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Polystyrene Bioremediation: A Perspective on Microbial and Environmental Constrained Interventions

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Abstract

The polystyrene is the specific kind of plastics based upon petroleum which is composed of monomers of vinyl benzene (styrene). Regardless of the attraction of polystyrene, multiple organizations and municipalities are enduring an emerging challenge during disposal of polystyrene products and packaging. It is appraised that the products of polystyrene accounts for not more than 1% of the overall burden of landfill resources. It could be a greater health threat for humans also who are on the topmost level of food chain and farsighted styrene monomers of the plastics used in engineering polystyrene has been categorized as a potential humanoid carcinogen

by the International Agency for Research on Cancer (IARC) and the US National Institutes of Health (NIH). As a few researchers have been published on the degradation of polystyrene plastic but the key point addressed in this review is the recognition of microbial and fungal enzymes which are known at present to be involved in polystyrene monomer plastic degradation. The major bacterial and fungal enzymes involved within the degradation reaction of vinyl side chain include styrene monooxygenase, styrene oxide isomerase, styrene monooxygenase, flavin adenine dinucleotide (FAD) reductase, styrene isomerase and phenyl acetaldehyde dehydrogenase. Omics"-based approaches revitalized the study of PAH catabolism by permitting for an integrative assessment of the biochemical mechanism in charge to degrade PAH including polystyrene on the polluted locations. The applications of such enzymes in procedures that would permit the degradation of polystyrene plastics contaminating niches is a dare for future cohorts of microbiology experts.

Keywords: polystyrene, plastic pollution, microbial applications, waste management

Introduction

The polystyrene is the specific kind of plastics based upon petroleum which is composed of monomers of vinyl benzene (styrene) (Figure 1). In 1931, polystyrene was produced very first time on commercial scale (Wang et al., 2016). At the current time, it has been used for the extensive ranges of marketable, packing and construction purposes and developed to be one of the most universal household stuffs throughout the world (Hussain et al., 2012). Most of the polystyrene is functional to make rigid and resilient items, such as appliances and cabinets of computer systems and televisions along with for food packaging purposes. It is also used as general-purpose polystyrene (GPPS) for example single-usable drinking cups, disposable plates and cutlery, higher impact products like yoghurt containers as well as expanded polystyrene foam for manufacturing meat trays, cups and coolers (Baker,2010).

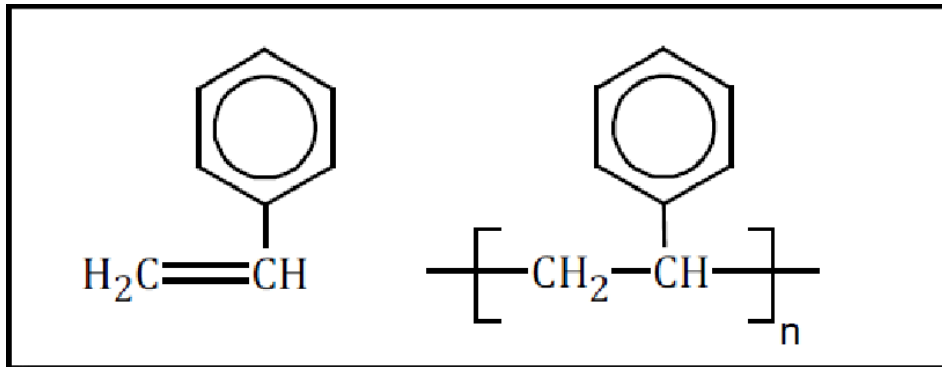


Figure.1. Molecular Structure of vinyl benzene (styrene) (Source (Rochman, Manzano, Hentschel, Simonich, & Hoh, 2013)

The closed-cells pre-expanded beads of foams are used for designing the expanded polystyrene. The engineering procedure comprises implementation of the styrene polymerization in precipitations suspended within the water (Verma et al., 2016). It leads to the establishment of polystyrene beads which are valuable due to ideal as an exceptional insulator such as beneficial for developing the chilly bins and line cool boxes (Hussain et al., 2020). It can absorb the shocks and therefore useful for molding or bead-based packaging materials for carrying fragile consignments. It is also used for a widespread scale of food contact packing, for instance egg cartons, clamshells, meat trays and fast food basins. Expanded polystyrene is mostly stated as its commercial symbol 'Styrofoam' designed in 1941 by Dow Chemical (Rader & Langer, 2019).

1. Polystyrene Waste Challenges:

Regardless of the attraction of polystyrene, multiple organizations and municipalities are enduring an emerging challenge during disposal of polystyrene products and packaging (Adane & Muleta, 2011). Due to massive and huge size, polystyrene adopt considerable space in waste bins which resources to become bins filled more speedily and thus prerequisites to be emptied more frequently (Aspin, 2012). Polystyrene is less weight material comparative to its volume consequently it subjugates lots of valuable landfill plans and can be propelled in surroundings which cause an irritation in the adjacent zones. Though few corporations have recycling strategies, most of the polystyrenes still get its channel into landfill locations throughout the sphere. As per conventional

approximations, thousands of millions of tons of polystyrene waste is released across the globe and referred to landfills every year (Shukla & Gottschalk, 2013).

It is appraised that the products of polystyrene accounts for not more than 1% of the overall burden of landfill resources, the proportion of landfill spaces it adopts is much greater considered that it is very low weight. Additionally, it is fundamentally non-biodegradable, taking thousands maybe millions of years for decomposition (Min et al., 2019). Yet on already disposing of in the landfills, polystyrene can certainly be moved by the winds and clutter the paths or culminate in polluting aquatic physiques. On breaking apart the polystyrene foams, the little polystyrene constituents can be swallowed by animals that can cause clogging or intestinal obstruction. Polystyrene can also be eaten by fishes when it disrupted in the marine bodies. Oceanic animals higher up the food chains in which the fishes can eat such polystyrene waste and hence concentrating the pollutants (Miandad et al., 2017).

It could be a greater health threat for humans also who are on the topmost level of food chain and farsighted styrene monomers of the plastics used in engineering polystyrene has been categorized as a potential humanoid carcinogen by the International Agency for Research on Cancer (IARC) and the US National Institutes of Health (NIH) (Vazquez & Barbosa, 2017). As the styrene is the derivative from either natural gas or petroleum, both of them are nonrenewable and are quickly being exhausted, producing sustainability hitches for environment (Figure 2) (Poletto et al., 2011). More lately, on 22nd April 2016, the Office of Environmental Health Hazard Assessment OEHHA included polystyrene to the chemical's list acknowledged to the condition of causing cancer disorders (Prathiba et al., 2018).

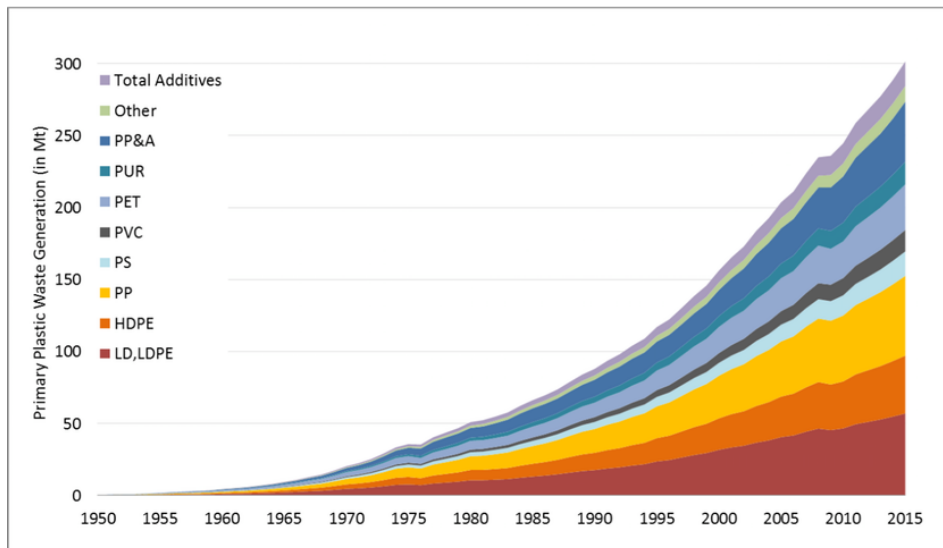


Figure.2. Global statistics of plastic waste including styrene (Source (Brems, Baeyens, & Dewil, 2012))

The Environmental Protection Authorities of USA and UK determines that polystyrene is probably carcinogen to human beings and categorizes the chemical as perilous because of its risky characteristics such as flammability, acutely toxicity, assumed anthropoid mutagen, carcinogenic, human developmental and reproductive toxin, deadly to organ systems, much exotoxin in the water atmosphere for crustaceans and algal species, faintly injurious to fishes and terrestrial vertebrates (Williams & Bagri, 2004). The categorization of polystyrene as 'toxin' is explicit as, by reason of its higher chemical reactivity, it interacts with cellular systems and cause extensive metabolic impairment (Parasuram et al., 2013).

Above all, styrene can react chemically with particular components of DNA and consequential in mutations, which disturb the rate of cell division (Kim et al., 2003). Such alterations are the foundation of chemical carcinogenesis and elucidate how polystyrene is a carcinogenic for animals and practically anticipated in human beings (Zhang et al., 1995). The IARC's declaration that styrene is a potential human carcinogen is emphasized in proclamations issued by the several environmental protection organizations of the world. They accounts the environmental threats

linked with styrene as the very exotoxin for algae, fishes and crustacean in the aquatic bodies along with terrestrial vertebrates and designed them for biocidal actions (Ayse & Filiz, 2016).

2. Polystyrene Chemical Hazards:

Furthermore, the European Union placed styrene recently in the Category 1 in the list of highly disruptor chemicals for endocrine system. These developmental classifications align with an emergent worldwide interest in the perilous nature of the constituents used in the manufacturing of plastic products (Savoldelli et al., 2017). Stimulatingly, according to a model of vulnerability ranking issued by the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals United Nations, the chemical constituents of over 50 percent of plastic products have been concluded to be hazardous nowadays (Liu et al., 2000). Irrespective of few community individuals and an emerging number of autonomous researchers currently treat polystyrene as a harmful waste product. After entirely polymerization of styrene during the manufacturing procedure to make polystyrene, the reactivity of the styrene polymer constituents removed absolutely as the chemical bonds which form between the units of monomers modifies the reactive nature of moieties of the styrene molecules. It justifies how polystyrene is very slightly toxic for mammalian species (Sabaa & Ravindrarajah, 1997).

Furthermore, the monomer units of styrene are bonded much toughly with each other which show that styrene monomers cannot be discharged in an ecological setting in its polymerized form. The microbial degradation of polystyrene in landfills does not liberate styrene, however relatively produces elements for example 4-phenylvaleric acid that is less toxic in nature. Meanwhile, if the process of styrene polymerization for the manufacturing of polystyrene is incomplete, the monomers of styrene maybe contaminate the polystyrenes which are known as residual monomers. Such styrene can travel into food packaging in styrene-contaminated polystyrenes. As styrene is rationally fat soluble and 103.6 times more fats soluble comparative to water, movement is higher in fatty foodstuffs like milk in polystyrene containers.

These molecules can leach from polystyrene and pollute both the products like stored food items and environment (Gregory, 1996). Irrespective to either styrene is entirely polymerized or not within the manufacturing process of polystyrene, all polystyrene waste have substantial consequences on the environment. It is for the reason that the entire environmental degradation with polystyrene is very slow and releases en route little particles of polystyrene (Rutkowski &

Kubacki, 2006). These particles involve imperative effects on environment as they accumulate in atmospheres such as in marine and aquatic bodies (Gondal & Siddiqui, 2007). Along with the physical threats they impose, polystyrene like hydrophobic plastics are the most perilous in marine ecosystems and fresh water due to their capability for adsorbing insistent organic pollutants which can be discharged following digestion of tiny polystyrene particles by aquatic creatures (Figure 3) (Singh & Sharma, 2016).

The pathway by which plastic enters the world's oceans Our World in Data

Estimates of global plastics entering the oceans from land-based sources in 2010 based on the pathway from primary production through to marine plastic inputs.

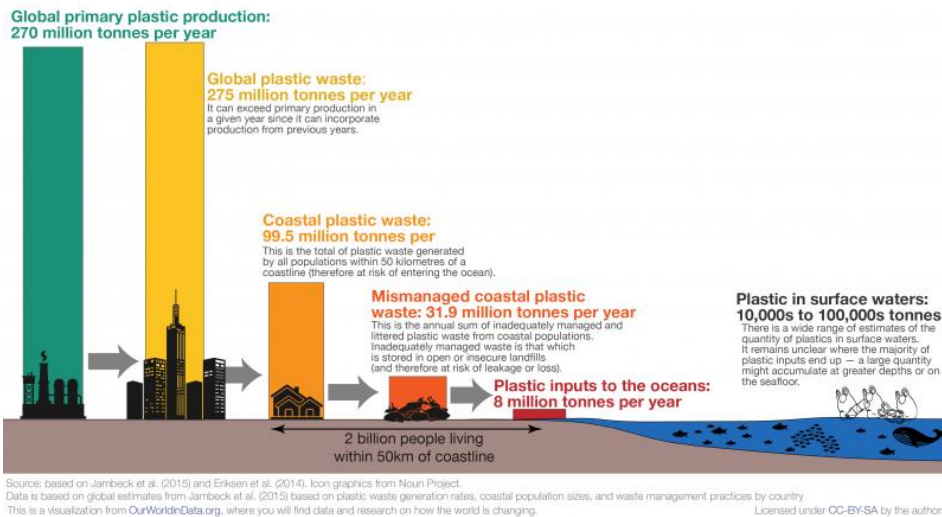


Figure.3. Facts of Plastic Pollution in Marine Life (Source: (C. Guo et al., 2018)

As polystyrene is extremely appreciated for its slight weight, thermal insulation, shock absorbance and strength attributes, it's manufacturing and discarding exposed imperative extortions to the atmosphere (Brems et al., 2012). Nowadays, most of the plastic garbage travels to the landfills and related chemical substances leach and pollute groundwater and soil. According to the US EPA reports, every year only residents of USA dispose of 25,000,000,000 Styrofoam made cups per minute (Rochman, et al., 2013). It is approximation that even after 500 years; the polystyrene made products people used daily is expected to endure intact in earthly landfills. The US EPA reported on solid waste management in 1986 and declared manufacturing of polystyrene as the 5th largest resource of lethal waste in the USA (Najafi, 2013).

Furthermore, the manufacturing of polystyrene is energy concentrated, generating high proportion of greenhouse gases like carbon dioxide along with solid and liquid wastes. Based upon such life cycle of harmful effects of polystyrene to the environment and its petroleum based manufacturing, it is regarded as non-sustainable and seriously poisoning source of environmental pollution. Subsequently, the environmental production costs of polystyrene have made it worst form of waste by several integrated waste management administrations (Guo et al., 2018). According to the World Economic Forum reports 2016, it is predicted that the percentage of plastics in the marine life is predictable to be 1:3 by 2025. At present, 60 to 80 percent of waste originate in aquatic settings is plastic, and in 2014, it was assessed that over 226,796 tons of plastic is presently submerged in maritime environment. Due to its low weight, polystyrene is extremely mobile and can carry hostile species throughout the marine frontiers (Pivnenko et al., 2017).

Up to now, with respect to only the trash blotch of Pacific Ocean, over 1.8 trillion plastic pieces have been accumulated with an approximated weigh of 80,000 tons with nothing impending. As a few researchers have been published reviews by centering on the degradation of polystyrene plastic but the key point addressed in this review is the identification of microbial and fungal enzymes which are known at present to be involved in polystyrene monomer plastic degradation (Guo et al., 2018).

3. Microbes and Microbial Enzymes of Polystyrene Degradation:

There are several enzymes reported in multiple studies that can degrade the polymers of higher molecular weight. The first report was published recently by Krueger and associates on the recognition of brown rot fungi capable to break polystyrol through applying Fenton reactions derived by hydroquinone (Tischler, 2015). Initially, during this research, *Gloeophyllum trabeum* (DSM 1398) and *Gloeophyllum striatum* (DSM 9592) exhibited considerable depolymerization subsequent to twenty days of incubatory period (Oelschlägel et al., 2012). Approximately 50 percent reduction in molecular weight of polystyrol was reported by the most active strains of *Gloeophyllum*. In another research, the white rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus*, the brown rot fungi *Gloeophyllum trabeum* were associated with the polystyrene depolymerization on incubating them along with lignin (Crabo et al., 2017).

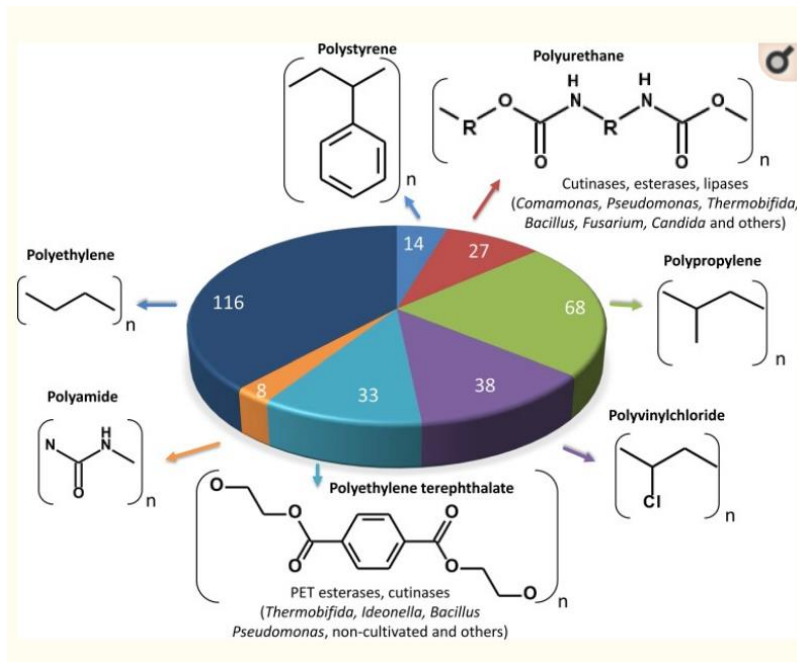


Figure.4. Microbial species for degradation of polystyrene and related plastics (Source (Parasuram, Karthikeyan, & Sundram, 2013))

As these were the first and auspicious information about the degradation of the polystyrene polymers of higher molecular weight, the enzymes linked with these reactions of depolymerizing still need to be explained (O’Leary et al., 2014). As earlier outlined mentioned, weight loss might have been instigated with the decaying of chemical additive substances (Ward et al., 2006). Likewise, a number of bacterial strains have been existed to make either individually or as supporters of consortium biofilms on polystyrene particles and films, hence decaying the polymers. In such researches, primarily weight loss of polystyrene has been examined. Unluckily, in no one of these studies were enzymes associated to the presumed depolymerization (Table 1) (Savoldelli et al., 2017).

Table.1. List of Microbial and Fungal enzymes for plastic degradation

Species	Plastic Types	Microbes	Enzymes	Ref.
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Bacterial	PCL	<i>Rhizopus delemar</i>	Lipase	(Habraken, Peeters, Thornton, Koning, & Heise, 2011)
	PHA	<i>Pseudomonas stutzeri</i>	Serine Hydrolase	(Sakuma et al., 1997)
Fungal	PCL	<i>Aspergillus flavus</i>	Glucosidases	(Neurath & Strick, 1981)
		<i>Aspergillus Niger</i>	Catalase, protease	(Sekhar et al., 2016)
		<i>Fusarium</i>	Cutinase	(Lee, Doi, & Ha, 2001)
	Polyurethane	<i>Trichoderma sp.</i>	Urease	(Tahir et al., 2013)
		<i>Pestalotiopsis microspora</i>	Serine hydrolase	(Peng et al., 2019)
	Polyethylene	<i>Phanerochaete chrysosporium</i>	Manganese peroxidase	(Krueger et al., 2017)
Insects		Plesiophthalmus davidis larvae		
		Zophobas atratus		
		Tribolium castaneum (Red Flour Beetle)		

Commented [H1]: Only include polystyrene plastic based studies, add one more column which highlight the technique/method, medium, degradation obtained.

Commented [H2]: Add insects based biodegradation

3.1. Operon

The vinyl side chain is attacked by the styrene monooxygenase to secret epoxystyrene that is subjected afterwards into isomerizing for the formation of phenyl acetaldehyde (Chauhan et al., 2018). The oxidation occur on the last product which form phenylacetic acid with the contribution of a dehydrogenase (Mooney et al., 2006). In *P. putida*, the activation of phenylacetic acid occurs to form phenylacetyl-coenzyme A (CoA) which is directed towards β -oxidation for yielding acetyl-CoA that is transferred directly into the Krebs cycle (O'Leary et al., 2002). The relevant genes for the oxygenation of side-chains are commonly located in a distinct cluster of conserved genes, mostly referred as *styABC(D)*. Thus, the styrene monooxygenase complex activated through the encoding of *styA* and *styB* genes (Tischler, 2015).

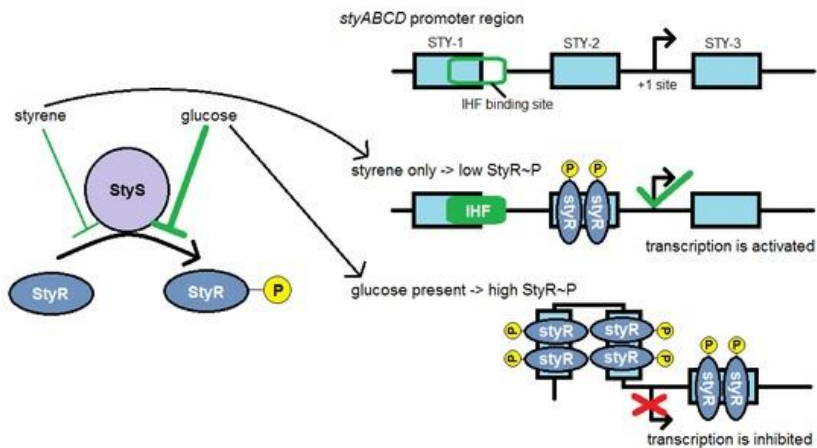


Figure.5. Mode of Action of Microbial and Fungal polystyrene degradation (Source (Tahir et al., 2013))

3.2. Phenyl acetaldehyde Metabolism

Moreover, there are large numbers of bacterial species are reported which are able to metabolize the styrene monomers as an individual carbon source (Ullmann et al., 1985). The chemistry of biological metabolism of styrene degradation is well known in bacteria including *Corynebacterium*, *Rhodococcus*, *Pseudomonas* and *Xanthobacter* (D. Guo, Zhang, Pan, & Li, 2017). It seems to be an extensive metabolic reaction while styrene is oxidized under aerobic conditions through 2 different pathways, specifically; (1) it attacks the vinyl side chain and (2) relatively bonding with non-specific aromatic ring, thus formed mainly the intermediates 2-phenylethanol, phenylacetic acid and 3-vinylcatechol (Milstein et al., 1992). These intermediates are directed into the Krebs cycle after cleavage of ring. The reaction of 3 vital enzymes involves within the degradation reaction of vinyl side chain including styrene monooxygenase, phenyl acetaldehyde dehydrogenase and styrene oxide isomerase (Ho et al., 2018).

Commented [H3]: Write about their mode of action

1.1. Cleavage pathways

The direct cleavage of styrene ring is started by a dihydroxylation of the aromatic rings and catalysis of this reaction performed by 2, 3-dioxygenase by following through 2, 3-dihydrodiol

dehydrogenase (Oelschlägel et al., 2012). The crucial products which are produced are 3-vinylcatechol and styrene *cis*-glycol. The degradation of former afterward can be accomplished by succeeding ortho or meta- cleavage to produce acrylic acid, pyruvate and acetaldehyde (Crabo et al., 2017). The pathways are relatively non-specific for the common degradation of several aromatic complexes, for example toluene and phenols (Figure 6). The formed phenyl acetaldehydes are relevant to multiple manufacturing activities, because they can be deliberated as the building blocks to produce diverse pharmaceutical composites and reasonable chemicals (O’Leary et al., 2014).

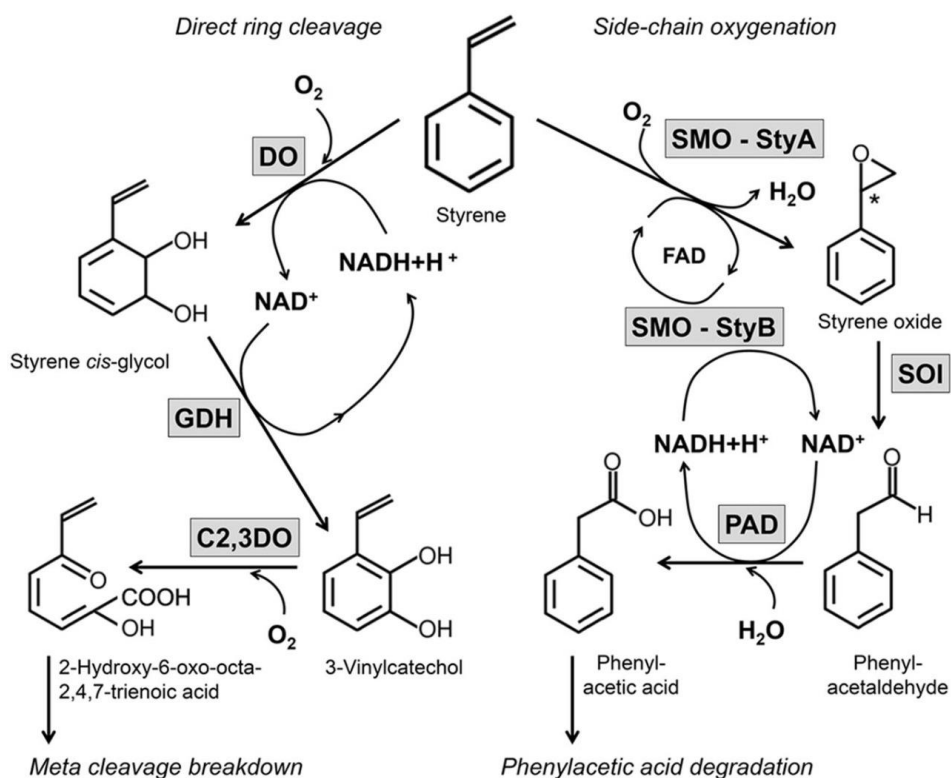


Figure.6. Pathways of Microbial and Fungal polystyrene degradation (Source (Liu, Qian, & Wang, 2000))

1.2. Monooxygenase

The FAD and NADH-dependent epoxidation of styrene is catalyzed through two-component flavoprotein styrene monooxygenase which transferred into styrene oxide (Tischler et al., 2009). *StyA* protein is a definite monooxygenase, while *StyB* protein is responsible to work as flavin adenine dinucleotide (FAD) reductase that delivers the electrons from NADH to FAD⁺ for supplying *StyA* with the mandatory electrons (Velasco et al., 1998). The *styC* protein encodes styrene isomerase, while *styD* is the gene termed as phenyl acetaldehyde dehydrogenase. The regulation of gene expression of conserved clusters is managed via either LysR-type regulators or a two-component regulatory system (Morrison et al., 2013).

Few other investigations have also revealed that *Rhodococcus zopfii*, *Pseudomonas putida*, and other Gram-negative bacteria can transform polystyrene (specifically styrene oil) into the polyhydroxyalkanoate biodegradable polymers or regarding appreciated composites Ward et al., 2006). The methodology comprises as a first phase of polystyrene pyrolysis particularly for styrene oils that is transformed to the second phase into polyhydroxyalkanoate and similar components. Whereas the general perception of such two-phase mechanism is stimulating, it might not be realistic on a huge scale, as the pyrolysis is a course which runs at 520°C and it is actively very challenging (Savoldelli et al., 2017).

2. Omics approaches;

2.1. Metagenomics,

There are remarkably several evolving integrative “Omics”-based approaches which can support to explore the genomic, transcriptomic, proteomic and metabolomic characteristics of living organisms and even mixed populations. They have opened innovative prospects to interpret molecular mechanisms of polycyclic aromatic hydrocarbons (PAH) including polystyrene degradation in polluted atmosphere. In an environmental sample, the metagenome, metatranscriptome, metaproteome, and metametabolome address the complete complement of DNAs, mRNAs, proteins and metabolites, correspondingly (Ellis, 2013). Metagenomics can recognize the toxicity and functional potentials of all organisms but produces no evidence as for the real active members of the population (Tripathi & Nailwal, 2020).

Metagenomics contains two different flavors, (1) shotgun metagenomics in which genomic DNA is extracted and sequenced to explore functionality and taxonomic hierarchy with the help of homology databases searching, (Ruuskanen et al., 2020) and (2) functional metagenomics in which larger pieces of DNA are introduced in different vectors and sequentially expressed in subjective host cells which are after that screened for activity within the clones that is sequenced based upon the desired activities (Yang, 2013). The combination of such approaches can support to determine that which organism is in fact carrying out particular functions in situ and on what extent these functions are expressed in the specific environmental samples (Zampolli, Zeaiter, Di Canito, & Di Gennaro, 2019).

In addition, Amplicon sequencing comprise in the marker genes based next-generation sequencing (for example, the 16S rRNA gene, cpn60, the ITS region, rpoB) is also broadly functional to recognize the diversity and composition of microbial community in an environmental sample (Malla et al., 2018). On combining with other approaches, such as amplicon sequencing and stable-isotope probing (SIP) can also generate evidence on community participants metabolizing a specific substrate (Jaiswal, Sharma, & Shukla, 2020). The principle of RNA and DNA-SIP is to feed a microbial community with a characterized substrate (such as, C13-naphthalene) and retrieve another substrate RNA or DNA with the help of density gradient centrifugation of nucleic acids (Breton-Deval et al., 2020).

In terms of polystyrene degradation, such as, by means of SIP combined with 16S rRNA gene amplicon sequencing, it is promising to associate taxonomic group character to polystyrene degradation (Shahsavari, Schwarz, Aburto-Medina, & Ball, 2019). This approach facilitated to highlight the concept that complicated organic pollutants are mineralized or metabolized using microbial groups within the fields, which greatly vary from obtained outcomes under laboratory settings (Zhu et al., 2011). Recently, “Omics”-based approaches revitalized the study of PAH catabolism by permitting for an integrative assessment of the biochemical mechanism in charge to degrade PAH including polystyrene on the polluted locations (Guan & Zhang, 2017). It has been revealed that the disclosure of soil microbial populations to phenanthrene led to an upsurge in the richness of gene transcripts associated to dioxygenase, detoxification and stress responses (Viggor et al., 2020).

Likewise, the growing willows rhizosphere within the contaminated soil was considerably improved in transcripts linked with PAH polystyrene degradation that were consequently revealed to be principally connected to the members of orders Rhodospirillales, Rhizobiales, Actinomycetales, Burkholderiales, Solirubrobacterales, Alteromonadales, and Caulobacterales (Lim, Teh, & Thong, 2017). Proteomics approaches have also been effectively used to explain microbial degradation of high molecular weight PAH plastics including polystyrene (El Amrani, Dumas, Wick, Yergeau, & Berthomé, 2015).

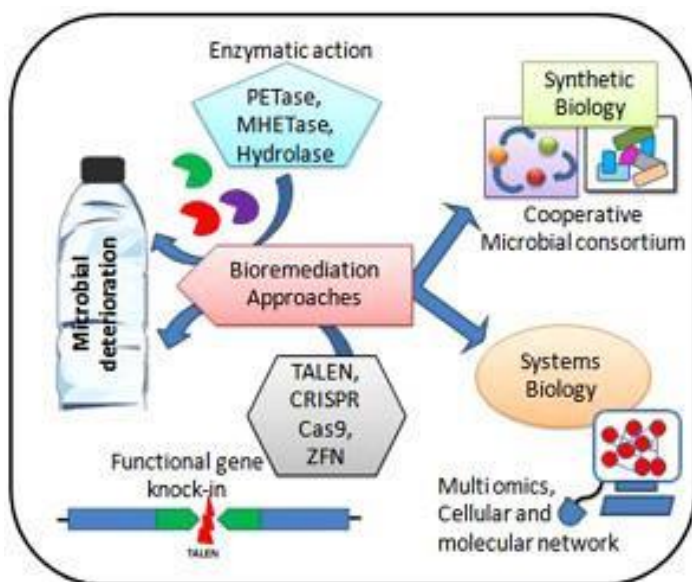


Figure.7. Multi Omics and Biodegradation Interaction (Source (Aydin et al., 2017)

For example, within 1122 proteins, different studies recognized 54 and 27 proteins crucial in the wide-ranging degradation of fluoranthene and pyrene respectively (Persyn et al., 2019). An additional recent study linked metagenomic and metaproteomic profiles, with regard to interactions and diversity between community members which brought about in the renovation of the naphthalene degradation pathway for particular groups of microbes from multifaceted microbial communities (Sadeghinejad et al., 2017). Such types of explicit pathway reconstruction on the basis of taxonomic and metaproteomic data is assisted through the development of novel bioinformatics tools (Deng et al., 2019).

2.2. Proteomics.

2.3. Bioinformatics

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Conclusion and Recommendations:

Humans have the aptitude to release polystyrene waste and it is hard to get away when there will no longer landfills available as they are functional for short duration. However, at present consumption rates are about 33 billion tons of plastic projected to be produce by 2050. The variety of known microbes and regarding enzymes act on synthetic polymers is still relatively inadequate. Thus, imminent research has to deal to identify the organisms on the most leading polystyrene polymers. Additionally, the application of enzymes in procedures that would permit the degradation of plastics contaminating ecological niches is a dare for future cohorts of microbiology experts. Since present technologies have not yet ensued in the recognition of extremely active enzymes for polystyrene plastics, the variety of microorganisms including fungi and the derived proteins and enzymes offer an auspicious source for the detection of such biological catalyts. Therefore, the further expansion to explore smart algorithms for mining innovative methods is certainly a worthwhile mission.

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