INT. J. BIOAUTOMATION, 2022, **26**(1), 93-108 doi: 10.7546/ijba.2021.26.1.000727

The Highlighting of a Biological Process for the Treatment of Leachate from a Public Discharge

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Received: August 11, 2019 **Accepted: August 28, 2021**

Published: March 31, 2022

Abstract: *Due to the composition and their impact on the environment, landfill leachate is a serious environmental and public health problem. Our physicochemical and microbiological study has shown that leachate is highly loaded with minerals including iron, Mg, Cd, etc.) and pathogenic microorganisms hence the need for effective and sustainable treatment. Our present study enters this preoccupation we have highlighted a biological process allowing the transformation of leachate by way of fermentation, being based on leaven having fermenting, acidifying and antimicrobial power. Microbiological analysis showed that almost all the pathogenic flora was removed showing the biological treatment efficacy. In addition, the stable product obtained after 15 days of fermentation was used as a base in a formula of a bio-fertilizer. Application trials in different crops (wheat, peas, corn, etc.) have shown satisfactory results.*

Keywords: *Leachate, Biological treatment, Fermentation starter.*

Introduction

The environment is an environment in which the persistence of a normal life is closely related to the availability of air, water, land, natural resources, flora, fauna and their interrelations. Thus, the protection of the environment has become a major issue in international relations and national and public policies. In recent decades, waste production has increased drastically as a result of population growth and excessive changes in consumption patterns [28, 29]. Keeping the largest developments of the agroecological system functioning promotes the optimization of agricultural economic activities [61].

The environmental situation in Morocco is characterized by a number of problems, the main ones of which are: the growing scarcity and degradation of the quality of the water resources, Insufficient treatment of solid waste and urban and industrial wastewater, increasing air pollution, increasing soil erosion, deforestation and increasing desertification.

Like most developing countries, the most widely adopted mode of solid waste management in Morocco is the landfilling of municipal waste. These are mainly open and uncontrolled landfills, where all types of wastes are untreated and mixed: urban, industrial, hospital and agricultural [1, 2, 53]. However, one of the major problems of this method is the generation of leachate [52].

Leachate is the result of the infiltration of water through the waste that has undergone aerobic and anaerobic microbial decomposition [16, 25, 47]. Leachate can contaminate groundwater, rivers, lakes and soils so it has the potential to negatively affect local habitats, resources and human health [5, 44].

Typically, leachate consists of a heterogeneous mixture comprising refractory organic compounds, heavy metals, inorganic contaminants, humic and fulvic acids, and high concentrations of nitrogen [59]. Depending on the stage of biological evolution of the waste, three types of leachates were distinguished:

- The so-called young leachates

  Less than 5 years old which are characterized by a high biodegradable organic load. These leachates can be loaded with metals (up to 2 g/L), their pH is close to 6.5. They correspond to the first non-methanogenic phases of the evolution of a landfill.
- Intermediate leachates

  As the landfill ages and the waste stabilize, the organic load decreases and volatile fatty acids become scarce (20 to 30% of the leachate load) in favor of high molecular weight compounds. The emergence of these compounds tends to decrease the biodegradability of leachate. As a result, the pH is close to neutral, and the metal charge becomes negligible. These leachates correspond globally to the stable methanogenic phase.
- Stabilized leachates (> 10 years)

  They are characterized by a low organic load, composed mainly of humic substances (fulvic and humic acids) of high molecular weight (the chemical oxygen demand (COD) exceeds 3000 mg/L) [38], which is refractory to biodegradation (DBO5/COD < 0.1). These stabilized leachates correspond to the maturation phase of the landfill. In summary, the leachates from an old landfill are therefore generally less loaded than those of a young landfill but more difficult to treat. Apart from the evolution over time, the composition of leachates obviously depends on the nature of the buried waste, the presence or absence of the fermentable organic matter and the climatic conditions combined with the mode of exploitation of the site.

The control of the waste, and the choice of an appropriate treatment of leachate produced, is essential for the protection of the environment. However, their treatment is the main management issue facing the discharge operators [15].

Various treatment techniques have been used to remove leachate pollutants, including flotation [4, 19], coagulation, flocculation [42, 56], adsorption [31], nanofiltration, reverse osmosis [34], chemical precipitation [31], electrochemical [49, 51] and biological process [9, 54, 60, 62].

Because of their cost-effectiveness and low cost, biological processes are usually used primarily to remove the organic fraction contained in the leachate. These processes are very efficient due to the presence of a high fraction of recalcitrant organic compounds, mainly humic substances [48, 50].

In the present work we have studied the possibility of treating and transforming leachate by a biotechnological process based on the use of a fermenting leaven based on bacteria and yeasts with strong fermenting and acidifying properties. This biotechnological technique is simple and easy to implement and especially less expensive.

Materials and methods

Origin and characterization of leachate

Sampling

Leachate samples are collected from the Kenitra City Ouled Berjal public landfill, from the first leachate collection basin coming directly from the waste dumped in the bin via the drainage system. The samples are taken simultaneously at 5 different points in lover and downstream of the leachate basin to be homogeneous and representative in polyethylene bottles with labels mentioning the conditions, date and sampling site, the bottles are then put into an insulated briefcase at 4 °C and transported directly to the laboratory for analysis.

Physico-chemical and microbiological characterization

In order to determine the impact of leachates on the environment and public health, physicochemical and microbiological analyzes were carried out.

Physico-chemical characterization

Physical parameters such as temperature, electrical conductivity and pH were measured using a conductivity meter and a multi-parameter pH meter.

All chemical leachate parameters were determined using standard methods for wastewater analysis.

The COD is analyzed by oxidation by excess of potassium dichromate at 148 °C in an acidic medium. The biological oxygen demand (BOD5) by the respiratory method using a BOD-meter (OxiTop). For suspended solids, the method used was Sertorius MGC Glass Microfibre Filter Filtration (NBN EN 872 (2005)).

Kjeldahl total nitrogen was measured by the Kjeldahl method [45]. The phosphors are measured by the spectrometric method with amonium molybdate. Potassium was measured by flame spectrophotometer; Fe, Cu, Zn, Mn – by flame atomic adsorption.

Microbiological analyzes

The physicochemical characterization of leachates and the possible plume of pollution due to their infiltration into the subsoil has been the subject of many studies [17], the data relating to pathogenic bacterial communities. Colonizing leachates are very rare. However, the development of these microorganisms and their transport by leachates can be at the origin of a contamination of ground water, and by extension of reservoirs intended for the supply of drinking water.

To qualitatively and quantitatively determine the microbial concentration of leachates, microbiological analyzes were carried out.

They are focused on the flora of hygienic interest, pathogenic flora and toxinogenic flora.

Ecotoxicological analysis

To assess the potential of the physicochemical, chemical and biological stressors to influence the ecosystems [63], the ecotoxicity of some samples collected were determined using selected biotests.

Preparation of dilutions

Ten ml of the sample to be analyzed are collected in a 250 ml Erlenmeyer flask containing 90 ml of sterile physiological saline. A dilution of 10^{-1} is thus obtained. From the latter is diluted from 10 to 10^{-7} using sterilized pipettes and into tubes containing 9 ml of sterile physiological saline.

Total Aerobic Mesophilic Flora (FMAT) is listed on Plate Count Agar (PCA) after incubation for two days at 30 °C.

Total and fecal coliforms are counted on deoxycolate citrate lactose agar (DCL) respectively after incubation at 37 °C and 44 °C for 24 hours.

The *Salmonellae* are isolated after enrichment on selenite sodium medium on Salmonella-Schigella medium (SS), after incubation for 24 hours at 37 °C.

The *Clostridia* are counted on reinforced clostridium agar (RCA) after incubation for 24 hours at 44 °C. The *Staphylococci* are isolated on Chapman selective medium after incubation for 24 hours at 37 °C.

The *Enteropathogenic cholera vibrios* are counted on thiosulfate-citrate-bile-sucrose (TCBS) medium after incubation at 37 °C for 24 hours.

The *Lactic bacteria* are counted on Man’s medium, rogosa and sharpe agar (MRS), after incubation at 37 °C for 48 hours. The yeasts are counted on PDA medium (Potato Dextrose Agar) after incubation for three days at 30 °C.

Biological process of leachate transformation

Our biotechnological process is based on the use of microorganisms allowing the reduction of the organic load, the reduction of the mineral elements in particular the heavy metals and especially the hygienization of the leachate by the inhibition of the growth and the development of pathogenic microorganisms.

Preparation of fermentation leaven

The choice of micro-organism used in the hygienization, fermentation and stabilization of the product was based on several criteria, the fermentative, acidifying, enzymatic and antibacterial production, in particular lactic acid producing bacteria, acetic acid, d citric acid and formic acid and production of bacteriocin. This leaven has been formulated from the strains of the laboratory micro-library, which have been selected and used for the treatment of other waste [23, 27, 39].

Fermentation and stabilization of waste:

The conduct of the fermentation process was carried out according to the diagram in (Fig. 1). Ten L of leachates are put in closed plastic cans of 20 L capacity each. These leachates are mixed with the fermentation starter at a rate of 0.6%. The cans are placed in a room at room temperature and subjected daily to manual agitation.

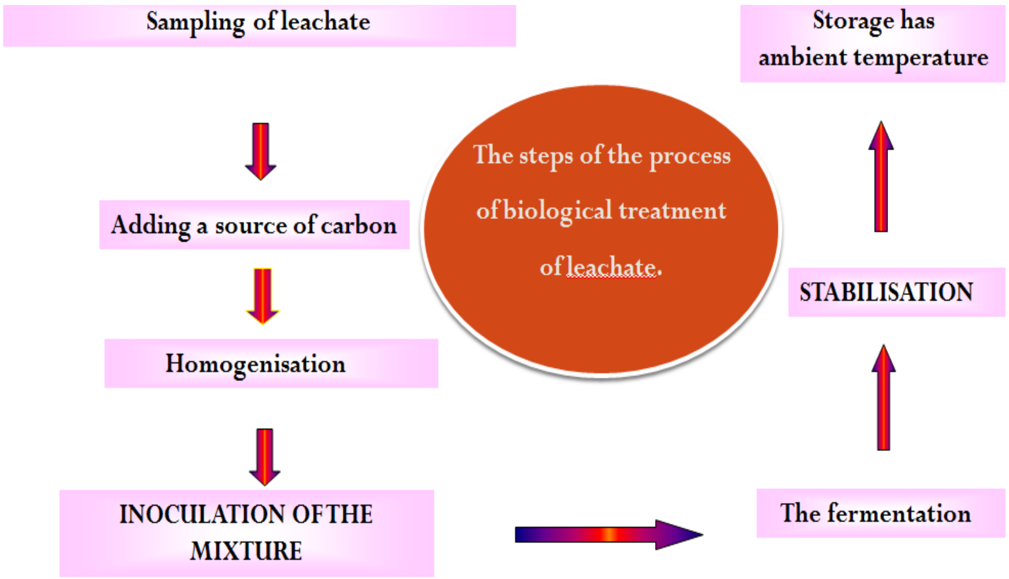

Fig. 1 General scheme of biotechnological treatment process [22]

Samples are taken throughout the fermentation for pH analyzes, while all physicochemical and microbiological analyzes are performed before and after stabilization of the finished product.

Results and discussion

Physicochemical characteristics of leachates

Leachate evolves over time according to a succession of degradation phase which depends, the quality of household waste (proportion of biodegradable material), and the volume of waste, their deposit mode, and weather conditions. This study aims to study the evolution of physicochemical parameters of leachates. The observed values reveal that the hydrogen potential is alkaline. This revealed basic character is in accordance with that obtained by [22] on the public dump of Rabat, [35-37]. In addition, Kjeldsen [33] showed that as the landfill ages, the leachate becomes depleted in volatile organic compounds. This will then cause the pH to rise to 7 or higher.

As for the electrical conductivity, the average value is 41,000 µS/cm. This value is greater than that recorded for previous jobs [3]. In fact, 50% of leachate has a value below 3450 µS/cm the high values were recorded in the dry season. On the other hand, the low conductivities were obtained during the rainy season.

COD represents the amount of oxygen consumed by chemically oxidizable materials in the water. It is representative of most of the organic compounds and oxidizable mineral salts [43]. The average content recorded is 4900 mg/O_2/L (Table 1). This content is significantly higher than the reference value [35, 37, 46]. The high value of COD indicates a high organic load. This difference could be related to the age, nature and quantity of the waste as well as the different climatic factors such as rainfall, air humidity and temperature. Indeed, according to [17], these different factors are at the base of the variability of polluting loads. While the average value of BOD5 obtained is estimated at 190 mg/O_2/L, it remains lower than that found in the literature with a mean of 843 mg/O_2/L. However, 50% leachate has a value of less than 84 mg/O_2/L.

The BOD5/COD ratio is a good indicator of the biodegradability of an influent. The resulting ratio is estimated at 0.04. A BOD5/COD ratio of less than 0.1 is an indicator of low biodegradability, whereas if this ratio is greater than 0.3 shows significant biodegradability. It should be noted that it has been observed time and time again that the industrial origin of leachate does not exclude good degradability [18]. The values obtained remain in the range of those recorded by the previous work of El Khamlichi [22, 35, 37].

Table 1. Physico-chemical characteristics of leachate

Settings	Leachate
pH	8.6
Odour	bad
Color	Blackish
Suspended matter, (g/L)	8.9
Conductivity, (ms/cm)	41
COD, (mg/L)	4900
BOD5, (mg/L)	190
BOD5/COD	0.04
Organic matter, (mg/L)	136
NTK, (mg/L)	1532
Orthophosphates, (mg/L)	50
Potassium (K^+), (mg/L)	1780
Calcium (Ca), (mg/L)	56,67
Magnesium (Mg), (mg/L)	146,50
Iron (Fe), (ppm)	13
Copper (Cu), (ppm)	0.6
Zinc (Zn), (ppm)	0.2
Manganese (Mn), (ppm)	0.7

ppm: part-per-million

In addition, the recorded temperatures oscillate between 22 °C and 37 °C. These values would be favorable for maintaining colonies of “mesophilic” microorganisms that develop at a temperature of between 20 °C and 40 °C. The dry matter represents 34.12 mg/L.

As far as organic matter is concerned, the leachate shows a high degree of richness with an organic content (136 mg/L). For Kjeldahl nitrogen (NK), it is estimated to be 1532 mg/L at high levels. The mineral composition is dominated by potassium (1780 mg/L) whereas the concentration of PO4 phosphate is only (50 mg/L). This value remains largely identical to those of the reference limit values, however, remains much lower than the values found by Kouassi [37] which oscillate between 103.07 and 148.68 mg/L.

- Secondary elements: Ca estimated at 56.67 mg/L, Mg of 46.50 mg/L;
- Micronutrients: Fe is estimated at 13 mg, Mn at 0.2 mg, Zn at 0.2 mg, Cu at 0.6 mg.

The analysis of the results obtained shows a high concentration of potassium and a low concentration of calcium. These high values of the cations in leachates indicate a strong presence of mineral matter. The relatively high amount of potassium in leachates could be explained by the burial of calcined products and food products that undergo biological and physicochemical evolutions.

Microbiological characteristics

The objectives of this study are to monitor bacterial evolution in the leachates of a garbage dump and their impact on the surrounding waters. This case study provides new data on bacterial numbers and their capacity for development and survival in specific environments: leachates from garbage dumps. In addition, monitoring these fecal bacteria will allow this tool to be used as a natural tracer for recent contamination.

The determination of the microbial load was carried out on 10 leachates samples. The results obtained are shown in Table 2.

Table 2. Microbiological characteristics of leachate

Sprouts/ Sample	**FMAT, cfu/g**	**Colif, cfu/g**	**Staphy, cfu/g**	**Strep, cfu/g**	**Clost, cfu/g**
1	1.5×10^{12}	3.0×10^{5}	5.0×10^{4}	110	670
2	2×10^{12}	3.1×10^{5}	3.2×10^{4}	110	680
3	4.3×10^{12}	1.1×10^{6}	7.0×10^{4}	110	370
4	3.8×10^{12}	3.0×10^{4}	3.0×10^{4}	140	520
5	9×10^{12}	4.7×10^{4}	2.1×10^{4}	145	670
6	2×10^{12}	3.0×10^{5}	8.3×10^{4}	140	207
7	3.5×10^{12}	2.7×10^{5}	1.7×10^{4}	145	211
8	1.2×10^{12}	1.1×10^{5}	3.2×10^{4}	110	750
9	0.7×10^{12}	7.0×10^{5}	4.0×10^{4}	140	547
10	1.1×10^{12}	4.1×10^{5}	1.7×10^{4}	110	320
11	2.5×10^{12}	2.2×10^{5}	2.4×10^{4}	145	432
12	3.2×10^{12}	4.2×10^{5}	2.1×10^{4}	110	322
Average	2.9×10^{12}	3.5×10^{5}	3.6×10^{4}	126.25	475

1. Total aerobic mesophilic flora (FMAT)

The leachate samples analyzed showed a high level of total aerobic mesophilic flora, which ranged from 0.7×10^{12} cfu/g to 9.0×10^{7} cfu/g with an average of 2.9×10^{12} cfu/g.

2. Enterobacteriaceae

2.1. Total coliforms

Coliforms are strongly represented in these by-products. The results of enumeration of this flora show that more than 75% of the samples have a coliform charge greater than 10^{5} cfu/g while about 17% have a load greater than 10^{4} cfu/g with an overall average of 3.5×10^{5} cfu/g.

2.2. Salmonella

Our samples showed the presence of *Salmonella*. Three species have been identified, *Salmonella thyphi*, *Salmonella parathyphi A*, and *Salmonella entredis*, and their presence is a serious public health problem.

Salmonella is considered among the microorganisms of an epidemic nature.

2.3. Fecal streptococci

Enterococci those are responsible for food poisoning range from 110 to 145 cfu/g, with an average load of 126.25 germs/g. Most *Streptococci* are identified as Group D *Streptococcus*.

2.4. Staphylococcus

The number of *Staphylococci* shows a small variation between 1.7×10^4 and 8.3×10^4 cfu/g, with an average of 3.6×10^4 cfu/g. The suspected strains have been identified. The results showed that most strains are catalase +, coagulase +, DNase and phosphatase +. They are identified as *Staphylococcus aureus*.

2.5. Clostridiums

Clostridiums are a problem of food poisoning, their presence in Table 3, shows a significant load that varies between 207 and 750 cfu/g with an average of 475 cfu/g.

Table 3. Physicochemical parameters of leachate before and after treatment

Parameter	Before treatment	After treatment
pH	8.6	3.9
Odour	Bad	Improved
Color	Bad	Clear
Suspended matter	8.3	19
Conductivity, (ms/cm)	41	21
COD, (mg/L)	4900	7600
BOD5	190	316
Organic material, (%)	19	19.4
NTK, (mg/L)	1532	1601
Orthophosphates, (mg/L)	50	51
Potassium (K^+), (mg/L)	1780	1780.3
Iron (Fe), (ppm)	13	38
Copper (Cu), (ppm)	0.6	0.6
Zinc (Zn), (ppm)	0.2	0.2
Manganese (Mn), (ppm)	0.7	0.7

The microbial load far exceeds the required standards.

The presence of coliforms may indicate the presence of faecal coliforms, traditional indicators of faecal pollution [57]. Only non-fecal coliforms are able to multiply under environmental conditions [58]. *Escherichia coli*, commensal of the digestive tract of many animals, is not saprophyte in a temperate environment. It is the most appropriate coliform bacteria to indicate fecal pollution of warm-blooded animals [8, 10], it is the most common [32] and the most resistant extreme environmental conditions [21]. *Escherichia coli* is an opportunistic pathogen capable of causing poisoning (entero-haemorrhagic strain) and diarrhea [14, 55], as well as infections [26, 28]. *Enterococci* are ubiquitous bacteria found in wastewater, freshwater, seawater, soil and plants. They have a longer lifespan than coliforms [18, 20, 45, 58], and even equivalent to that of viruses [13] but are not able to multiply in the environment [11, 12]. All this makes them very good indicators of faecal contamination and the presence of viruses. In humans, *Enterococci* are opportunistic pathogenic bacteria responsible for many infections. The three bacterial species *Pseudomonas aeruginosa*, *Salmonella* and *Staphylococcus aureus* are not involved in public health problems related to drinking water but rather indicative of dirty water. *Pseudomonas aeruginosa* is the bacterial species with the largest habitat. It lives saprophyte in water and wet soils. Its presence is constant and abundant in wastewater [41]. *Pseudomonas aeruginosa* can multiply in the environment and therefore has no value indicative of recent fecal contamination.

On the other hand, no epidemiological study has shown the existence of an association between the presence of this bacterium in drinking water and the appearance of disease cases. Its presence is therefore not a current public health problem [6, 24, 30]. *Salmonella spp.* are widespread in the environment and can survive for several weeks in dry conditions and several months in the water. Non-typhoid salmonella is abundant but rarely cause poisoning (gastroenteritis) by consumption of drinking water. Finally, *Staphylococcus aureus* is a commensal of the skin and mucous membranes of humans and animals. Eliminated in the outdoor environment, this bacterium can survive for a long time. Its presence in leachates and groundwater has been identified. Its ubiquitous character and its increasing resistance to antibiotics are at the origin of frequent and serious infections (suppurations, septicemia, toxi-infections and toxic shocks). Its effective presence in drinking water has never been the source of infection [7, 40].

2.6. Sulphito-reducers

Their natural habitat is the soil or the large intestine of humans or animals. Most species are saprophytic organisms in the soil. Their spores can survive for long periods in feces, soil, dust and water.

In terms of conclusion, the leachates show a significant microbial load, indeed this study reveals that the leachates harbor several pathogenic bacterial species, including the *Enterobacteriaceae* reputed among the most pathogenic and epidemic germs, toxigenic bacteria including *Staphylococci*, *Streptococci* and especially *Clostridiums* are a food poisoning hazard. This is a serious environmental and public health problem, especially since there is also the presence of the alteration flora involved in two types of risks, the production of toxins that can induce intoxication and the production of unwanted odors, hence the need for effective and sustainable treatment. However, leachates show a wealth of organic and mineral matter which constitutes a qualitative and quantitative potential for a possible valorization and exploitation in the agricultural production.

This work is part of this concern which aims to highlight a biotechnological process for the treatment, transformation and stabilization of leachates and their development in a balanced formulation of bio-fertilizers for agricultural application.

Our biotechnological process is based on the use of microorganisms possessing an acidifying, antibacterial and fermentative power allowing on the one hand the hygienization of leachate by the inhibition of the growth and the development of pathogenic microorganisms and on the other hand will allow the transformation and stabilization of leachate.

The choice of microorganism used in the hygienization, fermentation and stabilization of the product was based on several criteria, the fermentative, acidifying and antibacterial power, especially lactic acid, acetic acid and citric acid producing bacteria, and formic acid and especially the production of bacteriocin. The general scheme of the transformation and fermentation process is summarized in Fig. 1.

Fermentation and stabilization of leachates

10 liters of leachates are poured into a barrel and then inoculated with a high-performance fermentation leaven at a rate of 0.6 g/L. The fermentation is monitored by measuring pH, acidity, evolution of microbial flora at room temperature until product stabilization. The results obtained (Fig. 2) show that the inoculum has accelerated the fermentation process.

The pH gradually decreases according to the microbial growth during 18 days of fermentation. It goes from pH 9 to pH 3.9 to stabilize at pH 4.9 at the end of the fermentation.

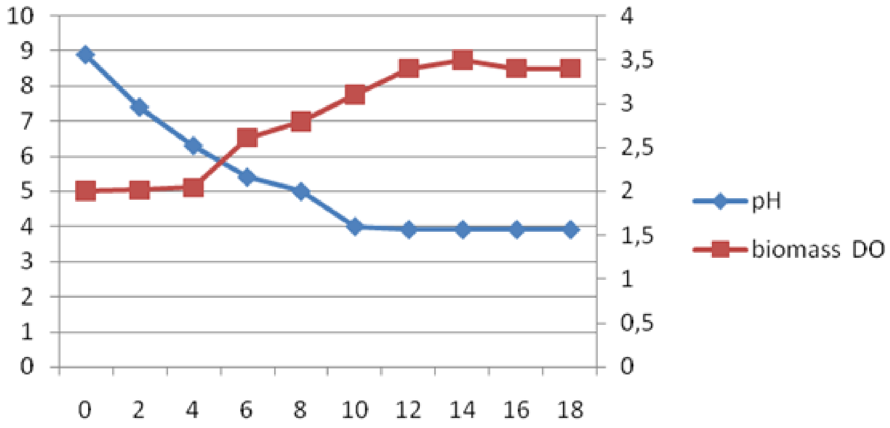

Fig. 2 Evolution of pH and biomass as a function of time (days)

Characteristics of the finished product

The physicochemical composition is slightly modified (Table 3). An improvement in color and odors was noticed; we noted a decrease in pH to 3.9. The phosphorus and potassium levels are kept stable. A slight increase in the total nitrogen content, organic matter was found the microbiological analysis (Table 4), showed that the FMAT increased from 2.9×10^{12} to 7.2×10^{18}, this increase is due to the multiplication and proliferation of the arsenal of lactic acid bacteria that is our fermentation leaven. There is a very large reduction of groups of pathogenic bacteria, so coliforms, *Staphylococci* are reduced and *Clostridiums*, *Salmonellae* are eliminated after 15 days of fermentation.

Table 4. Microbiological leachate analysis before and after treatment

Samples	**FMAT**	**Lactic bacteria**	**Yeasts**	**Total coliforms**	**Fecal coliforms**	**Streptococci**	**ST aureus**	**Clostridia**	**Salmonella**
Before treatment	2.9×10^{12}	0.3×10^{2}	2.1×10^{3}	2.1×10^{10}	4.2×10^{7}	3.1×10^{7}	4.5×10^{6}	+	+
After treatment	7.2×10^{18}	8.7×10^{15}	2.3×10^{5}	6.2×10^{2}	2.1×10^{2}	2.3×10^{2}	2×10^{2}	-	-

Conclusion

The physico-chemical and microbiological analyzes have shown that these wastes are rich in mineral elements and organic matter and are loaded with pathogenic microorganisms, which constitutes a serious ecological and public health problem.

Leachate fermentation is possible by demonstrating a process based on the use of a fermenting leaven composed of microorganisms with a strong fermenting, acidifying and antibacterial power and allowing stabilization and hygienization of leachate by eliminating

Pathogenic bacteria (*Enterobacteria, Streptococcus, Staphylococcus* and *Clostridium*) as well as the spoilage microorganisms and nematodes.
Thus, the stable product obtained has the following characteristics:

- Disappearance of the unpleasant odor replaced by a fermentation odor.
- The finished product showed a composition in macro and trace elements, richness in nitrogen, and especially an excellent hygienic quality.
- Long-term storage capacity of the finished product.

The process of biological transformation of the byproducts of this technique turns out to be original, rapid and leads to the formation of substances which enter the composition of different liquid bio stimulants.

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