Fermentation

Science and Technology

Joaquin Weber

Fermentation: Science and Technology

This book outlines the processes and applications of fermentation in detail. It gives detailed information about the science and technology behind this process. Fermentation is the process that converts the sugar present in an object into gas, alcohol or acid. It is a metabolic process and it generally occurs in yeast and bacteria. The book explores the crucial processes that make this field vital for industries like food processing. Such selected concepts that redefine this subject have been presented in this text. Also included in it is a detailed explanation of the various concepts and applications of fermentation. This textbook is an essential guide for both academicians and those who wish to pursue this discipline further.

Joaquin Weber pursued his Masters in Food Safety and Microbiology from the Purdue University, Indiana, United States of America. He has spent more than 8 years in the food production industry, and has authored numerous articles and books, which have been published worldwide. Weber has also been the recipient of two awards for his research work in the field of zymology. He is a renowned lecturer of undergraduate programs and travels extensively for educating students across the globe.

ISBN 978-1-63549-119-7

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ISBN: 978-1-63549-119-7 (Hardback)

Larsen & Keller
Published by Larsen and Keller Education,
5 Penn Plaza,
19th Floor,
New York, NY 10001, USA

Cataloging-in-Publication Data

Fermentation : science and technology / edited by Joaquin Weber.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-63549-119-7
1. Fermentation. 2. Biochemical engineering.
I. Weber, Joaquin.
TP156.F4 F47 2017
660.284 49--dc23

Printed and bound in China.

For more information regarding Larsen and Keller Education and its products, please visit the publisher's website www.larsen-keller.com

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Preface

This book outlines the processes and applications of fermentation in detail. It gives detailed information about the science and technology behind this process. Fermentation is the process that converts the sugar present in an object into gas, alcohol or acid. It is a metabolic process and it generally occurs in yeast and bacteria. The book explores the crucial processes that make this field vital for industries like food processing. Such selected concepts that redefine this subject have been presented in this text. Also included in it is a detailed explanation of the various concepts and applications of fermentation. This textbook is an essential guide for both academicians and those who wish to pursue this discipline further.

A foreword of all Chapters of the book is provided below:

Chapter 1 - Some organisms like yeast and certain bacteria metabolize sugar to produce acids, gases and/or alcohol and this is known as fermentation. Fermentation also occurs in fatigued muscle cells and is called lactic acid fermentation in this case. Fermentation has been used to produce food like yoghurt, pickles, wine etc. This chapter provides an extensive initiation to the process of fermentation and provides an overall approximation of the metabolic process; **Chapter 2 -** Sugar is converted by chemical reactions like electron transport chain and oxidative phosphorylation and energy, in the form of adenosine triphosphate (ATP), is released with by-products like alcohol, acids and gases. In this section the chemical reactions are discussed in minutiae using details like the chemical mechanism, transport chain and inhibitors among others; **Chapter 3 -** There are various organisms that utilize fermentation to produce energy and based on the type of organism and the end products, fermentation can be categorized as lactic acid fermentation, mixed acid fermentation and butanediol fermentation. The content of this section explains these fermentation pathways, the organisms that are responsible for each type and the conditions and metabolites of each type; **Chapter 4 -** Yeast is a single cell eukaryote that can be classified as facultative anaerobic organism as it undergoes respiration in the presence of oxygen and ferments when presented with oxygen-deprived conditions. Pyruvic acid, which is the product of glycolysis in the absence of oxygen is broken down into lactate in animals and ethyl alcohol in plants and microbes. This chapter discusses the important molecules that contribute to fermentation like pyruvic acid, acetyl-CoA and the citric acid cycle or the Krebs cycle; **Chapter 5 -** Glycolysis is the oxygen dependent biochemical pathway that metabolizes glucose to produce pyruvate, energy, water and two positively charged hydrogen ions. This section explores the metabolic pathway of glycolysis and also delves into allosteric regulation and the role of the enzyme phosphofructokinase 1 in the process of glycolysis; **Chapter 6 -** Fermentation can be made to occur under certain conditions to aid in the preparation of food like wine, pickles, yoghurt, stinky tofu, vinegar etc. Fermented foods are full of beneficial

enzymes and various strains of probiotics that help keep the human gut functioning well. This chapter expounds about the role of fermentation in food processing, wine making and brewing. It also studies food microbiology that is concerned with the microorganisms that thrive in food and are responsible for food contamination or food spoilage.

I would like to thank the entire editorial team who made sincere efforts for this book and my family who supported me in my efforts of working on this book. I take this opportunity to thank all those who have been a guiding force throughout my life.

Editor

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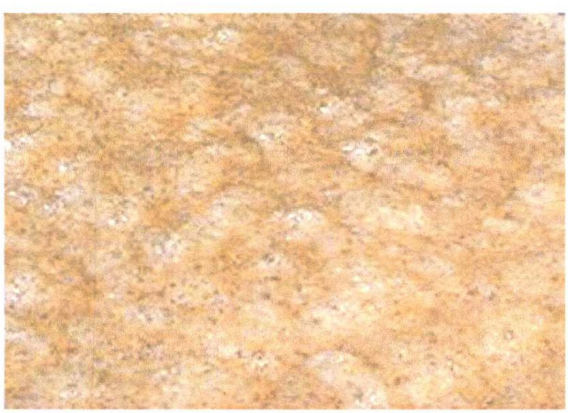
Introduction to Fermentation

Some organisms like yeast and certain bacteria metabolize sugar to produce acids, gases and/or alcohol and this is known as fermentation. Fermentation also occurs in fatigued muscle cells and is called lactic acid fermentation in this case. Fermentation has been used to produce food like yoghurt, pickles, wine etc. This chapter provides an extensive initiation to the process of fermentation and provides an overall approximation of the metabolic process.

Fermentation

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.



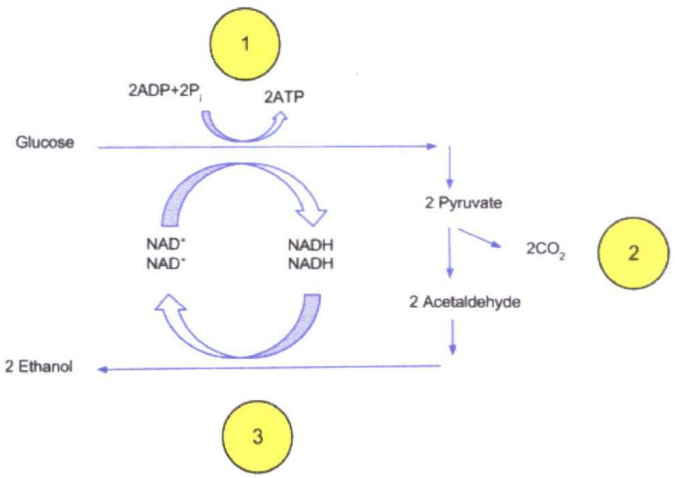
Fin progress: Bubbles of CO_2 form a froth on top of the fermentation mixture.

Fermentation takes place when the electron transport chain is unusable (often due to lack of a final electron receptor, such as oxygen). In this case it becomes the cell's primary means of ATP (energy) production. It turns NADH and pyruvate produced in glycolysis into NAD^+ and an organic molecule (which varies depending on the type of fermentation). In the presence of O_2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen.

The first step, glycolysis, is common to all fermentation pathways:

$$C_6H_{12}O_6 + 2\,NAD^+ + 2\,ADP + 2\,P_i \rightarrow 2\,CH_3COCOO^- + 2\,NADH + 2\,ATP + 2\,H_2O + 2H^+$$

Pyruvate is CH_3COCOO^-. P_i is inorganic phosphate. Two ADP molecules and two P_i are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD^+ are also reduced to NADH.



Overview of ethanol fermentation. One glucose molecule breaks down into two pyruvate molecules (1). The energy from this exothermic reaction is used to bind inorganic phosphates to ATP and convert NAD+ to NADH. The two pyruvates are then broken down into two acetaldehyde molecules and give off two CO2 molecules as a waste product (2). The acetaldehyde is then reduced into ethanol using the energy and hydrogen from NADH; in this process the NADH is oxidized into NAD+ so that the cycle may repeat (3).

In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

Humans have used fermentation to produce drinks and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation can even occur within the stomachs of animals, such as humans.

Definitions

To many people, fermentation simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, one might say it was 'off' or fermented. Here are some definitions of fermentation. They range from informal, general usage to more scientific definitions.

1. Preservation methods for food via microorganisms (general use).

2. Any process that produces alcoholic beverages or acidic dairy products (general use).
3. Any large-scale microbial process occurring with or without air (common definition used in industry).
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions (becoming more scientific).
5. Any metabolic process that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (most scientific). fermend star is een maymun

Examples

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). The antibiotic activity of hops also inhibits aerobic metabolism in yeast.

Fermentation reacts NADH with an endogenous, organic electron acceptor. Usually this is pyruvate formed from the sugar during the glycolysis step. During fermentation, pyruvate is metabolized to various compounds through several processes:

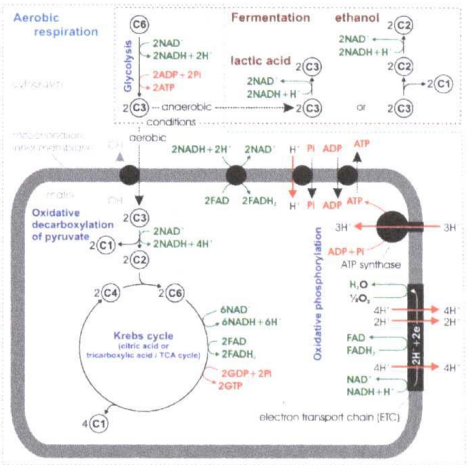
- ethanol fermentation, aka alcoholic fermentation, is the production of ethanol and carbon dioxide
- lactic acid fermentation refers to two means of producing lactic acid:

1. homolactic fermentation is the production of lactic acid exclusively
2. heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide, and hydrogen gas (H_2). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

Chemistry

Fermentation products contain chemical energy (they are not fully oxidized), but are considered waste products, since they cannot be metabolized further without the use of oxygen.



Comparison of aerobic respiration and most known fermentation types in eucaryotic cell. Numbers in circles indicate counts of carbon atoms in molecules, C6 is glucose $C_6H_{12}O_6$, C1 carbon dioxide CO_2. Mitochondrial outer membrane is omitted.

Ethanol Fermentation

The chemical equation below shows the alcoholic fermentation of glucose, whose chemical formula is $C_6H_{12}O_6$. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

C_2H_5OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This is known as glycolysis.

Lactic Acid Fermentation

Homolactic fermentation (producing only lactic acid) is the simplest type of fermentation. The pyruvate from glycolysis undergoes a simple redox reaction, forming lactic acid. It is unique because it is one of the only respiration processes to not produce a gas as a byproduct. Overall, one molecule of glucose (or any six-carbon sugar) is converted to two molecules of lactic acid: $C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$ It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some kinds of bacteria (such as lactobacilli) and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste. These lactic acid bacteria can carry out either homolactic fermentation, where the end-product is mostly lactic acid, or

Heterolactic fermentation, where some lactate is further metabolized and results in ethanol and carbon dioxide (via the phosphoketolase pathway), acetate, or other metabolic products, e.g.: $C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

If lactose is fermented (as in yogurts and cheeses), it is first converted into glucose and galactose (both six-carbon sugars with the same atomic formula): $C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6$ Heterolactic fermentation is in a sense intermediate between lactic acid fermentation, and other types, e.g. alcoholic fermentation. The reasons to go further and convert lactic acid into anything else are:

- The acidity of lactic acid impedes biological processes; this can be beneficial to the fermenting organism as it drives out competitors that are unadapted to the acidity; as a result, the food will have a longer shelf life (part of the reason foods are purposely fermented in the first place); however, beyond a certain point, the acidity starts affecting the organism that produces it.
- The high concentration of lactic acid (the final product of fermentation) drives the equilibrium backwards (Le Chatelier's principle), decreasing the rate at which fermentation can occur, and slowing down growth.
- Ethanol, into which lactic acid can be easily converted, is volatile and will readily escape, allowing the reaction to proceed easily. CO_2 is also produced, but it is only weakly acidic, and even more volatile than ethanol.
- Acetic acid (another conversion product) is acidic, and not as volatile as ethanol; however, in the presence of limited oxygen, its creation from lactic acid releases additional energy. It is a lighter molecule than lactic acid, that forms fewer hydrogen bonds with its surroundings (due to having fewer groups that can form such bonds), thus is more volatile and will also allow the reaction to move forward more quickly.
- If propionic acid, butyric acid, and longer monocarboxylic acids are produced (see mixed acid fermentation), the amount of acidity produced per glucose consumed will decrease, as with ethanol, allowing faster growth.

Aerobic Respiration

In aerobic respiration, the pyruvate produced by glycolysis is oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and is not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways.

Hydrogen Gas Production in Fermentation

Hydrogen gas is produced in many types of fermentation (mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glyoxylate fermentation), as a way to regenerate NAD^+ from NADH. Electrons are transferred to

ferredoxin, which in turn is oxidized by hydrogenase, producing H_2. Hydrogen gas is a substrate for methanogens and sulfate reducers, which keep the concentration of hydrogen low and favor the production of such an energy-rich compound, but hydrogen gas at a fairly high concentration can nevertheless be formed, as in flatus.

As an example of mixed acid fermentation, bacteria such as *Clostridium pasteurianum* ferment glucose producing butyrate, acetate, carbon dioxide and hydrogen gas: The reaction leading to acetate is:

$$C_6H_{12}O_6 + 4\ H_2O \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + 4\ H^+ + 4\ H_2$$

Glucose could theoretically be converted into just CO_2 and H_2, but the global reaction releases little energy.

Methane Gas Production in Fermentation

Acetic acid can also undergo a dismutation reaction to produce methane and carbon dioxide:

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad \Delta G^\circ = -36\ kJ/reaction$$

This disproportionation reaction is catalysed by methanogen archaea in their fermentative metabolism. One electron is transferred from the carbonyl function (e^- donor) of the carboxylic group to the methyl group (e^- acceptor) of acetic acid to respectively produce CO_2 and methane gas.

History of Human Use

The use of fermentation, particularly for beverages, has existed since the Neolithic and has been documented dating from 7000–6600 BCE in Jiahu, China, 5000 BCE in India, Ayurveda mentions many Medicated Wines, 6000 BCE in Georgia, 3150 BCE in ancient Egypt, 3000 BCE in Babylon, 2000 BCE in pre-Hispanic Mexico, and 1500 BC in Sudan. Fermented foods have a religious significance in Judaism and Christianity. The Baltic god Rugutis was worshiped as the agent of fermentation.

The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications appeared by C. Cagniard de la Tour, T. Swann, and F. Kuetzing, each of whom independently concluded as a result of microscopic investigations that yeast is a living organism that reproduces by budding. It is perhaps because wine, beer, and bread were each basic foods in Europe that most of the early studies on fermentation were done on yeasts, with which they were made. Soon, bacteria were also discovered; the term was first used in English in the late 1840s, but it did not come into general use until the 1870s, and then largely in connection with the new germ theory of disease.

Louis Pasteur (1822–1895), during the 1850s and 1860s, showed that fermentation is initiated by living organisms in a series of investigations. In 1857, Pasteur showed