

Bacteriological quality of effluents from the University Clinics of Kinshasa treated in UASB

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Abstract- The analysis of wastewater from the University Clinics of Kinshasa has shown that they are filled in considerable proportion the total germs, total coliforms, faecal coliforms, Staphylococci (*Staphylococcus sp.* and *Staphylococcus aureus*); the Enterobacteriaceae (*Entérobacter agglomerans*, *Entérobacter cloacae*, *Providencia*, *Klebsiella pneumoniae*, *Proteus vulgaris*) multi-resistant to antibiotics, responsible for nosocomial infections. As a result of a treatment by UASB, it was noted a total reduction of germs (total germs from 10.6×10^6 CFU/L to 32×10^4 CFU/L; 2.1×10^6 CFU/L of total coliforms to 0 CFU/L and from 1.5×10^6 CFU/L of faecal coliforms to 0 CFU/L), and an elimination to 100% of Entérobacter which proves the effectiveness of the UASB system for the treatment of effluents from the hospital environments.

Key words: UASB, Bacteriological Quality, Effluents, nosocomial infections, Enterobacteriaceae

Introduction

Linking health and the environment is a highlight for the opinion, but it is still a challenge for anybody looking for reliable and accurate information. The Environmental health therefore relies partly on the assessment and risk management from where the emergency of the precautionary principle that it is now seeking to introduce in addition to the protection and prevention health (Fatiha Elmoumen, 2010). All health institutions produce a large quantity of waste. It will be observed in the framework of the DRC, that the hospital wastes are a threat to the environment. The hospital waste are all biological waste or not, eliminated without any intention to be reused (Kasuku and Kitambala, 2016).

The problem of discharges of hospital effluents becomes increasingly significant. Indeed, these institutions generate large volumes of liquid effluents that contain specific substances and are likely to spread of pathogenic germs. These effluents are usually evacuated in urban networks without prior treatment, in the same way as traditional domestic wastewater (Boillot Clotilde, 2008).

The microbiological pollution, toxicological, and genotoxic, added to the importance of volumes of effluent products (of the order of $1 \text{ m}^3/\text{day}/\text{active bed}$) lead to raise several questions on their potential risk to man and his environment on the one hand and on their negative influence on the biological treatment in Step on the other hand (Jehannin, 1999). Leprat (1998), indicates that a level of global pollution higher than household effluent, associated with the systematic presence of germs having acquired the characters of resistance to antibiotics and to the presence of ad hoc typically strains in hospital. On the microbiological level, the concentrations of pilot germs are lower in the hospital effluents than in urban effluents, which is probably related to higher concentrations of disinfectants and antibiotics. One finds an average between 10^4 and 10^6 germs/ml.

The level of contamination is very variable depending on the time, day, or the flow rate at the sampling time. That is why it is important to keep a extreme vigilance in regards to the security of the internal network. The hospital strains are characterized by their resistance to antibiotics. Their survival in the environment is poorly known; research of parasites is not currently practiced.

According to Tilley E. and al. (2014), the use of a UASB process in a treatment station is due to the very high organic loads discharged by the source of production in the sewage system. The UASB uses a anaerobic process while forming a coverage of granular sludge which is suspended in the tank. The wastewater flows upward through the cover and are processed (degraded) by the anaerobic microorganisms. The UASB reactors generally feet to dilute the currents of wastewater (TSS to 3% with a particle size $> 0.75 \text{ mm}$).

This research has had as objective to characterize the bacteriological pollution of the effluent from clinics, to count and identify the substances germs responsible for the biological pollution of these hospital effluents, and to see the effect of a treatment by UASB on

the Microbiological quality of hospital effluent hospital as well as the effect of a treatment by UASB on the Microbiological quality of the hospital effluent.

Study area, Material and Methods

Study area

The University Clinics of Kinshasa (CUK) have been created by the University Lovanium in 1957, currently the University of Kinshasa, placed under the Scientific Authority of the Faculty of Medicine, but having a clear management autonomy and dependent, to do this, directly to the Board of Directors of the University.

They are built on the site of the University of Kinshasa (UNIKIN) behind the Faculty of Medicine.

They have a triple mission: the dispensing of high quality care, the teaching practice of medicine intended as well to the students of the Faculty of Medicine as to the pupils of the nurses school and other paramedical, and, finally, that of scientific research.

Built for a capacity of 1,000 beds, they currently have 800 beds whose 545 effectively functional divided into ten departments and the occupation of beds varies from 50% to 70%. The duration of hospitalization is of ± 3 days for the maternity and ± 17 days in hospitalization. The CUK are classified in the highest level having the rank of the tertiary hospital of last reference for the country.

As soon as the opening of the University Clinics of Kinshasa, the sewage system unit was with three main sewer including two poured waters in front of the clinics to the FUNA valley and the third behind the technical building of the clinics laying the effluent into the Kemi river.

The sewers which were evacuating the technical waters and valves are out of use. A separatist system was introduced with the implementation of three septic tanks, which contain the technical waters, and valves of some services. Despite the presence of these pits, which are located elsewhere in a state of very advanced decay, these waters are poorly contained. Only the storm water continue to be evacuated through the old sewer.

Material

The effluents of the infectious and tropical diseases services from the university clinics of Kinshasa were collected in four sites of rejection and treated in the laboratory of Ecotoxicology and environmental Biotechnology of the Department of Environmental Sciences, Faculty of Sciences of the University of Kinshasa by the UASB system. The UASB reactor mounted in glass at the Faculty of Polytechnics of the UNIKIN had a volume of one litre. It was adjusted to a HRT of 13 hours by the technique of gravity.

The microbiological characterization of the effluents was held before and after treatment by UASB.

Sampling

These samples were taken at 4 main glances of evacuation of the whole of the wastewater of the clinics, in March 2017.

- the site 1 deals with the effluents of the central administrative services, the emergency rooms, the central pharmacy, physiotherapy, and on the upstairs the consulting rooms;
- Site 2 receives effluent from emergency services and the block containing the gynaecology and paediatrics;
- the site 3 collects the effluent from the internal medicine, the surgical blocks and general pharmacy;
- the Site 4 is the glance, which drains the effluents of the technical building;
- the 5th sample was made up of the whole of effluents from these 4 mixed sampling points;
- the 6th sample was obtained after treatment of all the samples in the Pilot UASB set up system.

For bacteriological analysis of effluents from CUK, samples were collected in glass bottles of 200 ml each previously sterilized. The sign S1, S2, S3, S4, S5, S6, identified each sample.

The samples were transported directly to the laboratory in an insulated box at a temperature of 4°C and kept in the fridge for bacteriological analysis.

In order to isolate the germs contained in the effluent from the university clinics, two culture media (Ando agar and nutrient agar) have initially been prepared. The technique of Multiple dilutions in tubes has been applied using the Sterile physiological water, up to the ten thousandth. In total, 6 samples E1 - E6 were treated with 4 repetitions, and diluted up to ten-thousandth.

Microbial growth is characterized by the appearance of the troubles in the dilution tubes.

Petri dishes containing culture media Nutrient agar were seeded with 100 microliters per sample. The medium nutrient agar was used to isolate to a thousandth the total germs and the Ando agar at a tenth near for total and faecal coliforms.

a) Culture Medium Lactose broth

On the quantitative level, it has been applied the approach or the most probable number method in achieving the successive dilutions of the samples from hospital effluent hospital and those treated in UASB, in the lactose broth, general environment in which grow all the germs.

The principle is to place a series of four tubes containing 9 ml of lactose broth, numbered T1, T2, T3, T4 by proceeding with the addition of 1 ml of sample from the medium to the T1, then from T1 to T2 and so on. A blank tube was planned, without adding sample.

b) test of confirmation by the culture medium in broth Brilliant green bile

After incubation at 37°C and check for the presence of gas in the tubes of Durham after 24 to 48 hours, it was proceeded to the insulation by the planting of the positive samples on the culture media Mac-Conkey and Mannhutul Salt Agar (MSA) in order to respectively isolate Enterobacteriaceae and Staphylococcus. The insulation of faecal coliforms was done on the Peptone Water medium

The identification of *Staphylococcus aureus* was made on the test of related free coagulation.

3.1. Results

Table 1: Bacteriological characterization (total coliforms and germs) of effluents from Cuk

Sample	First Laboratory			Second laboratory (presence)				interpretation n bact/ml
	Germs (CFU/l)			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	
	TG	TC	FC					
S1	8.10 ⁵	0	0	+	+	+	+	10 ⁴
S2	9.4.10 ⁶	2.1x10 ⁶	1.5x10 ⁶	+	+	+	+	10 ⁴
S3	10.6x10 ⁶	33.10 ⁴	12.10 ⁴	+	+	+	+	10 ⁴
S4	10.1x10 ⁵	0	0	+	+	+	+	10 ⁴
S5	9.10 ⁶	73.10 ⁴	11.2x10 ⁶	+	+	+	+	10 ⁴
S6	32.10 ⁴	0	0	+	+	+	-	10 ³

It is clear from this table that the site 3 presents 10.6x10⁶CFU/L of total germs, followed by the Site 4 with 10.1x10⁵ CFU/L, and the Site 2 with 9.4x10⁶ CFU/L. The mixed sample then comes with 9.10⁶ CFU/L, and finally the Site 1 with 8.10⁵ CFU/L.

With regard to the total coliforms, apart from the site 1 and 4 not having presented the CFU, the second site has presented 21,3.10⁵ CFU/L, followed by the mixed sample with 73.10⁴ CFU/L and the third site was 33.10⁴ CFU/L.

The Faecal coliforms were found in the mixed effluent with a concentration of 11.2x10⁶ CFU/L, the effluent of sites 2 and 3 respectively with 15,1.10⁵ CFU/L and 12.10⁴ CFU/L, whereas the Sites 1 and 4 were lacking. After treatment (S6), in addition to the total germs, which have been present with 32.10⁴ CFU/L, total and faecal coliforms were not isolated in our sample, which shows a processing efficiency of 60% for the total germs and 100% for faecal coliforms but one has assisted to the emergency of other germs.

Table 2: isolation of staphylococci

N°	Sample	MSA and coagulase test
1	1 st site	-
2	2 nd site	<i>Staphylococcus sp</i>
3	3 rd site	<i>Staphylococcus aureus</i>
4	4 th site	<i>Staphylococcus sp</i>
5	5 th sample (mixde)	<i>Staphylococcus sp</i> and <i>Staphylococcus aureus</i>
6	After treatment	-

The presence of *Staphylococcus* was noted in the Sites 2, 4, and 5.

Table 3: Isolation and identification of germs (Enterobacteria)

N°	Samples	Identification						germs
		Kligler			Gaz	Citrate	indole	
		gluc	lac	H ₂ S				
1	1 st site	+	-	-	+	+	+	<i>Entérobacter agglomerans</i>
2	2 nd site	+	±	-	+	+	+	<i>providencia</i>
3	3 rd site	+	-	-	-	-	-	<i>Klebsiella pneumoniae</i>
4	4 th site	+	-	+	+	+	+	<i>Proteus vulgaris</i>
5	mixed sample	+	±	+	+	+	+	<i>Klebsiella Pneumoniae et providencia cloaceae</i>
5	After treatment	-	-	-	-	-	-	-

After culture of the samples of effluent from CUK, certain biochemical parameters (tests) (glucose, lactose, H₂S, gas, citrate and indol) were observed whose some positive and the other negative. Using the Table of Identification of Enterobacteriaceae, the germs below were isolated according to the sampling site: *Entérobacter agglomerans* to the first, *Providencia* at the second site, *Klebsiella pneumoniae* at the third site, *Proteus vulgaris* at the fourth site; the mixed sample contained *Klebsiella pneumoniae* and *Providencia*, the *Entérobacter* were not found on the effluent after treatment.

Table 4: Isolation of faecal streptococci on the Bilsculine medium

N°	Samples	Bilsculine medium
1	1 st site	No blackening
2	2 nd site	Blackening
3	3 rd site	No blackening
4	4 th site	Blackening
5	After treatment	No blackening

The samples from sites 2 and 4 went black in the tube containing 5 ml of Bilsculine thus showing that there was growth of germs (faecal streptococci) in this environment.

Table 5: Isolation of *Escherichia coli* on the peptone water medium

N°	Samples	Peptone water medium
1	1 st site	presence of red ring
2	2 nd site	presence of red ring
3	3 rd site	presence of red ring

4	4 th site	presence of red ring
5	After treatment	No ring

The results in this table show the presence of *Escherichia coli* in all sites except for the treated effluent.

Discussion

The enumeration of total germs in the effluent of the University Clinics of Kinshasa has given respectively 10.6×10^6 CFU/L and 10×10^6 CFU/L at Sites 3 and 4. The study of KASUKU and KITAMBALA (2016), conducted on the effluents of the Department of Gynecology of the University Clinics of Kinshasa, gave a concentration of 3.1×10^3 CFU/L of bacterial flora.

With regard to the total coliforms, apart from the site 1 and 4 not having presented the CFU, the second site has presented 21.3×10^5 CFU/L, followed by the mixed sample with 73×10^4 CFU/L and the third site was 33×10^4 CFU/L. This is consistent with the idea of Fatiha Elmoumen (2010), which declares that the total coliforms are present in large numbers in the faeces of humans and animals (2×10^9 coliforms /day/capita) and it is considered as an indicator of the control of the general quality of the water. It is possible that they are found in large numbers in the effluent of a hospital. These are of indicator bacteria of pollution of microbial contamination (Edberg et al, 2000).

The analyzes on the faecal coliforms have revealed the presence of germs in the second and third site with respectively 15.1×10^5 CFU/L and 12×10^4 CFU/L. The presence of coliforms in water indicates that this water may be contaminated by pathogens. The coliform bacteria are used as indicators of the contamination of the water because many of them live in abundance in the digestive tract of man (Midian and Martinko, 2007).

Améziane and Benaabidate (2014) have obtained in their studies the annual mean values of the load of the traditional pilot bacteria of faecal pollution in the effluent from the hospital Mohamed V in the following way: TC: 10.6×10^7 CFU/L, FC: 7.44×10^6 CFU/L and FS: 2.28×10^6 CFU/L.

The results obtained after treatment, in addition to the total germs, which have been present (32.1×10^4 CFU/L), coliform bacteria were not isolated in this sample. The installation of our pilot UASB station has folded back the bacterial load where the coliforms were eliminated; the installation of a treatment plant within the hospital would reduce the load of the river in the city.

Finally it is even within the hospital that the fate of liquid waste must be supported, by sensitizing the staff on the impact of these discharges. Given the bacterial load of these effluents, the need for a treatment plant within the hospital is useful.

The culture of the CUK effluent samples made in the MSA and MC media has shown that the sample taken at site 1 and after treatment did not present germs in the MSA medium whereas the 2nd, 3rd, 4th have shown the presence of germs and all samples had a positive result in the Mac-Conkey. The bacteriological quality of the water is evaluated by the search for pilot bacteria of faecal contamination. The culture of effluents from the services of care presents pathogenic germs that are often polyrésistants to antibiotics such as *Salmonella*, bacteria responsible for nosocomial infections (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, etc.), viruses, parasites (Boillot C, 2008).

In the samples of effluent from CUK, bacteriological analyzes have highlighted the *staphylococcus* sp and the *Staphylococcus aureus*, responsible for nosocomial infections at the hospitals. These results confirm the hypothesis of Evans (2004), which indicates that the wastewater produced by the establishments of health care can serve as a vector to agents of transmission of nosocomial infections. As to Schlosser (1999), if for most of the researchers the hospital waters would not be more polluted than the urban wastewater, the specific species such as *Pseudomonas aeruginosa* and pathogenic *Staphylococci* would make exception (more than 10 times higher for *Pseudomonas*).

After culture of the samples from the CUK effluent, analyzes of some biochemical parameters (tests) have enabled using the Identification table for Enterobacteriaceae, isolate the germs such as the *Entérobacter agglomerans*, *Entérobacter cloacae*, the

providencia , *Klebsiella pneumoniae*, and *Proteus vulgaris*. The enterococci, Staphylococci, Enterobactériaceae and heterotrophic bacteria are used as indicators of the presence of multi-resistant bacteria to the antibiotics in the network of hospital sanitation (Evans, 2004).

The observation made on the results obtained after isolation of streptococci in the effluent of CUK is that, the samples in the site 2 and site 4 have presented the darkness in the tube containing the Bilsculine and this means the growth of germs (faecal streptococci) in this environment. The parasitological or bacteriological pollution of water remains the main problem of developing countries (Fatiha Elmoumen, 2010).

During the identification of *Escherichia coli* in the effluent of the CUK having to undergo a treatment by UASB, it was noted the presence of a red ring on the whole of the samples, which characterizes the growth of *Escherichia coli* except the treated sample. *Escherichia coli* is an enteric bacterium living in the digestive tract; when they are excreted in the water, coliforms eventually die. But they die less rapidly than several pathogens (Madigan and Martinko, 2007). In order to avoid that all pollutants from hospital effluent be found in the environment, they should be treated upstream. Due to the fact that the hospital effluents contain a lot of germs, they are part of infectious wastes (KASUKU and KITAMBALA, 2016).

Regarding the performance of the wastewater treatment plant, it is clear from the microbiological analyzes, that the amount of total coliforms and faecal coliforms were greatly reduced by the treatment in our pilot UASB unit.

The UASB process is complex but very effective to eliminate the organic content of wastewater (Tony Davies, 2005). The anaerobic treatment offers several advantages for developing countries because of its merits such as the high efficiency, profitability and simplicity in the construction and operation (Abdullah Yasar and Bari Amtul Tabinda, 2009).

In a study on the effluents of the Department of Gynecology of the University Clinics in Kinshasa, a concentration of bacterial flora 3.1×10^3 per 100 ml has been found (KASUKU and KITAMBALA, 2016). According to the authors, by lack of standards established in this regard in the DRC, they could not compare this value. In the identification, several colonies of faecal coliforms and streptococci were counted; and in the insulation the effluents have shown the presence of *Escherichia coli*, *Klebsiella*, *Salmonella* and *Enterococcus*.

Conclusion

Wastewater from the university clinics in Kinshasa have shown significant concentrations of bacteria responsible for nosocomial infections and multi-resistant to antibiotics such as *Klebsiella pneumoniae* and *Staphylococcus aureus*.

The highest concentration was that of total germs obtained at 10.6×10^6 CFU/L, faecal coliforms with 15.1×10^5 CFU/L and total coliforms with $21.3 \cdot 10^5$ CFU/L

The UASB treatment applied to these waters eliminates the coliforms in general to almost 60% and the *Entérobacter* to 100% but this gives rise to the establishment of a new bacterial community different from that of departure, due to anaerobic conditions that settle in the reactor.

The bacteriological analyzes carried out allowed to deduct that microbiological load of effluents from the University Clinics in Kinshasa constitutes a health risk that could potentially be hazardous especially where these effluents are used for the irrigation of crops consumed by the populations.

It is therefore necessary to limit the discharges of hospital effluents in the environment without prior treatment.

However, despite the fact that bacteriological analyzes represent only a fraction of the total load of contaminants from the Hospital, it is recognized the danger of microorganisms among all other contaminants from the CUK.

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