Biotransformation: Basics and Applied Perspectives

16

M. H. Yashavarddhan, Sania Bajaj, Sandeep Kumar Shukla, and Arpana Vibhuti

Abstract

Biotransformation is integral part of a living system. The system is built upon materials which are absorbed and transformed into the organic and inorganic building blocks and the unwanted or/and toxic materials are transformed and eliminated out of the system. These biotransformations are a part of multistep process containing specific enzymes and reactions catalyzed by them. Further these reactions and transformations are governed by biological factors, physiochemical properties, etc. of the system and materials which are taken in. Therefore, biotransformations can be used as technical strategy for pollution abatement, production of pharmaceutics, compounds especially for chemical and agricultural industry, etc. The current chapter delineates different phases of biotransformation process, the enzymes and their applications in industry and briefly sums up the current status of available research.

16.1 Introduction

The process by which a xenobiotic compound is transformed from one form to another within the living body is called biotransformation. This process can be completed by the involvement of an enzyme or multiple enzymes and/or organisms such as fungi, bacteria, etc.

M. H. Yashavarddhan
Defence Institute of Physiology and Allied Sciences, Defence Research and Development Organisation, Delhi, India

S. Bajaj · S. K. Shukla
Institute of Nuclear Medicine and Allied Sciences, Defence Research and Development Organisation, Delhi, India

A. Vibhuti (✉)
SRM University Delhi-NCR, Sonepat, Haryana, India

A. Singh et al. (eds.), *Environmental Microbiology and Biotechnology*,
https://doi.org/10.1007/978-981-15-7493-1_16

The Pharmacology and Experimental Therapeutics Department Glossary at Boston University School of Medicine propose the following definition:

Chemical alteration of an agent (drug) that occurs by virtue of the sojourn of the agent in a biological system (Pelikan 2004). This statement rightly explains and summarizes the true meaning of word Biotransformation.

In our day to day life we take in many compounds which are converted by our body into useful materials such as nutrients required for regular body functions. Due to metabolic activities, wastes are produced which need elimination. By virtue of biotransformation, the xenobiotic compounds entering the body and the body wastes are converted into less harmful substances that can be excreted out. Human body has an efficient biotransformation system for most of the xenobiotics and body wastes (Liska 1998). Hemoglobin, the oxygen-carrying iron–protein complex in red blood cells (RBCs) is one of the examples of body waste which should be eliminated after the RBCs complete their lifespan and the hemoglobin complex is broken into globin and heme. The heme gets fragmented into biliverdin, which is rapidly reduced to bilirubin, an orange-yellow pigment (Kuntz 2008). If the body is unable to excrete out the bilirubin via the liver due to a disease, medicine, or infection, it builds up in the body. As a result, the eyes and the skin may look yellow indicating the development of jaundice (Walker et al. 1990). The presence of high concentrations of bilirubin can cause irreversible brain injury in a newborn baby (Hansen and Bratlid 1986). However, liver break downs the lipophilic bilirubin molecule via biotransformation process into water-soluble hydrophilic metabolites which are converted into bile and eliminated through feces. Thus, the process of biotransformation is essential for survival of a living organism.

16.2 History of Biotransformation

Historically, most studied biotransformations were catalyzed by micro-organisms and a lot of research has been conducted on this subject in the 1970s. Due to expansion of science and technology, mechanisms and the probable agents of biotransformation could be explored in 1980s. With the introduction of enzyme technology, including enzyme and cell immobilization techniques, a much wider potential choice biocatalysts became available. During the 1980s there has been a much greater awareness that biological catalysts can also be used to transform reactants which have low solubility in aqueous media (Lilly 1992). Thus with the help of new technologies and knowledge, research shifted more towards searching for the mechanism at molecular level and exploring applications for handling the pollutants also. This makes it important to know about the process and its working in the organisms.

16.3 The Sites and Phases of Biotransformation Reaction

16.3.1 Sites for Biotransformation Reactions

Liver Predominantly, liver is the major and largest site in the body which contributes to both the pre-systemic and the systemic elimination of several xenobiotic compounds and the metabolism involved is called the hepatic metabolism. It has the largest concentration of enzymes required for transformation of compounds.

Intestinal Mucosa Cells The cells in the mucosal lining contain specific enzymes and microflora which convert the orally administered compounds and prepare them for complete conversion in the liver. The intestinal tract helps in the hepatic metabolism of the compounds as the blood from intestine flows directly into the liver via hepatic portal vein instead of going into general circulation (Pang and Kwan 1983; Liska 1998).

The other organs for extra-hepatic metabolism are kidneys and lungs which have only 10–30% liver's capacity. Skin, testes, and placenta have very low capacity for biotransformation (Vīna et al. 2013).

16.3.2 Different Phases of Biotransformation Process

Major contributions to the knowledge of metabolism and toxicology of drugs and xenobiotics were made by professor Richard Tecwyn William who is also known as founding father of drug metabolism. He not only studied the processes of transformation of several drugs but also classified these reactions and introduced the concept of phase I and phase II metabolic pathways in the second edition of his book "Detoxication mechanism: The metabolism of drugs and allied organic compounds" (Williams 1959). He suggested that in the first phase or phase I, oxidations, reductions, or hydrolysis or a combination of any of these three occurs, whereas in the second phase or phase II, it uses the metabolites of phase I and conjugates them with another molecule (Table 16.1). He also argues for making the distinction between phase I and phase II reaction. He suggested that the phase II conjugation reactions were always deactivating toxicity, whereas phase I reactions could either activate or deactivate depending on the compound (Williams 1959).

Table 16.1 Lists of major biotransformation reactions for xenobiotics broken into phase I and phase II reactions

Phase I	Phase II
Oxidation	Sulfate conjugation
Reduction	Glucuronide conjugation
Hydrolysis	Glutathione conjugation

After William's theory, many researchers proved his concept and end up developing the thought that phase I reactions must by necessity precede phase II. However, in some of the cases, the metabolic process can bypass all phase I chemical manipulations and undergo conjugation, i.e. phase II reaction directly (David et al. 2005).

16.3.2.1 Phase I Reactions

In general, phase I reactions are reactions which modify the chemical by adding a functional structure which is usually a small polar group which contains both positive (+ve) and negative (−ve) charges (Lech and Vodicnik 1985). The addition of a functional group permits the substance to fit into the enzymes of phase II reaction. The phase I reactions are further classified into:

1. Oxidation
2. Reduction
3. Hydrolysis

Oxidation

It is the most common reaction of phase I. It is correctly defined as a process where the substrate loses electrons. There are a number of reactions which have the ability to remove electrons from the substrate but because the addition of oxygen, or oxygenation, was the first discovered so it was named oxidation. However, most of the oxidation reactions do not involve oxygen (Lech and Vodicnik 1985; David et al. 2005).

Example Dehydrogenation is one of the examples of the oxidation reaction, which removes the hydrogen from the molecule. Most of the oxidizing reactions are explained by the name of the reaction or enzyme involved. Few of these oxidation reactions are as follows:

- Alcohol dehydrogenation
- Alkyl/acyclic hydroxylation
- Aromatic hydroxylation
- Deamination
- Desulfuration
- N-dealkylation
- N-hydroxylation
- N-oxidation
- O-dealkylation
- Sulfoxidation

The drugs which undergo oxidative metabolism include ropivacaine, paracetamol, omeprazole codeine, and phenothiazines.

Reduction

This is the second type of phase II reaction. The gain of an electron by the substrate in a chemical reaction is termed as reduction reaction. The reducing agent refers to the element that accepts electrons and in the reaction, one compound is oxidized and another compound is reduced. The reactions are most likely to occur in xenobiotics with low oxygen content. Reductions can take place across nitrogen–nitrogen double bonds, termed as azo reduction, or on nitro groups (NO_2). Commonly, the resultant amino compounds get oxidized to form toxic metabolites. In biological tissues, carbon tetrachloride can be reduced to free radicals which are very harmful to the body. So, the reduction reactions frequently result in inactivation of a xenobiotic rather than detoxification (Lech and Vodicnik 1985; David et al. 2005). An example of a reduction reaction in which the nitro group is reduced is illustrated in Fig. 16.1.

Most of the reduction reactions are explained by the name of the reaction or enzyme involved. Few of these reduction reactions are as follows:

- Azo reduction
- Dehalogenation
- Disulfide reduction
- Nitro reduction
- N-oxide reduction
- Sulfoxide reduction

Examples of drugs which undergo reduction include a prodrug called prednisone, which is reduced to the active glucocorticoid prednisolone; warfarin, an anticoagulant.

Hydrolysis

It is the final reaction of phase I. The hydrolysis reaction is breaking of toxicant into two fragments or smaller molecules when a water molecule is used to break the bond. The hydroxyl group (OH–) is attached into one fragment and the hydrogen atom is attached into the other fragment (Lech and Vodicnik 1985; David et al. 2005). These hydrolysis reactions mainly happen between an ion and water.

Example In a biotransformation reaction, the procaine compound produces two major smaller chemicals (Fig. 16.2).

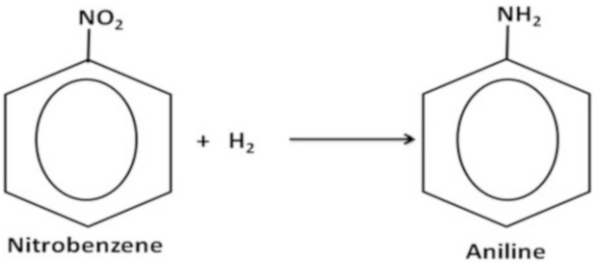

Fig. 16.1 Reduction reaction in which the nitro group is reduced (from NO_2 to NH_2)

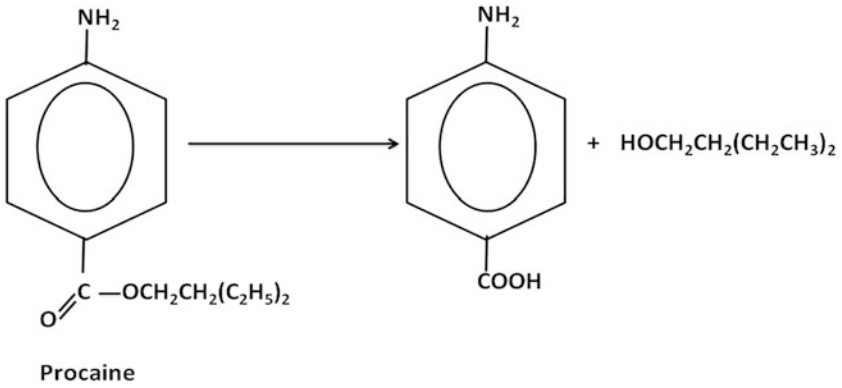

Fig. 16.2 Hydrolysis of procaine

Drugs which undergo hydrolysis reaction include prilocaine, an anesthetic agent; remifentanil, an analgesic drug.

In phase I biotransformation, toxicant converted into metabolites which are hydrophilic, or sufficiently ionized or to be either eliminated from the living body without further biotransformation or it can have the ability to convert the intermediate metabolites which were ready for phase II biotransformation process. In most of the cases, the intermediates forms due to phase I reaction may be very useful or it may be toxic than the parent (Lech and Vodicnik 1985).

16.3.2.2 Phase II Reactions

Phase II biotransformation reactions generally serve as a detoxifying step in metabolism. These reactions consist of those enzymatic reactions that conjugate the modified xenobiotic with another substance. Usually, the conjugated products are larger molecules than the substrate and polar or water-soluble in nature. These help in their rapid excretion from the body in bile or urine. The primary phase II reactions are as follows:

- Glucuronide conjugation
- Sulfate conjugation
- Acetylation
- Amino acid conjugation
- Glutathione conjugation
- Methylation

Some of the important phase II reactions were explained below.

Glucuronide Conjugation

The glucuronide conjugation is one of the most common and important types of phase II reactions. As the name suggests glucuronic acid molecule is used for conjugation in this reaction. The term was derived from glucose, which is the most common carbohydrate (sugar) and energy source for the cells inside the living body.

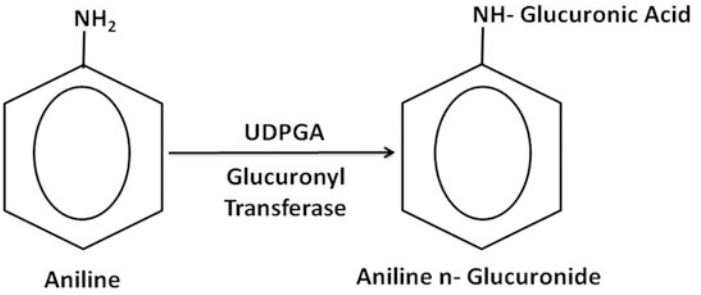

Fig. 16.3 Glucuronide conjugation of aniline

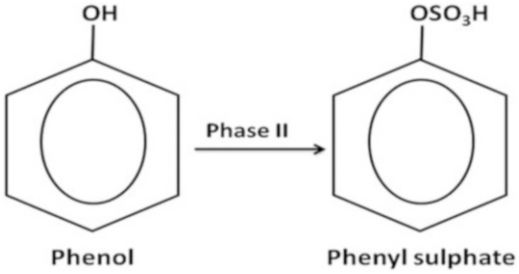

Fig. 16.4 Sulfate Conjugation of phenol

The substrate having nitrogen, oxygen, or sulfur bond is the site for the glucuronidation reactions and is applied to a wide array of xenobiotics as well as endogenous substances, such as thyroid hormones, steroid hormones, and bilirubin, etc. In glucuronidation reaction pathway, the conjugates xenobiotics are at a high capacity and the conjugation usually decreases the level of toxicity. However, there are some notable exceptions, for example, carcinogenic substances. These conjugates are generally hydrophilic in nature and are eliminated out by kidney or bile, depending upon the size of the conjugate (David et al. 2005; Jancova et al. 2010).One of the examples of glucuronide conjugation is conjugation of aniline to aniline *n*-glucuronide (Fig. 16.3).

Drugs which undergo glucuronidation are morphine, midazolam, etc.

Sulfate Conjugation

In phase II reaction, sulfate conjugation is another important reaction which occurs in most xenobiotics. The sulfation process helps in reducing the toxicity of xenobiotics. While the glucuronic acid conjugates are eliminated in bile (those with high molecular weight) and/or through kidney, the highly polar sulfate conjugates are excreted out from the body through urine. In some of the cases, the glucuronidation or the sulfation can conjugate the same xenobiotics. Generally, this sulfation pathway has a low capacity for the xenobiotic conjugation (David et al. 2005; Jancova et al. 2010). For example, sulfate conjugation of phenol to phenyl sulfate (Fig. 16.4).

The common drug which undergoes sulfation is paracetamol. But as described, only 40% of it is metabolized due to sulfation (Schonborn and Gwinnutt 2010).

16.3.2.3 Phase 0 and Phase III Concept or Transporters

The concept of two phase biotransformation reaction given by Professor RT William has been extended to four phases with transporters. Transporters guide the xenobiotics, drugs and their metabolites in entering and excreting out of the cells. In general, the xenobiotics or drugs are not able to overcome the phospholipid membrane barrier. Thus, these transporters help and guide the xenobiotics and drugs to overcome the phospholipid barrier. These transporters belong to two main clusters of transporter families, i.e. solute carrier (SLC) and the ATP binding cassette (ABC) carriers (Döring and Petzinger 2014).

The SLC comprises of 400 different transporters, organized into 65 families and are involved in drug uptake. Later on, this uptake process was termed as "phase 0 transporters or phase 0 reaction" of biotransformation (Fig. 16.5) (Döring and Petzinger 2014; Bai et al. 2017).

Similarly, the ABC transporters have 49 family members belonging to seven subfamilies (Hacker et al. 2009). These carriers operate as pumps at the expense of ATP splitting. In the year 1992, Ishikawa coined the term "phase III reaction" which

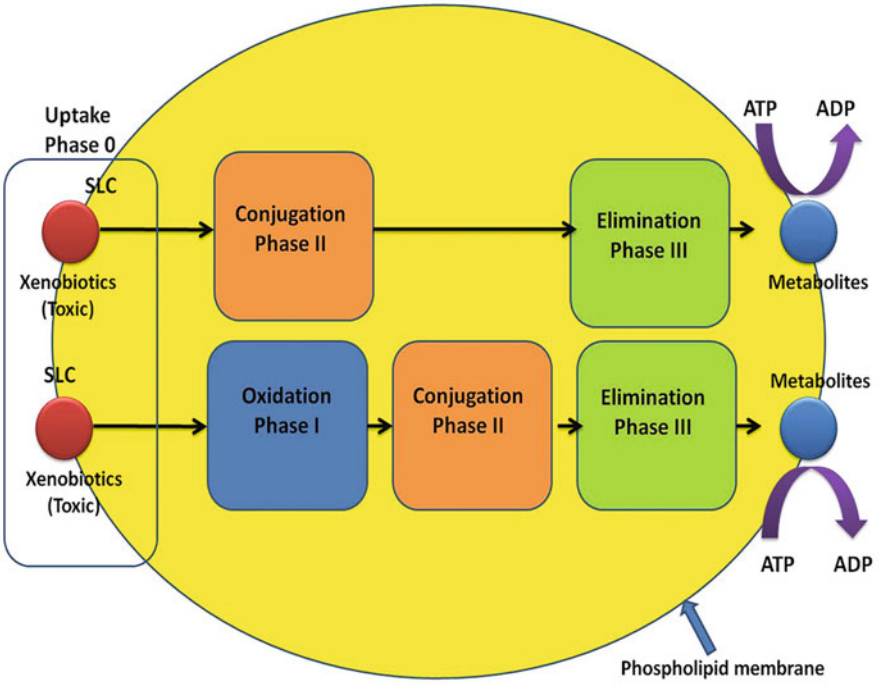

Fig. 16.5 Various steps involved in neutralization and/or elimination of xenobiotics or drugs from the cell inside the living body

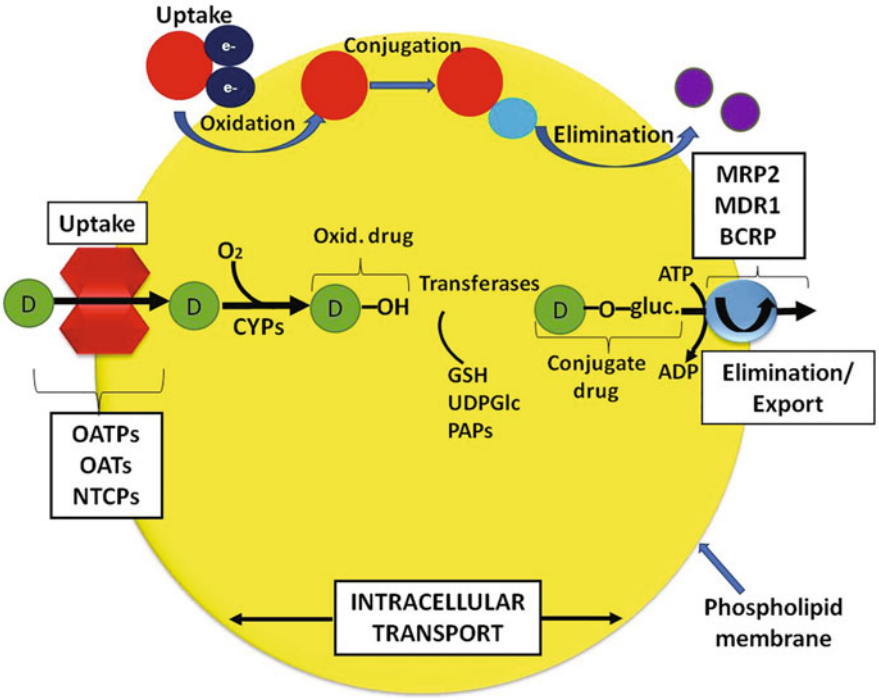

Fig. 16.6 Sequential steps of drug uptake from blood, metabolism inside the cell and its excretion by metabolism and membrane transport pathways via various carriers (Döring and Petzinger 2014; Modified)

includes ABC efflux pumps in the process of biotransformation for the export of drug material (Fig. 16.5).

These transporters for xenobiotics or drugs in eukaryotes generally operate in liver but they are also present in extra-hepatic tissues such as lung, the adrenal gland, intestine, and kidney. As tissues perform specific functions therefore according to their epithelial role (absorption or secretion), the cellular location of the transporters varies. In polarized cell, generally, the SLC transporters are located on blood-facing side of a cell, whereas ABC carriers occur on lumen side or on both sides (Petzinger and Geyer 2006).

The sequential transport steps of a drug molecule in a liver cell are given in Fig. 16.6.

Classification of Transporters

The biotransformation reaction needs transporters for the uptake and elimination process from the cell to extracellular fluid. There are 2 and 16% prokaryotic and eukaryotic genomes, respectively, which encode for the membrane transport proteins (Ren and Paulsen 2005; Hediger et al. 2013). According to current

knowledge, the transporters are divided into two types, i.e. phase 0 transporters and phase III transporters.

Phase 0 Transporters

These are transporters which generally initiate drug metabolism. Phase 0 transporters help in the uptake of drug molecules by using the SLC families. These SLCs operate non-actively as uniporters and act via facilitated biological diffusion, which allows an uptake of extracellular molecules inside the cells. It acts on the theory of concentration gradient (passive transport). The SLC-mediated drug transport might also be secondarily active via symport in the identical flux direction together with an organic substrate or an electrolyte, or in the opposite direction in the case of antiport (Fig. 16.7) (Döring and Petzinger 2014).

Phase III Transporters

Phase III-mediated elimination uses active transport. ABC carriers play an important role in the elimination of metabolites to extracellular fluids. ABC carriers generate a primary active transport process, which means uphill transport as against a concentration gradient of the transported substrate (transportate) using energy by converting

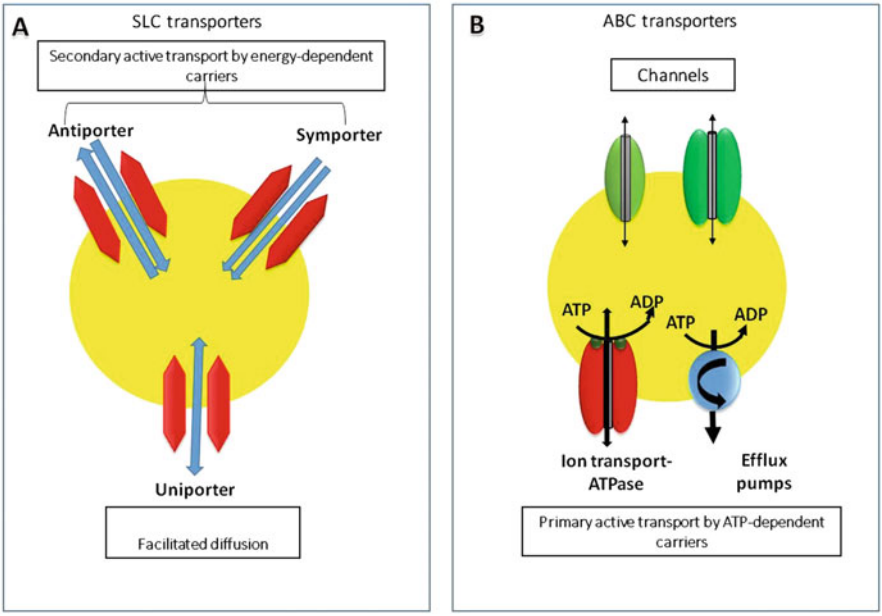

Fig. 16.7 Classification of transmembrane transporters and transport processes for organic compounds and electrolytes. (**a**) SLC carriers are facilitated diffusion carriers that allow non-active downhill transport (uniporter) but, in the case of sym- and antiporters, achieve uphill concentrative substrate transport as well (secondary active transport mechanism). (**b**) The ABC carriers use ATP for uphill substrate transport (primary active transport mechanism) (Döring and Petzinger 2014; Modified)

the ATP to ADP (Fig. 16.7). The ABC carriers including the GS-X pump named multidrug resistance-associated protein 1 (MRP1) (Fig. 16.6) and P-gp (Ishikawa 1992; Hediger et al. 2004).

16.4 Types of Biotransformation

16.4.1 Enzymatic Elimination

The use of different metabolizing enzymes of the body/cells for the process of biotransformation is known as enzymatic elimination. On the basis of compartmentalization of enzymes, it is broadly classified into non-microsomal biotransformation and microsomal biotransformation (Smitha et al. 2017).

16.4.2 Non-microsomal Biotransformation

A type of biotransformation in which the concerned enzymes are soluble and are present in the cytoplasm and mitochondria of cells in tissues including liver, intestine, plasma, etc. (Gaynes and Fiscella 1996). These enzymes catalyze mainly reductive and hydrolytic reactions, a few oxidative, and also conjugation reaction except glucuronidation. This category contains alcohol dehydrogenase, aldehyde dehydrogenase, monoamine oxidase, diamine oxidase, etc. (Gaynes and Fiscella 1996). The examples of reactions catalyzed by these enzymes include:

- Non-microsomal metabolism of catecholamines and noradrenaline via monoamine oxidase.
- Metabolism of ethanol into acetaldehyde by alcohol dehydrogenase.

As these enzymes are present in less quantity or a few are completely absent in neonates, they make neonates sensitive to a few drugs. For example, even though some drugs or syrups contain very less quantity of alcohol, they are still harmful to children as they cannot metabolize the drug completely (Sellers and Holloway 1978).

16.4.3 Microsomal Biotransformation

A type of biotransformation in which enzymes responsible are present within the lipophilic vesicle called microsome (Buhler and Williams 1988). These heterogeneous vesicles are formed from the endoplasmic reticulum when the cells are disrupted in vitro. These range from 20 to 200 nm in diameter and are isolated by differential centrifugation. They possess all functional, morphological properties of the endoplasmic reticulum. These have mainly three structural features: rough vesicles, smooth vesicles, and ribosomes & enzymes associated with it. In general,

the smooth endoplasmic reticulum is concerned with biotransformation and contains enzyme components while the rough endoplasmic reticulum is mainly concerned with protein synthesis (Buhler and Williams 1988).

These are mainly present in tissues like liver, kidney, lungs, and intestinal mucosa. These can be induced by drugs and/or diet. This category contains—Cytochrome P450 (CYPs), Flavin-containing monooxygenase (FMOs), UDP-glucuronosyltransferase (UGTs), etc. (Fig. 16.6) Enzymes of this category are non-soluble proteins. The microsome containing Cytochrome P450 (ferric, ferrous forms), NADPH (flavoprotein), membrane lipids, molecular oxygen is termed as microsomal mixed function oxidase system (Parkinson 2001; Timbrell and Marrs 2009).

The microsomes are very useful for researchers to mimic the properties of the endoplasmic reticulum in a test tube. They use hepatic microsomal preparations from various species for determination of phase I biotransformations of xenobiotics (drugs). The CYP enzyme family present in microsomes is responsible for phase I biotransformations and these tests are the primary source of information on the metabolism of any test compound. For this determination, the test compound is typically incubated in approximately 1.0 mg/ml microsomal protein, phosphate buffer, pH 7.4 at 37 °C for 30 min (Vrbanac and Slauter 2013). Its stability is then checked by an appropriate analytical method such as LC-UV or LC-MS (Vrbanac and Slauter 2013). Viable drug candidates should have a species-specific, pre-determined percentage remaining under these conditions.

16.5 Non-enzymatic Elimination

It is a process of spontaneous, non-catalyzed type of biotransformation which takes place at physiological pH for highly active, unstable compounds (Smitha et al. 2017).

The substrate can react with any of the following to result in a non-enzymatic reaction:

1. Reactions with endogenous nucleophiles: Endogenous water, thiols, cysteine act as endogenous nucleophiles. Example: Oxidation of nitrosobenzene in red blood cells.
2. Reactions with endogenous electrophiles: Biogenic aldehydes and ketones form endogenous electrophiles. Example: Formation of hydrazones from hydralazines.
3. Breakdown and rearrangement in acidic and neutral aqueous media. Example: non-enzymatic conversion reaction of 6-chloromethyl and 6-bromomethyl to 6-hydroxymethyl BP at physiological conditions. 80–90% of the incubated amount was transformed in 1 h.
4. Reactions between themselves (two drug or their metabolites): such reactions usually involve adduct formation. Example co-administration of acetylsalicylic acid and acetaminophen to model animals leads to the formation of salicylic acid

N,O-diacetyl-p-aminophenol. Example 2: At 37 °C and pH 2.5, norethindrone reacts with isoniazid form hydrazone XXI, in 6 min.

Examples Drugs which undergo non-enzymatic elimination:

- Mustin HCl converted into Ethyleneimonium
- Atracurium converted into Laudanosine and Quaternary acid
- Hexamine converted into Formaldehyde
- Clorazepate converted into Desmethyldiazepam

16.6 Factors Affecting Biotransformation

The process of transformation in vitro can be affected by numerous factors (Renton 1986) such as:

1. Physiochemical properties of the drug
2. Chemical properties of the drug
3. Biological factors

16.6.1 Physiochemical Properties of Drug

It includes the molecular size, acidity/basicity, pKa values, lipophilicity, interaction with drug metabolizing enzymes.

16.6.2 Chemical Properties of Drug

16.6.2.1 Enzyme Induction

Certain enzymes get induced or activated when they are exposed to a specific drug. These agents are called enzyme inducers. These can be several drugs and pesticides, or even cigarette smoke.

16.6.2.2 Enzyme Inhibition

Inhibition of enzyme activity due to exposure to a specific drug is a property which affects the biotransformation process. The inhibition can be due to a direct or indirect mechanism. Direct inhibition is further achieved by three mechanisms—competitive inhibition, non-competitive inhibition, product inhibition. Example: allopurinol inhibits xanthine oxidase; whereas indirect inhibition is achieved by two mechanisms, i.e. repression and altered physiology. Examples: actinomycin, puromycin.

16.6.2.3 Environmental Factors/Chemicals

The natural factors which affect the outcome of a biotransformation process can be the presence of halogenated pesticides, polycyclic aromatic hydrocarbons; organophosphate insecticides, and heavy metals in the environment. The temperature, altitude, atmospheric pressure, etc. are other forces which affect the process.

16.6.3 Biological Factors

16.6.3.1 Species Difference

Due to the difference in genetic makeup the drug metabolizing enzymes which are controlled by genes differ from species to species.

Example Dogs are deficient in acetyltransferase and cats are deficient in glucuronosyltransferase.

16.6.3.2 Strain Difference

The difference observed in response to the same drug within the individuals of the same species is called pharmacogenetics. The strain difference is also termed as the study of inter-subject variability in drug response. It can be monogenetically or polygenetically controlled. In humans, polygenic control is observed in fraternal twins, born from different eggs. But variations also arise due to ethnic cultural differences.

16.6.3.3 Sex Difference

When the regulation of drug metabolism is controlled by sex hormones, it causes variation in the process in different sexes of the studied population.

16.6.3.4 Age

As the body's growth status is at a different page in different age groups, the process of biotransformation varies among children, adults, and elderly. As mentioned before, the neonates and infants have underdeveloped microsomal enzyme system so they cannot metabolize commonly used drugs containing alcohol. But on the other hand, children can metabolize some drugs faster than adults. Whereas in elderly the reduced liver size and decreased hepatic blood flow also affect the biotransformation process.

16.6.3.5 Diet

The type of food intake forms a very important factor which affects the enzyme system in the body thereby affecting the whole process. The total protein intake; protein–carbohydrate ratio; fat-free diet; dietary deficiency of vitamins and minerals; starvation; alcohol ingestion are important parameters to consider for drug metabolism.

16.6.3.6 Altered Physiological Factors

The physiological conditions like being pregnant, diseased, or suffering from hormonal imbalances have drastic effects on the biotransformation process of several drugs. These states can reduce the individual's drug metabolizing ability due to high steroid hormones, or enhance half-life of almost all the drugs or may inhibit the activity of few enzymes while may induce others.

16.7 Biological Agents Used for Biotransformation

Biotransformation process has been witnessed by humans from the ancient times but was not understood then. The products of transformations have been extensively used and enjoyed for thousands of years for manufacturing bread, dairy products, and alcoholic beverages. Ethanol to vinegar was probably the first true biotransformation process applied in an industrial manner.

In a broad sense, biotransformation refers to the process in which microorganisms convert organic compounds into structurally related products. Microbes develop mechanisms to acclimatize to changing environmental conditions, thus proving very useful as biological agents or biocatalysts for biotransformation processes.

Certain reasons have been listed by researchers which make microbial cells as an ideal choice for biotransformation over conventional chemical methods (Smitha et al. 2017) such as:

1. *Surface–volume ratio*: Microbial biotransformation needs a high surface–volume ratio in the microbial cells.
2. *High growth rate*: The higher growth rate of microbial cells reduces the time of biomass transformation.
3. *High metabolic rate*: The higher rate of the metabolism in microbes leads to efficient transformation of the substrate.
4. *Toxic waste products*: Microbial biotransformation usually produces reduced levels of toxic waste products.
5. *Non-extreme conditions*: A highly selective biotransformation procedure can be performed with microbes at non-extreme pH and near room temperature.
6. *Desired stereoselectivity*: Use of specific microbes for regio- and stereoselective reactions, or to introduce chirality in end products.

These biocatalysts or the agents are not limited to microbes as whole cells. In fact, the enzymes are the principal biocatalysts. Their properties were recognized in the early 1900s during the phase of kinetic studies (Lin and Tao 2017).

16.7.1 Enzymes

These are key agents of biotransformation process. They are known to catalyze innumerable different chemical reactions. Therefore, these are named and classified into six classes accordingly by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) (McDonald et al. 2001).

1. *Oxidoreductases*: Enzymes catalyzing oxidation-reduction reaction, i.e. oxygenation of C–H, C–C, C=C bonds, or overall removal or addition of hydrogen atom equivalents belong to this category.
2. *Transferases*: Enzymes catalyzing the transfer of a group from one compound to another belong to this category. The different groups can be a methyl, aldehydic, ketonic, acyl, sugar, or phosphoryl.
3. *Hydrolases*: The enzymes which catalyze hydrolytic cleavage of C–O, C–N, C–C bonds. Hydrolases catalyze the removal of groups from their substrate to acceptor water molecules.
4. *Lyases*: These enzymes catalyze the addition or elimination of small molecules on C=C, C=N, C=O bonds.
5. *Isomerases*: These enzymes catalyze structural changes like racemization, epimerization, a rearrangement in a molecule.
6. *Ligases*: Ligases are enzymes catalyzing the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate.

All the above-mentioned enzymes participate in the defined phases of biotransformation. The physical state of these biological agents whether isolated enzyme systems or intact whole cells varies depending on the following factors (Doelle et al. 2009):

1. The type of reaction.
2. The requirement of co-factors.
3. The scale at which the biotransformation has to be performed.

16.7.2 Immobilized Enzymes

The enzymes restricted to a polymeric matrices or a carrier material by a physical or chemical treatment form an immobilized enzyme system. Although there is eventual loss of activity during immobilization of enzymes, these cell-free enzyme systems are commonly used in biotransformations, due to the following advantages:

1. The single desired product is mostly the end product as undesirable side reactions do not occur.
2. The desired products are stable and do not degrade.

3. As it is a cell-free system, there is no transport barrier such as cell membrane for the substrate or product.
4. The isolation and recovery of the product are simpler and easier.
5. Not only the product but the system itself is stable.

Example Production of glucose isomerase, penicillin acylase.

16.7.3 Whole Cells

Isolated immobilized enzyme systems are easily available commercially and have advantages of their own. However, the need of a cofactor for the specific reaction adds complications to the process. The whole cell systems have complete machinery for enzymes thus they overcome the disadvantage of co-factors. This system is also environmental friendly and cheaper than isolated enzyme systems. However, the major disadvantages of the system are then tiresome work-up due to large volumes, low productivity due to lower concentration tolerance, the formation of more than one product due to side reactions, and cell membrane as a barrier for biomolecule transportation.

Example The production of aromatic alcohol 2-phenylethanol (2-PE) from l-phenylalanine (l-Phe) in yeast. The use of whole cells for production of the suitable product can be performed in the form of growing cells or non-growing cells.

16.7.4 Growing Cells

Growing whole cell system is the most common type of production systems. Here the desired cells are cultivated in a suitable medium. A concentrated substrate is added to the culture only after a certain growth of the cells in the media. Sometimes, emulsifiers (Tween, organic solvents) are added to solubilize substrates and/or products as in case of steroid biotransformation. The substrate conversion to the product can be monitored by spectroscopic or chromatographic techniques. Biotransformation can be terminated when the product formation is optimum.

16.7.5 Non-growing Cells

During the non-growing cell biotransformation process, the cell growth (the enzyme manufacturing phase) and production phase are separated. Substrates are converted to the desired products by resting or non-growing cells. It is a preferred method for biotransformation reactions due to the following advantages:

1. A very high substrate concentration can be used as the cell growth stops at this concentration.

2. Low chances of contamination as cells can be washed and used again.
3. The conversion efficiency of the substrate to product is high.
4. Product isolation and its recovery are easy.

Example Free resting cells of *Mycobacterium* sp. NRRL B-3805 used for side-chain cleavage of β-sitosterol in biphasic systems containing bis(2-ethylhexyl) phthalate (BEHP).

16.7.6 Immobilized Cells

Biotransformations can also be carried out using the immobilized living cells with a specific set of enzymes. The same cells can be used again and again. The transformation mechanism can be a single or multistage reaction.

Example Commercial production of malic acid and L-alanine. The use of lipases by immobilization of fungal mycelium on biomass supports particles and the expression of lipases on the surface of microbial cells is another example.

16.8 Product Recovery in Biotransformation

In commercial times, the biotransformation is employed to yield a specific product, and in most of the cases, the end product is extracellular. It can be in the soluble state or in a suspended state. Accordingly, the product is recovered by using common techniques like extraction using solvents, precipitation with salts, adsorption, and ion exchange chromatography method. The volatile products can be recovered by direct distillation from the medium (Hellstén 2013; Fan et al. 2014).

16.9 Advantages of Biotransformation

- This technique is helpful in the production of those compounds which are otherwise difficult to be produced via chemical methods (Sultana and Saify 2013).
- Biocatalysts are highly substrate specific, stereo specific. They can easily incorporate desired structural changes than chemical reactions (Sultana and Saify 2013).
- Recombinant DNA technology can be easily applied to microbes for production of desired end product in biotransformation (Vasic-Racki 2006).
- Further, biotransformation with recombinant microbial enzymes has been widely used, including applications for the production of hormones, antibiotics, and specialty chemicals (Seeger et al. 2003).

16.10 Current Status of Biotransformation Technique

Scientists all around the world are exploring the biotransformation process for drug designing and development. The success of a safe drug designing programme lies in proper knowledge and combination of pharmacoproperties of molecule/s under consideration. Thus the drug metabolism research plays the most important role in its designing programme (Kebamo et al. 2015). Therefore, a lot of interest in the industrial research lies on the biotransformation of bioactive molecules in the body. Indeed very recently, the first commercial software was introduced in the market for automated catabolite and metabolite identification by SCIEX company which offers the flexibility to quickly identify metabolites, catabolites and enable in-depth metabolism or catabolism studies (Sicurella and Farrell 2017). The biotransformation information not only helps in designing and development but also in the production of several drugs such as steroid based pharmaceutical products, anticancer drugs, regio-, stereo-specific drugs from natural raw materials (Gao et al. 2013; Song 2018). The knowledge of this process also enables and strengthens the researchers to use microbes for production of bioactive metabolites, antifungal products, etc. under the concepts of white biotechnology and green chemistry (Bianchini et al. 2015). Also, with the help of metabolomics approach, researchers are trying to study the biotransformation biomarkers of metabolomic pathways related to certain diseases to get an insight for development of the disease.

Development and globalization in the countries around the world have produced various side effects on the environment in the form of pollutants. Various conventions have been signed and the governments are funding researches to build strategies to employ the biological processes for pollution abatement. The countries are guiding their industries to adopt and integrate methods to treat their effluents by natural process with environmental, economic, and social acceptability. Thus, biotransformation of environment pollutants like petroleum hydrocarbons, dye-contaminated industrial effluents, heavy metals like mercury and chromium, municipal waste, aquaculture effluents is looked up as vital solution for environment crisis. Various technologies have been patented which use the microbes like pseudomonas spp., thermus spp., *Archaeoglobus fulgidus*, *Microbacterium* sp., etc. in consortia for cleaning the environment (Peeples 2014). Researchers are thus continuing to evaluate this process as solution for providing more specific drugs, for disease development, reducing pollution, etc. and it is anticipated that biotransformation will be part of sustainable solution to a better future.

Acknowledgments We acknowledge all the authors whose articles have been cited in the text.

References

Bai X, Moraes TF, Reithmeier RA (2017) Structural biology of solute carrier (SLC) membrane transport proteins. Mol Membr Biol 34(1–2):1–32

Bianchini LF, Arruda MF, Vieira SR et al (2015) Microbial biotransformation to obtain new antifungals. Front Microbiol 6:1433
Buhler DR, Williams DE (1988) The role of biotransformation in the toxicity of chemicals. Aquat Toxicol 11(1–2):19–28
David JP, Peter GF, Miners JO (2005) "Phase I and phase II" drug metabolism: terminology that we should phase out? Drug Metab Rev 37(4):575–580
Doelle HW, Rokem JS, Berovic M (2009) Biotechnology: fundamentals in biotechnology. EOLSS Publications, Oxford, p 10
Döring B, Petzinger E (2014) Phase 0 and phase III transport in various organs: combined concept of phases in xenobiotic transport and metabolism. Drug Metab Rev 46(3):261–282
Fan LL, Li HJ, Chen QH (2014) Applications and mechanisms of ionic liquids in whole-cell biotransformation. Int J Mol Sci 5(7):12196–12216
Gao F, Zhang JM, Wang ZG et al (2013) Biotransformation, a promising technology for anti-cancer drug development. Asian Pac J Cancer Prev 14(10):5599–5608
Gaynes BI, Fiscella RG (1996) Biotransformation in review: applications in ocular disease and drug design. J Ocul Pharmacol Ther 12(4):527–539
Hacker M, Messer WS, Bachmann KA (2009) Pharmacology: principles and practice. Academic Press, Amsterdam
Hansen TWR, Bratlid D (1986) Bilirubin and brain toxicity. Acta Paediatr 75(4):513–522
Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Pflugers Arch 447(5):465–468
Hediger MA, Clémençon B, Burrier RE, Bruford EA (2013) The ABCs of membrane transporters in health and disease (SLC series): introduction. Mol Aspects Med 34(2–3):95–107
Hellstén S (2013) Recovery of biomass-derived valuable compounds using chromatographic and membrane separations. Acta Universitatis Lappeenrantaensis, Tampere
Ishikawa T (1992) The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci 17(11):463–468
Jancova P, Anzenbacher P, Anzenbacherova E et al (2010) Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154(2):103–116
Kebamo S, Tesema S, Geleta B (2015) The role of biotransformation in drug discovery and development. J Drug Metab Toxicol 6(196):2
Kuntz E (2008) Hepatology: textbook and atlas. Springer, Berlin, p 38
Lech JJ, Vodicnik MJ (1985) Biotransformation. In: Fundamentals of aquatic toxicology: methods and applications. Hemisphere Publishing Corporation, Washington, pp 526–557
Lilly MD (1992) The design and operation of biotransformation processes. In: Vardar-Sukan F, Sukan ŞS (eds) Recent advances in biotechnology, vol 210. Kluwer, Dordrecht, pp 47–68
Lin B, Tao Y (2017) Whole-cell biocatalysts by design. Microb Cell Fact 16(1):106
Liska DJ (1998) The detoxification enzyme systems. Altern Med Rev 3(3):187–198
McDonald AG, Boyce S, Tipton KF (2001) Enzyme classification and nomenclature. eLS 83:289–300
Pang KS, Kwan KC (1983) A commentary: methods and assumptions in the kinetic estimation of metabolite formation. Drug Metab Dispos 11(2):79–84
Parkinson A (2001) Biotransformation of xenobiotics. McGraw-Hill, New York
Peeples TL (2014) Bioremediation using extremophiles. In: Microbial biodegradation and bioremediation. Elsevier, Amsterdam, pp 251–268
Pelikan EW (2004) Glossary of terms and symbols used in pharmacology. University School of Medicine, Department of Pharmacology and Experimental Therapeutics, Boston
Petzinger E, Geyer J (2006) Drug transporters in pharmacokinetics. Naunyn Schmiedebergs Arch Pharmacol 372(6):465–475
Ren Q, Paulsen IT (2005) Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. PLoS Comput Biol 1(3):e27
Renton KW (1986) Factors affecting drug biotransformation. Clin Biochem 19(2):72–75

Schonborn JL, Gwinnutt C (2010) The role of liver in drug metabolism. Anesth Tutor Week 179:1–6

Seeger M, González M, Cámara B et al (2003) Biotransformation of natural and synthetic isoflavonoids by two recombinant microbial enzymes. Appl Environ Microbiol 69(9):5045–5050

Sellers EM, Holloway MR (1978) Drug kinetics and alcohol ingestion. Clin Pharmacokinet 3 (6):440–452

Sicurella S, Farrell P (2017) SCIEX announces new biotransformation solutions for routine and comprehensive metabolism and biologic catabolism studies. SCIEX, Thornhill

Smitha MS, Singh S, Singh R (2017) Microbial biotransformation: a process for chemical alterations. J Bacteriol Mycol 4(2):85

Song Z (2018) A bibliometric analysis of research updates and tendencies on steroid biotransformation. IOP Conf Ser: Earth Environ Sci 128:012132

Sultana N, Saify ZS (2013) Enzymatic biotransformation of terpenes as bioactive agents. J Enzyme Inhib Med Chem 28(6):113–1128

Timbrell JA, Marrs TC (2009) Biotransformation of xenobiotics. In: General, applied and systems toxicology. Wiley, New York

Vasic-Racki D (2006) History of industrial biotransformations—dreams and realities. In: Industrial biotransformations. Wiley, London, pp 1–35

Vīna I, Linde R, Patetko A, Semjonovs P (2013) Glucuronic acid from fermented beverages: biochemical functions in humans and its role in health protection. Int J Recent Res Appl Stud 14(2):217–230

Vrbanac J, Slauter R (2013) ADME in drug discovery. In: Faqi AS (ed) A comprehensive guide to toxicology in preclinical drug development. Academic Press, Amsterdam

Walker HK, Hall WD, Hurst JW (1990) In: Stillman AE (ed) Clinical methods: the history, physical, and laboratory examinations, 3rd edn. Butterworths, Boston

Williams RT (1959) Detoxication mechanisms: the metabolism and detoxication of drugs, toxic substances, and other organic compounds. Wiley, London