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Review

Molecular and biological techniques used in landfill investigations: A mini-review

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The purpose of this research paper was to review the different molecular biology techniques that are used in landfill investigations. The methods discussed include polymerase chain reaction (PCR), fluorescent *in situ* hybridization (FISH) and phospholipid fatty acid analysis (PLFA). Operation of landfills as bioreactors is now becoming a common practice, which involves the identification of different microbiological activities that facilitate the eventual breakdown of landfill wastes into useful and innocuous materials. In this review, the two important microbial activities that are discussed include methanotrophic process, carried out by methanotrophic bacteria, and methanogenic processes, carried out by methanogenic bacteria. Other bacteria encountered in landfills such as *Nitrosospira* and *Nitrosomonas* are also briefly discussed. As the name of these processes imply, methane oxidation and methane production by these microbial activities in landfills constitute another main focus of this paper. The application of these molecular biological techniques in real-time has also been demonstrated in studies involving the investigation of methanogenic diversity and activity in municipal solid waste landfill leachates and this is also discussed further. The results and conclusions of different research studies that focused on these techniques are hereby identified, discussed and summarized.

Key words: Bioreactors, polymerase chain reaction (PCR), fluorescent *in situ* hybridization (FISH), phospholipid fatty acid analysis (PLFA), methanogens, methanotrophs, landfilling.

INTRODUCTION

Landfilling still remains a common practice in municipal solid waste disposal. Reports have shown that approximately 55% of the 220 million tons of municipal waste generated in 1998 were disposed in landfills (Mehta et al., 2002). Increasing amounts of municipal solid wastes have also become a source of environmental problems over the past 15 years due to decreasing availability of landspace (Charlier et al., 2012; Srivastava and Nema, 2011; Hartlieb et al., 2003). A major problem with municipal solid waste landfills is the contaminants which are disposed into them. These contaminants include household hazardous wastes, wastes disposed prior to the 1980 enactment of U.S. hazardous waste disposal legislation, and biological and chemical transformation products of buried refuse (Li and Cleall, 2010; Lowry et al., 2008; Reinhart, 1993). The emission of methane (CH_4) from landfills to the atmosphere and the oxidation of CH_4 in the cover soil pose a concern for scientists and it has been reported that 6-12% of global methane emission are from municipal solid waste landfills (Stratis-Pavese et al., 2004; Barlaz et al., 2010; Einola et al., 2007; Frantz et al., 2000). The decomposition of the solid wastes in landfills is mediated by microbial processes involving different bacterial communities thus; the micro-biology of landfills is an important topic for discussion since landfilling continues to be the main means of waste disposal. With all these issues in mind, numerous research studies on landfills are now being conducted, which are focused on

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identifying the microbiological processes necessary for landfill sustainability. While most landfill research studies have focused on the utilization of the methane generated from landfills for power generation (Gupta and Morris, 2013; Mor et al., 2006; Sanchez et al., 2006; Copty et al., 2004; Frantz et al., 2000; Themelis and Ulloa, 2007), others have focused on methane production and oxidation and the production of other landfill gases (Tolaymat et al., 2010; Jung et al., 2009; Abichou et al., 2006; Hilger et al., 2000; Powelson et al., 2006; Nozhevnikova, et al., 2003; Borjesson et al., 1998; Borjesson et al., 2004).

However, the isolation and identification of the different microorganisms responsible for the production and oxidation of methane and other landfill gases from landfill cover soil, wastes, and liners are significant steps in understanding the environment under landfills. Two groups of microorganisms that have been isolated and identified in municipal solid waste landfills are fungi and bacteria (Providenti et al., 2004; Lockhart et al., 2006; Chen et al., 2003; He et al., 2008; Hanson and Hanson, 1996). However, the focus of this review will be on two groups of bacteria, which have been extensively studied in landfills, which include methanotrophic bacteria (methanotrophs) and methanogenic bacteria (methanogens). Methanotrophs are aerobic bacteria that are capable of utilizing methane as a sole source of carbon and energy (He et al., 2012; Vishwakarma and Dubey, 2010; Luesken et al., 2012; Hanson and Hanson, 1996; Mer and Roger, 2001). By oxidizing methane through methanol and formaldehyde to carbon dioxide, these bacteria are able to incorporate methane into their cell (Luesken et al., 2012; Stratis-Pavese et al., 2004). Examples of these methanotrophs include *Methylobacter*, *Methylomicrobium* and *Methylococcus*. Conversely, methanogens are obligate anaerobes which are capable of breaking down organic matter and inorganic substrates into methane (Zhou et al., 2011; Staley et al., 2012; Mer and Roger, 2001; Laloui-Carpentier et al., 2006; Garcia et al., 2000). Examples of these bacteria include the genera *Methanoculleus, Methanofollis* and *Methanosarcina* spp.

The molecular biology techniques used in landfill investigations involving the identification of these bacteria have been improved over the years especially in monitoring the different microbiological processes behind the generation, emission, oxidation and reduction of landfill gases (Henneberge et al., 2013; Latorre et al., 2012; Ran et al., 2010). The conventional microbiological technique for investigating the different microbial processes occurring in municipal solid waste landfills has been the incubation of the landfill cover soil and gas emissions using agar plate cultures. Although this method still remains in use, researchers have documented that as many as 99% of the existing microorganisms in the environment cannot be cultured (Sanz and Kochling, 2007; Mohammadi et al., 2005; Riesenfeld et al., 2004). Therefore, other new molecular biology techniques have been developed, which are more sensitive and specific in the detection of unculturable microbial population through nucleic acid probes, gene sequencing, or direct cloning from the environment (Van Dyke and McCarthy, 2002; Mojiri et al., 2013; Han and Kim, 2010). These new techniques include polymerase chain reaction (PCR), fluorescent *in situ* hybridization (FISH) and phospholipid fatty acid analysis (PLFA). The application of these methods in wastewater treatment technologies have been recently reviewed as well (McDonald et al., 2008, 2012; Han and Kim, 2009; Sanz and Kochling, 2007). Despite the attractiveness of these techniques for microbiological landfill investigations, information on their application is still lacking. However, few investigations on landfill sites have been identified in which different approaches involving these techniques were used. Therefore, the purpose of this paper was to review the findings of those research studies with respect to the type of molecular biology technique used and to also discuss the advantages and disadvantages associated with the use of these techniques.

POLYMERASE CHAIN REACTION (PCR) IN LANDFILL INVESTIGATIONS

In PCR, short DNA molecules isolated from a living organism are amplified exponentially without the use of the organism (Hunt, 2006). In this technique, a pair of DNA sequences, also called primers, is designed to facilitate the targeting of the sequence of different taxonomic levels such as strains, species and genus (Dorigo et al., 2005; Yu et al., 2005). The principle behind this technique is the extraction of nucleic acid, amplification and cloning of the 16SrRNA genes, which are sequenced and identified using a sequence database such as Genebank^{TM} (Dorigo et al., 2005)

Different research studies have revealed the application of this technique in identifying the microbial activities and diversity in landfill sites. In a study to evaluate and assess the performance and application of 16S rRNA based terminal restriction fragment length polymorphism (T-RFLP) for landfill site cover soils, Yang et al. (2011) and Stralis-Pavese et al. (2006) showed the application of T-RFLP as a method to study methanotroph communities using PCR amplification of the 16S rRNA from methanotrophic bacteria under a simulated landfill site. Through the extraction and purification of the DNA from two methanotrophs, type I and type II, the authors designed a forward primer (5'-CCTTCGGGMGCYGACGAGT-3') and a reverse primer (5'-GATTCYMTGSATGTCAAGG-3') of the methanotrophic bacteria. This process was carried out in 50 µl mixtures containing approximately 100 ng of soil DNA, a reaction buffer, 200 µM each of dATP, dCTP, dGTP and dTTP, 3 mM $MgCl_2$, 0.15 µM of each primer and 2.5 U Taq DNA polymerase. Through PCR, the forward and reverse primers were used to construct a 16S rRNA gene

library and the authors used these PCR products to identify the methanotophs through an ABI 373A automated sequencer. From this sequence analysis, a phylogenetic tree was constructed. Results of this study showed a dominance of the type I methanotrophs such as the closely related species of *Methylobacter* sp., *Methylcoccus* and *Methylomicrobium*, which were present in all samples. The analysis of the phylogenetic sequence of the 16S rRNA genes also showed the presence of methanotrophs related to *Methylosarcina* and a different group of *Methylobacter*. The sequence analysis also revealed the abundance of type II methanotrophs such as *Methylocystis* sp. (*Methylocystis echnoides* and *Methylocystis pyriformis*). In concluding the results of this study, Stralis-Pavese et al. (2006) noted that the methanotrophs, *Methylobacter* and *Methylocystis*, dominated the group of methanotrophs found at the rhizospheres of various plants under landfill simulated lysimeters. It was also concluded that type I methanotrophs such as *Methylobacter* are best adapted to grow in the presence of landfill gas in the upper layer. However, the results of the study failed to establish a correlation between methane oxidation capacity and the methanotroph communities in the landfill cover soil. From this study, it was shown that the efficiency of the T-RFLP technique in landfill studies depends on the accuracy of the DNA extraction and PCR amplification of the target gene sequence. Mnif et al. (2012) and Stralis-Pavese et al. (2004) also obtained similar results in a related study to optimize the diagnostic microarray for application in analyzing landfill methanotroph communities under different plant covers.

Working at two landfill sites that contain mainly household, construction, and industrial wastes in Sweden, Sundberg et al. (2007) investigated the treatment of landfill leachates by overland flow systems through potential nitrification. The study also involved investigating the structure of ammonia-oxidizing bacterial community in those landfills over a growing season. Through an 8 h watering and 16 h drying, the authors were able to stimulate nitrification and denitrification processes in the landfill leachates. Samples of leachates were collected at designated times for nutrient analyses as well. The potential ammonia oxidation and potential nitrite oxidation were also determined using seven replicates, which were then used for DNA extraction. Using PCR, two forward primers (CTO189fA/B-GC and CTO189fC-GC) and one reverse primer (CTO654r) were designed to amplify a 465 base pair sequence of the 16S rRNA gene. PCR was performed using a PTC -100^{TM} thermal cycler in a 50 µl mixture composed of 1.33 U of polymerase, 5 µl of buffer, 1.5 mM of $MgCl_2$, 200 µM concentration of each nucleotide, 0.5 µM of forward primer and 0.5 µM of reverse primer, 0.5 ng µl^{-1} of T4 gene 32 protein and 5 µl of DNA template. Denaturing gradient gel electrophoresis (DGGE) was also carried out, which involved the analysis of the PCR products using a DCode universal detection system. From the sequencing and phylogenetic analysis of the samples, results were obtained, which indicated the presence of microorganisms such as *Nitrosospira* sp. and *Nitrosomonas eutropha* in the samples. *Nitrosospira* and *Nitrosomonas* populations were also detected in the overland flow area while the PCR amplication and DGGE indicated the presence of *N. europaea* in high concentrations in the samples. The results of the study also showed the presence of ammonia oxidizing bacteria in all the ecosystems, which were detected by real-time PCR. It was concluded that the most diverse population of the ammonia oxidizing bacteria was found in landfill leachates with low levels of oxygen (Sundberg et al., 2007; Chen et al., 2007). The results of this study also indicate the relevance of PCR in analyzing environmental samples for other microorganisms apart from methanogens and methanotrophs in landfill leachates.

The application of real-time PCR has also been demonstrated in studies involving the investigation of methanogenic diversity and activity in municipal solid waste landfill leachates. Working with two leachate samples collected from different solid waste landfill sites in France, Laloui-Carpentier et al. (2006) used a 16S rRNA approach to characterize the presence and activities of archaeal microbial communities present in the leachates. Using PCR, the 16S rRNA genes were amplified using primers, *Archaea* 8aF5' TCY GGT TGA TCC TGCC 3' and 1114aR5'GGGTCT- CGCTC GTT RCC 3', which were specific for the target sequence. The mixture for the PCR analysis was composed of 10 ng of DNA, 50 pmol µl^{-1} of the primers, 25 mM each of 5 µl of deoxynucleoside triphosphate mix, 25 mM of $MgCl_2$, 5 µl of *Taq* buffer and 0.5 U of DNA polymerase. The cloning of the PCR product was also carried out using pGEM®-T Vector System into *Escherichia coli* JM109 competent cells and sequencing of the plasmid templates using pGEM®-T Vector System universal sequencing primers T7 and SP6 as well. This study also applied the fluorescent *in situ* hybriddization (FISH) technique in the 16S rRNA sequencing but details of this technique will be discussed later in this paper. Results of this study showed that a total of 239 archaeal sequences were recovered from the landfill leachate. This result was used to develop a phylogenetic tree, which showed that 89% of the sequences were closely related to the methanogenic family *Methanosaetaceae* while about 18% of the sequences were associated with the methanogenic genera *Methanoculleus* and *Methanofollis*. Other methanogenic genera identified from the study include *Methanosarcina*, *Methanospirillum*, the genus *Methanosaeta* and the order *Methanomicrobiales*. From these results, it was concluded that these microorganisms are the significant methanogens in municipal solid waste leachates and that the incubation of landfill leachates with methanol and hydrogen also resulted in methanogenetic activities in landfills (Laloui-Carpentier et al., 2006).

At this point, suffice to say that the applications of PCR

in landfill studies have shown that more information can be obtained about the microbial populations and metabolic activities in landfills. Furthermore, the results from these studies also show that methanogens and methanotrophs are the most studied microorganisms in landfills. Other studies have also used FISH in investigating these microorganisms as well as other parameters in landfills. The next section describes the FISH technique and its applications.

FLOURESCENT *IN SITU* HYBRIDIZATION (FISH) IN LANDFILL INVESTIGATIONS

In FISH, short sequences of single-stranded DNA, probes, are designed to bind to complementary target DNA sequences, which are tagged with a fluorescent material. This technique facilitates the observation of the location of the target DNA sequences therefore, it can be applied in studies involving microorganisms with non dividing cells and can be used for gene mapping and identifying chromosomal abnormalities as well (National Human Genome Research Institute, 2007). Generally, the principle behind this technique is the use of rRNA-targeted fluorescent probes to hybridize a specific DNA sequence from an organism, which is then viewed with an epifluorescent microscope (Dorigo et al., 2005). The application of FISH in landfill investigations have also been demonstrated by different researchers.

In a study involving the molecular detection and direct enumeration of methanogenic *Archaea* and methanotrophic bacteria in domestic solid waste landfill soils, Kallistova et al. (2007) and Chen et al. (2003) evaluated the activities of these bacteria in methane oxidation and production in a landfill cover soil. Working at a landfill site in Tainan, Taiwan, the authors collected a total of 1000 kg of four samples of the landfill cover soil from depths of 10 and 30 cm and four samples of the solid wastes from depths of 1 and 3 m. By carrying out a DNA extraction, PCR amplification, and cloning of the archaeal 16S rDNA from the samples, the authors determined the sequences of the rDNA clones using a Thermo Sequence fluorescence-labeled primer cycle sequencing kit and an automated sequence analyzer. For the FISH analyses, oligonucleotide probes such as Archaea-universal probe ARC915, Bacteria-universal probe EUB338, type I methanotrophs-specific probe 1041-5, and type II methanotrophs-specific probe 1034-Ser were designed and labeled with Cy-5 at the 5' terminal. To determine the optimal conditions for hybridization, cells of *Methylobacter luteus* (target of probe 1041-5), *Methylosinus trichosporium* (target of probe 1034-Ser) and *Methylococcus cupsulatus* (non-target of either probe), were used. Using the soil samples containing the cells, a number of laboratory processes including sonification, centrifugation, washing and drying were performed to prepare the cells for hybridization. Hybridization of the cells was performed overnight at 46°C after the cells suspended with water were immobilized on poly tetrafluoethylene (PTFE) membrane by filtration. To view the images of the probe cells and their target sites using an epifluorescent microscope, part of the PTFE membrane was cut out and stained for 3 min with 10 ng ethidium bromide (EtBr) μl^{-1}. Results of this study showed a phylogenetic identification of methanogenic archaea genera such as *Methanosarcina*, *Methanoculleus* and *Methanobacterium*. It was also reported that among the total clones used, 31 of them were 99.2% similar to the 16S rDNA of *Methanosarcina thermophila,* 20 belonged to the genera *Methanoculleus*; three belonged to *Methanobacterium* while six belonged to the genus *Methanosaeta*. A direct counting of the bacteria, archaea and methanotrophs in the landfill soils showed that bacterial cells made up about 6 to 15% of the total cells in the 10 and 30 cm cover soil but accounted for 37 to 62% of the total cells in the 1 and 3 m depth solid wastes. Using the FISH probe in the direct counting of the *archaea* showed that they made up only 0.3 to 1% of the total cells in the solid wastes samples while none was observed in the cover soils (Chen et al., 2003). Similar results were also obtained in another study carried out by Chen et al. (2003), which focused on the determination of the archaeal community compositions at different depths of a municipal landfill in Taiwan using 16S rDNA cloning analyses. An extensive application of FISH in the study of methanogens has also been demonstrated by Limam et al. (2010), Wang et al. (2010) and Nakamura et al. (2006). The conclusions from these studies indicate that FISH is an effective approach for a direct enumeration and the determination of methanogenic and methanotrophic activities in a municipal solid wastes landfill.

An analysis of a nitrifying bacteria community in a landfill leachate has been carried out using FISH. Working with samples collected from a landfill in Korea, Kim et al. (2006) studied the spatial distribution of nitrite oxidizing bacteria and ammonia oxidizing bacteria and their roles in these processes. In this study, FISH probes such as Eub338, Alf1b, Bet42a, Nso1225, Nsv443 and Nsm156 were designed for targeting the 16S rRNA of most bacteria*, Proteobacteria*, *Nitrosomonas* spp. and *Nitrosospira* spp. Results of the bacteria community analyses showed that 60% of the Nso1225 probe belongs to *Nitrosomonas* while the Nsv443 failed to detect the *Nitrosospira* that it was designed for. Hybridization of the cells using probes such as Nit3 and Ntspa662 indicated the abundance and distribution of *Nitrobacter* and *Nitrospira* in the samples (Kim et al., 2006). The study concluded that the distribution of the bacteria populations depended on products formed (nitrite and ammonia) at different layers of the samples, which also highlights the importance of the FISH technique in identifying these microorganisms in samples collected from landfill sites. As previously mentioned, the study carried out by Laloui-Carpentier et al. (2006), which was focused on investiga-

ting the methanogenic diversity and activity in a MSW landfills also applied the use of FISH in identifying the microorganisms present. Using fluorescently labeled rRNA targeted probes such as Arch915, Cren512, Eub338, EubII, MB1174, MC1109 and MX825, methanogens such as *Methanosaeta* spp., *Methanosarcina* spp. and *Methanococcus* spp. were also identified. Domingo et al. (1997) have also described the application of whole cell hybridization, another form of FISH, in studying the response of microbes to triethylphosphate in landfill leachates. These studies have also shown that FISH can be widely applied in a variety of landfill studies involving qualitative and quantitative microbiological analyses in landfill sites.

PHOSPHOLIPID FATTY ACIDS (PFLA) IN LANDFILL INVESTIGATIONS

The use of PFLA in landfill studies have been described by Henneberger et al. (2013), Watzinger et al. (2008), Mellendorf et al. (2010) and Borjesson et al. (2004). In a study focused on investigating the microbial oxidation of methane at different temperatures in landfill cover soils, the authors conducted a PFLA analysis in other to identify the different groups of microorganisms in the landfill. Working with soil samples collected from three different landfill sites in Sweden, PFLA analyses of the samples was performed at temperatures of 10 and 15°C. In these studies, the extraction and methylation of PFLA was initially carried out using a one-phase extraction method of lipids from soil and mild alkaline methanolysis, respectively (Borjesson et al., 2001, 2004; Ren et al., 2012). Derivatisations of the PFLAs were performed with dimethyl-disulphide and GC-MS analysis, which was used to determine the positions of double-bonds and quantification of the monounsaturated PFLAs. Quantification of the methylated PFLAs was carried out using a GC equipped with fused silica capillary column and a flame ionization detector (FID) and the identification of the PFLAs was performed using prepared standards and by GC-MS analysis as well. Results of the PFLA analysis showed that the total amount of the PFLAs in the samples was between 266 and 1382 nmol (g dw soil)$^{-1}$, which was uniform in the samples collected from three different landfill sites. The results of the PFLA analysis also indicated that the PFLAs were associated with methanotrophic bacteria, which was correlated with the rates of methane consumption in the samples. The PFLAs identified include 16:1 ω 8t, 18:1 ω 11t, 18:1 ω 10t, 18:1 ω 9t, and 18:1 ω 8t, 18:1 ω 7t microcosms and the methanotroph-specific PFLAs include, 16:1 ω 5t, 18:1 ω 8c, 16:1 ω 6c, and 16:1 ω 8c microcosms. It was reported that the specific methanotrophs identified through this technique include the type I methanotroph, *Methylosphaera hansonii* and *Methylosphaera capsulatus* (Borjesson et al., 2004). These studies show the significance of PFLA as biomarkers for methanotrophs and also show the response of methanotrophic activities in landfills to changing temperatures. Other studies using PLFA have shown the distribution of various methanotrophic bacteria in soil samples collected from landfill sites and incubated with 2 ppm $^{13}CH_4$. Among the bacteria identified *Methylococcus whittenbury, Methylococcus luteus, Methylomonas agile, Methylomonas methanica* and *Methylocystis echinodes* were dominant (Maxfield et al., 2011; Kong et al., 2013). A summary of these studies and the associated microorganisms obtained in the results is presented in Table 1. The effects of temperature on methanotrophic activities in tropical landfill cover soils have been studied including the effects of moisture content and methane concentration. However, none of these molecular biology techniques was applied in such studies, which limited the scope of the bacteria identified (Rachor et al., 2013; Urmann et al., 2009; Visvanathan et al., 1999). Data from PFLA analysis have also been used to establish a correlation between microbial activities such as sulfate reduction, microbial population in a landfill leachate, and other biogeochemical parameters in landfills (Pombo et al., 2005; Ludvigsen et al., 1997). Despite the results obtained from this technique with respect to landfill investigations, its application still remains limited due to the complexities of the approach.

From the discussions so far, it can be inferred that the application of PCR, FISH, and to some extent, PFLA, yields significant results with respect to the microbiological processes occurring in MSW landfill sites. These methods have shown to be efficient in the identification, quantification, and the determination of the activities of microorganisms such as methanotrophs, methanogens, and in some cases, fungi in landfills (Lockhart et al., 2006). From the extraction and amplification of the DNA sequence isolated from these microorganisms, it is now possible to identify about 99% of these organisms including their viable and nonviable forms and this is carried out independent of the organism. This development contrasts the results obtained through the traditional form of using agar plate cultures to grow organisms for identification and quantification, which only yields 1% of the expected results.

However, these molecular biology techniques also have their drawbacks. For example, the use of PCR for landfill investigation can be time consuming and laborious, therefore, its application in studies involving a large number of samples is difficult and the extraction of each representative DNA in solid waste samples can be tedious as well (Sanz and Kochling, 2007). Reports have also shown that the bands detected in DGGE are small, therefore, the number of species that can be identified is limited (Bodelier et al., 2005). In using FISH for the purpose of studying landfill microbiology, the goal of designing a specific probe for a certain group of microorganisms is not always achieved especially when a myriad of microbial groups are involved and quantification can also be tedious and complex. Studies have also shown that the design and optimization of conditions for

Table 1. Summary of various studies using the different molecular biological techniques with the associated microorganisms obtained from the results.

Molecular Biological Technique	Reference	Microorganisms identified
Polymerase chain reaction (PCR)	Yang et al., 2011 Stralis-Pavese et al., 2006	*Methylobacter* sp., *Methylcoccus*, *Methylomicrobium, Methylosarcina, Methylocystis* sp.
	Sundberg et al., 2007	*Nitrosospira* sp. *Nitrosomonas eutropha*, *Nitrosomonas europaea*
	Laloui-Carpentier et al., 2006	*Methanoculleus Methanofollis, Methanosarcina Methanospirillum*
Fluorescent *in situ* hybridization (FISH)	Kallistova et al., 2007 Chen et al., 2003	*Methylobacter luteus, Methylosinus trichosporium, Methylococcus cupsulatus, Methanosarcina thermophile*
	Kim et al., 2006	*Proteobacteria, Nitrosomonas* sp., *Nitrosospira* sp.
Phospholipid fatty acid (PLFA)	Borjesson et al., 2004	*Methylosphaera hansonii Methylosphaera capsulatus*
	Maxfield et al., 2011; Kong et al., 2013	*Methylococcus whittenbur Methylococcus luteus Methylomonas agile Methylomonas methanica Methylocystis echinodes*

hybridization can be difficult to attain. According to most researchers, the application of PFLA still remains cumbersome therefore, its limited application up to date.

CONCLUSIONS

In this paper, a brief review of the different molecular biological techniques involved in landfill investigations is presented. The identification of various methanotrophic and methanogenic bacteria in landfill soils and leachates through these techniques facilitates the understanding of the biochemical processes that are essential for sustainable landfill management. Despite the drawbacks discussed, these techniques remain as the preferred investigative tool in landfill studies and other areas of environmental science. Since these techniques have afforded many researchers a better understanding of environmental microbiology, more research studies are needed to improve their efficiency and accuracy.

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