

MICROBIOLOGICAL AND CHEMICAL EVALUATION OF BENTONITE AS A NEW TECHNIQUE FOR SEWAGE WATER TREATMENT, ASWAN CITY, EGYPT

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ABSTRACT

Bentonite clay technique was successfully applied to improve wastewater quality and upgrade its use in agricultural and industrial purposes. Bentonite is a natural clay characterized by possessing excess of negative charges on its lattice and swell into very large surface area when dispersed in water. Both BOD and COD were effectively reduced to 50 % of their original concentration by adding of bentonite for the first time. Additional reducing of BOD and COD to about 27 % of their original concentration was achieved using bentonite for the second time. Microbiological studies of the treated water using bentonite indicate getting ride of all the counted densities of microorganisms. The later includes *Total viable counts*, *Total coliform*, *Faecal coliform*, *Faecal Streptococci*, *Yeast*, *Candida*, *Aeromonas*, *Acid fast bacteria*, *Salmonellae*, *Shigella*, *Vibrios*, *Staphylococci* and *Listeria*. The treated sewage water is free from several densities of bacteria and pathogenic microorganisms and contains low content of BOD and COD under the standard allowed limits of World Health Organization (W.H.O.). So, the final treated water can be used safely in different agricultural and industrial purposes.

Key Words: activated sludge - bentonite - wastewater treatment - BOD - COD - microorganisms - bacteria - water pollutants.

1. Introduction

Sewage is the wastewater released by residence, businesses and industries in particles. Fresh sewage is a gray turbid liquid characterized by an earthy but has mostly offensive odor. It contains several numbers of diseases-causing organisms (Mara [1]). Wastes may classify according to their physical state into gases, liquids, solids or heterogeneous mixture, where both solid and liquid phases exist. Also, they can be classified according to the origin of particular wastes. The dominant components may define the type of waste such as industrial effluents, which contain hazardous halo-organic compounds or bio hazardous (Claudio *et al.* [2]).

The problem of recycling of wastewater is the presence of pathogenic organisms as well as metal pollutants; therefore, the sewage water should be treated before its ultimate disposal in a receiving water source in order to reduce the spread of communicable diseases (Abdalla *et al.* [3]). In view of upgrading the quality of wastewater before its recycling, different physical and chemical methods have been developed. Nevertheless, the microbiological and physicochemical statuses of such treated wastewater are still far from being satisfactory. Bentonite technique that applied during this study may offer a safe, effective and economic method for elimination of such pathogens present in wastewater before being recyclable.

The increase in human population leads to the utilization of available sources of water. Horizontal extension is one of the main targets for enlarging the cultivated area in Egypt. This could be easily achieved in the deserts surrounding the Nile course and Delta (96% of the country is desert). These soils either sandy or calcareous and their cultivation require the availability of a suitable source of water for irrigation. This could be achieved by the reuse of sewage water produced from the surrounding sites (El-Taweel [4]).

Wastewater characteristics play an important role in the designation of wastewater treatment facilities. The selection of wastewater treatment processes depends on waste-water composition, e.g. BOD, COD, pH, suspended solids, nitrogen, phosphorous, presence of toxic materials and bacterial population (Negm, *et al.* [5]). Wastewater may contain millions of bacteria per milliliter including coliforms, streptococci, staphylococci anaerobic, sporeforming 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 (Pleczar, *et al.* [6]). These characteristics of wastewater is 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.

Sewage treatment practices may be classified into three categories: 1) Primary treatment through which some of the suspended and floating solids are removed. 2)

Secondary treatment is designed to reduce the biological oxygen demand (BOD). 3) Final treatment, which involves disinfecting of liquid effluent and the disposal of the remaining solids with the control of heavy metals levels to comply with the Best Available Treatment Technology (BATT).

Activated sludge contains some disease-causing microorganisms. Chlorine gas is used in treatment of waste sludge can not completely get ride such organisms. The final treated water can be used only in irrigation of wooden trees. Due to such disadvantages, an effective and cheap material is urgently needed for treatment of sewage water.

The present study has been conducted to asses the impact of bentonite treatment technique to improve wastewater quality and upgrade its use in agriculture purposes. Bentonite is a natural clay of limited expense and being mined from Kurkur area south west of Aswan City (Abou Elmagd [7]). It is produced from weathering of basaltic rocks, and made up mineralogically of montmorillonite mineral in addition to secondary amounts of other clay and nonclay minerals. Montmorillonite mineral is formed of three layer structure with two silicate layers enveloping an alumina layer and characterized by excess of negative charges on its lattice (Grim and Guven [8]). Bentonite component is made up of relatively large flakes that disperse readily in water into very thin units, with remarkable swelling.

The biological indicators and some pathogenic bacteria were determined during the treatments stages. These organisms are total viable counts; total and faecal coliform, total faecal streptococci total yeasts, *Candida albicans*, *Aeromonas hydrophila*, acid fast bacteria, salmonellae, shigella, staphylococci, vibrios and listeria group.

2. Materials and Methods:

Samples of sewage water were collected during the different treatment stages from Aswan City treatment plant. Samples were collected in 10ml, sterile mouth, glass bottles, preserved in ice-box and examined within 2 – 6 hours of collection. A representative sample of bentonite was crushed and homogenized then mineralogically and chemically analyzed. X-ray diffraction analysis of this sample revealed that bentonite is formed of the following clay minerals; montmorillonite (85%), kaolinite (11%), chlorite (3%) and illite (1%). Nonclay minerals present also in secondary amounts and include quartz, calcite, gypsum and hematite. Chemical analysis of the sample using x-ray fluorescence revealed that is composed of SiO₂ (50.42 %), Al₂O₃ (30.0 %), Fe₂O₃ (0.5 %), CaO (0.42 %), MgO (0.02 %), TiO₂ (1.6 %), P₂O₅ (0.15 %), Na₂O (0.25 %), K₂O (0.45 %) whereas loss of ignition (L.O.I) attains (15.9 %). For treatment purpose, a 20 gm of bentonite sample were added to 1 liter of sewage water regularly in all stages.

For microbiological examination, the suitable number of each bacteria was reached, three different ten fold dilution were prepared from each sample and used for

the enumeration of each bacteria. For total viable counts, poured plate method according to APHA [9] was used, while Total and faecal coliforms as well as faecal streptococci were estimated on MacConky agar (Oxoid [10]). For enumeration of yeasts, Littman oxgall agar (pH 6.5) supplemented with chloramphenicol (5 mg/L), penicillin (50.000 units/L) and streptomycin (30mg/L) was used by a surface plate technique (El-Hawaary and Khalafalla [11]). For enumeration of *Candida albicans*, candida selective medium was used to enumeration and count of *Candida albicans*. Surface inoculated plates were incubated for 2 – 3 days at 22°C. Brown to black, smooth colonies were counted. Total count of *Aeromonas hydrophila* was detected by Tis agar (Shuzo [12]).

For enumeration of acid fast bacteria, each sample was mixed with equal volume of 2.5% oxalic acid for 10 minutes. The mixture was neutralized with 2% NaOH. Filter membrane filtration, membranes were placed onto the enriched TB agar medium and incubated at 37°C for 7 days. Filters supporting growth were gently heat dried and stained by Brook's acid fast stain (El-Hawaary and Khalafalla [11]). Pinks to red colonies consisting of acid-fast organisms were counted.

Detection of *Salmonellae* and *Shigella* was carried out according to ICMSF [13]. For enumeration and Identification of vibrios, surface plate technique using TCBS medium according to Monsur [14] was employed. Biochemical identification of vibrios was carried out by the method of Furniss *et al.* [15]. Total count of Staphylococcus carried out by surface plating technique by employing Baird Parker Agar medium. The suitable dilution was applied to the surface of plates and incubated for 48 hours at 37°C. Typical colonies were counted as staphylococci. For enumeration and Identification of Listeria, listeria selective agar medium was used in enumeration of listeria group. After suitable preparation of samples, 0.1ml was spread onto each plates of Listeria medium supplemented with 0.01% escualin and 0.05% ferric citrate. The inoculated plates were incubated for 1 – 2 days at 37°C. The typical listeria colonies were counted. Biochemical reactions were carried out for the Listeria identification according to Fenlon [16].

3. Results and Discussion:

a. Microbiological evaluation:

The microbiological analyses of wastewater were performed at different stages of treatments i.e. intake, primary treatment, aeration tank and end tank of treatment plant located at Aswan city, Egypt. The obtained results recorded in Table (1) revealed that a significant decrease in the densities of Classical bacterial indicators counts during different stages of treatment in Aswan station including *Total viable counts*, *Total coliform*, *Faecal coliform* and *Faecal Streptococci* are recorded by using bentonite than activated sludge technique. The results obtained in the Table (1) also indicated that it was no detection of Classical bacterial indicators in the end tank by using bentonite, meaning that the final treated water can be used in different agricultural and industrial purposes.

Wastewater treatments are processes in which microorganisms play crucial roles, and illustrate well some of the principles of biogeochemistry. Wastewaters are materials derived from domestic sewage or industrial processes, which for reasons of public health and for recreational, economic, and aesthetic considerations cannot be disposed of merely by discarding them untreated into convenient lakes or streams. Rather, the undesirable and toxic materials in the water must first be either removed or rendered harmless. Inorganic materials such as clay, silt, and other debris are removed by mechanical and chemical methods, and microorganisms participate only casually or not at all. If the material to be removed is organic in nature, however, treatment usually involves the activities of microorganisms, which oxidize and convert the organic matter to CO₂. Wastewater treatment usually also results in the elimination of pathogenic microorganisms, thus preventing these organisms from getting into rivers or other supply sources. As a result of the sewage treatment process, the effluent is stabilized and its content of toxic materials is reduced, it can thus be disposed into the environment with less difficulty. Alonso *et al.* [17] recorded that total and faecal coliforms were around 10⁷ in marine water mixed with raw sewage. Gambrill *et al.* [18] found that faecal coliform in the raw wastewater was 3.9 x 10⁷ as mean value. Lesne *et al.* [19] reported that faecal streptococci counts in raw wastewater were around 10⁶ to count /100ml either under warm or cold conditions.

The total counts per 1ml of yeasts, *Candida*, aeromonas, and acid fast bacteria during different stages of treatment in Aswan station were recorded Table (2). The obtained data indicated the advantage of the bentonite at different stages of treatments of the wastewater. It was almost no detection of counted bacteria in the end tank by using bentonite. Cook and Schlitzer [20] recorded that *Candida albicans* was enumerated in raw wastewater at counts ranged from zero to 4.0 x 10² cells/100ml. Hass *et al.* [21] reported that the density of yeasts in raw wastewater varied from 2.7 x 10³ to 3.9 x 10⁴ cells/100ml during his investigations.

Araujo *et al.* [22] reported that *Aeromonas* spp were isolated from all the domestic sewage samples at a constant count between 10⁷ to 10⁸ cell/100ml. Moreover, Alonso *et al.* [23] reported that motile *Aeromonas* were isolated from all samples taken from marine recreational waters polluted with sewage in a count ranged from 10⁴ to 10⁶ cells/100ml.

Table: 1) Classical bacterial indicators counts during different stages of treatment in Aswan station.

Level of the treatment	Total viable counts / 1ml		Total coliform		Faecal coliform		Faecal Streptococci	
	Activated sludge	Bentonite	A S	B	A S	B	A S	B
Intake	4.7×10^6	3.4×10^4	5.4×10^7	4.1×10^5	5.5×10^7	3.3×10^6	2×10^6	1.2×10^4
Primary treatment	4.3×10^5	2.3×10^2	4.9×10^6	3.6×10^3	4.3×10^6	2.5×10^4	1.3×10^5	1.1×10^3
Aeration tank	2.1×10^5	0.5×10^2	3.3×10^5	1.4×10^2	2.9×10^4	1.7×10^2	0.78×10^3	0.35×10^2
End Tank	3.2×10^2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

A S = Activated sludge, B = Bentonite, N.D. = Not detected

Table (2): Total counts per 1ml of yeasts, Candida, aeromonas, and acid fast bacteria during different stages of treatment in Aswan station.

Level of the treatment	Yeast/ 1ml		Candida/1ml		Aeromonas//1ml		Acid fast bacteria /1ml	
	Activated sludge	Bentonite	A S	B	A S	B	A S	B
Intake	5.3×10^7	4.2×10^6	3.1×10^5	2.3×10^4	3.9×10^7	2.2×10^6	4.4×10^3	3.1×10^2
Primary treatment	4.1×10^6	3.3×10^4	2.2×10^2	0.11×10^2	2.6×10^6	1.7×10^4	3.2×10^2	0.2×10^2
Aeration tank	3.6×10^5	1.2×10^2	0.4×10^2	N.D.	2.3×10^4	0.9×10^2	0.4×10^2	N.D.
End Tank	2.3×10^3	N.D.	N.D.	N.D.	1.9×10^3	N.D.	N.D.	N.D.

A S = Activated sludge, B = Bentonite, N.D. = Not detected.

Aeromonas hydrophila is common in aquatic environment; moreover it is considered as a human and animal pathogen (Burke *et al.* [23]). Generally, *A. hydrophila* concentration in water and wastewater was usually greater than coliform, they form up to 75% of the total bacterial population in the polluted ecosystems (Hazen and Esch [24]). Araujo *et al.* [22] reported that *A. hydrophila* can not be considered as of faecal origin, but its counts were similar to faecal coliform bacteria. Alonso *et al.* [17] reported that this group of organisms are able to multiply in and / or sewage polluted waters. Moreover, other investigators (Kaper *et al.* [25]) observed that the numbers of aeromonas often approached or exceeded *E. coli* in surface water and sewage. Engelbrecht *et al.* [26] and Hass *et al.* [21] found that the density of acid fast bacteria in the municipal wastewater was around 10^4 cells/100ml.

Pathogenic organisms represented by salmonellae, Shigella, vibrios, Listeria and total staphylococci were also enumerated (Table 3). Counts of pathogenic organisms during this study revealed that, it was no detection of pathogenic organisms in the end tank by using bentonite, recommending that the final treated water can be used safely in different agricultural and industrial purposes. Vibrios enumeration, detection and counting is still a hard task. Isolation and counting vibrios in wastewater is not common in the literatures (Roberts *et al.* [27]). Lesne *et al.* [19] reported that *Vibrio cholera* was detected in sewage at counts ranged from 1.7×10^3 to 5.1×10^4 /100ml. Ahtiainen *et al.* [28] reported that the counts of staphylococci in wastewater ranged between 1.0×10^3 to 1.6×10^3 cells/100ml.

Moreover, El-Hawaary *et al.* [29] recorded that *Listeria monocytogens* counts ranged between 10^5 and 10^7 cells/100ml for all tested raw sewage samples which approximately similar to our results. On the other hand, Watkins and Sleath [30] found that the counts of *L. monocytogens* after the primary settling of sewage ranged from 10^2 to more than 10^4 cells/100ml.

Egyptian sewage can be considered as a concentrated wastewater, regarding values of BOD and COD in addition to total solids content ranged from 300 to 540, 550 to 900 and 1038 to 1933mg/l in average, respectively (El-Gohary [31]). Thus it may be logic to obtain the high counts of the tested bacterial parameters number under the warm climate in Egypt, enhancing the important of the present study which has been conducted to assess the impact of bentonite treatment technique to improve wastewater quality and upgrade its use in agriculture purposes. Densities of some microorganisms are limiting factors for effluent discharge and / or safe reuse of the treated sewage. Thus the initial densities of these organisms as well as the rate of reduction are very important. Though, reduction between 4 and 5 logs from the initial load in raw wastewater is quite sufficient universally for sewage discharge and / or reuse. On the other hand, under Egyptian conditions, reduction between 5 and 8 logs were needed to reach the same acceptance. So, selection of treatment system (s) management and operation conditions must consider these data for safe discharge and / or reuse.

b. Chemical evaluation:

Oxidation of reduced forms of nitrogen, mediated by microorganisms, exerts nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, as clearly evidenced by the inclusion of ammonia in the dilution water. The interference from nitrogenous demand can now be prevented by an inhibitory chemical (Young [32]). If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands. The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. For samples from specific source, COD can be related empirically to BOD, organic carbon, or organic matter. The test is useful for monitoring and control after correlation correlation has been established (Jirka and Carter [33]).

The values of BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) have measured instrumentally in different stages of treatment of the sewage water before and after addition of bentonite, Table (4). These results revealed that the levels of uptaking were improved by about 50%. The treated sewage water was handled by bentonite for the second time which resulted in additional reducing of BOD and COD values within influent and primary tanks respectively, Table (4).

Temperature of the treated water ranges from 15 to 25°C whereas the hydrogenous exponent ranges from 6 to 9. It is concluded that the treatment of sewage water by bentonite showed satisfactory results of uptaking levels of both BOD and COD without leaving activated sludge. The better results of treatment may reach by reformation of the influent and primary tanks water using bentonite for the second time (secondary tanks). The last results of BOD and COD using bentonite lies within the limits of World Health Organization (W.H.O) [34], and the treated water is suitable for all agricultural and industrial uses.

c. Role of bentonite:

The impact of bentonite on wastewater may be related to:

- i) It is characterized by excess of negative charges on its lattice which cause modifying of oxidation state of the treated water and hence its suitability for microorganisms.
- ii) It is swelling into several times of its original volume when disperse in water resulting in very large surface area and hence improve its ability to adsorb large amounts of impurities and microorganisms.
- iii) Positive sodium and calcium ions in the montmorillonite layers may act as exchange ions with cationic elements contained in wastewater which reflects on modifying of oxidation state. The same bentonite exhibits high removal of iron cations from ground water exploited from Aswan area (Taha and Abou Elmagd [35]).

Table (3): Total counts of pathogenic bacteria during different stages of treatment in Aswan station.

Level of the treatment	Salmonellae / 1ml		Shigella/1ml		Vibrios /1ml		Staphylococci /1ml		Listeria /1ml	
	Activated sludge	Bentonite	A S	B	A S	B	A S	B	A S	B
Intake	3×10^5	2.1×10^4	3.8×10^4	1.8×10^3	2.5×10^3	1.1×10^2	1.4×10^6	1.2×10^5	3.7×10^7	2.4×10^6
Primary treatment	2.7×10^4	$.09 \times 10^2$	2.6×10^3	0.8×10^2	1.4×10^2	0.3×10^2	1.3×10^5	1.19×10^3	2.6×10^6	1.9×10^4
Aeration tank	$.06 \times 10^2$	N.D.	0.7×10^2	N.D.	0.2×10^2	N.D.	0.9×10^3	0.3×10^2	1.8×10^4	1.7×10^2
End Tank	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.4×10^2	N.D.	1.5×10^3	N.D.

A S = Activated sludge, B = Bentonite, N.D. = Not detected

Table: 4) Determination of BOD and COD in different treatment levels using bentonite.

Level of the treatment	BOD (mg/l)			COD (mg/l)		
	Activated sludge	Bentonite (first time)	Bentonite (second time)	Activated sludge	Bentonite (first time)	Bentonite (second time)
Influent	290	150	80	350	190	90
Primary treatment	250	120	60	280	110	80
Aeration tank	100	90		50	40	
Final Tank	60	40		40	30	

4. Summary and Conclusion:

- 1- Bentonite clay technique was successfully applied for treatment of sewage water in Aswan City. This technique accesses several advantages including best removal of the targeted pollutants, low sludge production and limited cost.
- 2- Both BOD and COD were effectively reduced to about 50% of their original concentration using bentonite for the first treatment.
- 3- Second time treatment of sewage water of the influent and primary tanks using bentonite resulted in additional reducing of BOD and COD to about 27% of their original values.
- 4- BOD and COD content are effectively reduced to acceptable levels; 60 mg/l and 80mg/l respectively. These results lie under the limits allowed by the Egyptian and international specifications of recycled wastewater standards.
- 5- The quantity of used bentonite is 20 gm/l which refer to a cheap and effective method of treatment.
- 6- Microbiological studies of the treated water using bentonite technique revealed disappearing of all the counted densities of microorganisms including: *Total viable counts*, *Total coliform*, *Faecal coliform*, *Faecal Streptococci*, *Yeast*, *Candida*, *Aeromonas*, *Acid fast bacteria*, *Salmonellae*, *Shigella*, *Vibrios*, *Staphylococci* and *Listeria*.
- 7- A high count of the tested bacterial parameters number in the studied sewage water is related to the concentration of BOD, COD and solids in addition to the warm climate in Egypt.
- 8- Bentonite clay have negative charge on its surface should affect its capacity to adsorb different waste materials. It is also expected to be effective in adsorbing heavy metals and other pollutants from the treated wastewater.
- 9- The treated sewage water is free from several densities of bacteria and pathogenic microorganisms and contains low content of BOD and COD. So, the final treated water can be used safely in different agricultural and industrial purposes.

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