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Waste Water Microbes and Environmental "Clean Up": Roadmap to Environmental Sustainability

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ABSTRACT: Micro-organisms being ubiquitous in nature are supposed to be the principal agents, which can clean and modify the complex organic compounds which are xenobiotic in nature, to simple water soluble products by a process of biomineralization. This capability largely depends upon the selective microbial community as well as on the structural and functional groups of toxic compounds. Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The micro-organisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment.

KEYWORDS: Clean up, Environment, Microbial diversity, Sustainability, Waste Water Treatment Plant

I. INTRODUCTION

Unprecedented rise in population and rapid industrialisation has captivated developing industries and is leading to serious environmental problems which subsequently lead to generation of hazardous wastes which may be released in the form of effluents or may be categorized as solid wastes Lopes et al. [1]. The major constituents of wastes are xenobiotic compounds which owe their origin to different natural and anthropogenic activities Gienfrada and Rao, 2008 [2]. Waste water is generally rich in organic matter primarily rich in nitrogen and phosphorus Fredrikson, [3]. The characteristics of waste waters from industries vary so greatly in both flow and pollution strength. In general, industrial waste waters may contain suspended, colloidal or dissolved (mineral and organic) solids. The organic material would decompose producing bad smelling gases and the high amount of nutrients would lead to eutrophication with algal blooms followed by oxygen depletion and death of species requiring oxygen, such as fish. Wastewater also contains pathogenic bacteria and viruses from human faeces and these would be a health risk if left untreated. For these reasons, wastewater must be treated before it is discharged into a body of water. In addition, a river or a lake that is receiving the wastewater from one community may be the drinking water source for another community. In such cases, a reliable and functional wastewater treatment is even more important. Consequently, increased wastewater treatment availability can improve public health significantly Naik and Stenstrom, [4]. A typical Waste water system has following stages (Figure 1).

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II. HOW DOES A WASTE WATER SYSTEM LOOK LIKE?

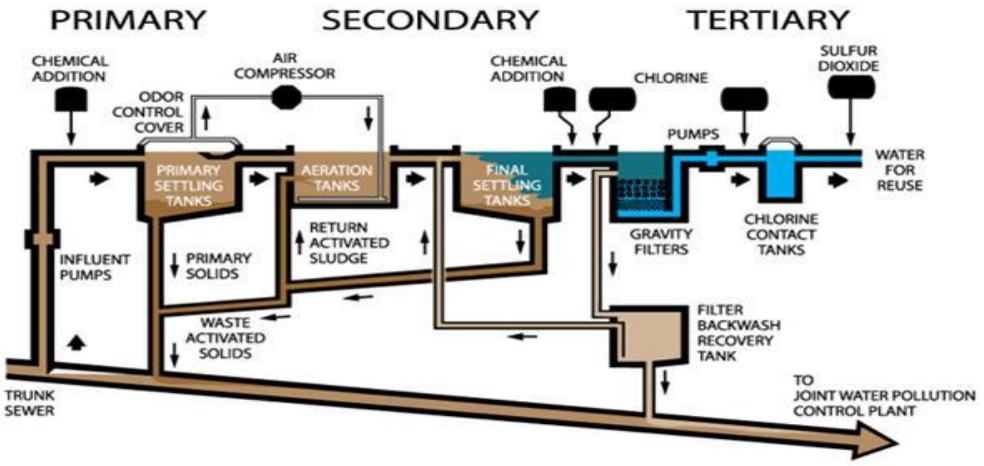

Figure 1: Schematic representation of a typical Waste Water Treatment Plant
(http://www.lacsd.org/wastewater/wwfacilities/moresanj.asp

Waste water systems present excellent example of a typical biotechnological process Lopes et al. [4] which are helpful in eliminating sewage born diseases like cholera Crockett, [5]. The operation of wastewater treatment technologies relies on a combination of physical, chemical and biological factors. For many decades, these systems have been based mainly on information about chemical and physical parameters, having been quite successful in using the advantages of the amazing metabolic potential of microbial communities without detailed available knowledge about the organisms involved Gilbride et al. [6]. Secondary treatment (Activated Sludge process) is one of the key components of a wastewater treatment plant. It involves the biological reduction of organic matter, suspended solids and toxicity of industrial wastewaters, together with the production of low nutrient effluents. These functions are carried out by the resident microbial (mainly bacterial) community, which is considered the foundation of the secondary treatment process. It involves the biological reduction of organic matter, suspended solids and toxicity of industrial wastewaters, together with the production of low-nutrient effluent. Besides, prokaryotes, eukaryotes like protozoa and metazoa also inhabit the system. The predation of bacteria by metazoans improves the effluent quality as free bacterial flocs may affect the population dynamics of the system (Pinto and Love, [7]. The role of Archea for nitrogen removal has been investigated in an activated sludge process (Daims et al., [8]; Wells et al. [9].

In most cases, secondary treatment follows primary treatment and involves the removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes. Aerobic biological treatment is performed in the presence of oxygen by aerobic microorganisms (principally bacteria) that metabolize the organic matter in the wastewater, thereby producing more microorganisms and inorganic end-products (principally CO_2, NH_3, and H_2O). Several aerobic biological processes are used for secondary treatment differing primarily in the manner in which oxygen is supplied to the microorganisms and in the rate at which organisms metabolize the organic matter.

High-rate biological processes are characterized by relatively small reactor volumes and high concentrations of microorganisms compared with low rate processes. Consequently, the growth rate of new organisms is much greater in high-rate systems because of the well controlled environment. The microorganisms must be separated from the treated wastewater by sedimentation to produce clarified secondary effluent. The sedimentation tanks used in secondary treatment, often referred to as secondary clarifiers, operate in the same basic manner as the primary clarifiers described previously. The biological solids removed during secondary sedimentation, called secondary or biological sludge, are normally combined with primary sludge for sludge processing.

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Common high-rate processes include the activated sludge processes, trickling filters or biofilters, oxidation ditches, and Rotating Biological Contactors (RBC). A combination of two of these processes in series (e.g., biofilter followed by activated sludge) is sometimes used to treat municipal wastewater containing a high concentration of organic material from industrial sources.

One of the treatment alternatives that can be applied for waste treatment is biological treatment known as biodegradation. Biodegradation defined as a process of oxidation of organic compound by microorganisms, in the soil, water, or on the installation of waste water treatment Reynold, [10]. The biological process of waste treatment is often chosen because it requires relatively little cost and the small amount of sludge compared with the chemical or physical process Meitiniarti et al. [11]. One type of biodegradation process is activated sludge which is defined as a process in the biological treatment of waste liquid, where the mixing of the liquid waste with the active sludge is on a aeration tank, for then be aerated.

Commercial and industrial wastewater systems are of great microbiological interest, in terms of both community structure and function, yet many of the component bacteria and their metabolic activities are poorly understood Seviour and Blackall, [12]. Cultivation-based studies have been carried out in an attempt to isolate and identify the important bacteria present . These studies have demonstrated the diversity that exists, but because of their selective nature they cannot provide a true indication of the organisms present.

III. AIMS OF WASTE WATER TREATMENT

Following are the primary aims of a waste water treatment process Moura et al. [13].

- Reduction of Biochemical Oxygen Demand (BOD)
- Removal of potential recalcitrant trace organic compounds
- Removal of toxic chemicals
- Nitrate , phosphate reduction so as to ascertain environmental sustainability
- Removal or inactivation of pathogens

Secondary aim of a waste water treatment is to convert waste into a reusable form as a drinking water form (Rodriguez et al., [15] or generation of energy from sludge Applels et al. [14]. Utilisation of sludge as potential source of biofertilizer production has been investigated in recent past (Sengupta and Pandit, [15]. Hierarchy of treatment stages in a typical waste water treatment plant is represented in figure 2.

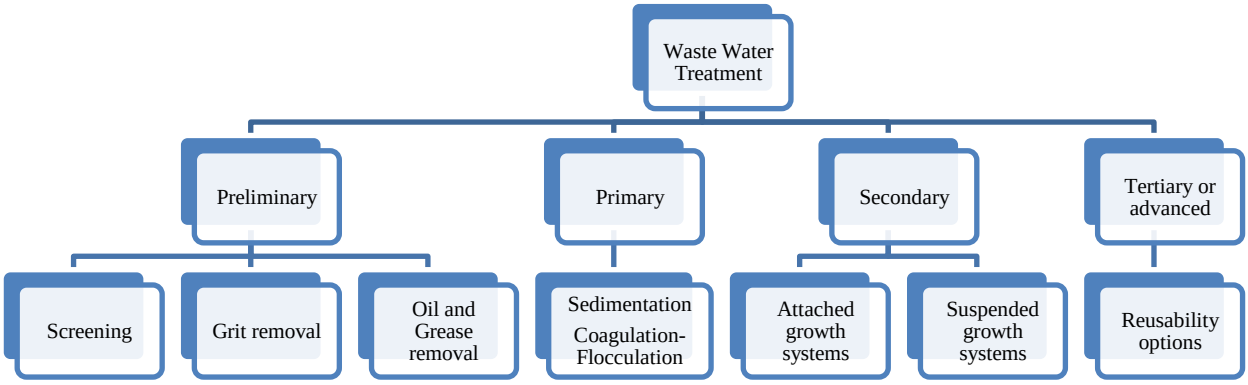

Figure 2: Hierarchy of stages involved in a typical waste water treatment plant

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IV. BIOLOGICAL TREATMENT AND MICROBIAL DIVERSITY: EXPLORING THE UNTAPPED POTENTIAL

Microbes have always formed a major component of global biodiversity, either as producers (e.g., phototrophic blue-green algae) or decomposers (e.g., heterotrophic bacteria). Furthermore, in the future, they may serve as producers of useful alternative energy sources Ohnishi et al. [16]. Traditionally, microbiologists have used culture-dependent approaches for the detection and isolation of environmental microbes, and the methods currently in use are based on those developed in the late 19th century Okabe et al. [17]. There is growing interest in research and development to develop novel tools to study, detect, and characterize microbes and their communities in industrial environments. Bacterial detection, identification, and typing from industrial samples remain an important task. The wealth of new techniques applicable to environmental microbiology is starting to uncover the variety of sequences relevant in biodegradation and biotransformation, as well as conditions in which these are active in their natural niches. Correlating phylogenetic analysis of communities with metabolic traits relevant for decontamination is increasingly important, because slight phylogenetic variation can be associated with different degradative specificities. Approaches aiming at characterizing microbial communities in the environment, be it by large-scale sequencing of metagenomes or by looking at biodegradative potential by mRNA-type techniques, are likely to go hand in hand with pure culture systems for detailed functional studies of biodegradative functions, to yield information about microbial enzymatic specificity and processivity. In view of the dearth of new enzymes discovered since the late 1970s, the ultimate goal of the ongoing efforts to explore the biodegradation and/or biotransformation gene pool is to open new avenues to our sustainability as industrialized societies Galvao et al. [18].

To circumvent the problem of isolating the different species by growth condition preferences, DNA-based molecular techniques over the last few decades have been developed and had revealed an enormous reservoir of unexplored or unculturable but viable microbes. Un-culturability is a condition that encompasses: (i) Lack of specific growth requirements (nutritional, temperature and aeration); (ii) slow-growing strains, out-competed in the presence of fast- growers and (iii) injured organisms, which are incapable of overcoming the stressful conditions imposed by cultivation. These categories may not represent specific taxonomic positions but account for about 99% of the environmental bacterial diversity. This large genetic diversity can potentially be used as a bioresource, leading to development of novel biotransformation, bioremediation processes and bioenergy generation Kalia et al. [19-20]. Culturing these unexplored microbes in controlled laboratory environment requires extensive knowledge of their fastidious growth requirements. This has in fact been the driving force for development of new methods to access this vast microbial wealth Wanger and Loy, [21] Handelsman, [22]; Green and Keller, [23]. However, the development of culture-independent methods and the commercialization of next-generation sequencing technology Mardis, [24] have yielded powerful new tools in terms of time savings, cost effectiveness, and data production capability. Methods such as 16S rDNA gene clone libraries, Fluorescence In Situ Hybridization (FISH) or Denaturing Gradient Gel Electrophoresis (DGGE) are being used to explore the bacterial diversity in waters have been reported judiciously Zwart et al. [25]; Bottari et al,. [26] ; Von Mering et al. [27]; Revetta et al. [28]; Gabriel, [29]. More recently, the potential of the high-throughput 454 pyrosequencing to explore the environmental diversity has been emphasized Roh et al.[30].

16S rDNA gene diversity specifies the idea of species richness (number of 16S rDNA gene fragments from a sample) and relative abundance (structure or evenness), which are reflective of relative pressures that construct diversity within biological communities Manefield et al [31]; Paul et al. [32]. 16S rDNA based molecular identification aims at identification, by virtue of it's universal distribution among bacteria and the presence of species-specific variable regions. Figure 3 represents characterization of microbial diversity by 16S rDNA sequencing strategy.

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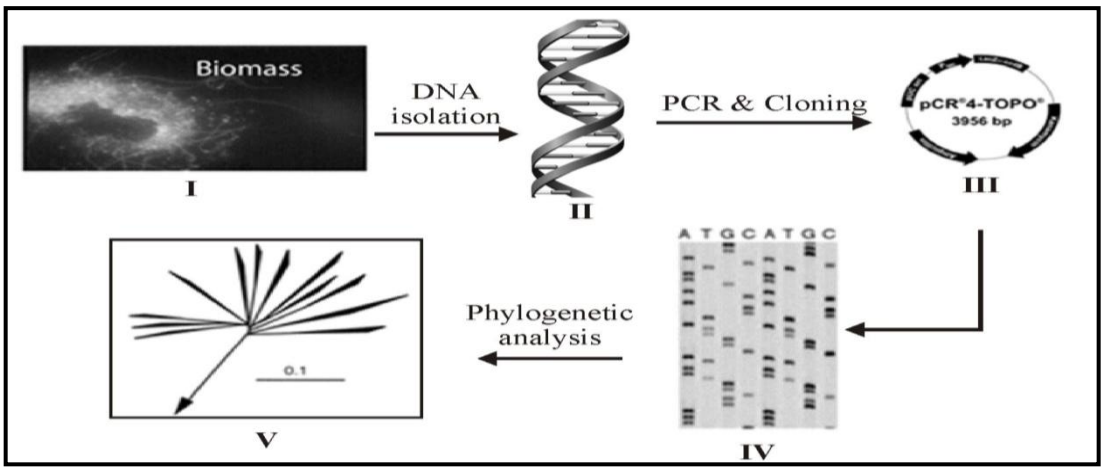

Figure 3: Flowchart depicting characterization of microbial diversity by 16S rDNA analysis Hobel, [33]

Some studies have been based on specific bacterial groups, such as the family Enterobacteriaceae Vacca et al. [34] or methanotrophic bacterial populations DeJournett et al. [35]. Examination and characterization of activated sludge have proved difficult, but adaptation of conventional techniques may provide further insights into this complex microbial ecosystem. This fact holds true because activated sludge has been a phenomenal model ecosystem for diversity based studies. Currently, molecular fingerprinting methods such as Denaturing Gel Gradient Electrophoresis (DGGE) have been used to investigate population dynamics Boon et al. [36] and terminal fragment length polymorphism (t-FLP) Saikly et al. [37]. Recently, PCR based 454 pyrosequencing has been applied to investigate microbial population of activated sludge and in full scale bioreactors Sanapareddy et al. [38]; Kwon et al. [39]; Kim et al. [40]; Ye et al. [41]; Zhang et al. [42].

The microbial communities in ETP exist in dynamic consortial forms, the understanding of which could be made through the knowledge of different co-existing microbial populations which is non uniform with changing operational conditions of the reactor Kapley et al. [43]. Their contribution to overall degradation of pollutants is likely to provide un paralleled control over the bioremediation of the effluents. In wastewater treatment, microbial molecular ecology techniques have been applied mainly to the study of flocs (activated sludge) and biofilms that grow in aerobic treatment systems like trickling filters (Sanz and Kochling,[44]. Prokaryotes are among the most important contributors to the transformation of complex organic compounds in WWTP. Forster et al. [45] has reported the importance of bacterial assemblages to the proper functioning and maintenance of treatment plants.

Molecular approaches such as 16S rDNA clone libraries Seviour and Blackall et al. [12] ; McGarvey et al. [46] Ribosomal Intergenic Spacer Analyses (RISA) Baker et al.[47] 16S-restriction fragment length polymorphism (16S-RFLP) Repetitive Extragenic Palindrome PCR (REP-PCR Bjornsson et al. [48] have already been applied to the study of wastewater-associated microbial communities. Figure 4 represents assessment of microbial diversity through culture dependent and culture independent approaches Jain et al, [49] and Figure 5 represents multidisciplinary approach to identify microbial diversity Hugenholtz [50].

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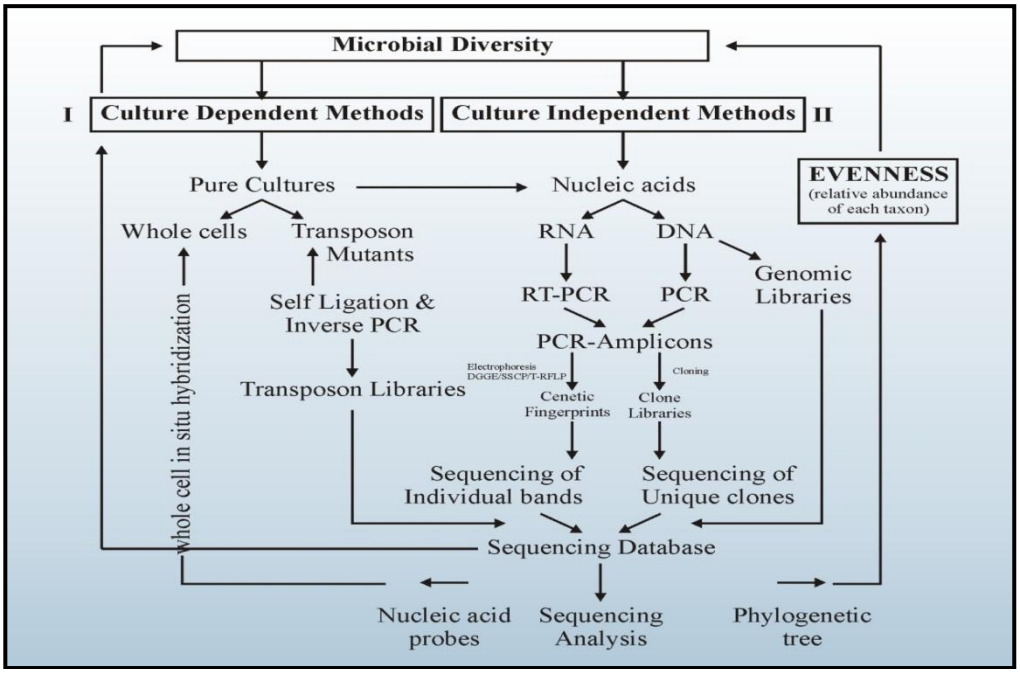

Figure 4: Assessment of microbial diversity through culture dependent and culture independent approach Jain et al. [49]

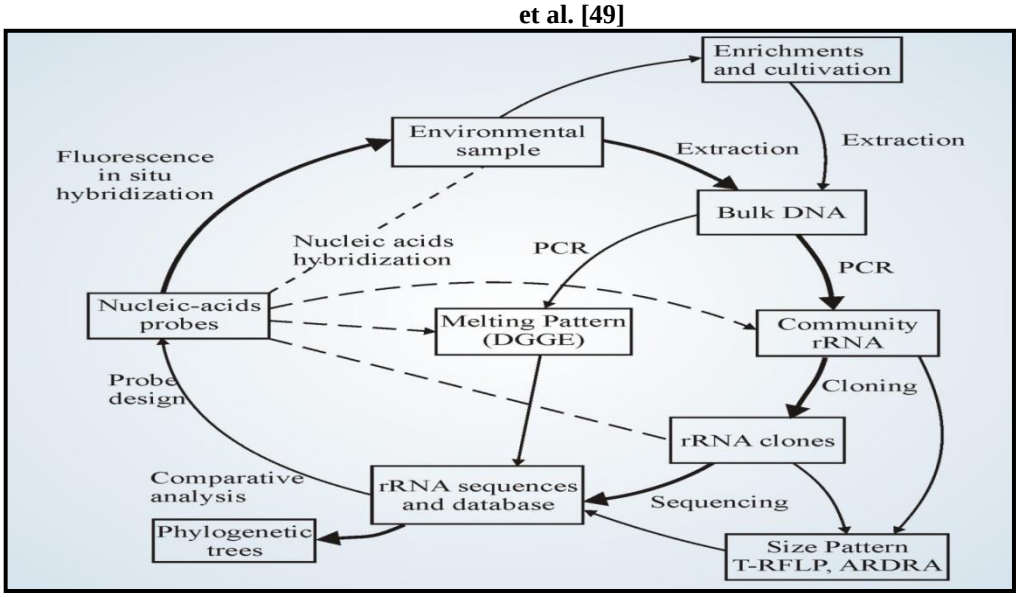

Figure 5: Multidisciplinary approach to analyze and characterize the microbial diversity through culture independent approach Hugenholtz [50]

PCR–DGGE has been successfully implemented in many fields of microbial ecology to assess the diversity and to determine the community dynamics in response to environmental variations. DGGE-based approach has been used to study bacterial diversity in wastewaters using reactors systems Liu et al. [51]; Rowan et al. [52] and activated sludge Ibekwe et al.[53] ; Gilbride et al.[54] revealing the presence of highly complex bacterial communities. Bacterial diversity of aerated lagoons from WWTP so far have not been substantially assessed where the degradation of organic matter takes place. Cloning had been employed to establish with precision the phylogenetic position of filamentous bacteria in granular sludge Sekiguchi et al [55]. or to determine the prevalent sulfate reducing bacteria in a biofilm Ito et al. [56]. The study was extended by Yamada et al. [57] who found the most prevalent sulfate reducer belonging to

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Chloroflexi subphylum. This prokaryote has been associated with bulking of sludge in treatment plants. The microbial communities residing in reactors for treating several types of industrial wastewater have also been determined by means of 16S rDNA cloning and sequencing. Egli et al. [58] examined the microbial composition and structure of a rotating biological contactor biofilm for the treatment of ammonium-contaminated wastewaters. The study revealed the sequences of several previously undetected and uncommon microorganisms. Phylogenetic analysis of the sequences obtained showed a narrow range of diversity, with most of the screened microorganisms belonging to the *Methanosarcina sp.* Studies conducted by Zhang et al [42] elucidated the efficacy of cloning approach in conjunction with in situ hybridization analysis in methanogenic reactor adapted to phenol degradation.

This essentially requires designing of new specific primers and gene probes for detection and/or quantification of microorganisms. On the similar lines, Crocetti et al. [59] extracted genomic microbial DNA from a sequencing batch reactor cloned the bacterial 16S rDNA and identified *Rhodocyclus sp. and Propionibacter pelophilus* as the microorganisms responsible for the polyphosphate accumulation taking place in the reactor. Futhermore, the same research group, designed probes for these species that could correlate phosphorous removal and the number of hybridized cells in different sludges. Alpha-proteobacteria, whose role in anaerobic/aerobic-activated sludge phosphorous removal plants has been analysed were characterized by new probes for in situ hybridization with information provided by 16S rDNA gene library sequences and DGGE analysis Beer et al. [60] . A similar approach had been considered three years earlier by the same authors, but Kong et al. [61] utilised the combination of cloning, DGGE, and FISH for identification of predominant microorganisms in an anaerobic sequencing batch reactor (SBR) without probe design and phosphorus removal. A comparative analysis of further insights into molecular biology tools for detection of microbial diversity are represented in Table 1.

Table 1: Comparative analysis of further insights into molecular biology tools for detection of microbial diversity Sanz and Kochling, [43]

S.No	Method	Outline	Advantages	Disadvantages
1	tRFLP	16 S based, relies on differences in polymorphism	Relatively simple procedures	Heterogenous size of fragments makes phylogenetic analysis less confident
2	RISA	Phylogeny with intergenic spacer region between 23S and 16S rDNA sequences	High sensitivity, down to sub-species level	Database for comparative analysis small in comparison to 16S sequences
3	PCR with genes	Microorganisms containing enzymes involved in the biodegradation process are detected	Direct detection of the presence of degradative microorganisms. Subtyping on strain level is possible	Global profiling of microbial community missing
4	PLFA	Profiling of microorganisms by characteristic fatty acid content	Molecular characterization of microorganisms not relying on genes. Complementary information to 16S based assays	Not a good choice as a standard alone method
5	DNA microarray	Multi sample hybridization method	High sample throughput Parallel analysis of different parameters	Expensive equipment, difficult handling

V .CONCLUSION

Microorganisms are ubiquitous in nature. They occupy every possible ecological niche. Microbial Diversity is composed of two main elements species richness and evenness. These elements represent the selective pressures which are essential in community dynamics. The detailed analysis of microbial diversity within an environment can be divided into two broad categories; culture dependent and culture independent approach. Waste water systems have proved to be model ecosystems for assessment of microbial diversity. Waste water treatment facilitates sequential removal of organic and inorganic pollutants. The biological process is selectively governed by the resident micro-flora

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primarily the prokaryotes. These prokaryotes are unique in biodegrading the pollutant of interest through their immense metabolic potential. Studies based at assessing the bacterial diversity has been envisaged in recent past. Different molecular methods have been proved phenomenal in assessing the community dynamics of these model ecosystems. The molecular approaches have unlocked the black box (Microbial Diversity) through construction of metagenomic libraries. Bioprospecting is one such field which has gained momentum in recent past. Different enzyme systems and genes responsible to carry out the degradative bioprocesses in these waste water systems has been focal theme of many environment research groups. Diversity based studies will definitely prove to be a breakthrough in microbial ecology and environmental biotechnology domains.

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