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Microbial Indicator as a Performance Evaluation Tool for STP: Case Study

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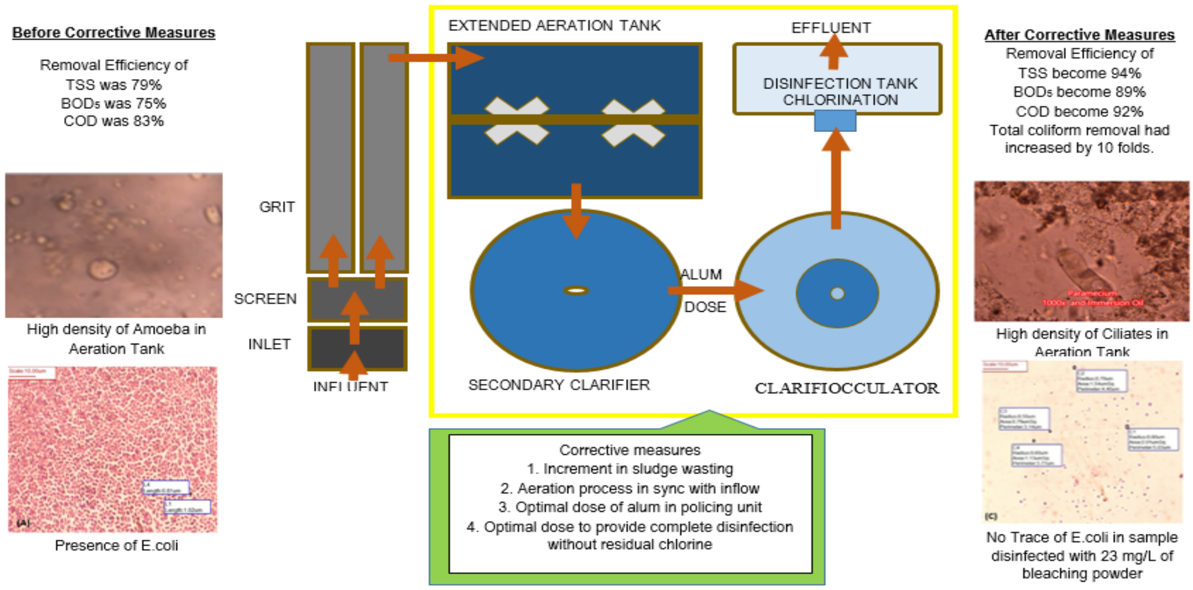
Graphical Abstract



Abstract

Mixed community of microorganisms is key to sewage treatment and few of them may be used as an indicator organism to evaluate the performance of sewage treatment plants (STPs). The aeration tank in the activated sludge process (ASP) forms an artificial ecosystem consist of bacteria and protozoans. Protozoans and E.coli can be used as a reliable microbial indicator to evaluate the performance of sewage treatment plant. In the present research the existing performance of Sanjauli Maliana Sewage Treatment Plant had evaluated in terms of removal efficiency of Total Suspended Solids (TSS), 5-day Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD). The operational parameter of extended aeration ASP has been determined which showed low food/microorganism (F/M) and high mean cell residence time (MCRT) but still the Dissolved oxygen (DO) of aeration tank was very low. In contrary to that, microscopic analysis suggested high food/active microorganism ratio and low MCRT that explained the low DO. The corrective measures were implemented successfully to elevate the performance of STP. The performance evaluation of sewage treatment had been done after 4 weeks and 6 weeks of successful implementation of corrective measures. The removal efficiency of TSS, BOD_5, and COD had increased to 94%, 89%, 92% from 79%, 75% and 83% similarly the total coliform removal has increased by 10 folds after six weeks of implementation of corrective measures. The relative density of ciliates had increased significantly and the relative density of amoeba had decreased. An optimal dose of bleaching powder had been evaluated which may ensure the optimized complete disinfection to reduce the risk of Hepatitis E Virus (HEV) epidemics as well as no stress to the aquatic life. In developing countries like India, these findings may increase the reliability and adaptability of microbial indicators to evaluate the performance of STP which is currently done predominately by physicochemical parameters.

Keyword: Microbial indicators; Ciliated protozoans; Total coliform and E.coli; Sewage treatment, Removal efficiency

1. Introduction

Microorganisms are symbiotically present in almost all types of ecology. Microbes play a vital role in biochemical processes and act as a performance indicator. Removal of sewage pollutants through activated sludge process (ASP) is performed by a mixed community of microbes (Tyagi et al., 2008). The aeration tank in ASP forms an artificial ecosystem of microorganisms to reduce the organic matter (Liu et al., 2008). The protozoans are extensively used to assess the effluent quality. Chahal et al. (2016) has reported that protozoans are nearly ten times larger than the average size of bacteria thus microscopic analysis become a reliable promising technique. Protozoans population density act as an indicator of effluent quality, mean cell residence time (MCRT) and settleability which makes them suitable microbial indicator of sewage treatment (Faiz et al., 2022).

Microbiological indicators have a key role in environmental risk assessment caused due to faecal contamination and pathogens (Lyons et al., 2015; Sánchez-Alfonso et al., 2020). Total coliform and E.coli are indicator organisms to determine the faecal (Narayan et al., 2023).

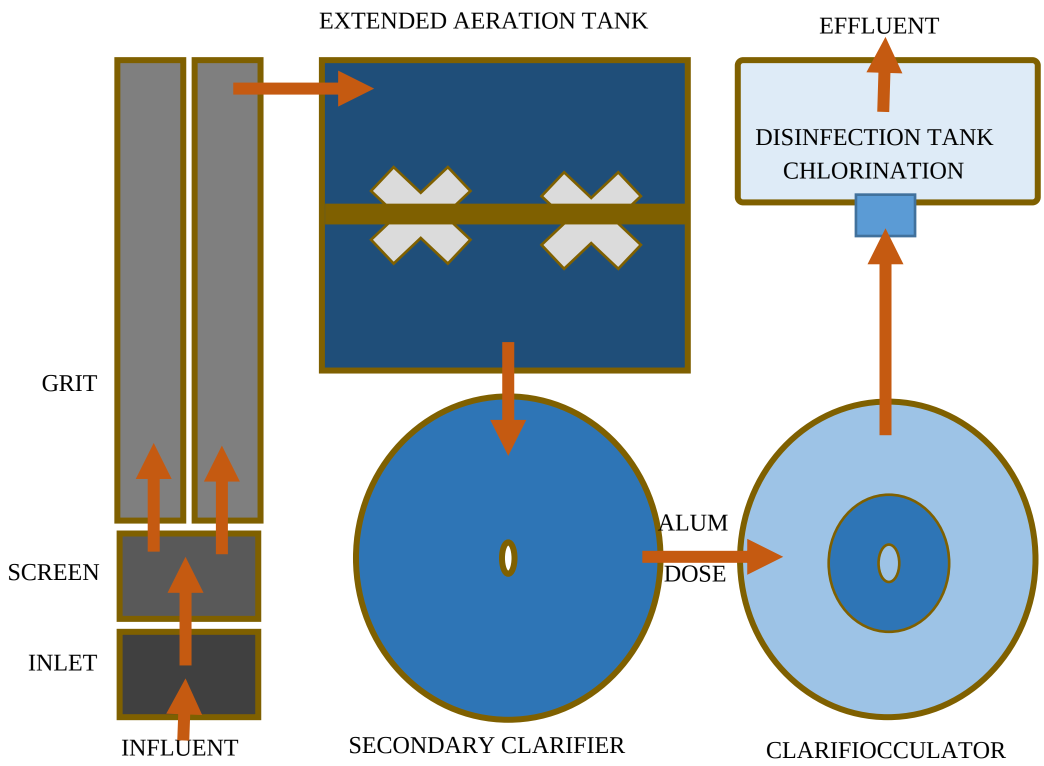


Fig.1. Layout of the flow diagram of the Sanjauli Maliana sewage treatment plant

contamination and risk ofpathogen. The removal of total coliform and E.coli may used to measure the degree of disinfection (Thwaites et al., 2018), (Narayan et al., 2023).

The present study had applied microbial indicator as a biomonitoring tool for performance evaluation of Sanjauli Maliana sewage treatment plant located in Shimla, India. There was a series of jaundice outbreak occurred caused due to Hepatitis E Virus (HEV) in Shimla. Masclaux et al., 2013 showed no significant correlation of HEV either with total coliform or E.coli but epidemiological research indicates that an infected person discharges a high amount of HEV through excreta during the incubation period (Harsha, 2010). Total coliform is an indicator of faecal contamination and HEV infection caused due to faecal oral route thus they have indirect relation. Complete disinfection of effluent using chlorination can be achieved but any excess chlorine can risk the survival of aquatic life in the discharge stream. Viruses are usually associated with the organic matter present in the sewage water and chlorine may form chloramines with organic matter thus significant inactivation of HEV have been observed in urban sewage (Girones et al., 2014). Effluent from secondary clarifier contains microorganism associated with bioflocs. Policing unit of clariflocculator with optimal alum dose may play a vital role in intensification of effective chlorination. In addition to the clear effluent the clariflocculator reduces the pathogens.This study aims to find the ease of acceptance of the microbial indicator as a tool to evaluate the performance of STP. In India, most of the sewage treatment facility intensively uses physiochemical parameters as a performance indicator. Although there is a plethora of research are available in biomonitoring of STP using indicator microorganism but this study will check the adoptability of these findings when applied with the regular operation of the STP. The finding of this research may increase the reliability over microbial indicators to assess the treatment process at STPs, (Narayan et al., 2023).

2. Material and methods

2.1. Description of the treatment facility

The in-situ study had performed at Sanjauli Maliana sewage treatment plant located on the outer side of hill station Shimla. The STP is situated at seven hundred meters lower elevation with respect to the city. High undulation causes chaotic flow at inlet in terms of sewage characteristics and inflow. The fig.1 shows the schematic layout of the Sanjauli Maliana sewage treatment plant. The plant has sequential operations of screen, grit, extended aeration ASP. The clariflocculator using alum is employed as the policing unit. Post disinfection, effluent allowed to outfall into Ashwini Khad inland surface water stream, (Mall et al., 2022), (Mall et al., 2023).

2.2. Sample collection and testing

Spatial and temporal variation had been taken with the utmost care to obtain the replicative and representative sample. The daily samples of each process are taken to evaluate the performance of performance of the sewage treatment plant. The physiochemical test had been carried out at in-situ STP laboratory. Weekly effluent samples were collected and transported to environmental engineering laboratory at Centre for Energy & Environmental Engineering, National Institute of

Technology Hamirpur (NITH) and the microbial analysis had been carried out. Ambient temperature and sample temperature had been noted while taking the sample. The samples were taken from mid-august to mid-February to study the effect of low temperature. (Srivastava et al., 2022), (Sawhney et al., 2022).

2.3. Physiochemical and biochemical analysis

Total Suspended Solids (TSS), 5 days Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) had been evaluated for all the influent and effluent samples, Winkler's method and closed reflux method using COD digestor (Merck Spectroquant® TR 320) respectively as per standard methods (APHA, 2012). Eutech pH Tutor (Glass electrodes) was used to determine the pH. Dissolved oxygen (DO), MLSS and mixed liquor volatile suspended solid (MLVSS) had obtained for the samples collected from extended aeration ASP reactor as per (APHA, 2012). F/M ratio, MCRT, Sludge Volume Index (SVI) and oxygen required (with altitude correction) for the extended aeration ASP reactor has been obtained. Settling test had been conducted as discussed in Metcalf and eddy. Jar test has been carried out to determine the health of the physiochemical policing unit as mentioned in APHA (2012). Idometric titration method as explained in APHA (2012) had been used to determine efficacy of disinfection. (Mutyalaiah et al., 2022).

2.4. Microscopic analysis

Identification and relative quantification of microbiological indicator protozoans were determined through a bright field digital microscope (Magnus MLXi) with an installed camera. The relative density had been estimated by the enumeration of protozoan observed under the microscope in 50µL sample sucked through micropipette after vigorous shaking of the sample as discussed by Vincigurerra and Petruccioli (2012). The sample has been observed at 100x, 400x 1000x. The video and picture had clicked at 400x and 1000x. The average enumerations is used to report the relative density of protozoans. The relative density of protozoans was compared before and after the implementation of corrective measures.

2.5. Total coliform and E.coli.

Total coliform and E.coli are widely used microbial indicators to detect faecal contamination and the presence of pathogens (Barrios-Hernández et al., 2020; Lyons et al., 2015; Sánchez-Alfonso et al., 2020; Tyagi et al., 2008,). Most probable number (MPN) techniques was used in quantification of Total coliform (APHA, 2012; Bailey et al., 2018). The fermented sample had cultured to apply gram staining technique. The microscopic analysis done through a bright field digital microscope (Magnus MLXi) with an installed camera at 1000x to detect and confirm the presence of non-spore forming gram-negative bacilli bacteria. Total coliform was evaluated for the disinfected sample with variable dose of bleaching powder to optimize the disinfection process and residual chlorine had been measured for each dose after disinfection. The optimal dose was evaluated through which no traces of E.coli observed. This optimal dose may ensure complete disinfection and nonappearance of excess disinfectant in the effluent.

3. Result and discussion

3.1. Sewage treatment characteristic

The performance of the plant had been evaluated in terms of removal efficiency of TSS, BOD_5 and COD. Fig. 2 shows the removal efficiency of TSS, BOD_5 and COD for treatment of sewage through each process and overall STP. The removal efficiency was found significantly below the normal range (Metcalf and eddy). The pH of all the influent samples was in the range of 7.4-7.9 while the effluent sample in the range of 7.8-8.1. The fig. 3 shows the effect of lowering the temperature in winter over the overall efficiency STP in terms of TSS, BOD_5 and COD. There was a drop of 10 to 15 percent observed in the removal efficiencies that maybe account for the inhibition of biochemical activity in the winter season. The very similar temperature effect over removal efficiency has been reported by Singh and Viraraghavan (2003), Lew *et al.* (2003), Uemura and Harada (2000). The high tendency of forming filamentous bacteria and its bulking at low temperatures might be the cause of lower removal efficiency in the winter season (Fan et al., 2019). The lower removal efficiency of the physiochemical process (coagulation-flocculation) in the policing unit may be attributed as the non-optimal alum dose and increased TSS due to the bulking of filamentous bacteria.

3.2. Operational problem and corrective measures

Operational parameters of extended aeration ASP had calculated as mentined in Metcalf and eddy, (2003). The obtained values were within the normal range but still the efficiency was significantly less. Settling test has shown contradictory result in itslef. The MLSS and MLVSS and MCRT were found in normal range as well as the F/M was very low but still DO of aeration tank was 0-0.05 mg/L. In contrary to that, the microscopic analysis had shown that there are very less free-swimming ciliates, moderate flagellates and high amoeboid which suggested high F/M, low MCRT and poor settling (Metcalf and Eddy, 2003). This type of situation suggests straggler flocks formation. Very high BOD and COD had been observed which might have caused shock load. Seetha et al., 2010 has reported the disappearance of certain types of bacteria during a controlled shock load inside the laboratory. In the present case the shock loads occurred every morning during peak inflow thus microbial growth had helplessly may get affected. This hypothesis was confirmed by considerable low sludge wasting found during further investigation. A similar finding has been explained by (Salvado, 1994). The plant is located in the hilly terrain of Shimla and due to space-constrained there were no sufficient sludge drying beds. The filter press belt was not able to reduce the optimum moisture content from excess sludge and the cloudy cold climate was not supporting the sludge drying through evaporation under sun. The operational parameters were failed to provide the real picture of the active microorganism present in the extended aeration ASP reactor while microscopic analysis had helped to identify the operational problem which shows higher reliability of microbial indicators for performance evaluation of STP.

The role of policing unit becomes more important during the winter season. The formation of large bioflocs get inhibited.

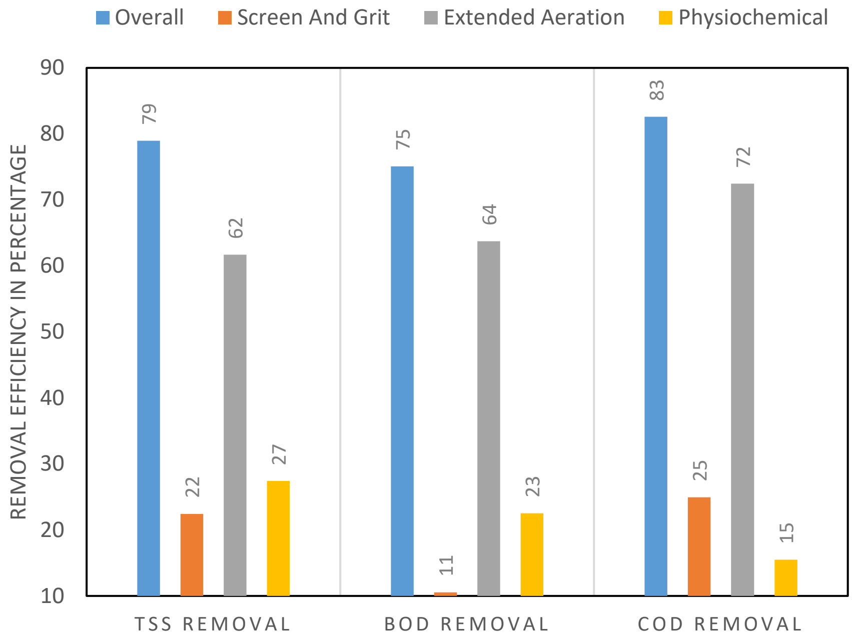


Fig. 2. Removal efficiency of TSS, BOD_5 and COD for treatment of sewage through each process and overall STP.

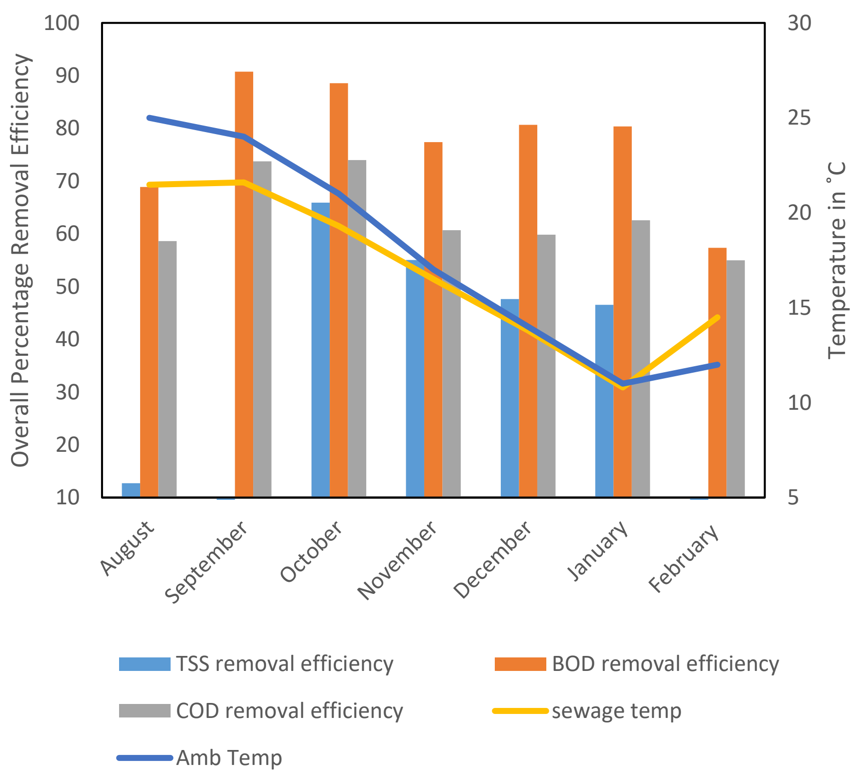


Fig. 3. Month wise removal efficiency of STP in terms of TSS, BOD_5 and COD with respect to ambient and sewage temperature.The operational problem and corrective measures are shown in table 1.

Table 1
Operational problem and corrective measures.

Operational Problem	Cause	Corrective measure
Insignificant active biomass in Recycle Sludge	Sludge is not wasted as per design parameter.	Optimiation of sludge wasting, construction of new sludge drying bed and third party disposal.
Shock load	Peak flow flushing of settled material in sewers during less flow.	The scheduling of operation of the sludge recycle pump and aerators.
Clarifloculator efficiency is low	Inappropriate dosage of Alum	Optimal dose
Total coliform in effluent is high	Inappropriate dosage of beaching powder	Optimization of disinfected samples.

3.3. Relative density of protozoans

Micro fauna inside the reactor should have given equal importance in the management of sewage treatment plants because protozoan's density is an indicator of the quality of effluent and efficient biochemical treatment. The protozoans are sensitive to operational parameters (Singh et al., 2016). Tyagi et al., 2008 have reported that if MCRT is greater than 10 days than slower-growing protozoan's rotifers and nematodes come under the microscopic view. In the present case, the rotifers and nematodes were absent although the MCRT obtained through formula was 27 days this implies that actually MCRT is low which was confirmed with the presence of high density of amoeba, moderate density of flagellates and very less density of ciliates (Metcalf eddy, 2003; Tyagi et al., 2008). The relative density of protozoans before and after the implementation of corrective measures is reported in table 2. The images captured during the identification enumeration of protozoan before and after 6 weeks of the implementation of corrective measures are represented in fig. 4. The DO of aeration tank was measured after 4 weeks and 6 weeks of successful implementation of the corrective measures and those were 0.1 mg/L and 0.14 mg/L respectively. The removal efficiency of BOD_5 through extended aeration process had elevated to 79% from 64% and similarly the overall removal efficiency of TSS, BOD_5 and COD had increased to 94%, 89% and 92% respectively within 6 weeks of implementation of corrective measures.

3.4. Removal of total coliform and E.coli

Total coliform and E.coli are measures of faecal contamination and risk of pathogen in sewage. E.coli is considered as a mandatory faecal indicator by the United States Environmental Protection Agency and European Union for wastewater discharge (Chahal et al., 2016). The removal of total coliform and E.coli indicates the effect of any process over the reduction of pathogenicity. Total coliform removal before and six weeks after implementation of corrective measures with and without disinfection is reported in table 3.

Table 2
Relative density of protozoans before and after implementation of corrective measures in extended aeration ASP reactor.

Classification of protozoans	Before corrective measure	4 weeks after corrective measure	6 weeks after corrective measures
Free swimming ciliates	+	+++	++++
Crawling ciliates	+	++	+++
Stalked ciliates	ND	+	+++
Flagellates	++	++	+
Amoeba	+++++	++	++
Rotifers	ND	ND	ND
Nematodes	ND	ND	ND

Relative density notation
ND Not detected + very low ++low +++moderate ++++high +++++very High

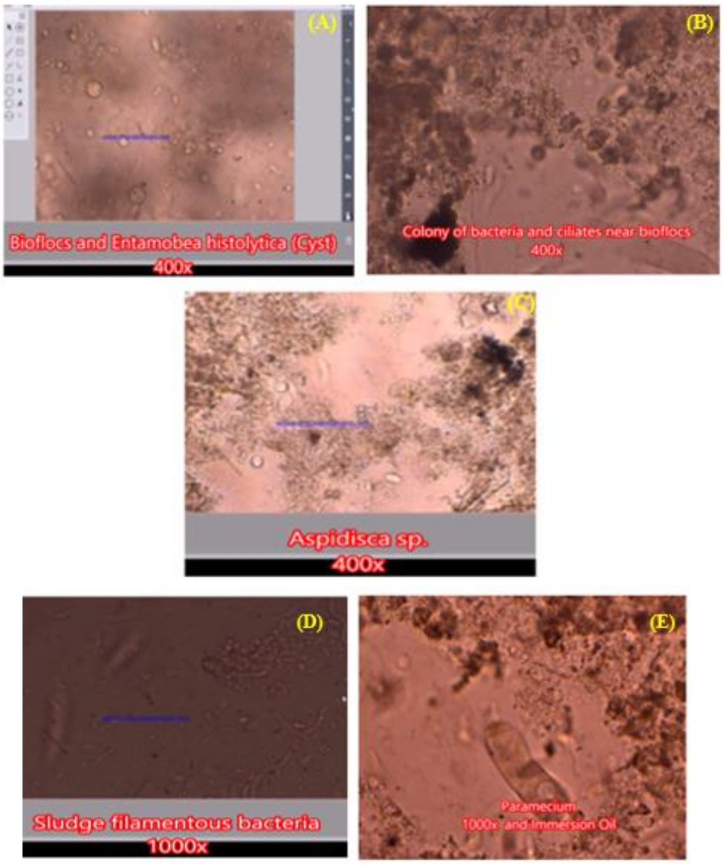


Fig. 4. (A) Very high density of Amoeba before the implementation of corrective measures (B) High density of free-swimming ciliates 6 weeks after implementation of corrective measures (C) Moderate density of crawling ciliates 6 weeks after implementation of corrective measures (D) Sludge filamentous bacteria before the implementation of corrective measures (E) Free swimming ciliates and crawling ciliates 6 weeks after implementation of corrective measures.

Table 3
Total coliform removal before and six weeks after implementation of corrective measures with and without disinfection.

Particulars	Before corrective measures	Six weeks after corrective measures without disinfection	Six weeks after corrective measures with Disinfection using Bleaching Powder				
			5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
Total coliform/100ml	4.8×10^4	3.5×10^3	1600	920	540	120	0
Residual Chlorine (mg/L as Cl)	-	-	0	0	0	0	0.1

Girones et al. (2014) have reported that HEV gets inactivated after disinfection with chlorine in the sewage sample. The available chlorine in bleaching powder stored at the sewage plant was 3.9 percent. The optimal dose of chlorine was found 0.875 mg/L as Cl which corresponds to 22.43 mg/L (taken as 23 mg/L) of bleaching powder. The similar high chlorine value in similar condition has reported by Hutchison et al. (2019) which suggest the hypothesis that during winter season pathogens become tolerant to chlorine because of experiencing the low-temperature stress. Fig. 5 shows the presence of E.coli in the effluent sample and absence after disinfection with 23 mg/L bleaching powder.

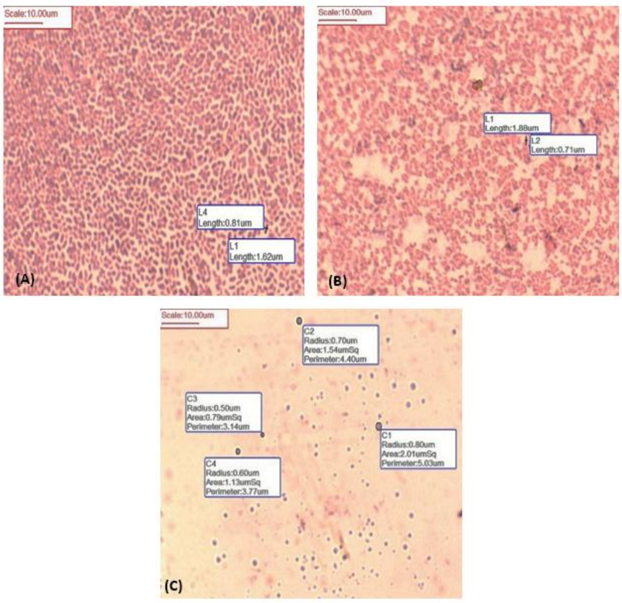


Fig. 5. (A) E.coli in the sample without disinfection (B) E.coli cell wall ruptured in the sample with a dose of 5 mg/L bleaching powder (C) E.coli absent in the sample disinfected with a dose of 23 mg/L bleaching powder.

4. Conclusion

Protozoans were used as an indicator of active microorganism. The protozoan's relative density had increased after the optimization of sludge wasting. The protozoans qualifies as a more reliable performance indicator against the physiochemical parameters. Sludge wasting is an important parameter and it is as equally vital as sludge recycling. Operation of plant equipment operation in sync with temporal physiochemical parameters is a key step to enhance performance of plant. Implementation of corrective measures had increased efficiency of clariflocculator. Removal of total coliform and E.coli can be treated as a measure of the safe disinfected effluent disposal. The optimized complete disinfection will reduce the risk of pathogens like HEV as well as reduce the risk of endangering aquatic life. Microscopic analysis of the elementary level can be employed on sewage treatment plant with the skilled operator having basic knowledge of microbial indicators as protozoans and E.coli. These findings may increase the reliability and adaptability of microbial indicators that will help to reduce chemical usage for the determination of physicochemical parameters.

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