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Waste water process assessment and waterborne pathogens removal using bentonite filtration as an eco-friendly technique in Tlemcen city, Algeria

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ARTICLE INFO	ABSTRACT/RESUME
	Abstract: Like cities in other arid and semi-arid African countries, Tlemcen is facing population growth problems, water stress, and pollution. *This study summarises the findings from assessing a plant's role and technology in meeting the public health criteria through the waterborne pathogens identification and enumeration, a complete bacteriological analyses were performed and the count of bacteria has been compared between the three studied processes.* *Furthermore, this study is dedicated to the study of the bentonite clay technique, which consists of the targeted pollutants removal at limited cost and a better understanding and control of waste water quality.* *The results of the assessment revealed that activated sludge (ASP) treatment plant recorded the highest coliform concentrations with an average of 785.67 MPN/100 ml vs. 1.28 MPN/100 ml for sequential batch reactor (SBR) and 0 MPN/100 ml for membrane bioreactor (MBR) treated water.* *The study disclosed the MBR technology as the most efficient in the pathogenic micro-organisms removal, however, the well-known high cost of this technology compared to other processes allowed us to experiment the efficiency of the bentonite clay to get rid of all the pathogens.* *Thus, the results of influent and effluent bacterial studies, using bentonite filtering showed a total removal of all counted densities of microorganisms. The latter included total viable counts, total coliform, faecal coliform, faecal Streptococci, Salmonellae, Shigella, Vibrio, Staphylococci, and Listeria.*

I. Introduction

Increasing water scarcity in dry climate regions, with agriculture-based economies forced people to use untreated wastewater for crops irrigation. The practices in developing countries of using untreated municipal wastewater for irrigation, using raw manure as fertiliser, and the habit of eating vegetables raw or undercooked are reported to result in a risk of infection with intestinal diseases [1]. Moreover, unhygienic sewage disposal and the absence of sewage treatment facilities poses potential health hazards through contaminating irrigated food crops with pathogens in urban and suburban areas of African countries [2-5].

Wastewater characteristics play an important role in the designation of wastewater treatment facilities.

The selection of wastewater treatment processes depends on wastewater parameters, e.g. biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, suspended solids, nitrogen, phosphorous, the presence of toxic materials, and bacterial population [6]. Wastewater may contain millions of bacteria per millilitre including *coliforms*, *Streptococci*, *Staphylococci* anaerobic spore-forming bacilli, the *Proteus* group, and many other types of organisms. Wastewater is also a potential source of many human pathogenic forms including bacteria, viruses, and protozoa. In addition, certain bacterial viruses (bacteriophages) are readily isolated from wastewater [7]. These characteristics of wastewater greatly vary with the source of potable water supply, the sewerage system, the season, the nature of industrial discharge into the system, the amount of flow, and the living standards of people. Therefore, the determination of the typical microbiological load of wastewater is essential for each individual treatment works.

The present study has been conducted to assess the impact of the bentonite filtration water treatment technique to improve wastewater quality and upgrade its use in water treatment for agricultural purposes. Bentonite is a natural clay of minimal expense mined from Maghnia in Tlemcen Province, Algeria. It is produced through the weathering of basaltic rocks and made up mineralogically of montmorillonite mineral in addition to secondary amounts of other clay and non-clay minerals. Montmorillonite mineral is formed of a three-layer structure, with two silicate layers enveloping an alumina layer, and characterised by an excess of negative ion charges on its lattice [8-9].

Another objective of this study is to list the pathogens species that can be found in the treated wastewater of three different technologies, and to demonstrate which of the technologies is the most efficient at removing these pathogens.

II. Materials and methods

Samples of sewage water were collected during the different treatment stages (intake, primary treatment, aeration tank and end tank) from ASP, SBR, and MBR treatment plants. Samples were collected in 10 ml sterile-mouth glass bottles, preserved in ice-box, and examined within 2–4 hours of collection. For treatment purpose, a 10 mg of bentonite powder was added to 500 ml of sewage water at each stage, which was then stirred at a constant speed of 40 rpm at 25 °C for 20 min. Each experiment was repeated three times.

II.1. Bacterial identification

Total coliforms and *Escherichia coli* were identified using SMWW 21st Edition 2005 – 9223 based on membrane filtration method. *Faecal streptococci* were estimated on MacConkey agar and *Salmonellae* and *Shigella* detection was carried out according to ICMSF.
Vibrio Enumeration and identification was performed using the surface plate technique using TCBS medium.
Staphylococcus estimation was carried out by employing Baird-Parker agar medium. The suitable dilution was applied to the surface of plates. Typical colonies were counted as *Staphylococci* after incubation for 48 hours at 37 °C.
Listeria-selective agar medium was used for *Listeria* enumeration and identification,. After suitable preparation of samples, 0.1 ml was spread onto each plate of *listeria* medium, supplemented with 0.01% esculin and 0.05% ferric citrate. The inoculated plates were incubated for 1–2 days at 37 °C. The typical *listeria* colonies were counted.

III. Results and discussion

III.1. Comparison study

III.1.1. *Bacterial load study*
According to Figure1, activated sludge treatment plant results recorded the highest *coliforms* concentrations with an average of 785.65 MPN/100 ml vs. 1.28 MPN/100 ml for SBR, and 0 MPN/100 ml for MBR.
According to Ramnath Lakshmanan et al., [8], When the *coliforms* concentrations rise above 25 CFU/100ml, then recreation activities (i.e. swimming or full body exposure) are prohibited according to the goals described in protection for public health. As a consequence, higher concentrations affect the gastrointestinal tract in fish and children less than 11 years old are at greater risk from exposure [9]. In most of the countries *E.coli* is regarded as a cheap way to indicate the existence of parasites in water. In the present case, the MBR process is an efficient technology for secondary wastewater treatment considering the pore size (0.2μm) and removal efficiency of the present membrane filter; it does achieve 100% of bacteria removal.
Pathogenic organisms represented by *Salmonellae, Shigella, Vibrio, Listeria,* and *total staphylococci* are also enumerated in Table 2. The pathogenic organisms count during this study revealed that no pathogens were detected in the end tank when using bentonite, recommending that the final treated water can be used safely in different agricultural and industrial purposes.
The role of this assessment is to present a valuable information background and establish the measures

to be taken when selecting and applying Best Available Techniques (BAT) for different wastewater technologies especially in the developing countries in which the operational cost versus water quality for the reuse of the treated water for crop irrigation is primordial [10].
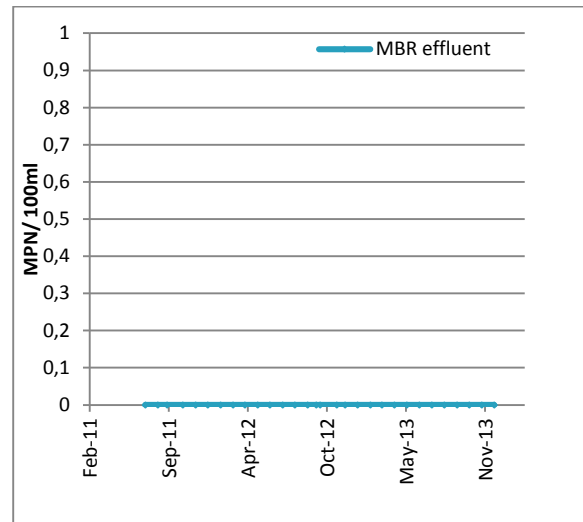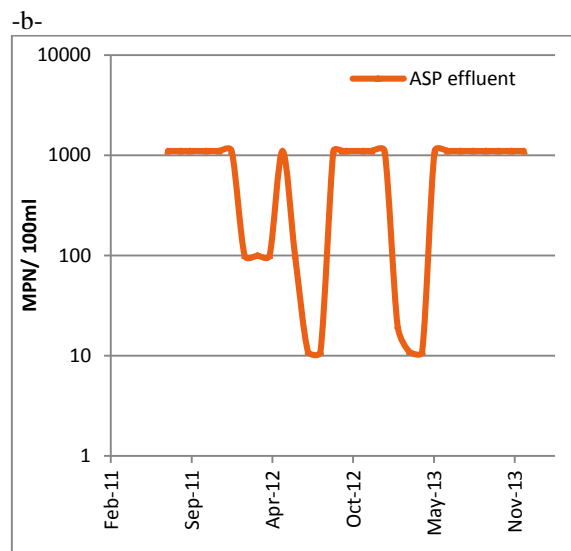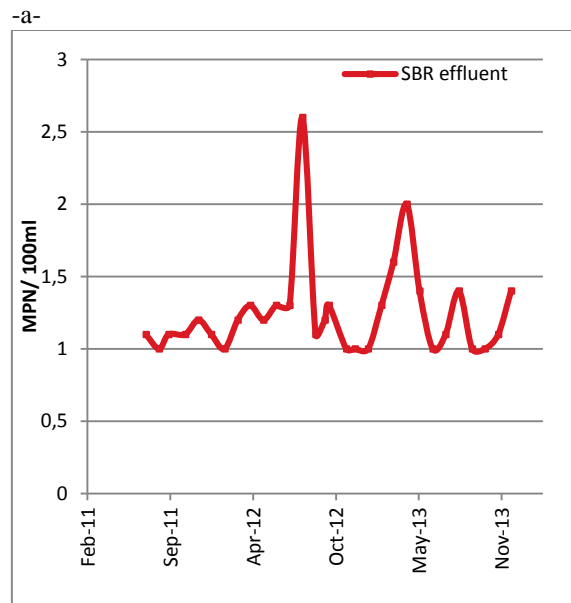
-a-

-b-

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Figure 1(a,b,c). *Coliform concentrations in the three treatment plants*

III.1.2. Bentonite efficiency study

The microbiological analyses of wastewater were performed at different stages of treatments i.e. intake, primary treatment, aeration tank, and end tank of the three treatment plants. The obtained results are recorded in Table 1. revealing a significant decrease in the bacterial indicators count densities during different stages of treatment in Ain Al Hout station (Tlemcen) – including total viable counts, *total coliform, faecal coliform* and *faecal Streptococci* – are recorded by using bentonite instead of the activated sludge technique. The results obtained in Table 1 also indicate that no common bacterial indicators were detected in the end tank when using bentonite, meaning that the final treated water can be used in both agricultural and industrial purposes.

Same findings have been reported by Malovanyн [11], with higher concentration of sorbent higher treatment efficiency can be reached. The best results are obtained for bentonite. Efficiency of microorganisms removal with bentonite was 99,8%, with glaukonite – 97,7%, and 83,1% when zeolite was used.

Table 1. Common bacterial indicators count during different stages of treatment

Level of treatment	Total counts/ml		Total coliforms/ml		Faecal coliforms /ml		Faecal streptococci/ml	
	Activated sludge	Bentonite	AS	B	AS	B	AS	B
Intake	4.5×10^6	5×10^7	5×10^7	4.1×10^6	4.1×10^6	1.1×10^4	2×10^6	2×10^6
Primary treatment	4.2×10^5	3.2×10^2	4.5×10^6	2.5×10^4	4×10^5	1.1×10^3	1.5×10^5	1.1×10^5
End tank	3.2×10^3	1.5×10^2	ND*	ND	ND	ND	ND	ND

ND*: not detected .

Table 2. Total counts of pathogenic bacterial indicators during different stages of treatment

Level of the treatment	*Total viable Salmonella/ml*		*Shigella/ml*		*Staphylococci/ml*		*Listeria/ml*	
	AS	B	AS	B	AS	B	AS	B
Intake	4×10^6	4×10^6	4.5×10^7	4.5×10^7	2.5×10^6	2.5×10^6	2.5×10^6	2.5×10^6
Primary treatment	3.7×10^5	2×10^2	3.5×10^6	2.5×10^4	1.5×10^5	1.15×10^3	1.5×10^4	1.5×10^5
Aeration tank	2×10^5	ND	0.6×10^5	ND	0.1×10^3	ND	0.3×10^3	0.6×10^3
End tank	3.2×10^3	ND	ND	ND	0.2×10^2	ND	ND	1.5×10^2

The results in Tables 1 and 2 demonstrate that bacterial removal using bentonite indicate all the counted densities of microorganisms have been eliminated, including total viable counts, *total coliform, faecal coliform, faecal streptococci*, *Salmonellae, Shigella, Vibrio, Staphylococcus,* and *Listeria*.

Vibrios enumeration, detection, and counting remains a difficult task. Isolation and counting *Vibrios* in wastewater is not common in the literature [12]. Lesne etal., [13] reported that *Vibrio cholerae* was detected in sewage at counts ranging from 1.7×10^3 to 5.1×10^4/100 ml. Ahtiainen et al., [14] reported that the counts of *staphylococci* in wastewater are ranged between 1.0×10^3 to 1.6×10^3cells/100 ml.

Moreover, El-Hawaary et al.,[15] recorded that *Listeria monocytogenes* counts are ranged between 10^5–10^7cells/100 ml for all tested raw sewage samples, which is approximately similar to our results. On the other hand, Watkins and Sleath [16] found that the counts of *L. monocytogenes* after the primary settling of sewage are ranged from 10^2 cells to more than 10^4 cells/100 ml.

Mogens and Joergen [17] have studied the natural bentonite clays effect with respect to *faecal indicator* removal, as well as selected enteric bacterial pathogens removal, in water purification through a traditional method used in Sudan. Employing various types of water, a primary bacterial reduction of 1–3 log units (90–99.9%) was obtained within the first 1–2 h of flocculation. During the 24 h observation period, bacterial multiplication in the water phase occurred consistently for *Vibrio cholerae* and test organisms belonging to the *Enterobacteriaceae* group, but not for *Streptococcus faecalis* and *Clostridium perfringens*. Some of the conditions influencing the hygienic effects obtained were examined, and the potential and limitations of the method as a local alternative in water treatment have been discussed.

III.2. Statistical analysis

Statistical differences ($p < 0.05$) deduced from Table 3 in the removal of bacterial pathogens were highly dependent on the type of process technology (Table 3).

Table 3. *Fisher's test and standard deviation*

	Unit	P	F	LSD	HSD	Scheffe	STDV
Coliforms	**MPN/100 mL**	**0.000**	**0.05**	**± 4.43**	**168.45**	**87.53**	**785.67**

Table 4 analyses results from the three treatment plants, which allowed us to reject the null hypothesis that there is no significant difference between them. Indeed, the post-hoc test allowed us to confirm significant differences in the total coliforms between the three treatment plants

(p = 0.000, F = 82.523, ddl = 92, Scheffe test = 87.53).

This concludes that each technology has its own limit efficiency with regard to bacteriological performance.

Table 4. *ANOVA (comparative analyses)*

					Coliforms		
					Variance		
	SS	df	MS	F	MBR	SBR	ASP
Between Groups	12730909	2	6365454	82.52353			
Within Groups	6942152	90	77135.02				
					0	0.158624	231404.9
Total	19673061	92					

IV. Conclusion

The present study was designed to assess the sewage treatment plant's role in meeting public health criteria. The study disclosed the occurrence of a variety of bacteria, which represents a certain range of health risk. The study also provided recommendations to improve the treatment plants performance in terms of pathogens elimination.

The activated sludge treatment plant recorded the highest coliforms concentrations, with an average of 785.67 MPN/100 ml vs. 1.28 MPN/100 ml for SBR, and 0 MPN/100 ml for MBR treated water, resulting in the conclusion that MBR technology is the most efficient but not the only one recommended due to its operation and maintenance high cost. The quantity of used bentonite is 10 gm/500 ml which indicates a cheap and effective method of treatment. Microbiological studies of the treated water using the bentonite technique revealed the removal of all counted densities of microorganisms including: total viable counts, total coliform, faecal coliform, faecal streptococci, *Salmonellae, Shigella, Vibrio, Staphylococcus,* and *Listeria* which were also occasionally found were also eliminated. The bentonite clay technique was successfully applied for treatment of sewage water in Tlemcen city. This technique accesses several advantages

including best removal of the targeted pollutants at limited cost. Usage of natural sorbents in water treatment technologies does not require their regeneration, and polluted modified sorbents can be used in other chemical and construction industry or applied in agriculture. Therefore the wastewater treatment using sorbents is a promising and relatively inexpensive method.

Positive sodium and calcium ions in the montmorillonite layers may act as exchange ions with cationic elements contained in wastewater, which reflect the modifying of oxidation state. Therefore, it seems essential to include a tertiary treatment step in Ain Al Hout treatment plant (ASP-based process) to ensure that the purification process results in bacterial concentrations remaining in compliance with discharge and crop irrigation standards.

Finally, microbial (i.e. *E.coli, Coliforms* and *total viable count*) analyses are crucial in regular basis to achieve high proficiency with the ASP process or bentonite clay.

V. References

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