful to the course's students and instructors, we will feel that our efforts have been well rewarded.

Chapter 1
Inoculum Preparation & Sterilization Technique

At the end of this chapter, students will be able to:

1. Select appropriate sterile techniques for bacterial culture bacteria (P1)
2. Prepare isolated microbial colonies from an inoculums (P2)
3. Build the growth profile of Saccharomyces cerevisiae (P3)
4. Construct and determine the exponential phase of growth profile (P4)

1.1 INTRODUCTION/THEORY/BACKGROUND

This experiment is conducted to prepare the inoculum for the bacterial growth, and also to determine the four phases of its growth profile. Bacterial cultivation technique involves the technique of transferring inoculums. Inoculums is a small amount of microorganisms used in cultivation to produce more microorganisms and product. Inoculum transfer is performed aseptically using sterile loop. In this experiment, student will be exposed to several standard techniques of bacteria cultivation.

A quick technique for qualitative isolation is the streak plate approach. The methods frequently utilized for isolating discrete colonies first call for a reduction in the inoculum's organism content. It essentially involves spreading a loopful of culture over the top of an agar plate, which is a dilution technique. Because of the consequent decrease in population size, it is guaranteed that after inoculation, individual cells will be spaced apart enough on the surface of the agar medium to cause a separation of the various species present. The four-way or quadrant streak is the most common process, despite the fact that many different kinds are carried out.

A sterile loop or swab is used in the streaking method to obtain an uncontaminated microbial culture. When isolated colonies are transferred from an agar plate to a new agar or gelatin plate using a sterile loop or needle, the procedure is known as "picking colonies". Following that, an agar surface is streaked with the inoculating loop or needle. Numerous microorganisms are deposited on the first region of the streak, leading to confluent growth or the growth of culture over the whole surface

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Chapter 2
Bioreactor Configuration

2.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Identify the important components of the bioreactor (P1).
2. Assemble and dissemble completely the components of the bioreactor components (P4).

2.1 INTRODUCTION/ THEORY/ BACKGROUND

This experiment is conducted to identify the components of the bioreactor and also to hands its operational procedure accordingly. Fermentation is the act or process of fermenting; a slow decomposition process of organic substances induced by microorganisms or by complex nitrogenous organic substances (enzymes) of vegetable or animal origin, usually accompanied by evolution of heat and gas, e.g. alcoholic fermentation of sugar and starch, and lactic fermentation. The term fermentation can be used to describe any process involving the production of organic products by the mass culture of a microorganism (bacteria, yeast and fungi).

In order to run a fermentation process, bioreactor plays a vital role. The main function of bioreactor is to provide a controlled environment for the growth of a microorganism or a defined mixture of microorganisms, to obtain a desired product. Most important factor in bioreactor design for industrial application must be low in capital and operating cost. However, no single system adequately meets the needs of all biological systems. Steps involve in fermentation process:

1. The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermentor.
2. The sterilization of the medium, fermenters and ancillary equipment.
3. The production of an active, pure culture in sufficient quantity to inoculate the production vessel.

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Chapter 3

Setting Up Bioreactor and Media Preparation

3.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Assemble, dissemble and setting up the bioreactor correctly (P4).
2. Organize the workflow for the preparation of fermentation process and its technique effectively (P2).
3. Prepare the fermentation media based on the specific formulation (P2).

3.1 INTRODUCTION

A 3L or 10L stirred tank bioreactor is used for this experiment. Two six-bladed turbine impellers mounted on the agitator are used for agitation. For aeration, sterile air is sparged through the air sparger placed just below the impeller. The fermenter is equipped with temperature, dissolved oxygen and pH controllers. During fermentation, agitation is fixed at 300 rpm and the temperature within the fermenter was controlled at 30°C.

Culture media are the nutrient solutions used to grow microorganism. A very simple defined medium will be used in this experiment. The formulation of a defined medium is often a tedious process of trial and error. However, a well formulated defined medium can support the healthy growth and maintenance of cells as effectively as, or sometimes superior to, a complex one. Most practical industrial fermentation processes are based on complex media because of the cost and the choice of the nutrients and the ease of nutrient reparation. The use of complex media is discouraged in the fundamental studies of fermentation kinetics because of the possibility of variations in the nutrient composition from run to run. For example, the exact content of a yeast extract preparation is not known, and its nutritional quality may vary from batch to batch. On the other hand, a defined medium can be reproduced time after time to ensure the reproducibility of biochemical experiments.

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Chapter 4

Shake Flask Fermentation

4.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Study and sketch the growth profile of Saccharomyces cerevisiae grown in shake flasks (P4, C4)
2. Determine and get the cell concentration, substrate utilization, growth kinetics and product yield of Saccharomyces cerevisiae grown in shake flasks (P2, C4)

4.1 INTRODUCTION/ THEORY

Fermentations can be carried out either as submerged (liquid medium) or solid state (solid or semi solid medium) fermentation process. More than 90% of industrial processes are carried out as submerged fermentation. Shake flask is used for small scale cell cultivation and higher O_2 transfer rates. Shaking breaks liquid surface & provides greater surface area for O_2 transfer. Increased rates of O_2 transfer are also achieved by entrainment of O_2 bubbles at the surface of the liquid [1].

Shake flasks are usually applied for medium optimization, as they are easy to handle even when operated in parallel (Figure 4.1). Compared to the production process, which is commonly performed in stirred-tank bioreactors, shake flasks have limited oxygen supply and no control of important process variables such as pH. In face of the simplicity of shake flask, their lack of representative results for the relevant production process is often neglected for medium development. Rather, an optimal medium identified in shake flasks is considered to perform equally well in the large-scale process [2].

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Chapter 5

Production of Biomass (Baker's Yeast) Using a Batch Bioreactor

5.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Study and sketch the growth profile of Saccharomyces cerevisiae grown in fermenter (P4, C4).
2. Determine and get the cell concentration, substrate utilization, growth kinetics and product yield of Saccharomyces cerevisiae grown in fermenter (P2, C4)

5.1 INTRODUCTION AND THEORY

Yeast has been in use since the beginning of human civilization. It is still a very versatile microorganism widely used in a wide range of fermentation industries. For example, the carbon dioxide released as a result of carbohydrate metabolism is used to raise dough in baking; the ethanol produced supports a multi-billion dollar alcoholic beverage industry; single cell protein is used to supplement animal feed. Although fermentation was practiced even before recorded history, the fact that microorganisms were responsible for the leavening and brewing actions was not realized until the last century. Yeast biomass is produced not only for the baking industry but also for pharmaceutical and microbiological purposes and is used for animal feed in the farming industry.

The Baker’s yeast, Saccharomyces cerevisae, is used in this experiments. This is hybrid yeast produced for commercial Baker’s, which is developed for their capacity to quickly ball on the dough with gas, especially when primmed with sugar and milk. Baker’s yeast die in an oven before they have chance to digest much of the actual starch, producing a loaf that inevitably tastes of them and raw flavour-something the Baker’s attempt to mitigate by sweetening the bread with malted barley. However, when yeast is given time to digest the starch, the result is a more nutritious loaf (additional protein and B vitamin) and one with superior texture and richer, sweet flavour.

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Chapter 6
Analytical Method

6.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Obtain and analyze the cell concentration, substrate utilization, growth kinetics and product yield of Saccharomyces cerevisae grown in shake flasks and fermenter (P2, C4).
2. Complete the fermentation process and determine cell concentration, substrate utilization, growth kinetics and product yield of Saccharomyces cerevisae with correct procedure (P4, C4).

6.1 INTRODUCTION

The products of a fermentation process may be few, as in the case of the classical yeast fermentation, or they may be tremendously diverse, which is typical of much bacterial fermentation. Therefore, the analysis of fermentation presents tasks ranging in magnitude from the relatively simple to the highly complex, involving the separation, identification, and quantitation of a broad spectrum of compounds. Therefore, there are various methods available to be employed to assess fermentation process and its efficiency.

One of the vital factors in determining the productivity of fermentation process is the stability of cells as well as its viability. In order to assess these parameters, several methods of quantitative cell enumeration such as automated cell counting systems, stain-based microscopic method using haemocytometer as well as sample absorbance using spectrophotometer are employed.

In addition, since the fermented cells will need carbon as the source of energy to multiply and grow, carbohydrate (sugar) is supplied. The most abundant and cheap fermentation fuel is glucose. Hence, the measurement of glucose consumption as an indicator for cell growth. Conventionally, the presence of reducing sugars (such as glucose, fructose, glyceraldehyde, lactose, arabinose and maltose) can

6.4 RESULTS AND ANALYSIS

Obtain the cell concentration, substrate utilization, growth kinetics and product yield of Saccharomyces cerevisae grown in:

(i) shake flasks
(ii) bioreactor

Compare those results.

ACTIVITY 6

1. Why do we need to scale up the inoculum before going to the large scale?
2. What is the important process you must know?

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Chapter 7

K_LA Measurement

7.0 LEARNING OUTCOMES

At the end of this chapter, students will be able to:

1. Understand and perform the dynamic gassing out techniques during fermentation process (P4, C1).
2. Complete the gassing out technique and determine K_La value of a fermentation system (P4, C4).
3. Investigate and manipulate the effect of air flowrate, agitation speed and salt on K_La value (P4, C3).

7.1 INTRODUCTION/ THEORY

K_La is considered as a single parameter which known as volumetric or overall oxygen transfer rate (OTR). Kca represents the oxygen transfer rate per unit volume. Whilst O_2 transfer rate is dependent on K_La & concentration driving force. Oxygen is an important substrate in aerobic fermentations. Since oxygen is sparingly soluble in water, it may be the growth-limiting substrate in these fermentations. For bacteria and yeast cultures, the critical oxygen concentration is about 10% to 50% of the saturated DO (dissolved oxygen concentration). Determination of K_La in a fermenter is important to establish its aeration efficiency and quantify effects of operating variables on oxygen supply. It is also used to compare fermenters before scale up or scale down.

K_La can be determined using either chemical method or dynamic gassing out method. In chemical method using sulphite oxidation technique, O_2 transfer rate is controlled by physical adsorption by sampling and titration of unreacted sulphite. This is inaccurate in the presence of even low level of surface-active contaminants.

In the dynamic gassing out method, the increase DO concentration of solution is monitored during aeration & agitation. It is necessary to decrease the O_2 first to the low value. Two methods have been developed which are non-fermentative

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