Biodegradation of Synthetic and Natural Plastic by Microorganisms

Fatimah Alshehrei[*]

Department of Biology, Faculty of Applied sciences, Umm AlQura University, Makkah, Saudi Arabia
*Corresponding author: comup.fm@hotmail.com

Abstract Plastic disposal is one of the greatest problems facing the environment today, as vast amounts of synthetic plastic remain non degradable. A number of microorganisms have the ability to degrade different types of plastic under suitable conditions, but due to the hardness of these polymers and their non-solubility in water, biological decomposition is a slow process. Natural plastics are made from plant and animal sources, or produced by a range of microorganisms, must be introduced. Some bacterial strains can produce and store bioplastics using carbon sources under suitable fermentation conditions. Such biomaterials are called polyhydroxyalkanoates (PHA) or biological polyester. They are safe, have no toxic by-products and can be degraded easily by microorganisms.

Keywords: biodegradation, synthetic plastic, natural plastic, PHA, biodegradability tests

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1. Introduction

Plastic is a synthetic polymer. It consists of carbon, hydrogen, silicon, oxygen, chloride and nitrogen. It is derived from different sources such as oil, coal and natural gas. Plastics are extensively used because of their stability and durability. They are different types such as polyethylene (PE), Poly Ethylene Terephthalate (PET), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) [1]. Due to the absence of efficient methods for safe disposal of these synthetic polymers, they often end up accumulated in the environment, posing an ever-increasing ecological threat to flora and fauna [2].

In Saudi Arabia, approximately 12 million tons of municipal solid wastes are produced annually, consisting of 40% organic wastes, 20% paper wastes, and 12–15% of plastic products [3]. According to the 2014 statistics of the Holy Mecca Municipality, about 82,933 tons of municipal solid wastes were produced, 26% of which were plastics [4]. Figure 1 illustrates the percentages of the different types of solid wastes produced by this municipality in 2014.
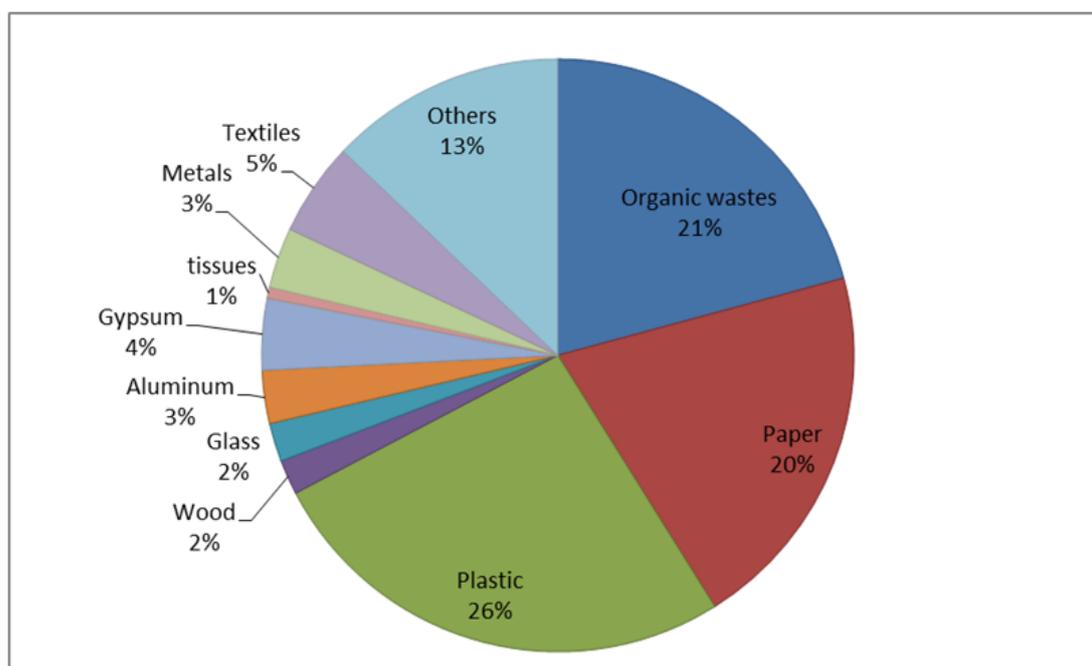

Figure 1. The percentage of municipal solid wastes in Makah city according to the Holy Makah Municipality report (2014)

Due to the presence of plastics in municipal wastes, many countries do not allow the incineration of these wastes. Instead, plastics are disposed of through open, uncontrolled burning and land-filling. Various health problems can be present as a result of open burning of these wastes which release pollutants into the air. In addition, the burning of Polyvinyl chloride (PVC) plastics produces persistent organic pollutants known as furans and dioxins, and the burning of polyethylene, polyurethane, polyvinyl chloride and polystyrene produces toxic irritant products that lead to immune disorders and lung diseases, and are classified as possible human carcinogens [5].

Plastic can degrade by a variety of mechanisms such as chemical, thermal, photoxidation and biodegradation, all of which take an extremely long time depending on the molecular weight of polymer, it could take up to 1000 years for some types of plastics to degrade [6].

Microorganisms can also play a vital role in this process, as over 90 genera of bacteria, fungi and actinomycetes have the ability to degrade plastic [7]. Generally, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms can't degrade certain plastics [8].

Biodegradable plastics are materials designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities, and thus open the way for new waste management strategies [9]. Some strains of Microorganisms can produce Polyhydroxy Alkonates (PHA), a bio plastic that is safe, has no toxic effects and can be easily biodegraded [10]. This study focuses on the role of microorganisms in the biodegradation of synthetic and natural plastics polymers, and describes the biodegradation pathways.

2. Categories and Classification of Plastics

There are various types of plastics, classified according to their properties and chemical structure.

2.1. Thermal Properties

Based on the plastic's thermal properties, plastics can be dividing in two groups: thermoplastics and thermosetting polymers.

2.1.1. Thermoplastics

Thermoplastics are polymers cannot change in their chemical composition when heated, and can therefore undergo moulding multiple times. These polymers are different types such as Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polytetrafluoroethylene (PTFE). They are also known as common plastics, range from 20,000 to 500,000 AMU in molecular weight and have different numbers of repeating units derived from a simple monomer unit [11].

2.1.2. Thermosetting Polymers

Thermosetting polymers are different types of plastics. Thermosetting polymers remain solid and cannot be melt and modified. The chemical change here is irreversible, and hence these plastics are not recyclable because they have a highly cross-linked structure, whereas thermoplastic

are linear [12]. Examples include phenol–formaldehyde, polyurethanes, etc…

2.2. Design Properties

Plastics are also classified based upon their relevance to the manufacturing process and design. Different parameters can be used, such as electrical conductivity, durability, tensile strength, degradability and thermal stability.

2.3. Degradability Properties

The chemical properties of plastics can be used as criteria for differentiating them into degradable and non-degradable polymers [13]. Non-biodegradable plastics, usually known as synthetic plastics, are derived from petrochemicals. They have a lot of repetitions of small monomer units; make them a very high molecular weight.

In comparison, biodegradable plastics are made from renewable resources that are completely biodegrade in their natural forms, such as components of living plants, animals and algae as source of cellulose, starches, protein and algal materials. They can also be produced by a range of microorganisms [14]. Biodegradable plastics usually break down upon interaction with UV, water, enzymes and gradual changes in pH. There are four types of degradable plastics: Photodegradable bioplastics, compostable bioplastics, bio-based bioplastics and biodegradable bioplastics [15].

Photodegradable bio plastic has light sensitive groups connected directly into the backbone of the polymer. Ultraviolet Radiation exposure for a long time can disintegrate their polymeric structure, rendering them open to further bacterial degradation. Landfills, however, typically lack sunlight, thus keeping these plastics non-degraded [15].

Bio-based bioplastics are defined as "plastics" in which 100% of the carbon is derived from renewable agricultural and forestry resources, such as corn starch, soybean protein and cellulose.

Compostable bioplastics are decomposed biologically in a composting process that occurs at a similar rate to other compostable materials, without leaving visible toxic remainders. In order to designate a plastic as bio-compostable, its total biodegradability, its disintegration degree and the possible ecological toxicity of its degraded materials must be determined by standardized tests.

Biodegradable bioplastics are fully degraded by microorganisms, without leaving visible toxic remainders. The term "biodegradable" refers to materials that can disintegrate or break down naturally into biogases and biomass (mostly carbon dioxide and water) as a result of being exposed to a microbial environment and humidity [16].

Polyhydroxyalkanoic acids (PHAs) are a significant type of biodegradable plastics, since they possess properties similar to conventional plastics. They are completely biodegradable but may be melted and modelled, making them ideal for use in consumer products. Figure 2, displays the typical PHA structure, as well as the structures of most important PHAs: poly (3-hydroxybutyrate) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) [17].
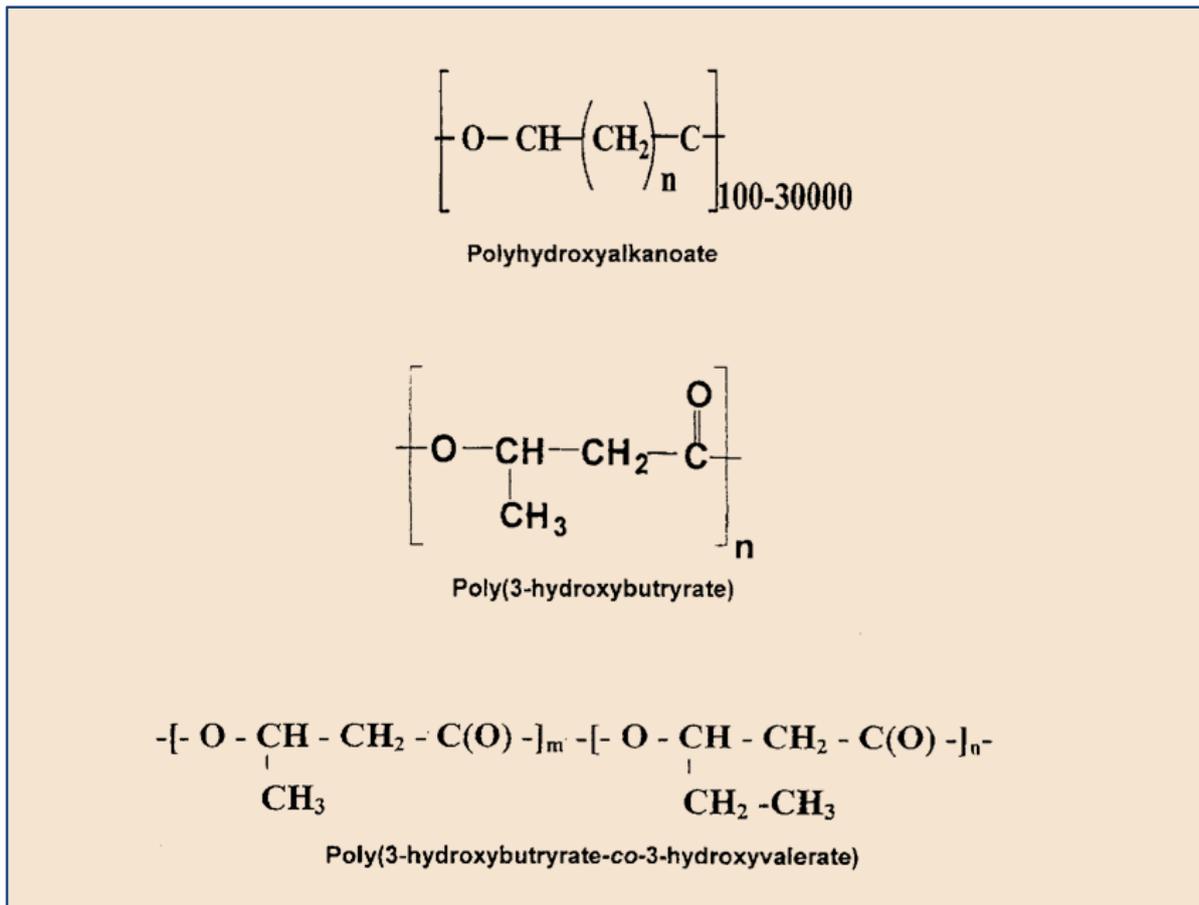
Figure 2. Structure of biodegradable plastic polyhydroxyalkanoates (PHA) and its derivatives poly(3-hydroxybutyrate) PHB and poly(3-hydroxybutryrate – co-3-hydroxyvalerate) Adapted from Shah *et al*., 2007 [18]
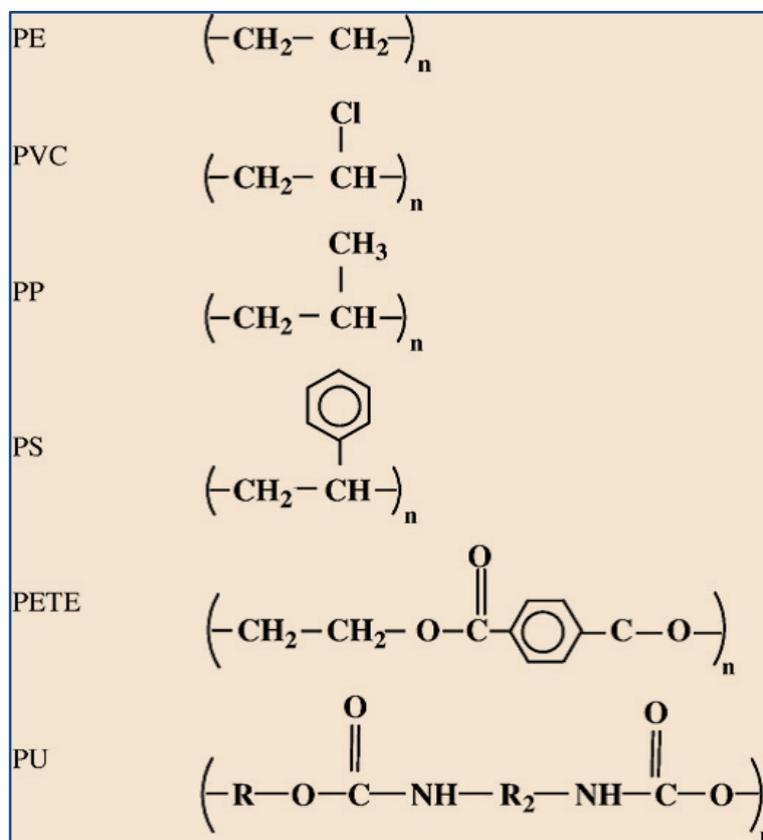

Figure 3. Chemical structures of petrochemical plastics Polyethylene (PE), Polyvinyl chloride (PVC), Polypropylene (PP), Polystyrene (PS), Polyethylene Terephthalate (PET) and Polyurethane (PU). Adapted from Shah *et al*., 2008 [18]

2.4. Chemical Structure

Synthetic Plastics are classified according to the characteristics of the reactions by which they are formed. If all atoms in the monomers are incorporated into a polymer, the polymer is called an *addition polymer*; if some monomer atoms are released into small molecules, such as water, the polymer is called a *condensation polymer*. Most addition polymers are made from monomers containing a double bond between carbon atoms. Such monomers are called *olefins*, and most commercial addition polymers are *polyolefins*.

Condensation polymers are made from monomers that have two different groups of atoms that can join together, such as ester or amide links. They include polymers like polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyurethane and polyethylene terephthalate, shown in Figure 3.

2.5. Manufacturing and Uses

Plastics are relatively very low-cost, durable, and very easy to manufacture. The following table (Figure 4) describes some commonly-used plastics and their applications [19].

Plastic Recycling Symbol	Plastic Name	Where to Find This Plastic in Your Home	This Plastic is Valued For
1 PETE	Polyethylene Terephthalate	water and soda bottles	clarity, strength, impermeability to gas and moisture
2 HDPE	High Density Polyethylene	milk jugs, grocery bags and toiletry bottles	stiffness, strength, resistance to moisture, permeability to gas
3 V	Polyvinyl Chloride	water pipes, blister packaging for non-food items	strength, ease of blending with other materials, versatility
4 LDPE	Low-density Polyethylene	food bags, squeezable bottles, cling films, disposable cups	flexibility, ease of processing, ease of sealing, barrier to moisture
5 PP	Polypropylene	microwaveable containers, yogurt cups, disposable plates / cups	strength, resistance to heat, chemicals, oils and moisture
6 PS	Polystyrene	disposable plates, cups, cutlery, containers and packing peanuts	clarity, versatility, molding ease
7 OTHER	Other (often Polycarbonate or ABS)	beverage bottles, CD's, lenses for glasses, riot shields	properties dependent upon the mixture of polymers, may contain BPA

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**Figure 4.**

3. Biodegradation

Plastics can degrade via different mechanisms: thermal, chemical, photo and biological degradation. The degradation of plastics is a physical or chemical change in polymers that occurs as a result of environmental factors, like light, heat, moisture, chemical conditions or biological activity [20]. Biodegradation is a bio-chemical process that refers to the degradation and assimilation of polymers by living microorganisms, to produce degradation products [21].

3.1. Biodegradation of Plastics

Biodegradation is defined as any physical or chemical change in a material caused by biological activity. Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics. Plastics are usually biodegraded aerobically in nature, anaerobically in sediments and landfills and partly aerobically in compost and soil. Carbon dioxide and water are produced during aerobic biodegradation, while anaerobic biodegradation produces carbon dioxide, water and methane [22].

3.1.1. Aerobic Biodegradation

Also known as aerobic respiration, aerobic biodegradation is an important part of the natural attenuation of contaminants in many hazardous waste sites. Aerobic microbes use oxygen as an electron acceptor, and break down organic chemicals into smaller organic compounds. CO_2 and water are the by-products of this process [23].

$$C \text{ plastic} + O_2 \rightarrow CO_2 + H_2O + C \text{ residual} + Biomass$$
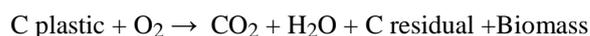
3.1.2. Anaerobic Biodegradation

Anaerobic biodegradation is the breakdown of organic contaminants by microorganisms when oxygen is not present. It is also an important component of the natural attenuation of contaminants at hazardous waste sites. Some anaerobic bacteria use nitrate, sulphate, iron, manganese and carbon dioxide as their electron acceptors, to break down organic chemicals into smaller compounds.

$$C \text{ plastic} \rightarrow CH_4 + CO_2 + H_2O + C \text{ residual} + Biomass$$

Microorganisms are unable to transport the polymers directly through their outer cell membranes, into the cells where most of the biochemical processes take place, since polymer molecule are long and not water-soluble. In order to use such materials as a carbon and energy source, microbes developed a strategy in which they excrete extracellular enzymes that depolymerize the polymers outside the cells [24].

Anaerobic and aerobic biodegradation mechanism pathways are given in Figure 5. Extracellular and intracellular depolymerize enzymes are actively involved in biological degradation of polymers. During degradation, microbial exoenzymes break down complex polymers, yielding short chains or smaller molecules like oligomers, dimers and monomers. These molecules are small enough to be water-soluble, and can pass through the semi-permeable outer bacterial membranes to be used as carbon and energy sources. This initial process of breaking down polymers is called depolymerization; and when the end products are inorganic species (e.g., CO_2, H_2O, or CH_4), the degradation is called mineralization [24].
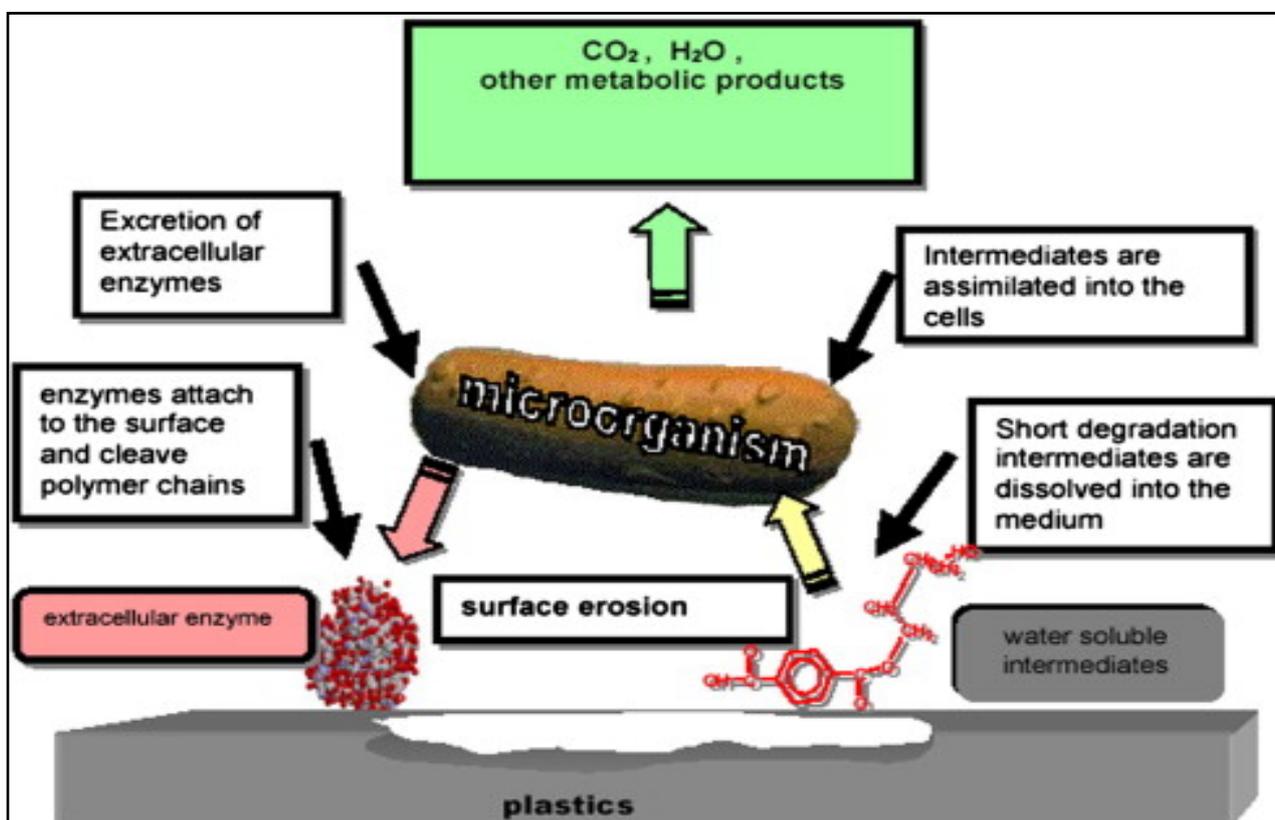

Figure 5. The General Mechanism of Plastic biodegradation under Aerobic Conditions [24]
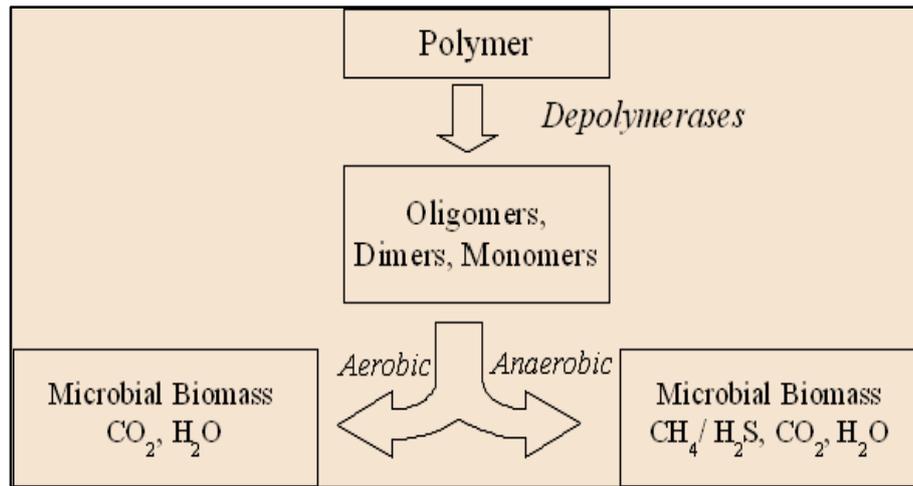
Figure 6. Reaction pathways during biodegradation of polymers

3.2. Mechanism of Biodegradation

Biodegradation of polymers involves following steps:
1. Attachment of the microorganism to the surface of the polymer.
2. Growth of the microorganism, using the polymer as a carbon source.
3. Ultimate degradation of the polymer.

Microorganisms are able attach to a polymer's surface, as long as the latter is hydrophilic. Once the organism is attached to the surface, it is able to grow using the polymer as its carbon source. In the primary degradation stage, the extracellular enzymes secreted by the organism cause the main chain to cleave, leading to the formation of low-molecular weight fragments, like oligomers, dimers or monomers. These low molecular weight compounds are further used by the microbes as carbon and energy sources. Small oligomers may also diffuse into the organism and get assimilated in its internal environment. These reaction pathways are illustrated in Figure 6 [25].

3.3. Factors Affecting Biodegradation of Plastics

The biodegradability of a polymer is essentially determined by the following physical and chemical characteristics:
1. The availability of functional groups that increase hydrophobicity (hydrophilic degradation is faster than hydrophobic).
2. The molecular weight and density of the polymer (lower degrades faster than higher).
3. The morphology of TM: amount of crystalline and amorphous regions (amorphous degrades faster than crystalline).
4. Structural complexity such as linearity or the presence of branching in the polymer.
5. Presence of easily breakable bonds such as ester or amide bonds. Chain coupling (ester > ether > amide > urethane).
6. Molecular composition (blend).
7. The nature and physical form of the polymer (e.g., films, pellets, powder or fibers).
8. Hardness (Tg) (soft polymers degrade faster than hard ones) [26,27,28].

3.4. Biodegradation of Synthetic Plastics

3.4.1. Polyethylene (PE)

Polyethylene is a stable polymer that consists of long chains of ethylene monomers; it cannot be degraded easily by microorganisms. However, it has been reported that lower molecular weight PE oligomers (MW=600–800) can be partially degraded by *Actinobacter* spp. upon dispersion, while high molecular weight PE could not be degraded [13].

The biodegradation of PE is a very slow process. A wide variety of Actinomycetes like *Streptomyces* strain and fungi like *Aspergillus* and *Penicillium* have been used in research to facilitate this process. El-Shafei *et al.* (1998) investigated the ability of fungi and Streptomyces strains to attack degradable polyethylene that consisted of disposed-of polyethylene bags containing 6% starch [29]. They isolated eight different strains of Streptomyces and two fungi *Mucor rouxii* NRRL 1835 and *Aspergillus flavus.*

Yamada-Onodera *et al.* (2001) studied a strain of fungus, *Penicillium simplicissimum* YK, that can biodegrade polyethylene without additives [30]. Ultraviolet light or oxidizing agents, such as a UV sensitizer, were used at the beginning of the process to activate an inert material, polyethylene. Polyethylene was also treated with nitric acid at 80°C for six days before cultivation with inserted functional groups that were susceptible to microorganisms. With fungus activity, polyethylene with a starting molecular weight in the range of 4000 to 28,000 was degraded to units with a molecular weight of 500, after a month of liquid cultivation. This indicated the successful biodegradation of this polyethylene. Overall, polyethylene degradation is a combined photo- and bio-degradation process. First, either by abiotic oxidation (UV light exposure) or heat treatment, essential abiotic precursors are obtained, allowing selected thermophilic microorganisms to degrade the low molar mass oxidation products.

The biodegradation of polyethylene is known to occur by either Hydro-biodegradation and Oxo-biodegradation. These two mechanisms can be used because of two additives, starch and pro-oxidant, used in the synthesis of biodegradable polyethylene. Starch blended polyethylene has a continuous starch phase that makes the material hydrophilic, and therefore allows it to be catalyzed by amylase enzymes. Microorganisms can easily access,

attack and remove this section, thus the polyethylene with the hydrophilic matrix continues to be hydro-biodegraded. If a pro-oxidant additive was used, biodegradation occurs following photodegradation and chemical degradation. If the pro-oxidant is a metal compound, after transition-metal catalyzed thermal peroxidation, biodegradation of low molecular weight oxidation products occurs sequentially [31]. The process is depicted in Figure 7.

3.4.2. Polypropylene (PP)

Polypropylene is a thermoplastic that is commonly used for plastic mouldings, stationary folders, packaging materials, plastic tubs, non-absorbable sutures, diapers, etc. It can be degraded by exposure to ultraviolet radiation from sunlight, and it can also be oxidized at high temperatures. The possibility of degrading PP with microorganisms has also been investigated [33].

Even though PP is a polyolefin, and thus prone to oxidative degradation like PE, the substitution of methyl for hydrogen in the ß position makes it more resistant to microbial attacks, as previously discussed in the section dealing with factors that affect biodegradability (namely structural complexity). The decreasing order of susceptibility of polymers to degradation in soil mixed with municipal refuse was PE > LDPE > HDPE. This was revealed by analyzing sample weight loss, CO_2 evolution, changes in tensile strength, and changes in FTIR and bacterial activity in the soil.

Studies reported on biodegradation of PP, many microbial communities such as certain fungal species like *Aspergillus niger* and bacteria such as *Pseudomonas* and *Vibrio* have been reported to biodegrade PP. A decrease in viscosity and the formation of new groups, namely carbonyl and carboxyl, were observed during the degradation process [34].
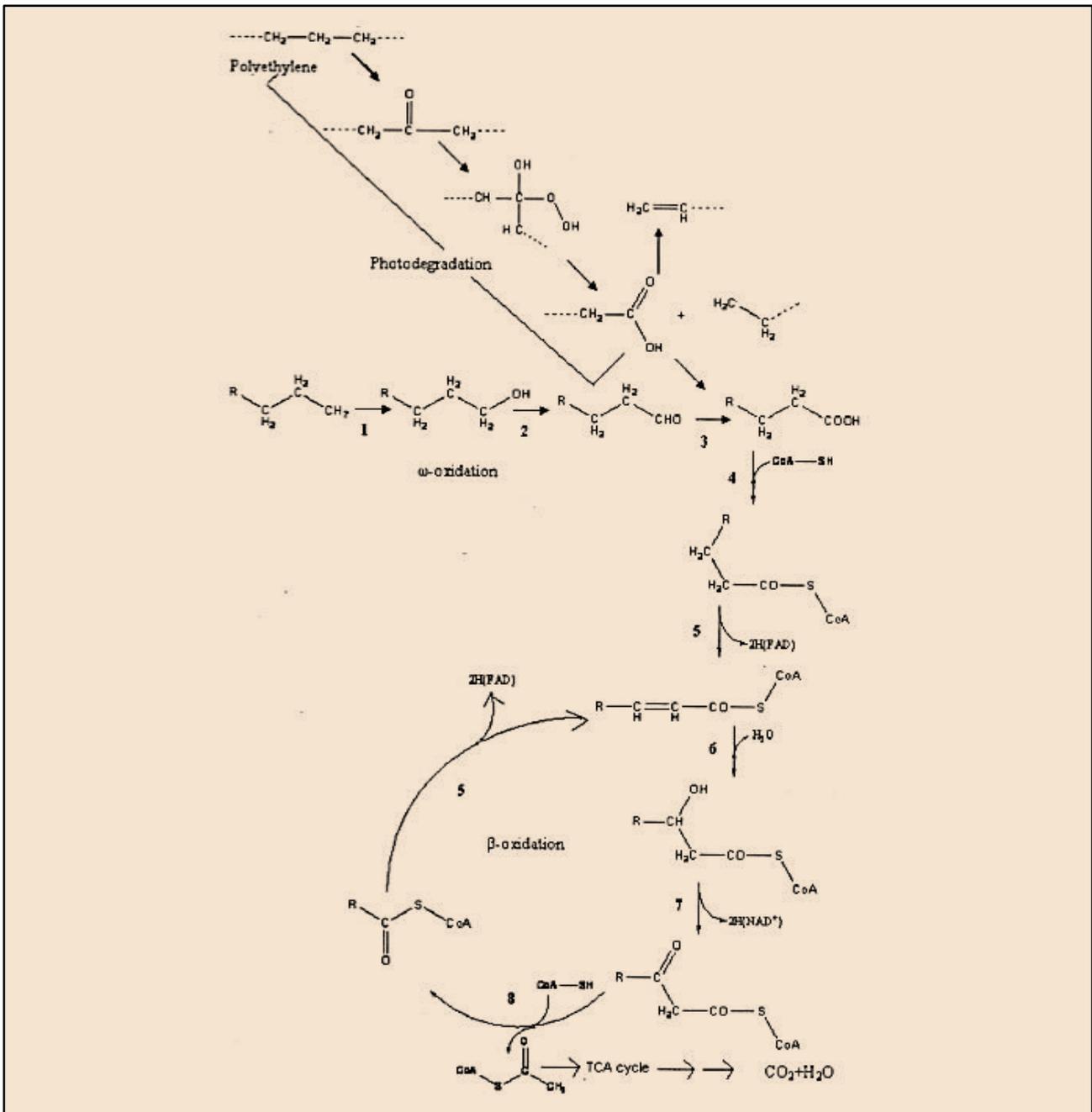

Figure 7. The Mechanism of biodegradation of polyethylene. Adapted from Vasile, 1993 [32]

3.4.3. Polystyrene (PS)

Polystyrene is a synthetic hydrophobic polymer with a high molecular weight. It is recyclable, but not biodegradable; and although it has been reported that PS film was biodegraded by an Actinomycetes strain, the degree of this biodegradation was very low [35].

Kaplan *et al*. (1979) investigated the biodegradation of Polystyrene with seventeen species of fungi capable of degrading plastics. They found low decomposition rates, even though the addition of cellulose and minerals increased these decomposition rates significantly [36]. Lyklema *et al*. (1989) studied the adhesion of an *Arthrobacter* species, *E.coli,* Micrococcus and *Psuedomonas* on Polystyrene film. Adhesion was followed microscopically and radio metrically [37].

3.4.4. Polyvinyl Chloride (PVC)

Polyvinyl chloride is a strong plastic that resists to different factors such as abrasion and chemicals, and has low moisture absorption; there are many studies about thermal and photo degradation of PVC, but only a few reports on its biodegradation. According to kirbas *et al*. (1999), PVC's low molecular weight can be exposed to biodegradation by white rot fungi [38].

3.4.5. Polyethylene Terephthalate (PET)

Polyethylene terephthalate has different properties. It is a semi crystalline polymer ,and chemically and thermally is a stable .The molecular weight of this polymer range from 30,000 to 80,000 $gmol^{-1}$ (39). Sharon *et al.* (2012) studied the degradation of PET transparency sheets by microbes and Esterase enzyme, and detected important chemical changes of polymeric chains by X-ray photoelectron spectroscopy (XPS) analysis. Microbial degradation affect crystalline structure and a presence of microbes inside the polyethylene terephthalate were seen as well, using scanning electron microscopy (SEM) micrographs [40].

3.5. Biodegradation of Natural Plastic

Biomaterials are natural products, synthesized and catabolized by different microorganisms, which have been found to have broad biotechnological applications. They can be assimilated by many types of species (biodegradable) and do not cause toxic effects in the host (biocompatible).

Bioplastics are a special type of biomaterial. They are polyesters produced by different microorganisms, and cultured under different nutrient and environmental conditions [41]. These polymers, usually lipid in nature, are accumulated as storage materials and allowing microbial survival under stress conditions. The number and size of the granules, the monomer composition, macromolecular structure and physicochemical properties vary widely, depending on the producer organism. As depicted in Figure 8, they can be observed intracellularly as light refracting granules or as electron lucent bodies, which, in overproducing mutants, cause a striking alteration of the bacterial shape [42].

3.6. Biodegradation of Polyhydroxyalkanoates (PHB & PHBV):

Microorganisms utilize Poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-valerate) (PHBV) as energy sources, thus allowing these polymers to be biodegraded in microbial active environments. Microbes colonize the polymer surface and secrete enzymes that degrade PHB into HB (hydroxybutyrate) and PHBV into HB and HV (hydroxyvalerate) segments. These fragments are used as a carbon source by the cells, for the purpose of growth.

The rate of polymer biodegradation depends on a variety of factors, including surface area, microbial activity of the disposal environment, pH, temperature, moisture and the presence of other nutrient materials. Aerobic and anaerobic microorganisms that degrade PHA, particularly bacteria and fungi, have been isolated from various environments [43].

Luzier (1992) isolated different strains of soil bacteria and fungi, such as Acidovorax facilis, Aspergillus fumigatus, Comamonas spp., Pseudomonas lemoignei and Variovorax paradoxus [44]. Moreover, Alcaligenes faecalis and Pseudomonas have been isolated from activated sludge, Comamonas testostroni has been found in seawater and Llyobacter delafieldii is present in the anaerobic sludge. PHA degradation by Pseudomonas stutzeri has also been observed in lake water. Since a microbial environment is required for degradation, PHA is not affected by moisture alone and is indefinitely stable in air.

Lee *et al*. (2005) investigated the degradation of PHB by fungi samples collected from various environments. PHB depolymerization was tested in vials filled with a PHB-containing medium, which were inoculated with isolates from the samples. The degradation activity was detected by the formation of a clear zone below and around the fungal colony. In total, 105 fungi were isolated from 15 natural habitats and eight lichens, among which 41 strains showed PHB degradation [17].

Most of these were deuteromycetes (fungi imperfect) resembling species of *Penicillium* and *Aspergillus,* and were isolated mostly from soils, compost, hay and lichens. Soil-containing environments were the habitats from which the largest numbers of fungal PHB degraders were found, but other organisms involved in PHB degradation were also observed. A total number of 31 bacterial strains out of 67 isolates showed clear zones on assay medium. Protozoa, possible PHB degraders, were also found in several samples from ponds, soil, hay, horse dung and lichen. Lichen, a fungi and algae symbiosis, was an unexpected sample from which fungal and bacterial PHB degraders were isolated [17].

Tokiwa and Jarerat (2003) investigated the distribution and phylogenetic affiliation of polymer-degraders among actinomycetes obtained from culture collections. PHB-degraders were widely distributed among the families of Pseudonocardiaceae and the related genera micromonosporaceae, Thermonosporaceae, Streptosporangiaceae and Streptomycetaceae [45]. Finally, Tansengco and Tokiwa (1998) investigated the biodegradability of *Bacillus* spp. TT96, Thermotolerant *Aspergillus* ST-01 and *Strptomyces* strain MG respectively, against the PHAS [46].
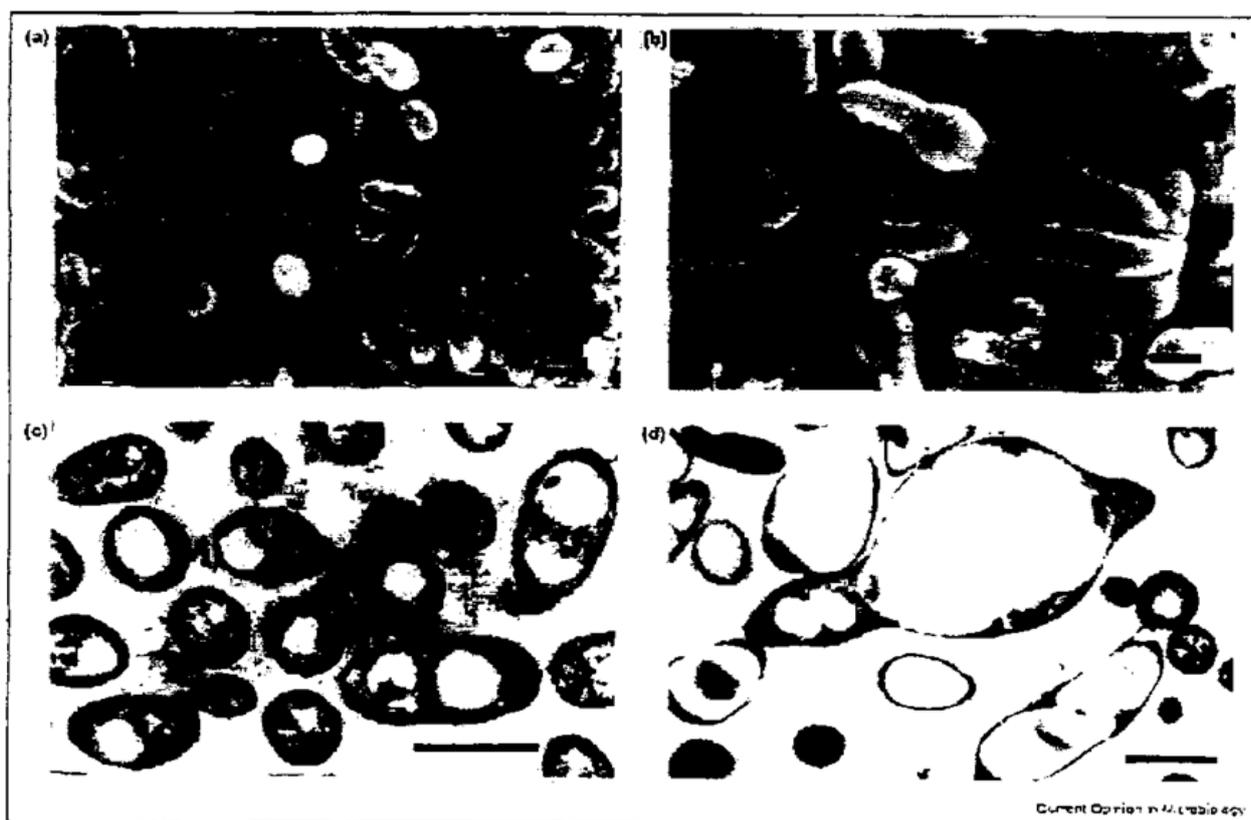
Figure 8. Scanning (a,b) and transmission (c,d) electron micrograph of *Pseudomonas putida* U (a,b) and its *fad*BA β-oxidation mutant (b,d) cultured in a chemically defined solid medium containing 7-phenlhepanoic acid (5mM) as a source of aromatic PHAs and 4-phenlhepanoic acid (5mM) as an energy source. Bar = 1 μm (*Luengo* et al. 2003)

3.7. Enzymatic Degradation of Bioplastics

Microorganisms that produce and store PHA under nutrient-limited conditions can degrade and metabolize it when the limitation is removed [47]. However, the ability to store PHA does not necessarily guarantee the ability to degrade it in the environment [48]. Individual polymers are too large to be transported directly across the bacterial cell wall, therefore, bacteria need to secrete extracellular hydrolases capable of converting the polymers into corresponding hydroxyl acid monomers [47,48].

The product of PHB hydrolysis is R-3-hydroxybutyric acid (49), while the extracellular degradation of PHBV yields both 3-hydroxybutyrate and 3-hydroxyvalerate [44]. The monomers are water soluble, but small enough to passively diffuse through the cell wall, where they are metabolized by β-oxidation and tricarboxylic acid cycle (TCA), to produce carbon dioxide and water under aerobic conditions [50]. Under anaerobic condition, methane is also produced [44]. In general, no harmful intermediates or by-products are generated during PHA degradation [43], as depicted in Figure 9.

The enzymatic degradation of polymers by hydrolysis is a two-step process, where the enzyme binds to the polymer substrate, and then subsequently catalyses Intracellular and Extracellular depolymerases in PHB/PHBV-degrading bacteria and fungi. Intracellular degradation is the hydrolysis of an endogenous carbon reservoir by the accumulating bacteria themselves, while extracellular degradation refers to the utilization of an exogenous carbon source but not necessarily by the accumulating microorganisms [51].

Polyhydroxyalkanoate-degrading PHA microorganisms secrete PHA depolymerases, which hydrolyze the polymer extracellularly to water-soluble products and utilize the hydrolysis products as carbon and energy sources for growth [52,53].

3.8. Biodegradability Tests

The analytical tools used to monitor the biodegradation process include:
1. Visual observations: The evaluation of visible changes in plastics can be performed in almost all tests. Effects used to describe degradation include the roughening of the surface, formation of holes or cracks, de-fragmentation and changes in colour or formation of biofilms on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack. To obtain information about the degradation mechanism, more sophisticated observations can be made using either scanning by using SEM, transmission optical microscopy or atomic force microscopy AFM [55].
2. Change in the physical properties of a polymer such as density, contact angle, viscosity, Molecular weight distribution (using GPC), melting temperature (Tm), glass transition temperature (Tg) with TGA and DSC and changes in the crystalline and amorphous regions by using X-ray diffraction, SAXS and WAXS [56]. Biodegradability is also evaluated by weight loss, tensile strength loss, changes in present

elongation and change of polymer molar mass [57,58].

3. Changes in the chemical properties of the polymer in synthetic media, including the formation or disappearance of functional groups as determined by FTIR, can be measured. The molecular weight and molecular weight distribution of the degraded products or intermediates are observed by techniques such as TLC, GCMS and NMR [34].

4. CO_2 evolution / O_2 consumption: Under aerobic conditions, microbes use oxygen to oxidize carbon, and form carbon dioxide as a major metabolic end product. Consequently, the consumption of oxygen (respirometry test) or the formation of carbon dioxide (strum test) is the most often used method to measure biodegradation in laboratory tests, as it gives direct information on the bio-conversion of the carbon backbone of the polymer to metabolic end product [59].

5. Anaerobic microorganisms predominantly produce a mixture of CO_2 and methane, called biogas, as an extracellular product of their metabolic reactions. This can be tested by using Gas Chromatography, or manually by titrating with barium hydroxide [60,61].

6. Radiolabeling is used particularly for investigating slowly degradable materials in a matrix containing carbon sources other than the plastics [62].

7. Biological tests: A very simple semi-quantitative method is the 'clear-zone' test. This is an agar plate test in which the polymer is dispersed as very fine particles within the synthetic medium agar, which results in the agar having an opaque appearance. After inoculation with microorganisms, the formation of a clear halo around the colony indicates that the organisms are at least able to depolymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain polymer [63,64]. Measurements of clear-zone formation in agar plates and the metabolic activity of the cells in the culture and in the biofilm can be done by ATP assays, protein analysis and FAD analysis [34].
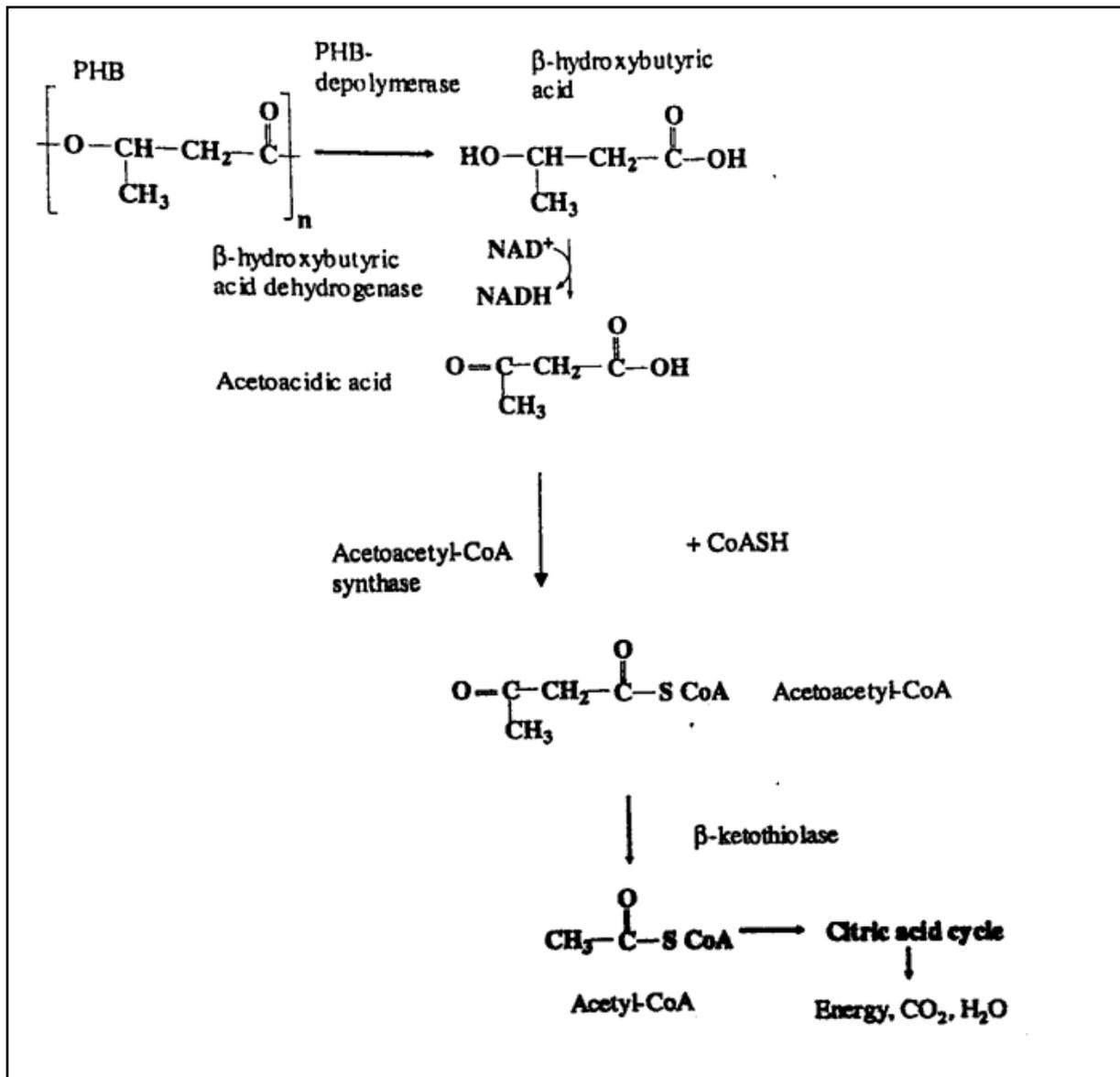

Figure 9. Biodegradation of Poly hydroxy butyric acid [54]

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