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Biodegradation of PEGs: A review

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ABSTRACT

A variety of applications involving animals and humans make use of polyethylene glycol (PEG). Because the toxicity of various PEGs is becoming increasingly apparent and has been documented several times, their removal from polluted regions is urgently required. Several studies have shown and advocated that PEG biodegradation by microorganisms may be a viable approach for remediating PEG-contaminated landfills. As a result of Malaysia's huge number of landfills, which is likely to expand in the future, it is believed that PEG contamination would rise as well, and the use of PEG-degrading microorganisms may benefit the country. This review aimed to summarize and update information on PEG-degrading microorganisms so that the information compiled can benefit local researchers in isolating more efficient PEG-degraders. The absorption of PEGS into energy under aerobic and anaerobic systems includes a variety of enzyme chains, which will present a challenge that needs to be addressed in future isolation of PEG-degraders and the elucidation of the degradation mechanisms.

INTRODUCTION

Polyethylene glycols is utilised in various areas are hydro-soluble polymers with a common HO (CH_2CH_2O)nCH_2CH_2OH, which vary in molecular weights solely from one another. These polymers in industrial goods are so widespread that they are regarded a significant contaminant along with household trash in the environment [1]. As a result, the number and quality of human sperm, genital anomalies and the incidence of breast cancer attributable to oil-based polymers have decreased. One of the chemical by-products thought to be transmitted to women feeding their boys via breast milk is dioxin, a highly carcinogenic and poisonous by-product of the process of plastics production. The combustion of plastics, in particular PVC releases dioxins and furans into the environment. Dioxin may also be absorbed in soil and water and the water is contaminated and harmful. Therefore, traditional plastic is a significant environmental issue from production through disposal. The need for biodegradable polymers continues to grow with rising awareness of waste issues and their effects on the environment [2–10].

In addition, some researchers are looking for microorganisms capable of degrading current non-biodegradable polymers such as polyvinyl chloride, polyethylene, etc. The method in which nature recycles trash or breaks organic matter into nutrients which may be utilised by another creature is biodegradation. "Degradation" implies decay, and the prefix of the organic signifies a wide variety of dead matter-eating, recycled bacteria, fungus, insects, worms and other creatures. In nature, there is no waste since everything is recycled where waste products of one organism are food for another and provide nutrients and energy when organic waste is broken down. It is a cyclical process, in which organic elements break down much

more quickly than others, but ultimately everything will deteriorate [11–17].

The biodegradation of PEG by microbes has been documented and has been suggested as the future method to remediate PEG-contaminated landfills [1,18]. Since landfills in Malaysia is numerous and would continue to increase, the contamination of PEG s expected to be increased as well. Thus, bioremediation organism must be prepared. However, the usage of foreign microbes for bioremediation has potential ecological problems and is usually outcompeted by indigenous bacteria and thus is not recommended. Hence, screening of local PEG-degrading microbes must be carried out. In this thesis, the first PEG-degrading bacterium from Malaysian soils is presented.

Polyethylene Glycol

In the European pharmaceutical sector polymerization of ethylene oxide (EO, Ethylene Glycol), which is known as the macrogols, are produced as an initial material under alkaline catalysis, either water and mono-ethylene glycol or diethylene glycol. The reaction is stopped by acid neutralising the catalyst once it has achieved the appropriate molecular weight, typically controlled via viscosity measurements as in-process control. Lactic acid is usually utilised, although it may also be found in acetic acid or others. The result is a relatively basic structure of chemical: N-H where (n) the number of e-units is HO-[CH2-CH2-O] n-H [19–22].

Nomenclature of Polyethylene Glycol

Although polyethylene oxides should technically be referred to, polyethylene glycols are usually used to refer to the substantial impact of hydroxyl end groups on the chemical characteristics and the physical properties of these molecules for products with medium molecular weights of 200 to 35,000. Polyethylene oxides are referred to only products produced by polymerisation of ethylene oxides in solvents and with molecular weight up to a million. The word "PEG" in conjunction with a number value is used as an acronym for polyglycols. The number shows the mean molecular weight within the pharmaceutical business, whereas the number in the cosmetics industry shows the number (N) of EO-units in the molecule [23–25].

As ethylene oxide is 44 in weight, the PEGs are given round values of N*44 for average molecular weight values. Unfortunately, for certain PEG molecular weights, many pharmacopoeia use alternative names. **Table 1** includes the nomenclature of British Pharmacopeia II from 1993, in addition to the European, United States and Japanese monographs. The terminology is frequently still used today, even though the monograph is no longer valid. It is anticipated that a distinct and less complicated nomenclature will be created via the worldwide harmonisation of the pharmacopoeia [26].

Table 1. Nomenclature of Polyethylene Glycol (PEG) (from [27])

Mean Mwt.	Clariant Polyglykol	Nomen-clature European Ph. 2001	Nomen-clature USP 24/NF 19	Nomen-clature Japanese Ph. XIII	Nomen-clature British Ph. II
1500	1500 S	1500	1500	-	1540
3000	3000 S or P	3000	3000	-	-
3350	3350 S or P	3350	3350	4000	4000
4000	4000 S or P	4000	4000	-	-
6000	6000 S or P	6000	6000	-	-
8000	8000 S or P	8000	8000	6000	-

Polyethylene Glycol Properties

Non-volatile fluids at room temperature are polyethylene glycols with medial molecular weight up to 400. PEG 600 at 17 to 22°C is liquid at room temperature but pasty at lower ambient temperature, whereas PEGs with a medium molecular weight of 800 to 2000 are pasty, low melting materials. The polyethylene glycols are solid over 3000 molecular weight and are not only accessible as flak but as powder. Its solubility in water is the most significant characteristic of all PEGs, which makes them excellent for usage in many applications. In any ratio of water, liquid PEGs up to PEG 600 is miscible. However, even solid grades of PEG have high water solubility. Although the molar mass decreases somewhat, even 50% (w/w) of the PEG 35,000 may be dissolved in water at normal temperature. The solution solubility and viscosity of the electrolytes, since the PEGs are noionic compounds, are not impacted [19,20,28,29].

In hard water or other aqueous solutions of different sales, PEGs are very soluble. In some PEGs, precipitates such as phenol, cresol, resorcinol, salicylic acid, tannins, iodine, tetraiodide, or mercury chloride are formed together with other compounds. Some of these reactions may be used to identify or quantify polyethylene glycols. Furthermore, liquid PEGs may be utilised to remove some of the harmful chemicals from burnt skin as antidotes. The solvent capacity of many compounds which are water-sparingly soluble is another extremely significant characteristic. The development of a compound between polyethylene glycol and the active chemical may be attributed to this activity. Polyethylene glycol has excellent acute and chronic oral toxicity, embryotoxicity or compatibility of skin. For many years they have been utilised in the cosmetics, food and pharmaceutical sector. All relevant pharmaceuticals are registered. Acceptable daily intake of polyethylene glycols (ADI) in foodstuffs with a maximum body weight of up to 10 mg/kg [21,28–30].

The Applications of Polyethylene Glycol

Polyesthers have a long history as speciality polymers used in their primary form in lubricants, anti-colding agents, ink and cosmetics as raw materials for synthesisation. They either are water-soluble or oily fluids that ultimately enter the systems of environment or wastewater. PEG is known as polyesters in large quantities and is produced in polyesters, most of which are utilised in order to synthesise nonionic surfactants as well as in various industrial products such as anti freezing agents, pharmaceutical products, cosmetics as well as lubricants as commodity chemicals. PEG is produced in copolymers in blocks and utilised as surfactants amphoteric.

Aliphatic ether bonds are very stable from a chemical point of view. In addition, the xenobiotic molecules PEG and other polyesters are genuine, given that in nature there is no tight relationship. Fortunately, no reports were discovered on polyester buildup in nature, indicating that microbes are completely metabolised [23,31]. In the pharmaceutical business, polyethylene glycol plays an essential function. The comparatively small melting point promotes a method for sintering or compression. The PEG also has a plastifying action which makes the tablet weight easy to form and may counteract capping during the compression process. In tablet coatings, solid PEGs are also often utilised. PEG is also directly tolerogenic in the transplanted organ on donor antigen [32].

Toxicity of Polyethylene Glycol

Polyethylene Glycols are neutral, molecule-soluble polymers available in a broad range of molecular weight. Many researchers have employed them as an osmotic agent for whole plants or for plant tissues, cells and organelles. The use of PEG has become a common method for reducing nutrient solutions' water potential for predefined water stress without plant consumption. Many of the researchers successfully used it to different animals where a reduction in osmotic potential without any harmful adverse consequences was ascribed to PEG responses. However, its toxicity to plants was a fairly frequent issue with PEG. Sometimes this kind of toxicity has been attributed to metal ions such as aluminium or an ionic organic molecule. While ion exchange resins, gel filtration or dialysis may be used to remove these pollutants, toxicity was not always avoided. Plant roots may not be totally impermeable for PEG, and the toxicity of the roots may be caused by intake have shown that the transport takes place without breaking down and molecular size defines the pace and location of its transport and is hypothesised that PEG impede the water route and thus promote drying. Inhibited phosphorus transport throughout the xylem root was ascribed to toxic effects of PEG [33–36]. Mexal et al. indicated a poor O2 solubility even in diluted PEG levels and a delayed transfer of O2 to root produced the major harm to plants induced by PEG [37].

Besides their toxicity on entire plants, polyethylene glycols seem to be sluggish to act parasympathomimetic substances in general. When taken intravenously, the propensity of blood to coagulate is increased and, when administered quickly, the cells may get clumped and embolization leads to death. Long contact with skins of PEG 1500 and 400 in the rabbit's skin was not harmful at doses 10 g/kg of body weight, and little, if any, of the matter was absorbed by the skin.

While early Smtyh et al. study in the 1950s [38] demonstrated skin sensitivity in a few humans and in guinea pigs tested with specific polyethylene glycols, subsequent tests revealed that materials presently manufactured are neither irritating or sensitising [39,40]. In a patient who has severe allergic reactions to several drugs on a regular basis, the cause of anaphylaxis based on an extensive diagnostic workup including skin prick tests, intradermal tests (IDT), and a double-blind oral challenge are carried out. An IDT confirmed hypersensitivity to the additive polyethylene glycol in this case, implying that the reaction was caused by an Immunoglobulin E-dependent mechanism [41].

Biodegradation

Biodegradation is a natural process in which micro-organisms are transformed into biological agents. In a basic way, it is a microorganism degradation process, a biological disruption of complex materials into a simpler inorganic component such as CO_2, H_2O, NH_3, Cl, SO_4 etc [42–46]. The method enables basic inorganic chemicals to be recycled. These atoms may be bonded in their complex structure without this process because they are non-biodegradable and unable to re-join the cycles of the ecosystems.

The actions of microorganisms may be helpful in the removal or at least the reduction of toxicity to organisms for dangerous chemicals. Soil factors such as temperature, moisture, aeration, pH and organic matter have a negative impact on microbial development and activity. microbiological degradation rate [47–53]. Compared to actinomycetes, fungus, alga, and protozoa, bacteria constitute the most prevalent soil microflora. This bacterium is found on the ground surface and multiply quickly and may be adapted to different surroundings. Therefore, natural and manufactured compounds present in the environment are essential to decompose and convert. In degrading dangerous substances like oil spill, polyaromatic hydrocarbons, heavy metals and others, the bacteria themselves play a major role. Many research have been conducted and a few xenobiotics degrading-bacteria are identified including *Pseudomonas* sp., *Ahcromobacter* sp., *Arthrobacterium* sp., *Ahcromobacter* sp., *Arthrobacter* sp. and many more were mentioned [47–53].

Fungi are the principal microorganisms found in the forest and acid surface layers, breaking down organic materials and producing the bulk of soil and organic biomass. As a potentially feasible biodegrading agent, fungi are able to convert a broad range of dangerous substances. Inhabitants of soils include two kinds of fungus, unicellular creatures called yeasts and multicellular filament fungi (for example moulds, smuts, rods and mildews. *Phanerochaete* sp., *Penicilium* sp. and *Aspergillus* sp. are the common fungus that are found in the biodegradation of hazardous xenobiotics like aromatic hydrocarbon, pesticides and dyes [54–62]. Actinomycetes seem like bacteria, but is also like filamentous fungus, with their thin branch filaments. In contrast to actinomycetes, the bacteria and fungus controlled the majority of soils, however, actinomycetes may break down animal and plant-tissue resistant compounds including cellulose, chitine and phospholipids and are engaged in the production of humic substances such as green manure, compost heaps, animal dung and other plant-based materials [63–70].

Bioremediation

A big environmental issue is generated by the fast industrial advances in the globe along with the widespread usage of synthetic goods. Human neglect and lack of care are damaging to the earth's ecology. In fact, azo dyes, heavy metals, polyaromatic carbon and chemical effluents accumulate and contaminate the environment as well as xenobiotics such pesticides for agriculture, oil spill offs and industrial wastes. The remediation of this issue is part of a decision to detoxify or at least reduce toxicity for chemicals in our environment. The treatment is based on bioremediation.

Bioremediation is a biodegradation concept that has been modified and developed using technology to clean up polluted areas. It is a natural process that includes living creatures, mostly microbes, in order to reduce the danger to the environment. This may extend from the simple degradation of hazardous chemicals in natural circumstances by indigenous microbes or the isolation from non-indigenous areas, giving optimum conditions to promote the degradation of substances by microorganisms [46,71–75]. As the knowledge of the whole topic improves, the efficacy and application of bioremediation is increasing quickly. More is known about microorganisms' interacting with the physical medium nowadays and methods for GMOs are constantly developing. Constantly optimised are the techniques used to discover and isolate biodegradation enzymes.

Dealing with Plastics Waste Management

On the average, each Malaysian produces approximately 0.8 kg rubbish daily with higher figures of about 1.5 kg rubbish per day for those living in towns. According to the statistical data produced by the Ministry of Housing and Local Government in 2001, 16,247 metric ton of rubbish is produced daily. The state of Selangor leads other states in Malaysia producing about 2,995 metric ton of wastes every day. This is followed by Kuala Lumpur, which produces 2634 metric ton per day. Johor is third with an amount of 2002 metric ton per day, Perak (1596/day) and Kedah (1383/ day) [76].

From the data mentioned above, the production of wastes or solid wastes are dependent on the size of the area, the number of residents and their activities. The type of wastes being produced depends on the area itself. The solid wastes that are being produced in the town area are more complex compared from the suburban area. The products of waste are in the form of papers, glass materials, woods, plastic materials, food wastes, aluminium and so forth. Many of the trash is either transported to the waste dump site or to incinerators with 161 waste disposal settlements in Malaysia. Both systems in Malaysia are extremely popular. There is the greatest number of sites in the state of Sarawak with 28 sites. Following are Johor (26 locations), Perak (18 locations), Pahang (14 locations) and Kelantan (12 sites). Although Malaysia has a lot of garbage deposits, the growing volume of waste is still not sufficient, and the majority of sites will be overwhelmed within two years. The site will raise the danger to the environment and to the people living around the site itself. In this specific region, for example, the waste dumps may influence the land system and water [77–79].

Currently, Malaysia uses a burning technique to solve the rubbish problem caused by the limited space. Currently there are 7 mini burning centers located at four islands namely Langkawi, Tioman, Pangkor and Labuan with each of these centers has a capacity to treat about 3-10 ton/day/unit. Approximately 1,500 tons of solid wastes can be managed by this future center daily. Meanwhile, Labuan also has another thermal center which is uses the "Thermal Oxidation" method and which can handle about 40 tons of solid wastes per day. The existence of this thermal or burning center could overcome the problems of solid wastes in Malaysia [77,80].

The ability of the thermal center to burn the rubbish is faster than the open dumping system. About 99 % of the solid waste are burnt using the high temperature of between 500-600 º Celcius. Through this method, half of the solid waste can be burnt. The ash that is being produced is approximately 99 % safe and can be buried in any landfill sites. Besides that, the thermal or burning method also destroys the microorganism inside the wastes to avoid any spread of diseases. At the same time, this system was built to take into other considerations other factors such as haze, bad smell and also the dioxin gas which will be attributed to the environment [77,80]. It is no wonder that more researchers all over the world are searching for new biodegradable polymer as well as scrambling for an answer to accelerate the biodegradation of existing recalcitrant wastes especially petroleum-based polymeric wasters more commonly known as plastics.

Microorganism and general degradation of polymer

The polymers of heterotrophic microorganisms, including bacteria and fungus, are known as potential substrate. Polymer biodegradability relies on the molecular weight, crystallinity and physical shape. An increase in molecular weight leads to a decrease in micro-organism polymer degradation. In contrast, a polymer repeating unit's monomers, dimers and oligomers are destroyed and extracted very readily. A significant decrease in solubility owing to the large molecular weight makes microbial attacks undesirable. The reason is that in bacterium, the PEG has to be absorbed through the membrane of the cell and then destroyed by cellular enzymes. It is important to note out that the deterioration of polymers may be facilitated by a competitor biological activity [81–88].

Extracellular and intracellular depolymerases are two categories of enzymes that are engaged in biological breakdown of polymers. During the depolymerization, microorganism exoenzymes break down complex polymers in order to produce short chains or smaller molecules like oligomers, dimers and monomers which are small enough to pass through semi-permeable bacterial external membrane and are then used as carbon and energy sources [89]. In contrast, deterioration in inorganic species such as CO_2, H_2O or CH_4 are known as mineralization in finishing goods. A widely known guideline is to breakdown and mineralize more closely a polymeric structure is comparable to a natural molecule. Polymers like cellulose, chitin and pullusan are bio-syntotic and may be totally and quickly biodegraded in a broad variety of natural environments via heterotrophic micro-organisms. In addition, natural circumstances include situations in which anaerobic activities are the most important breakdown of a polymer. The biodiversity and breakdown of polymer substrates may rarely approach 100% owing to the tiny part of the polymer that is integrated in the microbial biomass, humus and other natural products [81–88].

Often environmental factors influence the dominant species of bacteria and the degrading trajectories related with polymer degradation. Aerobic microorganisms with microbial biomass, CO_2, and H_2O, the end products, are mainly responsible when O_2 is present for the breakdown of complex material. Anaerobic microbe consortia are responsible, however, for the degradation of polymers in anoxic circumstances. In certain circumstances or H_2S, CO_2 and H_2O under sulfidogenic conditions the main products will be microbial biomass, CO_2, CH_4 and H_2O. Aerobic processes generate energy that is greater than anaerobic. Aerobic energy is able to sustain a larger population of microorganisms than anaerobic ones, since thermal O_2 is more efficient than SO_4 and CO_2 electron acceptors. These circumstances are quite common in natural settings and may be induced with suitable inoculations in the laboratory. Aerobic and strictly anaerobic micro-organisms are used for degrading polymers [3,84,86,90–93] (**Fig. 1**).

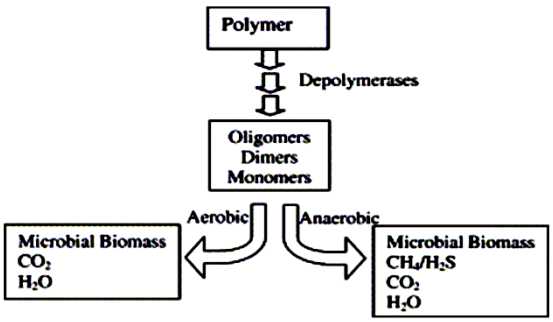

Fig. 1. Schematic diagram of polymer degradation under aerobic and anaerobic conditions.

Microbial degradation of PEG

Polyethers microbiological breakdown on PEG using aerobic bacteria was investigated [1]. There have been several reports of PEG aerobics using bacteria on PEG of different molecular sizes, well-described examples of which may be found in the sphingomonad genus. Fincher and Payne reported on a PEG 400-using bacteria for the first time in 1962. Diversity occurs for bacteria in regard of the maximal size of the degradable PEG molecule [18]. Different microorganisms recognised or not identified and activated sludges were absorbed into PEG up to 4,000. A limited number of species assimilated the PEG with a molecular weight of more than 4,000: *P. aeruginosa* up to 20,000; soil bacteria up to 6000; *P. stutzeri* up to l3,500 *Pseudomonas* spp. and *Sphingomonas* species down to 20,000. PEG was assimilated by a limited number of species. The PEG

of more than 6,000 has been fully digested by *Sphingomonas terrrae* and its associate consortia (Rhizobium, Agrobacterium and Methylobacterium spp.) [94]. [95]. Instead of lipopolysaccharides in most Gram-negative bacteria, sphenolipids are present in their outer membranes. Various lipophilic, xenobiotic, assimilating, lipophilic, α-hexachlorocyclohexane, 2,4-dichloro-phenoxyacetic, dibenzo-p-dioxine and diphenyl ethereal, were included in the genus: (poly)chlorofenol, polycyclic aromatic hydrocarbons [96].

Although there may be a barrier to the macromolecules for polyethers, the distinctive membrane structures of this genus may be linked with the use of membranes to take up big PEGs. This has also been supported by results of the fact that *Rhodopseudomonas acidophila* M402 can use its alcohol dehydrogenase to oxidise PEGs to a certain size, but cannot be grown on these compounds, which allows the dehydrogenation of high PEGs (6,000 and 20,000) that could not be used as single energy and carbon extracts of 400-1,000 or 4,000-deposit bacteria [97]. Kohlweyer et al. reported that *Pseudiocardia* sp. strain K1, a Gram-positive actinomycete was also identified to grow at 4,000 and 8,000 PEGs, initially being insulated as a tetrahydrofuran degrader. It is an intriguing issue whether actinomycete has the same metabolic track and mechanism of larger PEG absorption, as is discovered in sphingomonads, and the only Gram positive bacteria that can metabolize PEG [98].

Three research groups reported anaerobic assimilation of PEG. The *Alcaligenas faecalis* var. denitrifiers from the PEG bacteria were isolated from Fincher and Payne [99]. However, anaerobic growth as a denitrifier has been observed, at the cost of certain free ether glycols up to PEG 300 [100]. Dwyer and Tiedje have isolated methanogenic consortia from sewage sludge [101]. The ethylene glycol monomer and polymers of as big as PEG 20,000 were degraded by the methanogen's consortiums. Schink and Stieb [102] reported the latest group to perform the anaerobic absorption of PEG. *Pelobacter venetianus* is shown to breakdown PEG up to 20,000 in size [103]. More recently, a PEG 4000-degrading and Hexavalent Molybdenum-reducing Pseudomonas putida strain Egypt-15 has been isolated.

Growth on PEG 4,000 was optimum at concentrations from 600 to 800 mg/L and higher concentrations are inhibitory. At 800 mg/L, about 75% degradation of PEG 4,000 occurred. A lag period was observed during growth on PEG of about two days. Others have also discovered a lag period in the breakdown of PEG by bacteria, which is generally shorter (about one to two days) under aerobic settings and longer (>10 days) under anaerobic conditions [104,105]. The growth was adequately modelled using the modified Gompertz model, which give parameters such as maximum specific growth rate of 2.216 d^{-1} and a lag period of 1.45 day. Growth on PEG was optimum at pH 7.5 and 30 °C [106].

Metabolism Of Polyethylene Glycol

In 1975 the hydrolytic breakdown of PEG 20,000 to oligomers was suggested by Haines and Alexander [22]. But early investigations of PEG aerobic breakdown have previously shown that the metabolization must be carried out via oxidation [1]. Initially, three enzymes were believed to be necessary: a dehydrogenase of alcohol and the aldehyde dehydrogenase, conversion into carboxylic acid group of the terminal alcohol groups, and an enzyme breaking ether producing glyoxylate (GOA), as illustrated in **Fig. 2** as the end product. PEG dehydrogenase (PEG-DH) and an ether bonding enzyme have been purified and described accordingly [96]. This differs greatly from the findings from Obradors and Aguilar [107], which had dehydrogenation of PEG and diglycolic acid as a single periplasmic protein (DGA). PEG-using bacteria most likely get usable energy from the oxidation stages and GOA is the basis of carbon absorption pathways. However, it has been shown that PEG-DH is unusable in bacteria that do not grow on PEG but generate dehydrogenase quinohemoprotein alcohol (QH-ADH, soluble type).

The computer modelling of a 3-D structure indicates the size of the active place is big enough to accustom a large molecule of the supports such as PEG to oxidise the alcohols and aldehydes to the corresponding acids [108]. However, since it is not yet discovered in a PEG-use organism, QH-ADH may be designed to produce alcohol dehydrogenase but probably by mistake it is called a "PEG dehydrogenase." An ether bonding enzyme seems to be dehydrogenase of β-hydroxy-acid, as detailed below. These findings may address the issue of the existence of or acquisition of PEG capability via mutations. Although the origin of the PEG metabolism machine is clear, there is still another matter of how the large molecule may reach the metabolising machine through the natural outer and inner membrane barrier. A alternative route was suggested [109] but wasn't experimentally verified, using desaturation and hydrating in the form of a hemiacetal derivative.

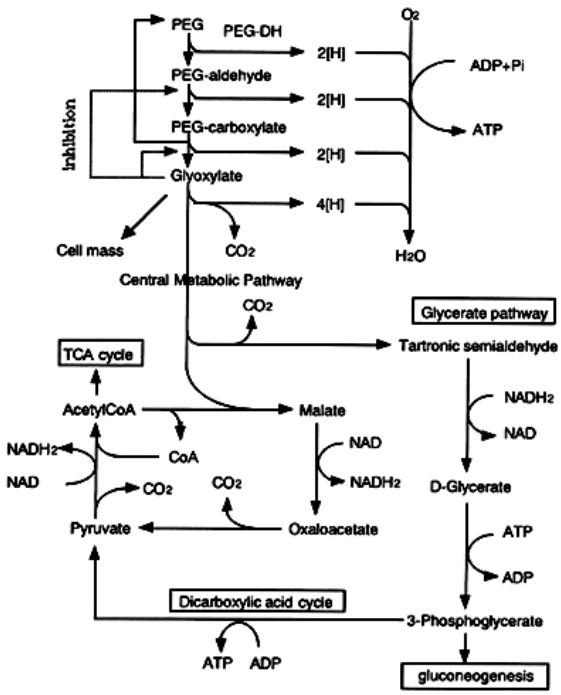

Fig. 2. Aerobic metabolic pathway for polyethylene glycol (PEG) (from [1])

Gram-Negative bacteria were until recently solely isolated as microorganisms for PEG use. On PEG 4000 or 8000, a gram-positive actinomycete was reported to develop as tetrahydrofuran-degrader [98]. It is necessary to clarify whether the actinomycete has the same metabolic trajectory and absorption mechanism as sphingomonads with the big PEG. PEG seems to be carried straight into cytoplasmic space. PEG can control the metabalic genes for anaerobic bacteria as is proposed in the cytoplasmic space. However, not big PEGs themselves, but another PEG-transduced signal, must be regulated by metabolic genes of aerobic bacteria that seem to be barrier to large molecules, as well. The genuine regulatory components for PEGs

must be found in either aerobics or anaerobics. Because derivatives such as free peg and ppg may be assimilated, but dialkyl peg/monoalkyl ppg acetate can't be used in PEG/PPG applications, at least one free alcohol group to be used for metabolism is needed. PEG/PPG [95]. As many studies bby Kawai (1987) have indicated in oxidative metabolism of PEG, the oxidised metabolites were made up of dipropylene glycol. The findings indicate that similar metabolic processes in PEG and PPG work: alcohol dehydrogenases associated with a microbial respiratory chain first attack a terminal alcohol group and then divide a terminal ether bond [110–113].

PPG must contain atactic structures since it is randomly polymerized with optically active propylene oxide. Since nearly all PPGs were added to the carbon supply, all isomers had to be digested [18]. Preliminary studies on PPG-DH showed that it exists in membrane, periplasmic and cytoplasmic fractions, even if it has not explained the relevance of these sites. PPG-dH is purified, and it should be determined that the isomers can be metabolised by a broad stereo and enantioselectivity or by various PPG-DHs.

In crude extracts of *Alcaligenes denitrificans*, the enzyme activity of poly(oxytetramethylene) glycol (PTMG) dehydrogenisation has also been discovered [114], but additional investigations have not been conducted. The terminal structure of PTMG [R-O-$(CH_2)_4$Oh] may be oxidised to R-O-$(CH_2)_3$COOH and subsequently split into R-O-CH_2COOH by β-oxidation, a structure similar to that of oxidised PEG. This may indicate that the metabolism utilises the same mechanism as PEG.

In addition, both aerobe and anaerobic bacteria have to vary in the metabolic mechanism for polymers [96]. PEG acetaldehyde lyase was the only one enzyme that might participate in PEG metabolism, as proposed by [103]. This enzyme is similar to the diol dehydratase process and divides acetaldehyde as a result, as illustrated in **Fig. 3** [115]. The cytoplasm fraction showed activity of the enzyme. These authors studied and indicated that at least an un-masked terminal hydroxyl group was required for the production of hemiacetal intermediate hydroxylation, the ether-cycled enzymes in cell free extracts from *Acetobacterium* sp. and *Pelobacter venetianus*.

This idea for the breakdown of PEG by an aerobic bacterium was originally proposed by Pearce and Heydeman [116]. Acetaldehyde is referred to by anaerobic bacteria as the ultimate result of PEG breakdown. The carbon and energy source of these creatures is most likely the acetaldehyde molecule. Dwyer and Tiedje [101] further indicated that Bacteroides and Desulfovibrios were a direct metabolite of PEG. A difference was proposed, but never tested, with the breakdown of oligomers via an unknown depolymerisation process and conversion into acetaldehyde.

PEG-dehydrogenase

Research on PEG-utilisating Sphingomonads have revealed that the PEG alcohol function is oxidised by a dye-linked, membrane-closed dehydrogenase. The enzyme possesses large suspension characteristics, however PEG dehydrogenase is named in light of its oxidation capacity (PEG-DH). PEGDHs originally were reported to be a quinoprotein (PQQ-Containing) enzyme from *Sphingomonas terrae* and *Sphingomonas macrogoltabidus* [18]. However, PEG-DH sequencing showed that the enzyme was part of the flavoprotein GMC group, and one FAD molecule was linked to the homodimeric-protein protein monomer.

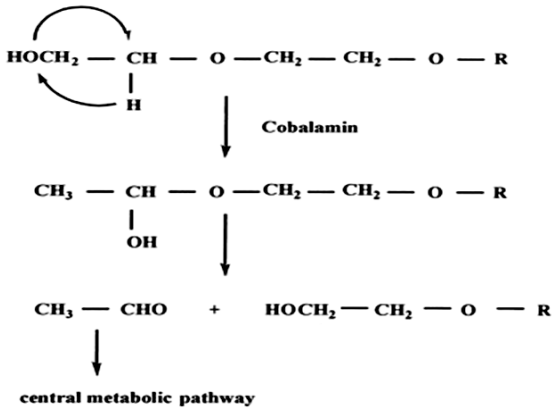

Fig. 3. Anaerobic metabolism of polyethylene glycol (PEG) (from [1])

Polyethylene Glycol-Degrading Enzymes

The kinetic resolution of racemic alcohols has previously revealed an intriguing characteristic of PEG-DH [117]. Applications in bioconversions as well as in analytical chemistry of these enzymes (organisations) may be envisioned. PEG-DH participation in PEG metabolism for several additional microorganisms that use PEG was indicated [1]. A dehydrogenase, which had low activities toward PEG homologs (dimer ~PEG 600), was developed by *Alkaligenes* sp. PE18, which was isolated as an Ethylene glycol (EG) monomethyl ether-utilisating bacterium [118]. Ether-alcoholic dehydrogenase reliant on NAD generated in cell-free *Alcaligene* sp MC11 not only oxidised ether-alcohol, but also PEG homologues (n=1~3 and PEG 400) [1].

Quinohemoprotein alcohol dehydrogenase

Alcohol dehydrogenases (QH-ADHs) of quinohemoprotein may be classified into soluble (type I) and membrane-bound (type II) groups. Type II of QH-ADH can be found exclusively in Gluconobacter and A*cetobacter* bacteria and is not accessible information on the capacity to oxidise PEG [119]. However, certain kinds of I QH-ADH purified by other PEG-uncomplicated bacteria act as alcohol dehydrogenases (ADH) for PE G: *Comamonas testosteroni* ADH [120] Vanillyl alcohol dehydrogenase from *Rhodopseudomonas acidophila* strain M402 [121].

C. testosteronii N-terminal portion of the enzyme is very comparable to other enzymes containing PQQ, especially methanol dehydrogenases [108] whereas C-term C does not include hemocytic. Based on this fact and on the 3-D structure of dehydrogenase methanol, modelling studies have revealed that *C. testosteroni* QH-ADH has a wide active location, explaining PEG and secondary alcohol oxidation capabilities for polyvinyl alcohol dehydrogenase [108]. In addition, three kinds of I QH-ADH identified in *Pseudomonas putida* have not been active in PEG [122]. These findings indicate that certain enzymes gained a major active site via a partial mutation to broaden their xenobiotic PEG substrate selectivity.

Dehydrogenase of liver alcohol

PEG, which may be causing mono- and dicarboxylated PEG, is known to produce a deadly condition when it's absorbed [123]. The in vivo metabolism of PEg was indicated by the presence of organic PEG acids in the blood of toxic patients and an animal model.

The PEG homologs' oxidation (n=1~8) was catalysed by horse liver alcohol dehydrogenase: it is hypothesised that the syndrome may have been induced by successive oxidising of ADH and aldehyde dehydrogenase. Through different alcohol dehydrogenases, PEG may be oxidised with various routes [123] in various bacteria.

Enzyme for aldehyde oxidation

Not only primary alcohols, but also aldehydes, are the source of PEG-DH activity. The cloned PEG-DH activity towards aldehydes, however, was much weaker than towards the equivalent alcohol under optimal reaction conditions; in fact it was not significant. On the other hand, PEG, both seeming membrane-associated, was produced by separate dehydrated aldehyde dehydrogenases (AL-DH, DCIP-dependent and NAD-dependent). While the purification failed, it was found that three distinct enzymes were involved in the PEG metabolism.

Splitting enzymes Ether-bond

In general, different enzyme processes may separate the link from the ether **[1,124]**. However, only instances relating to the splitting of an ether bond in PEG or to its derivatives where the enzymes are separated or where such an enzyme is highly probable to result from extract activity are addressed in this study.

Dehydrogenase of PEG-carboxylic acid or diglycolic acid

Studies on PEG sphingomonad degradation showed an enzyme capable of oxidising GOA-forming PEG-carboxylic acid [1]. The DGA-DH enzyme was also known as a good substratum. DGA-DH was termed a DGA dehydrogenase. The purification of the enzyme in lauryl maltoside showed that the enzyme is obviously different from PEG-DH since it was not a substrate for either PEG or any other alcohol (including diols) [125]. Apparently, for the oxidation at uterine location in the molecule it is essential to have a terminal carboxylic acid group. The fact that GOA and GA are also substrates for DGA-DH is not necessary, however.
The oxidation process may thus be comparable to that of the peroxisomal, flavoprotein glycolate (EC 1.1.3.l) and flavoprotein -hydroxide dehydragenases, by transferring hydride, by electron transfer, or by covalent catalysis in the adduct of the (carbanion) substratum and cofactor molecule [126]. In all cases, the process has to be accompanied by an H_2O molecule attack so that the rearrangement of the oxidised ether bond may be carried out under the formation of both the terminal Aldéhyde (GOA) group and the end-group Alcohol Group since no O2 use was identified for the conversion of dicarbonate PEG acid (in the PEG1). Elucidation of the mechanism and the co-factor identifiers involved must wait till significant quantities of DGA-DH are purified.

A flavoprotein DGA oxidase [127], which has no periplasmic space, was found to be present in DG A-using *Rhodococcus* sp. 432. Because ferricyanides and DCIP are significantly higher than O_2, the enzyme may be a cytoplasmic dehydromide oxidizer of DGA on the membrane and could utilise O_2 as an in vitro electron accepter. Since both GA and GOA (but not PEG) are also used for the enzyme, glycolate oxidase may also be comparable. While structural resemblance with DGA-DH is apparently excluded by several characteristics of DGA oxidase, further research is undertaken to verify that.

In DCIP-linked dehydrogenases from a *Pseudomonas stutzeri* strain that oxidises PEG directly under GOA production, there is the same ambiguity about similarities with DGA-DH [107]. Based on PAGE and activity stained by the authors, the solubilization of a detergent was not necessary for a single enzyme oxidising PEG as well as for DGA and purifying the enzyme. Although this suggests significant differences with the sphingomonad system, comprising two distinct enzymes (PEG-DH and DGA-DH), which require addition of solubilization detergents, no conclusions can be drawn at the moment without any additional information on the molecular characteristics of the enzyme. The initial stage of the metabolism was to produce dodecanol from *Pseudomonas* sp. strain SC25A from whole cells or cell-free extracts that may grow on PEG dodecyl ether. The split mechanism has not been investigated, nevertheless [128].

PEG acetaldehyde Lyase

PEG-degrading extracts from the anaerobic bacteria have shown diol-dehydrate and the enzyme PEG-degrading that produces acetaldehyde [115]. Unfortunately, the two enzymes seemed to be highly oxygen-sensitive, which is deemed prohibitive for purification by the authors. Since some corrinoids have been promoted by PEG degradation, they hypothesised that ether bond breaking happens in the bacterium using a PEG acetaldehyde lyase similar to diol dehydratase [102,115]. The development of acetaldehyde and PEGn-1 as products was supposed to lead to the change of position (a real mutase reaction, intramolecular rearrangement) of the terminal OH PEG group to the product, in order to create an unstable hemiacetal group.

Although the idea is validated by *Pelobacter venetianus* [102], to confirm, the enzyme needs to be purified and characterised. Furthermore, these enzymes are situated on a cytoplasm; the transportation of PEG into this compartment needs to be explained. In *P. venetianus* mentioned above two porins were discovered, however were not suitable for transporting high molecular PEG across the external cell membrane [108]. Schink discovered that phenoxyethanol is stoichiometrically metabolises by *Acetobacterium* sp. (LuPhet), while phenoxyacetic acid is not [103]. The ether connection to yielding acetaldehyde was closed as a reaction product by the cell-free extracts that clearly indicated that the chemical was destroyed by the same method as PEG anaerobic degradation.

CONCLUSION

PEG is utilised in many instances involving animal and human purposes. As the toxicity of some PEGs is becoming evident and numerously reported, its removal from contaminated areas is urgently needed. PEG biodegradation by microorganisms has been reported and proposed as a potential technique for remediating PEG-contaminated landfills. Because Malaysia has a large number of landfills and is projected to grow in the future, PEG contamination is expected to rise. Various PEG-degrading bacteria have been isolated and characterized. The route of assimilation into energy in aerobic and anaerobic system involves diverse chains of enzymes and present a challenge to those investigating the degradation of PEGs.

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