Artículos

Genome-scale metabolic modeling for the development of rational strategies applied to bioremediation

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Abstract

The *omics era* has eased the access to high throughput biological data such as annotated genomes, transcriptomes, proteomes, metabolomes, etc. Genome-scale data can be integrated into metabolic reconstructions to obtain an integrated picture of the physiological state of a specific organism through gene-reaction-protein associations. Genome-scale models (GEMs) allow the improvement of strain metabolism using both, non-genetic engineering, and genetic engineering techniques and metabolic fluxes simulations, resulting in optimized processes of metabolite production, media formulation, growth rate, or compound degradation rate (*i.e.,* pollutants). The applications of GEMs enlarge as the access to omics data increases. Bioremediation applications of GEMs are particularly interesting due to the design of rational strategies that are more effective than trial and error traditional approaches. The aim of this review is to describe the advances made in bioremediation using GEMs of individual strains and microbial communities in order to guide design of metabolic engineering strategies applied to bioremediation. We further discussed opportunities in oil bioremediation integrating the different approaches of GEMs use here reviewed.

Key words: Genome-scale models, metabolic engineering, bioremediation, microbial community engineering, oil spill water contamination.

Resumen

La era de las ciencias ómicas ha facilitado el acceso a información biológica de alta calidad, como genomas anotados, proteomas, metabolomas, etc., estos datos pueden ser integrados en modelos metabólicos a escala genómica (GEMs) para obtener una mejor perspectiva del metabolismo de un organismo específico a través de asociaciones gen-reacción-proteína. Los GEMs permiten el mejoramiento de cepas utilizando técnicas tradicionales y de ingeniería genética, así como simulaciones de flujos metabólicos, resultando en procesos optimizados de producción de metabolitos, formulaciones de medios de cultivo, tasas de crecimiento, o tasas de degradación de compuestos (como contaminantes). Las aplicaciones de los GEMs van en aumento conforme el acceso a información ómica incrementa. El uso de GEMs en bioremediación es particularmente interesante debido al diseño de estrategias racionales que son más efectivas que los enfoques tradicionales de prueba y error. El propósito de esta revisión es describir los avances hechos en bioremediación mediante la aplicación de GEMs en cepas individuales y comunidades microbianas, así como guías para la toma de decisiones en las estrategias de ingeniería metabólica aplicadas a la biorremediación. Asimismo, discutimos oportunidades en la biorremediación de hidrocarburos mediante la integración de las diferentes aplicaciones de los GEMs cubiertas en esta revisión.

Palabras clave: Reconstrucciones metabólicas a escala genómica, ingeniería metabólica, biorremediación, ingeniería de comunidades microbianas, contaminación de agua por hidrocarburos.

Introducción

Mexico's economy heavily depends on the oil industry, most activities in the country use fossil fuels, and a considerable proportion of government budget is destined to oil (Bermudez Delgado et al., 2018). However, Mexico follows the global trend regarding use of oil; according to Wu and Chen (2019) crude oil constituted approximately 34.21% of the total primary energy supply in 2017, having the United States, China, South Korea, Japan and Canada as the main users. Beyond representing an economic concern, the extensive use of oil and its derivatives constitutes an enormous risk to the environment. Oil handling (including its exploitation, use, transport and disposal) represents an environmental threat due to accidental spills; it is estimated that more than 7 million tons of oil have been released to the environment since the first reported oil spill in 1907 (Li et al., 2016).

Mexico has been affected by the two largest accidental marine oil spills in history, the *Deepwater Horizon Disaster* (DWH) in the Gulf of Mexico (585,000 tons), and the *Ixtoc I* oil spill in Campeche Bay (480,000 tons) (Rafferty, 2021). Oil spills consequences span the social, environmental, and economic spheres. Two of the most famous spills, *Deepwater Horizon* and *Exxon-Valdez* disasters, had serious impacts; for instance, approximately more than 250,000 seabirds died after the latter occurred, and the former costed more than US$61 billion (Li et al., 2016). *Deepwater Horizon Disaster* repercussions persist more than 10 years later, affecting the biodiversity in the Gulf of Mexico, health and socioeconomic wellness of Mexico and USA shore population (Sandifer et al., 2021).

Several approaches help remediation and prevention of oil spills, including physical, chemical, thermal, and biological methods. While widely used, the physical and chemical methods have limitations that decrease their efficacy and reliability (Ndimele et al., 2018). Physical methods are mainly focused on containing the oil with using physical barriers; this involves a significant amount of work force. While chemical methods certainly diminish human intervention, they are subject of public concern due to health risks associated to their use. A study found increased neurological symptoms in workers exposed to chemical dispersants compared to workers that were not exposed to it during the DWH disaster (Krishnamurthy et al., 2019).

Biological methods, also called "bioremediation", are the most promising and advantageous water and soil clean-up techniques for heavy metals and organic pollutants released to the environment (Tyagi et al., 2011). Bioremediation is the elimination of toxic compounds from the environment by speeding up the normal metabolism of bacteria, fungi, algae, plants (or endogenous enzymes) to transform pollutants into less toxic compounds such as carbon dioxide (CO_2), water, inorganic salts, microbial biomass, and other by-products (commonly, secondary metabolites) (Kumar et al., 2018). There are two main strategies in bioremediation: biostimulation and bioaugmentation. The former consists in adding or supplementing nutrients and other compounds that enrich the polluted zone with an ideal pH, water content, oxygen, temperature to promote microbial growth and metabolism (Kumar et al., 2018; Tyagi et al., 2011). In bioaugmentation, indigenous or non-indigenous microorganisms with better pollutant degradation capability are added to the contaminated site, they can also be genetic modified strains with increased degradability potential (Kumar et al., 2018). However, due to the *in situ* bioremediation progress is directly associated with the catabolic potential of the microorganisms present and the bioavailability of the contaminants, biological treatment processes still remains a challenging task (Antizar-Ladislao et al., 2006; Szulc et al., 2014).

Most of the bioremediation techniques are based on intuition and experience, following trial and error experiments (Xu et al., 2019). These approaches are costly and time consuming because the performance evaluation of the microorganism is laborious, requiring long iterative cycles to obtain a desired biodegradability rate. For instance, diesel degradation percentages using biostimulation and bioaugmentation were lower than with natural attenuation in a study lasting 12 weeks (Bento et al., 2005); in another study, 57% hexadecane degradation took 24 days, reporting around 30 days in previous studies (Zhao et al., 2017). In addition, bacteria may not respond as expected, such as the dramatic CFU (colony forming units) decline found in the phenanthrene degrading GFP-tagged bacterium *Novosphingobium* sp. LH128 4 hours after inoculation in soil (Fida et al., 2017). Overall, this supposes an impediment to bioremediation, delaying its application. A better understanding of the physiology of the bacteria used for this purpose could accelerate the application of bioremediation approaches (Xu et al., 2019). Biostimulation and bioaugmentation techniques require a deep understanding of the bacterial metabolism (individually and as consortium) and the interactions with biotic and abiotic factors (pH, water content, oxygen/air availability, temperature, carbon sources, etc.). Genome-scale models (GEMs) are one of the most relevant *in silico* approaches that help contextualization of phenotypic behavior of microorganisms and represent a valuable tool to understand the pollutant degradation capability and survival of the bacteria.

Genome scale models (GEMs) are bioinformatic tools that connect genes, proteins, and reactions encoded in a genome. They give the entire picture of the metabolism of an organism allowing for simulation of metabolic routes to predict metabolic flux changes with Flux Balance Analysis (FBA) and other algorithms to optimize the production of a metabolites (Fang et al., 2020). Due to the increasing availability of genomes and GEMs building software the number of GEMs that have been reconstructed has augmented, by February 2019, 6239 organisms had GEM reconstructions (Gu et al., 2019).

GEMs have proved to be useful in bioremediation, several applications successfully removed pollutants using model and non-model microorganisms, as well as individual and community models (Dhakar et al., 2021). The use of non-model and environmental microorganisms is a challenge in the bioremediation process because there is a significant physiological-data gap, and as environmental strains respond notably different to model strains, the mass production yields of bioremediation valuable metabolites are deficient. More importantly, GEMs serve as tools to obtain better comprehension of the physiological response of a given microorganism towards different milieu conditions to develop cost-effective bioremediation procedures that surpass current techniques (Ofaim et al., 2020). Here, we review and discusses the effectiveness and benefits of GEMs applied to the design of rational bioremediation strategies using individual strains and microbial communities.

GEMs and their relevance in the study of organisms

GEMs have been used for different applications including optimization of metabolic pathways for utilization of carbon sources such a as glucose, xylose, and acetic acid in *Escherichia coli i*ML1515 (Monk et al., 2017); the genome scale metabolic model of *Streptomyces coelicolor* was analyzed to predict growth rate in 62 carbon sources and two nitrogen sources identifying essential reactions, the model also helped to fill gaps in genome annotation (Borodina et al., 2005). Eukaryotic models such as *Saccharomyces cerevisiae* GEM Yeast v6.0 are also useful, the model was reconstructed with the aim of rigorous metabolic examination (Aung et al., 2013). GEMs have served to carry out studies in pathogenic organisms to understand their metabolic reactions, for instance, a study evaluated antibiotic pressure responses using a GEM of *Mycobacterium tuberculosis* (Kavvas et al., 2018); the recent GEM of *Pseudomonas aeruginosa* PA14 includes reactions to decipher metabolic mechanisms of drug resistance (Dahal & Yang, preprint). Furthermore, GEMs are used to improve the production of metabolites of interest, for example, an enhanced erythromycin production was achieved performing predictions with the metabolic reconstruction of the actinomycete *Saccharopolyspora erythraea* NRRL23338, the authors found 5 predicted amino acids capable of improve the yield, *in vitro* testing confirmed 4 out of the 5 amino acids to increase erythromycin titers (Licona-Cassani et al., 2012). Moreno-Avitia et al. (2020) analyzed the feasibility of maximizing the production of phenazine-1-carboxamide using an experimentally validated metabolic reconstruction of *Pseudomonas chlororaphis* ATCC 9446. Thus, GEMs allow the metabolic analysis and simulation of several organisms of all the domains of life under different conditions (Table 1); this is one important reason for keeping metabolism databases updated.

Table 1. Examples of organisms with a reported genome-scale model (GEM)

Organism	Model	Description	Reference
		Bacteria	
Escherichia coli	*i*ML1515	Contains information on 1515 open reading frames, it also has some specific contexts, for example *i*ML1515-ROS version has additional reactions associated with the generation of reactive oxygen species.	(Monk et al., 2017)
Bacillus subtilis	*i*Bsu1144 *i*YO844	It was developed by incorporating thermodynamic information for the reactions to improve intracellular parameters. Version that integrates principles of GECKO (GEM with Enzymatic Constraints using Kinetic and Omics data) to improve the prediction accuracy of central carbon flux.	(Kocabas et al., 2017) (Massaiu et al., 2019)
Mycobacterium tuberculosis	*i*EK1101	Provides knowledge about the metabolism of this pathogenic microorganism and it has been used to evaluate responses to antibiotics.	(Kavvas et al., 2018)
Streptomyces coelicolor		Metabolic model for improvement of antibiotic yields, also used for the annotation of several genes.	(Borodina et al., 2005)
Saccharopolyspora erythraea		A model actinomycete for antibiotic production, used for erythromycin enhanced synthesis by optimizing the metabolic route based on the GEM developed. They formulated a medium with glucose and four amino acids to obtain high erythromycin yields.	(Licona-Cassani et al., 2012)
Pseudomonas aeruginosa **PA14**	*i*SD1511	An updated GEM with high accuracy for substrate utilization data, strain specific reactions (e.g., phenazine transport and redox metabolism, cofactor metabolism, carnitine metabolism, oxalate production, etc.) and can simulate data in aerobic and anaerobic conditions.	(Dahal & Yang, preprint)
Pseudomonas chlororaphis **ATCC9446**	*i*MA1267	The first experimentally validated GEM for *Pseudomonas chlororaphis* ATCC9446. Used to analyze strategies to maximize PCN production, to study the denitrification process and describe the ability of the strain to consume several carbon sources.	(Moreno-Avitia et al., 2020)
		Archaea	
Methanosarcina acetivorans	*i*ST807	It has information about the methanogenesis pathway of *M. acetivorans* and represents a useful resource for the study of unusual reactions.	(Peterson et al., 2016)
		Eukarya	
Saccharomyces cerevisiae	Yeast 7	Constructed by an international collaborative effort to include consensus metabolic networks. The version Yeast 7.Fe includes information on iron metabolism.	(Aung et al., 2013) (Dikicioglu & Oliver, 2019)
Mus musculus		A model that employed genetic tools for data extraction, storage and flux simulation that can be used as example for developing a model. The model can simulate basic growth and metabolic functions.	(Quek & Nielsen, 2008)
Caenorhabditis elegans	WormJam	The reconstruction involves metabolic compounds distributed across four different compartments, cytosol, mitochondria, nucleus, and extracellular secretions.	(Witting et al., 2018)
Arabidopsis thaliana		A model organism for plants that predict accurate fluxes, and it considers the transport costs associated with nutrient uptake and protein translocation between organelles.	(Cheung et al., 2013)
Homo sapiens	Recon3D *i*Adipocytes1809	It has GPR associations and structural information on metabolites and enzymes. It is the latest version of a *Homo sapiens* GEM. An adipocyte-based metabolic reconstruction. Contains information on subcellular localization and tissue specific gene expression based on the Human Protein Atlas database.	(Brunk et al., 2018) (Mardinoglu et al., 2013)

Metabolic reconstruction approaches.

Reconstruction of GEMs complexity varies depending on the approach followed and the use of software, however it is a laborious work, it involves many hours of database research, coding, and manual curation. The average time required for the reconstruction of a GEM goes from 6 months to two years, conditioned to the genomic size and study status of the bacteria (Thiele & Palsson, 2010). There are two main approaches to reconstruct GEMs, bottom-up and top-down. The former consists in building the model from scratch, and the latter in constructing it from an already made universal model. Some software, such as CarveMe, use the top-down strategy, providing a draft model in short time; nevertheless, most software employ the bottom-up strategy, requiring more manual curation (Machado et al., 2018; Mendoza et al., 2019). Both approaches need an annotated high quality genome as starting material. In the traditional or bottom-up strategy, the annotation is input in a software that automatically generates a draft model by comparing reactions databases and the annotated genome (by homology or orthology) (Machado et al., 2018). Then, the user manually cures the draft to obtain the final model.

Top-down strategy is usually faster, the whole process is described somewhere else (Machado et al., 2018), briefly, a universal model is created by integrating all models available in a database, namely BiGG, then it is manually curated. This universal model serves as a template for the new model; hence, the annotated genome of a given organism is compared to the universal model by orthology/homology using a software, during this process reactions present in the organism are included (together with metabolites) in the model, the unnecessary reactions are removed. The output is a functional curated model; however, further manual curation is needed. A key step in the reconstruction process is the correct establishment of orthology and homology, it is of utmost importance to take in consideration the precise selection of related and reference strains, particularly when working with environmental strains. The quantity and quality of information found in reaction databases relies mostly in this step (Thiele & Palsson, 2010). Manual curation also depends on the collected data; in cases where there is not enough information of a given organism, physiological and experimental data have to be compared with model reconstruction to ensure model accuracy (Thiele & Palsson, 2010).

GEMs are constantly growing and evolving as new data for the given organism is available. Though metabolic models do not cover the whole metabolism, predictions obtained are helpful approaches and guidelines to design rational strategies that are more efficient, reliable, and accurate than trial and error scheme. In the case of environmental strains, metabolic models give a powerful insight to make conscious decisions.

Genome scale metabolic modeling to improve bioremediation using individual species

Several studies have successfully used GEMs for biostimulation and bioaugmentation. As of January 2013, only seven species including *Acinetobacter baylyi*, *Dehalococcoides ethenogenes*, *Geobacter metallireducens*, *G. sulfurreducens*, *Pseudomonas putida*, *Rhodococcus erythropolis*, and *Shewanella oneidensis* and nine GEMs were constructed for bioremediation applications, these are discussed in another review (Xu et al., 2013). To date, the number of GEMs applications in bioremediation is growing and more interest is being put in them as they show high efficacy.

GEMs can be reconstructed for individual strains and microbial communities, most GEMs in bioremediation are for individual strains, moreover they are mainly used for biostimulation purposes due to the challenges bioaugmentation imply; these, along with microbial community GEMs, will be further discussed. They are helpful in non-model and environmental organisms, such as *Paenarthrobacter aurescens*; Ofaim and collaborators reconstructed a metabolic model of the TC1 strain to study the degradation of the pollutant herbicide atrazine with 100 different compounds to supplement the medium Ofaim et al. (2020). They found the optimal combination of compounds to enhance degradation, further tested the predictions *in vitro* and confirmed them, highlighting the importance of GEMs to capture complexities that go beyond biochemical knowledge and to screen hundreds to thousands of compounds with no cost. In another study, a reconstruction of a toluene-degrader strain *Pseudomonas veronii* 1YdBTEX2 was reported. *P. veronii* is a bacterium used in bioaugmentation, the GEM reported included REMI (Relative Expression and Metabolomics Integrations), a method to integrate omics data into metabolic models and specific-context models, to predict the growth of the strain during transition from exponential to stationary phase and adaptation from liquid medium to sand (Hadadi et al., 2020). The optimization of microbial activity of *Variovorax* sp. SRS 16 through simulation of linuron degradation outcomes under several conditions was performed reconstructing a GEM (Dhakar et al., 2021). *Ralstonia eutropha* H16 GEM RehMBEL1391 was reconstructed to analyze its metabolic capacities, the strain is able of degrading several aromatic compounds (Park et al., 2011).

Model organisms such as, *E. coli* str. K-12 substr. MG1655 and *Pseudomonas putida* KT2440 have been used in bioaugmentation. Iman and colaborators used the *E. coli* GEM *i*JO1366 to study the degradation of 2,4,6-trinitrotoluene (TNT) and to design mutant strains with higher TNT degradation yields than wild type strain (Iman et al. 2017). *Pseudomonas putida* KT2440 is a non-pathogenic soil bacterium with the metabolic capacity of degrading toluene, lignin and other xenobiotics. The first GEM *i*JN746 and the most recent and curated *i*JN1462 model have been used to analyze the biodegradability of such contaminants as well as the strain potential as a biofactory to produce polyhydroxyalkanoate (PHA) (Nogales et al., 2008, 2020).

Microbial communities engineering strategies to enhance bioremediation methods

As mentioned earlier, micro-environmental conditions are a key factor to be considered for the design of bioremediation strategies. There is a lack of information about how the inoculated bacteria will be affected by the new environmental conditions. In other words, an engineered strain may not behave similarly under laboratory compared to real environmental non-controlled conditions causing physiological stress and decreasing its performance. The results are mixed and the inoculation goals are commonly not achieved; inoculated bacteria may not survive or express the desired metabolic activity (Morales et al., 2021). Therefore, bioaugmentation techniques are commonly accompanied by biostimulation to aid during the adaptation stage and increase the pollutant removal, these include the use of a microbial community or nutrients (Nikolopoulou et al., 2013).

In nature, microorganisms coexist taking advantage of their produced metabolites and interchanging nutrients. Thus, in bioaugmentation methods it is important to study the interaction of the inoculated bacteria with the indigenous bacteria to increase the efficacy of the technique. Studying the metabolism of the microbial community by performing enzymatic tests and growth curves in different media *in vitro* could be time and money consuming. Microbial communities metabolic modeling represents an approach that has allowed to know which reactions are relevant to drive microbial interactions within specific environments. For example, the context-specific metabolic reconstruction of two communities present in polyaromatic contaminated soil allowed the analysis of naphthalene degradation with and without biostimulation, these results can aid in the development of better bioremediation strategies (Tobalina et al., 2015). A possible outcome of studying microbial communities is the design of synthetic consortia.

Synthetic consortia are constructed through microbial community engineering, which allows the accomplishment of highly complex tasks that pose a challenge for monocultures (Tsoi et al., 2019). Genome-scale metabolic modeling has proved to be a successful tool to design synthetic communities with applications in bioremediation. For example, a study reconstructed GEMs of the atrazine-degrading bacterium *Arthrobacter aurescens* TC1 (*Paenarthrobacter aurescens* TC1) and other four non-atrazine degrading soil bacteria to analyze the community interactions (Xu et al., 2019). In this study, the model was used to predict community performances considering growth, atrazine degradation, and specific exchange fluxes. The main contribution of such simulations was the increase in atrazine-degrading efficiency by specific interactions of a synthetic consortium of *Arthrobacter*, *Halobacillus* and *Halomonas*. It was also possible to present a model of which metabolites are degraded (and used) by the community, describing the metabolic pathways involved. Zhuang and collaborators also studied the bioremediation of uranium using community GEMs, they first analyzed the competition for nutrients between *Geobacter sulfurreducens* and *Rhodoferax ferrireducens* (two bacteria coexisting in uranium polluted sites) in order to explain how these interactions may influence *in situ* uranium bioremediation (Zhuang et al., 2011). Further, they studied a microbial community composed of *Geobacter sulfurreducens* and sulfate-reducing bacteria (SRBs) to design a rational strategy for uranium bioremediation, they found that the concentration of *G. sulfurreducens* could be kept low with a constant supply of acetate and Fe(III) (Zhuang et al., 2012). Additionally, (Zomorrodi et al., 2014) introduced d-OptCom, a tool for the dynamic metabolic modeling of microbial communities, and designed an improved synthetic consortia of *Geobacter sulfurreducens*, *Rhodoferax ferrireducens*, and *Shewanella oneidensis* to reduce uranium; they found an increased uranium remediation with the addition of lactate.

Genome-scale metabolic modeling and metabolic engineering for bioremediation

Indigenous bacteria and known pollutant degraders can naturally degrade the contaminants at their site, however their metabolic pathways are not always suitable for large scale degradation, decreasing the bioremediation efficiency (Dangi et al., 2019). The use of improved strains through metabolic engineering can solve this problem. Metabolic engineering comprises the use of already existing metabolic pathways to rewire them, or the introduction of exogenous pathways to optimize the production of specific metabolites. Metabolic engineering requires a detailed comprehension of the metabolic pathways of the microorganism used, GEMs are a powerful tool to design rational strategies.

GEMs are broadly used in metabolic engineering, for instance, predicting gene modification strategies to overproduce desired compounds accelerating the process, like the achieved by metabolic rewiring in *Pseudomonas putida* KT2440 for indigoidine production (Banerjee et al., 2020). In the study they used the MCS (minimal cut set) to determine the targets to enhance the production, however, most optimizations are performed with FBA and related algorithms like OptKnock (Burgard et al., 2003).

Despite the extended use of GEMs in metabolic engineering, the limitations of introducing modified strains into natural habitats hamper the development of this field (Ofaim et al., 2020). Therefore, only few studies have reported the use of metabolic engineering and GEMs in bioremediation. Izallalen et al. (2008) applied the metabolic reconstruction of the bacterium *Geobacter sulfurreducens* to predict gene deletions to increase the respiration rate using OptKnock. They performed the predicted deletions to do metabolic engineering in a strain and successfully increased the respiration rates and diminished the cell growth and biomass yield. This engineered strain has applications in bioremediation of uranium as it is preferred a rapid biodegradation with low biomass.

Furthermore, the *in silico* strain design of *E. coli* was possible through the GEM *i*JO1366 to obtain increased 2,4,6-trinitrotoluene (TNT) degradation rates than the wild type strain, the results could be used as a future reference for the design of better bioremediation techniques (Iman et al., 2017). Additionally, other studies pointed out the feasibility of using their metabolic reconstructions in metabolic engineering to improve bioremediation strategies (Aggarwal et al., 2011; Sohn et al., 2010).

Future directions: The potential use of genome-scale metabolic modeling as a mean to improve oil spill bioremediation

Contaminated sites are constantly changing as pollutants vary in concentration and dispersion over time; some polluted zones, such as oil spills move faster due to the oceanic currents and wind (Li et al., 2019), therefore response time is crucial to diminish the ecological impact. The GEMs applications discussed in this review are promising approaches to develop enhanced oil spill bioremediation strategies.

The GEM-guided synthetic microbial consortia design is particularly interesting because of the feasibility of using these consortia in different sites. The characterization of the autochthonous communities living in oil-polluted zones as well as oil degraders is essential to develop a synthetic consortium that can be applicable to different scenarios. The *omics era* has made oil-degrader microorganisms identification faster and more accurate. Recent studies mention the existence of more than 79 genera with petroleum hydrocarbons degradation capability, including *Pseudomonas*, *Dietzia*, *Aquamicrobium*, *Alkanindiges*, *Staphylococcus*, *Sphingomonas*, *Mycobacterium*, *Methylobacterium*, *Burkholderia*, *Achromobacter*, *Streptobacillus*, *Kocuria*, *Acinetobacter*, *Chryseobacterium*, *Marinobacter*, *Paraburkholderia*, *Arthrobacter*, *Enterobacter*, *Streptococcus*, *Bradyrhizobium*, *Alteromonas*, *Pandoraea*, *Leifsonia* and *Rhodococcus*, among others (Geng et al., 2022; Xu et al., 2018). Furthermore, studies have analyzed the composition of microbial communities present in oil contaminated soil (Cai et al., 2020) and water (Neethu et al., 2019). This information allows for the design of synthetic consortia with increased petroleum degradation using GEMs to analyze the interactions between degraders and non-degraders. Recently, the GEM-guided design of a synthetic consortium composed by hexadecane-degrading bacterium *Dietzia* sp. strain DQ12-45-1b and non-degrader *Pseudomonas stutzeri* SLG510A3-8 successfully removed 85.54% diesel oil in 15 days (Hu et al., 2020). Using the GEM *i*BH1908, authors found that *P. stutzeri*, despite not being able of degrading C_{16} (hexadecane), could survive using the metabolic intermediaries produced by *Dietzia* sp when metabolizing acetate and glutamate produced by *P.stutzeri*.

Synthetic consortia have demonstrated to achieve high bioremediation yields, however the combination of synthetic consortia from autochthonous bacteria with biostimulation is a promising strategy to maximize the biodegradation. A study analyzed the use of biostimulation (addition of KNO_3 and KH_2PO_4, or uric acid and lecithin), a pre-adapted indigenous population, and rhamnolipids, to degrade spilled oil (Nikolopoulou et al., 2013). They mixed the variables and found that 3 of the 6 treatments showed the best results; seawater + crude oil + KNO_3 + KH_2PO_4 + rhamnolipids + pre-adapted indigenous population (NPKMR), seawater + crude oil + uric acid + lecithin + rhamnolipids (ULR), and seawater + crude oil + uric acid + lecithin + rhamnolipids + pre-adapted indigenous population (ULRM) obtained a degradation of 99%, 97% and 88%, respectively, within 15 days.

Additionally, findings by Nikolopoulou et al. (2013), serve as a guide for the design of bioremediation strategies using GEMs, for example, the design of a synthetic consortium biostimulated with biosurfactants, such as rhamnolipids. Biosurfactants have attracted some research groups for bioremediation because the amphiphilic nature of these compounds, promoting the partitioning of the hydrophobic contaminants into internal cores of micelles and detaching the pollutants to the sediments and increase their bioavailability (Dell' Anno et al., 2021). Biosurfactants have advantages such as being environmentally friendly, with reduced toxicity and high biodegradability compared to chemical surfactants (Mnif et al., 2017). For instance, rhamnolipids are the most studied biosurfactants, they are glycolipids that consist in a β-hydroxy (3-hydroxy) fatty acid glycosylated with either one or two rhamnose residues (mono- and di-rhamnolipids, respectively) produced by some *Pseudomonas* species (Thomas et al., 2021; Varjani & Upasani, 2017). Several studies show the efficacy of rhamnolipids in the removal of hydrocarbon pollutants. A diesel degradation yield of 70% was achieved by the rhamnolipids produced by *Pseudomonas aeruginosa* AHV-KH10 (Pourfadakari et al., 2021). Another study reports removal of up to 80% petroleum hydrocarbons using a mixture of rhamnolipids, biochar and nitrogen (Wei et al. 2020). Thomas and collaborators found that rhamnolipids have similar capacity as chemical surfactants (Superdispersant 25, Finasol OSR 52, and Slickgone NS) to remove hydrocarbons (Thomas et al. 2021).

Despite rhamnolipids are the biosurfactants with higher yields, their commercialization and applications are limited due to the low level of production of *Pseudomonas* strains (Soberón-Chávez et al., 2021). Several studies have achieved increased production of rhamnolipids by metabolic engineering, utilization of different substrates and culture media. Nonetheless, results are far from achieving process economic feasibility - for a more detailed insight of these studies check the review by Soberón-Chávez and collaborators (Soberón-Chávez et al. 2021). Recently, the use of pyocyanin, a blue pigment exclusively produced by *Pseudomonas* strains, was found to assist rhamnolipids emulsification of petrol, hexadecane and diesel (Das & Ma, 2013). They observed that strains with higher production of pyocyanin increased hydrocarbon emulsification. While tests were performed using cell-free supernatants, the results may indicate the role of pyocyanin as an enhancer of the emulsification by rhamnolipids (Das & Ma, 2013). This poses the opportunity of using GEM for the fine tuning of the biosynthesis of an optimal ratio of rhamnolipids and pyocyanin to use them as biostimulators (Gonçalves & Vasconcelos, 2021).

The use of pyocyanin and rhamnolipids for bioremediation is complicated because of the complexity of their metabolic routes, they derive and intersect from central metabolism pathways; moreover, *Pseudomonas* possesses an elaborated *quorum sensing* (QS) system in which both metabolites are involved (Soberón-Chávez et al., 2021). As a future perspective, GEMs can overcome the problems discussed and furthermore, be used to design an improved synthetic consortium of hydrocarbon degraders biostimulated with rhamnolipids and pyocyanin to speed up oil removal. The metabolic reconstruction of a non-virulent *Pseudomonas aeruginosa* strain with optimized pyocyanin and rhamnolipids co-production could guide the metabolic engineering strategies to solve the problems metabolic pathways suppose. However, the modeling of quorum sensing is more difficult, at the moment of writing this review, few attempts have been done to incorporate regulation systems (Chung et al., 2021) and quorum sensing pathways into GEMs (Botero et al., 2020); hence, similar strategies could be followed to obtain a well-nurtured GEM. This system of oil bioremediation can be designed to be universally applicable.

Further research is needed to extend the applications of GEMs and to overcome current limitations, such as viability, cellular regulation, genetic engineering, among others. Withal, GEMs have a plethora of advantages and applications, their versatility, as one reconstruction can be reutilized for different purposes, increases the possible outcomes, and allows the simulation of thousands of scenarios, making them a great tool to improve bioremediation.

References

Aggarwal, S., Karimi, I. A., & Lee, D. Y. (2011). Reconstruction of a genome-scale metabolic network of *Rhodococcus erythropolis* for desulfurization studies. *Molecular BioSystems*, *7*(11), 3122–3131. https://doi.org/10.1039/c1mb05201b

Antizar-Ladislao, B., Lopez-Real, J., & Beck, A. J. (2006). Degradation of polycyclic aromatic hydrocarbons (PAHs) in an aged coal tar contaminated soil under in-vessel composting conditions. *Environmental Pollution*, *141*(3), 459–468. https://doi.org/10.1016/j.envpol.2005.08.066

Aung, H. W., Henry, S. A., & Walker, L. P. (2013). Revising the Representation of Fatty Acid, Glycerolipid, and Glycerophospholipid Metabolism in the Consensus Model of Yeast Metabolism. *Industrial Biotechnology (New Rochelle, N.Y.)*, *9*(4), 215–228. https://doi.org/10.1089/ind.2013.0013

Banerjee, D., Eng, T., Lau, A. K., Sasaki, Y., Wang, B., Chen, Y., Prahl, J.-P., Singan, V. R., Herbert, R. A., Liu, Y., Tanjore, D., Petzold, C. J., Keasling, J. D., & Mukhopadhyay, A. (2020). Genome-scale metabolic rewiring improves titers rates and yields of the non-native product indigoidine at scale. *Nature Communications*, *11*(1), 5385. https://doi.org/10.1038/s41467-020-19171-4

Bento, F. M., Camargo, F. A. O., Okeke, B. C., & Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, *96*(9), 1049–1055. https://doi.org/10.1016/j.biortech.2004.09.008

Bermudez Delgado, N. A., Bermudez Delgado, E., & Saucedo, E. (2018). The relationship between oil prices, the stock market and the exchange rate: Evidence from Mexico. *The North American Journal of Economics and Finance*, *45*, 266–275. https://doi.org/10.1016/j.najef.2018.03.006

Borodina, I., Krabben, P., & Nielsen, J. (2005). Genome-scale analysis of *Streptomyces coelicolor* A3(2) metabolism. *Genome Research*, *15*(6), 820–829. https://doi.org/10.1101/gr.3364705

Botero, D., Monk, J., Rodríguez Cubillos, M. J., Rodríguez Cubillos, A., Restrepo, M., Bernal-Galeano, V., Reyes, A., González Barrios, A., Palsson, B. Ø., Restrepo, S., & Bernal, A. (2020). Genome-Scale Metabolic Model of *Xanthomonas phaseoli* pv. manihotis: An Approach to Elucidate Pathogenicity at the Metabolic Level. *Frontiers in Genetics*, *11*, 837. https://doi.org/10.3389/fgene.2020.00837

Brunk, E., Sahoo, S., Zielinski, D. C., Altunkaya, A., Dräger, A., Mih, N., Gatto, F., Nilsson, A., Preciat Gonzalez, G. A., Aurich, M. K., Prlić, A., Sastry, A., Danielsdottir, A. D., Heinken, A., Noronha, A., Rose, P. W., Burley, S. K., Fleming, R. M. T., Nielsen, J., … Palsson, B. O. (2018). Recon3D enables a three-dimensional view of gene variation in human metabolism. *Nature Biotechnology*, *36*(3), 272–281. https://doi.org/10.1038/nbt.4072

Burgard, A. P., Pharkya, P., & Maranas, C. D. (2003). Optknock: A bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. *Biotechnology and Bioengineering*, *84*(6), 647–657. https://doi.org/10.1002/bit.10803

Cai, P., Ning, Z., Liu, Y., He, Z., Shi, J., & Niu, M. (2020). Diagnosing bioremediation of crude oil-contaminated soil and related geochemical processes at the field scale through microbial community and functional genes. *Annals of Microbiology*, *70*(1), 36. https://doi.org/10.1186/s13213-020-01580-x

Cheung, C. Y. M., Williams, T. C. R., Poolman, M. G., Fell, David. A., Ratcliffe, R. G., & Sweetlove, L. J. (2013). A method for accounting for maintenance costs in flux balance analysis improves the prediction of plant cell metabolic phenotypes under stress conditions. *The Plant Journal*, *75*(6), 1050–1061. https://doi.org/10.1111/tpj.12252

Chung, C. H., Lin, D.-W., Eames, A., & Chandrasekaran, S. (2021). Next-Generation Genome-Scale Metabolic Modeling through Integration of Regulatory Mechanisms. *Metabolites*, *11*(9), 606. https://doi.org/10.3390/metabo11090606

Dahal, S., & Yang, L. (2021). Genome-scale modeling of *Pseudomonas aeruginosa* PA14 unveils its broad metabolic capabilities and role of metabolism in drug potentiation [Preprint]. Systems Biology. https://doi.org/10.1101/2021.04.15.439930

Dangi, A. K., Sharma, B., Hill, R. T., & Shukla, P. (2019). Bioremediation through microbes: Systems biology and metabolic engineering approach. *Critical Reviews in Biotechnology*, *39*(1), 79–98. https://doi.org/10.1080/07388551.2018.1500997

Das, P., & Ma, L. Z. (2013). Pyocyanin pigment assisting biosurfactant-mediated hydrocarbon emulsification. *International Biodeterioration & Biodegradation*, *85*, 278–283. https://doi.org/10.1016/j.ibiod.2013.07.013

Dell' Anno, F., Rastelli, E., Sansone, C., Brunet, C., Ianora, A., & Dell' Anno, A. (2021). Bacteria, Fungi and Microalgae for the Bioremediation of Marine Sediments Contaminated by Petroleum Hydrocarbons in the Omics Era. *Microorganisms*, *9*(8), 1695. https://doi.org/10.3390/microorganisms9081695

Dhakar, K., Zarecki, R., van Bommel, D., Knossow, N., Medina, S., Öztürk, B., Aly, R., Eizenberg, H., Ronen, Z., & Freilich, S. (2021). Strategies for Enhancing in vitro Degradation of Linuron by *Variovorax* sp. Strain SRS 16 Under the Guidance of Metabolic Modeling. *Frontiers in Bioengineering and Biotechnology*, *9*, 602464. https://doi.org/10.3389/fbioe.2021.602464

Dikicioglu, D., & Oliver, S. G. (2019). Extension of the yeast metabolic model to include iron metabolism and its use to estimate global levels of iron-recruiting enzyme abundance from cofactor requirements. *Biotechnology and Bioengineering*, *116*(3), 610–621. https://doi.org/10.1002/bit.26905

Fang, X., Lloyd, C. J., & Palsson, B. O. (2020). Reconstructing organisms in silico: Genome-scale models and their emerging applications. *Nature Reviews. Microbiology*, *18*(12), 731–743. https://doi.org/10.1038/s41579-020-00440-4

Fida, T. T., Moreno-Forero, S. K., Breugelmans, P., Heipieper, H. J., Röling, W. F. M., & Springael, D. (2017). Physiological and Transcriptome Response of the Polycyclic Aromatic Hydrocarbon Degrading *Novosphingobium* sp. LH128 after Inoculation in Soil. *Environmental Science & Technology*, *51*(3), 1570–1579. https://doi.org/10.1021/acs.est.6b03822

Geng, S., Qin, W., Cao, W., Wang, Y., Ding, A., Zhu, Y., Fan, F., & Dou, J. (2022). Pilot-scale bioaugmentation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil using an indigenous bacterial consortium in soil-slurry bioreactors. *Chemosphere*, *287*, 132183. https://doi.org/10.1016/j.chemosphere.2021.132183

Gonçalves, T., & Vasconcelos, U. (2021). Colour me blue: The history and the biotechnological potential of pyocyanin. *Molecules*, *26*(4). Scopus. https://doi.org/10.3390/molecules26040927

Gu, C., Kim, G. B., Kim, W. J., Kim, H. U., & Lee, S. Y. (2019). Current status and applications of genome-scale metabolic models. *Genome Biology*, *20*(1), 121. https://doi.org/10.1186/s13059-019-1730-3

Hadadi, N., Pandey, V., Chiappino-Pepe, A., Morales, M., Gallart-Ayala, H., Mehl, F., Ivanisevic, J., Sentchilo, V., & Meer, J. R. van der. (2020). Mechanistic insights into bacterial metabolic reprogramming from omics-integrated genome-scale models. *NPJ Systems Biology and Applications*, *6*(1), 1. https://doi.org/10.1038/s41540-019-0121-4

Hu, B., Wang, M., Geng, S., Wen, L., Wu, M., Nie, Y., Tang, Y.-Q., & Wu, X.-L. (2020). Metabolic Exchange with Non-Alkane-Consuming *Pseudomonas stutzeri* SLG510A3-8 Improves n-Alkane Biodegradation by the Alkane Degrader *Dietzia* sp. Strain DQ12-45-1b. *Applied and Environmental Microbiology*, *86*(8), e02931-19. https://doi.org/10.1128/AEM.02931-19

Iman, M., Sobati, T., Panahi, Y., & Mobasheri, M. (2017). Systems Biology Approach to Bioremediation of Nitroaromatics: Constraint-Based Analysis of 2,4,6-Trinitrotoluene Biotransformation by *Escherichia coli*. *Molecules (Basel, Switzerland)*, *22*(8), E1242. https://doi.org/10.3390/molecules22081242

Izallalen, M., Mahadevan, R., Burgard, A., Postier, B., Didonato, R., Sun, J., Schilling, C. H., & Lovley, D. R. (2008). *Geobacter sulfurreducens* strain engineered for increased rates of respiration. *Metabolic Engineering*, *10*(5), 267–275. https://doi.org/10.1016/j.ymben.2008.06.005

Kavvas, E. S., Catoiu, E., Mih, N., Yurkovich, J. T., Seif, Y., Dillon, N., Heckmann, D., Anand, A., Yang, L., Nizet, V., Monk, J. M., & Palsson, B. O. (2018). Machine learning and structural analysis of *Mycobacterium tuberculosis* pan-genome identifies genetic signatures of antibiotic resistance. *Nature Communications*, *9*(1), 4306. https://doi.org/10.1038/s41467-018-06634-y

Kocabas, P., Calik, P., Çalık, G., & Özdamar, H. (2017). Analyses of extracellular protein production in *Bacillus subtilis* − I: Genome-scale metabolic model reconstruction based on updated gene-enzyme-reaction data. *Biochemical Engineering Journal*, *127*. https://doi.org/10.1016/j.bej.2017.07.005

Krishnamurthy, J., Engel, L. S., Wang, L., Schwartz, E. G., Christenbury, K., Kondrup, B., Barrett, J., & Rusiecki, J. A. (2019). Neurological symptoms associated with oil spill response exposures: Results from the Deepwater Horizon Oil Spill Coast Guard Cohort Study. *Environment International*, *131*, 104963. https://doi.org/10.1016/j.envint.2019.104963

Kumar, V., Shahi, S. K., & Singh, S. (2018). Bioremediation: An Eco-sustainable Approach for Restoration of Contaminated Sites. In J. Singh, D. Sharma, G. Kumar, & N. R. Sharma (Eds.), *Microbial Bioprospecting for Sustainable Development* (pp. 115–136). Springer Singapore. https://doi.org/10.1007/978-981-13-0053-0_6

Li, P., Cai, Q., Lin, W., Chen, B., & Zhang, B. (2016). Offshore oil spill response practices and emerging challenges. *Marine Pollution Bulletin*, *110*(1), 6–27. https://doi.org/10.1016/j.marpolbul.2016.06.020

Li, Y., Yu, H., Wang, Z., Li, Y., Pan, Q., Meng, S., Yang, Y., Lu, W., & Guo, K. (2019). The forecasting and analysis of oil spill drift trajectory during the Sanchi collision accident, East China Sea. *Ocean Engineering*, *187*, 106231. https://doi.org/10.1016/j.oceaneng.2019.106231

Licona-Cassani, C., Marcellin, E., Quek, L.-E., Jacob, S., & Nielsen, L. K. (2012). Reconstruction of the *Saccharopolyspora erythraea* genome-scale model and its use for enhancing erythromycin production. *Antonie Van Leeuwenhoek*, *102*(3), 493–502. https://doi.org/10.1007/s10482-012-9783-2

Machado, D., Andrejev, S., Tramontano, M., & Patil, K. R. (2018). Fast automated reconstruction of genome-scale metabolic models for microbial species and communities. *Nucleic Acids Research*, *46*(15), 7542-7553 https://doi.org/10.1093/nar/gky537

Mardinoglu, A., Agren, R., Kampf, C., Asplund, A., Nookaew, I., Jacobson, P., Walley, A. J., Froguel, P., Carlsson, L. M., Uhlen, M., & Nielsen, J. (2013). Integration of clinical data with a genome-scale metabolic model of the human adipocyte. *Molecular Systems Biology*, *9*, 649. https://doi.org/10.1038/msb.2013.5

Massaiu, I., Pasotti, L., Sonnenschein, N., Rama, E., Cavaletti, M., Magni, P., Calvio, C., & Herrgård, M. J. (2019). Integration of enzymatic data in *Bacillus subtilis* genome-scale metabolic model improves phenotype predictions and enables in silico design of poly-γ-glutamic acid production strains. *Microbial Cell Factories*, *18*(1), 3. https://doi.org/10.1186/s12934-018-1052-2

Mendoza, S. N., Olivier, B. G., Molenaar, D., & Teusink, B. (2019). A systematic assessment of current genome-scale metabolic reconstruction tools. *Genome Biology*, *20*(1), 158. https://doi.org/10.1186/s13059-019-1769-1

Mnif, I., Sahnoun, R., Ellouz-Chaabouni, S., & Ghribi, D. (2017). Application of bacterial biosurfactants for enhanced removal and biodegradation of diesel oil in soil using a newly isolated consortium. *Process Safety and Environmental Protection*, *109*, 72–81. https://doi.org/10.1016/j.psep.2017.02.002

Monk, J. M., Lloyd, C. J., Brunk, E., Mih, N., Sastry, A., King, Z., Takeuchi, R., Nomura, W., Zhang, Z., Mori, H., Feist, A. M., & Palsson, B. O. (2017). IML1515, a knowledgebase that computes *Escherichia coli* traits. *Nature Biotechnology*, *35*(10), 904–908. https://doi.org/10.1038/nbt.3956

Morales, M., Sentchilo, V., Hadadi, N., & van der Meer, J. R. (2021). Genome-wide gene expression changes of *Pseudomonas veronii* 1YdBTEX2 during bioaugmentation in polluted soils. *Environmental Microbiome*, *16*(1), 8. https://doi.org/10.1186/s40793-021-00378-x

Moreno-Avitia, F., Utrilla, J., Bolívar, F., Nogales, J., & Escalante, A. (2020). Metabolic reconstruction of *Pseudomonas chlororaphis* ATCC 9446 to understand its metabolic potential as a phenazine-1-carboxamide-producing strain. *Applied Microbiology and Biotechnology*, *104*(23), 10119–10132. https://doi.org/10.1007/s00253-020-10913-4

Ndimele, P. E., Saba, A. O., Ojo, D. O., Ndimele, C. C., Anetekhai, M. A., & Erondu, E. S. (2018). Chapter 24—Remediation of Crude Oil Spillage. In P. E. Ndimele (Ed.), *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem* (pp. 369–384). Academic Press. https://doi.org/10.1016/B978-0-12-809399-3.00024-0

Neethu, C. S., Saravanakumar, C., Purvaja, R., Robin, R. S., & Ramesh, R. (2019). Oil-Spill Triggered Shift in Indigenous Microbial Structure and Functional Dynamics in Different Marine Environmental Matrices. *Scientific Reports*, *9*(1), 1354. https://doi.org/10.1038/s41598-018-37903-x

Nikolopoulou, M., Pasadakis, N., & Kalogerakis, N. (2013). Evaluation of autochthonous bioaugmentation and biostimulation during microcosm-simulated oil spills. *Marine Pollution Bulletin*, *72*(1), 165–173. https://doi.org/10.1016/j.marpolbul.2013.04.007

Nogales, J., Mueller, J., Gudmundsson, S., Canalejo, F. J., Duque, E., Monk, J., Feist, A. M., Ramos, J. L., Niu, W., & Palsson, B. O. (2020). High-quality genome-scale metabolic modelling of *Pseudomonas putida* highlights its broad metabolic capabilities. *Environmental Microbiology*, *22*(1), 255–269. https://doi.org/10.1111/1462-2920.14843

Nogales, J., Palsson, B. Ø., & Thiele, I. (2008). A genome-scale metabolic reconstruction of *Pseudomonas putida* KT2440: I JN746 as a cell factory. *BMC Systems Biology*, *2*(1), 79. https://doi.org/10.1186/1752-0509-2-79

Ofaim, S., Zarecki, R., Porob, S., Gat, D., Lahav, T., Kashi, Y., Aly, R., Eizenberg, H., Ronen, Z., & Freilich, S. (2020). Genome-scale reconstruction of *Paenarthrobacter aurescens* TC1 metabolic model towards the study of atrazine bioremediation. *Scientific Reports*, *10*(1), 13019. https://doi.org/10.1038/s41598-020-69509-7

Park, J. M., Kim, T. Y., & Lee, S. Y. (2011). Genome-scale reconstruction and in silico analysis of the *Ralstonia eutropha* H16 for polyhydroxyalkanoate synthesis, lithoautotrophic growth, and 2-methyl citric acid production. *BMC Systems Biology*, *5*, 101. https://doi.org/10.1186/1752-0509-5-101

Peterson, J. R., Thor, S., Kohler, L., Kohler, P. R. A., Metcalf, W. W., & Luthey-Schulten, Z. (2016). Genome-wide gene expression and RNA half-life measurements allow predictions of regulation and metabolic behavior in *Methanosarcina acetivorans*. *BMC Genomics*, *17*(1), 924. https://doi.org/10.1186/s12864-016-3219-8

Pourfadakari, S., Ghafari, S., Takdastan, A., & Jorfi, S. (2021). A salt resistant biosurfactant produced by moderately halotolerant *Pseudomonas aeruginosa* (AHV-KH10) and its application for bioremediation of diesel-contaminated sediment in saline environment. *Biodegradation*, *32*(3), 327–341. https://doi.org/10.1007/s10532-021-09941-2

Quek, L.-E., & Nielsen, L. K. (2008). On the reconstruction of the *Mus musculus* genome-scale metabolic network model. *Genome Informatics. International Conference on Genome Informatics*, *21*, 89–100.

Rafferty, J. P. (2021). 9 of the Biggest Oil Spills in History. In *Encyclopedia Britannica*. https://www.britannica.com/list/9-of-the-biggest-oil-spills-in-history

Sandifer, P., Ferguson, A., Finucane, M., Partyka, M., Solo-Gabriele, H., Hayward Walker, A., Wowk, K., Caffey, R., & Yoskowitz, D. (2021). Human Health and Socioeconomic Effects of the Deepwater Horizon Oil Spill in the Gulf of Mexico. *Oceanography*, *34*(1), 174–191. https://doi.org/10.5670/oceanog.2021.125

Soberón-Chávez, G., González-Valdez, A., Soto-Aceves, M. P., & Cocotl-Yañez, M. (2021). Rhamnolipids produced by *Pseudomonas*: From molecular genetics to the market. *Microbial Biotechnology*, *14*(1), 136–146. https://doi.org/10.1111/1751-7915.13700

Sohn, S. B., Kim, T. Y., Park, J. M., & Lee, S. Y. (2010). *In silico* genome-scale metabolic analysis of *Pseudomonas putida* KT2440 for polyhydroxyalkanoate synthesis, degradation of aromatics and anaerobic survival. *Biotechnology Journal*, *5*(7), 739–750. https://doi.org/10.1002/biot.201000124

Szulc, A., Ambrożewicz, D., Sydow, M., Ławniczak, Ł., Piotrowska-Cyplik, A., Marecik, R., & Chrzanowski, Ł. (2014). The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: Feasibility during field studies. *Journal of Environmental Management*,*132*,121–128. https://doi.org/10.1016/j.jenvman.2013.11.006

Thiele, I., & Palsson, B. Ø. (2010). A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nature Protocols*, *5*(1), 93–121. https://doi.org/10.1038/nprot.2009.203

Thomas, G. E., Brant, J. L., Campo, P., Clark, D. R., Coulon, F., Gregson, B. H., McGenity, T. J., & McKew, B. A. (2021). Effects of Dispersants and Biosurfactants on Crude-Oil Biodegradation and Bacterial Community Succession. *Microorganisms*, *9*(6), 1200. https://doi.org/10.3390/microorganisms9061200

Tobalina, L., Bargiela, R., Pey, J., Herbst, F.-A., Lores, I., Rojo, D., Barbas, C., Peláez, A. I., Sánchez, J., von Bergen, M., Seifert, J., Ferrer, M., & Planes, F. J. (2015). Context-specific metabolic network reconstruction of a naphthalene-degrading bacterial community guided by metaproteomic data. *Bioinformatics*, *31*(11), 1771–1779. https://doi.org/10.1093/bioinformatics/btv036

Tsoi, R., Dai, Z., & You, L. (2019). Emerging strategies for engineering microbial communities. *Biotechnology Advances*, *37*(6), 107372. https://doi.org/10.1016/j.biotechadv.2019.03.011

Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. C. R. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation*, *22*(2), 231–241. https://doi.org/10.1007/s10532-010-9394-4

Varjani, S. J., & Upasani, V. N. (2017). Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresource Technology*, *232*, 389–397. https://doi.org/10.1016/j.biortech.2017.02.047

Wei, Z., Wang, J. J., Gaston, L. A., Li, J., Fultz, L. M., DeLaune, R. D., & Dodla, S. K. (2020). Remediation of crude oil-contaminated coastal marsh soil: Integrated effect of biochar, rhamnolipid biosurfactant and nitrogen application. *Journal of Hazardous Materials*, *396*, 122595. https://doi.org/10.1016/j.jhazmat.2020.122595

Witting, M., Hastings, J., Rodriguez, N., Joshi, C. J., Hattwell, J. P. N., Ebert, P. R., van Weeghel, M., Gao, A. W., Wakelam, M. J. O., Houtkooper, R. H., Mains, A., Le Novère, N., Sadykoff, S., Schroeder, F., Lewis, N. E., Schirra, H.-J., Kaleta, C., & Casanueva, O. (2018). Modeling Meets Metabolomics—The WormJam Consensus Model as Basis for Metabolic Studies in the Model Organism *Caenorhabditis elegans*. *Frontiers in Molecular Biosciences*, *5*, 96. https://doi.org/10.3389/fmolb.2018.00096

Wu, X. F., & Chen, G. Q. (2019). Global overview of crude oil use: From source to sink through inter-regional trade. *Energy Policy*, *128*, 476–486. https://doi.org/10.1016/j.enpol.2019.01.022

Xu, C., Liu, L., Zhang, Z., Jin, D., Qiu, J., & Chen, M. (2013). Genome-scale metabolic model in guiding metabolic engineering of microbial improvement. *Applied Microbiology and Biotechnology*, *97*(2), 519–539. https://doi.org/10.1007/s00253-012-4543-9

Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., & Yu, H. (2018). Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Frontiers in Microbiology*, *9*, 2885. https://doi.org/10.3389/fmicb.2018.02885

Xu, X., Zarecki, R., Medina, S., Ofaim, S., Liu, X., Chen, C., Hu, S., Brom, D., Gat, D., Porob, S., Eizenberg, H., Ronen, Z., Jiang, J., & Freilich, S. (2019). Modeling microbial communities from atrazine contaminated soils promotes the development of biostimulation solutions. *The ISME Journal*, *13*(2), 494–508. https://doi.org/10.1038/s41396-018-0288-5

Zhao, D., Kumar, S., Zhou, J., Wang, R., Li, M., & Xiang, H. (2017). Isolation and complete genome sequence of *Halorientalis hydrocarbonoclasticus* sp. Nov., a hydrocarbon-degrading haloarchaeon. *Extremophiles*, *21*(6), 1081–1090. https://doi.org/10.1007/s00792-017-0968-5

Zhuang, K., Izallalen, M., Mouser, P., Richter, H., Risso, C., Mahadevan, R., & Lovley, D. R. (2011). Genome-scale dynamic modeling of the competition between *Rhodoferax* and *Geobacter* in anoxic subsurface environments. *The ISME Journal*, *5*(2), 305–316. https://doi.org/10.1038/ismej.2010.117

Zhuang, K., Ma, E., Lovley, D. R., & Mahadevan, R. (2012). The design of long-term effective uranium bioremediation strategy using a community metabolic model. *Biotechnology and Bioengineering*, *109*(10), 2475–2483. https://doi.org/10.1002/bit.24528

Zomorrodi, A. R., Islam, M. M., & Maranas, C. D. (2014). d-OptCom: Dynamic multi-level and multi-objective metabolic modeling of microbial communities. *ACS Synthetic Biology*, *3*(4), 247–257. https://doi.org/10.1021/sb4001307