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Studies of Microbiological Analysis and Identification of Microorganisms in High TDS and Low TDS Waste Water of CETP Naroda

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Abstract

Industrial wastewater from chemical industries contains a wide range of pollutants and microorganisms which can have adverse effects on the environment and public health. This study investigates the microbial and physicochemical characteristics of wastewater collected from different treatment stages of Common Effluent Treatment Plant (CETP), Naroda, Ahmedabad. Parameters like pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Chloride and Ammoniacal Nitrogen were analysed using standard methods. The presence of microorganisms in the raw wastewater sample despite the TDS concentration (25,000 to 15000ppm) indicates that they can decompose organic matter present in the polluted water and their identification was analysed using various agar media.

Microbiological analysis was performed using culture-based techniques on selective and differential media including Nutrient Agar, McConkie Agar, EMB Agar, XLD Agar, PDA, Rose Bengal Agar, Robertson's Cooked Meat Medium and high-TDS media. The results revealed heterotrophic bacteria such as Pseudomonas and Bacillus species, indicator organisms such as Escherichia coli, pathogenic Salmonella species, halophilic bacterial and fungal isolates mainly belonging to Mucor species. These findings shed light on the microbial diversity of wastewater entering CETP.

Key words: Waste Water, Microorganisms, Pathogenic Bacteria, Selective and Differential media.

Introduction

Rapid industrialization and urban expansion have resulted in the generation of large amounts of wastewater from chemical, pharmaceutical, textile, dye, industries. Industrial wastewater is characterized by high concentrations of organic and inorganic pollutants such as dyes, acids, alkalis, salts, heavy metals, suspended solids, and toxic compounds. If untreated or inadequately treated in natural water bodies, these wastewaters can significantly degrade water quality, disrupt aquatic ecosystems, and pose serious risks to human and environmental health (Metcalfe and Addy, 2014; WHO, 2017)

Chemical wastewaters alter the physicochemical characteristics of receiving water bodies by increasing biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), and nutrient loads. Elevated BOD and COD reduce dissolved oxygen levels, leading to anaerobic conditions that are harmful to aquatic organisms (APHA 20217). For this, wastewater from industries is treated and properly managed.

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Microorganisms play a key role in the treatment and stabilization of industrial wastewater. Biological treatment processes rely on diverse microbial communities that can degrade, transform, or detoxify organic pollutants through enzymatic and metabolic activities (Prescott et al., 2017). Bacteria such as Pseudomonas and Bacillus (Fig 2 & 6) species are commonly found in wastewater treatment plants due to their metabolic versatility and resistance to toxic compounds (Madigan et al., 2018). These microorganisms contribute significantly to reducing organic loads and improving wastewater quality.

However, industrial and mixed wastewaters may also contain pathogenic and indicator microorganisms, including Escherichia coli (Fig 9&11) and Salmonella (Fig 7) species. The presence of these organisms indicates faecal contamination and inadequate sanitation or treatment efficiency, posing a potential risk for waterborne diseases (Tortora et al., 2019). Such pathogens are able to survive harsh environmental conditions and can even survive treatment processes if not effectively controlled (WHO, 2017).

Fungal populations are frequently found in wastewater environments due to their extreme pH, high organic content, and tolerance to toxic substances. Fungi such as Mucor species play an important role in the biodegradation of complex organic compounds and contribute to natural detoxification processes (Atlas and Bartha, 2005). Furthermore, industrial effluents with high salt concentrations support the growth of halophilic and high TDS-tolerant bacteria, which have specific adaptive mechanisms to survive under osmotic stress (Brock et al., 2018).

Common effluent treatment plants (CETPs) are designed to treat combined wastewater from multiple industries through a combination of physical, chemical, and biological processes. Regular monitoring of both physicochemical parameters and microbial populations at various treatment stages is essential to assess treatment efficiency and environmental safety (APHA, 2017).

Therefore, the present study focuses on the microbial and physicochemical analysis of wastewater from CETP Naroda, Ahmedabad, and to assess the microbial diversity of the CETP Naroda wastewater for biological treatment of the wastewater.

Methodology

Study Area and Sample Collection

The present study was carried out on wastewater samples collected from the Common Effluent Treatment Plant (CETP), Naroda, Ahmedabad, Gujarat. The CETP receives combined effluents from various chemical and allied industries, making it an important site for studying industrial wastewater characteristics. Wastewater samples were collected from different stages of the treatment process, namely the collection tank (raw influent), activated treatment units (ACT-1 and ACT-2), activated sludge process (ASP), and the final treated effluent outlet (Fig.no 1).

Samples were collected in sterile, properly labelled containers following standard sampling guidelines to avoid external contamination. The containers were rinsed with sample water prior to collection. All samples were transported to the laboratory in an insulated container and analysed within a short period to ensure reliability of results.

Fig .1 Different type of water Samples

Physicochemical Analysis of Wastewater

Physicochemical parameters of wastewater samples were analysed according to standard procedures described by the American Public Health Association (APHA). The parameters analysed included pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), chloride concentration, and ammoniacal nitrogen. (Table no.1)

The pH of the samples was measured using a calibrated digital pH meter. BOD was determined by the 5-day incubation method at 20 °C, which measures the oxygen consumed by microorganisms during organic matter decomposition. COD was analysed using the dichromate reflux method, indicating the amount of chemically oxidizable organic matter present in the sample.

TDS was estimated by the gravimetric method after filtration and evaporation of the filtrate, while TSS was measured by filtering a known volume of sample and drying the retained solids at 103–105 °C. Chloride concentration was determined using the DPD colorimetric method, and ammoniacal nitrogen was estimated by the distillation and titration method. All measurements were carried out in triplicate to ensure accuracy.

Microbial analysis of waste water

Serial Dilution Technique

To determine microbial load, serial dilution was performed. One millilitre of each wastewater sample was aseptically transferred into 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Further serial dilutions were prepared up to 10^{-6} by transferring 1 mL of the previous dilution into fresh sterile diluent. Dilutions were mixed thoroughly to ensure uniform distribution of microorganisms.

Isolation and Enumeration of Microorganisms

Isolation of microorganisms was carried out using standard microbiological techniques such as pour plate, spread plate, and streak plate methods. Different selective and differential media were employed to isolate specific groups of microorganisms.

Nutrient Agar was used for the enumeration of total heterotrophic bacteria. MacConkey Agar was used for the detection of coliform bacteria, while Eosin Methylene Blue (EMB) Agar (Fig no.5) was employed for the identification of Escherichia coli (Fig no.9) Xylose Lysine Deoxycholate (XLD) Agar was used for the isolation of Salmonella species (Fig no.7). Robertson's Cooked Meat (RCM) medium (Fig no.8) was used for the growth of anaerobic bacteria. High TDS medium was prepared to isolate salt-tolerant and halophilic bacteria. For fungal isolation, Potato Dextrose Agar (PDA) (Fig no.3) and Rose Bengal Agar (Fig no.4) were used.

Bacterial plates were incubated at 37 °C for 24–48 hours, while fungal plates were incubated at room temperature. Colony forming units (CFU/mL) were calculated using the standard plate count method.

Microscopic Examination and Identification

Bacterial isolates were subjected to Gram staining to determine Gram reaction, cell shape, and arrangement. Fungal isolates were examined by lactophenol cotton blue staining to observe hyphal structures and spore morphology.

Identification of microorganisms was carried out based on colony morphology, including shape, size, margin, elevation, pigmentation, surface texture, and opacity, along with microscopic characteristics. The observed features were compared with standard microbiological descriptions for presumptive identification.

Data Analysis

All observations and experimental results were recorded systematically. Physicochemical and microbiological results were tabulated and compared across different treatment stages to evaluate wastewater treatment efficiency and microbial diversity.

Results and Discussion

Physicochemical analysis of wastewater from different stages of CETP Naroda showed clear variation in pollution load during the treatment process. In which the sample analysis showed very high values of COD, BOD, TDS, TSS, Chloride and Ammoniacal Nitrogen, indicating heavy organic and inorganic contamination typical of chemical industrial effluents. The initial COD value of the wastewater was 4000 ppm, which gradually decreased after the treatment stages. Overall, a 94.75% reduction in COD was observed from the initial to the final stage. The initial TDS concentration of the wastewater in the PST sample was 25,000 ppm. After primary treatment (ACT-I), the TDS was 19,000 ppm, with no significant change observed between ACT-I and ACT-II stages. The maximum was recorded in the ASP stage, where TDS was 3,000 ppm, in the final sample, TDS is 15,000 ppm and BOD 97.5% degradation is observed in the initial stages which is a very good result (Table 1). A gradual decrease in COD and BOD values was observed from ACT-1 and ACT-2 to the activated sludge process and the final outlet, indicating the effectiveness of biological treatment in reducing organic pollution. The pH of all samples remained almost neutral to slightly alkaline, which is favourable for microbial activity during treatment.

Microorganisms play an important role in the degradation of dissolved organic matter contributing to TDS. During biological treatment, heterotrophic bacteria use dissolved organic compounds as a source of carbon and energy, converting them into simple end products such as carbon dioxide, water and biomass. This microbial metabolic activity leads to a reduction in the organic fraction of TDS. At high TDS conditions, salt-tolerant and adapted microorganisms survive and contribute to partial degradation of the waste, while at low TDS conditions, a wide range of microorganisms actively participate in biodegradation.

Microbiological analysis revealed high microbial load in raw and partially treated wastewater. Growth on nutrient agar showed the presence of heterotrophic bacteria such as Pseudomonas and Bacillus species, which are generally involved in the biodegradation of organic matter. Selective media such as MacConkey agar, EMB agar and XLD agar confirmed the presence of indicator organisms including Escherichia coli and Salmonella species.

Isolation of fungi on PDA and Rose Bengal agar revealed the presence of Mucor species, indicating the ability of fungi to survive in high organic content and adverse wastewater conditions. Furthermore, the growth of microorganisms on high TDS media confirmed the presence of salt-tolerant bacteria adapted to high dissolved solids concentrations. Overall, the results indicate that CETP treatment significantly reduces the pollution load and microbial density; however, the presence of indicator and resistant microorganisms found to be helpful for biological treatment of wastewater emphasizes the need for continuous monitoring and effective wastewater management.

Conclusion

The study demonstrated that wastewater contains high levels of organic and inorganic pollutants along with a diverse microbial population. Physicochemical analysis revealed elevated values of COD, BOD, TDS, TSS, chloride, and ammoniacal nitrogen. Microbiological analysis confirmed the presence of heterotrophic bacteria such as Pseudomonas and Bacillus, indicator organisms including Escherichia coli, pathogenic Salmonella species, high TDS-tolerant bacteria, and fungal isolates mainly belonging to Mucor species. These findings highlight that wastewater supports a complex microbial ecosystem and emphasize the importance of microbiological and physicochemical assessment for understanding wastewater characteristics. high TDS in CETP wastewater is primarily caused by industrial chemicals, mixed salts, heavy metals, and dissolved organic compounds. The microbiological analysis of CETP wastewater revealed the presence of salt-tolerant and stress-adapted bacteria rather than true halophilic marine microorganisms. Bacillus and pseudomonas are common species that we are find in high or low TDS water and they also present in sea water.

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Table 1: **Physicochemical Parameters of Waste Water**

Name of sample	COD (ppm)	TDS (ppm)	TSS (ppm)	PH	BOD (ppm)	Ammoniacal Nitrogen(ppm)
PST (collection)	4000	25000	600	7.5	800	100
ACT-1	2000	19000	800	7.8	515	60
ACT-2	1500	19000	900	8.0	380	40
ASP	100	3000	2000	7.5	50	10
FINAL	210	15000	80	7.5	20	N.D
Degradation (%)	94.75	-	86.66	-	97.5	Nil

Table 2: Colony Characteristics of Waste Water

MEDIA	SHAPE	SIZE	MARGIN	ELEVATION	COLOUR	SURFACE	OPACITY	INTERPRETATION
N agar	Irregular Or Circular	Small	Entire	Flat to raised	Pale greenish Or Off white	Moist to dry	Opaque	Presence of pseudomonas or bacillus (Fig 2)
Mac-Conkey agar	Circular	Small	Entire	Convex	Red, Yellow	Smooth	Opaque	Presence of E. coli and salmonella (Fig 7 & 9)
EMB agar	Irregular	Small to medium	Irregular to diffuse	Flat to slightly raised	Greenish-black pigmented spot	Rough	Opaque	Presence of p E. coli (Fig 11)
XLD agar	Circular	Small	Entire	Convex	Red colony with black centre	Smooth glossy	Opaque	Presence of salmonella (Fig 7)
PDA agar	Circular	Small to medium	Entire	Raised to convex	Dry chalky to powdery	Dry	Opaque	Presence of mucor spp. (Fig 10)
Rose Bengal agar	Filamentous	Small to medium	Irregular	Raised to convex	Pink or creamy white	Smooth and moist	Opaque	Presence of mucor (Fig 10)
High TDS	Circular	1-4mm	Entire	Raised	Creamy	Smooth	Opaque	Halophiles Like bacillus spp. (Fig 6)

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Fig -2N agar/ Pseudomonas


Fig-3 PDA agar


Fig-4 Rose Bengal agar

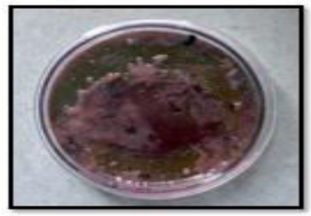
Fig -5 EMB agar


Fig -6 High TDS

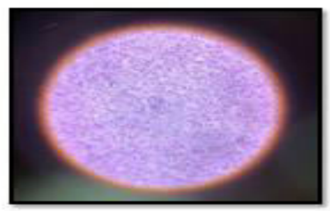
Fig -7 Salmonella


Fig -8 RCM medium

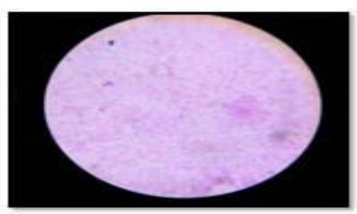
Fig -9 E. Coli

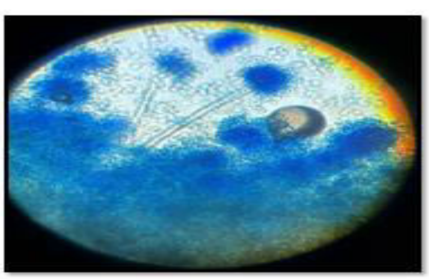
Fig -10 Mucor

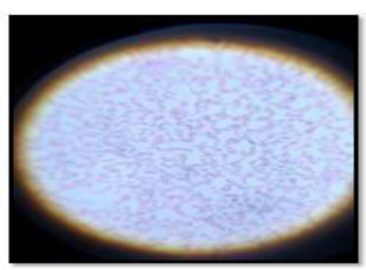
Fig-11 E. Coli

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