Advanced Fermentation and Cell Technology

Urvashi Swami and
Kunwar Digvijay Singh Thakur

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5′-GMP	guanosine 5′-monophosphates
5′-IMP	inosine 5′-monophosphates
AA	acetic acid
ADH	alcohol dehydrogenase
AldDH	aldehyde dehydrogenase
ASS	adenylosuccinate synthetase
ATP	adenosine triphosphate
BCG	*Bacillus* Calmette-Guérin
BOD	biological oxygen demand
CAGR	compound annual growth rate
CBHs	cellobiohydrolases
CBM	carbohydrate-binding module
CEs	carbohydrate esterases
CGD	chronic granulomatous disorder
CHO	Chinese hamster ovary
CO_2	carbon dioxide
COD	chemical oxygen demand
CPT	camptothecin
DAPA	diaminopimelic acid
DNA	deoxyribonucleic acid
DSP	downstream processing
EMP	Embden-Meyerhof pathway
EPS	exopolysaccharide
ER	endoplasmic reticulum
FAD	flavin adenine dinucleotide
GA	glucoamylase
GHs	glycoside hydrolases
GI	gastrointestinal tract

GMP	good manufacturing practices
GO	glucose oxidase
GOS	galactooligosaccharides
HPLC	high-performance liquid chromatography
HPV	human papillomavirus
HSD	homoserine dehydrogenase
IDH	isocitrate dehydrogenase
IFN-α	α-interferon
IMP	inosine monophosphate
LA	lactic acid
LC-MS	liquid chromatography-mass spectrometry
LF	liquid fermentation
LOX	lipoxygenases
LPF	liquid phase fermentation
MAb	monoclonal antibodies
MPF	mixed-phase fermentation
MSM	microbial synthetic metabolites
NH_3	ammonia
NH_4^+	ammonium
O_2	oxygen
PDC	pyruvate decarboxylase
PLA1	phospholipase A1
PLA2	phospholipase A2
PLC	phospholipases C
PLD	phospholipases D
RER	rough endoplasmic reticulum
rRNA	ribosomal ribonucleic acid
SCID	severe combined immune deficiency
SCP	singled cell protein
SER	smooth endoplasmic reticulum
SMF	submerged fermentation
SPF	solid-phase fermentation
SSF	solid-state fermentation
SSI	site-specific immunotherapies

STD	sexually transmitted disease
TCA	tricarboxylic acid
TPC	total phenol content
VLPs	virus-like particles
WBC	white blood cells
WWT	wastewater treatment

PREFACE

Many traditional enzyme and microbial systems are included in fermentation and cell culture technologies that have been employed in the pharmaceutical, biochemical bioenergy, and agri-food industries. Novel technologies like protein engineering, systems biology, genetic engineering, and plant cell and mammalian cell systems, as well as the requirement for biomaterials, pharmaceuticals, bioenergy, and sustainable bio ingredients, are fast advancing. Industrial practitioners, students, and researchers must develop and implement new fermentation methods, and a range of cell technologies as the biobased economy continues to drive innovation.

Written by an expert in fermentation and cell technology, this comprehensive book contains information about the plant cells, mammalian cells, and various microbial technologies used in the contemporary biochemical processes. This convincing textbook illustrates the association between cell culture biopharmaceutical actives and food fermentation in conjunction with the crucial features of advanced fermentation and cell technology. Comprehensive chapters of this book have been divided into different that explain plant and animal cell technology, microbial cell technology, new biotechnologies' safety concerns, and the use of microbial fermentation in food items, pharmaceuticals, and chemicals.

This book is composed of eight chapters, and all these chapters explain a particular topic. To start with Chapter 1, readers will find a thorough introduction of fermentation and cell technology along with animal and plant cell cultures. Chapter 2 gives a detailed explanation of microorganisms in the fermentation process with their methods and diversity.

In Chapter 3, readers will be familiarized with the concept of fermentation miniaturization. Chapter 4 discusses the concept of aerobic and anaerobic solid-phase fermentation (SPF) with their advantages and disadvantages, phase control, and bioreactors.

Chapter 5 offers the explanation of the scaling-up of the industrial microbial process with its procedures. Chapter 6 thoroughly discusses the dietary uses of microbial fermentation along with a detailed explanation of several enzymes. Chapter 7 covers food quality and explains the role of microbial fermentation in food quality enhancement. This book ends with Chapter 8 that explains the medical applications of industrial fermentation, its various types, stages, and products.

Advanced Fermentation and Cell Technology is an ideal reference for technologists, researchers, and food scientists through the food industry, mainly in the fermented beverage, bakery, and dairy sectors, and is a valuable source for students of microbiology, biotechnology, food science, agricultural sciences, biochemistry, and biochemical engineering.

Chapter

1 Introduction to Fermentation and Cell Technology

CONTENTS

1.1. INTRODUCTION

To produce enzymes, biomass, pharmaceuticals, and chemicals, the processes of fermentation may be utilized. In such culture processes, traditionally used cell types are fungi, bacteria, and yeasts. Withal, lately, industrial fermentation processes incorporating plant and animal cells are being introduced (Marino and Gaggìa, 2013). Reactor configurations are required by the aerobic fermentation incorporating microbial cells which expedite oxygen transfer and aeration. These include rotating-drum, air-lift reactors, stirred-tank reactors, and tray systems concerning solid substrate fermentation, and several other specialized systems. A simpler bioreactor design is required by the anaerobic fermentation as there is no need for aerated facilities. Minerals, nitrogen, carbon, and at times growth factors, oxygen, and water (if aerobic) are required by the microorganisms as energy sources for cell maintenance and biosynthesis, and as constituents for cell biomass. Growth factors are not required by some of the microorganisms in the medium, whereas others need complex media involving some nucleotides, vitamins, or amino acids. The design of the media and the parameters of alternate fermentation is in such a way that optimizes both product formation and cell growth. Chemical and physical environmental conditions affect the yield and cell growth rate, involving pH, temperature, toxic metabolites production, and the presence of growth-limiting substrates. Medium constituents (e.g., inducers, precursors, inhibitors, repressors), morphology, growth rate, temperature, oxygen/carbon dioxide (CO_2) concentration, rate of substrate assimilation, pH, and secretion of by-product and other factors may affect the formation of product. They need conditions of gentler agitation in culture and are very fragile as mammalian cells are inadequate to a tough outer cell wall (against microbial cells).

Moreover, their nutritional needs are too complicated, and cells are very receptive to change in parameters like pH, temperature, CO_2, and dissolved oxygen. Based upon cell type, animal cells can grow in monolayers cultures connected to surfaces (i.e., anchorage-dependent) or being suspended cells (i.e., anchorage-independent). The contact inhibition property is shown by some animal cells, that is, they quit raising on the contact surface with other cells (Fröhlich, 2018). These properties dramatically affect environmental conditions and the nature of media used in the culture of an animal cell. Sometimes, air-lift or gently stirred fermenters concerning the growth of suspension cultures are involved in the reactor types, which incorporates the usage of microcarrier beads concerning anchorage-dependent cells. For the elimination of sensitivity of cells to shear, cell encapsulation has been

utilized. Semi-permeable membranes, in other systems, are used to regulate concentrations of nutrients, toxic metabolites, and gases through perfusion. Usually, newborn calf-, fetal calf-, calf-, or horse-serum (5–10% v/v) involved in the animal cell culture media creates a supply of growth factors. Where for a specific cell type, there is an optimization of basal media, where the serum content can be minimized to 1 to 2%. Usually, these basal media involve 13 to 22 amino acids, 6 to 12 types of inorganic salt, glucose, 9 to 13 vitamins, and alternate compounds. The detection and supply of the key serum-containing growth factors are required by the formulation and development of serum-free media for usage in mammalian cell culture (Jayme and Blackman, 1985). In suspension culture, there is a growth of several plant cells in chemically defined media involving a nitrogen source like nitrate, and organic carbon source like sucrose, growth regulators, and inorganic salts (Mir et al., 2018).

1.2. MICROBIAL FERMENTATION PROCESSES EXAMPLES

1.2.1. Alcohol Production

Perhaps, the most significant example of anaerobic fermentation is the formation of alcohol using fermentable sugar. The oxidative enzymes of the cytochrome system and the tricarboxylic acid (TCA) cycle are repressed catabolite at higher than 250 mg/l glucose concentrations, and yields of yeast biomass remain little with many sugars being transformed to alcohol under aerobic conditions. Alcohol yields are increased further by the maintenance of anaerobic conditions and minimized biomass production because of the phenomenon called the Pasteur effect (Gualdrón et al., 2012). Efficient recycling of minimized NADH under such conditions, formed at the glyceraldehyde-3-phosphate dehydrogenase phase in glycolysis is concluded from the ensuing reduction of acetaldehyde to ethanol. The 0.49 g CO and 0.51 g ethanol are the theoretical yields by 1 g glucose. Practically, the glucose of around 10% is transformed to biomass. Therefore, yields of CO and ethanol, may attain theoretical values of 90%. Whereas alcoholic fermentations are highly anaerobic, a little oxygen is required to facilitate the yeast to unsaturated fatty acid membrane elements and synthesize little sterols. For the supplementation of the fermentation medium with oleanolic or oleic acid, this need for oxygen vanishes.

Around 12% to 14% of ethanol concentrations can be achieved by several yeast strains. However, choosing strains has been shown to be able to produce alcohol of 18 to 20%, the rate of fermentation is reduced greatly with the increase of ethanol concentration (Contreras et al., 2014). The yeast's alcohol tolerance is affected greatly by the phospholipid composition of the yeast plasma membrane. Increased alcohol tolerance is observed when membrane unsaturated fatty acid (incomplete sentence).

Figure 1.1(a) illustrates the time course of a conventional batch molasses fermentation. The density of the initial yeast cell comes to about 5 to 10 billion cells per liter; it increases to around 100 billion at the completion of fermentation (Collett, 1851). The primary growth stage ends at around 15 hours, with the accumulative fermentation consuming approximately 30 hours. Around 80% cells are recycled in the process of Melle-Boinot (Logomasino, 1949), thus, the density of the initial yeast becomes 80 billion cells per liter, consequent to a greater rate of the production of the ethanol with a too minimized time of fermentation. Figure 1.1(b and c) illustrates the instance patterns of alcohol production and yeast growth patterns in methods for the production of white wine and Bourbon, respectively. It must be noted that the lower levels of the yeast biomass turn to protracted fermentation times. Generally, variations in the alcohol fermentation rate in a broad range of processes can be associated with the variables like the fermentation pH, the fermentation composition of broth nutrients, and the yeast concentration and temperature.