

Current Trends in Bioremediation and Biodegradation: Next-Generation Sequencing

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Next-Generation Sequencing (NGS) obviously ignited a real revolution in environmental sciences, and it triggered the spread of its novel, cutting-edge disciplines e.g. metagenomics and metatranscriptomics. Bioremediation and biodegradation entered into an *omics* era [1], and nowadays the state-of-the-art technologies play a common role in several molecular microbiological laboratories. Modern NGS platforms are capable of producing gigabases of monoclonal and digital DNA data in a massively parallel fashion. In line with the decreasing sequencing prices, more and more convenient solutions and tools are offered for the community of the environmental science.

It is well-known that at least 95% of microbes are uncultivable in the laboratory applying standard culture conditions, which hampered their investigation for a long time during bioremediation studies. However, by using *comparative metagenomic* approach, millions of individual microbes can be identified and quantified based on their PCR-amplified 16S or 18S rDNA segments in a single run. Long-read sequencing of two hyper variable regions enables the species-level taxonomic characterization of bacteria, archaea, fungi, protozoa, algae etc. Currently, the gold standard in exact microbial diversity analysis is the highly accurate technology of Roche Diagnostics, but the concurrent Ion Torrent appears to be rather competitive with the new 400 base pair (bp) chemistry and its significantly lower sequencing cost per bp. NGS-based *molecular ecology* can easily be framed into *on-site* bioaugmentation or biostimulation regimes. During regular sampling of the test site, not only chemical and physical, but also comprehensive microbiological datasets can be gained enabling the scientists to create multidimensional matrices, where coherent dynamics and association networks can be determined [2]. In this way, the microbial key players and relevant interactions in the niche can be identified. Another useful tool to study the given complex phenomena in lab scale is the application of *Stable Isotope Probing* (SIP) technique. After addition of labeled substrate to the microcosm, the heavy fraction of synthesized gDNA can be isolated and the microbial distribution of the functionally enriched metagenome can also be determined in analogous way [3].

By using the SIP methodology, not just targeted sequence information, but the entire genomic data of the microbial degraders can be achieved as well. Whole metagenome amplification followed by *shotgun sequencing*, assembly and annotation may reveal catabolic enzymes involved in substrate depletion. Metagenomic or metatranscriptomic analyses are practically obligatory when the subjects of investigations are uncharacterized, non-cultivable microbial consortia, which are typically present in subsurface soil and aquifer. Due to their high throughput and low running cost, Illumina and SOLID platforms are often chosen for these scientific purposes. NGS technology can also enhance functional genomic research, when the microorganism is unknown but cultivable. After physical or enzymatic fragmentation of its DNA, short segments may undergo uniform and accurate sequencing. These aligned strings of bases, the so-called reads, can be assembled and annotated even in the absence of a reference genome. This methodology, the *de novo* sequencing, may help to explore novel catabolic pathways, mutations, peculiar genetic arrangements in chromosomes or in cryptic plasmids.

NGS is a very robust, straightforward technology and with its progress the cost will decrease, whilst the accuracy and throughput will increase dramatically. The bottleneck of NGS is the proper IT support, unfortunately the high performance scientific computing and big data research are still in their infancy. For the meantime, a close cooperation among technologists, biologists, bioinformaticians and data mining experts need to be built in the field of bioremediation and biodegradation in order to gain reliable scientific results.

References

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