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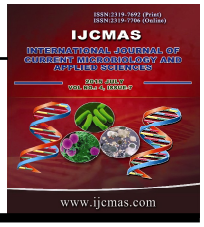

Original Research Article

Bacteriological and Physico-Chemical Assessment of Municipal Wastewater from Buldana District, India

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Keywords

Municipal wastewater, Isolation, Physico-chemical analysis, BOD, COD

A B S T R A C T

The present study was conducted for bacteriological and physicochemical analysis of municipal wastewater. The bacteria were isolated from municipal wastewater samples as well as sludge samples. Total 44 bacteria were isolated on nutrient agar medium. The isolated bacteria were belonging to genera of *Enterobacter*, *Bacillus*, *Paenibacillus*, *Klebsiella*, *Escherichia*, *Micrococcus*, *Salmonella*, *Pseudomonas*, *Proteus*, *Flavobacterium*, *Alcaligenes*, *Serratia*, *Aerobacter*, *Stenotrophomonas*, *Planococcus* and *Staphylococcus*. The most of them were industrially important enzyme producer whereas wastewater also contains pathogenic bacteria. The physicochemical analysis of four cities of Buldana district revealed that pH of municipal wastewater ranging from 6.7 to 7.2. The BOD and COD values were ranging from 72 mg/L to 238 mg/L and 115 mg/L to 480 mg/L respectively. The nitrate values were ranging from 44.5 mg/L to 210 mg/L. The concentration of phosphate was ranging from 0.98 mg/L to 2.1 mg/L whereas ammonical nitrogen was ranging from 28.5 mg/L to 67 mg/L. These results reveals that pH and phosphate were within the permissible limit whereas BOD, COD, ammonical nitrogen and nitrate were above the permissible limits in accordance with World Health Organization standards.

Introduction

Domestic wastewater is the water that has been used by a community and which contains all the materials added to the water during its use. It is composed of human body wastes i.e. faeces and urine together with the water used for flushing toilets and sullage. It is objectionable in appearance and hazardous in content, mainly because of the number of disease-causing pathogenic organisms (Mara, 2004).

The rapidly increasing human population and industrialization have placed tremendous pressure on the natural water resources and their quality (Sinha and Shrivastava, 1995). Because of such problems, the river water usually receives untreated sewage, domestic waste, industrial and agricultural effluents that results in pollution of several rivers in India. During the last several decades the water quality of

the Indian River has been deteriorating due to continuous discharge of industrial waste and domestic sewage (Krishnan *et al.,* 2007; Smitha *et al.,* 2007).

WHO estimated that about 80% of water pollution in developing country, like India is carried by domestic wastes. The improper management of water systems may cause serious problems in availability and quality of water. The water quality characteristics is denoted by knowing the physicochemical parameters like pH, BOD, COD, Ammonical nitrogen, nitrate, phosphate etc. (Subba Rao and Subba Rao, 1995).

In India, municipal and industrial waste is not properly treated, especially excreta and other liquid waste from households and the community which leads to a serious health hazard and the spread of infectious diseases which ultimately leads to unhygienic conditions and thereby to rise in the health problems. Kulkarni *et al.* (1996) reported out break enteric fever in village Katkalamba, in Nanded district, Maharashtra in 1995. This was attributed to fecal contamination of water. Sathe *et al.* (1983) reported an explosive epidemic of typhoid fever occurred in Sangli Town in Maharashtra State, India, between December 1975 and February 1976. In 1955, an outbreak of hepatitis in Delhi, India and in 1817, major epidemic of cholera occurred in Calcutta, India (U.S. Environmental Protection Agency Washington, D.C. *PIPELINE* – Summer 7:3). The aim of present study was microbiological and physicochemical analysis of municipal wastewater.

Materials and Methods

Sample collection and site: The sludge and wastewater samples were collected for isolation of bacterial from various places from Buldana district. Zip-lock plastic bag was used for sludge sample which was taken from 4-5cm deep from the surface by the help of spatula. The wastewater samples were collected in clean polythene bottle fitted with screw caps. Both zip-lock plastic bag and spatula were autoclaved before used and polythene bottle was washed with distilled water. The wastewater samples were collected for physicochemical analysis from open drainage of four cities namely Buldana, Chikhli, Khamgaon and Mehkar of Buldana district. The samples were collected from February 2012 to April 2012 according to standard procedures from American Public Health Association (APHA, 1998). The samples collection time was from 8.00 to 10.0 am at morning.

Isolation and identification of bacterial isolates: The sludge samples and wastewater samples were serially diluted and inoculated on the nutrient agar medium by spread plate method. The plates were incubated at 37^0C for 24 h. The morphologically different colonies were isolated and purified and subculture on nutrient agar slants. These slants were kept at 4^0 C for preservation. Bacterial strains were examined for their colony and cell morphology, motility, Gram and spore staining and standard biochemical tests such as Catalase, Oxidase, IMViC tests, fermentation of carbohydrates, Nitration reduction and H_2S. Along with this, the enzyme (amylase, protease and lipases) producing activity was also checked. The isolated bacteria were identified according to Bergey's Manual of Determinative Bacteriology.

Physicochemical analysis of wastewater: The wastewater samples were collected from four cities such as Buldana, Chikhli, Mehkar and Khamgaon from Buldana district. The samples were analyzed for

various physicochemical characters such as pH, BOD, COD, ammonical nitrogen, nitrate and phosphate. The experiments were performed in triplicate sets. The physicochemical analysis was done according to APHA (1998).

Results and Discussion

In present study, total 44 bacteria were isolated on nutrient agar medium. Out of these, 21 bacteria were isolated from wastewater samples. Among these, 9 bacterial isolates were Gram positive whereas 12 bacterial isolates were Gram negative. All the 21 bacterial isolates were catalase positive. Out of 21 bacterial isolates, 11 isolates were oxidase positive whereas 10 isolates were oxidase negative. The total 10 bacterial isolates were spore former while 11 were non spore former. All the isolates were tested for enzyme production such as amylase, protease and lipases. Among these, 8 bacterial isolates were amylase producer, 13 isolates were protease producer and 11 bacterial isolates were lipase producer. These 21 bacterial isolates were *Enterobacter intermedius* (1), *B. subtilis* (2), *B. cereus* (1), *B. licheniformis* (2), *Paenibacillus* sp. (1), *K. pneumoniae* (2), *E. coli* (2), *Enterobacter* sp. (1), *Bacillus* sp. (2), *Micrococcus varians* (1), *Salmonella* sp. (1), *Klebsiella* sp. (1), *Pseudomonas* sp. (1), *Proteus* sp. (2) and *Flavobacterium* sp. (1).

In present study, total 23 bacteria were isolated from sludge samples. Among these bacterial isolates 7 isolates were Gram positive whereas 16 isolates were Gram negative. All the isolated 23 bacteria were catalase positive. Among 23 bacterial isolates, 11 isolates were oxidase positive whereas 12 isolates were oxidase negative. The total 8 bacterial isolates were spore former while 15 were non spore former. Out of these 23 bacterial isolates, 6 bacterial isolates were amylase producer, 19 isolates were protease producer and 20 bacterial isolates were lipase producer. The 23 bacterial isolates were *B. cereus* (2), *Paenibacillus borealis* (1), *Alcaligenes faecalis* (1), *E. coli* (1), *Serratia ficaria* (1), *Serratia liquefaciens* (1), *Enterobacter aerogenes* (1), *Bacillus* sp. (1), *Aerobacter hydrophilia* (2), *Pseudomonas aeruginosa* (1), *Stenotrophomonas maltophilia* (1), *Planococcus salinarum* (1), *Alcaligenes* sp. (1), *Klebsiella* sp. (1), *Pseudomonas* sp. (2), *S. aureus* (3), *S. epidermidis* (1) and *Proteus* sp. (1).

Wan Ishak *et al.* (2011) isolated *B. licheniformis* from activated sludge and compost for municipal solid waste treatment system. Al-Zubeiry (2005) reported isolation of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, E. coli* and *Salmonella* sp. from raw sewage, secondary effluent and dewatered sludge. Similarly Garode and Sonune (2014) reported *Paenibacillus borealis* isolated from domestic wastewater. Many researchers (Mendes and Nascimento, 1991; Mitchel, 1976; Ponamareva *et al.,* 1994; Wilcox *et al.,* 1983) reported that the activated sludge from municipal and industrial wastewater contain great number of microorganisms and the genera found most frequently are *Pseudomonas, Bacillus, Achromobacter, Enterococcus, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Escherichia, Salmonella, Proteus, Streptococcus, Staphylococcus, Micrococcus, Corynebacterium, Clostridium* and *Penicillium.*

In present study most of the bacterial isolates showed enzyme producing ability such as amylase protease and lipases. Such enzyme producing bacterial isolates are industrially important as well as they may be

used for bioremediation of municipal wastewater. In our study, *B. subtilis*, *B. cereus*, *B. licheniformis*, *Paenibacillus* sp. and *Pseudomonas* sp. etc were found to be enzyme producer. Garode and Sonune (2013) also reported amylase producing *Bacillus* sp. and *Paenibacillus* sp. from domestic wastewater. Similarly Mobarak-Qamsari *et al.* (2011) also reported lipase producing *Pseudomonas aeruginosa* from wastewater of an oil processing plant.

Physicochemical analysis of wastewater from Buldana city:

Total five samples were collected from Buldana city. The sampling was done at alternate days. The results from table1 suggested that pH of wastewater from Buldana city was ranging from 6.8±0.01 to 7.0±0.12 and it was within the permissible limit. The lowest value of BOD was 76±9.96 mg/L whereas highest value was 144±18 mg/L. All the BOD values were above the permissible limit. The COD values were ranging from 134±13.0 mg/L to 264±22.27 mg/L. Out of the five samples one sample showed high COD value than permissible limit and it was 264±22.27 mg/L. The other COD values were within the permissible limit showed in table 1. The ammonical nitrogen was ranging from 46±14.18 mg/L to 67±12.0 mg/L. Out of the five samples; two samples showed less concentration than permissible limits i.e. 46±14.18 mg/L and 48±4.0 mg/L. The remaining three samples showed higher concentration than permissible limit and they were 67±12.0 mg/L, 55.5± 19.37 mg/L and 66.5±10.04 mg/L. The nitrate was ranging from 96.5±13.44 mg/L to 166±5.0 mg/L. All the nitrate values were above the permissible limit. The phosphate values of all samples were below the permissible limit and ranging from 1.087±0.23 mg/L to 2.05±0.11 mg/L.

Physicochemical analysis of wastewater from Chikhli city:

The samples from Chikhli city was done at alternate days and total five samples were collected and analyzed for their physicochemical characters. The physicochemical results of municipal wastewater from Chikhli were showed in table 2.

The pH of wastewater from Chikhli were ranging from 6.8±0.01to 6.9±0.02. All the five values of pH were below the permissible limit. The BOD values of all samples were above the permissible limit. The BOD was ranging from 78±5.88 mg/L to 136±10.2 mg/L. The minimum value of COD was 115±9.55 mg/L whereas the maximum value was 261±16.34 mg/L. Out of the five samples, one showed higher value the permissible limit and it was 261±16.34 mg/L. The other four samples showed less value than permissible limit and they were 156±24.52 mg/L, 215±12.54 mg/L, 220±25.14 mg/L and 115±9.55 mg/L. The ammonical nitrogen was ranging from 34±6.8 mg/L to 54±1.2 mg/L. In these cases, one value was above the permissible limit i.e. 54±1.2 mg/L whereas remaining four values were within the permissible limit and they were 38±5.05 mg/L, 46±4.54 mg/L, 34±6.8 mg/L and 43±3.42 mg/L. The nitrate was ranging from 44.5±5.5 mg/L to 145±14.0 mg/L. The lowest value of nitrate was 44.5±5.5 mg/L and highest value was 145±14.0 mg/L. All the samples showed higher values of nitrate than permissible limit. The phosphate was below permissible limit and ranging from 1.01±0.08 mg/L to 2.1±0.14 mg/L.

Physicochemical analysis of wastewater from Mehkar city:

The physicochemical results of municipal wastewater were given in table 3. Total five

samples were collected from Mehkar at alternate days. The pH of the five samples was within the permissible limit, ranging from 6.7±0.05 to 7.1±0.01. The minimum value of BOD was 72±9.33 mg/L whereas maximum value was 121.5±11.66 mg/L. The BOD values of all samples were higher than permissible limit. The COD was ranging from 125±8.32 mg/L to 194±15.48 mg/L. The COD values of all samples were within the permissible limit. The ammonical nitrogen was ranging from 28.5±3.06 mg/L to 67±4.47 mg/L. Out of the five samples, two samples showed higher concentration of ammonical nitrogen than permissible limit and they were 67±4.47 mg/L and 66.5±8.77 mg/L. The remaining three samples values showed in table 3 were below the permissible limit.

Physicochemical analysis of wastewater from Khamgaon city:

The results of physicochemical analysis of municipal wastewater from Khamgaon were given in table 4. The total five samples were collected at alternate days. All the five samples showed pH value within the permissible limit, ranging from 6.7±0.02 to 7.1±0.12. The BOD and COD values of all samples were above the permissible limit. The BOD and COD values of samples were ranging from 130±11.08 mg/L to 238±35.04 mg/L and 278±24.11 mg/L to 480±38.11 mg/L respectively. The ammonical nitrogen was ranging from 41±4.87 mg/L to 66±8.91 mg/L.

The two samples showed high value of ammonical nitrogen than permissible limit whereas three showed less value than permissible limit. The nitrate was ranging from 129±8.82 mg/L to 188±18.25 mg/L. All the five samples showed higher values of nitrate than permissible limit. The minimum concentration of Phosphate was 1.45±0.21 mg/L whereas maximum concentration was 2.09±0.11mg/L. These phosphate values were within the permissible limit.

The sampling for physico-chemical analysis was done at morning from 8.00 to 10.0 am because maximum house holding activities are occurring at morning than afternoon and evening. The physicochemical analysis of four cities reveals that pH of municipal wastewater ranging from 6.7 to 7.2. These pH values were within the permissible limit in accordance with World Health Organization standards.

Table.1 Physico-chemical characteristics of municipal wastewater from Buldana

Sr. No.	pH	BOD (mg/L)	COD (mg/L)	Ammonical Nitrogen(mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	6.9±0.08	144±18	264±22.27	46±14.18	153±14.11	1.087±0.23
2	7.2±0.02	120±22.91	188±19.05	67±12.0	96.5±13.44	1.87±0.35
3	6.8±0.01	76±9.96	134±13.0	55.5±19.37	132±10.39	1.46±0.11
4	6.9±0.02	110±10.21	214±10.51	48±4.0	156.5±0.5	1.34±0.04
5	7.0±0.12	82±6.93	156±1.73	66.5±10.04	166±5.0	2.05±0.11

± = Standard Deviation

Table.2 Physico-chemical characteristics of municipal wastewater from Chikhli

Sr. No.	pH	BOD (mg/L)	COD (mg/L)	Ammonical Nitrogen(mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	6.8±0.2	127±22.08	261±16.34	38±5.05	86±12.2	1.453±0.05
2	6.8±0.11	98±8.2	156±24.52	46±4.54	122±24.66	1.22±0.02
3	6.9±0.02	124±16.04	215±12.54	34±6.8	145±14.0	1.32±0.11
4	6.8±0.04	136±10.2	220±25.14	54±1.2	94±4.8	1.01±0.08
5	6.8±0.01	78±5.88	115±9.55	43±3.42	44.5±5.5	2.1±0.14

± = Standard Deviation

Table.3 Physico-chemical characteristics of municipal wastewater from Mehkar

Sr. No.	pH	BOD (mg/L)	COD (mg/L)	Ammonical Nitrogen(mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	7.1±0.01	72±9.33	187±28.55	67±4.47	134±16.22	1.54±0.05
2	7.0±0.01	108±10.12	180±20.15	66.5±8.77	145±13.55	1.04±0.34
3	7.0±0.04	121.5±11.66	194±15.48	34±2.08	210±26.11	1.82±0.14
4	6.8±0.01	88±8.90	125±8.32	42.8±5.41	120.5±9.65	2.08±0.4
5	6.7±0.05	95.5±4.85	160.5±18.14	28.5±3.06	130±10.55	0.98±0.02

± = Standard Deviation

Table.4 Physico-chemical characteristics of municipal wastewater from Khamgaon

Sr. No.	pH	BOD (mg/L)	COD (mg/L)	Ammonical Nitrogen(mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
1	6.7±0.02	238±35.04	423±34.86	41±4.44	129±8.82	2.04±0.03
2	7.0±0.0	190±16.78	480±38.11	56±6.08	131±15.6	1.66±0.12
3	7.1±0.12	155±27.0	315±18.16	66±8.91	176±7.65	2.09±0.11
4	6.9±0.14	130±11.08	278±24.11	48±1.66	144±12.14	1.88±0.05
5	6.9±0.02	210±15.15	380±25.52	41±4.87	188±18.25	1.45±0.21

± = Standard Deviation

The permissible limit for pH is 6.00 to 9.00 of wastewater which must be discharged into the sea or environment (WHO, 2006). The pH values of our study were similar to Shirin and Yadav (2014). The BOD and COD were ranging from 72 mg/L to 238 mg/L and 115 mg/L to 480 mg/L respectively. The wastewater samples from Khamgaon city showed higher BOD and COD values as compared to Buldana, Chikhli and Mehkar city. The probable reason for the higher BOD and COD is high organic load present in wastewater by various house holding activities as higher population of the city than other cities.

Kandhasamy and Santhaguru (1994) reported that the higher BOD may be due to higher organic load. In our study, BOD and COD values were similar to Dubey (2013) and Shirin and Yadav (2014). The nitrate was ranging from 44.5 mg/L to 210 mg/L. Our nitrate values were similar to Singh *et al.*, (2012) and higher than Dubey (2013).

The phosphate values were ranging from 0.98 mg/L to 2.1 mg/L. In present study, our phosphate values were similar with Sarkinnoma *et al.,* (2013) and Sonune *et al.* (2015). The ammonical nitrogen was ranging from 28.5 mg/L to 67 mg/L. Similar results were showed by Binu Kumari *et al.* (2006). The high organic load and its degradation resulted in marked increase in ammonical nitrogen (Das *et al.,* 2003)

The present study reveals that municipal wastewater from Buldana district contains various pathogenic bacterial genera such as *Klebsiella*, *Escherichia*, *Salmonella*, *Proteus* etc. The results of physicochemical analysis of municipal wastewater from four cities showed that pH and phosphate were within the permissible limit in accordance with World Health Organization standards whereas the BOD, COD, ammonical nitrogen and nitrate were above the permissible limits, indicating high organic load present in the wastewater. Such wastewater should required treatment before discharged it into environment.

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