Journal of Applied and Natural Science
14(2), 660 - 676 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Review Article

Autochthonous antimicrobial microorganisms: application in wastewater treatment

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Article Info
https://doi.org/10.31018/jans.v14i2.3462
Received: April, 10, 2022
Revised: June 8, 2022
Accepted: June 13, 2022

How to Cite

Bhatnagar, A. and Kalra, S. (2022). Autochthonous antimicrobial microorganisms: application in wastewater treatment. *Journal of Applied and Natural Science*, 14(2), 660 - 676. https://doi.org/10.31018/jans.v14i2.3462

Abstract

In the previous two decades, people's lifestyles have changed as a result of industrialization, urbanization, and modernity, resulting in a rise in pollutants in daily sewage wastewater output. Less than half of the sewage generated is processed in a sewage treatment facility, while the remaining gets discharged into rivers untreated, deviating physio-chemical parameters of river water from the standards and thus causing harm to aquatic ecosystems. Sewage water contains autochthonous bacteria such as *Pseudomonas fluorescens*, *Bacillus sp., Acinetobacter sp.* and *Rhodococcus sp* that are effective in decontaminating wastewater. They employ a variety of mechanisms to consume pollutants, including biosorption, bioaccumulation and enzyme-mediated bioremediation, and thus can be used in bioremediation schemes. Bacteria possessing antimicrobial activity as well as protease production can be isolated from the wastewater and employed in the sewage treatment plant. The bacterial consortium has also been shown to be successful in wastewater treatment due to the synergistic degradation capabilities of the co-cultivated bacterial strains, which enhance the uptake rate of pollutants as nutrients. Environmental factors such as temperature, pH, oxygen, and nutrition availability at the site all affect the process outcome. The major focus of this review is to emphasize the bacterial capacity to clean wastewater as a single bacterial culture or as part of a bacterial consortium and the factor affecting the degradation process to achieve the requirement of a safer environment.

Keywords: Antimicrobial, Autochthonous, Bacterial consortium, Biodegradation, Sewage water

INTRODUCTION

Due to the exponential rise of the world's population and the need to meet expanding demands for irrigation, residential, and industrial usage, many areas' accessible water resources are decreasing, and the quality of their water is worsening, Thus, Water contamination is one of the most serious issues that have arisen as a result of these factors. Water pollution occurs from three vital sources: domestic sewage (encompass effluent from toilets, baths, showers, kitchens and sinks), industrial effluents (organic and inorganic chemicals, hydrocarbon-containing compounds, heavy metals, dyes, radioactive substances and many xenobiotic substances) and run-off from agricultural fields (crops residue, NPK (nitrogen, phosphorous, potassium) rich fertilizers, pesticides). Mass bathing and religious activities also contribute to the addition of water pollutants in the aquatic ecosystem (Bhatnagar *et al*., 2016; Devi *et al.,* 2019). The Waste material from these sources dissolved or suspended in water is termed as “Sewage”. Aquatic bodies incessantly receive large tons of sewage per day from these sources and thus depleting the water quality parameters such as DO (dissolved oxygen), BOD (biochemical oxygen demand), COD (chemical oxygen demand) and heavy metal content etc. (Saha, 2014). Almost all of India's water supplies are contaminated to some degree by biodegradable and non-biodegradable contaminants, rendering them unfit for human consumption unless treated, making it the most remarkable environmental complication and threat to public health in both rural and urban areas.

India generates roughly 38000 million litres of sewage per day; however, our treatment capability is only about 12000 million litres per day (ENVIS 2021). About 30% of total sewage comes from urban areas. Only about 20% of our country's sewage production is treated and utilized daily, with the other about 80% being untreated and unutilized. Pindihama *et al.* (2011) & Kaur *et al.* (2012) illustrated in their case study of wastewater treatment facilities in South Africa's Limpopo Province. They reported that wastewater is hardly treated to guidelines and less than half of the population of the country receives treated water with acceptable standards and the prime reason was the dumping of untreated sewage water from chief sources to the rivers. Similarly, Matta (2014) monitored the physio-chemical characteristics of the river Ganga at Rishikesh, Hanif *et al*. (2020) evaluated the Kapotaksha River water pollution status at Bangladesh. Seanego and Moyo (2013) determined the parameters of the water of Sand River in Limpopo, South Africa while Kalgapurkar (2018) worked on physio-chemical parameters of Mutha river, Pune, Chopra *et al*. (2021) evaluated the water quality index of the Kali Bein which is one of the main tributaries of River Beas. Likewise, Vaz *et al.* (2016) monitored Rivers from the region of Catalão, Southeast Goiás State, Brazil and they all observed that values of water parameter of samples collected from their respective sites show a greater difference from the values given by World Health Organization (2006) and Bureau of Indian Standards (2020) standards as these site receives loads of polluted commercialized wastewater. Thus, the water quality of these rivers was not satisfactory. By restricting food sources, eroding spawning grounds, and impairing gill function, the discharge of improperly treated wastewater directly influences its users, fish, and other aquatic life. Among the many types of sewage, water pollutants are pathogenic organisms, oxygen-demanding wastes, soil nutrients, synthetic organic compounds, inorganic compounds, microplastic particles, sediments, radioactive elements, oil, toxins, and a diversity of other pollutants. As a result, suitable measures to reduce future pollutant loads entering the river should be taken (Akpor *et al.,* 2014).

Sewage water treatment is the primary procedure, and it entails several issues that require extensive documentation owing to the sewage water's significant environmental effect. As the lack of clean water intensifies, better management of limited water resources is needed. In general, chemical and biological techniques can be used to treat sewage effluent. Chemical wastewater treatment methods include precipitation, adsorption, ion exchange method, neutralization (Yadav *et al.,* 2021) and disinfecting with chlorination or dechlorination agents or using radiations whereas mycoremediation, phytoremediation, vermifilteration, vermicomposting, and other biological wastewater treatment technologies include oxidation ponds, aeration lagoons, aerobic and anaerobic bioreactors, activated sludge, trickling filters, and biological filters (Samer, 2015; Ahmed *et al.,* 2021). Bioremediation is a process for eliminating or transforming toxic pollutants such as heavy metals or organic chemicals into less harmful substances like carbon dioxide, water, nitrogen gas, and so on. *In-situ* and *ex-situ* procedures can be used to apply bioremediation to soil and water. The essential premise of bioremediation is biodegradation, which refers to the continuous mineralization of organic pollutants into carbon dioxide, water, inorganic materials, and cell protein (Shishir *et al.,* 2019). Pollutant biodegradation can take place on three different levels. The first method is natural degradation, in which local microorganisms destroy contaminants without the need for human intervention. The second method is to employ nutrients and oxygen to speed up the biodegradation process (biostimulation). The third process is the indulging of special microorganisms which carry specific pollutant catabolic capability (bioaugmentation) (Shishir *et al.,* 2019).

The greater the deviation from norms, the more expensive water treatment for drinking and food processing becomes. The estimated cost of installing a treatment system for all sewage effluent was anticipated to be roughly Rs. 7,560 crores. The expense of operation and maintenance would be in addition to this (Central Pollution Control Board (CPCB), 2021). These costly treatments require ample space as well and prolonged-time, which are the major reason for the failure of this process. There is a need of some alternative solutions for these issues. Finding a new natural and cost-effective treatment can break these water pollution-related issues. Thus, for maintaining the sewage water quality, there is need to conduct studies related to characterization of sewage water and determining its quality parameters such as BOD, COD, DO, TSS, TDS, alkalinity, total nitrogen content, total phosphate, calcium and magnesium content, pH, heavy metal content and total coliform level etc. Studies related to their impact on ecosystem and their treatment using the economically feasible and efficient technique are also needed of the hour (Chopra *et al.,* 2021). Standard values for these parameters according to ENVIS (2021) and CPCB (2021) are given in Table 1.

Sewage water aspects (physical, chemical and biological)

Physical attributes of wastewater include total suspended solid, total dissolved solid, colour, temperature, turbidity and odor. The temperature usually remains higher than surface water due to the natural biodegradation process. The colour of freshly created sewage is light grey or brown, but it darkens after a considerable bacterial breakdown. The main component of sewage water is hydrogen sulphide, although additional compounds such as skatol, indol, cadaverin, and mercaptan, which are produced in anaerobic conditions or found in the effluents of pulp and paper mills, can also cause an unpleasant odour in sewage water (Woldeamanuale, 2017). Venkatesh *et al.* (2009); and Velusamy and Kannan (2016) observed the pH value of the sewage water that was somewhat alkaline, the presence of dissolved salts and particles in the sewage

Table 1. Permissible limit of important physio-chemical parameters of sewage water prescribed by ENVIS (2021) and CPCB (2021)

S. No.	Water parameter	Permissible Concentration of sewage discharge	Desirable concentration of sewage discharge
1.	Suspended solids mg L^{-1}, max.	600	300
2.	pH value	5.5-9.0	7-8
3.	Total residual chlorine, mg L^{-1}max	50	20
4.	Ammonical nitrogen, mg L^{-1}, max	50	20
5.	BOD (3 days at 20˚C) mg L^{-1}, max	350	
6.	COD, mg L^{-1}, max		250
7.	Arsenic mg L^{-1}	0.2	
8.	Mercury, mg L^{-1}, max	0.01	
9.	Phenolic compounds mg L^{-1}, max.	5.0	
10.	Cadmium mg L^{-1}, max.	2	1
11.	Cyanide mg L^{-1}, max.	2.0	0.1
12.	Lead mg L^{-1}, max.	0.1	
13	Fecal Coliform (FC) (Most Probable Number per 100 milliliter, MPN/100ml	1000	500

caused it to be electrically conductive. Turbidity in wastewater was created by unclean dishwater, bathroom water, paper, vegetable skins, and fruit skins etc. Carbonates, calcium and magnesium bicarbonates derived from kitchen wastewater contributed to the alkalinity. Proteins in food wastes were used to produce ammonical nitrogen and TKN (Total Kjeldahl Nitrogen). Kitchen wastewater and urinal waste were responsible for making phosphate. BOD and COD were generated by organic materials in sewage, primarily in the form of food waste.

Chemically, the sewage water has high alkalinity, BOD, COD, calcium, magnesium and heavy metal content (Woldeamanuale, 2017). Carbohydrates, lignin, lipids, soaps, synthetic detergents, proteins, and their metabolic byproducts are among the organic compounds found in sewage. Inorganic substances include arsenic, cadmium, chromium, copper, lead, mercury, zinc, etc. The composition of sewage water varies with the seasons, with nutrients such as total nitrogen, phosphates, and salts such as nitrates, chlorides, and sulphates being more prevalent during the pre-monsoon season and physical characteristics such as TSS being more prevalent during the post-monsoon season (Velusamy and Kannan, 2016).

Biological components of sewage water include viruses, harmful and useful bacteria, algae, protozoa and helminthes, which can be pathogenic or non-pathogenic. They use oxygen to metabolize the sewage in which they live. Mostly soil-inhabiting and intestinal bacteria are present in sewage water and their common examples are coliforms, *Streptococci, Clostridia, Micrococci, Pseudomonas,* and *Lactobacilli* (Samer, 2015; Bhatnagar *et al.,* 2017). Proteobacteria has emerged as the most common phylum (21-62%), Betaproteobacteria is the most common class found in municipal wastewater treatment plants and primarily involves organic and nutrient removal. The subdominant phyla are Acidobacteria, Bacteroidetes, and Chloroflex (Zhang *et al.,* 2014). The bacterial content of textile wwtps (Waste Water Treatment Plants) was investigated, and it was discovered that nitrifying and denitrifying bacteria, as well as phosphate-accumulating bacteria, were observed in greater abundance in municipal Wastewater treatment systems, whereas sulfate-reducing bacteria were almost exclusively found in textile Wastewater treatment systems (Meerbergen *et al.,* 2017).

Protozoa such as amoebae, ciliates, and flagellates are present throughout the sewage water treatment process. They remove superfluous bacteria while also stimulating their growth and promoting flocculation. By consuming the free bacteria, they assist to lower the turbidity of the effluent, as well as its BOD and suspended matter content (Dadrasnia *et al.,* 2017). Parasites that are commonly found in sewage include *Cryptosporidium*, *Giardia* and roundworms. Viruses like hepatitis A can also be found in sewage wastewater. Algae like *Chlorella phormidium, Ulothrix*etcare are used in trickling filters in sewage treatment plants (Samer, 2015).

Source wise composition of sewage water

Domestic sewage, industrial discharges, and run-off from farmlands are the three primary sources of water contamination. Sewage water from the household, which is often sourced from the kitchen and bathroom, is composed of yellow water, i.e., human urine, brown water, i.e., human faeces with flushed water and grey water, which is water from sinks, showers, laundry etc.

Agricultural wastewater is mainly composed of inorganic salts, crop residue, pesticides, herbicides, fertilizers rich in NPK content etc. (Maji *et al.,* 2020) which get drained into rivers along with surface runoff. DDT (Dichlorodiphenyltrichloroethane), Aldrin, Dieldrin, Malathion, Hexachloro Benzene, and certain other pesticides are routinely employed (Zhang *et al.,* 2018). Surface runoff from agricultural fields, dispersion from spraying, precipitation washing down, and direct sprinkling and splattering of pesticides in low-lying zones all contaminate aquatic bodies. The composition of industrial wastewater is determined by the enterprises that produce it and the nature of the raw materials they utilize. It may contain biodegradable materials or non-biodegradable materials, e.g., textile industries effluent consists of dyes natural dyestuff, gum thickener (guar) wetting agents, pH buffers and dye retardants. In the paper and pulp industry, cellulose, lignin, colouring agents, and inorganic materials such as acids, alkalis, bleaching agents, and other chemicals are frequently used. Emulsifying substances such as phosphorus, borax, alkyl benzene, sulphonate, and others can be detected in detergent of industrial effluent (Manasa and Mehta, 2020). Chemical and pharmaceutical industries often emit drugs such as endocrine disruptors that need to be treated before being discharged into subsequent water bodies (Tijani *et al.,* 2016).

Effect of sewage water discharge on the aquatic and terrestrial environment

Nitrogen and phosphorous-rich fertilizers and pesticides from agricultural runoff dumped into the sewerage system and then entering into ground water sources often nourishes algae's growth, thereby causing eutrophication (Maji *et al.,* 2020). Excess eutrophication blocks the path of light penetration in the water and leads to the death of submerged aquatic biota. Pesticides like DDT (dichloro-diphenyl-trichloroethane) reach humans through the food chain, directing them towards biomagnifications. The vast majority of them are non-biodegradable and will last for a significant length of time. Microorganisms use excessive oxygen to break down sewage, resulting in a hypoxic (oxygen-depleted) situation in the water (Singh *et al.,* 2020). Thus, aquatic organisms get surrounded by oxygen-deficient water and fail to acquire threshold oxygen for respiration.

Textile dyestuffs in wastewater, for example, devalue the aesthetic quality of water bodies, increase BOD and COD, obstruct photosynthetic activity reactions, inhibit plant growth, enter the food chain, provide recalcitrance, and accumulate in the body, and can be noxious, mutagenic, and cancerous in nature (Lellis *et al.,* 2019). Metals are introduced into the sewage due to a variety of human activities involving mining, processing, industrial, agricultural, pharmaceutical, domestic effluents and via the use of metals or substances containing metal contaminants. Heavy metals enter the aquatic food chain through two primary mechanisms: direct food and water consumption through the digestive system and non-dietary pathways such as muscles and gills (Rajeshkumar and Li, 2018). As a result, their concentrations in fish usually correspond to those found in the sediment and water of the aquatic habitat from where they are collected. Because fish are on the food web and can serve as a transfer medium to humans, they are an obvious focus for toxicant biomagnification. Epidermal carcinoma, fin/tail rotting, gill illness, hypertrophy, liver cirrhosis, and ulceration (Santos and Dos Reis Martinez, 2021) are among the alleged pollution-related ailments seen in fish. Many studies have demonstrated that polluted aquatic environments have a higher proportion of diseased fish than non-polluted aquatic habitats.

Toxic organic compounds, heavy metals, and chemical wastes can alter cellular organelles and other components, such as genomic DNA and mitochondrial DNA. These may also suppress various DNA repair enzyme, resulting in irreversible cellular damage and conformational changes that can lead to cell cycle disruption and unprogrammed death (Briffa *et al.*, 2020). Chromium ions can alter the activity of enzymes by their carboxyl and thiol groups. Aluminum ions result in the formation of superoxide radicals that are responsible for DNA damage. Copper ion catalyzes the production of reactive oxygen species (Igiri *et al.,* 2018).

Infectious diseases like typhoid, cholera, gastroenteritis, bacterial dysentery, jaundice, and amoebic dysentery are the most prominent diseases caused by contaminated sewage water in humans (Tripathi *et al.,* 2021). Table 2 shows the most common species of bacteria, fungi, protozoan and viruses found in sewage waste water and their related disease.

Conventional methods of sewage water treatment

Generally, sewage water undergoes primary, secondary and tertiary treatment. Primary treatment, also known as physical treatment, entails only separating solid refuse from sewage water. This process cannot separate the dissolved materials. Secondary treatment, such as biological treatment, can help with this. This methodology includes treating wastewater using microbes like algae, fungus, or bacteria under aerobic or anaerobic conditions, during which organic content in the wastewater is oxidized or integrated into cells that the sedimentation process may have retrieved. In tertiary treatment, chemical compounds that can react with a fraction of the xenobiotic and heavy metals, allowing

Table 2. Hazardous pathogens identified in municipal wastewater, along with diseases or symptoms linked to them (Gerba and Smith, 2005; Cui *et al*., 2019)

Pathogens	Name of the pathogen	Major disease or symptoms
Bacteria	*Vibrio cholera*	Cholera
	Salmonella spp.	Salmonellosis, typhoid
	Campylobacter jejuni	Gastroenteritis
	Shigella spp.	Bacillary dysentery
	Escherichia coli	Gastroenteritis
Viruses	Adenovirus	Respiratory infection and gastroenteritis
	Astrovirus	Gastroenteritis
	Rotavirus	Acute gastroenteritis with severe diarrhea
	Hepatitis A virus	Infectious hepatitis
	Polio virus	Poliomyelitis
	Hepatitis E virus	Infectious hepatitis, death
Protozoa	*Balantidium coli*	Balantidiasis
	Toxoplasma gondii	Toxoplasmosis
	Entamoeba histolytica	Acute amoebic dysentery
	Cryptosporidium spp.	Cryptosporidiosis
	Giardia duodenalis	Giardiasis
Helminths	*Trichuris trichiura*	Diarrhea, anemia, weight loss
	Ascaris lumbricoides	Ascariosis
	Necator americanus	Hookworm disease
	Taenia saginata	Insomnia, anorexia

for fast removal, are utilized (Patel *et al.,* 2021). The fact that xenobiotics and a considerable amount of heavy metals remain intact is a major drawback of this approach. Due to the high cost of chemical substances and the difficulties of disposing of chemical sludge, this treatment method was also less reliable. As a result, biological treatment on a large scale with changes in microbe composition is required. In essence, the microbes feed on the dissolved organic elements, minimizing the volume of sludge that will be chemically treated. As a result, greater research into effective microbial species capable of digesting hazardous chemicals is needed.

Several other physico-chemical approaches to treating domestic and industrial wastewater include adsorption, chlorination, coagulation, flocculation, solvent extraction, membrane process, etc. This can reduce the pollution load of the aquatic water bodies (Patel *et al.,* 2021). Conventional methods, on the other hand, have drawbacks such as slow and ineffective removal, the production of contaminated sludge that requires considerable disposal, high cost and energy input into the operations, membrane constriction, and the propagation of secondary pollutants that are more noxious than the parent pollutants (Shreshtha *et al.,* 2021)

***In vitro* studies on the degrading potential of the bacteria**

Bacteria contribute to the degradation process of toxins in wastewater. Therefore, different strains have been used to boost the metabolic pathway at diverse scales and disintegrate a variety of target contaminants in the same wastewater. The utilization of carbon dioxide and the generation of valuable products while cleaning up polluted surroundings (Athar *et al.,* 2022) are two of the major benefits of using these bacteria in bioremediation, which have both environmental and economic implications (Idi *et al.,* 2015). When garbage enters a treatment plant, bacteria are in charge of settling it. These bacteria work together to form floc particles, bacterial clusters that help break down waste. The floc particles also act as absorbents for garbage that will be decomposed later. Furthermore, filamentous bacteria create trichomes, or chain-like filaments that serve as a backbone for floc particles grow in size, and endure catabolic action during the treatment process. The first component to encounter metallic ions or dissolved substances present in wastewater is the bacterial cell wall, which concentrates them on the surface or within the cell wall structure (Nanda *et al.,* 2019).

Many different bacterial strains have been found for their versatile biodegradation capacity based on their target substrate. Several reports on the *in vitro* studies regarding the treatment of wastewater with autochthonous bacteria, mixed bacterial culture and commercial bacterial strains have been presented in this review.

Isolation and identification of bacteria possessing biodegrading capacity from sewage water samples

The serial dilution agar plate method was used to isolate bacterial strains from wastewater (Hesnawi *et al.,* 2014). The water sample suspension was made by diluting 1 mL of the sample in 9 mL of sterilized distilled water. The diluted water sample was spread on a Petri dish containing nutrient agar medium in aseptic conditions. The Petri plates were then incubated for 24-36 hours at 37˚C. On agar plates, several colonies emerged. The streaking procedure was used to obtain the pure colony, in which a single established colony was subcultured on a fresh agar plate (Nadeem *et al.,* 2021). To prevent isolation of the same bacterial isolates several times, colonies with comparable culture properties were acquired by repeatedly streaking diluted solutions from isolated bacterial colonies (Ahsan and Lin, 2021). On nutritional agar slants, the purified bacterial isolates were kept at 4°C. All bacterial isolates were subcultured after a 30-day interval. Fig. 1 outlines the steps for isolating bacteria from the water sample.

The identification of bacteria was made by morphological tests such as gram staining, biochemical tests such as amylase, catalase, citrate, gelatin, methyl red, etc. Using 16S rDNA based Molecular Technique, bacterial strains were further identified (Nadeem *et al.,* 2021). Based on the morphology of the bacteria and the staining attributes (Gram-positive produces purple colour, Gram-negative gives pink or red colour), a Gram stain aids in the identification of bacteria or offers an indication of the type of bacteria present (Saha and Santra, 2014). A Gram stain may also be used to identify bacterium combinations, determine which agar plates to utilize for subsequent cultures, and evaluate culture findings (Lee *et al.,* 2018). Biochemical tests are used to determine sugar consumption, amino acid decarboxylation, catalase, protease, and oxidase production, nitrate reduction, hydrogen sulphide formation, and starch, casein, and urea hydrolysis (Saha and Santra, 2014). According to Bergey's Manual of Systematic Bacteriology (2012), these tests were used to identify the bacterium strains.

Bacteria that were to be isolated should possess a large yield, able to secrete an ample amount of protease and bear antimicrobial activity (Zhang *et al.,* 2015). Protease-producing bacteria are major participants in the natural nitrogen breakdown and nutrient recycling of the aquatic environment. Proteases are enzymes that dissolve the peptide bonds that connect amino acid residues to break down massive protein chains into smaller components (Josephine *et al.,* 2012). The bacteria were streaked over casein hydrolyzed media and cultured for 24 hours at 35˚C. A clear zone surrounding the growth suggests that the strains are proteolytically active (Ethica *et al.,* 2018). There was a discernible difference in the size of the clearing zone for proteolytic activity.

A slight modification to the agar-overlay technique was used to assess the antipathogenic activity of isolated bacteria by Asagabaldan *et al.* (2017). These bacteria isolates were inoculated on an agar medium and incubated for 18–20 hours at 37°C, the optimal temperature for bacteria development. Over the isolated strains, 3.5 ml of soft agar was gently coated with a pathogenic strain (*Escherichia coli*). A freshly prepared culture of pathogenic bacteria strain (0.45 in OD_{600}) was inoculated in 100 ml soft agar (0.75 % agar) and mixing it thoroughly to make the soft agar. The overlay plates were then incubated for 24 hours at 37˚C, and the zone of inhibition (measured from the colony's edge to the clear zone's edge) was recorded. The disc-diffusion method (Swenson *et al.,* 2004) was used to test the antimicrobial susceptibility of *Acinetobacter* isolates, in which agar plates were inoculated with bacteria whose antimicrobial property was to be carried out, and then a disc of filter paper containing the test microorganism or compound whose antimicrobial activity was to be carried out and left for incubation at favorable environment so that antimicrobial agent diffuse into agar plate and inhibit the growth of the test organism and the presence of a zone of inhibition would indicate that test organism possess the antimicrobial activity.

Mechanism of pollutants degradation by bacteria

Bacteria employ several mechanisms to revamp pollutants into non-harmful products, namely biosorption, bioaccumulation, bio leaching, biomineralization and

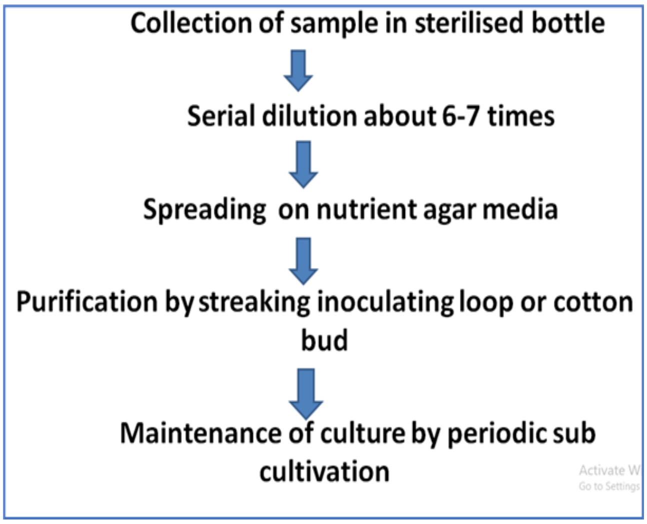

Fig. 1. *Steps for isolation of bacteria from the water sample*

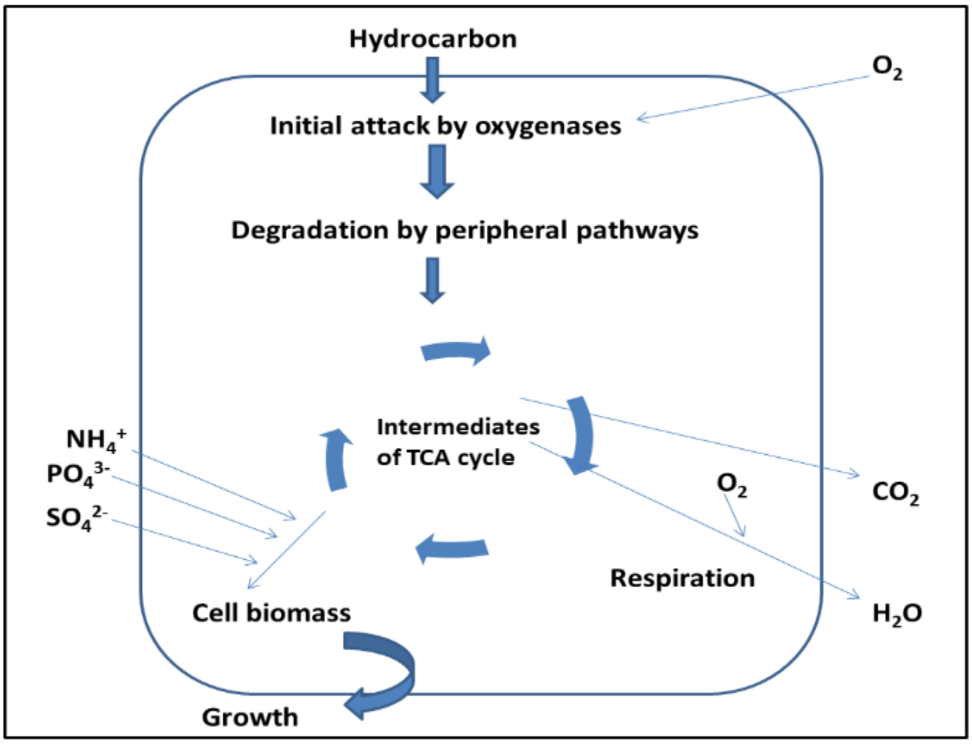

Fig. 2. *Main principle of aerobic degradation of hydrocarbons by microorganisms (Das and Chandaran, 2011; Mabachu et al., 2020)*

enzyme-linked biotransformation (Begum *et al.,* 2021). Bacteria can eliminate harmful metal ions by permitting activities in enzymatic and nonenzymatic systems that need energy from redox reactions. Silver (1996) investigated two pathways involved in the emergence of resistance in bacteria. One is detoxification, in which poisonous metals are revamped into less toxic forms, and the other is actively extrusion, in which toxic metal ions are pumped out of the cell. Through the biosorption approach, heavy metals interact with binding sites in the cellular structure of bacteria. Extracellular Polymeric Substances (EPS) found in bacterial cell walls have essential metal adsorption components, including synthesising complexes via specialized processes for metal precipitation (Zeng *et al.,* 2020). The biosorption of these solutes is mediated by a variety of functional groups found on the cell wall of bacteria, including amine, hydroxyl, carboxyl and phosphonate groups. These functional groups are negatively charged and ubiquitous. Therefore, they actively engage in the binding of positively charged cations. Several dye compounds that reside in wastewater as dye cations are also drawn to carboxyl and other negatively charged functional groups (Vijayaraghavan and Yun, 2008). *Synechococcus sp*. has been reported to accumulate heavy metals through the production of metal-binding proteins and peptides, with the smtA gene revealed to be responsible (Vashishth *et al.,* 2019).

Heavy metals are oxidised by bacteria, which give up electrons and then it gets absorbed by electron acceptors such as nitrate, sulphate, and ferric oxides. In aerobic circumstances, oxygen operates as an electron acceptor, whereas in anaerobic contexts, it oxidises inorganic pollutants. The catalysis of intracellular enzymes is primarily responsible for the breakdown of organic pollutants by microorganisms. Various enzymes involved in degradation of pollutants, their substrate and the reaction involved are given in Table 3.

During these procedures, organic pollutants are completely mineralized into a carbon compound. Oxidation is the first intracellular attack on hydrocarbons, with oxygen incorporation being the key enzymatic step mediated by oxygenases and peroxidases (Fig. 2). Organic pollutants are transformed into central intermediary metabolic precursors, such as the tricarboxylic acid cycle intermediates, using peripheral degradation mechanisms one step at a time (Mbachu *et al.,* 2020). These essential precursor metabolites, including acetyl-CoA, succinate, and pyruvate, are required for cell biomass production (Das and Chandaran, 2011). Biphenyl dioxygenase, 2, 3-dihydroxybihenyl dioxygenase, dihydrodiol dehydrogenase and hydrolase are some of the additional enzymes involved in PCB (polychlorinated biphenyl) breakdown (Jariyal *et al.,* 2020).

Role of autochthonous bacteria in sewage water treatment

Natural microorganisms in wastewater play a vital role in wastewater treatment (Adebayo and Obiekezie, 2018). Many diverse organisms live inside the

Table 3. Various microbial enzymes, their substrate and mechanism involved (Karigar and Rao, 2011; Bhandari *et al.,* 2021)

Sr. no.	Enzyme	Substrate	Reaction
1.	Monooxygenases	Saturated hydrocarbon, steroids, fatty acids and aromatic compounds	Desulfurization, dehalogenation, denitrification, and ammonification are all processes that include the incorporation of an oxygen atom into a substrate and the use of the substrate as a reducing agent.
2.	Dioxygenases	Aromatic compounds	Intradiol cleaving and extra diol cleaving occurs when two oxygen atoms are introduced to the substrate, yielding an aliphatic product.
3.	Laccase	Ortho and paradiphenols, aryldiamines, aminophenols, polyphenols and lignins	Oxidation and decarboxylation of substrate.
4.	Lignin peroxidase	Halogenated and aromatic compounds	Substrate level oxidation in the presence of co-substrate H_2O_2 and mediator like veratryl alcohol.
5.	Lipase	Organic pollutants such as oil spill	Breakdown of triacylglycerol to glycerols and free fatty acids.
6.	Cellulase	Cellulosic substance	Metabolize the complex substrate to simple carbohydrates
7.	Protease	Proteins	Enzymes that breakdown and hydrolyze peptide bonds of protein in aqueous environment
8	Alkaline serine proteases	Proteins present in pharmaceutical effluent, detergents etc.	Enzymes that breakdown and hydrolyze peptide bonds of protein in aqueous environment

wastewater itself, known as autochthonous microorganisms, because they aid in the decomposition of specific organic substances. Bioaugmentation with one or more species of specialised microorganisms is frequently used to improve the performance of biological processes (Raper *et al.,* 2018). When a population of a particular species is present in lower concentrations and can be cultivated under laboratory conditions, this strategy can be used. It can also increase the success rate since that species is isolated from that location and has already acclimated to that habitat.

Degradation potential of some autochthonous bacteria such as *Micrococcus luteus* and *Staphylococcus aureus* were evaluated in which these were isolated from the sewage effluent and were found to be efficient degrader of the components in the sewage and therefore could be efficiently be employed for the treatment of sewage water (Sinha and Paul, 2014). Similarly, Li *et al.* (2018) isolated three bacteria strains from a coking wastewater treatment plant, later identifying them as *B. cereus, P. synxantha,* and *P. pseudoaligenes*, all of which have high dehydrogenase activity, indicating a strong ability to degrade the organic matter. All three strains exhibited a strong ability to degrade naphthalene, which is commonly present in coking effluent. Other complex chemical compounds, such as phosphoric acid triphenyl ester and 4H-1-benzopyran-4-one and the generation of simpler compounds like alcohols and aliphatic hydrocarbons like 1-heptacosanol, docosane, and hexadecane, may be successfully removed from wastewater by *Serratia sp.* (Gupta and Thakur, 2015). Similarly, removal of synthetic nitrogen was studied by Medhi *et al*. (2019) in *Paracoccus denitrificans* ISTOD1 in the formed ammonia from synthetic water. The addition of *Acinetobacter sp.* and *Rhodococcus sp.* may effectively eliminate phenolic chemicals such as 2-methoxyphenol, 4-chlorophenol, 2, 4-dichlorophenol, and pentachlorophenol (Paisio *et al.,* 2014). *P. stutzeri* N2 and *Rhodococcus qingshengii* FF might be effective biotechnological tools for the efficient degradation and mineralization of diverse effluents' phenolic compounds showed a broad range of adaptability in detoxifying these complicated matrices, even when supplemented with high phenol concentrations (Bai *et al.,* 2021).

Bacillus was found to be the most dominant bacterial genus in sewage sludge, accounting for 69 % of the bacterial population (Niu and Li, 2022). *B. subtilis, B. licheniformis, B. cereus, B. coagulans,* and members of the genus *Phenibacillus,* such as *P. polymyxa*, are notable examples of bacteria appropriate for bioremediation of organic detritus. However, because their typical home is silt, they are not generally present at the appropriate levels in the water column. Kafilzadeh *et al.* (2011) discovered *Bacillus*, and other bacterial species, including *Corynebacterium, Staphylococcus, Streptococcus, Klebsiella, Escherichia, Acinetobacter, Alcaligenes, Shigella,* and *Enterobacter*, for eliminating crude oil pollution from polluted cities in the world. Biphenyl, naphthalene, camphor, and phenanthrene may all be broken down by these microorganisms. The majority of research has focused on isolating gram-negative aerobic bacteria from the genera *Ralstonia, Pseudomonas, Sphingomonas,* and *Achromobacter.* Gram-positive bacteria, including *Bacillus, Microbacterium, Janibacter, Rhodococcus,* and *Paenibacillus* have also been proven to be successful in PCB breakdown on polluted locations in various studies (Xiang *et al.,* 2020). *Dehalobacter*, *Dehalococcoides*, and *Desulfitobacterium* are potent PCB degraders during anaerobic conditions (Khalid *et al.,* 2021).

Heavy metal content in wastewater has increased dramatically in last few years due to metal plating facilities, mining activities, fertilizer industries, batteries, paper industries etc. Heavy metals are persistent in nature and can be toxic or carcinogenic. Bacteria have employed metals as terminal electron acceptors in anaerobic respiration. Additionally, bacteria may have metal-resistance-inducing reduction mechanisms apart from respiration. For example, Cr(VI) can be reduced to Cr (III) in aerobic or anaerobic conditions, Se(VI) to elemental Se, U(VI) to U(IV), and Hg(II) to Hg(III) under anaerobic conditions (0). Heavy metal ions can be captured by biosorbing them onto binding sites in the cellular structure. They can penetrate through the cell membrane and enter the cell during the metabolic cycle. Bacterial species such as *P. fluorescens* strain was found to be resistant to some of the major heavy metals and chemical compound namely $Cd^{2+,}$ Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, BHC (Benzene hexachloride), 2,4-D (2, 4-Dichlorophenoxyacetic acid) and phenols (Khan and Ahmad, 2006). As a result, this strain appears to have a high capability for detoxifying these heavy metal contaminants. *Pseudomonas sp.* was also used in effectively treating wastewater effluent from rubber processing industry (Shruthi *et al.,* 2012) and has the potential to degrade fluorine and phenanthrene derived from the effluent of the chemical industry (Ma *et al.,* 2012). As a result, *Pseudomonas* is the most effective bacterial genus for hazardous chemical disintegration. The capability of these bacteria to break down these chemicals is dependent on the amount of time they have to encounter the drug, the environment in which they grow, and their physiological adaptability.

Polychlorobiphenyls (PCBs) and chlorinated herbicides (e.g s-triazines) are persistent organic pollutants (POPs) that are widely disseminated in the environment. These are deadly compounds that can cause cancer and function as endocrine disruptors. Seeger *et al.* (2010) identified a variety of bacteria capable of degrading s-triazines and PCBs. Anaerobic and aerobic bacteria can convert PCBs to non-toxic compounds by hydrolytic processes mediated by aminohydrolases, which are encoded by the atz genes. Anaerobic bacteria dehalogenate higher chlorinated PCBs through reductive dehalogenation. Aerobic bacteria oxidise lower chlorinated biphenyls. Genome studies of PCB-degrading bacteria have improved our understanding of their metabolic capacities and stress tolerance. Malathion one of the widely used organophosphorus insecticides globally, was eliminated by *Azospirillum lipoferumis,* a free living nitrogen fixer (Kanade *et al.*, 2012).

It is important to highlight the various bacterial communities that can be found within effluents of industries. Many researchers determined the bacterial community from various industrial effluents. Table 4 includes the studies done by various researchers on the role of autochthonous bacteria in biodegrading pollutants.

Table 4. Different isolated bacterial strains and their target substrate

Bacterial strain	**Target Pollutant**	**Reference**
Pseudomonas fluorescens	Cr^{6+}, Ni^{2+}, $Cd^{2+,}$ Pb^{2+}, Cu^{2+}, BHC, 2,4-D and phenols	Khan and Ahmad (2006)
Acinetobacter johnsoni and *Pseudomonas beteli*	Sodium dodecyl sulphate	Hosseini *et al.* (2007)
Rhodobacter sphaeroides	Organic compounds	Madukasi *et al*. (2010)
Escherichia, Bacillus, Corynebacterium, Staphylococcus, Klebsiella, Acinetobacter, Shigella. Streptococcus, Enterobacter, Alcaligenes	Biphenyl, Naphthalene, Camphor and Phenenthrene	Kafilzadeh *et al.* (2011)
Azospirillum lipoferum	Malathion	Kanade *et al.* (2012)
Acinetobacter sp. and *Rhodococcus sp.*	Phenolics compounds (2,4-dichlorophenol and pentachlorophenol, 2-methoxyphenol)	Paisio *et al.* (2014)
Bacillus cereus, Pseudomonas pseudoaligenes and *Pseudomonas synxantha*	Naphthalene	Li *et al.* (2018)

Role of mixed bacterial culture in treatment of sewage water

A microbial consortium is a group of two or more microbial or bacterial species that live together in a symbiotic relationship. In contrast to degradation operations that employ a single bacterial culture, microbial consortia in biodegradation processes have expanded substantially due to their reported synergistic metabolism, which boosts the degeneration efficiency of hydrocarbons and other chemicals (Kumar *et al.,* 2013). The use of consortia accelerates and improves the biodegradation process for a variety of reasons: i) another bacteria can utilized the metabolic intermediate formed by one bacteria as an energy source (Forgacs *et al.,* 2004) ii) From a bioremediation standpoint, the process becomes significantly quicker, and iii) full degradation as well as degradation of a variety of compounds may be achieved. Chen *et al.* (2009) compared the usage of consortium and pure bacteria culture. They tested single strain and bacterial consortium treatments on municipal wastewater and discovered that bioaugmentation with three different strains of bacteria such as *E. cloacae, Gordonia* and *P. putida* was more successful at removing total organic carbon than single strain treatments. Dhall *et al*. (2012) constructed sewage wastewater treatment consortia using isolated *B. pumilus, Brevibacterium sp,* and *P. aeruginosa*. COD (chemical oxygen demand), BOD (biochemical oxygen demand), MLSS (mixed liquid suspended solids), and all decreased dramatically after treatment. Consequently, they concluded that forming such consortia can assist sewage treatment plants overcome the inefficiencies of current biological treatment centers (Bhatt *et al.,* 2021).

Bacterial consortiums can dramatically reduce phosphate and nitrate levels in wastewater. A bacterial consortium comprised of *Bacillus sp, Pseudomonas sp*, and *Enterobacter sp* recovered phosphate from a synthesized phosphate solution and MSM (mineral salt medium) (Krishnaswamy *et al.,* 2009). Some of the most prevalent nitrate-reducing bacteria are *Pseudomonas, Bacillus, Micrococcus*, and *Alcaligenes.* Consequently, researchers determined that a bacterial consortium made up of *Pseudomonas sp*. and *Bacillus sp.* was successful with in decreasing nitrate. A bacterial consortium composed of *Pediococcus acidilactici*, *P. pentosaceus*, *L. planta*, and *B. subtilis* removes high % of COD, BOD, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), ammonia, nitrate, total Kjeldahl nitrogen (TKN), oil, and grease from domestic wastewater (Bilen Ozyurek and Seyis Bilkay, 2020). In contrast to using a bacterial consortium, using a pure culture has a number of advantages, including predictable bacterial action and detailed knowledge of degradation pathways, as well as increased assurance that biodegradation of pollutants will result in environmentally safe end products under a given array of external conditions (Ghosh *et al.,* 2016). Table 5 represents the various studies on the bacterial consortium and their bioremediation effect.

Table 5 Study on various bacterial consortiums and their effect in wastewater.

Bacterial consortium	**Bioremediation Effect**	**Reference**
Enterobacter cloacae, Gordonia and Psuedomonas putida	Reduced total organic carbon	Chen *et al.*(2009)
Bacillus pumilus, Brevibacteriumsp, and *Pseudomonas aeruginosa*	Reduced COD, BOD, TSS, and MLSS	Dhall *et al.* (2012)
Bacillus sp, *Pseudomonas sp* and *Enterobacter sp*	Reduced the phosphate content	Krishnaswamy *et al.* (2009)
P. aeruginosa LP$_{602}$, *Bacillus sp*. B$_{304}$ and *A. calcoaceticus*	Lipid content reduction	Mongkolthanaruk and Dharmsthiti (2002)
Pseudomonas sp. and *Bacillus sp.*	Nitrate reduction	Rajkumar *et al.* (2008)
P.acidilactici, Pediococcus pentosaceus, Lactobacillus plantarum, and *Bacillus subtilis*	Reduced BOD, COD, TS, TSS, TDS, TKN	Ibrahim *et al.,* (2020)

Sewage water treatment by commercial bacteria

The use of indigenous bacterial strains for treating waste water has several detriments as these are ineffectual in completely eliminating each type of contaminant present in the wastewater. Thus, there was a need to formulate such a consortium that contained selected species based on their target pollutant and degrading potential as well as affirmative for the particular physiochemical characteristics of the wastewater. Commercially available bacterial inoculums are now widely used (Bhatt *et al.,* 2021). These commercial consortiums vary in makeup, cell density, bacterial strains combinations, advised dosing rate, stabilizers, nutrients, etc. These factors must be considered while formulating inoculums. Commercial inoculums have been shown to provide a short-term solution to an acute treatment problem; however, because such inoculums are manufactured and tested under controlled laboratory circumstances. Such settings do not mirror the normal situation for wastewater from various sources, decreasing the inoculums' capacity to survive in that new environment.

A commercially available bacterial consortium contains specific species of bacteria that can degrade specific chemical compounds (Uma and Gandhimathi, 2019). As a result, it has been demonstrated that formed mixed culture seems to be more efficacious in wastewater treatment and might even overcome the constraint of partial degradation (Feng *et al.,* 2021). Hesnawi *et al.* (2014) compared the potential of commercially available SludgeHammer and some local isolated strains in treating the degradation of synthetic and real municipal wastewater. TOC (total organic carbon) removal efficiency in synthetic wastewater for the SludgeHammer, *B. subtilis, B. laterosponus*, and *P. aeruginosa* were 54 %, 52 %, and 42%, respectively. When compared to other strains, TOC breakdown studies using a blended bacterial culture of *B. subitlis* and *P. aeruginosa* strains enhanced treatment efficiency by 6 to 16%. They took longer to reach the maximum breakdown rate than the SludgeHammer bacteria. From a commercially available bacterial culture, *Pseudomonas aeruginosa* LP602, *Bacillus sp.* B304, and *Acinetobacter calcoaceticus* LP009 were used. Protease and amylase were produced by B304, while lipases were produced by LP602 and LP009, lowering BOD and lipid content (Mongkolthanaruk and Dharmsthiti, 2002).

Factors affecting the biodegradation potential of bacteria

When the environmental circumstances are ideal for bacteria to work, the outcome of each degradation process and the biodegradation rate are at their highest. As a result, before the remediation procedure can begin, it is vital to get information about the biotic and abiotic attributes of the contaminated areas. Basically, three types of factors that influence bacterial degradation include physiological factors (pH, temperature, solubility, nutrient availability, incubation period) (Luka *et al.,* 2018), biological factors (population density of bacterial species, types of bacterial species, consortium) and environmental factors (oxygen level, CO_2 level). Lack of knowledge regarding the factors influencing the bioremediation process can often reduce the efficiency of the process whenever implemented. Microbes possess the inherent ability to adapt according to the environment but have certain limitations. Bioaugmentation fails due to low inoculum survival and/or competition with indigenous microbial populations if factors inside the treatment process are not understood. These characteristics influence the acclimatization duration of microorganisms to the substrate.

Nutrient availability

One of the most significant elements influencing bacterial activity is the capacity and accessibility of reduced organic matter to serve as source of energy. The average oxidised state of the carbon in the substance determines whether a contaminant will serve as an appropriate energy source for an aerobic heterotrophic cell. Lower energy outputs are associated with higher oxidation states, reducing the energetic motivation for microbial breakdown (Naik and Duraphe, 2012). For cellular metabolism, bacteria require additional elements such as nitrogen, potassium, phosphorus, and carbon (Luka *et al.,* 2018). Due to quick metabolism, wastewater with high levels of organic pollutants frequently has low levels of mineral nutrients. Thus, these sites should be treated with nitrogen and phosphorus to speed up bioremediation (Sihag *et al.,* 2014). The addition of other nutrients and sufficient oxygen to the biostimulation process aids indigenous microorganisms in producing the required enzymes to break down contaminants.

Temperature

Temperature significantly impacts the microorganism's ability to degrade (Saxena *et al.,* 2020). The ideal temperature for the metabolic cycle of bacterial enzymes involved in the degradation process is not the same at all temperatures. Indeed, biodegradation rate is approximately halved for every 10°C reductions in temperature. The bulk of oil-degrading bacteria is active in the mesothermic range from 20 - 35 °C, where they provide superior degradation. Because bacterial enzymes may denature at these temperatures, much higher temperatures can also limit the rate of disintegration (Sihag *et al.,* 2014). The rate of disintegration in cold water is generally slower than in warm water.

pH

Biodegradation could occur at any pH, although in most aquatic and terrestrial systems, a pH of 6.5 to 8.5 is optimal for biodegradation (Saxena *et al.,* 2020). Many locations have pH levels that are not ideal for bioremediation. *Pseudomonas spp.* can thrive at temperatures of 27°C and 37°C in a pH range of 4-10; however, pH 8 and 37°C was optimum pH and temperature (Monica *et al.,* 2011). The pH of a microbiological solution is a crucial factor to consider while preparing it. Biosorption is a pH-dependent process in which the isoelectric point of a solution is influenced by the negatively charged ligands (carboxyl, phosphoryl, and amino acid groups) on the microbial cell surface. Metals with higher oxidation states are soluble and must be transformed by the bacterium to an insoluble form. This process is pH-dependent as well. As a result, the metal ions must attach to the microbial cell surface at a lower pH.

Biological factors

It has been observed that microbes, especially bacteria, often possess different scents for different substrates due to the presence of plasmid-encoded genes that encode the specific enzymes. Single bacteria can not completely degrade the contaminants. Various intermediated products are produced, which further break down during the biodegradation process by other species of bacteria. Diversified bacteria or bacterial communities can lead to complete biodegradation rather than single species of bacteria. Other biological interactions such as competition between indigenous and foreign bacteria for limited carbon sources, antagonistic interactions, and protozoa and bacteriophage predation also contribute to the bioaugmentation outcomes.

Oxygen

Dissolved molecular oxygen is required for microorganisms throughout the degradation process (Saxena *et al.,* 2020). It is one of the basic requirements for biodegradation. Degrading 1 ml of hydrocarbons to carbon dioxide and water usually requires 3-4 ml of dissolved oxygen. The amount of oxygen accessible in the system determines whether it is aerobic or anaerobic (Sihag *et al.,* 2014). *Mycobacterium, Pseudomonas, Alcaligenes, Rhodococcus*, and *Sphingomonas* are examples of aerobic bacteria well known for their degrading ability. Pesticides and hydrocarbons, including alkanes and polyaromatic chemicals, are degraded by these bacteria on several occasions. Under aerobic circumstances, hydrocarbons are easily destroyed (Saxena *et al.,* 2020), but chlorinated compounds are only decomposed under anaerobic conditions. Many of these bacteria rely only on the pollutant for carbon and energy. Anaerobic bacteria are less common than aerobic bacteria. Anaerobic bacteria are being widely utilized as bioremediation of PCBs in river sediments and for dechlorination of chloroform and trichloroethylene (TCE).

Conclusion

Various sources of wastewater generate tons of sewage water per day, of which only 70% are treated via sewage treatment plants. Sewage water comprises high BOD, COD and heavy metal content. Various chemical components such as pesticides, fertilizers, and detergents are also present. These contaminants are noxious, mutagenic, and cancerous in nature. Biological components of sewage water include viruses, harmful and useful bacteria, algae, protozoa and Helminthes, which can be pathogenic or non-pathogenic. Useful Microbes such as bacteria play a significant role in healing our ecosystems and the sustainability of environmental cycles throughout the bioremediation process. The majority of contaminants can be easily eliminated by using microorganisms. A significant amount of waste water may be purified in a short span of time. Researchers have used a variety of microbes to purify sewage water so far. These microorganisms can be found in nature or bought commercially. They can also form a part of a single strain or consortia of strains. *P. fluorescens*, *Bacillus sp., Acinetobacter sp.* and *Rhodococcus sp* are examples of autochthonous bacteria resistant to varied toxicants. These bacteria possess the protease producing capacity, hence used to degrade sewage water's organic and inorganic content. These bacteria can be inoculated in pure culture or in consortia based on the requirements. Although the biodegradation efficiency of these bacterial strains has been assessed *in vitro*, several limiting variables might stymie the process in the natural environment, such as nutrient availability, presence of other microorganisms, oxygen content, optimum temperature and pH. Thus the selection of bacterial strains based on their unique enzyme-producing capabilities, on the other hand, can be effective in wastewater treatment. At the same time, suitable environmental conditions should be maintained.

Future prospective

A substantial amount of sewage water is generated daily due to human and inhuman activities. This wastewater directly affects the biotic communities and thus decries the quality of life on earth. Microorganisms such as bacteria have been evaluated for their potential to decay these pollutants from water many years ago. Although proteolytic enzymes producing bacteria contribute to the breakdown of organic nitrogen and the recycling of nitrogen in marine sediments, little is understood regarding their diversity and extracellular proteases production. In addition, there is a scarcity of information on the factors that influence and control the speed of biodegradation and the development of an effective bacterial consortium for treating sewage water before it is discharged into fresh water bodies. Thus, there is a need to isolate further and investigate a more significant number of bacteria that possess a high degrading capacity and show resistance to xenobiotics. Also, there is a call to evaluate their synergistic effect with other strains on the catabolism of pollutants. Researchers are now focusing on developing new strategies to enhance the participation of bacteria in the wastewater treatment process. Further studies can be done to investigate the effect of using these bacteria beyond the laboratory scale in the natural environment.

ACKNOWLEDGEMENTS

The authors are grateful to Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0) for support via the Centre for Applied Biology in Environment Science (CABES), Kurukshetra University, Kurukshetra.

Conflict of interest

The authors declare that they have no conflict of interest.

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