OPINION

# Issues about application of bioremediation for cleaning up of contaminated sites

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**Abstract:** Bioremediation as a cleaning up technology is less predictable and efficient for application on s ite. An apparent gap is still evident between the laboratory results with pure culture or mixed culture of microorganisms and their biochemical capability including genes and enzymes involved, and the effectiveness at cleaning up the pollutants in soil and sediment on site. Associated issues include the characteristics of the site, ageing of the chemical pollutants and sequestration into soil as one, and activity and competitive of the degradative microorganisms under the actual contaminated conditions of natural environment. Because of these, the favorable conditions for the active growth of degradative microorganisms have not been investigated well enough with the available technology, so a simple inoculation of the pure culture effective under laboratory conditions cannot guarantee an expected efficiency and positive results at on site t esting. In addition, the soil or sediment physical and chemical conditions under the natural environment play an important role in the removal or mineralization of organic pollutants, but inadequate attention has been given to these factors involved from ecology. It is the objectives here to bring the attention to the bioavailable concentration of the pollutants and also the active metabolism of the organisms *in situ* to advance and demonstrate the effective cleaning up of contaminated sites.
**Keywords:** Sequencing, scientific questions, science, publication, spirit of science

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"*Everything flows; nothing stays.*"

— Heraclitus

Environmental contamination and pollution are widely detected in different compartments of the ecosystems from aquatic waters of fresh and marine ecosystems to sediments and ground aquifers (Schwarzenbach et al., 2006). There are also highly contaminated sites locally from industrial production and establishment, military and also aviation and maintenance operations (Atlas, 1995). All these pose serious hazards and ecotoxicity to the local flora and fauna of the ecosystems, including human on sites or living in close vicinity. Chemical spills and discharge of not-thoroughly-treated wastewater are other forms of environmental pollution which can accumulate resulting in negative effects over time. All these contaminated sites are proposed for cleaning up by the (bio)remediation technology as an effective option for removal of the toxicants (Atlas, 1995), but toxic organic pollutants are generally hydrophobic and easily accumulated in both biota and also sequestrated into the sediments by reacting with macromolecular humic-similar substances and adsorption onto surfaces of clay minerals, resulting a diminishing bioavailable concentration of them over time of ageing (Alexander, 1995). This important and critical factor on diminishing bioavailability and toxicity of pollutants over time in the environments have not been given enough attention even though it deserves to further a better understanding and knowledge of this fundamental issue based on chemistry and biology, and instrumental analyses for bioremediation and its success in operation (Gu, 2020, 2023). Almost all research conducted on this subject are based on freshly introduced pollutants by spiking without prior ageing and the results are not applicable to the remediation operation at contaminated sites with a history of pollutant release over time because the ecotoxicity of aged chemicals is less than the fresh ones (Alexander, 1995).

Efficient biodegradation of organic pollutants requires the microorganisms possessing the responsive genes and enzymes for biochemical metabolizing and mineralizing the organic pollutant carbon as a source of energy and carbon under the laboratory conditions to confirm the fundamental sci-

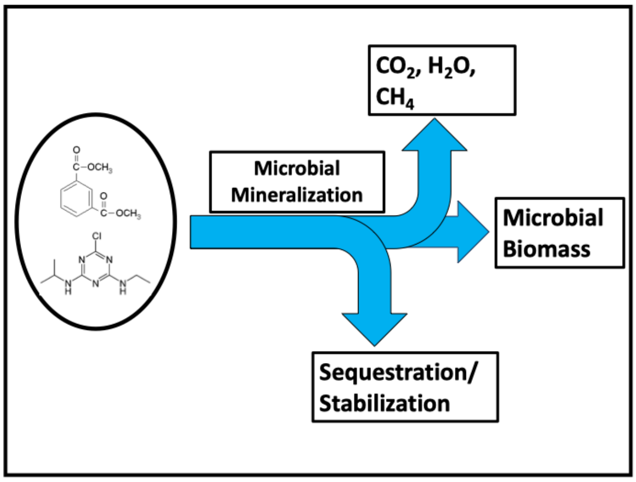


**Figure 1.** A schematic graph showing the competition between microorganisms for the bioavailable fraction of pollutant and also sequestration by soil inorganic clay minerals and organic humic-like substances.

ence and potential applications (Figure 1) (Evans, 1977; Liu and Suflita, 1993; Gao and Gu, 2021; Gu, 2016). Through such approach, the microorganisms, genes and/or enzymes responsible for specific steps of the biochemical reactions can be elucidated to allow the construction of the biochemical degradation pathways (Evans, 1977; Alexander, 1999; Gu, 2016, 2023). However, after the release of the toxic chemicals into the physical environment, especially through interactions with clay minerals and humic substances, the fate of the specific chemicals in the environment is at least a two-way competition between degradation of the soluble fraction by microorganisms for one, and, simultaneously, sequestration of them into the aggregates of organo-minerals to be less or non-available to organisms as residual fractions in sediment for the other (Gu, 2023). Several issues are still not resolved well enough facing this challenging situation: First, there is no available or standard method to determine the bioavailable concentration or fraction of any organic pollutants in soils/sediments. The current analytical methods used for measuring concentration of them in soils as an example are purely based on extraction by solvent, purification and then quantification of the concentration of interested fractions by sophisticated instruments, which are analytically valid, but have no direct connection to the physically status or form in the *in situ* environments or bioavailability in the sediment (Alexander, 1995; Gu, 2023). Then, because both the concentration of the pollutant and the degrative population of the microorganisms in soil are dynamics and changing constantly over time according to the local ecological conditions, a direct relationship between them is not linear or cannot be established easily for predication with high confidence, so a cause-and-effect relationship is almost impossible to establish without more sophisticated techniques are used, e.g., tracing with radioactive or stable isotope to monitor the substrates in soils and also the responsible degradative population. For an active growth of the capable microorganisms in the sediment or soil, available water or water activity is an important factor in addition to oxygen and nutrients available. To promote the microorganisms in degradation of specific chemical pollutants, it is necessary to consider several factors to achieve the dominance of the responsible microorganisms if possible. This is not an easy or simple task because available nutrients are mainly limited under natural conditions and survival of these microorganisms is a difficult task. In addition, more information shows that microbial degradation in complex environmental conditions involved metabolic network than a mineralization pathway by a single microorganism (Liu and Suflita, 1993). This natural or ecological way of life, though not being accepted widely or implemented, the success of the bioremediation trials is heavily dependent upon the reality of the soil microbiome and their activity on metabolizing the specific pollutants.

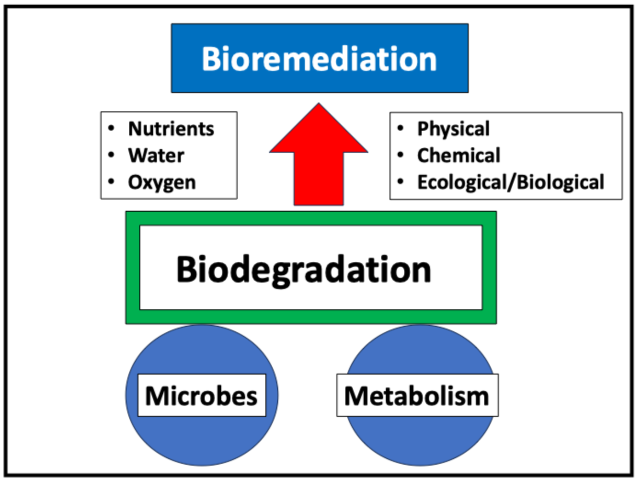


**Figure 2.** An illustration of the framework or structure from biodegradation research based on microorganisms and their metabolic activity to bioremediation as the goal.

More importantly, bioremediation shall be tested under the most realistic conditions of the *in situ* soils or natural conditions than in laboratory incubation in flasks or reactors to justify for bioremediation (Gu, 2021). It is possible that bioreactors can be used for bioremediation and the success of the operation system has to be supported by both the activity of the responsible organisms and also the availability of the pollutants including the transformation of the pollutants through chemistry (Figure 2). When dealing with soils, it is common that the water regime of the microcosms, mesocosm or field condition testing is mandatorily set to a number of personal choices without a good understanding of the water state and water activity or bioavailability to the microorganisms. In other words, soil water holding capacity, not the absolute content, is a relatively comparable parameter among soils of different texture and composition. The water content of practical application and use is a proportion of the water-holding capacity of the specific soil and this term is variable from soils to soils, but the water content based on water-holding capacity is a more reliable number (McGill et al., 1981). A 65% of the water-holding capacity yields the soils containing a balanced proportion of water and air to occupy the pores in soil so that aerobic process can be actively carried out as the dominant one. In addition to the water and aeration or available oxygen, nutrient requirement is fundamental for active microbial metabolism, common nutrients of N, P and S are required for actively growth and increase of the biomass, and the nutrient ratio shall be balanced for microorganisms (McGill et al., 1981). This, though ignored in many laboratory or reactor investigations than field trials, is basic to the ecosystem function of the microorganisms. Generally speaking, micronutrients are not a major issue for the soil environments.

The active microorganism and their biochemical capability are important for successful bioremediation, but such information is lacking for most of the studies, especially in the genomics era with the convenience of such technology inexpensively (Chen and Gu, 2022; Gu, 2022b). Without conformation on the microbial capability, it is premature to carry out the bioremediation testing no matter in labratory scale or field testing. In field trial, ecological conditions of temperature are hardly altered, but the available water and oxygen can be manipulated to benefit the growth of the microorganisms in soils. To be frankly, field testing for bioremediation for petroleum contaminated soils in Yukon Territories of northern Canada showed that aeration by tillage coupling with fertilizer addition promote better bioremediation results (McGill et al., 1981). Therefore, the physical, chemical and biological properties of the soils for remediation shall be examined closely from the points of view in soil science and microbiology to serve the purpose well.

Organic pollutants are in competition between microbial degradation when available and sequestration into clay minerals and humic-like substances to be residue remaining in soils permanently. These two processes are driven by the soil clay mineral types and their physical properties mostly; at the same time microbial transformation and mineralization are also operative (Figure 2). Under such scenario, study has been rarely carried out to look into this key issue and condition for a good information and comprehensive understanding of the fate of pollutants.

Bioremediation is good in theoretical concept, but its success in applications or practice is challenging and highly dependent upon the understanding of the pollutants-sequestration-microorganisms for bioavailability of the pollutant and also the *in situ* physically and chemically conditions to enhance the microbial mineralization process (Gu, 2023). It is clear that bioremediation in practice requires a good understanding of soil science, microbial ecology and practical information of the bioavailable pollutants, microbial physiological requirements of nutrients and the Minimum Law to advance the technology in application (Madsen, 1991).

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## Conflict of Interest

Author declares that there is no conflict of interest in the information presented here.

## Ethical approval

This article does not contain any studies with human participants or animals performed by the author involved.

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