

Chapter 3 Relevance of gene expression studies to understand pollutants biodegradation

Capítulo 3 Importancia de los estudios de expresión génica en la comprensión de la biodegradación de contaminantes

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Abstract

Nowadays, pollution is a global problem that affects the environment and human health. The primary pollutants are hydrocarbons, plastics, heavy metals and pesticides, all of which are essential for basic human needs. For this reason, research into environmentally friendly and viable degradation methods has become key, e.g., biodegradation. Biodegradation is a technology that uses the enzymes or metabolism of an organism to hydrolyze pollutants but is limited by factors such type of substrates, environmental conditions and organism physiology. The study of gene expression, i.e., protein production from genetic information at a specific time and condition of biodegradation, provides valuable information about the genes and enzymes expressed during the degradation process, the response to stress and the pathways involved. This information can be applied to increase biodegradation efficiency, find new enzymes, improve enzyme activity, or optimize metabolic pathways. Gene expression studies can be performed by applying omics technologies. This chapter aims to describe the importance of studying the gene expression of organisms used in the pollutant biodegradation process.

Gene expression, Biodegradation, Pollutants

Resumen

Hoy en día, la contaminación es un problema global que afecta el medio ambiente y la salud humana. Los principales contaminantes son hidrocarburos, plásticos, metales pesados y pesticidas, todos ellos esenciales para las necesidades básicas de los seres humanos. Por esta razón, las investigaciones de métodos de degradación viables y amigables con el medio ambiente han adquirido relevancia, por ejemplo, los estudios de biodegradación. La biodegradación es una tecnología que utiliza las enzimas o el metabolismo de un organismo para hidrolizar contaminantes, pero se ve limitada por factores como el tipo de sustrato, las condiciones ambientales y la fisiología del organismo. El estudio de la expresión génica, es decir, de la producción de proteínas a partir de la información genética en un momento y condición específica de biodegradación, proporciona información valiosa sobre los genes y enzimas expresadas, la respuesta a estrés y las vías metabólicas involucradas en el proceso de biodegradación. Esta información puede ser utilizada para incrementar la eficiencia de la biodegradación, encontrar nuevas enzimas, mejorar la actividad enzimática u optimizar vías metabólicas. Los estudios de expresión génica se pueden realizar aplicando tecnologías ómicas. Este capítulo tiene como objetivo describir la importancia de estudiar la expresión génica de los organismos utilizados en los procesos de biodegradación de contaminantes.

Expresión génica, Biodegradación, Contaminantes

1 Introduction

Environmental pollution is defined as introducing the environment (air, water or soil) of substances harmful in higher than usual concentrations that reduce the quality (Manisalidis *et al.*, 2020). Some critical substances toxic, named pollutants or xenobiotics, are hydrocarbons, synthetic polymers (plastics), heavy metals and pesticides. When contaminants found into air, water or soil, they can produce various adverse environmental impacts and human health effects. Xenobiotics are necessary for basic human needs to make medicines, plastics, detergents, chemicals, herbicides, combustibles and various other products (Mishra *et al.*, 2019).

Therefore, the research into pollute degradation methods that are environmentally friendly and economically viable, such as biodegradation, is relevant. Biodegradation is a promising technology, but now it is limited by scarce evidence of gene expression changes in the organisms used. This work aims to describe the importance of studying the gene expression of organisms in the biodegradation pollutants process.

The following sections first show the environmental impacts caused by the primary pollutants (hydrocarbons, plastics, heavy metals and pesticides). Then, the biodegradation is defined as well as their process and some microorganisms used. Subsequently, is presented the general process of gene expression. After, the use of science omics in the study of gene expression and its application in biodegradation research is exposed. Finally, some examples of successful biodegradation research using gene expression studies are shown.

2 Environmental impacts

The list of pollutants in the world is highly extended. Among the most abundant and important contaminants are hydrocarbons, plastics, heavy metals and pesticides, due to their environmental and human health impacts (Kour *et al.*, 2021). As illustration of its importance, the study of Polidoro *et al.* (2016) in coastal streams and sediments of America Samoa showed a presence of approximately 0.12% w/w of heavy metals (Cd, Co, Cr, Cu, Hg, Ni, Pb, Sn and Zn) in sediments samples, and presence closely to 20 ppb of pesticides (benthiocarb, diazinon, ethion, fenitrothion and parathion), 0.2 ppb of total PAH's and 9 ppb of phthalates (diethyl phthalate) in water samples. We will refer to these four pollutants in the subsequent.

The anthropogenic sources of pollutants include household, agricultural, industrial, transportation and others (Mishra *et al.*, 2019). Hydrocarbons, for example, can be introduced in ecosystems by spills, pipes in bad conditions, lousy manipulation practices, or runoff of rain and rivers. Due to poor waste management and environmental education, the plastics arrive in the ecosystems by sewage and industrial water discharge or even by human hand. On the other hand, heavy metal and pesticide contamination are principally from metallurgical industrial, farming activities and illegal dumping effluents (Thakare *et al.*, 2021). Table 3.1 shows some important pollutants nowadays, prevalence (air, water or soil), the primary source and the more relevant environmental and health human impacts.

Table 3.1 Main environmental and health human impacts caused by relevant pollutants

Pollutant	Example	Prevalence	Source	Environment and human health impact	Reference
Hydrocarbons	Naphthalene Fluorene Phenanthrene Anthracene Xylene Toluene	Water: sediment, column and surface Soil Air	Petrochemical industry Oil leaks Industrial activities as asphalt production Vehicle exhaust gases Forest fires	Toxic effects on flora and fauna Bioaccumulation along the food chain Carcinogenic, teratogenic and mutagenic They have been found in the human liver, kidney, lung, plasma and tissues Cardiovascular disorders	Marris <i>et al.</i> , 2020 Ahmed and Fakhruddin, 2018 Alegbeleye <i>et al.</i> , 2017
Synthetic polymers (plastics)	Polyethylene (PE) Polyethylene terephthalate (PET) Polyvinyl chloride (PVC) Polystyrene (PS) Microplastics (<5 mm)	Water: sediment, column and surface Soil	Public waste: food packaging, bottles, textile fibers, hygiene products, detergents Sewage, river and stormwater discharges Aquatic transportation Commercial and recreational fishing equipment	Fauna trapped or suffocated Ingestion and bioaccumulation in organisms Toxic to reefs Adverse effects on growth, photosynthesis, reproduction and immune system of organisms Release of toxic compounds	Ganesh-Kumar <i>et al.</i> , 2019 Chae <i>et al.</i> , 2018 Rhodes, 2018
Heavy metals	Mercury Copper Zinc Nickel Lead Cadmium Chromium Cobalt Arsenic	Water: underground and surface Soil Air	Metallurgical and glass industry Carbon-burning and other fuels Vehicle exhaust gases Municipal and industrial wastewater Paints Volcanic eruptions	Decrease in biodiversity Damage to the membrane, proteins and DNA of organisms Interference with enzymatic activity impacting germination, development, photosynthesis and reproduction of organisms Through plants, they can reach humans Carcinogenic, neurotoxic and nephrotoxic Affect prenatal development and childhood Cause of cardiovascular disease, immune and reproductive disorders and alteration in blood composition	Thakare <i>et al.</i> , 2021 Zwolak <i>et al.</i> , 2019 Vareda <i>et al.</i> , 2019 Vardhan <i>et al.</i> , 2019
Pesticides	Carbamates Organophosphates Organochlorines Pyrethroids Triazines	Water: underground, surface Soil Air	Agriculture and irrigation Pest control activities (insecticides, fungicides, herbicides, rodenticides) Maintenance of private gardens Seeping Industrial wastewater Volatilization	Disruption depredator-prey interaction affect earthworms, parasitoids, and pollinators (bees, beetles and birds) They affect small fish directly and indirectly by decreasing their food (algae and plankton) Interference with soil fertility, properties of microflora, nitrogen fixation, nitrification, ammonification and mineralization Mutagenic, carcinogenic and neurodegenerative Cause of tumors, nervous system disorders, pulmonary dysfunction, immune system deficiency, cardiovascular, respiratory, kidney, endocrine, reproductive, and blood disorders	Hassaan <i>et al.</i> , 2020 Kaur <i>et al.</i> , 2019 Yadav and Devi, 2017

The excessive use of xenobiotics has caused several environmental and healthy human impacts, due to their toxicity and non-biodegradable nature (Kour *et al.*, 2021). Consequently, searching for new economic and ecologic degradation processes is necessary; in this sense, an alternative can be biodegradation.

3 Biodegradation

Biodegradation is a biochemical process that refers to the broken down of various pollutants into more minor compounds caused by the metabolic potential of different organisms (Kour *et al.*, 2021; Alshehrei, 2017). Biodegradation is often used to describe a variety of microbial processes such as mineralization, detoxication or cometabolism (Riser-Roberts, 2020). Bacteria, archaea and fungi are typical biological factors (Abatenh *et al.*, 2017). On the other hand, bioremediation is the application of biodegradation to hydrolyze environmental contaminants (in soil, sediments, groundwater) to reduce levels below concentration limits established by regulatory authorities (Singh *et al.*, 2014; Kensa, 2011). Bioremediation is then an efficient, cost-effective and eco-friendly cleanup tool (Kour *et al.*, 2021). Bioremediation can divide into two types: phytoremediation and microbial bioremediation (An *et al.*, 2020).

It is well known that microorganisms are capable of degrading a wide range of organic compounds (Riser-Roberts, 2020). Microbial biodegradation has received significant attention as an efficient biotechnological strategy to decontaminate the environment (Kour *et al.*, 2021). Microorganisms such as bacteria, fungi, algae are reported for their ability to degrade pollutants (Table 2). Still, biodegradation efficiency depends on many factors such as the type of pollutants, environmental conditions, and microorganisms used, which have to be deeply studied.

In the case of hydrocarbons biodegradation, there are many fundamental factors for a successful process. For example, hydrolysis rates are strongly influenced by substrate characteristics (availability, volatilization, type and length of hydrocarbons), involved microorganisms (cell metabolic pathways) and environmental conditions (pH, temperature, salinity) (Varjani, 2017).

On the other hand, the polymers synthetic biodegradation is determined by polymers characteristics such as functional groups increasing hydrophobicity, the molecular weight, density, branching, amount of crystalline or amorphous region and form (films, pellets, fibers) (Alshehrei, 2017). The plastic films compared with pellets and fibers, facilitate the adherence of cells, leading to considerable changes in the plastics morphology (Taniguchi *et al.*, 2019).

Heavy metals can be effectively remediated using plants and microorganisms (bacteria, fungi, microalgae) with tolerance to toxicity and capable of converting heavy metals into a less hazardous state (Table 2) (Thakare *et al.*, 2021; Ojuederie and Babalola, 2017). Factors that influence the heavy metals bioremediation efficiency are biomass concentration, temperature, pH, metal ion concentration, redox potential and climatic conditions (Jacob *et al.*, 2018).

Microorganisms (bacteria, fungi, actinomycetes) and plants have been used to help remove or detoxify pesticides. The factors that affect the degradation of the pesticides are the molecular weight, the structure, type and number of substituents of pesticide molecule, besides environmental factors as temperature, pH, salinity and viscosity (Ye *et al.*, 2018; Parte *et al.*, 2017).

Table 3.2 shows some reports about microorganisms with degradation capacity and the general biodegradation mechanisms. Even though the list is extensive, it is essential to mention that there are still pollutants without biodegradation studies.

Table 3.2 Examples of pollutant-degrading organisms and principal degradation mechanisms

Pollutant	Organism	Substrate	Reference	Mechanisms
Hydrocarbons	<i>Acinetobacter</i> sp.	Total petroleum hydrocarbons	Cai <i>et al.</i> , 2021	The initial attack is generally through attachment to the substrates or production of biosurfactants/bioemulsifiers. The intracellular attack is an oxidative process (oxygenases and peroxidases), then peripheral degradation pathways convert HC into intermediates of central metabolism: β -oxidation and tricarboxylic acid cycle (Varjani, 2017).
	<i>Pseudokirchneriella subcapitata</i>	1-methylphenanthrene and 3,6-dimethylphenanthrene	Luo <i>et al.</i> , 2020	
	<i>Klebsiella pneumoniae</i>	Alkanes C ₁₀ -C ₂₀ in petroleum	Ozyurek and Bilkay, 2017	
	<i>Pseudomonas aeruginosa</i>	Total petroleum hydrocarbons	Varjani and Upasani, 2016	
	<i>Bacillus</i> sp.	Anthracene, naphthalene, benzene, toluene, xylene	Bisht <i>et al.</i> , 2014	
	<i>Aspergillus terreus</i>	Naphthalene and anthracene	Ali <i>et al.</i> , 2012	
Synthetic polymers (plastics)	<i>Tenebrio molitor</i>	Polyvinyl chloride	Peng <i>et al.</i> , 2020	Microorganisms attack the polymer surface, and the extracellular enzymes secreted cause the main chain to cleave. The lower molecular weight compounds formed can be used by the microorganisms as carbon and energy source (Alshehrei, 2017).
	<i>Streptomyces albogriseolus</i>	Polyethylene	Shao <i>et al.</i> , 2019	
	<i>Zophobas atratus</i>	Polystyrene	Yang <i>et al.</i> , 2019	
	<i>Brevibacillus</i> sp. <i>Aneurinibacillus</i> sp.	Polypropylene	Skariyachan <i>et al.</i> , 2018	
	<i>Aspergillus nidulans</i>	Polyethylene terephthalate and polybutylene succinate, polycaprolactone, polylactic acid	Peña-Montes <i>et al.</i> , 2017	
	<i>Ideonella sakaiensis</i>	Polyethylene terephthalate	Yoshida <i>et al.</i> , 2016	
Heavy metals	<i>Bacillus cereus</i>	Cd, Cu, Ag, Zn	Al Azad <i>et al.</i> , 2020	Two mechanisms exist the neutralization of metals to non-toxic forms by the enzymatic attack (oxidoreductases, oxygenases, peroxidases) and bioaccumulation of heavy metals inside cellular components with non-apparent toxic effect (Ojuederie and Babalola, 2017).
	<i>Saccharomyces cerevisiae</i>	Pb, Cd, As, Hg	Massoud <i>et al.</i> , 2019	
	Mixed culture: <i>Desmodesmos</i> sp., <i>Chlorella</i> sp., <i>Scenedesmus</i> sp.	Al, Cu, Fe, Mn, Zn	Aslam <i>et al.</i> , 2019	
	<i>Robinia pseudoacacia</i>	Zn, Cd, Pb	Fan <i>et al.</i> , 2018	
	<i>Gemella</i> sp., <i>Micrococcus</i> sp., <i>Hafnia</i> sp.	Cd, Cr, Pb	Marzan <i>et al.</i> , 2017	
	<i>Penicillium simplicissimum</i>	Mo, V, Mn, W, Zn	Anahid <i>et al.</i> , 2011	
Pesticides	<i>Chlamydomonas reinhardtii</i>	Trichlorfon (TCF)	Wan <i>et al.</i> , 2020	Degradation by extracellular enzymes is through oxidized, dehydrogenation, reduction or hydrolysis. Degradation by intracellular enzymes begins with the adsorption of pesticides on the surface of microbial cells. The membrane permeability determines its penetration of the cell and can be degraded by mineralization or partially degraded by co-metabolism (Ye <i>et al.</i> , 2018).
	<i>Aspergillus flavus</i>	Malathion	Derbalah <i>et al.</i> , 2020	
	<i>Pseudomonas nitroreducens</i>	Chlorpyrifos	Aswathi <i>et al.</i> , 2019	
	<i>Pleurotus ostreatus</i>	Aldrin, dieldrin	Purnomo <i>et al.</i> , 2017	
	<i>Stenotrophomonas</i> sp.	1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT)	Pan <i>et al.</i> , 2016	
	<i>Trichoderma viride</i> FRP3	Glyphosate	Arfarita <i>et al.</i> , 2016	

Several microorganisms with biodegradation capability have been reported; it is known that the expression of their specific enzymes affects the rate of contaminant degradation (Abatenh *et al.*, 2017). Understanding the conduct of liable degrader is an effective approach to evaluating the efficiency of various biodegradation approaches (Aydin *et al.*, 2016). Therefore, gene expression studies provide valuable biological information that can be used to improve the biodegradation process.

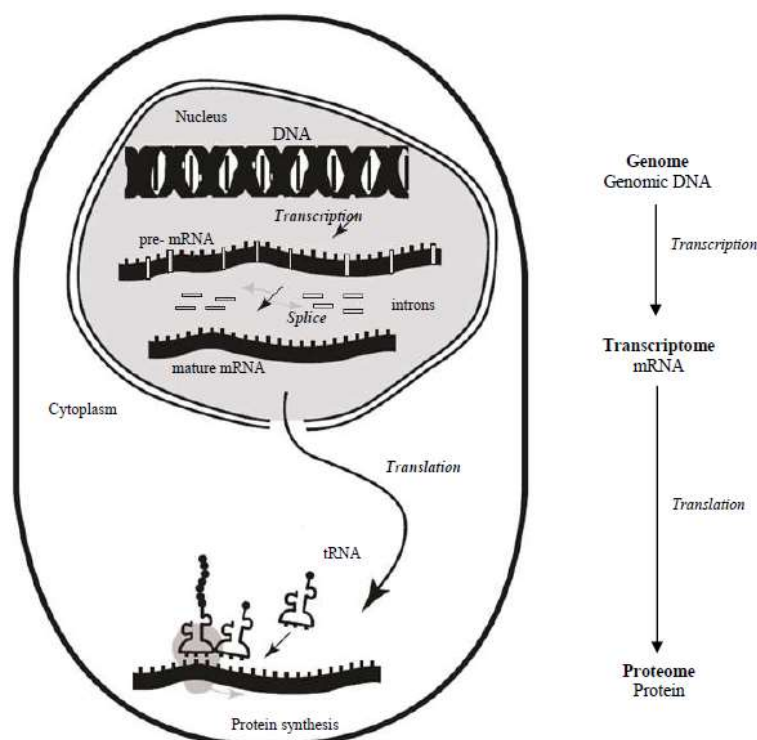
4 Gene expression studies and their regulation

4.1 Gene expression

Gene expression is the relationship between deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins, and it is named the central dogma of molecular biology. Expression of the genes changes under stress situations as the presence of toxic compounds (Qian *et al.*, 2021). Genes contain the information necessary for cells to survive and reproduce (Shafee and Lowe, 2017). The genetic information is encoded in the DNA. During the gene expression process, this information is copied from DNA into messenger RNA (mRNA) during transcription. Then, proteins are built from mRNA; this process is called translation (Figure 3.1) (Selzer *et al.*, 2018).

DNA is a double-stranded molecule, one of the strands encodes information that the RNA polymerase reads to produce protein-coding mRNA. This strand runs in the 5' to 3' direction where the numbers refer to the backbone's carbon atoms' ribose sugar (Shafee and Lowe, 2017). RNA polymerase starts to copy in promoter DNA sequence and finished in the terminator sequence (Paniagua *et al.*, 2003). The organization of genes is different in prokaryotes and eukaryotes. The most striking difference is that prokaryotic gene information is encoded on a continuous DNA stretch, whereas in eukaryotes, coding exons are interrupted by non-coding introns. Therefore, eukaryotic transcription of DNA to mRNA (derived only from exons) requires several steps (Selzer *et al.*, 2018). Exon regions are retained in the final mature mRNA molecule, while intron regions are spliced out during post-transcriptional processing. One spliced together; exons form a single continuous protein coding region. Eukaryotic post-transcriptional processing adds a 5' cap to the start of the mRNA and a poly-adenosine tail to the end. These additions stabilize the mRNA and direct its transport from the nucleus to the cytoplasm (Figure 1). In comparison, prokaryote genes are often grouped into a polycistronic operon transcribed in the same mRNA (Shafee and Lowe, 2017). Three mRNA bases (named codon) determine the incorporation of an amino acid into the protein chain. The protein synthesis or translation starts with the codon AUG (adenine-uracil-guanine) that codifies the amino acid methionine. The amino acids are supplied by the specific transfer RNA (tRNA). The tRNA is united to the small ribosomal subunit, and then the large ribosomal subunit is joined. The tRNA supplies the following amino acid, and the amino acids are bonded with the help of the enzyme peptidyl transferase. The end of the protein synthesis is determined by terminato sequence (UAA-UAG-UGA), and finally the protein is liberated (Figure 1) (Paniagua *et al.*, 2003). Thus, the flow of genetic information generally proceeds from the genome (entirety genomic DNA) over the transcriptome (total pool of mRNA) to the proteome (entire pool of proteins).

Figure 3.1 The general process of eukaryotes gene expression



Reference: Selzer *et al.*, 2018

4.2 Gene expression regulation

The gene expression is managed by networks of regulatory mechanisms and is acquired during a life span due to specific changes. (Mazaira *et al.*, 2018; Tomanek *et al.*, 2020). Gene expression can be regulated in response to extracellular and intracellular signaling to cope with cells' metabolic needs and adapt to the changing environment (Li *et al.*, 2018). There are different steps where gene expression can be regulated: chromatin remodeling, transcription, post-transcription, translation and post-translation (Liang *et al.*, 2019; Spriggs *et al.*, 2010).

Gene expression in eukaryotes is a highly complex and tightly regulated process. The first level is the organization of the genome into chromatin (Shandilya and Roberts, 2012). DNA in eukaryotic cells is packaged into chromatin. Different chromatin modifications regulate chromatin structure, which modulates the accessibility of DNA and subsequently contributes to the regulation of gene expression (Li *et al.*, 2018).

In eukaryotes, all protein-coding genes are transcribed by RNA polymerase II (Pol II). The regulation of Pol II transcription requires various transcription activators and repressors (Soutourina, 2018). Multiple genes with different functions are temporally regulated by transcriptional “on” and “off” switches that express specific genes that cells need in response to external stimuli (Carpenter *et al.*, 2014).

The regulation also involves post-transcriptional points occurring at the level of splicing, capping, polyadenylation, stability, and export of each mRNA. These mechanisms, modeling the duration of the new response to an abrupt environmental change for adapting the system in a timely and efficient manner (Carpenter *et al.*, 2014).

Translational control of gene expression is essential in stress responses, as it allows a rapid change in the proteins without any lag. At the same time, new mRNA is transcribed and processes together with reprogramming protein synthesis to elicit an appropriate response to the type of stress-induced (Spriggs *et al.*, 2010). Post-translational modification alters the functional diversity of the proteome by phosphorylation, dephosphorylation, ubiquitination or sumoylation. This mechanism is critical for the rapid reprogramming of cells for defense signaling (Withers and Dong, 2017; Kang and Han, 2011).

Eukaryotic genes typically have more regulatory elements to control gene expression compared to prokaryotes. The regulation process of prokaryotes cells is often regulated at the transcriptional level. The operator sequence next to the promoter is the main regulatory element. Repressor bound to the operator sequence physically obstructs the RNA polymerase, preventing transcription. Various transcriptional regulators usually control promoters of target genes in response to environmental stimuli (Eckweiler *et al.*, 2018). Riboswitches are another important regulatory sequence commonly present in prokaryotic. These sequences switch between alternative secondary structures in the RNA depending on the concentration of key metabolites (Shafee and Lowe, 2017).

Microorganisms have an impressive metabolic ability and can easily grow in a wide range of environmental conditions (Abatenh *et al.*, 2017). Once they are exposed to pollutants, the microorganisms try to adapt to these pollutants by modulating their gene expression (Paliwal *et al.*, 2012). These specific genes could be turned on or turned off depending on environmental conditions (Baez-Rogelio *et al.*, 2017). Different states of contamination affect gene expression.

The expression of genes involved in biodegradation may be fundamental to obtain the beneficial effect (Baez-Rogelio *et al.*, 2017). Expression patterns provide valuable information for deducing the physiological roles played by the microorganisms (Yang *et al.*, 2017).

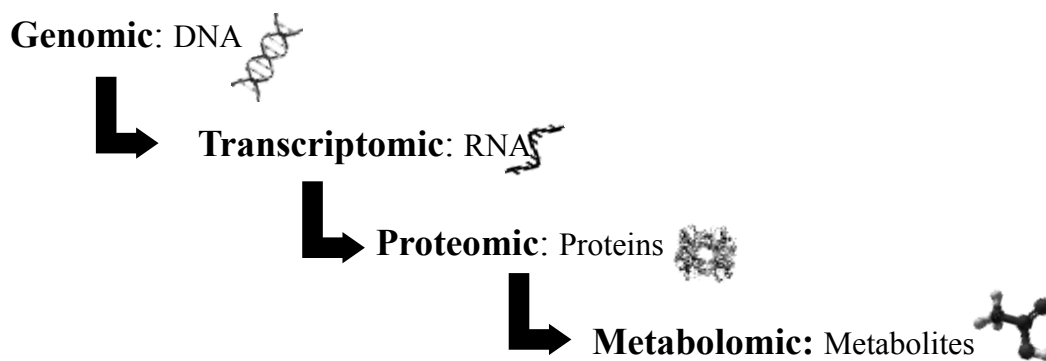
The application of advanced molecular biology techniques has recently provided a new perspective for a better interpretation of the biodegradation process, for example, the omics sciences. The omics sciences study the whole genome, transcriptome, proteome, and metabolome of the organisms involved in a metabolic process such as biodegradation (Wang *et al.*, 2019).

5 Omics sciences: a tool for gene expression study

A better understanding of gene expression of the microorganism used to remediate contaminated sites is required to overcome biodegradation limitations and achieve successful implementation (Rodríguez *et al.* 2020). Omics sciences can help in the study of gene expression. The suffix “-omics” mainly indicates studies on a genome-wide scale of a specific biological system. Omics are principally used to study the whole genes (genomics), RNA transcripts (transcriptomics), proteins (proteomics) and metabolic products (metabolomics) (Marvasi *et al.*, 2019; Zampolli *et al.*, 2018).

The integration and interaction of the different components of the omic studies have given rise to the holistic view of the biological processes as the orchestration of biomolecules network of genes, transcripts, proteins and metabolites (Bedia, 2018). Data generated through each omic are analogous to an information cascade in a cell (Figure 3.2) (Rawat and Rangarajan, 2019).

Figure 3.2 Schematic representation of the omic cascade



Reference: Pandey *et al.*, 2019; Bedia, 2018; Jansson and Baker, 2016

The omic technologies have considerably impacted life and environmental sciences and have generated important insights to increase our understanding in many research areas. Genomics, transcriptomics, proteomics and metabolomics offer remarkable promise as tools to address questions regarding the mechanism involved in the pollutant biodegradation process (Rodríguez *et al.* 2020).

Genomic. Omic science that studies the entire DNA sequence from a given sample obtains information on the entire genome, including phylogenetic and functional genes (Jansson and Baker, 2016). Genomic is static by nature and does not give information about the biological mechanisms encoded within it (Bedia, 2018). When genomic is applied to environmental investigations is based on scanning the genome of a single organism with degradative capacity (Rawat and Rangarajan, 2019). On the other hand, metagenomic, also known as environmental genomics, analyzing the genome of an entire collective microbial in an environmental sample (Desai *et al.*, 2010). The major difference between genomic and metagenomic is that genomic determines gene sets of an organism. In contrast, metagenomic involves analyzing the genome sequence of an entire community inhabiting the same environment (Pandey *et al.*, 2019).

The whole-genome sequence from microorganisms pertinent to bioremediation has been helpful to determine the gene pool of enzymes involved in the degradation of pollutants (Desai *et al.*, 2010). Metagenomic is employed to know the taxonomic community composition and predict the degradation of contaminants (Chandran *et al.*, 2020). The most widely used techniques in genomic are quantitative polymerase chain reaction (qPCR), real-time polymerase chain reaction (real-time PCR), DNA microarrays and next-generation sequencing (NGS) (Pandey *et al.*, 2019; Bedia, 2018).

Transcriptomic. Transcriptomic detect, quantify, study and analyze the transcriptome that is the complete set of mRNA expression (coding or non-coding proteins) in an organism under specific circumstances (dos Santos *et al.*, 2016; Pandey *et al.*, 2019). The transcriptome is more dynamic than the genome due to the continuous transcription processes that reflect cell's activity and their response to external stimuli (Bedia, 2018).

Transcriptomic in environmental science is used to identify and decipher the mRNA expression profiles of genes upregulated or downregulated in microorganisms exposed to pollutants (Desai *et al.*, 2010). But transcriptomic data alone cannot provide information about the activity of degradative enzymes (Rawat and Rangarajan, 2019).

It is accepted that there is a strong correlation between mRNA levels and protein abundance; however, some studies indicate that they are different. This difference results from factors such as protein regulation, post-transcriptional regulation, or possible functional requirement for protein binding. The integration of transcriptomics and proteomics allow to understand this discrepancy (Rodríguez *et al.* 2020). Principal methodologies used to obtain the transcriptional profile are RNA microarrays, RNA sequencing (RNA-Seq), quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and next-generation sequencing (NGS) (Rodríguez *et al.* 2020).

Proteomic. Omic technology is the branch of science that studies the proteome, the entire set of proteins expressed in an organism in a specific place and time (Rodríguez *et al.* 2020; Pandey *et al.*, 2019). Proteomic aims are to provide information about proteome profile, protein phosphorylation, protein trafficking, comparative expression analysis of two or more samples, identification of post-translational modifications and the study of protein interactions (Bedia, 2018).

In environmental studies, proteomic provides information about changes in the protein profiles as a result of the exposition of organisms to pollutants; this identification can facilitate the understanding of which genes are involved in bioremediation processes and how they are regulated (Rodríguez *et al.* 2020). Metaproteomic studies the complete protein profile content of microbial communities residing in a given habitat (Chandran *et al.*, 2020; Pandey *et al.*, 2019).

Proteomic provides information about the mechanisms of adaptation, metabolic pathways, the physiological responses of microbes to pollutants, temperature and other stressors during the biodegradation process (Malla *et al.*, 2018). In the protein analysis process, there are two key steps. The first is the extraction and separation by two-dimensional gel electrophoresis (2DE-GE), two-dimensional difference gel electrophoresis (2D-DIGE), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or high-performance liquid chromatography (HPLC). The second key step is the identification of proteins by mass spectrometry (MS) based approaches. Four types of MS-bases approaches are generally used: quadrupole (Q), quadrupole ion trap (QIT), linear ion trap (LIT or LTQ), time of flight mass analyzer (TOF) and Fourier transform ion cyclotron resonance mass analyzer (FTICR). Also, MS ionization techniques have improved, such as electrospray ionization (ESI) and matrix-assisted laser desorption (MALDI). Nowadays, a diversity of mass analyzers exists in single (MS) or tandem (MS/MS) (Rodríguez *et al.* 2020; Bedia, 2018; Horgan and Kenny, 2011).

Metabolomic: Metabolomic is the quantitative and qualitative study of the metabolome or global metabolite profile produced by an organism in response to defined conditions and time (Rodríguez *et al.* 2020). Metabolome consists of a mixture of thousands of molecules (primary and secondary metabolites), for example, sugars or lipids (Bedia, 2018; Desai *et al.*, 2010). The metabolome study provides information about the biochemical activity of microorganisms and allows the establishment of a relationship between the genetic and phenotypic profiles (Rodríguez *et al.* 2020).

Metabolomic differs from genomic, transcriptomic, and proteomic. A direct connection between metabolites and genes cannot be established because the cell metabolism changes if there is a slight change in the environment (Pandey *et al.*, 2019). In the environmental area, metabolomic allows understanding the dynamics of the microbes and their functional contributions to the environments in which they live and explores the functional roles of these metabolites (Malla *et al.*, 2018). Metabolomics approaches have been used to investigate the responses of microorganisms to various environmental stressors such as heavy metals or temperature and to provide information about the regulatory events in a cell (Chandran *et al.*, 2020; dos Santos *et al.*, 2016).

Extraction and separation of metabolites can be performed by mass nuclear magnetic resonance spectrometry (NRM) or mass spectrometry (MS) based approaches, for example, liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE) (Rodríguez *et al.* 2020). The main MS ionization methods are electrospray ionization (ESI) and electronic impact (EI). Nowadays, a diversity of mass analyzers exists in single (MS) or tandem (MS/MS) (Bedia, 2018).

The information generated by a single omics study is crucial in environmental research but is not always enough to understand a complex biological process as microbial biodegradation (Rodríguez *et al.* 2020). Combining omics enables a better understanding of the mechanisms occurring in the biodegradation process and provides much richer information for predictive models (Rawat and Rangarajan, 2019; Jansson and Baker, 2016).

Integration of genomic and transcriptomic technologies is required to decipher the biodegradation pathways and understand how the contaminant's presence regulates the gene expression. Besides, it is possible to elucidate if the involved genes are constitutive, inducible, downregulated or upregulated (Rodríguez *et al.* 2020).

Combined genomic and proteomic data can provide important information about the microbial enzymes associated with the biodegradation pathway (Pandey *et al.*, 2019). Studies of combined of transcriptomic and proteomic are especially favorable for analyzing the differences in gene expression and their regulation in response to environmental conditions (Aydin *et al.*, 2016). The omics studies mentioned, genomic, transcriptomic and proteomic; will help to discover novel genes, proteins, and underlying pathways for the biodegradation of pollutants (Pandey *et al.*, 2019).

Thus, a multi-omics approach would enable answering biological questions such as which genes are expressed into RNA (transcriptomics) and translated into proteins (proteomics) and which metabolites are present (metabolomic) under specific conditions (Jansson and Baker, 2016). If the aim is to understand the cellular gene expression, transcriptomics and proteomics should be employed (Dangi *et al.*, 2018)

6 Successful biodegradation research applying the study of gene expression

Recently, different omics-based approaches are being used for bioremediation (Pandey *et al.*, 2019). These studies provided relevant information that could be used to understand the biological process, additionally, can help in the implementation and improvement of bioremediation technologies. Table 3.3 displays some research examples where gene expression studies using omic tools were applied, besides the relevant contributions to the scientific community.

Table 3.3 Successful biodegradation research applying the study of gene expression

Pollutant	Organism used	Omic studies	Results	Contributions	Reference
Hydrocarbons (HC) Monocyclic aromatic hydrocarbon (MAH): aniline	<i>Delftia</i> sp. K82 isolated from the Gyeonggi province of Korea in 1992	Genomic: Next-generation sequencing (NGS) Transcriptomic: NGS Proteomic: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) For transcriptomic and proteomic, two cultures were prepared: Luria-Bertani media (LB) and aniline media (ANI)	Genomic: 6327 genes 6117 protein-coding genes Transcriptomic: 3919 genes were identified as differentially expressed genes Proteomic: ANI media 472 proteins LB media 409 proteins	Enzymes of the aniline degradation pathway and aniline-induced novel proteins were identified. ANI cultured was composed of 14 aniline degradation enzymes (11.9% of all proteins). Aniline oxygenase complex was induced more than 2-fold in aniline presence, transcriptionally and translationally. Among 95 proteins belonging to the cell wall, 12 were significantly induced in aniline media. Membrane proteins play essential roles in the protection against extracellular stress. <i>Delftia</i> sp. K82 has two different complete aniline degradation pathways.	Lee <i>et al.</i> , 2021
Synthetic polymer (plastic) Forms of polyethylene (PE): 1. PE4K: commercial powder of PE 2. PE4K-OX: PE4K thermo-oxidized during 14 days at 120°C 3. PEfi: PET films 4. PEfi-OX: oxo-degradable film, which has been stored at room temperature for 10 years	<i>Rhodococcus ruber</i> C208 (environmental strain)	Transcriptomic: NGS Metabolomic: Lipidomic strategy by nano-electrospray ionization mass spectrometry (nano-ESI MS) Condition reference: Mannitol	Transcriptomic: Genes were upregulated in PE conditions (PE4K 28, PE4K-OX 33, PEfi-OX 22) 11 transcripts were commonly overexpressed in the presence of the three types of PE 39 transcripts could be directly assigned to alkane degradation and β -oxidation 34 transcripts encoding putative cytoplasmic oxidase A diacylglycerol kinase was detected Metabolomic: Three main lipid species could be observed	In terms of the number of induced genes: the oxidized forms of PE were more efficient than non-oxidized PE in inducing the mineralization. The most upregulated pathways in the presence of PE are alkane degradation and β -oxidation of fatty acids. The alkane degradation pathway is a central node in the degradation of the PE fragments generated by abiotic oxidation. Oxidases could well participate in the intracellular fragmentation of oxidized PE and extracellular oxidases to reduce the molecular mass of external PE. The redistribution of the phospholipid pattern and the presence of the enzyme diacylglycerol kinase suggests that PE fragments might serve as substrates for the β -oxidation pathway. Metabolic limiting steps were identified which could be fruitfully targeted for optimized PE consumption by <i>R. ruber</i> .	Gravouil <i>et al.</i> , 2017

<p>Heavy metals</p> <p>Plants were irrigated with 1000 mL of heavy metals contaminated water containing Cd, Pb, Cu and Ni at a concentration of 10 ppm. Reference plants were irrigated with tap water.</p>	<p><i>Sorghum bicolor</i> (Sorghum) growing in an open glasshouse at Assiut University Experimental Farm, Assiut, Egypt</p>	<p>Transcriptomic: Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) in leaves of 12-weeks old plants irrigated either with tap-water or heavy metals contaminated water</p>	<p>Transcriptomic: The expression levels of all 15 genes were highly upregulated in response to heavy metals stress</p>	<p>The roles of the genes found are:</p> <p>SbZFP17, SbZFP346 and SbZFP6 genes are zinc finger proteins that are highly expressed in response to the stress imposed by heavy metals. SbPPR1 gene plays a substantial role in heavy metals stress tolerance too.</p> <p>SbLysMR1 plays a role in recognizing symbiotic bacteria, and it is induced in Cd, Cu, and Cr response.</p> <p>LAC9 (laccase gene) is expressed in response to the high concentration of Cu, Pb and Cd and is implicated in response to plant development and stresses.</p> <p>MAPKK gene plays a role in signal transduction of abiotic and biotic stress, and their expression is upregulated under higher concentrations of Cd and Cu.</p> <p>SbAVPL1 regulates solute transport across the vacuolar membrane of plant cells and plays a crucial role in accumulating heavy metals.</p> <p>The genes indicate that these clusters may provide a conserved evolutionary mechanism that might be implemented in common metabolic pathways, produce a protein complex through direct interaction, or serve as receptors in signaling cascades.</p>	<p>Abou-Elwafa <i>et al.</i>, 2019</p>
<p>Pesticide</p> <p>Hexaconazole 98% (triazole fungicide) dissolved in acetone at a concentration of 50 mg L⁻¹.</p>	<p><i>Sphingobacterium multivorum</i> from sewage activated sludge and soil from a pesticide factory producing hexaconazole</p>	<p>Genomic: NGS</p> <p>Transcriptomic: RT-PCR and NGS</p> <p>Metabolomic: Ultra performance liquid chromatography quadrupole-time of flight mass spectrometry (UPLC/Q-TOF MS)</p>	<p>Genomic: The genome contained 5523 genes</p> <p>Transcriptomic: It was detected the presence of 864 differential genes between the hexaconazole treatment and control, 337 upregulated genes and 527 down-regulated genes. Aldehyde dehydrogenase, monooxygenase, RND transporters and ABC transporters were upregulated</p> <p>Metabolomic: Three interesting metabolites were identified 2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexane-2,5-diol; 2-(2,4-dichlorophenyl) hexane-1,2-diol and 1H-1,2,4-triazole</p>	<p>Differential genes are mainly related to metabolism; most of them are concerned with carbohydrate metabolism (39), energy metabolism (36) and amino acid metabolism (30). There are also six genes about xenobiotic biodegradation and metabolites.</p> <p>The reactions of oxidation, hydroxylation and substitution were involved during the degradation of hexaconazole.</p> <p>Hexaconazole can be oxidized, which may be due to the participation of monooxygenase.</p> <p>RND transporter may involve the exportation of toxic metabolites to maintain homeostasis of strain.</p> <p>ABC transporter may provide the essential phosphoric acid and amino acid to survive under a high concentration of hexaconazole.</p> <p>The results of these studies could provide a reference for in-situ remediation of hexaconazole.</p>	<p>An <i>et al.</i>, 2020</p>

7 Remarks

Gene expression analysis using multi-omics is a valuable tool for the elucidation of unknown or hidden metabolic diversity of organisms, screening novel genes for a biodegradation response, identifying novel enzymes for biodegradation, and knowing the enzyme synergy (Lee *et al.*, 2021).

In the specific case of biodegradation processes, knowing what happens at the molecular level from the moment the biological system comes into contact with pollutants helps understand the whole process. After the entire process is known, the following optimization studies will be more straightforward. For example, all the identified enzymes involved in the biodegradation process may be cloned, isolated, and used as a biological tool in specific contamination situations.

Gene expression studies generate large amounts of information about the root of biological behavior, serving to apply to a specific industrial sector or other research groups with different objectives.

8 Conclusions

Environmental pollution is a serious and global problem. Hydrocarbons, plastics, heavy metals and pesticides are priority pollutants. They arrive at the environment by human activities first and negatively impact the environment and human health. Biodegradation is a process that could be helpful to eliminate or reduce pollutants in an economically and ecologically way. Several organisms with degradative capacity have been reported, but the efficiency is affected by different factors. For that, it is necessary a better understanding of the complex behavior of the organisms in detail.

The study of gene expression of degradative microorganisms using omics technologies provides relevant information that could be used to implement or improve the biodegradation process on a large scale.

These studies have offered crucial information about biodegradation that can be used to know or understand: genes transcribed, enzymatic activities, novel enzymes, survival mechanisms, secondary metabolites, hidden biodegradation pathways, exact metabolic pathways, and modifications of existing pathways under conditions of stress caused by pollutants. These aspects are important to optimize the success of the biodegradation approach.

The knowledge about the degradative organisms will help design or improve remediation strategies by manipulating the pathways adding or deleting one or more genes, incorporating new metabolic pathways into organisms, or modifying enzyme specificity and affinity. Also, the enzymes could be used to produce other high-value-added metabolites such as precursors for biotechnological or pharmaceutical products.

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