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Effective microbial bioremediation via the multi-omics approach: An overview of trends, problems and prospects

Yunusa, Y.R.[1] **and Umar, Z.D.**[2]

[1]Department of Microbiology, School of Postgraduate Studies, Umaru Musa 'Yar'adua University, Katsina, Nigeria.

[2]Department of Microbiology, Faculty of Natural and Applied Sciences, Umaru Musa 'Yar'adua University, Katsina, Nigeria.

*Corresponding Author: yahayayunusariko@gmail.com; +234(0)7063966453

Abstract

Techno-industrial advancements the world over had led to the generation of hazardous environmental pollutants. Microbial bioremediation offers the best alternative for the removal of these pollutants. The most recent advancements in microbial bioremediation were catalyzed by the advent of various tools that enable the study microbes at levels of sophisticated detail, including genome analysis tools (genomics), protocols for analyzing expressed proteins and enzymes or proteomes (proteomics), techniques of analyzing ribonucleic acids (RNAs) transcriptomes (transcriptomics), and tools for analyzing metabolic end products/metabolomes (metabolomics). The twenty first century is witnessing an outpour of developments in the application of omics approaches in effective microbial bioremediation, thus, this paper attempts to review some of the most significant insights gained from relatively recent studies over a period of two decades (2000-2020) in the applications of multi-OMICS in microbial bioremediation, including trends and cutting-edge researches. We aim to highlight, particularly, the challenges that need to be overcome before OMICs approaches are successfully enshrined in microbial bioremediation, especially in developing countries. The strategies for overcoming such challenges, and the prospects achieved were also outlined. In the coming years, we envision further researches involving the application of multi-OMICs approach in microbial bioremediation potentially revolutionizing this field, opening up research avenues, and leading to improvements in bioremediation of polluted environment.

Keywords: Biodegradation, Bioremediation; Genomics; Multi-OMICs, OMICs techniques.

INTRODUCTION

Bioremediation can be defined as the application of living organisms, such a plants, algae and microorganisms, instead of physico-chemical and mechanical approaches in the cleaning of pollutants from contaminated environments, thereby returning the polluted environments back to their normal condition (Umar *et al.*, 2019). Bioremediation has assumed a status of necessity and urgency, in the face of environmental hazards associated with pollutants accumulating into the environments on a daily basis (Umar, 2017). Microbial bioremediation is more advantageous and applicable than phytoremediation, due to their cosmopolitan ubiquity and diverse metabolic potentialities, especially at extremes of environmental conditions where plants cannot survive (Desai *et al.*, 2009; Umar *et al.*, 2018a; 2018b).

Current studies in the field of microbial bioremediation focus on the use of molecular tools and OMICs techniques to study microbes at levels of sophisticated detail (Desai *et al.*, 2009; Chikere, 2013; Haroon *et al.*, 2013; Colin *et al.*, 2015; Plewniak *et al.*, 2018; Joye & Kostka, 2020). Previously, studies on bioremediation tend to centre on the isolation and identification of microorganisms capable of biodegradation; screening them for biodegradation ability; quantitative evaluation of the degradation ability of the microbes; determining the chemical kinetics of the biodegradation process and ascertaining the intermediate and final products of biodegradation (Konneke *et al.*, 2005; Das & Chandran, 2010; Ma & Zhai, 2012; Ajao *et al.*, 2014; Karthika *et al.*, 2014; Agarry & Latinwo, 2015; Hazrati *et al.*, 2015; Gieg & Toth, 2017; Overman *et al.*, 2017; Birch *et al.*, 2018; Hammershoj *et al.*, 2019; Musa, 2019; Umar *et al.* 2020a; 2020b).

Nonetheless, despite the great achievements recorded in the field of microbial bioremediation using these approaches, mainly the isolation and characterization of novel microbes with potential degradation abilities from different places, globally (McDonald *et al.*, 2006; Adebusoye *et al.*, 2007; Singh & Lin, 2008; Das & Chandran, 2010; Nikhil *et al.*, 2013; Rehman *et al.*, 2015; Yetti *et al.*, 2016). There had been a major shortcoming: despite the fact that microbial capability of utilizing metabolites and pollutants is virtually boundless, there are certain compounds that had been observed to be recalcitrant, and those that have not been reported to be degraded before. This leads researchers to pose questions, regarding whether new microbes and microbial capabilities for remediating these recalcitrant compounds can be isolated and identified (Esteve-Nunez *et al.*, 2001; Singh and Nagaraj, 2007). However, the use of -omics techniques (genomics, transcriptomics, proteomics, and metabolomics), spearheaded bywhole genome sequencing technology data makes possible the identification of microbes and elucidation of functional genes degrading hitherto unreported contaminants and certain recalcitrant compounds (El-Amrani *et al.*, 2015; Basak & Dey, 2016). Specifically, this approach has resulted in the elucidation of novel Archaea such as the first nitrifying Archaeon (Schleper *et al.*, 2005); the only strain in acid mine tailings capable of fixing nitrogen (Tyson *et al.*, 2005) and an iron-oxidizing strain grouped into the novel genus called *Ferrovum* (Ulrich *et al.*, 2016).

The term, meta-omics was coined to describe the applications of omics in environments, directly. This was necessitated by the widespread observations that biodegradation determinants in controlled microcosms, i.e. biodegradation experiments in the lab or *in vitro* and results of application of microbial communities *in situ*, i.e. during field trials, differ to some extent, which was attributed to spatio-temporal changes (Ma & Zhai, 2012). Among the omics technologies, proteomics and transcriptomics are associated with the direct indicators of changes in physiological responses elaborated by microbes in response to different phenomena in the environment, either through initiation of production of responses (transcriptomics), or through the production of translation products that can directly impact the environments called proteomics (Singh & Nagaraj, 2006)

These techniques widened, and are still widening the horizons of understanding of the molecular basis of microbial bioremediation; increasing the efficiency of *in situ* bioremediation techniques, and helping the redesigning of more effective techniques and approaches to microbial bioremediation of contaminated sites (Wood, 2008).

Moreover, the application of OMICs techniques had led to the discovery of novel microorganisms not previously cultured in the lab, the annotation of the genomes, metabolomes and proteomes of these microbes, and their applications in biotechnology, for enhanced microbial bioremediation (Overmann, 2010; Gutleben *et al.*, 2018).

Through the OMICs approach, current knowledge of microbial bioremediation has increased a great deal. For instance, researchers have confidently established, through these approaches, certain structural-functional relationships of microbial cells or communities and applied these to bioremediation. Moreover, it had been found that a correlation exists between the abundance and expression of the functional genes in polluted areas, and the rates of microbial degradation or transformation of the pollutants, thus paving the way for improved designs of models for predicting the kinetics of microbial bioremediation (Zhou & Fields, 2006).

Understanding the OMICs

The term OMICs refers to the holistic identification and quantification of repositories of biomolecules responsible for microbial physiology and metabolism, structure and dynamics, through various techniques such as genomics, proteomics and metabolomics. The term is derived from the phrase omic which is affixed to the end of the techniques mentioned above. Moreover, the subjects of studyof each field are described as the OMEs of the OMICs, i.e. genomes, proteomes and metabolomes, for the three OMICs techniques stated before, respectively. The OMICs also include lipodomics, which is the study of microbial lipids; interactomics, which study gene-gene and protein-protein interactions; and microbiomics, which studies the genomes of whole microbiomes, i.e. microbial communities in a particular locale (Lederberg and McCray, 2001; Holtorf *et al.*, 2002;Rathoure & Dhatwalia, 2016;Kumari & Kumar, 2021).

Conceptualizing the OMICs Approach

The OMICs technologies, as described earlier, encompass many aspects, including these seven major branches:

1. ***Genomics:*** Genomics involves the utilization of high-throughput and deep sequencing tools to sequence complete microbial genomes, with rapidity and precision. Data from genomics illustrates the fine details regarding the metabolic potentialities of particular microbes and their roles in diverse ecosystems, e.g. their hydrocarbon degradation potential. Upon subsequent robust laboratory investigations, it is possible to focus on specific metabolic activities and functions of individual microbial genes (Lovely, 2003; Pan *et al.*, 2015; Imperato *et al.*, 2019;Oyewusi, 2021).

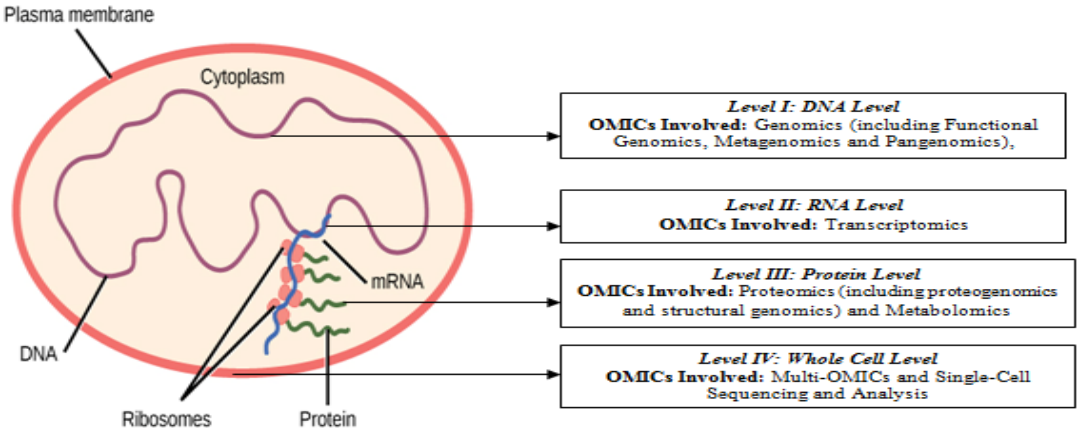

Figure 1: A simplified schematic diagram of a typical bacterial cell highlighting the areas where specific 'OMICs' are applicable. Modified from: Brainborder.com (2020)

Functional genomics, as a branch of genomics, involves the study of the functions of individual genes and their relationships with expressed metabolic products. It was said to arise due to the discovery of various genes whose function was unknown, in many genome-sequencing projects (Pan *et al.*, 2015; Shao *et al.*, 2019; Xiang *et al.*, 2020). Metagenomics, on the other hand, is the genomic analysis of environmental samples, while Pangenomics studies the whole genes or genomes associated with a particular species (Singh and Nagaraj, 2007; Marchesi & Ravel, 201; O'Connell *et al.*, 2017).

2. ***Transcriptomics:*** This involves "studying the profiles of microorganisms by analyzing their RNA sequences". Here, gene expression and its relationship to environmental features and or turbulences are revealed. As such, with such knowledge, the active microbial players in particular microcosms, their genetic composition and their responses to environmental phenomena can be discerned. Usually, environmental disturbances, such as oil spills, trigger changes in the RNA profiles of microorganisms, and studying these changes will illustrate how microbes respond to such conditions (Singh & Nagaraj, 2007; Yoneda *et al.*, 2016; Gu *et al.*, 2018).

3. ***Methylomics:*** This encompasses the investigation of how DNA methylation occurs, as it relates to the epigenetic regulation of DNA patterns, the study of which is known as epigenomics. Combined, these two switch specific genes or gene cascades on or off, in response to environmental changes or stress (Marchesi &Ravel, 2015).

4. ***Proteomics:*** Proteomics is a terminology coined in 1995 (Wasinger *et al.*, 1995). This involves the use of mass spectrometry analysis to disclose the protein content of cells, and how these can be affected by posttranscriptional modifications, such as phosphorylation. These changes are major mechanisms that switch many proteins on or off, which can dramatically affect their cellular functions. Subsumed under this is proteogenomics, which studies the application of proteomics for annotating genes, and structural genomics, which elaborates the 3-dimensional structures of proteins through experimentation and modeling (Zhao and Poh, 2008).

5. ***Metabolomics:*** This branch of OMICs deals with the application of a spectrum of advanced analytical chemistry techniques, including nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry, such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). These techniques are used to generate metabolite fingerprints of expressed metabolic currencies available to, produced by, and exchanged between microbes. A branch of the metabolomics is metabonomics, a quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification (Joye & Kostka, 2020).

6. ***Meta-OMICs:*** Meta-OMICs is a holistic approach that combines all aspects of cell metabolism, from gene sequences to expressed metabolites, with a view to revealing the roles and functions of microbial communities synergistically interacting with one another beyond individual efforts of microbes. Furthermore, meta-OMICs gives cues regarding the relative abundance, and indirectly, roles, of species of microorganisms, their genes, protein expression products within communities in the environment (Gutleban *et al.*, 2018).

7. ***Single-cell Sequencing and Analysis:*** Finally, in this ultimate approach, individual cells and species of microorganisms are studied; and particular genes, transcripts, etc., and associated ecosystem functions are linked to specific individuals within the microbial community, thus, the identification of most prominent hydrocarbon degraders is readily possible (Woyke *et al.*, 2017; Joye & Kostka, 2020; Kaster & Sobol, 2020).

Prospects of the OMICs Approach in Microbial Bioremediation

As Zhou & Fields (2006) have highlighted, microbial bioremediation stands to benefit from theapplication of these OMICs techniques in many respects. For example, the sequence-based metagenomics approach can serve as an index of observing microbial diversity and community dynamics of polluted environments. Likewise, proteomic approaches function as tools for the quantitative evaluation of microbial roles in the contaminated areas. The populations of microorganisms and the presence of certain genes of interest are detectable using functional gene arrays, generated via application of metagenomics, as high throughput and quantitative tools with great precision. Metaproteomics, the quantitative identification of hundreds if not a thousand proteins is also now employed (Bargiela *et al.*, 2015; Bozinovski *et al.*, 2016; Xu & Zhao, 2018).

OMICs approaches in general can as well delineate the interrelationships between biomarker measurements and microbial roles in polluted environments. Moreover, prospectively, data chunks from OMICs studies can be used to hypothesize for behaviors of microbial populations, communities, etc. (Denaro *et al.*, 2005; Roling *et al.*, 2010; Seifert *et al.*, 2013) The efficiency of metagenomic analyses can be increased by factoring in biochemical pathway(s) of interest and environmental conditions such assulfate-reduction, methanogenic, and iron-reduction (Hadadi *et al.*, 2020). In the near future, OMICs techniques can be utilized in improving the ability to predict geo-ecological phenomena in subsurface ecosystems, with regards to on-the-field bioremediation (McLean, 2013; Qiao *et al.*, 2013; Ali *et al.*, 2020). Ultimately, utilizing the profiles of functional microbial ability generated from OMICs studies into geochemical models can have enhanced predictive properties. Knowing the dominant microbial communities in degraded environments allow the adjustment of environmental conditions to favor the desired populations (Zhou & Fields, 2006; Umar *et al.*, 2016).

Focus on Specific OMICs and their Application in Bioremediation

I: Genomics, Functional Genomics & Metagenomics

Genomics approaches have helped microbiologists in the molecular study of the microbial communities involved in biodegradation in great detail, and describing microbes with potential degradation ability that were not previously discovered (Liu & Liu, 2013; Umar *et al.*, 2017;Joye & Kostka, 2020). A typical example of a research involving this approach is that of Liu and Liu (2013), in which the researchers described the previously unreported potential of some members of the *Bacteriodes* genera to carry out microbial biodegradation.

Furthermore, metagenomic studies can be targeted towards using environmental DNA (E-DNA)which had been amplified using specific primers to search the presence of microorganisms capable of performing biodegradation (Vakhlu and Gupta, 2013). For instance, genes such as methane monooxygenase, methanol dehydrogenase, and ammonia monooxygenase genes are used in the identification of methanotrophic and chemolithotrophic ammonium oxidizing bacteria (Henckel *et al.*, 2000; Singh & Singh, 2017; Gaby *et al.*, 2018; Thulasi *et al.*, 2018; Wright *et al.*, 2020). Similarly, catechol 2,3-dioxygenase, chlorocatechol dioxygenase and phenol hydroxylase genes are used in screening microorganisms for biodegradation potential (Marsachi *et al.*, 2000;Futamata*et al.*, 2001; Lilis *et al.*, 2010; He *et al.*, 2016).

The use of metagenomics can also reveal species succession in polluted environs (Rodriguez-R *et al.*, 2015). Using metagonomics studies spread across specific time intervals, *Alcanivorax* and *Marinobacter* spp., both belonging to the *Gamma proteobacteria,* that had earlier dominated the microbial community of a Deepwater spill buried in a Florida beach, for at least three months, degrading mostly the alkanes, were shown to be succeeded by members of the *Alphaproteobacteria*, specifically, *Hyphomonas* and *Parvibaculum*, which are capable of biodegrading aromatic

compounds. Biodegradation was finished in a year, with the beach's population then turning back to its original form, with the biodegraders suppressed to the low levels they were in hitherto the pollution (Huettel *et al.*, 2018)

Functional genomics have also highlighted the role of Marine Oil Snow (MOS) in the biodegradation of spilled petroleum hydrocarbons in marine environments. Marine Oil Snows form from the aggregation of microbial communities, typically bacteria, around oil particles, encasing them in biosurfactants or transparent exopolymers and biomass, forming 'snows' which then descend to the seafloor (Ziervogel *et al.*, 2012). This mechanism played an important role in the removal of oil from the water surface during the Deepwater spill (Vonk *et al.*, 2015). The lipase activity of MOS particles is thought to confer on them the potential to serve as ready avenues for oil degradation (Gutierrez *et al.*, 2018). The MOS contains microbial communities that are distinct from the surrounding water column and specialized for breaking down oil (Umar and Bashir, 2014). In particular, *Colwellia* and *Marinobacter* spp. (as well as other *Alteromonas* spp.) are prevalent in these particles, with the unique capacity to rapidly degrade oil in cold, deep marine environments (Gutierrez *et al.*, 2018).

Another application of functional genomics is the elucidation of candidate genes from microbial consortia for previously obscure roles, an example of which is the study by Shin *et al.* (2019) which described the anaerobic degradation of polycyclic aromatic hydrocarbons via anaerobic pathways, such as sulfate reduction, from enriched microbial consortia from the Gulf of Mexico.

The use of genomic approaches can also reveal the coupling of certain elements involved in biogeochemical cycling with microbial biodegradation, such as nitrogen, phosphorus, sulphur, iron, and other trace metals, the genes for the metabolism of which are shown to be enriched during biodegradation (Bashir *et al.*, 2014; Rodriguez-R *et al.*, 2015). This insight is useful in at least two ways, one is devising approaches for 'fertilizing' contaminated environments with those elements to facilitate biodegradation, and two, as evidence for the roles of certain metal cofactors, such as lanthanides, in serving as cofactors in biodegradation of methylotrophs (Shiller *et al.*, 2017).

Another area for potentially applying metagenomics is in the characterization of genes involved in biodegradation, as it is thought that 30-50% of those genes remain uncharacterized. Identifying these genes is particularly important as they may be involved in degrading a broader range of substrates, a smaller subset of substrates or perform an entirely new function (Igeno *et al.*, 2019; Joye & Kostka, 2020; Salvador *et al.*, 2020; Santero & Diaz, 2020).

II. Transcriptomics

The transcriptome describes the total of the transcription products of a microorganism, as such, they are critical bridges between genomics, proteomics, and cellular phenotypes. Transcriptomics is affected by changes in the genome, due to regulations in genes, manifested by the microbe, to adapt to varying environmental conditions, and the study of these is best done using DNA microarray studies, which enable, by and large, studies of individual messenger RNAs (mRNAs) (Dharmadi& Gonzalez, 2004; Diaz, 2004).

Practical, *in situ* applications of transciptomics in bioremediation include the use of DNA-microarray technologies in studying soils artificially contaminated with naphthalene (Cho and Tiedje, 2002).These DNA-microarray studies can generate the whole genome transcribed products. Subsequently, the data gathered can be assembled into a diagram of the regulatory mechanisms governing bioremediation in microbes (Seshadri *et al.*, 2005; Ladezma-Villaneuva *et al.*, 2018; Wang *et al.*, 2020). Furthermore, transcriptomics studies can involve the analysis of gene expression products of microbial communities following changes in certain environmental factors (Dennis, 2006), for instance, varying oxygen concentration results in more than 100 genes in *Bacillussubtilis* cultivated anaerobically (Ye *et al.*, 2000).

Greene and Voordouw (2003), in the same vein, utilized transcriptomics in studying how environmental microbial communities respond to stress, by studying functional 'stress genes'. Another study by Kuhner *et al.* (2005), studied the microbial bioremediation properties of anaerobic toluene and ethyl benzene degradation, where a global gene expression analysis helps in the elucidation of mechanisms for regulating many unidentified genes involved in the catabolism of alkylbenzenes. In another study, it was shown that upon exposure to the toxic compound o-xylene, *Rhodococcusopacus*R7 elaborated 542 differently expressed genes for stress tolerance, osmotic regulation and central metabolism (Zampoli *et al.*, 2020).

In another study, the same bacterium, and another member of the same genus, *R.aetherivorans* had also been reported to survive a wide spectrum of stresses ranging from osmotic and oxidative stress to the presence of antibiotics, metals and other toxic compounds (Capelletti *et al*., 2016).

III: Proteomics

Proteomics presents a comprehensive summary of the expressed phenotype of a microbial species, better than the genome can (Singh and Nagaraj, 2007). Traditionally, the tools used for proteomics analyses include multidimensional Poly Acrylamide Gel Electrophoresis and Mass Spectrometry, however, recent improvements have resulted into the advent of multidimensional protein identification technology (mudPIT) as a technique for proteomics (Paolett*iet al*., 2004).

Proteomics had been shown to play a key role in the detection of biomarkers for identifying environmental pollutants, such as polycyclic aromatic hydrocarbons (Aardema & McGregor, 2002). It is known that in the bioremediation of polycyclic aromatic hydrocarbons, alterations to the microbe involved in the process results in drastic changes in cell surface receptors and proteins, hence, by monitoring these using proteomics, significant changes can readily be detected (Singh and Nagaraj, 2007). When the peptides have been successfully removed from the soil environment, they are then identified by subjecting them to Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF), where the respective peptide fingerprints will be translated into specific proteins. Additionally, it is now possible to use MALDI-TOF-MS directly *in situ*, using an alternative developed called Surface-Enhanced Laser Desorption Ionisation Tine-of-Flight Mass Spectrometry, where the MALDI apparatus is incorporated onto a chip that can directly interact with sample fractions from environments (Knigger *et al*., 2004).

Another technique being considered is Liquid-Chromatography Mass Spectrometry (LC-MS), which is of particular use in aquatic analyses, to detect contaminants that can possibly pollute the water, however, in the microbial bioremediation arena, this technique has been extended to be used in analyzing the metabolites signaling biodegradation of pesticides, surfactants, pharmaceutical wastes, etc. (Joo and Kim, 2005).

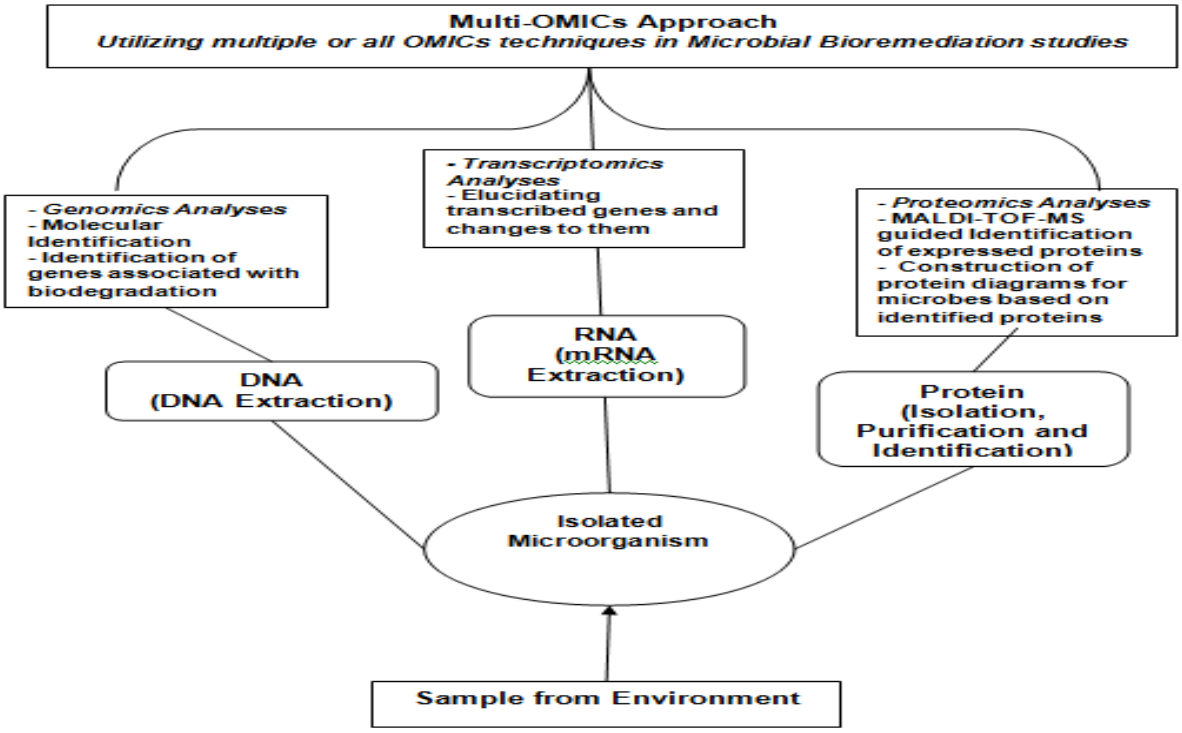

Figure 2: Proposed scheme for an OMICs approach to microbial bioremediation (Modified from: Singh and Nagarajan, 2007).

Proteomics is directly linked to microbial biodegradation because protein expression, at least in some genes, is regulated by certain responses to external environmental stimuli, such as presence of pollutants (Wilkins *et al*., 2001; Kim *et al*., 2002).

Separate, but related studies had practically demonstrated this. For instance, Wang *et al*. (2000) demonstrated that in the degradation of pyrenes, a protein of mass 81kDa is involved, which resembles a catalase-peroxidase enzyme complex. Khan *et al*. (2001), on the other hand, reported that growing a *Mycobacterium* sp. on phenanthrene, dibenzothiopene and pyrene led to the induction and overexpression of six major proteins, which the authors identified using 2-D-Gel Electrophoresis.

In another study, various proteins were indicated as potential indicators of enzymatic degradation of pyrene, e.g. two-ring hydroxylating doxygenases, by a species of *Mycobacterium* (Krivobok *et al.*, 2003). Moreover, Kim *et al*. (2004) were able to generate a library of about 20 proteins elaborated by a strain of *Mycobacterium vanbaalenii*, when cultured in a high molecular weight PAHs-rich environment.

In yet another study, Santos *et al*. (2004) constructed a protein reference map for *Pseudomonas putida* KT2440 grown in high, but tolerable concentrations of phenol, where 81 proteins where expressed, with 68 being up-regulated (including proteins involved in general and oxidative stress response, cell envelope and fatty acid biosyntheses, energy utilization, cellular transport, among others), and 13 being down-regulated (nucleotide biosynthesis and cell motility proteins).

In the future, it has been suggested that proteomics studies should target how the expression of proteins changes with variations in environmental factors, presence of toxins, etc. Such studies may hopefully elucidate signature proteins key in biodegradation activities of specific microorganisms. Furthermore, the combination of transcriptomics and proteomics has the potential to illuminate new pathways for aerobic and anaerobic biodegradation, and this can be doneusing protein microarray–based approaches (Namasivayam, 2013).

Finally, proteomics researches are receiving significant impetuses through the development of cutting-edge techniques, such as the recent approach of studying the translation products of microorganisms through the BONCAT approach (Bioorthogonal Noncanonical Amino Acid Tagging), which has the potential to be coupled with multi-OMICs to facilitate acquisition of news insights into patterns of microbial biodegradation of pollutants Hatzenpichler *et al*. (2016).

IV: Metabolomics

Through metabolomics-related studies, the diversity of microorganisms and their previously unknown capacities for microbial biodegradation can be understood. Such studies can also lead to understanding roles that are previously unknown for specific microbial genera or species. For instance, the bacterium, *Cycloclastus* sp. is previously known to degrade polycyclic aromatic compounds at low temperatures, (Dubinsky *et al.*, 2013), however, later, it was shown to have the capability of degrading ethane, propane and butane via aerobic oxidation; at both the surface and sediments, showing a more versatile tolerance to temperature fluctuations (Joye & Kostka, 2020).

Recent studies (for instance, Huettle *et al.*, 2018; American Association of Microbiology, 2020) had also enabled the appreciation of certain species having diversified metabolomic profiles, such as *Marinobacter* sp. (Huettel *et al.*, 2018), and in particular, *Marinobacter hydrocarbonoclasticus*, which has been shown to possess a repertoire of metabolic capabilities for growth in high and low salinity environments, using both oxygen and nitrates or nitrites as electron acceptors, and oxidizing both alkanes and aromatics. This biogeochemical opportunitroph, as it has been described, can exist in a spectrum of environments and carryout numerous functions (Gaby *et al.*, 2018; Joye & Kostka, 2020).

Likewise, metabolomics have helped in elucidating novel species of microorganisms widely distributed across the globe, and sharing remarkable functions. One such organism is the bacterium, "*Candidatus* Macondimonas diazotropica", (Karthikeyan *et al.*, 2019) which has been shown to account for about one-thirds of the microbial community degrading oil spilled on shores during the mid to late phases of the degradation (Weiman *et al.*, 2021;Karthikeyan *et al.*, 2019). A key feature of the bacterium is its ability to fix nitrogen, thus, it can survive even in low-nitrogen environs (Karthikeyan *et al.*, 2019).

Table 1: Summary of some applications of OMICs approaches in microbial bioremediation studies

OMIC Technologies and Instances of their Practical Applications in Microbial Bioremediation	Reference
I. Genomics, Functional Genomics & Metagenomics: Genetic material of organisms that reveal identity and functional genes	
1. Explanation of how environmental DNA which had been amplified using specific primers can be used to search the presence of microorganisms capable of performing biodegradation, e.g. using genes such as methane monooxygenase, methanol dehydrogenase, and ammonia monooxygenase genes to identify methanotrophic and chemolithotrophic ammonium oxidizing bacteria; and catechol 2,3-dioxygenase, chlorocatechol dioxygenase and phenol hydroxylase genes can be used in screening microorganisms for biodegradation potential.	Mesarch *et al*. (2000) Henckel *et al*. (2000) Futamata *et al*. (2001) Vakhlu & Gupta (2013)
2. Revealing microorganisms with previously unknown biodegradation potential	Liu &Liu (2013)
3. Illumination of how certain elements involved in biogeochemical cycling (e.g. nitrogen, phosphorus, sulphur, iron, and other trace metals), are coupled with microbial biodegradation, how genes for their metabolism are enriched during biodegradation; potential for fertilizing contaminated sites and their roles as cofactors (e.g. in biodegradation of methylotrophs).	Rodriguez-R *et al*. (2015) Shiller *et al*. (2017)
4. Elucidation of succession between microbial species (e.g. *Alcanivorax*, *Hyphomonas*, *Parvibaculum* and *Marinobacter* spp.), how they evolve over time in polluted environments, their enrichment, how they biodegrade alkanes and oraromatics and how they become suppressed after the completion of the biodegradation, through metagenomics analyses.	Rodriguez-R *et al*. (2015) Huettel *et al*. (2018)
5. Description of the role, formation and function of Marine Oil Snow (MOS) in biodegradation of spilled petroleum hydrocarbons in marine environments, the microorganisms involved in the process, (*Colwellia* and *Marinobacter* spp.; *Alteromonas* spp.) and the roles they play in bioremediating the Deepwater spill.	Ziervogel*et al*. (2012) Vonk*et al*. (2015) Gutierrez *et al*. (2018)
6. Highlighting of candidate genes from microbial consortia from the Gulf of Mexico which carryout previous undescribed functions, e.g. the anaerobic degradation of polycyclic aromatic hydrocarbons via anaerobic pathways, such as sulfate reduction.	Shin *et al*. (2019)
7. Characterization of genes involved in biodegradation – 30-50% of which remain uncharacterized – including those potentially involved in degrading a broader or smaller range of substrates, a smaller subset of substrates, or perform entirely new degradation functions.	Joye & Kostka (2020)
II. Transcriptomics: studying the gene expression products, in form of mRNAs, to determine changes to genomes, and genome functions.	
1. Application of DNA microarrays in studying soil artificially contaminated with naphthalene.	Cho & Tiedje, (2002)
2. Studies of mRNAs to elucidate previously unidentified genes involved in the degradation of alkylbenzenes.	Khuner*et al*. (2005)
III. Proteomics: Study of the translation products of expression of genes, their modification, and how they affect cellular response, with regards to environmental alterations	

1.	Characterization of an 81kDa protein resembling catalase-peroxidase enzyme complex elaborated in the presence of pyrenes.	Wang *et al*. (2000)
2.	Induction and overexpression of six major proteins identified using 2-D-Gel Electrophoresis, from a *Mycobacterium* sp. grown on phenanthrene, dibenzothiopene and pyrene.	Khan *et al*. (2001)
3.	Generation of a library of some 20 proteins elaborated by a strain of *Mycobacterium vanbaalenii* cultured in a high molecular weight PAHs-rich environment.	Kim *et al*. (2004)
4.	Construction of a protein reference map for *Pseudomonasputida* KT2440 grown in high, but tolerable concentrations of phenol, and monitoring the expression, up and down regulation of the proteins.	Santos *et al*. (2004)
5.	Analysis of metabolic intermediate and final products indicative of biodegradation of pesticides, surfactants, pharmaceutical wastes, etc.	Joo & Kim (2005)
6.	Description of changes in protein expression and how they vary with environmental factors, presence of toxins, etc., as such identifying signature proteins key in biodegradation activities of specific microorganisms.	Namasivayam, (2013)
7.	Combination of transcriptomics and proteomics to illuminate new pathways for aerobic and anaerobic biodegradation using protein microarray–based approaches.	Hatzenpichler *et al*. (2016)
8.	Elucidation of how translation products of microorganisms are studied through the BONCAT approach (BioorthogonalNoncanonical Amino Acid Tagging), coupled with multi-OMICs to facilitate acquisition of news insights into patterns of microbial biodegradation of pollutants.	Hatzenpichler *et al*. (2016)
IV.Metabolomics: Studying the comprehensive, expressed metabolic fingerprints of microorganisms or microbial communities		
1.	Revealing the diversity of microorganisms, and explaining previously unknown capacities of microbes for microbial biodegradation, with specificity up to the genera and even species level, e.g. *Cycloclastus* sp., previously known to degrade polycyclic aromatic compounds at low temperatures, had been shown to be capable of degrading ethane, propane and butane, aerobically or anaerobically, across a wide temperature range.	Dubinsky *et al*. (2013)
2.	Identification of diversified metabolomic profiles of microbes such as *Marinobacter* sp. and in particular, the 'biogeochemical opportunitroph':*Marinobacterhydrocarbonoclasticus*, which has been shown to possess a repertoire of metabolic capabilities for growth in high and low salinity environments, using both oxygen and nitrates or nitrites as electron acceptors, and oxidizing both alkanes and aromatics).	Gaby *et al*. (2018). Huettel *et al*. (2018)
3.	Elucidation of novel species of microorganisms widely distributed across the globe, having remarkable biodegradation capabilities, e.g. the bacterium "*Candidatus*macondimonasdiazotropica", which can take up to about one-thirds of the microbial community degrading oil spilled on shores during the mid to late phases of the degradation, and is also capable of nitrogen fixation, hence can survive even in low-nitrogen environs.	Karthikeyan *et al*. (2019) Weiman *et al*. (2021)

V. Multi-OMICs: Harmoniously leveraging the various OMICs techniques in concordance, towards better studies of microorganisms involved in bioremediation, and their metabolism.	
1. Studying the interrelated links between metabolic pathways for degradation of petroleum hydrocarbons, and how each specie in this 'microbial conglomerate' specifically contributes to 'division of labor'.	Mason *et al*. (2012)
2. Tracing the fate of a specified substrate within a diverse microbial community, thus characterizing pathways for its degradation, potentially new microbial species, and the unknown functions of known degraders, through Stable Isotope Probing (SIP).	Gutierrez *et al*. (2013)
3. Explaining how microbial communities involved in biodegradation change before and after the occurrence of spills, i.e. before spills, these communities may be present in low amounts, however, spills trigger their rapid proliferation, domination of the microbiome of the polluted place, and expression of multiple metabolic pathways depending on the nature of the pollution and other environmental factors.	Dubinsky *et al*. (2013) Rodriguez-R *et al*. (2015) Shin *et al*. (2019)
4. Identification of pollution-related biomarkers including predictive biomarkers (e.g. first responders in spill-affected environs that are indicative of potential for spills, leaks, etc.), diagnostic biomarkers (which indicate the presence of contamination in environments) and therapeutic biomarkers (which are used in restoration and reclamation of spill-affected environments)	Joye & Kostka (2020)
5. Studying soil microbial species' diversity or consanguinity, their genetic capabilities and potential, their inherent and expressed metabolic profiles; and their potential application in diverse environments.	Dini-Andreote*et al*. (2012) Fierer *et al*. (2012) Umar *et al*. (2020)

V: Multi-OMICs: The Combination of Multiple OMICs Techniques

The application of multi-OMICs in bioremediation can best be illustrated by the aftermaths of the case of the Deepwater Horizon spill. For instance, Mason *et al*. (2012) used metagenome, meta-transcriptome and single-cell sequencing analyses to reveal the overall microbial response of the multitude of microorganisms to the spill, revealing the spread of metabolic pathways across multiple community members, and using metatranscriptome and metagenome data to identify the role and function of each species in the pathway.

The application of multi-OMICs approach is also seen in Stable Isotope Probing (SIP), where the fate of a specified substrate is traced within a diverse microbial community, thus characterizing pathways for its degradation, potentially new microbial species, and the unknown functions of known degraders (Gutierrez *et al*., 2013). The technique is typically applied in identifying active microorganisms without having to resort to cultivation, and this can be important in screening microbes with potential to be used in bioremediation (Chen *et al*., 2010). This technique has proven particularly successful in the identification of uncultivable bacteria capable of degrading pesticides (Jiang *et al*., 2018).

The multi-OMICs approach have also helped in understanding the evolution of microbial communities involved in biodegradation before and after the occurrence of spills. Before spills, these communities may be present in low amounts; however, spills trigger their rapid proliferation, domination of the microbiome of the polluted place, and expression of multiple metabolic pathways depending on the nature of the pollution and other environmental factors (Dubinsky *et al*., 2013; Rodriguez-R *et al*., 2015; Shin *et al*., 2019).

Biomarkers are also identified using multi-OMICs approach, including predictive biomarkers (e.g. first responders in spill-affected environs that are indicative of potential forspills, leaks, etc.), diagnostic biomarkers which indicate the presence of contamination in environments and therapeutic biomarkers which are used in restoration and reclamation of spill-affected environments (Joye & Kostka, 2020).

Summarily, microbiologists were able to apply the OMICs tools and directly study the intricacies of microbial interrelationships in their habitats, from both oil-contaminated and uncontaminated water and sediment samples. These enable scientists to know the microbial reactions to hydrocarbon contamination and the methodological underpinnings of microbes-assisted environmental reclamation, in unparalleled detail (Chikere, 2013; Rathoure & Dhatwalia, 2016; Kaster & Sobol, 2020; Kumari & Kumar, 2020; Santero & Diaz, 2020).

Problems/Challenges of OMICs Approaches

Despite the numerous benefits and prospects of the application of the OMICs in microbial bioremediation, there are still certain challenges needing to be addressed before the OMICs can reach their full potential, especially in developing countries. Such challenges, as highlighted by Gutleban *et al*. (2018), Chakraborty *et al*. (2012), Ma & Zhai (2012); Zhou & Fields, (2006) & Lemos *et al*. (2003), include:

1. There is the need to standardize reference chemicals for OMICs studies, particularly metabolomics. Corollary to this is the establishment of reference databases, to obviate the danger of generation of metabolites whose structure might be unknown.
2. In transciptomics, the presence of contaminants may prevent perfect hybridization, degrade the quality of the RNA and prevent successful isolation of the mRNA from soil.
3. These techniques and approaches are still in their introductory and developmental phase. Thus, the consistent demonstration of their applicability in various environments remains to be done, and needs time, and research efforts.
4. OMICs application in microbial bioremediation is challenged by ecological questions that are interwoven with other bioremediation approaches, such as discrepancies in microbial diversity, functional redundancy, stability, environmental micro-heterogeneity, genetic micro-diversity, and ecotypes. Such variations may lead to differences in bioremediation rates between organisms, which need to be separately and consistently evaluated.
5. Microbial diversity, association with adaptation of various microorganisms to multifarious conditions, especially in soil and aquatic environments, may present a challenge to OMIC approaches, especially in terms of tremendous data generation and information overload.
6. Not all genes are detected by transcriptomic studies, only genes from species and populations contributing to >5% of the microbiome are detectable, thus, certain functional genes not reaching this critical threshold are not detectable.

Accurate generalization from data of specific populations needs the careful observance of many desired populations, concurrently and covering large areas, over a long period. This endeavor is tasking to both the researcher and resources.

7. Factors that may affect the distribution of microorganisms in spoilage-affected environments, apart from their ability to tolerate the contaminants, e.g. inter-specie interactions, such as competition, need to be taken into consideration.
8. Novel biomarkers need to be developed for enhancing the identification of microbial populations in spoilage-affected environs.
9. There is the need for further researches to make OMICs techniques robust, convenient and affordable.
10. There is a shortage of computational resources especially in developing countries, without with ever-increasing amounts of data cannot be sufficiently crunched, thus hindering overall bioremediation prospects.
11. Many microorganisms whose DNA, mRNA products, etc. were isolated from environments, however, they cannot be cultured in the lab, even though significant progress is being made in this area.
12. Certain microorganisms exhibit low rates of biodegradation in the environment, even though OMICs studies have characterized their potentialities.

Strategies for Ameliorating these Drawbacks

1. Generation of Standard Operation Procedures (SOPs) for genomic and metabolomics analyses, including appropriate protocols for genomic extraction and exact chemicals to be used in metabolomics analyses, to ensure methodological uniformity and homogeneity.

2. Consistent updating of reference databases to ensure that discovered proteins and enzymes are appropriately matched.

3. Accurate kits that minimize the interference of environmental contaminants and ensure precise genomic extraction should be employed for recovery of genetic material. Kits such as Power Lyzer Power Soil Power Microbiome RNA Isolation Kit, Master Pure RNA Purification Kit, RNA Power Soil Total RNA Isolation Kit, *One Step* PCR Inhibitor Removal Kit, RNeasy Mini Kit need to be improved (Lim *et al.*, 2016).

4. Continuous research in variegated environments is necessary in establishing the validity and suitability of all OMICs approaches to diverse microbial groups. This is especially necessary for transcriptomics, metabolomics and multi-OMICs.

5. There is the need for widening the focus of OMICs approaches areas beyond few strains of microorganisms, to counteract microbial species diversity. Likewise, samples and culture conditions should reflect wide physiochemical ranges (temperature, pH, amongst others)

6. Especially in developing countries, genetic and bioinformation data crunching facilities should be provided, and microbiologists should be brought up-to-date with bioinformatics techniques, to ensure a hitch-free analyses of the data generated from OMICs studies.

7. Specificity of detection technologies should be enhanced to ensure that genes below current minimal thresholds are detectable and analyzable. This will create an avenue for elucidation of novel genes with yet unknown properties and capabilities.

8. Bioremediation studies involving OMICs approaches need to cover large areas, over expanded time periods, and need to be followed up in installments. This requires increased funding from the government and concerned agencies, sustained focus and use of improved and up-to-date methodologies from the researchers.

9. Microbial ecology of contaminated sites needs to be studied in details, to accommodate inter and intra species interactions, mutualism and competition, as these factors can directly hinder or bolster bioremediation *in situ* and *ex situ*.

10. Development of novel and diverse biomarkers for enhanced environmental monitoring is necessary in OMICs studies, as currently available biomarkers are not sufficient.

11. Data sharing and comparison need to be encouraged across different laboratories, experiments, sites and research groups the world over. Thus, national and regional databases may be setup across the nation and region which can be distributed in an open-access.

12. Development of novel culture media for culturing microbes using OMICs techniques to enhance understanding of microbes in bioremediation for the potential applications in future.

13. The OMICs tools should be utilized in strain improvement techniques and microbial consortia, as opposed to single strains for enhanced bioremediation potentials.

14. Increased funding for bioremediation researches. Governmental and non-governmental organizations need to collaborate like the National Oil Spill Detection and Response Agency, Federal Ministry of Environment among others.

15. Conferences, workshops, retreats, seminars, symposia and related events dealing with OMICs approaches to biodegradation should be organized to facilitate sharing of knowledge, presenting new protocols, techniques, insights, apparatuses, inventions amongst others.

CONCLUSION

The application of OMICs in bioremediation is a current technique, full of promising potentials. Such include improved geo-prediction properties, enhanced bioremediation potentials. Future trends in bioremediation are expected to be fueled by synthesis of data from current studies, modeling and biosimulation analyses, standardization of methodologies, tools and techniques, use of novel biomarkers and leveraging the multi-OMICs approach toward superior bioremediation studies. Following the use of a complementary approach that harnesses OMICs tools vis-à-vis cellular biochemistry, microbial ecology, physiology and metabolism, in the near future,it is envisioned that OMICs tools will propel microbial bioremediation into a revolutionary phase characterized by detailed data gathering and enhanced efficiency in bioremoval of contaminants from polluted environments.

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