Can the black box be cracked? High throughput sequencing to understand wastewater bioremediation

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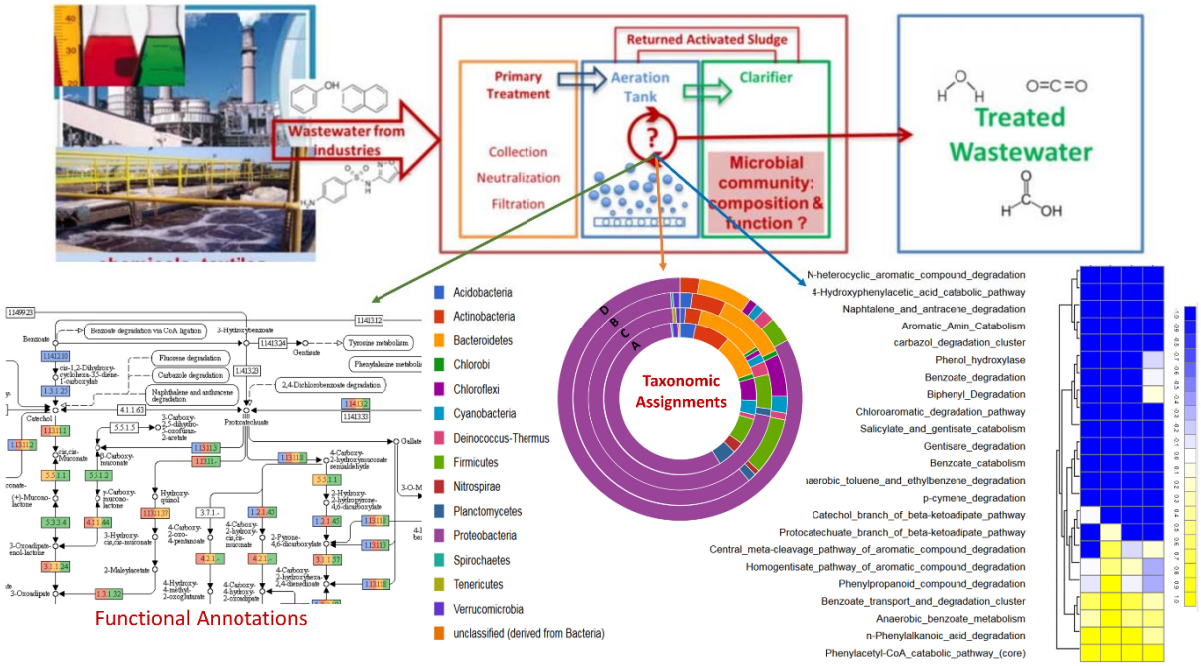

Abstract

The resilience of microbial populations to sustain disturbances of anthropogenic environments is governed by community composition and abiotic factors. This study aims at understanding the microbial populations involved in bioremediation of organic molecules in wastewater treatment plant, using the metagenomic approach. The whole metagenome sequencing of activated biomass sample revealed the dominating bacterial phyla namely Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes etc. Functional annotations revealed the presence of degradation pathways for 23 diverse molecules in 4 distinct wastewater samples studied. Comparative metagenomic analysis revealed the conserved and distinct functional features of the biomass. Environmental ecosystems harbour functionally potent phylotypes with respect to required ecosystem functions such as carbon metabolism, nitrification, denitrification and aromatic compound degradation.

Themes— water

1 Introduction

The discharge of industrial wastewaters from the urban sectors in cities threatens environmental and ecological safety. Present day technologies for wastewater treatment involves physical, chemical and biological methods. Of these methods either used individually or in combination, the aerobic biodegradation of organic molecules in wastewaters using the activated sludge is quite prevalent. This method is based on the principle of uptake of organic molecules as an energy source in metabolism in turn causing conversion of complex molecules into simpler forms. This renders bioremediation as an environmentally friendly and high-process-efficiency strategy for removing organic load of influent in wastewater treatment plants.

In this study we used the metagenomic approach to understand the microbial community effective in biodegradation of mixture of complex molecules acted upon by the microbial biomass in the aeration treatment. The microbial composition of the activated biomass is a significant aspect of the aerobic biodegradation, but very less understood in terms of the effective populations and the genetic makeup of the biomass functional in the chemical conversions of toxic compounds. The metagenomic approach enables us to analyse the microbial communities in detail, as it eliminates the need to culture the microbes using specific growth conditions.

2 Method

Whole metagenome analysis approach was undertaken to understand the microbial populations and the functional genes/pathways involved in bioremediation of the organics being treated in the influent of the wastewater treatment plant. 4 distinct samples were selected for this study to analyse the features conserved throughout distinct ecologies and unique to these sites.

2.1 Sampling sites

Sampling was performed as reported earlier [1] from an industrial wastewater treating plant. This sample is referred to as sample A in this study. Taxonomic and functional gene annotations were analyzed using MG-RAST server [2]. Comparative analyses were carried out using datasets generated in our earlier studies [3,4]. B: high Total Dissolved Solids (TDS) treating and C: low TDS treating represent activated biomass samples from petroleum refinery treating wastewater in Mumbai India. Sample D represents activated biomass sample from a plant treating wastewater from South India.

2.2 Metagenomic Analysis

The total metagenome extraction was performed as reported earlier [4]. Whole metagenome sequencing was performed using Illumina sequencing platform as reported earlier [3].Metagenomic and comparative analysis were carried out using MG-RAST annotations as reported earlier [3].

3 Results/ Discussion

We sampled the activated sludge biomass from an industrial wastewater treating plant in Gujarat, India. The Chemical Oxygen Demand (COD) of sample ranged from 2500-3000 mg/l. GCMS analysis of the samples revealed the presence of N-cyclohexyl-2-piperazin-1-yl-acetamide, 2'-methylenebis, biphenabid, sulfathiazole, pyridin-2-methyl ester, N-(4-acetylphenyl)-N-phenylurea, pyridinium, acetophenone, 3cyclohexene 1 methanol, benzaldehyde 2, 4 dimethyl, cyclohexane isothiocyanate,Benzene, 1,3 bris, 1-amino-4-methyl-chloride, benzothiazole., octacosane, 4-chloro-3-methylphenol like molecules. The whole metagenome analysis of sample A revealed the effective microbial population in bioremediation. To analyse the catabolic potential of the sample, we carried out comparative analysis using the datasets from 2 other locations studied earlier.

Taxonomic analysis was carried out for sequence features at 1000 abundance cutoff which revealed that genera *Acidovorax,Burkholderia,* and *Pseudomonas* were common to all 4 samples. Bacterial genera such as*Truepera, Stenotrophomonas, Flavobacterium, Methylobacillus, Mesorhizobium, Parvibaculum* were unique to sample A. Functional gene annotations revealed the presence of pathways such as benzoate, biphenyl, chloroaromatics, naphthalene, anthracene, phenol, carbazol, salicylate, gentisate, quinate, toluene, ethylbenzene, hydroxybenzoate, in all 4 samples.

4 Conclusions

The insights derived from this study help understand the role of microbial communities in bioremediation organic pollutants in the industrial wastewater treatment process. Bacterial genera *Acidovorax, Pseudomonas* and *Burkholderia* are most effective in bioremediation of wastewaters. The genes required for transformation of complex molecules of influent were traced through metagenomic mining and the efficiency of conversion could be understood for phenolic, polychlorinated, PAHs, and benzoate compounds. Our analysis help understand the dominating catabolic populations and diverse catabolic genes in wastewater bioremediation.

A Appendix

The high throughput Illumina platform was used for whole metagenome sequencing using Illumina TruSeq Nano DNA HT Library Preparation Kit. The paired end reads generated were assembled using CLC genomics workbench 6.0.

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