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SCHOOL OF COMPUTER SCIENCE ENGINEERING (SCOPE)

BIOREMEDIATION DATABASE: A Review

Biological Databases (BIT2002) Review - 3 Report

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1. Abstract

Bioremediation is the process of using microorganisms as initiators of the degradation pathways of contaminants in soil, water, etc. Often these organisms metabolize or mineralize the contaminants, hence helping in the *remediation* of the natural region. While research on bioremediation pathways, and microorganisms (and complex microbe cultures) has been ongoing for more than 4 decades now, there is yet to exist a consolidated database that compiles the information with bioremediation as the primary focus. With the NCBI MetaRouter no longer available, and the deprecated resources in other databases, the need for a modern, organized dataset for bioremediation, phytoremediation (using plant strains for remediation), and other variants is required. Our effort is to manually curate a database for Bioremediation, by compiling research articles, review papers, research papers, and other available publications to isolate the information that fit our parameters and make it publicly available to the community. The database is organized by contaminant, and contains information about the microorganism, the strain, the gene sequence information, the pathway graphic (if available), the enzymes involved in the reaction (or produced by the microorganism), the process parameters required for the reaction (pH and temperature), phytoremediation options for the same contaminant, and the referred resources.

2. Introduction

2.1 Bioremediation

Bioremediation is a branch of biotechnology that uses species of bacteria, fungi, and other microorganisms to break down pollutants in water, soil, sludges, solids, etc. Bioremediation often involves a redox reaction inside the cell of the microorganism, which happens in the form of an electron exchange. These reactions fuel the biological processes of the microorganism and hence they have resistance to the contaminant considered.

Bioremediation process experiments can happen *in situ* i.e. on the site of contamination, but often require careful monitoring of control conditions and may limit the ability of the experimenter in changing these conditions. If the process is examined *ex situ* i.e. outside of the actual site of contamination (wastewater, soil etc.) there is greater scope for research and more conclusive research, however this process can be more expensive.

For any bioremediation pathway to be deemed useful or successful, it is important to refine the context of the environment where the reaction will occur first. Factors like the geochemistry of the region, the natural temperature, the oxidation-reduction conditions etc, have to be considered while determining the microbial complex or microorganism that can initiate the pathway. This also prevents other undesirable reactions from taking place within the environment that can cause further harm by generating by-products.

2.2 Types of Bioremediation

2.2.1 Phytoremediation

Phytoremediation is the process of using plants to remove pollutants from soil and groundwater, or to help toxins degrade into a less hazardous state. Some plants have the ability to extract and concentrate specific elements from the environment, providing a long-term solution. Plant tissue that has collected pollutants can be harvested and treated safely. Remediation can also occur when pollutant species are degraded by bacteria on the plant's roots, or when the roots bring polluted ground fluid closer to the surface, exposing contaminant species to microbes in a more oxygen-rich environment.

2.2.2 Bioaugmentation

The introduction of specially selected or genetically altered microbe strains to a contaminated place is referred to as bioaugmentation. Exogenous microorganisms with the requisite biochemical capabilities can be introduced to successfully degrade specific waste compounds if site studies reveal that species of indigenous bacteria are unable to decompose target contaminants.

2.2.3 Biostimulation

The supply of oxygen and/or inorganic nutrients to indigenous microbial populations in soils and groundwater is referred to as biostimulation. To encourage biodegradation of pollutants, in situ or ex situ procedures might be used.

The majority of the pathways described in the database fall under the category of bioaugmentation, however there are few methods of biostimulation also explored, which will be reviewed in the literature survey. Phytoremediation pathways/processes have not been elaborated in detail, but there is scope in the database for the same.

3. Literature Survey

There exists a vast volume of literature, and associated experimental data around bioremediation methods and processes, and our primary effort is to compile the same. A primary strategy used for the bioremediation of soil pollutants, is the application of the Pseudomonas genus, especially the bacterium *Pseudomonas aeruginosa*. Considering the bacterium is increasingly gaining clinical relevance to its antibiotic resistance, efforts have been made to test its resistance to contaminants like Mercury, Chromium (VI), Cadmium, and even aromatic compounds like Naphthalene. Strains of the bacterium are reported to resist Lead, Nickel, Cadmium, Chromium, Copper, Cobalt, Arsenic and

Mercury, in varying concentrations between 50-100 mg/L. The rate of reduction increases as the concentration of the solute increases, until the threshold limit provided. Chromium was seen to have the highest resistance, upto 400 mg/L. Further, strains of *Pseudomonas stutzeri*, *Pseudomonas putida*, and *Pseudomonas fluorescens*, have been reported to catalyze the oxidation of aromatic compounds (Naphthalene, SGZ, PVA etc.) in the presence of multicomponent dioxygenase enzymes. *Pseudomonas Rhizophila* has been used in the oxidation of Polyvinyl Alcohol in the presence of oxidases, that involves the breaking of one of the aromatic rings. The *putida* strain has also been identified to help in the degradation of caffeine in water, in a methylation as well as an oxidation pathway.

The Bacillus sp. Genus has also shown great merit in the degradation of hydrocarbons, primarily by generating degradation enzymes for the initiation or intermediate steps in the pathways. *Bacillus subtilis* has been proven to have a large uptake of metals such as cadmium, lead, and work exists to report its efficiency in deproteinization reactions in the breakdown of crustacean wastes in water. *Bacillus megaterium* has been used for the metabolism and hydrolysis of ether pollutants like PCCD (polychlorinated dibenzo-pdioxins) and its variants. *Bacillus thuringiensis* can be used for the degradation of pharmaceuticals like Ibuprofen and Diazepam, by initiating the given pathways. *Bacillus cereus* has been identified to break down Olive oil components like oleuropein by generating select lipases.

Considering the Bacillus organisms mentioned, as well as most Pseudomonas bacteria are strictly aerobic, the reaction pathways are also aerobic, with a general pH between the range of 6-9. The ideal temperature for the organism to multiply the most, or the enzyme to perform the best is highlighted in most studies, and generally falls within the range of 25-35. These parameters are altered continuously, along with the concentration of the contaminant, to arrive at the optimal temperature, pH, and concentration. Some studies limit the temperature to the natural local temperature, however most studies do not prefer to do so.

Studies in phytoremediation are more limited to soil remediation, and are not as extensive as the literature for mycoremediation or other forms of bioremediation but curated data exists for the phytoremediation of trace metals like Lead, Cadmium, Mercury etc. as well as other hydrocarbons and aromatic compounds.

4. Materials and Methods - Protocol

The database is manually curated, by referring to experimentally verified data in publications, published during the time period of 2005 to 2020. The Biodegradation-Biocatalysis database - EAWAG-BBD, which is integrated with KEGG maintained by the University of Minnesota consists of microbe enzyme catalyzed pathways, which was used as the base resource for contaminants and microbes, however some of the pathways contributed are dated to 1995 or even older, which needed to be updated. Further, not all pathways contributed are bioremediation focused, and the process parameters are

unavailable, even in the research that led to the addition of the pathways. There exist practically no phytoremediation databases that compile research by contaminant and strain, and none that integrate different applications and types of bioremediation.

In our attempt to curate the database, we have sorted the database according to the contaminant, and identified the organism, the particular strain and gene information, and linked the sequence information through NCBI accession numbers in the sheet. The UniProt taxon identifier of the organism is also provided and linked in the sheet to allow a greater amount of cross-referencing. The graphics of the pathways have been stored in a locally hosted Google Drive Folder, and each graphic card has been linked separately with free access to the viewer. Most graphics images that have been included have been sourced from later research in an effort to update older pathways in existing databases. If no specific pathway has been mentioned in the research publication itself, the default pathway of the degradation of the contaminant, according to older research, has been favored, and these links have also been specified. The enzyme involved in the reaction has been listed, as have the other process parameters according to the research reviewed for each paper. The temperature mentioned is done so on the celsius scale.

The Phytoremediation options for each contaminant have been mentioned, although there is room for thorough expansion of the research involved in phytoremediation as well.

The links to all the research reviewed for the experimental data, the pathways and the strain information has been linked in the sheet for the reference of the user. ATCC strains have been linked to their respective product pages, and if there is no information regarding any constraint in the database, the field has been left blank or empty. If there is uncertainty regarding any of the entries, a * is placed next to the name of the contaminant.

5. Interpretation and Results

On reviewing literature to curate the database, it is evident that the literature is scattered, and the focus of every piece of literature may vary. When research on a particular pathway is saturated, there exists a large volume of data about process parameters, limitations of the reaction, and real-time implementation of the pathways. However, not every bioremediation process can be scaled from smaller samples, to full implementation over large swathes of pollutants, especially when experimentally verified data about that process is limited. Further, not every such process is cheap, and might require funding to scale. Our database has mostly focused on bioremediation by specific species of bacteria, some cases of mycoremediation, and phytoremediation options. The resulting curated raw data, in xlsx format, can be easily used to build an application, or can be referenced directly by students, and other members of the community. Due to constraints of time, the number of entries in the data is restricted to around 100, but the literature left to be reviewed remains vastly untouched. This database also provides an appropriate set of techniques and parameters to continue expanding the database, and the constraints set clear direction for the same.

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