J. Integr. Sci. Technol. 2023, 11(3), 516 Article

Journal of Integrated
SCIENCE & TECHNOLOGY

Identification and characterization of Microbial Consortia present in sewage samples collected from Sewage Treatment Plant, Jaipur, (Raj.)

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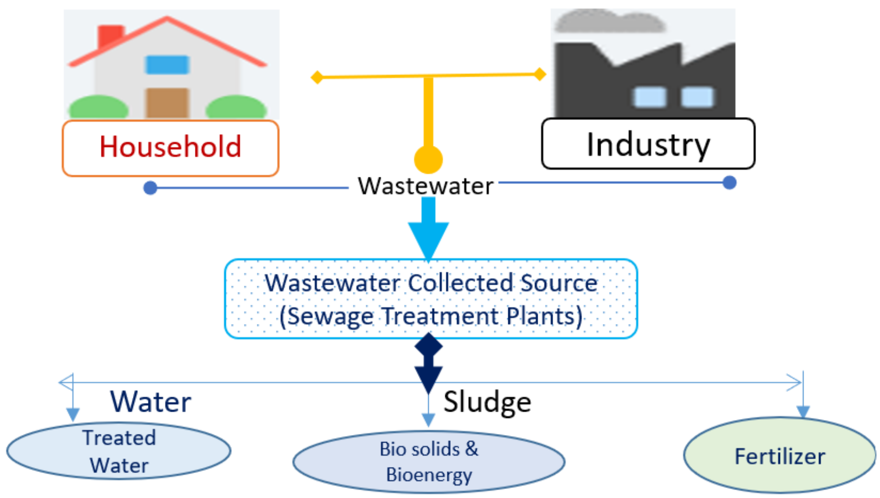
Received on: 17-Jan-2023, Accepted and Published on: 20-Mar-2023

ABSTRACT



Conventional wastewater management methods utilizing the municipal and industrial waste water in strict aerobic conditions are quite expensive in terms of consumption of excess amount of energy and available resources which consciously results in creation of big quantity of secondary sludge mostly containing many of the toxic pollutants. On this panorama several physico-chemical parameters can act as validation marker to estimate the amount of bioremediation and clearance out of pollutants within the sewage treatment plants. The present study demonstrated the identification of available microbial consortia along with the estimation of several physico-chemical parameters for the Sewage samples, collected from sewage treatment plant. The study showed the presence of specific microbes such as *Salmonella Spp., Escherichia Spp., Pseudomonas Spp., Alcaligens Spp., Staphylococcus Spp., Streptococcus Spp., etc.* in the sludge samples and *Salmonella Spp., Bacillus Spp., Escherichia Spp., Staphylococcus Spp., etc.* in the waste water sample**.** Moreover, it also validated the efficiency of microbial fuel cells within the sewage plant; as samples collected from different chamber compartments have showed significant reduction in many physico-chemical parameters such as BOD, COD, hardness, Organic carbon, Nitrate, etc.

Keywords: *Biological Oxygen Demand, physico-chemical, sewage analysis, wastewater management,*

INTRODUCTION

Most of the anthropogenic needs associated with the use of ground water leads to release of higher quantities of xenobiotics, that are mixed with toxic pollutants being directly discharged to the waterbodies.[1] These pollutants, residues and other harmful particles are being supplied to the water source as partially degraded, converted or completely non-degraded compounds with higher toxicity that can lead to several acute and chronic diseases.[2,3] Most of the industrial setups utilize the power of physico-chemical protocols like flocculation[4] and sedimentation or absorption for removal of heavy metals from wastewater.[5] However, these methods add up to production of more sludge discharged into the environment which becomes a source of secondary pollution.[6,4] Apart from this, the use of humongous chemicals questions its cost effectiveness.[7] Even though these chemical methods are popular, the co-ordinated and synergistic amalgamation of all the available physico-chemical techniques is not sufficient enough for removal of all pollutants.[8,9] On this aspect the sustainable utilisation of microbial consortium and/or plant species through bioremediation can mediate the complete removal of toxic sources[10,11,12] without production of any

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Cite as: *J. Integr. Sci. Technol., 2023, 11(3), 516.*
URN:NBN:sciencein.jist.2023.v11.516

 ISSN: 2321-4635
http://pubs.thesciencein.org/jist

associated sludge or secondary pollutant source.[13] Most of the physical, chemical methods utilised for such processes contain several setbacks like expensive protocol, secondary pollutant generation and interconversion of pollutants from one state to another state.

Hence a specialised, holistic approach is needed that can regulate the process gracefully without any harm to the environment for complete removal of the concerned pollutants.[14] For such case the microbial samples were collected through sewage samples from sewage treatment plants in Jaipur city (Rajasthan), evaluated for identification of available microbial consortia in both sludge and waste water samples and analysed for physico-chemical analysis for process validation.

MATERIALS AND METHODS

2.1. Sample collection

For validation of microbial strains efficacy in degradation of sewage pollutants, microbial samples were collected from Jawahar Circle sewage treatment plant situated in Jaipur city (Rajasthan). The microbial samples were collected from five sampling sites of Jawahar Circle sewage treatment plant. Out of this three samples were waste water samples collected from various sites of tank and the remaining two are sludge samples from other sites (Table 1). All studied samples (viz. waste waters and sludge samples) were collected from chambers (tanks) from sewage treatment plant of Jawahar Circle in Jaipur city (Rajasthan) for physio-chemical analysis process.

Table 1: Sites of sample collection

S. no.	Name of the sample collecting tank	site of sample collection
	Waste water samples	
1.	Raw/Grit tank	Inlet
2.	MBBR tank	secondary treatment tank
3.	Tertiary tank	treated water
	Sludge samples	
4.	Grit chamber	Inlet
5.	MBBR chamber	secondary treatment tank

2.2. Sample pre treatment

Samples were gathered in sample collector bottles. Triplicate samples per location were collected Temperature, color and pH of water samples measured. These were labeled properly and stored in the refrigerator at 4℃. Waste water and sludge samples were collected, processed and validated for their quality check in terms of different physico-chemical analysis.[15]

2.3. Reagents used

Manganese sulphate, Sodium hydroxide, Potassium iodide, $CaCl_2$, Potassium Di Hydrogen Phosphate, Ammonium chloride, Potassium dichromate, Mercuric sulphate, Ferrous ammonium sulphate, Phenanthroline monohydrate, Magnesium salt of EDTA, Eriochrome black T dye, Hydroxylamine hydrochloride, Silver nitrate, Aluminium potassium sulphate, Di sodium hydrogen phosphate, 2, 6-bis (4-methoxyphenyl)-4-phenyl pyrylium perchlorate, Di Potassium Hydrogen Phosphate, Boric acid, Phosphoric acid, Naphthalene, Sodium azide, Potassium iodide, Potassium hydroxide, Sodium thiosulphate, Starch indicator, Sulphuric acid, Azomethine H were purchased from Sigma Aldrich, India and Merck, India.

Similarly, Methanol, Anthrone, Standard glucose, Sodium carbonate, Copper sulphate, Sodium potassium tartarate, Folin-ciocaltue reagent and Sodium hydroxide were purchased from Merck life sciences, India.

2.4. Microbiome study

All collected sewage and waste water samples will be analysed for identification and biochemical characterization of isolated microbes from both the sewage and sludge samples. The morphological identification was carried out taking the microbes in a dual group series such as without heat shock (Series A) and with heat shock (Series B). In both cases, the microbial shape, size, elevation, margin, surface, opacity and pigment contents were evaluated. However, in case of biochemical evaluation, oxidase, indole, citrate, urease, catalase, MR, VP, Coagulase and gram staining was analysed to identify individual microbe strains.[16]

2.5. Physio-chemical analysis parameters

All collected sewage and waste water samples will be analysed for validation of physical parameters such as pH, temperature, hardness, Total Solids (TS), sulphate, Total volatile solid, Total Dissolved Solids (TDS), Turbidity (NTU), Chemical Oxygen Demand (COD), total chloride, nitrate, Biological Oxygen Demand (BOD), phosphate, Total Suspended Solids (TSS), total kjeldahl nitrogen (TKN), protein and Electrical Conductance (EC). All these parameters were measured according to standard protocols of APHA.[15]

2.6. Statistical validation

All the results were expressed as mean ± SD for three replicates. The data obtained from the water samples were analysed by using the GraphPad Prism software. For significant value, least significance difference was utilised for mean comparison at 5 % level using t-test. Statistical analysis was used to show significant differences at P value lower than 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

3.1. Morphological identification of microbes

The isolated microbial strains from both sewage samples and waste water samples were identified for morphological analysis. The samples from sludge sources are evaluated in two steps (with and without heat shock) and designated with codes like AKS 1 to AKS 15 for without heat shock and BKS 1 to BKS7 for with heat shock. Similarly, from waste water sources are evaluated in two steps (With and without heat shock) and designated with codes like CKW 1 to CKW 6 for without heat shock and DKW 1 to DKW 6 for with heat shock. In all cases the morphological parameters checked included shape, size, elevation, margin, surface, opacity and pigment contents. All the series retained shapes such as circular, puntiform, and irregular with medium size, wavy and/or even margin, opaque nature and colour varying from creamy white to light yellow.

3.2. Biochemical characterization of microbes

In the sludge samples, most of the samples with and without heat shock had shown negative results to oxidase, indole and citrate test. Even the urease, MR, VP and Coagulase test also showed maximum negative results. The sludge samples without heat shock through the biochemical test suggested the microbes to be *Salmonella Spp*., *Escherichia Spp*., *Pseudomonas Spp*., *Alcaligens Spp., Streptococcus Spp., Bacillus Spp.* and *Proteus Spp*. Similarly, the sludge samples with heat shock through the morphological and biochemical analysis were suggested to be *Bacillus Spp., Escherichia Spp., Staphylococcus Spp., Salmonella Spp. and Klebsiella Spp.*.

In the waste water samples, most of the samples with and without heat shock had shown negative results to oxidase, indole, urease and citrate test. Even the catalase, MR, VP and Coagulase test showed maximum positive results. The waste water samples without heat shock through the biochemical test suggested the microbes to be *Salmonella Spp*., *Escherichia Spp*., *Klebsiella Spp*. and *Shigella Spp*. Similarly, the waste water samples with heat shock through the morphological and biochemical analysis were suggested to be *Bacillus Spp*., *Escherichia Spp*., *Staphylococcus Spp*., *Streptococcus Spp. and Klebsiella Spp.*

3.3. **Physio-chemical analysis of waste water and sludge samples**

For understanding biochemical potency of the collected water and sludge samples, certain physico-chemical parameters like color, pH, Temperature, Electric Conductivity (EC), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Dissolve Solids (TDS), Total solid (TS), Hardness, Chemical Oxygen Demand (COD), Nitrate, phosphate, sulphate, total chloride and total kjeldahl nitrogen (TKN) were estimated (Table 2, Figure 1). For sludge samples, only colour, pH, temperature, electric conductivity (EC), total solid (TS), organic carbon and total kjeldahl nitrogen (TKN) were estimated (Table 3, Figure 2).

Though several traditional mechanisms are available for properly treating the waste relinquished from industrial, farm and domestic sectors; still due to the poor waste disposal techniques and negligence and greed of human beings, maximum of the toxic pollutants remain unresolved or form intermediate substances. With light brown colour of the wastewater system, it can be opined that the pollutants are affecting the water bodies quite recently; grey colour depicts certain degree of degradation and dark black depicts the septic nature of the wastewater due to formation of complexes of sulphides[10]. Compounds like indole, hydrogen sulphide and mercaptan are being developed in industrial sector in anaerobic conditions and are responsible for foul odour. In our study, the color of collected waste water samples from tank 1 showed dark yellow water color, the tank 2, water was found to be light yellow in color. Similarly, the tank 3 water was found to be colorless, as depicted in CPCB standard guidelines. Similarly, the colour of collected sludge samples, from tank 1 showed dark blackish sludge color, the tank 2 water was found to be Blackish/Brownish in color. The significant difference in the waste water treatment tank was clearly depicted through change in its color and the P value was found to be significant ($P < 0.0014$) for the tank 3 waste water sample at 95% confidence interval. Similarly, when the normal pH range for a standard water sample was found to be 6-8.5; all the experimentally analyzed waste water samples showed pH value in a range of 6.8-7.233. While the normal pH ranges for a standard sludge samples showed pH value in a range of 6.75-7.21. From the selected samples, all showed basic pH. When temperature of all the experimental samples was measured, mostly each sample had slightly higher temperature than the normal range (25-30°C). The waste water sample collected from tank 1 had a temperature of 30.37°C, while tank 2 waste water samples with 29.8°C and finally the tank 3 with 28.13°C. The P value was found to be $P = 0.0027$ at 95% confidence level. Considering the electric conductivity, it was found to be in a range of 715.33-803.33 s/cm. The electric conductivity for waste water was found to be 750 s/cm (described as permissible conductivity as depicted in CPCB, India) and that of sludge sample was in the range of 761-748 s/cm. Significant variation at $P < 0.0014$ was being observed.

Table-2: Physico-chemical analysis of waste water samples

Physicochemical Parameters	Permissible limit	TANK 1	TANK 2	TANK 3
pH	6-8.5	6.8±0.1	7±0.1**	7.233± 0.057**
Colour	Colorless	Dark Yellow	Light Yellow	Colorless
Temperature (°C)	25-30°C	30.37±0.15**	29.8.±0.35 **	28.13±0.25 **
Electrical Conductivity (EC) (s/cm)	750	803.33±10.41***	748.33±3.51	715.33±4.51***
Turbidity (NTU)	1-5	8.34±1.05***	5.4±1.5***	1.71±0.34
Hardness (mg L-1)	100	200±1.6***	106±5.3***	95±5***
BOD (mg L-1)	30	43.33±5.77****	34.7±5.77	24.33±3.05 ****
COD (mg L-1)	50	62.33±11.7****	48.33±5.7 ****	42.24±4.77 ****
Total Chloride (mg L-1)	250	270±4	176.87±6.4	125±1.4
TDS (mg L-1)	50	58±2***	46±5.3***	36.7±6.6***
TS (mg L-1)	100	100±20	91.3±6.51	78±3.6
TSS (mg L-1)	20	32.33±0.6	21.7±1.15	15±5
Nitrate	45	48.33±3.51	40.67±1.53	37.33±1.53
Sulphate	200	39.52±2.2	29.82±0.12	20.9±0.03
TKN (mg L-1)	20	32.82±0.02	17.77±0.3	1.503±0.015

**** $P < 0.0001$; *** $P < 0.0014$; ** $P = 0.0027$

Most of the microorganisms facilitate the degradation process of several pollutants and thereby lowering the BOD, COD, TDS amounts within the swage through the application of numerous metabolic changes occurring within the microbial cells due to development of certain metabolic enzymes within them.[17] Several widely accepted methodologies for wastewater treatment systems include Sewage Treatment Plants (STP), Effluent Treatment Plants (ETP) and Combined Effluent Treatment Plants (CETP).[16] Wastewater treatment is an important initiative which has to be taken more seriously for the betterment of the society and our future.[19,20] The COD removal rate along with Moving Bed Biofilm Reactor (MBBR) reactor activity rate is found to be interlinked inversely.[21] Even MBBR organic loading rates per carrier area (gm COD/m²Xd) also affect the MBBR reactor efficiency. Larger area elevates the MBBR efficiency.[22]

Biological oxygen demand generally indicates the organic waste present in water samples. When BOD amount in the collected waste water samples were analyzed, tank 1 water samples showed 43.33mg/l of BOD value, while tank 2 samples showed 34.7mg/l of biologically dissolved oxygen and tank 3 samples showed the lowest values of 24.33mg/l of dissolved oxygen. Due to deposition of toxic metals in waste water samples of tank 1 in more amounts, hence BOD value in this waste water sample was found to be highest than the permissible amount of 30mg/l. Significant variation at $P < 0.0001$ was being observed. COD is the measure to check oxygen amount utilized by reactions in aqueous solution. Range of COD in collected waste water samples ranged from 62.33-42.24 mg/L and as permissible amount of 50 mg/L. Significant variation at $P < 0.0001$ was being observed. Even the results were corroborated with other studies having similar results. During the use of urban wastewater, COD value was found to be 600 mg/L with generation of 1.67–2.33 kWh/m3 energy for hydraulic retention time of 18-192 h.

Total Dissolved Solids basically denotes amount or types of different minerals present in aqueous solution. Range of TDS in collected waste water samples ranged from 58-36.7 mg/L. Significant variation at $P < 0.0014$ was being observed. In the present study the hardness values in waste water samples ranged from 200-95 mg/L. Similarly, total hardness amount was found to be 200 mg/L in tank 1 waste water samples, while tank 2 samples showed 106 mg/L of hardness value. Similarly tank 3 samples showed 95 mg/L of total hardness values. The results depicted significant P value ($P < 0.0014$) for the tank 3 waste water sample at 95% confidence interval.

The total kjeldahl nitrogen (TKN) basically denotes amount of both organic and inorganic nitrogen values present in aqueous solution. Range of TKN in collected waste water samples ranged from 1.503-32.82 mg/L and sludge samples ranged from 13.87-12.5 mg/L. While tank 1 waste water samples showed 32.82 mg/L of TKN value, tank 2 samples showed 17.77 mg/L and finally tank 3 samples showed 1.503 mg/L of TKN. Similarly tank 1 sludge samples showed 13.87 mg/L of TKN value, tank 2 samples showed 12.5 mg/L of TKN. All the results depicted significant P value ($P < 0.0004$). For microbial mediated remediation of nitrogenous waste several steps are being followed within the bacterial fuel cells including ammonia passage through the proton

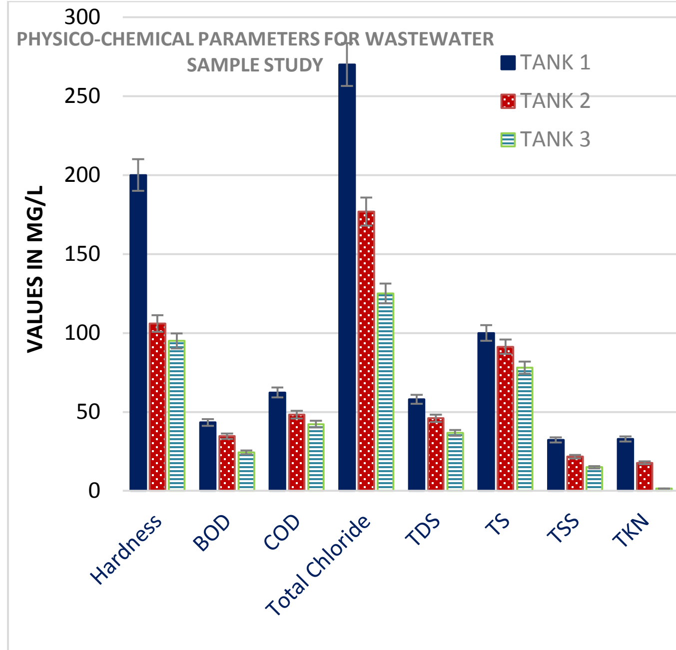

Figure 1. Physico-Chemical analysis of collected waste water samples from different sites

exchange membrane actively in form of NH_4^+, conversion of ammonium to ammonia, microbial mediated nitrification for conversion of ammonium into nitrogen via nitrate production and ammonium incorporation in substrate biomass to facilitate the microbial growth. In the case of nitrate reduction through bacterial fuel cells, the electron is directly diffused towards the denitrifying bacteria. In our study both nitrate content and kjeldahl nitrogen content was evaluated for both waste water and sludge samples. The total kjeldahl nitrogen (TKN) basically denotes amount of both organic and inorganic nitrogen values present in aqueous solution.

Table 3: Physico-chemical analysis of sludge samples

Physicochemical Parameters	Permissible limit	TANK 1	TANK 2
pH	6-8.5	7.21±0.06	6.747±0.015
Colour	colourless	Dark Blackish	Blackish/Brownish
Electrical Conductivity (EC) (s/cm)	750	761±0.17	748±2.82
Total Solid Content (Moisture Content)	100	99.25±0.55****	95.52±0.02****
TKN	20	130.9±0.67***	120.5±0.44***

**** P < 0.0001; *** P < 0.0014; ** P = 0.0027

Total solid content generally indicates the solid present in sludge samples. When total solid content amount in the collected sludge samples were analyzed, tank 1 sludge samples showed 99.2 of total solid content value, while tank 2 samples showed 95.5 of total solid content. Due to deposition of organic and minerals in sludge samples of tank 1 in more amounts, hence total solid content value in this sludge sample was found to be highest. Similarly, the total solid (TS) value in collected waste water was found to be in a range of 100-78 mg/L. All the results depicted significant P value ($P < 0.0004$). Similarly, total suspended solid (TSS) basically denotes amount of soluble and insoluble solute values. The range of TSS in collected waste water samples ranged from 32.33-15 mg/L. Turbidity generally indicates the presence of both organic and inorganic matters in water samples. When turbidity amount in the collected waste water samples were analyzed, tank 1 waste water sample showed 8.34 NTU of turbidity value, while tank 2 waste water samples showed 5.4 NTU of turbidity while tank 3 samples showed the lowest values of 1.71 NTU of turbidity.

Chloride, phosphate, sulphate and Nitrate concentration is vital to check the quality of waste water samples. In the present study the Chloride concentration in waste water samples ranged from 270-125 mg/L. While tank 1 waste water samples showed 270 mg/L of Chloride concentration, tank 2 samples showed 176.87 mg/L of Chloride concentration and finally tank 3 waste water samples showed 125mg/L of Chloride concentration. The value of total chloride in each tank was found to be within the standard range. In the present study the sulphate concentration in waste water samples ranged from 131.5-125 mg/L. The value of total sulphate in each tank was found to be below the standard range. Similarly, the Nitrate concentration in waste water samples ranged from 48.33-37.33 mg/L. While tank 1 waste water samples showed minimum 48.33 mg/L of nitrate concentration, tank 2 samples showed 40.67 mg/l of nitrate concentration and finally tank 3 samples showed 37.33 mg/L of nitrate concentration. According to the methodology utilised for phosphate removal include removal of phosphate in the cathode chamber from the

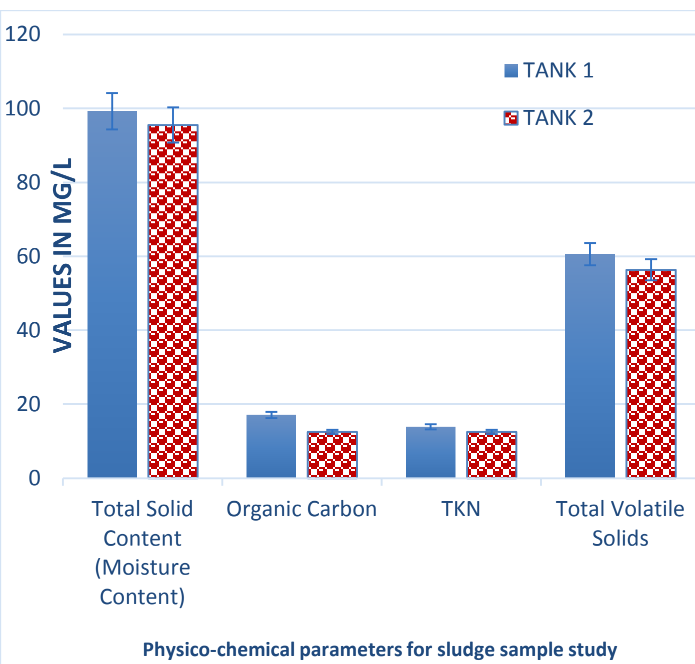

Figure 2. Physico-Chemical analysis of collected sludge samples from different sites

gradual break down of iron phosphate liberated from semi hydrolysed sewage directly or through methylene blue followed by electron release to anode chamber[23]. Considering the values of phosphate content in waste water samples in our study, it ranged in between 1.882-0.525 mg/L considering the values of phosphate content in waste water samples, it ranged in between 1.882-0.525 mg/L Chloride, phosphate, sulphate and nitrate concentration is vital to check the quality of waste water samples. In major anaerobic conditions, microbes utilised these sulphates in replacing the oxygen to facilitate the production of hydrogen sulphide. These hydrogen sulphides are toxic to fishes and in un-ionised form can accumulate toxicants within them and in water bodies also.

Organic carbon is the measure to check carbon amount present in sludge. Range of organic carbon in collected sludge samples ranged from 12.5-17.1. While tank 1 sludge samples showed 17.1 of organic carbon value, tank 2 samples showed 12.5 organic carbons. In the present study the total volatile solid values in sludge samples ranged from 56.35-60.61 mg/L. The amount of increased waste water resources, the increased organic matter content within them, it's easy availability and the higher efficacy of microorganisms in response to metabolically degrade them readily has paved the path for two way benefits from one targeted source. At one point the waste water can be treated effectively with remediation of produced toxic pollutants. The systematic understanding of bioremediation of toxicants within waste water depicts their ability in degradation of the pollutants through their physiological activities, bio-compounds produced and many more.[24,25] The bacterial cells facilitate not only in bioelectricity generation, but also enhance the removal of several toxic pollutant removals from the waste water along with nitrogenous, sulphur related toxicant removal.[26,27]

Conclusion

The use of traditional methods for treatment by using physicochemical methods is unfavorable because of its financial ineffectiveness as well as for addition of secondary pollutants to the water bodies. Bioremediation which is the process for treatment of toxic pollutants from the environment through the utilisation of plants, microbes and microbial products is effective in terms of its financial as well as eco-friendly nature. The present study is used for identification of available microbial consortia in both waste water and sludge samples. The study showed the presence of microbes such as *Salmonella Spp.*, *Escherichia Spp.*, *Pseudomonas Spp.*, *Staphylococcus Spp.*, *Streptococcus Spp.*, *Klebsiella Spp.*, and *Bacillus Spp.* found commonly in both waste water and sludge samples. It also demonstrated certain physico-chemical parameter estimation of selected Sewage samples, collected from sewage treatment plant, Jaipur, Rajasthan. The study showed the efficiency of microbial fuel cells as samples collected from different chamber compartments have showed significant reduction in many physico-chemical parameters such as BOD, COD, hardness, organic carbon, nitrate, chloride, total suspended solid, turbidity etc.

Acknowledgements

Authors are thankful to the college for providing necessary facilities to carry out this research.

Conflict of interest

The authors declare that they have no conflict of interest.

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