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Analyzing Enzymatic Potential of Bacteria from Wastewater Samples to Evaluate their Role in Bioremediation

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Abstract

Bioremediation is a durable and friendly method of eliminating the pollutants in the environment based on the metabolism of microorganisms. Wastewater ecosystems have diverse microbial communities, which can produce extracellular enzymes that catalyse the degradation, transformation and detoxification of multifaceted organic and inorganic contaminants. To find the enzymatic potential of indigenous bacterial populations, the wastewater samples were collected from various parts of Maharashtra, India, namely Satara, Sangli, Solapur, Karad, and other related sub-sites. The samples were screened for significant hydrolytic and metabolic enzymes involved in bioremediation reaction such as gelatinase, lipase, urease, protease, proteolytic activity, citrase, amylase, and for production of hydrogen sulfide. Enzyme activity was assessed on the basis of standard microbiological and biochemical assays by using substrate selective media and indicator-based detection systems. Most wastewaters samples showed great enzyme activities in various assays, which showed that the microbial consortia had a high functional diversity. All the regions showed the consistent presence of gelatinase, lipase, urease, protease and amylase activities. The activity of citrase was not observed in few samples of Sangli and Solapur which indicated the regional differences in the metabolic processes. Protease activity was measured quantitatively by spectrophotometry analysis which indicated that the enzyme levels were comparatively higher for Sangli sites and lower for Satara and Satara-Solar. These results indicate that the bacteria found in wastewater produce a variety of enzymes that are required for the disintegration of proteins, lipids, polysaccharides, and nitrogenous substances. The enzymatic diversity herein points out the inherent bioremediation potential of wastewater microbiota and points out the application of the microbiota in the sustainable approaches of environmental management.

Keywords: Bioremediation; Protease; Amylase; Gelatinase; Urease

Introduction

Wastewater ecosystems have diverse microbial communities, which can produce extracellular enzymes that catalyse the degradation, transformation and detoxification of multifaceted organic and inorganic contaminants (Bedekar et al., 2014, Jadhav et al., 2024). To find the enzymatic potential of indigenous bacterial populations, the wastewater samples were collected from various parts of Maharashtra, India, namely Satara, Sangali, Solapur, Karad, and other related sub-sites. (Masurkar et al., 2023, Bhargav et al., 2019). Exponential increase in human population, industrialization, urbanization and intensive farming activities have significantly risen the amount and intricacy of the wastewater that is discharged to natural habitats (Kato and Kansha 2024, Lin et al., 2022, Sathya et al., 2022). The secrete of domestic effluents, industrial waste, hospital and agricultural runoffs contribute to a broad range of organic and inorganic pollutants in water. Such pollutants are proteins, fats, carbohydrates, detergents, phenolic compounds, heavy metals, pesticides and petroleum-related hydrocarbons most of which are toxic, persistent and can undergo bioaccumulation (Akpor et al., 2011; Ali et al., 2019). When unmanaged, these pollutants interfere with aquatic life, decrease the quality of water, enhance the process of eutrophication, and create significant threats to human and animal lives (Varjani, 2017).

The traditional wastewater treatment methods are hinged on physical and chemical treatment that includes coagulation, filtration, adsorption, oxidation and sedimentation (Molinos-Senate et al., 2010). Although the methods are effective in minimizing the pollutant load, they are commonly either costly, energy-consuming and pollute the environment (Gregorio et al., 2018; Tak et al., 2006). In addition to that, most physicochemical methods produce a secondary waste as sludge or chemical residues which have to be further processed and disposed of. Such restrictions have led to the quest of alternatives of pollution control that are sustainable, less costly, and do not harm the environment (Fu et al., 2011).

Bioremediation has come out as a plausible alternative that takes advantage of natural metabolic processes of microorganisms to break down, remodel or even de-toxify toxic substances (Bhargav et al., 2019). The biochemical synthesis is amazingly versatile in microorganisms and they can use complex organic compounds to get carbon, nitrogen and energy. This ability is mostly mediated by both extracellular and intracellular forms of enzymes that catalyze hydrolytic, oxidative and reductive reactions (Shah and Maulin 2014; Karigar et al., 2011). Microorganisms transform complex pollutants into simpler and less toxic products which are usually mineralized through enzymatic activity e.g. carbon dioxide, water, ammonia as well as inorganic salts. Hydrolytic enzymes are among other enzymes that are used in the process of biodegradation and they are central in the initial breakdown of macromolecules. Proteins are broken down into peptides and amino acid by proteases and gelatinases; fat and oil are broken down into fatty acids and glycerol by lipases; starch is broken down into simple sugars by amylases; urease breaks urea down to ammonia and carbon dioxide; and an enzyme called citrases allows the utilization of citrate as a source of carbon (Gianfreda et al., 2004; Rao et al., 1998). The advertisement of Hydrogen sulfide production is a measure of sulfur metabolism, and it shows the existence of an anaerobic or facultative pathway involved in changing sulfur-containing compounds. These enzymatic activities together constitute essential biochemical pathways that are being involved in the breakdown of organic waste materials in polluted areas (Desouza et al., 2010).

The wastewater systems are dynamic microbial ecosystems with high consequences of organic matter and with changing environmental conditions. The environments apply immense selective pressure on constituent microorganisms thereby enhancing those with wide metabolic and enzymatic proficiencies. Consequently, wastewater has various populations of bacteria that are naturally programmed to digest complex substances. The native microbes are a rich and underutilized resource to be used in biotechnology in the environmental management (Barton et al., 2009). The research on enzymatic capability of bacteria in wastewater offers insightful information on the operational functions of these bacteria in natural self-treatment systems, and the applicable aspects of the bacteria in artificial bioremediation systems. In addition to indicating the degradative ability of the microbial communities, enzyme profiling enables the determination of areas with high bioremediation potential and patterns of spatial differences in the metabolism of microbes. This kind of knowledge is also needed in the development of location-specific and economical biological treatment rehabilitation plans (Wu et al., 2019; Weber et al., 2009).

In this regard, the following study is aimed at assessing the enzymatic potential of bacteria found in wastewater samples of various areas in Maharashtra such as Satara, Sangali, Solapur, Karad, and sub-sites to screen Gelatinase, Lipase, urease, protease, citrase, amylase and Hydrogen sulfide production, Standardized microbiological tests were performed to screen the samples for a variety of enzyme activities. Through the distribution and intensity analysis of the enzyme activities, the study will evaluate the diversity of functions in the wastewater microbiota, and explain their contribution to bioremediation. Knowledge of the enzymatic potential of wastewater bacteria not only enhances our explores naturally occurring biodegradation processes, but also provides the foundation of the development of sustainable biologically-based wastewater treatment technologies. The results of the study emphasize the ecological and practical relevance of microbial enzymes and confirm the possibility of wastewater ecosystems to serve as bioremediating microorganisms reservoirs (Tyagi et al., 2011; Sharma et al., 2018).

Materials and methods

Sampling Strategy and Area of Study

Wastewater samples were obtained from various urban and semi-urban locations of the state of Maharashtra in India which constituted Satara, Sangali, Solapur, Karad, and related sub-sites in Satara, KB, Satara-Solar, and Satara-ST. These sites were chosen to reflect geographically different areas with different anthropogenic sources like domestic sewage discharge, small-scale industrial effluents and agricultural runoffs. Higher organic loads and variable physicochemical states are known to support metabolically diverse communities of microorganisms in such environments (Alexander and Strete, 2001; Wu et al., 2019).

Open drainage channels and wastewater outlets were sampled with sterile polypropylene containers aseptically. About 250-500 mL of the wastewater was sampled from every location. The containers were also sealed off, labelled and loaded to the laboratory in insulated boxes in order to reduce temperature variations. The samples were stored in a time frame of 24 hours after collection to maintain the viability of the microbes and the activity of

the enzymes. Samples were next inverted and homogenized gently before analysis as a method of assuring homogeneity of distribution of microorganisms.

Experimental Design

The objective of the study was to understand the enzymatic potential of wastewater-based bacterial populations with the intent of quantitatively and qualitatively analyzing the results. All the samples were subjected to filtration under sterile conditions to identify the presence of extracellular enzymes that were pertinent in bioremediation and they included: Gelatinase, Lipase, Urease, Protease (quantitative assay), Plate-based proteolytic performance, Citrase, Amylase and Production of Hydrogen sulfide (H_2S). These enzymes were chosen due to their involvement in some of the key biochemical pathways involved in protein, lipid, polysaccharide, nitrogenous waste, and sulfur waste degradation in wastewater (Kasana et al., 2008; Gupta et al., 2002; Demain and Adrio, 2008). All the tests were conducted by standardized microbiological tests with slight modifications. The negative controls were uninoculated media.

Gelatinase Activity

A total of 20 mL of wastewater was placed in the test tubes containing 20 mL of 12% gelatin to inoculate them and allowed to remain in the test tubes at room temperature during seven days. The tubes were incubated, then chilled at 4^0C for 30 minutes and observed concerning liquefaction. The fact that the liquid retained its state after cooling was taken as a positive outcome pointing to the gelatin hydrolysis.

Table 1. Preparation of gelatin agar medium artificially (300 mL)

Distilled Water	300mL
Yeast Extract	0.3g
Gelatin	4.5g
Peptone	1.2g
Ph (adjusted)	7.2

Gelatin hydrolysis is a traditional proteolytic potential indicator, which indicates the ability of microorganism to digest complex proteinaceous substrates (Hankin and Anagnostakis, 1975; Jalgaonwala and Mahajan, 2011).

Lipase Activity

Tween 20 agar medium was prepared according to Hankin and Anagnostakis (1975). The peptone (10 g/L), NaCl (5 g/L), $CaCl_2H_2O$ (0.1 g/L), and agar (16 g/L) were put to the basal medium and the pH was adjusted at 6.0. Tween 20 (1% v/v) was placed in a sterile bottle and it was added following the autoclave process. Aliquots of the wastewater samples were placed on the medium surface and allowed to incubate at room temperature between 24-48 hours. The precipitate was formed around the bacterial colonies, as a sign of lipid hydrolysis and presence of lipase activity. Lipases are normally important in the breakdown of fats and oils, which are significant components of domestic and industrial wastewater.

Hydrogen sulphide Production

The hydrogen sulphide generation was compared by Tryptone Yeast Extract Agar, which was enriched with the sulphur sources and iron salts to identify the formation of sulfide.

Table 2. Medium Composition (for 200mL)

Peptone	6g
Beef Extract	0.6g
Ferrous ammonium sulfate	0.04g
Sodium thiosulfate	0.005g
Agar	0.6g
Distilled Water	200ml
pH	7.2+- 0.2

Plates were inoculated and incubated for 7 days. Formation of greenish-brown colour, brown colour or black colour in the medium was a sign of the production of H_2S. This is a sulphur metabolism assay, linked to the conversion of sulphur containing organic matter (Muyzer and Stams, 2008).

Urease Activity

Urease activity was assessed by using urea agar medium which was mixed with Phenol red as a pH indicator (Muyzer and Stams, 2008). 20% aqueous urea solution was aseptically filtered and put into the basal medium.

Table 3. Urea agar composition (for 1 L)

Peptone	1.0g
Sodium chloride	5.0g
Potassium phosphate	2.0g
Glucose	1.0g
Phenol Red (0.02%)	6.0mL
Urea (20% solution)	100Ml
Distilled Water	1000mL
pH	6.8

Plates that were inoculated were allowed to incubate for 24 hours. A change in color between yellow and pink was a sign of presence of ammonia as a result of hydrolysis of the urea and this was counted as positive urease activity.

Test for Protease Activity (Quantitative Assay)

Protease activity was measured by performing an albumin assay of hydrolysis. ASOS Test isolates were inoculated in a YEM broth and placed at room temperature. After six days of incubation, 0.2 mL of culture suspension was combined with 1 mL of 1% albumin suspension and incubated for one hour at 37°C. After incubation, 1 mL of 12 % trichloroacetic acid (TCA) was added for precipitation of protein. This mixture was kept in rapid cooler, then centrifuged and the protein concentration of the supernatant was determined by Biuret method. The absorbance was taken at 540 nm. Reduction of protein concentration as compared to the control was a sign of protease activity (Gupta et al., 2002).

The activity of the proteolytic growth was also verified by incubating nutrient agar with 0.4% gelatin at pH 6. 0. 8% gelatin solution was sterilized independently and 5 mL of it was put in each of 100mL of medium.
Plates were incubated at 37°C during 24-48 hours after which the saturated ammonium sulfate solution was flooded into it. Gelatin degradation and extracellular production of proteases was observed as clear colonies and had clear halos (Kasana et al., 2008; Demain, and Adrio, 2008).

Citrase Activity

Citrase was evaluated by the Sodium Citrate-amended YEMA medium with the bromothymol blue as a pH indicator. Mannitol was substituted with 1% sodium citrate and it was used as the only source of carbon. Plates were incubated for 24-48 hours, at room temperature. When color was shifted to blue, it indicated a positive test as a result of citrate utilization. Metabolism reflects metabolic flexibility and along with the use of carbon route (Kasana et al., 2008).

Results

The wastewater samples of Satara, Sangali, Solapur, Karad, KB, Satara-Solar, and Satara-ST were screened to evaluate various extracellular enzymatic activities important in bioremediation. The assays reveal general distribution of enzymes at all locations of sampling indicating that that there are metabolically diverse bacterial communities that can degrade a wide variety of organic matter and other pollutants. A summary in Table 1 is displayed based on experimental observations as a comparative overview of microbial enzymatic activities. Most of the wastewater samples gave positive results showing presence of a wide variety of enzymatic activities such as gelatinase, lipase, urease, protease, as well as amylase and production of hydrogen sulfide. This homogeneity among geographically different locations underscores the usefulness of wastewater microbiota.

Table 4. Enzymatic activities of various test samples

TEST/SAMPLES	SATARA	SANGLI	SOLAPUR	KB	SST	SATARA-SOLAR	KARAD
1.Gelatinase Activity	✓	✓	✓	✓	✓	✓	✓
2.Lipase Activity	✓	✓	✓	✓	✓	✓	✓
3.Hydrogem-Sulphide Production	✓	✓	✓	✓	✓	✓	✓
4.Urease Activity	✓	✓	✓	✓	✓	✓	✓
5.Protease Activity	✓	✓	✓	✓	✓	✓	✓
6.Proteolytic Test	×	✓	✓	✓	✓	✓	×
7.Citrase Activity	✓	×	×	✓	✓	✓	✓
8.Amylolytic Activity	✓	✓	✓	✓	✓	✓	✓

Regional Distribution of Enzyme activities

All the wastewater samples exhibited gelatinase activity. Samples from Satara, Sangali, Solapur, Karad, KB, Satara-Solar and Satara-ST exhibited the permanent liquefaction following refrigeration as a confirmation of gelatin hydrolysis in action. This implies that the wastewater contains proteinaceous material that has been easily exploited by the bacteria that inhabit it. The gelatinase-making microorganisms play an important role in the degradation of the complex form of proteins into peptides and amino acids such that the nutrient can be recycled in the polluted water.

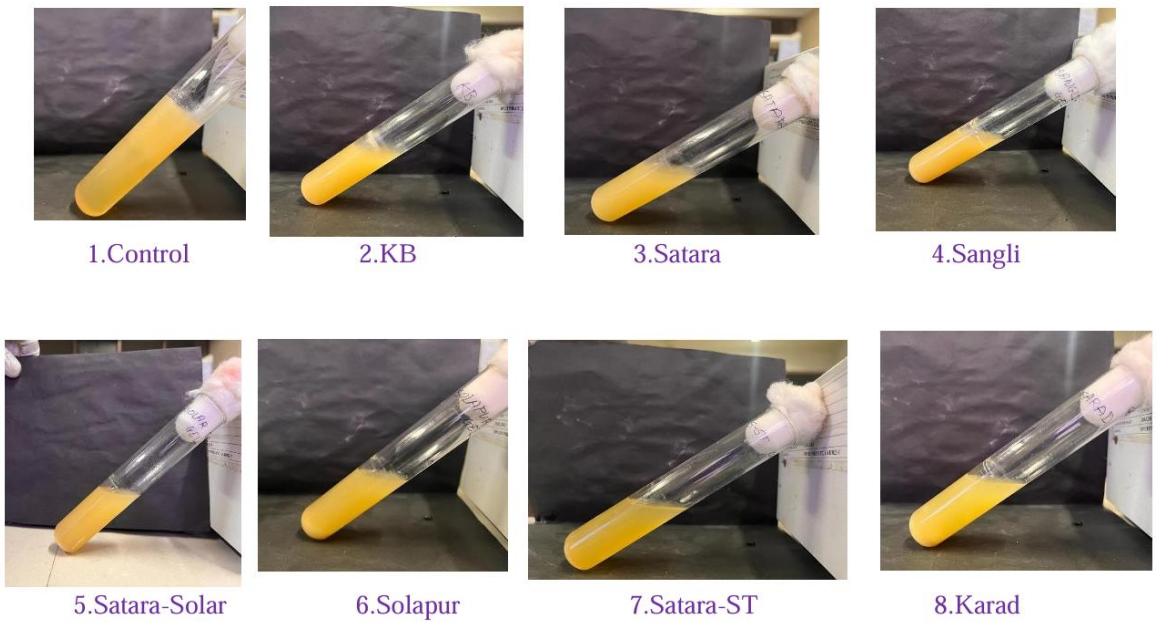

Fig. 1. Gelatinase activity

The activity of lipase was also positive in case of all the samples derived from all the regions. Lipid hydrolysis was detected by the clear halos and precipitated zones around the bacterial colonies on the Tween 20 agar. This indicates that wastewater habitats are enriched with lipase-degrading bacteria, which are prevalent in household and industrial effluent. Lipolytic activity plays a key role in inhibiting fats and blockage in results of natural and designed wastewater lines.

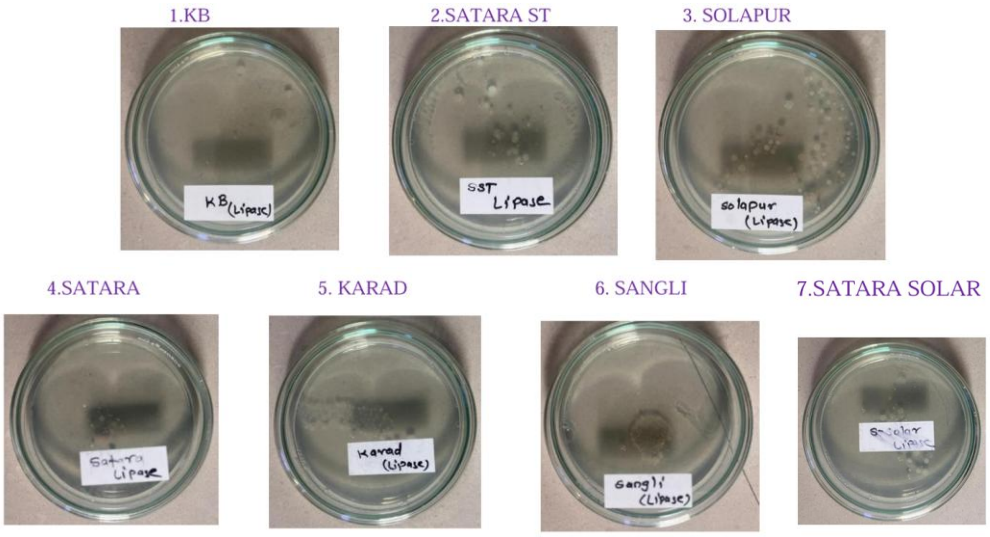

Fig.2. Lipase activity

The test for hydrogen sulfide production was positive for all samples as observed by formation of brown to black coloration on the Tryptone Yeast Extract Agar. This shows presence of active sulfur metabolism and availability of bacteria which can reduce sulfur containing compounds. H_2S production displays the conversion of organic sulfur into hydrogen sulphide and it also leads to mineralization of complex waste products under microaerophilic or anaerobic circumstances.

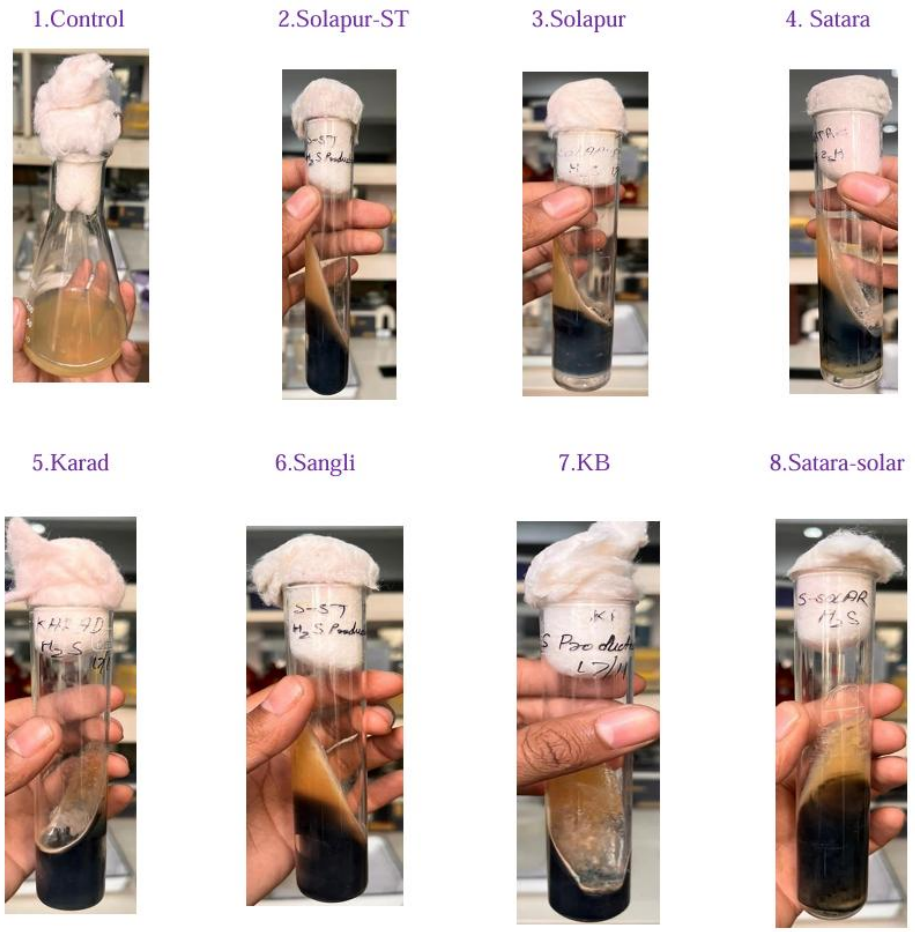

Fig. 3. Hydrogen sulphide production

Activity of urease was positive in all the samples of the wastewater. The urea agar underwent a specific colour change due to release of ammonia by urea hydrolysis activity which changes the colour of Phenol red to bright pink. This implies that wastewater contains nitrogenous wastes, which can be successfully utilized by the indigenous bacteria. The urease producing microorganisms are also vital in nitrogen cycling where urea is degraded into the bioavailable nitrogen.

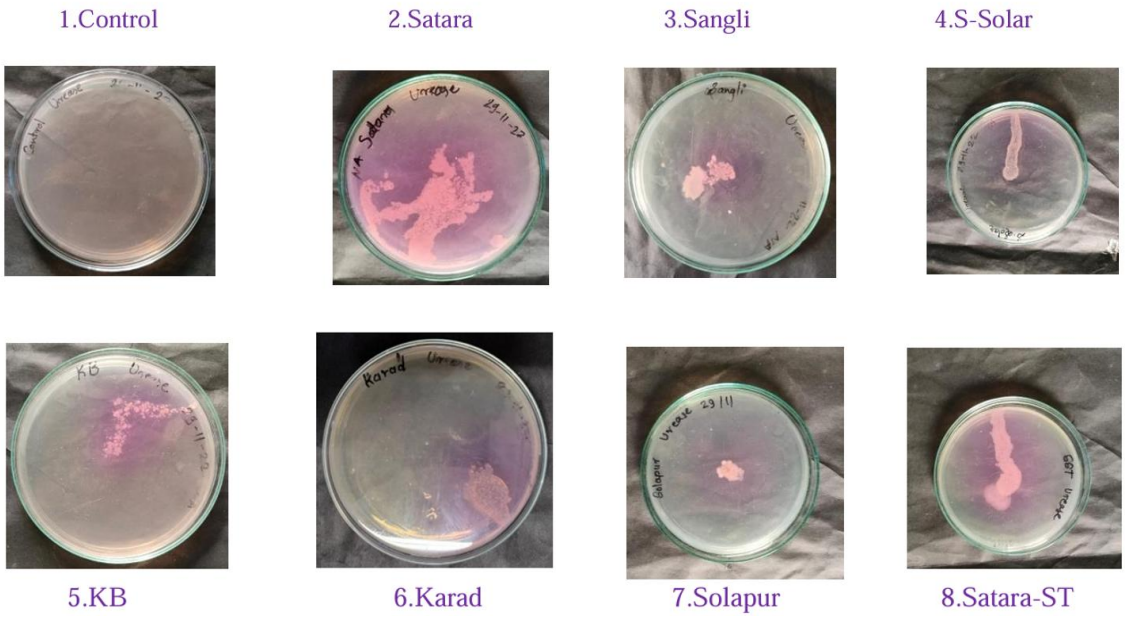

Fig. 4. Urease activity

Protease activity assessed qualitatively and quantitatively was ubiquitous at all locations. Plate based assays showed clear zones around colonies on gelatin supplemented media which confirmed extracellular secretion of proteases. This supports the gelatinase findings and additionally underlines the prevalence of protein-degrading bacteria in the wastewater settings.

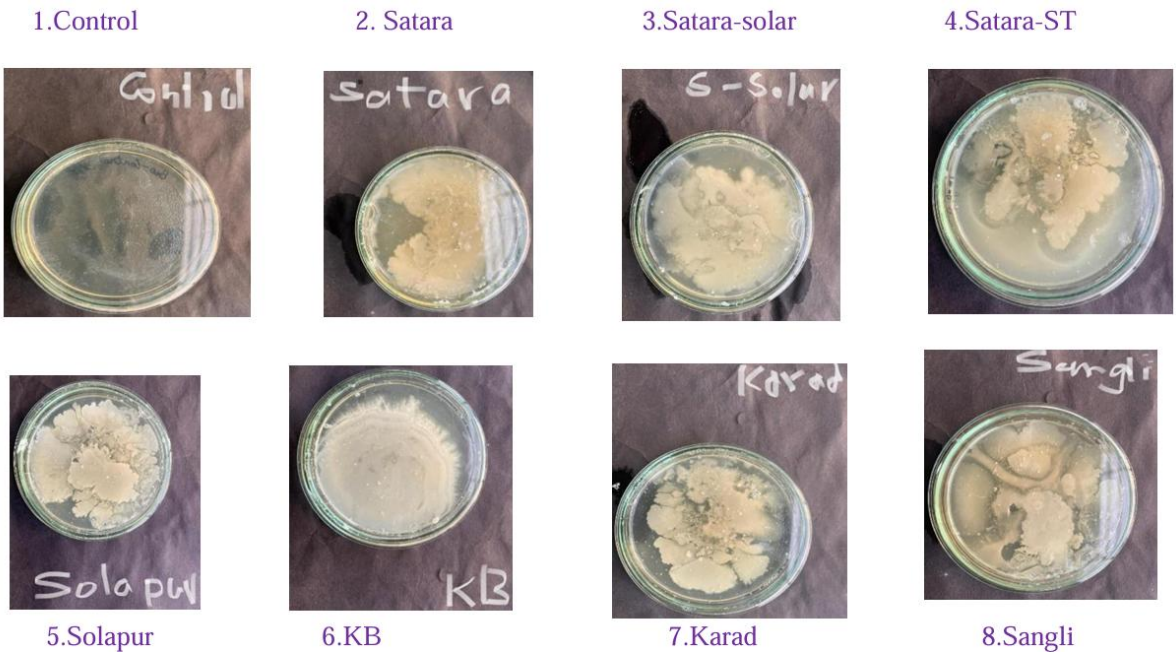

Fig.5. Protease activity

The activity of citrase was regionally different. Although samples of Satara, Karad, KB, Satara-Solar and Satara-ST had the change of green color to blue on the YEMA medium amended with citrate, samples of Sangali and Solapur did not change color, showing that citrate was not utilized in these areas. This is a different response that indicates metapleiotic differences across wastewater microbial communities as a result of space.

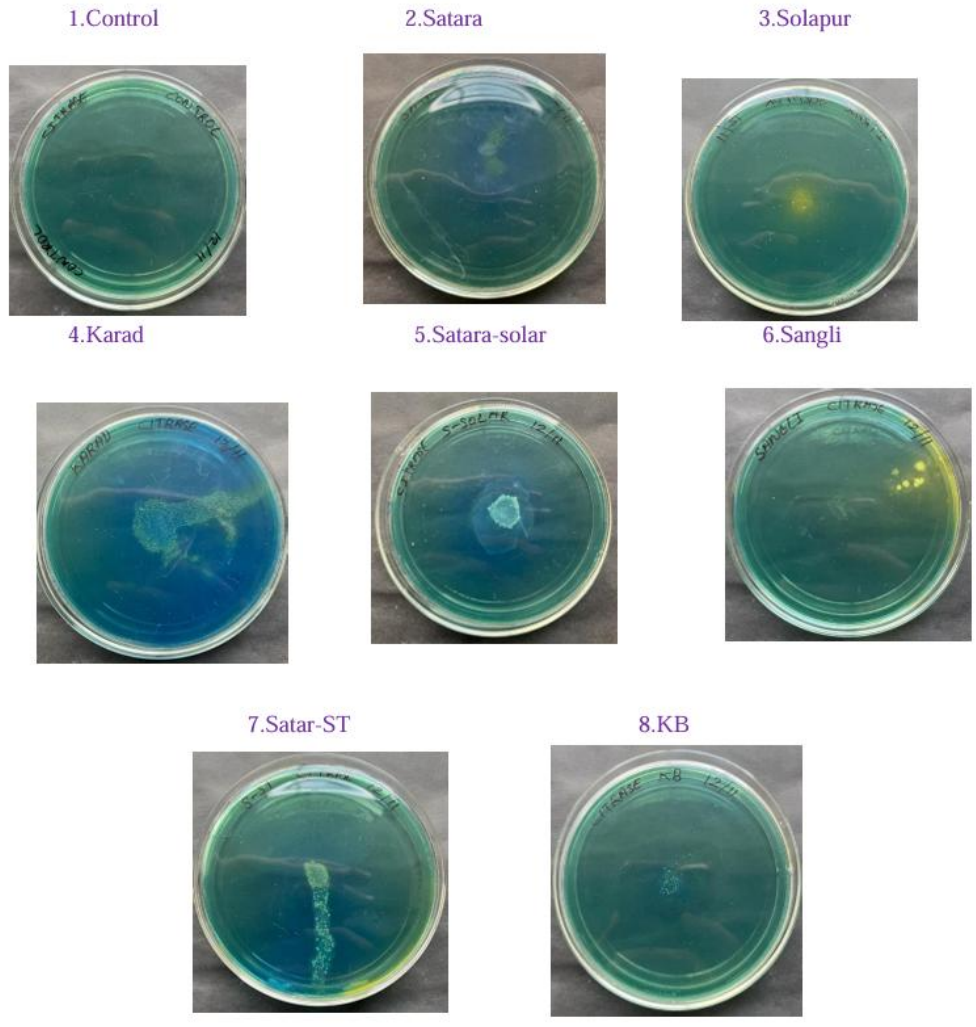

Fig.6. Citrase activity

All the samples exhibited positive amylase activity. When starch agar plates were flooded with iodine, clear halos appeared around colonies of all sites. This shows effective starch hydrolysis and outlines the ability of wastewater bacteria to breakdown food waste and plant residual
polysaccharides.

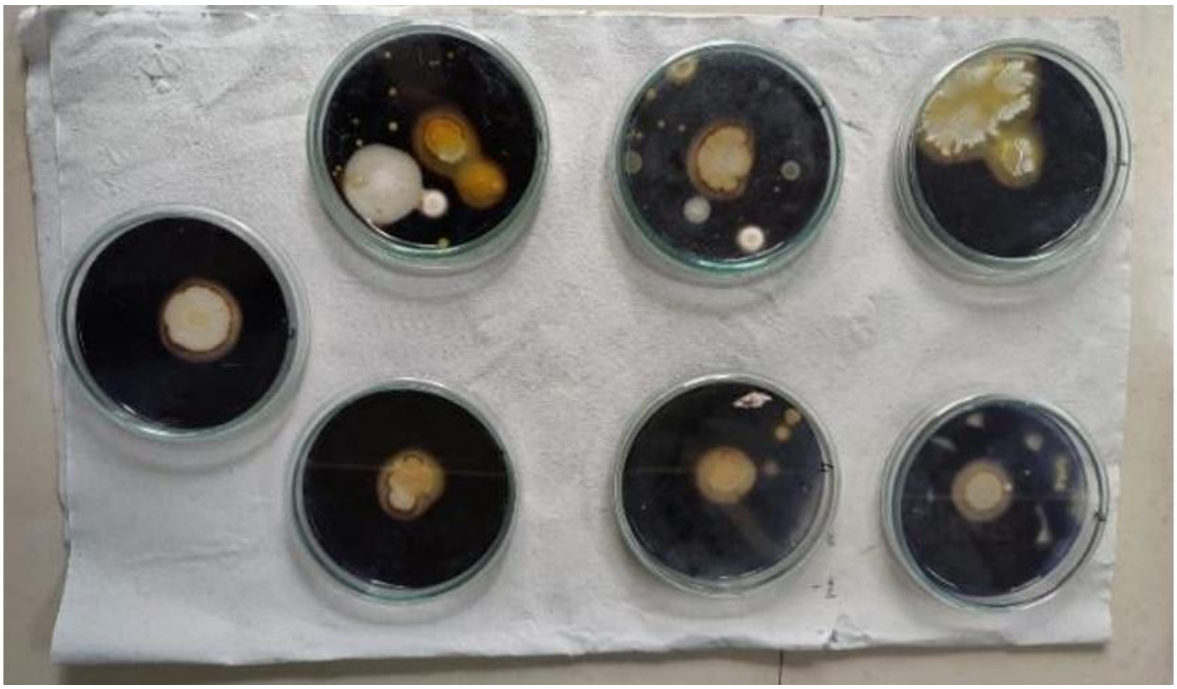

Fig. 7. Amylase activity

Protease Activity Quantitative Analysis

Protease activity was also measured by a spectrophotometric assay which was reliant on the albumin hydrolysis. Different values of ODs at 540 nm obtained for different regions due to differences in enzyme production (Table 2).

Table 5. Proteolytic activity

Sample Site	OD at 540 nm	
Karad	0.25	15.4
Sangali	0.28	15.07
Satara	0.24	12.5
Satara-ST	0.26	15.5
Satara-Solar	0.24	12.5
KB	0.26	15.5
Solapur	0.27	15.2

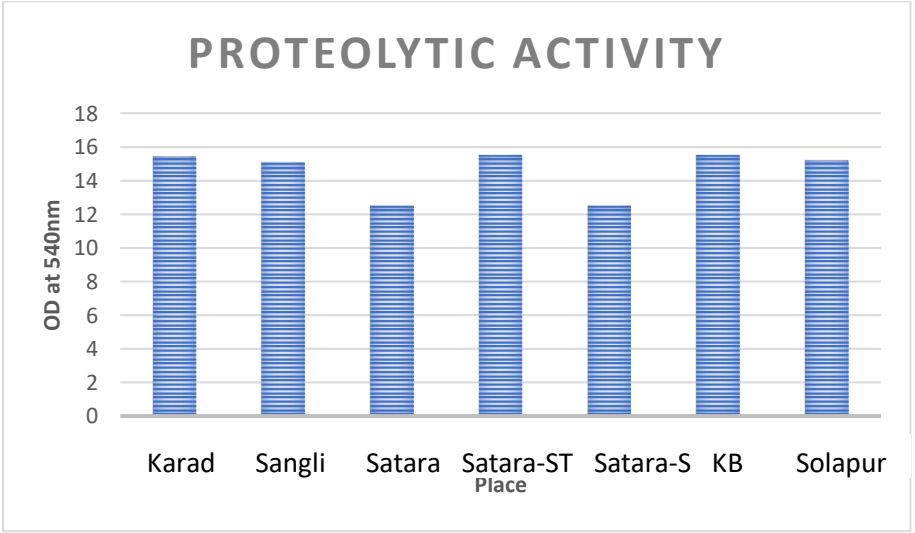

Fig. 8. The activity of Protease

Higher proteolytic activity was shown for the sample from Sangali (OD = 0.28), that was followed by Solapur (0.27) and Satara-ST and KB (0.26). Satara and Satara-Solar exhibited (0.24) activity. These differences show that protease activity is not uniformly distributed that varies among different parts of the environment, which may be because of the dissimilarity in the organic load, the presence of proteolytic substrates, and the structure of the microbial community. An increase in the values of OD is related to an increase in protein degradation implying that Sangali and Solapur wastewater contains more prolifically proteolytic populations. These areas can be especially well deployed as bioremediation solutions to a protein-laden waste stream.

Bioremediation Functional Implications

The overall presence of gelatinase, protease, lipase, amylase, urease and sulfur-transforming activities in all wastewater samples is indicative of a highly degradative strength. These enzymes work together to break out complex organic matter to simple molecules that can be assimilated or mineralized.

The breakdown of the proteinaceous waste takes place with the help of proteases and gelatinases.

- The break down of fats and oils occurs through the help of lipases.
- Starch and polysaccharides are hydrolyzed with the help of amylases.
- Nitrogenous waste is changed into types of waste that can be utilized.
- Transformation of sulfur is indicated by H_2S-producing pathways.
- Positive citrase activity increases the metabolic flexibility and carbon use.

Enzymatic diversity has been observed to indicate that the wastewater ecosystems act as natural bioreactors where microbial communities in a continuous way facilitate detoxification and the recycling of contaminants. Regional variations (e.g. lack of activity of citrase in Sangali and Solapur) also show the significance of site-specific characterisation during bioremediation design. All in all, the findings confirm that the bacteria in wastewater have extensive and strong enzymatic potential necessary in cleaning the environment. These facts confirm wastewater as an effective source of bio remedial micro-organisms and highlight the role they can play in the sustainable treatment and restoration of the environment in wastewater management programs.

Discussion

The current research proves that wastewater ecosystems are the home of acidic and metabolically plastic bacterial communities that can generate a wide range of extracellular enzymes (Demarche et al., 2012). The regular presence of gelatinase, protease, lipase, amylase, urease and hydrogen sulfide-producing activity in all sampled areas confirms that wastewater habitats operate as dynamic bioreactors where tricky organic subject matter is persistently broken down. The results confirm the idea that native microbiota of wastewater are key factors in the natural self-purification processes and can be taken as a promising source of biological resource in bioremediation works (Daims et al., 2006). The biochemical basis of microbial degradation in contaminated environments is comprised of extracellular enzymes. Proteins, lipids and polysaccharides are macromolecules and they cannot be directly introduced into the cells of the microorganisms as they need to be transformed in smaller molecules by hydrolysis. Gelatinases and proteases are common in all samples, which means that there is a high protein turnover. The fraction of proteins is significant in domestic sewage, food waste, and industrial effluents and their build-up of proteins is leading to increase biochemical oxygen demand (BOD). Proteolytic bacteria hence have a very vital role in decreasing the organic load and stabilizing the ecological balance (Schegolkoba et al., 2016).

The observed quantitative difference in the activity of proteases in different regions also emphasizes the importance of environmental conditions at that locality determining changes in the functionality of the microbes (Gupta et al., 2002). Sangali and Solapur recorded maximum activity of protease implying that there was more abundant or active protein degrading microorganisms. This can be an indication of higher organic input, variations in the wastewater structure or may be a long-term exposure to effluents that are rich in proteins (Weber et al., 2009). On the contrary, Satara and Satara-Solar had relatively smaller OD values and it can be concluded that proteolysis varies depending on the nutrient availability, pH and microbial colon structure. This kind of variability highlights the need of region-based evaluation in the model development of biological treatment systems. There was a consistent activity of lipase in all the samples indicating the ecological significance of lipid degradation in wastewater. A typical constituent of domestic and food-processing effluent includes fats, oils and grease which are also infamously hard to eliminate solely by physical means (Nybroe et al., 1992). They may become clogged and decrease the effectiveness of the treatment process. The large abundance of lipase-producing bacteria indicates that wastewater habitats tend to reward organisms with the capability to hydrolyze lipids. The former is an especially useful quality for bioremediation designs applied to oil- and grease-impacted waste streams.

The enzyme activity of amylase in all areas denotes effective decomposition of starch and polysaccharides produced out of food leftovers (Rao et al., 1998) and vegetative matter. Polysaccharide represents a large proportion of organic carbon in wastewater, its degradation through the breakdown of polysaccharides under the influence of enzymes is an obligatory procedure of carbon cycling. Being in the presence of amylase, protease and

lipase reveals the integrative character of microbial metabolism with several parallel enzymatic activities for breaking down of heterogeneous waste. The active metabolism of nitrogen is manifested by urease activity in all samples. One of the prevalent nitrogenous elements in the domestic sewage is urea. Hydrolysis of urea liberates ammonia which can later be taken up by microorganisms or can be worked out through nitrification and denitrification (Chipasa et al., 2006). Urease-producing bacteria would thus aid in the direct cycle of nitrogen and affect the chemical life cycle of the wastewater systems. Their availability is most pertinent when it comes to bioremediation strategies to mitigate the nitrogen pollution and the eutrophication.

The Hydrogen sulfide was produced in every sample, which represents active ways of transforming sulfur. Sulfur containing organic compounds are more frequently found in industrial and domestic wastes and their biochemical transformation is a part of biodegradation. Despite the frequent connections that H_2S has an odor and has corrosive nature, the generation of it also indicates the dissolution of higher sulfur derivatives in waste water and anaerobic or facultative metabolism (Mobley et al., 1989). This indicates the scope of the metabolic diversity of wastewater microbiota and their proficiency to operate in a wide range of redox environments. There was regional difference in the citrate utilization ability in which samples of Sangali and Solapur did not exhibit any citrase activity. Metabolic flexibility and the presence of potential to exploit alternative carbon sources shows consumption of citrate (Barton et al., 2009). No such activity was observed in some of the regions, which could be due to the variations in the accessibility of substrates as well as the composition of the microbes or the selective pressures of those wastewater systems. This ecological significance of such spatial heterogeneity is demonstrated by the fact that different wastewater environments are not functionally identical. In the case of applied bioremediation, this means that microbial consortia might have to be engineered or supplemented based on local metabolic constraints of any site. Altogether, the enzyme pattern in the present study demonstrates that the wastewater ecosystems are enriched with multi-functional microbial consortia that can break down an extensive array of organic substances (Bott, 1997). These bacteria do not use just one of the ways but rather a network of enzymatic reactions which act in synergy. This complementary redundancy and diversity give the system resilience so that biodegradation can still occur even in terms of variability in the composition of pollutants and environmental conditions.

The findings highlight the possibility of wastewater-grown bacteria to be used as engineered bioaugmentation and bioremediation systems. In contrast to laboratory strains, native microbes are already adapted to polluted system as well as tolerate high organic load, changing pH as well as toxic compounds. Such naturally selected communities can be used to harness them and make wastewater treatment processes more efficient and sustainable. Besides, the paper has identified the importance of enzyme-based screening as an easy but effective technique in assessing an existing bioremediation potential (Shah and Maulin, 2014). With mapping of the hallowed skills of enzymes, one is able to foretell functional positions, contrast sites, and pinpoint areas with a high degree of biodegradative aptitude. Such information could be used to develop region-specific approaches for biological treatment and it will facilitate to shift more towards the environmentally friendly wastewater management approach.

Conclusively, the multiple and ubiquitous enzymatic actions of the samples of wastewater in regions of Maharashtra suggest that the inherent bacterial population has a strong biodegradative capability. These microorganisms have been found to be the biochemical foundation of natural remediation and have huge potential in terms of technologies of sustainable environmental remediation and wastewater treatment (Kleerebezem et al., 2007).

Conclusion

The current research creates strict evidence that wastewater ecosystems possess highly active and metabolically diverse communities of bacteria with high bioremediation mechanisms. Wastewater samples of various regions of Maharashtra, Satara, Sangali, Solapur, Karad, KB, Satara-Solar and Satara-ST had a general expression of major extracellular enzymes, such as gelatinase, protease, lipase, urease, amylase as well as enzymes of hydrogen sulfide. These enzymes contribute to main biochemical pathways needed to be broken into proteins, lipids, polysaccharides, nitrogenous and-sulfur containing substances which are commonly present in the polluted fresh water (Singh et al., 2016). The uniform distribution of the various enzymatic activities among all the sampling sites is an indication of the fact that wastewater serves as a natural repository of competent microorganisms. Regional differences in protease activity indicate site-dependent disparity of microbial activities and organic load, which explains the heterogeneity of wastewater systems. The lack of citrate oxidation in the chosen regions also points to geometrical disparities in the metabolic capacity and above all to the significance of the local approach in ensuring bioremediation planning. All these findings establish that indigenous wastewater bacteria have the inherent ability to perform intricate biodegradation mechanisms. This is due to their enzyme versatility which allows them to carry out constant transformation and detoxification of organic waste. In more practical terms, the microorganisms are useful biological agents in developing low cost-sustainable wastewater management and environmental detoxification technologies. This study enhances the scientific rationale behind the use of naturally modified microbial communities in bioremediation as it reveals the functional enzymatic environment of wastewater microbial communities. Using and exploiting these biological systems can eliminate reliance on

energy-consuming physicochemical procedures and facilitate environmentally friendly practices of pollution control.

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