

Microorganisms Based Biological Agents in Wastewater Treatment and Agriculture: Potential Use and Benefits

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ABSTRACT

Human activities and population growth have placed the environment under increasing stress. Furthermore, indiscriminate use of natural resources is accompanied by increased local and global pollution levels, which are reflected in imbalances in our ecosystems. The generation of large quantities of wastewater with a high organic content and toxicants is one obvious product of excessive consumption. It has been known for many years that environmental discharges of high loads of organic matter can result in oxygen depletion in receiving waters due to stimulated microbial activity. This oxygen depletion and the presence of trace toxicants found in wastes also negatively influence ecosystems, including reduced biodiversity and environmental health. Therefore, negative environmental impacts have driven our need to understand the effect of pollution on water bodies and develop proper measures to reduce discharges, including treatment processes. Different technologies are available to treat wastes. However, biological wastewater treatment methods are most valuable because their economic benefits are high, especially when coupled with waste stabilization and resource recovery.

Keywords: Wastewater, microorganisms, treatment, agriculture, irrigation, environment.

INTRODUCTION

Biological wastewater treatment systems have been designed mostly from an engineering perspective, but in fact, many aspects of the ecology and dynamics of microbial communities should be taken into consideration (Cyzdik-Kwiatkowska and Zielinska 2016).

Biological processes are classified according to the primary metabolic pathways present in the dominant different microorganisms active in the treatment system. As per the availability and utilization of oxygen, the biological processes are classified as aerobic, anoxic, and anaerobic (Narmadha and Mary Selvam Kavitha 2012).

Many types of microorganisms can be found in the wastewater treatment system. However, the types of organisms that will dominate will be the ones that are best suited to the “environment” or conditions in the system. Wastewater treatment systems are designed to foster an “environment” that suits a certain type of microorganism. These microorganisms not only remove organic wastes from the water, but they also “settle out” as solid material for easy removal (Shaikh *et al.* 2013).

Wastewater treatment operators are required to maintain the right conditions in the treatment system for the right type of microorganisms. Biological degradation of organics is accomplished through the combined activity of microorganisms, including bacteria, fungi, algae, protozoa, and rotifers. To maintain the ecological balance in the receiving water, regulatory authorities have set standards for the maximum amount of the undesirable compounds present in the discharge water (Wagner and Loy 2002).

WASTEWATER CONSTITUENTS

Wastewater can be defined as the flow of use water discharged from homes, businesses, industries, commercial activities and institutions which are directed to treatment plants by a carefully designed and engineered network of pipes. This wastewater is further categorized and defined according to its sources of origin as domestic or sanitary wastewater and industrial wastewater (Table 1).

Table 1. Majors constituents of waste water (Narmadha and Mary Selvam Kavitha 2012).

Majors constituents of waste water	
Pathogens	Bacteria, viruses, prions and parasitic worms and Non-pathogenic bacteria
Organic particles	Faeces, hairs, food, vomit, paper fibers, plant material, humus
Soluble organic material	Urea, fruit sugars, soluble proteins, drugs, pharmaceuticals
Inorganic particles	Sand, grit, metal particles, ceramics

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Soluble inorganic material	Ammonia, road-salt, sea-salt, cyanide, hydrogen sulfide, thiocyanates, thiosulfates
Animals	Protozoa, insects, arthropods, small fish
Macro-solids	Sanitary napkins, nappies/ diapers, condoms, needles, children's toys, dead animals or plants
Gases	Hydrogen sulfide, carbon dioxide, methane, ozone
Emulsions	Paints, adhesives, mayonnaise, hair colorants, emulsified oils
Toxins	Pesticides, poisons, herbicides

MICROBIAL DIVERSITY IN WASTEWATER

Bacteria, protozoa, metazoan, filamentous bacteria, algae, fungi and helminthes are the major groups of organisms found in wastewater. Wastewater contains large quantities of bacteria, the majority of which are of fecal origin such as *Escherichia coli*, *Salmonella*, *Klebsiella* and *Enterococci* (Michael 2006; Baduru and Sai 2015).

A wide range of opportunistic pathogenic or pathogenic bacteria can be detected in wastewater. Among these bacteria are pathogens in general responsible for intestinal infections such as *Salmonella* or certain types of *E. coli* enteropathogens (Table 2). *Mycobacterium* spp., *Legionella* spp. and *Leptospira* are the most bacterial pathogen which causes non-enteric illnesses (João 2010).

Bacteria are the most common microbial pollutants in wastewater. They cause a wide range of infections, such as diarrhea, dysentery, skin and tissue infections, etc. Disease-causing bacteria found in water include different types of bacteria, such as *E. coli* O157:H7; *Listeria* sp., *Salmonella* sp., *Leptosporosis* sp., etc (João 2010).

The major pathogenic protozoans associated with wastewater are *Giardia* sp. and *Cryptosporidium* sp. They are more prevalent in wastewater than in any other environmental source (João 2010).

Viruses are among the most important and potentially most hazardous pollutants in wastewater. They are generally more resistant to treatment, more infectious, more difficult to detect and require smaller doses to cause infections. Because of the difficulty in detecting viruses, due to their low numbers, bacterial viruses (bacteriophages) have been examined for use in faecal pollution and the effectiveness of treatment processes to remove enteric viruses (Akpor *et al.* 2014).

Table 2: Some wastewaters microorganisms and their effect on human health (Roger 1982; João 2010; Jayakumar and Natarajan 2012).

Microorganisms	Diseases
Bacteria	
<i>Arcobacter</i> spp.	Bacillary dysentery, cholera, typhoid and paratyphoid, epidemic hepatitis, meningitis, gastroenteritis, vomiting, colitis, diarrhea (Enterotoxin), enteritis, erythema, tetanus, inflammation of the urinary tract, pus accumulation, respiratory tract inflammation, local infection, endocarditis, otitis and pneumonia.
<i>Campylobacter</i> spp.	
<i>Clostridium perfringens</i>	
<i>Pseudomonas aeruginosa</i>	
<i>E. coli</i>	
<i>Enterococci</i> spp.	
<i>Helicobacter</i> spp.	
<i>Klebsiella pneumoniae</i>	
<i>Legionella</i> spp.	
<i>Leptospira</i> spp.	
<i>Proteus</i> spp.	
<i>Salmonella typhi</i>	
<i>Salmonella typhimurium</i>	
<i>Salmonella paratyphi</i>	
<i>Shigella dysenteriae</i>	
<i>Staphylococcus aureus</i>	
<i>Streptococcus</i> spp.	
<i>Vibrio cholerae</i>	
<i>Yersinia enterocolitica</i>	
<i>Clostridium tetani</i>	
Fungi	
<i>Aspergillus</i> spp.	Broncho-pulmonary mycosis, mycosis of the nails, otitis, granuloma and mycosis of the skin.
<i>A. fumigatus</i>	
<i>A. niger</i>	

Trichophyton spp.

Nematodes

Ascaris lumbricoides *Ancltyostoma*
duodenale

Toxocara canis

Protozoa

Entamoeba histolytica

Giardia lamblia (*Lamblia intestinalis*)

Toxoplasma gondii

Cryptosporidium spp.

Sarcocystis spp.

Tapeworms

Taenia saginata,

Taenia solium

Echinococcus granulosus

Virus

Polio

Coxsackie

Echo

Hepatitis A, B and C

Enterovirus

Adenovirus

Coronavirus

Yeast

Candida albicans

Candida Crusei

Cryptococcus neoformans

Infect the small intestine, lungs, intestine and internal bodies.

Giardiasis, infect liver, intestine, biliary gall bladder and internal organs (liver, brain, heart), cryptosporidiosis.

Infect intestine, liver and lungs.

Poliomyelitis, fever, respiratory diseases, meningitis, myocarditis, hepatitis, encephalitis, eye inflammation and cold.

Disorders of the mucous membranes (mouth), lungs, meningoencephalitis, and granuloma.

BIOLOGICAL PROCESSES FOR WASTEWATER TREATMENT

The fundamental reason for the treatment of wastewater is to circumvent the effect of pollution of water sources and protect public health through the safeguarding of water sources against the spread of diseases. This is carried out through a variety of treatment systems, which could be onsite treatment systems or offsite treatment systems. This section is therefore aimed at describing the offsite (activated sludge, trickling filters, stabilization ponds, constructed wetlands, membrane bioreactors) wastewater treatment systems (Stottmeister *et al.* 2003).

These processes are based on the use of microorganisms for the conversion of organic contaminants into less toxic compounds (mineralization into carbon dioxide, water and inorganic salts in specific bioreactors). Several studies describe the biological treatment of contaminated water by various chemical pollutants such as perchlorates, bromates and polycyclic aromatic hydrocarbons (WHO 2006).

These processes have disadvantages such as the addition of additional energy and the pumping of the water to be treated. Moreover, these processes are generally not applicable to high concentrations of pollutants of high toxicity or very low biodegradability (WHO 2006).

Main Purifying Bacteria

Biological purification due to the action of the bacteria, allows the decomposition of the organic matter by nitrification in an aerobic zone, denitrification in the absence of oxygen and possibly an anaerobic zone at depth which ensures the digestion of other organic compounds phosphates and sulfates (Swiontek *et al.* 2014).

Carbonate-reducing Bacteria

They are anaerobic bacteria capable of oxidizing organic compounds, which are predominantly presented by methanogen producing methane CH₄, generating bacteria such as *Methanococcus*, *Methanosarcina*, sporulated *Methanobacillus* and non-sporulated *Methanobacterium* (Swiontek *et al.* 2014).

Nitrifying and Denitrifying Bacteria

Achromobacter alcalinigenens, *Azospirillum brasilense*, *Spirillum lipoferum*, *Sp. psychrophilum*, *B. azotoformans*, *B. licheniformis*, *Chromobacterium violaceum*, *Ch. lividum*, *Corynebacterium nephridii*, *Halobacterium marismortui*, *Kingella denitrificans*, *Neisseria sicca*, *N. flavescens*, *N. subflava*, *N. mucosa*, *Paracoccus halodenitrificans*, *Propionibacterium pentosaceum*, *Pr. cidipropionici*, *Peudomonas*, *Nirozomonas*, *Nitrobacter* and *Thiobacillus denitrificans* ...ect are participate in biological purification by reduce nitrogen accumulation in

wastewater, elimination of nitrate by denitrification and decrease the eutrophication of sewage water ecosystems (Abeliovich 1987; Richardson *et al.* 2009).

Dephosphatous Bacteria Acinetobacter

Acinetobacter baumannii, *A. lwoffii*, *A. junii*, *A. johnsonii*, *A. baylyi*, *A. bouvetii*, *A. grimontii*, *A. tjernbergiae*, *A. townneri*, *A. tandoii*, *A. gernerii*, *A. kyonggiensis*, *A. rudis* and *A. pakistanensis* are the main *Acinetobacter* species that dominants in sewage and raw wastewater, and play important role in activated sludge (Abbas *et al.* 2014; Al Atrouni *et al.* 2016).

Dephosphatation process is carried out by alternating the anaerobic/aerobic sequences by modifying the enzymatic equilibrium and subsequently induced phases of phosphorus accumulation. In anaerobic condition, the bacterium releases phosphorus, and then, as the oxygen concentration rises, it reabsorbs it. The assimilation of the polyphosphate by the bacterium can be used either as a reserve of energy or as phosphorus (Yazdani *et al.* 2009; Al Atrouni *et al.* 2016).

Sulfate-reducing Bacteria

Sulfate-reducing bacteria *Desulfovibrio desulfiricans*, *Desulfobacterium autotrophicum* and *Desulfobulbus propionicus* are anaerobic heterotrophs and autotrophs bacteria which play an interesting role in organic matter mineralization, biocorrosion and sulphur-cycle based wastewater treatment process by facilitate the reduction of sulfate to sulfide and decomposition of wastewater sediments (Kumar *et al.* 2011; Tingting and Dittrich 2016).

MICROORGANISMS

Protozoa

Ciliated protozoa are numerically the most common species of protozoa in activated sludge, but flagellated protozoa and amoebae may also be present. The species of ciliated protozoa most commonly observed in wastewater treatment processes include *Aspidisca costata*, *Carchesium polypinum*, *Chilodonella cinata*, *Opercularia coarcta*, *Opercularia microdiscum*, *Trachelophyllum pusillum*, *Vorticella convallaria* and *Vorticella microstoma* (Jayakumar and Natarajan 2012; Akpor *et al.* 2014).

Viruses

Viruses are also found in wastewaters, particularly human viruses that are excreted in large quantities in faeces. Viruses that are native to animals and plants exist in smaller quantities in wastewater, although bacterial viruses may also be present (Jayakumar and Natarajan 2012; Akpor *et al.* 2014).

Fungi

The most common sewage fungus organisms are *Sphaerotilus natans* and *Zoogloea* sp. A number of filamentous fungi are found naturally in wastewater treatment systems as spores or vegetative cells, although they can also metabolize organic substances. A number of fungi species, such as *Aspergillus*, *Penicillium*, *Fusarium*, *Absidia* and a host of others have been implicated in the removal of carbon and nutrient sources in wastewater (Jayakumar and Natarajan 2012; Akpor *et al.* 2014).

Algae

Algae can be found in wastewater because they are able to use solar energy for photosynthesis as well as nitrogen and phosphorus for their growth leading to eutrophication. Some types of algae that can be found in wastewater include *Euglena* sp., *Chlamydomonas* sp., and *Oscillatoria* sp. Algae are significant organisms for biological purification of wastewater because they can be able to accumulate plant nutrients, heavy metals, and pesticides, organic and inorganic toxic substances. The use of microalgae in biological wastewater treatment has gained a lot of importance over the years (Jayakumar and Natarajan 2012; Akpor *et al.* 2014).

Helminth

They are present in large numbers in secondary wastewater effluents, biofilters and biological contractors. A lack of nematode activity can be one of the bio-indicators of a toxic condition that may be developing in the treatment process (Jayakumar and Natarajan 2012; Akpor *et al.* 2014).

Table 3: Summary of 16S rRNA-based diversity surveys of waste water treatment (Wagner and Loy 2002)

Genus	Species
<i>Alphaproteobacteria</i>	<i>Rhodospirillum sodomense</i> <i>Paracoccus denitrificans</i> <i>Nostocoida limicolalike bacteria</i>
<i>Gammaproteobacteria</i>	<i>Moraxella osloensis</i> <i>Moraxella catarrhalis</i> <i>Acinetobacter baumannii</i> <i>Acinetobacter calcoaceticus</i> <i>Leucothrix mucor</i> <i>Thiothrix unzii</i> <i>Thiothrix nivea</i> <i>Thiothrix ramosa</i> <i>Thiothrix fructosivorans</i> <i>Thiothrix defluvii</i> <i>Moraxella catarrhalis</i>
<i>Betaproteobacteria</i>	<i>Sphaerotilus natans</i> <i>Variovorax paradoxus</i> <i>Leptothrix mobilis</i> <i>Leptothrix discophora</i>
<i>Bacteroidetes</i>	<i>Cytophaga columnaris</i> <i>Chryseobacterium balustinum</i> <i>Bergeyella zoohelcum</i> <i>Cytophaga lytica</i> <i>Runellas lithyformis</i> <i>Haliscomenobacter hydrossis</i>
<i>Planctomycetes</i>	<i>Gemmata obscuriglobus</i> <i>Isosphaera pallida</i> <i>Nostocoida limicola III</i>
<i>Chloroflexi and Thermomicrobia</i>	<i>Herpetosiphon aurantiacus</i> <i>Chloroflexus aurantiacus</i> <i>Nostocoida limicola-like bacterium AHW4</i> <i>Thermomicrobium</i>
<i>Actinobacteria</i>	<i>Janibacter thuringensis</i> <i>Terrabacter tumescens</i> <i>Nostocoida limicola II</i> <i>Rhodococcus rhodochrous</i> <i>Skermania pinensis</i> <i>Gordona amarae</i> <i>Nocardia asteroides</i> <i>Rhodococcus coprophilus</i> <i>Nocardia nova</i> <i>"Microthrix parvicella"</i>
<i>Firmicutes</i>	<i>Lactosphaera pasteurii</i> <i>Streptococcus suis</i> <i>Nostocoida limicola I</i> <i>Nostocoida limicola I</i>

USE OF TREATED WASTEWATER IN IRRIGATION

Drought, population growth, low water availability and the deterioration of freshwater quality by pollution resulting from the high quantity of waste released into the environment have led to the use of wastewater in irrigation in water-scarce countries, developing and the industrialized countries. Recycled wastewater is a high quality product that releases nutrients, organic matter, and fertilizers into the soil to promote plants growth and crop production (Baduru and Sai 2015; Djadouni and Madani 2016).

The wastewater management is contributes to the irrigation of crops, recycling of nutrients, reduces artificial fertilizer costs, minimizes toxic pollutants in soil and plants and reduce the pathogenic microorganisms in environment (FAO 2008; FAO 2010a).

Ignoring wastewater management leads to two principle water quality impacts, namely chemical contamination and microbial pollution. Pollution of healthy, undesirable chemical constituents, salinity effects on soil, crops contamination, microbial pathogens and waterborne diseases are the most environmental and

health risks associated with direct or indirect reuse of untreated wastewater for irrigation (Samuel 1996; Keiser and Utzinger 2005; FAO 2010b).

WASTEWATER IRRIGATION: DYNAMICS OF MICROORGANISM IN SOIL

Wastewater irrigation had a high influence on soil parameters and quality such as heavy metal (cadmium, copper, lead, selenium, Zinc, sodium) contents, nutrients variety and organic matter rate; and thus affect the microbiological parameters (microbial biomass, microbial activities and enzyme activities), dynamics of pathogen and indicator microorganism content in soil (Wang *et al.* 2010; Agnieszka and Magdalena 2016).

The activated sludge process is composed of aerobic and anaerobic microorganisms such as bacteria, archaea, fungi, and protists. It is capable of degrading organic compounds, including petroleum products, toluene, benzopyrene and neutralizing chemical pollutants such as toxicants and xenobiotics (Shumaila *et al.* 2013).

The organisms used in the activated sludge process include a specific variety of bacterial strains; these strains use oxygen to develop. They work in synergy to effectively degrade sludge, bioremediation and decontamination water from heavy metal (iron, sulphur, ammonium ferrous sulphate and ferrous sulphate, sulphate) (Michael 2006; Léon *et al.* 2009; Gopinath *et al.* 2015).

Five major groups of microorganisms are generally found in the aeration basin of the activated sludge process:

- Bacteria: aerobic bacteria remove organic nutrients.
- Protozoa: remove and digests dispersed bacteria and suspended particles.
- Metazoa: dominate longer age systems including lagoons.
- Filamentous bacteria: bulking sludge (poor settling and turbid effluent).
- Algae and fungi-Fungi: is present with pH changes and older sludge.

Some examples of bacteria which are commonly used in the activated sludge process include *Bacillus* and *Pseudomonas* species (Tsukasa *et al.* 2002; Lau *et al.* 2006; Kildea *et al.* 2008; Khan 2011).

Trichoderma harzianum, *Bacillus subtilis* QST 713, *B. pumilus* QST 2808, *B. megaterium* ATCC 14581 and *Pseudomonas chlororaphis* MA 342 are bacteria that act through a fungistatic and fungitoxic action (Abriouel *et al.* 2012; Devi and Natarajan 2015; Khaled and Balkhair 2016), but also through a stimulation of the plant's defenses, decomposition of cellulose and other organic waste components and produces bioactive substances that evolves the defenses against biotic stress (Abhilash *et al.* 2010; Amalraj *et al.* 2012; Shakir *et al.* 2017).

However, there are also ubiquitous microorganisms of water environments (storage basins) capable of causing non-enteric infections, such as bacteria of the genera *Pseudomonas*, *Aeromonas*, *Campylobacter*, *Legionella*, *Mycobacterium*, *Bacillus*, *Streptomyces* and *Leptospira*. Important roles of them are (i) improved nutrient uptake by the plant; (ii) improved bioavailability of nutrients in soil; (iii) stimulation of the degradation of organic matter; (iiii) produced a variety of biocontrol agents and bioactive compounds with antibacterial and antifungal properties (Chakraborty *et al.* 2006; Djadouni 2007; FAO 2010c; Benoît *et al.* 2012; Deepak *et al.* 2014).

WASTEWATER AND PLANTS

One of the most important vegetables irrigated with sewage water lettuce, mint, parsley, potatoes, cucumber, squash, pumpkin, watercress and radish; note that wastewater contains high levels of nutrients required for agricultural crop production and yield such as organic matter, inorganic compounds, micronutrients, nitrogen, phosphorus and microorganisms (WHOA 2005; Chun *et al.* 2015; Liang *et al.* 2016).

Wastewater nutrients will act as a sole source of soil fertilizers which contains dissolved minerals, phosphorus, nitrogen, sodium, potassium, iron, calcium and compounds such as fats, sugars and proteins; these substances are used by soil microorganisms as a foods and source energy for the synthesis of cell components and to maintain life processes (Wang *et al.* 2010; Zhang *et al.* 2011a; Narmadha and Mary 2012).

Treated wastewater is used to the irrigation of crops not intended for direct human consumption (restricted irrigation), and thus covers the irrigation of industrial crops (e.g., cotton, sisal, and sunflower); crops processed prior to consumption (e.g., wheat, barley, oats), and fruit trees, fodder crops and pastures. Unrestricted irrigation, on the other hand, refers to all crops grown for direct human consumption, including those eaten raw (e.g., lettuce, salads, cucumber etc.) and irrigation of sports fields and public parks (Lopez-Bucio *et al.* 2007; Wang *et al.* 2010).

CONCLUSIONS

The use of treated wastewater in agriculture can be beneficial to the ecosystems and agricultural production if it is careful planning and management. However, wastewater negatively affects humans and the surrounding environment; they contribute to the spread of microbes and pathogens, which is harmful to human health and increases the likelihood of being infected by incurable diseases. Bacteria in wastewater cause many diseases, inflammation of the intestine, small intestine ulcers, cholera, typhoid, respiratory diseases, fever and jaundice, while viruses cause intestinal infections, meningitis, paralysis, jaundice, respiratory diseases and unusual heart disease. The modesty of human infection with diarrhea and amoeba and epidemics of hepatitis and other diseases. Dissolves the consumption of oxygen in water by microbes, leading to the death of aquatic organisms, and the appearance of rot in water, and the spread of odors. Imbalance of biodiversity, pollution of soil when wastewater is on the run and reaching agricultural land.

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