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EFFICIENCY OF INDICATOR BACTERIA REMOVAL IN A WASTEWATER TREATMENT PLANT (ALGIERS, ALGERIA)

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Abstract
In order to preserve the quality of water masses and to reduce the natural environment deterioration, alternative water supplies should be required. The reuse of treated wastewater seems to be a good alternative. The objective of this study was to determine the pollution level of treated wastewater in Algiers region and its impact on aquatic environment. Water samples were taken at the inlet and outlet of the wastewater treatment plant (WWTP). The average concentrations decreased as follows: BOD from 60±7 $mg.L^{-1}$ to 3.1±0.7 $mg.L^{-1}$ and COD from 219±20 $mg.L^{-1}$ to 31±1 $mg.L^{-1}$. In the case of median values for microbial indicators, Total Coliform (TC) was decreased from $19.86x10^6$ to $0.986x10^6$ MPN.100 mL^{-1}, Faecal Coliform (FC) from $10.50x10^6$ to $0.401x10^6$ CFU.100 mL^{-1}, Faecal Streptococci (FS) from $11.62x10^6$ to $0.517x10^6$ MPN.100 mL^{-1}, spores of Sulfite Reducing Anaerobic Bacteria (SRA) from $79x10^3$ to $6.3x10^3$, *Pseudomonas aeruginosa* from $65x10^3$ to $2.8x10^3$ and *Staphylococcus aureus* from $80x10^3$ to $5x10^3$ CFU.100 mL^{-1}. Based on median values before and after purification, the overall performance of the wastewater treatment by activated sludge have permitted effectively removing of BOD_5 (94.7%), COD (85.4%), FS (95.7%), *Pseudomonas aeruginosa* (95.7%), TC (95.1%), FC (95.0%), SRA (93.0%) and *Staphylococcus aureus* (91.9%).

Keywords: Waste water treatment plant, microbiological analysis, removals efficiencies, effluent discharge standard, Algiers-Algeria.

1. INTRODUCTION

Population growth has resulted in increased water demand and greater contamination of water from the disposal of waters in many parts of the world, including Algeria. The water is one of the most privileged vehicles in the disease transmission to men. A major source of water pollution around the world is the discharge of inadequately treated domestic wastewater. This may cause severe problems such as anoxia and eutrophication in the recipient and a risk for spreading of diseases. One of the priorities in the treatment of wastewater is the removal of pathogenic microorganisms in order to comply with the required discharge standards for the treated effluent. Despite access to improved sanitation systems, most of these wastewaters collected through the sewer systems are inadequately treated, and discharge significant amounts of faecal pollution indicators and pathogenic microorganisms into the receiving water bodies, leading to a reduction in the quality of the water sources (Bahlaoui et al. 1997, Momba & Mfenyana, 2005). A more serious concern is that these water sources are used by communities for multiple purposes, which include drinking, recreational and agricultural purposes (Toze, 2004; Momba et al. 2006). Monitoring the quality of wastewater effluent before the discharge into the receiving water body might therefore assist water services authorities to trace the origin of an epidemic. Total coliforms (TC) and faecal coliforms (FC) are used as indicators of the possible presence of viral or bacterial pathogens in the effluent and receiving waters (Bitton, 2005). Coliform bacteria are reliable indicator of organic pollution because they are unable to survive in clean water beyond a limited time (Hiraishi et al. 1984). Coliform (FC or faecal streptococci) removal efficiencies in wastewater treatment plants are utilized as indicators of the ability of the process to effectively remove pathogenic viruses and bacteria (Kantachote et al. 2009).

For the enumeration of coliforms and enterococci, Colilert® and Enterolert®, had been developed, respectively. The MPN method is facilitated by use of a specially designed disposable incubation tray called the Quanti-Tray®. These methods require significantly less time than the MF (membrane filter) procedure and less quality control testing (Budnick et al. 1996). In 2009, it was adopted in Algeria by SEAAL (Société des Eaux et de l'Assainissement d'Alger). Two nutrient-indicators, ortho-nitrophenyl galactopyranoside (ONPG) and 4-methyl-umbelliferyl-glucuronide (MUG) are the major sources of carbon in Colilert, and can be metabolized by the coliform enzyme β-galactosidase and the *E. coli* enzyme β-glucuronidase, respectively. As coliforms grow in Colilert, they use β-galactosidase to metabolize ONPG and change it from colourless to yellow. *E. coli* use β-glucuronidase to metabolize MUG and create fluorescence (IDEXX Laboratories). The Enterolert test utilizes a nutrient indicator substrate, 4-methylumbelliferone- β-D-glucoside, that fluoresces when metabolized by enterococci. Methylumbelliferyl derivatives have the

advantage of being highly sensitive and specific, non-carcinogenic, and easily detected with UV light sources (Budnick et al. 1996).

The isolation of *Salmonella sp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Sulphite Reducing Anaerobic Bacteria (SRA) and *Vibrio* was done using standard Methods.

The present study aimed at assessing for the first time the effectiveness of Béni Messous wastewater treatment plant in the removal of faecal and pathogenic bacteria and the impacts of discharged effluents in the receiving water body.

2. MATERIEL AND METHODS

2.1. Study site

Monitored wastewater treatment plant namely Béni Messous is located at 20 km east of Algiers. Mechanical-biological wastewater treatment plant with sludge stabilizing tank started in 2007. The treatment plant was connected for Staouéli, Béni Messous, Chéraga, Ain Benian and one part of Dély Brahim. The wastewater plant contains primary and secondary levels. The primary level consists of a slot sand trap, coarse screen, storm tank and combined shallow reservoir. The secondary one is formed by the sludge stabilization tank. After treatment, the water is released into the Béni Messous River that joined Mediterranean Sea. The treatment capacity of the WWTP was about 50400 m^3 day^{-1} (Abdessemed et al. 2009).

2.2. Sampling and Microbiological Analysis

Sampling was done from February to August 2013 totalizing 12 samplings. Raw influent and final effluent samples were collected in 1 L sterile bottles and analyzed for the target micro-organisms using internationally accepted techniques. These wastewater samples were serially diluted in sterile water.

Total coliforms, *E. coli* and enterococci were enumerated by means of Colilert® and Enterolert® methods. One package of powdered Colilert or Enterolert reagent was then added to the vessel, and the sample-reagent combination was mixed and then poured into a Quanti-Tray®, a sterile plastic disposable panel containing 51 wells. The trays were then mechanically sealed, distributing the mixture into the wells, and the results read after 18h incubation at 35 ± 0.5 ºC for Colilert® and after 24 h incubation at 41 ± 0.5 ºC for Enterolert®. For Colilert®, yellow wells were interpreted as positive for coliforms (Fig. 1), then, the Quanti-Trays were checked for *E. coli*, in a dark environment by placing it under a 365 nm wavelength UV light (Fig. 2). Any fluorescence in a well was considered a positive reaction for that well and thus indicating the presence of *E. coli* on the Colilert test. The Enterolert® trays were also observed under the UV light and the fluorescent wells were considered positive for enterococci (Fig. 3). Based on the number of positive wells and the dilution factor, MNP tables are used to calculate coliforms, *E. coli* and enterococci density per 100 mL of sample.

For the isolation of *Pseudomonas aeruginosa*, water samples were filtered through a Millipore MF Filter. The filter was placed on the selective medium *Pseudomonas* cetrimide agar (PCA) using a spreading technique. Plates were incubated for 44 ± 4 hours and observed for suspected colonies of *P. aeruginosa.* Identification of *P. aeruginosa* was done by the routine bacteriological methods including colonial morphology, Gram stain, reactions on TSI agar, motility test, and catalase and oxidase tests incubated at 36 ºC for 48 h, according to the Standard Methods (UNE-EN 12780, International Standardisation Organisation (ISO) ISO 16266). Suspected colonies that showed the characteristic appearance and colour were inoculated on to cetrimide kanamycin nalidixic acid agar (CKNA) and incubated at 42 °C overnight. Isolates that showed growth were preliminarily identified as putative *Pseudomonas aeruginosa* (Khan et al. 2007). A single colony was selected for biochemical tests. *P. aeruginosa* is oxidase positive, hydrolyses casein, and produces pyocyanin and/or fluorescence. Occasionally a non-pigmented variant of *P. aeruginosa* can occur, therefore a pyocyanin negative, casein hydrolysis positive, fluorescence positive culture can also be regarded as *P. aeruginosa*. Pyocyanin non-producing colonies which fluoresce in the UV light (360 nm) were tryptone-soy agar cultivate to be identified by (+) oxidase test, (+) production of ammonia from acetamide, (+) fluorescence on King`s B medium, selective growth capacity at different temperatures (4, 22, 37 and 42 °C) (Fig. 4).

Clostridium were quantified by incorporation in plate count agar and beef liver agar, respectively, and incubated at 37 °C for 24 h (NF EN 26461-2 ISO 6461-2). The results are expressed in Colony Forming Units (CFU) per 100 mL.

Water samples were filtered through 0.45 micron filter paper, after filtration filter paper was aseptically transfer on Hectoen agar for the pathogens *Salmonella* and solid Chapman media for *Staphylococcus* (NF T 90 421). The *Staphylococcus* gender was subjected to biochemical identification tests by means of evidences such as coagulase and catalase (Fig. 5).
Isolation of *Vibrio* was analyzed following the procedure described in the ISO/TS 21872–1 by enrichment in alkaline peptone broth (pH 8.5) for 6–8 h at 37 °C.

The COD was determined by the method of potassium dichromate oxidizability (AFNOR, ISO 6060). The BOD was determined by method NF, ISO 5815 with a BOD-meter (SKALAR SP50). The removal efficiency of bacterial indicators was calculated using the following formula Kantachote et al. (2009):

$$\text{Removalefficiency} = \frac{\text{Number in influent - Number in effluentbrineff}}{\text{Number in influent}}.x100$$

2.3. Statistical analysis

The comparisons of the various parameters (before and after treatment) were performed using t-test for dependent samples; the Wilcoxon matched pairs tests were using in cases of severe violations to normality or heterogeneity of variances. The difference were considered significant at $p<0.05$. The Pearson's correlations analysis was performed to find out relationships among various characteristics.

3. RESULTS

The performance of the Wastewater Treatment Plant for removal of total coliforms, faecal coliforms, faecal streptococci, *Pseudomonas aeruginosa*, spores of Sulfite Reducing Anaerobic Bacteria (SRA), *Staphylococcus aureus*, COD and BOD_5 was studied from February to August 2013. The results from the microbiological analyses of raw and treated wastewater are detailed in Table 1.
Due to the variability of raw sewage quality, the influent contained higher average concentrations of total coliforms, faecal coliforms and faecal streptococci ($20.10x10^6$, $10.35x10^6$ and $13.47x10^6$ MPN/100 mL, respectively) but relatively lower average concentration of *Pseudomonas aeruginosa*, SRA and *S. aureus*: (84±22) $x10^3$, (171±79) $x10^3$ and (140±41) $x10^3$ CFU/100 mL, respectiveley.

Correspondingly, the treated effluent contained 952.10^3, 408.10^3 and 530.10^3 of total coliforms, feacal coliforms and faecal streptococci, respectively; and comparatively lower concentrations of *Pseudomonas aeruginosa,* SRA and *S. aureus* ($3.2x10^3$, $10.7x10^3$ and $10.9x10^3$ CFU/100 mL, respectively).
The pathogens such as *Salmonella sp.* and *Vibrio cholerae* were not detected in this study. There were probably not infected individuals in this area.

Table 1. Comparison between influent and effluent data using t-test or Wilcoxon Matched Pairs tests for Wastewater Treatment Plant of Béni Messous

Parameters		Min ($x10^6$)	Max ($x10^6$)	Median ($x10^6$)	Average+SE ($x10^6$)	Removal (%)	p
TC	Influent	10.46	24.20	19.86	20.10±1.27		
(MPN/100 mL)	Effluent	0.060	1.73	0.986	0.952±0.114	95.1	<0.0001
FC	Influent	0.130	24.20	10.50	10.35±2.06		
(MPN/100 mL)	Effluent	0.012	0.980	0.401	0.408±0.833	95.0	0.0004
FS	Influent	3.44	24.20	11.62	13.47±2.16		
(MPN/100 mL)	Effluent	0.064	0.870	0.517	0.530±0.074	95.7	<0.0001
		Min ($x10^3$)	Max ($x10^3$)	Median ($x10^3$)	Average+SE ($x10^3$)		
Pseudomonas aeruginosa	Influent	0	220	65	84±22		
(CFU/100 mL)	Effluent	0	9	2.8	3.2±0.9	95.7	0.0033
SRA	Influent	0	970	79	171±79		
(CFU/100 mL)	Effluent	0	66	6.3	10.7±5.2	93.0	0.0077

S. aureus (CFU/100 mL)	Influent	0	340	80	140±41		
	Effluent	0	26	5	10.9±3.0	91.9	0.0069
		Min	Max	Median	Average+SE		
COD ($mg.L^{-1}$)	Influent	129	390	217	219±20		
	Effluent	29	38	29	31±1	85.4	0.0022
BOD_5 ($mg.L^{-1}$)	Influent	25	110	56	60±7		
	Effluent	0.8	9.2	2.7	3.1±0.7	94.7	0.0033

TC: Total coliforms; FC: Faecal coliforms; FS: Faecal streptococci ; SRA: Spores of Sulfite Reducing Anaerobic Bacteria. *S. aureus*: *Staphylococcus aureus*

The results showed significant differences in mean values of microbial load (total coliforms, faecal coliforms and faecal streptococci) between wastewater (influent) and treated wastewater (effluent) from the sewage treatment plant.

The total coliform was significantly lower ($t=15.9$, $p<<0.05$) in effluent in comparison to raw. The other microbial indicators such as faecal coliform and faecal streptococci showed significantly lower values for effluent in comparison to the raw sewage ($t=4.95$ and 6.12, respectively, $p<<0.05$, Table 1).

The comparison of percentage efficiency rate showed significant differences among different microbial parameters. Removal efficiency rate was greater for faecal streptococci (95.7%) and *Pseudomonas aeruginosa* (95.7%) and least in *Staphylococcus aureus* (91.9%) (Table 1).

The values of COD and BOD are shown in Table 1. The influent had an average COD and BOD concentrations of 219±20 and 60±7 $mg.L^{-1}$, respectively. The effluent samples collected show moderate COD and BOD mean values of 31±1 and 3.1±0.7 $mg.L^{-1}$ respectively.

It is important here to note that low BOD content is an indicator of good quality water, while a high BOD indicates polluted water. BOD directly affects the amount of dissolved oxygen (DO) in rivers and streams.

The COD/BOD_5 ratio reflects the degree of treatment the wastewater has undergone. COD/BOD_5 ratio in the range from 3 to 7 indicated that the wastewater was moderately biodegradable. The influent wastewater of Béni Messous STP exhibited a COD/BOD_5 ratio range 2.4-6.7 (mean 3.9±0.5) before purification to ratio range 3.3-34.5 (mean 15.1±2.8) after purification indicating that the biodegradability of the effluent wastewater was reduced due to enhanced biological organic matter removal by the aerobic degradation process, that was involved in the facultative and aerobic ponds. Cumulative BOD reduction in the facultative and aerobic ponds reached 94.7%, in comparison to 85.4% reduction of COD which indicates that the wastewater was treated sufficiently.

The comparison between the average levels of BOD, COD, SRA, *Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from the influent and treated effluent of the Béni Messous wastewater treatment plant using the Wilcoxon matched pairs tests at significant level of 0.05 indicated that their removal was statistically significant (Table 1).

In the effluent samples, it was observed that in all samples collected, the COD and the BOD_5 values were very less than the maximum permissible limits according to WHO Standard (90 and 30 $mg.L^{-1}$ respectively) for the discharged of effluent into aquatic system, while they were much higher in influent. This indicated efficient removal from the STP wastewater.

The statistical analysis for correlations between COD and BOD_5 properties and indicator bacteria showed that SF exhibited a negative correlation with BOD_5 ($r=-0.66$, $p=0.028$) in influent. However, Faecal Coliform showed negative correlation ($r=-0.59$, $p=0.042$) with COD in effluent.

4. DISCUSSION

The removal efficiencies of 94.7% and 85.4% for BOD_5 and COD respectively, were reported by Mehrdadi et al. (2001), Abdessemed et al. (2009) and Sharafi et al. (2012) for activated sludge system. COD removal was not as good as BOD_5 removal. This was probably due to nature of COD which is representative of less biodegradable substances than BOD_5. In the current study, the bacterial loss significantly reduced the total microbial biomass, but did not result in a large reduction in the removal of COD. The calculation of Pearson's correlation coefficients between BOD_5, DCO and bacterial did not establish uniform pattern.

The ratio of feacal coliform: faecal streptococci (FC:FS) is an indication of the origin of contamination. A ratio 4 characterizes human faecal contamination, whereas a ratio 0.7 suggests animal

wastes (Hurst, 2002 in Tyagi et al. 2008). An average value of 0.77 was obtained as the FC:FS for the influent which was less than 4. This showed that animals were the major contributor to faecal pollution and significantly less faecal pollution was contributed by human wastes mainly due to discharges of breeding stations, runoff and leaching from agricultural land located near the Béni Messous WWTP. The FC load of treated effluents depended both on the quality of raw wastewater and the efficiency of the treatment. A rapid Colilert® and Enterolert® method was used as an alternative means to plate counts to estimate the bacteriological quality of sewage and effluent.

It is evident from the data that microbial load showed significant differences between the inlet and outlet, water samples nevertheless, these variations do not match as per WHO standards. The faecal coliform level defined in the WHO Guidelines as a water quality guideline have recommended that the acceptable guideline for irrigation with natural surface water, including river water containing wastewater discharges, be set at 1000 faecal coliforms per 100 ml (USEPA, 1973). The USEPA level is also consistent with the 1000-2000 faecal coliforms per 100 ml level used as a standard for bathing in Europe (WHO, 1989); therefore, this effluent could not be safely used for both unrestricted and restricted irrigation.

Efficiency rate showed significant differences between influent and effluent in all the microbial parameters. But the presence of pathogenic bacteria in treated wastewater effluent is a potential public health hazard, as this water source is directly discharged in receiving water bodies and may be used by communities for multiple purposes. However, the destruction of pathogens can also be achieved by chemicals stabilization, including lime, chlorine and its compound, quaternary ammonium, ozone, etc. The most common disinfectants are the oxidizing chemicals; among them chlorine is the most used (Tchobanoglous et al. 2003). So, it is essential tool to include tertiary treatment step in WWTP so that complete disinfection of effluent must be done.

The reduction of microbes also depends on settlement of suspended solids, inactivation due to sunlight, activity of bacteria and environmental conditions (Katsoyiannis & Samara, 2004). The results clearly showed that large amount of microbial load is retained even after the purification process. These results coincide with findings of Cohen & Shuval (1972); Payment et al. (2001) and Jamwal et al. (2009). Others mechanisms of microbial removal could be sedimentation (Quiñónez-Díaz et al. 2001; Karim et al. 2004) or predation (Decamp et al. 1999; Reinoso et al. 2008).

Clostridium is an important bacterial species, which sporulated in nature and survive in water for a comparatively longer period as compared to other faecal bacteria. Their presence in water is an indicator of faecal contamination of remote time (Sinha & Banerjee, 1987).

Presence of pathogenic bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* in effluent may cause acute to severe disease on getting suitable host and condition (Mishra & Tripathi, 2007).
There are few papers at present evaluating the efficiency of wastewater plant in indicator organisms and pathogenic bacteria. The data obtained in this wastewater treatment plant could be used as a baseline for other studies.

CONCLUSION

The presence of pathogenic bacteria in treated wastewater effluent is a potential public health hazard, as this water source is directly discharged in receiving water bodies and may be used by communities for multiple purposes. It is clear from our results that some amount of microbial load is retained even after the purification treatment process. So, it seems essential to include a tertiary treatment step in STP so that the purification process results in bacterial concentrations remain in compliance with discharge. Although the treatment plants succeeded in removing some presumptive pathogens from the influent, effluent discharges were only occasionally devoid of the organisms, thus constituting a potential threat of infectious diseases.

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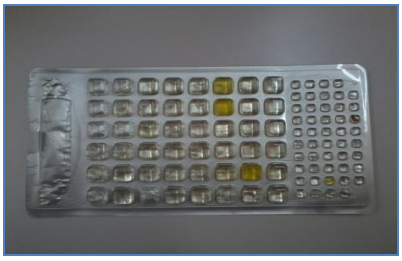

Figure 1. Enumeration of total coliforms by the method of Colilert-18.

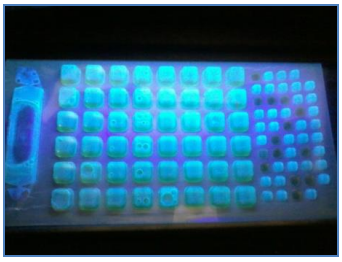

Figure2. Enumeration of faecal coliforms

Figure 3. Enumeration of Faecal streptococci by the method of Enterolert-18 (under UV)

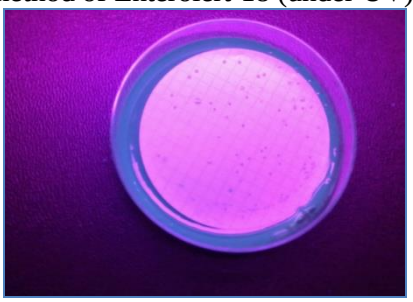

Figure 4. Results of *Pseudomonas aeruginosa*.

Figure 5. Results of *Staphylococcus aureus* with the oxidase test.

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