Journal of Water & Health

IWA PUBLISHING

Journal of Water and Health Vol 21 No 10, 1460 doi: 10.2166/wh.2023.115

Identification of some microbial flora contained in slaughterhouse effluent and likely to be effective in its treatment by biological process

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ABSTRACT

The specific contributions of the different bacterial flora during the biological treatment of slaughterhouse effluents remain unclear. The objective of this study is to identify the different microbial flora which mainly contribute to the efficiency of the biodegradation of the organic, nitrogenous and phosphate loads contained in the slaughterhouse effluent during its biological treatment. To achieve this, the effluent to be treated was sampled from three slaughterhouses in the city of Ngaoundéré (Cameroon). The various effluents underwent a physicochemical and microbiological characterization. The effluent was subjected to biological treatment. The biodegradation process (biological treatment) took place in two reactors, each operating in batch. One of the two reactors was supplied with oxygen (aeration). The effluent being treated underwent a physicochemical and microbiological characterization for 30 days. The results obtained show organic matter and ammonium contents >1,000 mgO_2/L in each of the three effluents. *Bacillus cereus* (69 × 108 CFU/mL), *Pseudomonas aeruginosa* (201 × 107 colony forming unit (CFU)/mL) and Yeasts (101 × 106 CFU/mL) globally constitute the majority of microbial groups among the seven microorganisms identified in the effluents of the three slaughterhouses. There is no real oxygenation effect of the medium on the growth of the three microbial flora during the treatment.

Key words: aeration, anoxia, biodegradation, microbial flora, slaughterhouse effluent

HIGHLIGHTS

- The presence of oxygen promotes the rate of elimination of the pollutant load without impacting microbial growth.
- The quality of the pollutant favours microbial growth more than the concentration of the pollutant.
- The concentration of the pollutant load is influenced by the microbial load during treatment.
- The pollutant load varies from one microorganism to another.

GRAPHICAL ABSTRACT

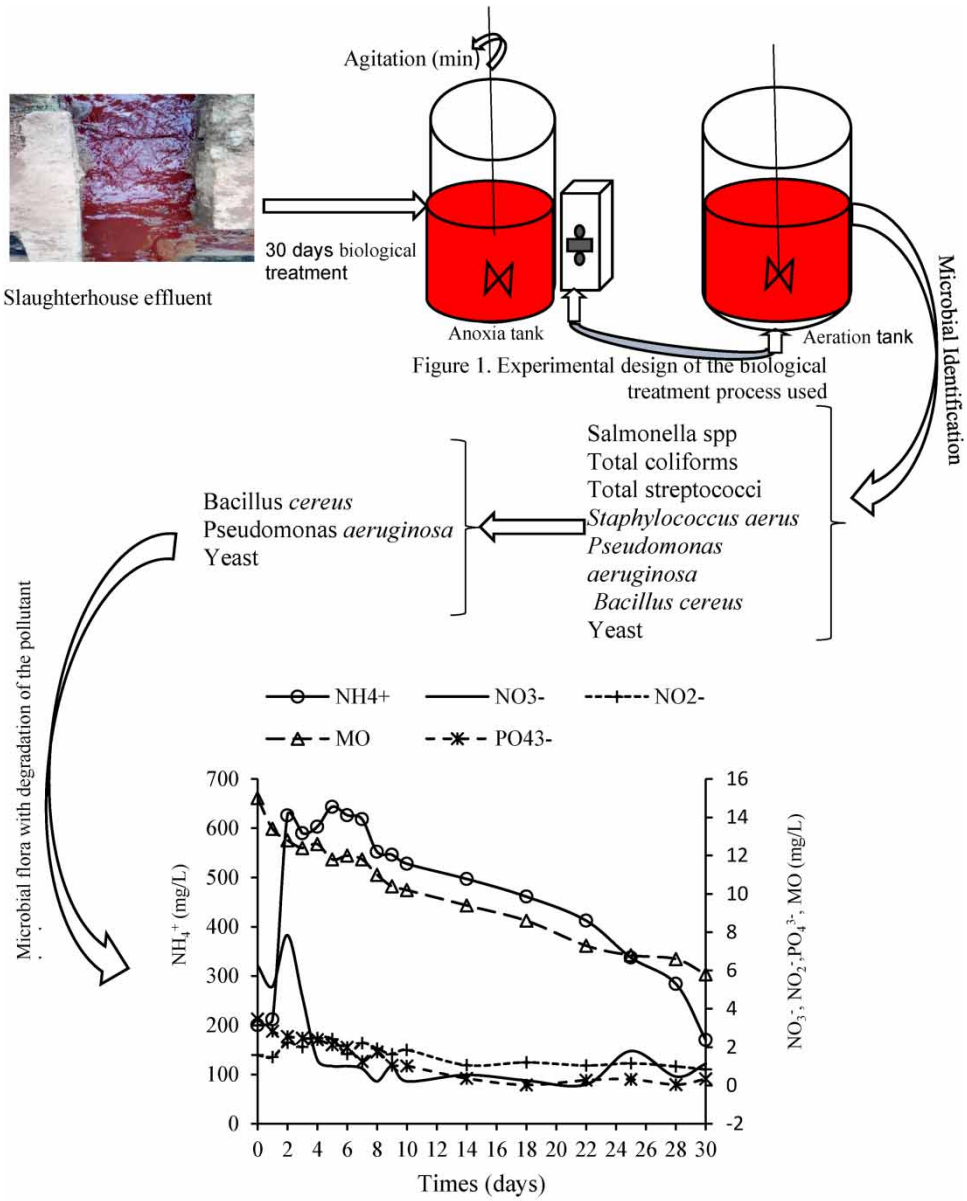

Figure 1. Experimental design of the biological treatment process used

Figure 2: Evolution of the reduction of polluting loads during the biological treatment of slaughterhouse effluent in a non-oxygenated

1. INTRODUCTION

Slaughterhouses are among the sectors of expansive industrialization because meat is included in the human diet (Dilara & Alper 2019). Depending on the number of animals slaughtered and the operating conditions of the facility, slaughterhouses can produce large quantities of significant wastewater from the production unit due to slaughter and cleanings (Ozturk *et al.* 2020). The physicochemical properties of the slaughterhouse wastewater vary regionally and depend on the size of the slaughterhouse and the water consumption (Akshay & Girish 2021). After use, slaughterhouse wastewater becomes a source of pollution for the natural environment if discharged without prior treatment. This pollution has multiple consequences on ecosystems, including eutrophication (Sengar *et al.* 2018; Abdallah *et al.* 2020; Cristina *et al.* 2020; Brian *et al.* 2021) and on human health through many waterborne diseases (Youssef *et al.* 2015). All this justifies the importance of its treatment.

Several authors have used different methods to treat this effluent. Gongwala *et al.* (2014), for example, subjected slaughterhouse wastewater to cold plasma treatment by glidarc and achieved a reduction rate of 41.55% for phosphates and 86.24% for nitrates. Dilara & Alper (2019) performed the electrochemical oxidation treatment and successfully removed 88% total carbon, 92.2% chemical oxygen demand (COD) and 93.5% total nitrogen. The high concentration of biodegradable organic material in this wastewater prompted other authors to use biological treatment (Carvalho *et al.* 2013; Ogbomida *et al.* 2016; Aziz *et al.* 2019; Kouakou *et al.* 2020; Akshay & Girish 2021).

However, biological treatment, which is based on the degradation of organic matter (OM) by microorganisms, proceeds very slowly due to the large fluctuations in concentrations. This prolongs the residence time of the effluent to be treated in the biological reactors. Faced with this difficulty, Gnowe *et al.* (2020) studied the influence of oxygenation of the medium and treatment time on the biodegradation of organic, nitrogenous and phosphate loads. They discovered that there is a minimum biological treatment time beyond which biodegradation is no longer effective. Except that at this reduced biological treatment time, the biodegradation of nitrogenous forms is not effective. To correct this other limit, Djonga *et al.* (2019) decided to carry out a simultaneous and batch combination of biodegradation and adsorption. They concluded that the efficiency would have been better if the reactors had been incubated with a specific microbial flora that is very active in the degradation of the target pollutants contained in the slaughterhouse effluent. It is with the aim of verifying this hypothesis that the authors of this work decided to identify the different microbial flora which would contribute mainly to the efficiency of the biodegradation of the organic, nitrogenous and phosphate loads contained in the slaughterhouse effluent during its biological treatment.

2. MATERIAL AND METHODS

2.1. Sampling of slaughterhouse effluent to be treated

The slaughterhouse effluent to be treated was sampled from the three slaughterhouses in the city of Ngaoundéré. These are the slaughterhouses of Baladji 2 (7°19′58°N; 13°34′11″E), Wakwa (7°22′76″N; 13°58′21″E) and Manwi (7°39′48″N; 13°55′17″E). Of the three slaughterhouses, only Wakwa carries out a so-called modern activity. That of Baladji 2 has the particularity of being more in demand (more active) than the other two. The different samples taken immediately underwent sieving, then a 100th dilution before undergoing physicochemical and microbiological characterization.

2.2. Chemical characterization of effluent

The chemical characterization of the effluent consisted of measuring the organic, phosphate and nitrogenous matter that it contained. OM was determined by the method described by Rodier (2005). Its principle is based on the hot and acidic oxidation of OM by potassium permanganate ($KMnO_4$) introduced in excess into the medium. The determination of phosphates was carried out by the method of Rodier *et al.* (2009). Its principle is based on the reduction of the phosphomolybdic complex by ascorbic acid of the phosphomolybdic complex to molybdenum blue following the orthophosphate anion, which reacts with ammonium molybdate in an acid medium. The analysis of absorbance of the orthophosphates with the ultraviolet (UV)-visible spectrometer was carried out measured at 690 nm.

Ammonium and nitrites were measured according to the methods described by Rodier (1978) and used by Gnowe *et al.* (2020). The principle of the method used for the determination of ammonium is based on the decomposition of alkaline potassium iodo-mercurate (Nessler's reagent) in the presence of ammonia with the formation of di-mercury-ammonium iodide. The principle of the method used to measure nitrites is based on the formation of a yellow complex, following the reaction of sulfonic acid with nitrite ions, in a hydrochloric medium and in the presence of ammonium ions and phenol. The absorbances of the ammonium and nitrites with the UV-visible spectrometer were measured at 420 nm (for NH_4^+) and 435 nm (for NO_2^-). The determination of nitrates was carried out by the sodium salicylate method according to Rodier (1978). Its principle is based on the formation of yellow-colored sodium para-nitro-salicylate, which is the product of the reaction of nitrites with sodium salicylate. Its absorbance was measured using a UV-Visible spectrometer at 420 nm. For each compound except OM, a standard solution was prepared and analyzed with $R^2 > 0.98$ and detection limits between 0.0010 and 0.0021 mg/L.

2.3. Microbiological characterization of the effluent

The identification and counting of the microorganisms present in the slaughterhouse effluent was carried out according to AFNOR (1996). It was first necessary to prepare a stock suspension, which consisted of the sample diluted 10^{-1} with sterile buffered peptone water (EPT). This suspension was then used to prepare decimal dilutions with EPT (20 g/L). The dilutions

obtained were used to inoculate the specific culture media. The agar culture media used are *Salmonella shigella* (SS), Eosin methylene blue (EMB), Bile Esculin agar (BEA), Mannitol salt agar (MSA), *Pseudomonas*, *Bacillus cereus* and Sabouraud.

Surface inoculation was carried out by spreading 0.1 mL of each dilution on the surface of 20 mL of agar culture medium previously prepared, sterilized and poured into a Petri dish. Then the covered and inverted dishes were incubated at 25 and 37 °C. After incubation, the colonies visible to the naked eye were counted by pointing at them with a marker on the bottom of the Petri dish. The number of colonies was then multiplied by the dilution rate and expressed as the number of colony-forming units per milliliter of slaughterhouse effluent (CFU/mL). The volume of inoculum seeded was also taken into account. The determination of the microbial load was carried out using the following equation.

$$N = n \times \frac{1}{D} \times \frac{1}{V} \quad (1)$$

where n is the number of colonies counted in a Petri dish for a given dilution, D is the dilution factor, V is the volume of the inoculum in mL seeded in the dish and N is the microbial load in CFU/mL of the sample for a specified germ group.

2.4. Biological treatment of the effluent

The slaughterhouse effluent was first diluted to 1/100 before being introduced into two different reactors. The two reactors are components of a pilot wastewater treatment unit using the principle of activated sludge (MP44) which operates continuously. However, the two reactors were used in the batch. Thus, 16 L of the diluted effluent was introduced into the first reactor which operates without supplying the medium with oxygen, while 38 L was introduced into the oxygen-fed reactor (Figure 1). The treatment was initiated with stirring (112 rpm) over 30 days, during which the evolution of the pollutant load and the microbial flora were monitored. Indeed, the slaughterhouse effluent was characterized every day for 10 days. After 10 days of treatment, it was characterized once every 4 days for 12 days. After 22 days of treatment, it was characterized once every 3 days, then once over 2 days during the last 8 days; therefore, the treatment was followed for a total of 30 days, as shown in Table 1.

3. RESULTS

3.1. Physicochemical and microbiological characteristics of slaughterhouse effluent

The characteristics of the different slaughterhouse effluents sampled are presented in Table 2.

The results in Table 1 show the overall existence of a real site effect on the physicochemical and microbiological characteristics of the slaughterhouse effluent. These effluents are generally basic. Their OM levels are greater than 1,000 mgO_2/L, with at least 100 mgO_2/L more in the effluent of Baladji 2, compared to the other two. The three effluents contained an

Figure 1 | Experimental design of the biological treatment process used. (a) Reactor without oxygen supply; (b) stirring blade; (c) oxygen-fed reactor; and (d) air pump.

Table 1 | Various effluent samples taken during treatment

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Levies	×	×	×	×	×	×	×	×	×	×	.	.	.	×	.
Days	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Levies	.	.	×	.	.	.	×	.	.	×	.	.	×	.	×

(×) indicates the sample.

Table 2 | Physicochemical and microbiological characteristics of effluents from three slaughterhouses in Ngaoundéré

Parameters	Baladji 2	Wakwa	Manwi
pH	7.73 ± 0.60^{a}	8.05 ± 0.05^{a}	$7{,}91 \pm 0{,}11^{a}$
MO (mg.O_2/L)	1500 ± 10^{a}	1270 ± 10^{c}	1360 ± 11^{b}
PO_4^{3-} (mg/L)	346 ± 24^{b}	410 ± 12^{a}	207 ± 10^{c}
NH_4^+ (mg/L)	8642 ± 128^{a}	1501 ± 11^{c}	1712 ± 11^{b}
NO_2^- (mg/L)	69.3 ± 1.6^{a}	$31.4 \pm 0{,}3^{c}$	43.4 ± 0.2^{b}
NO_3^- (mg/L)	312 ± 22^{b}	352 ± 10^{a}	155 ± 10^{c}
Salmonella spp (UFC/mL)	$79 \times 10^{7\ aB}$	$47 \times 10^{3\ bC}$	$97 \times 10^{3\ bD}$
Total coliforms (UFC/mL)	$33 \times 10^{7\ aB}$	$116 \times 10^{4\ bB}$	$216 \times 10^{3\ cD}$
Total streptococci (UFC/mL)	$9 \times 10^{5\ aD}$	$55 \times 10^{3\ bC}$	$75 \times 10^{3\ bD}$
Staphylococcus aureus (UFC/mL)	$21 \times 10^{4\ aE}$	$104 \times 10^{2\ cD}$	$94 \times 10^{3\ bD}$
Pseudomonas aeruginosa (UFC/mL)	$201 \times 10^{7\ aB}$	$32 \times 10^{5\ bA}$	$31 \times 10^{7\ aA}$
Bacillus cereus (UFC/mL)	$69 \times 10^{8\ aA}$	$7 \times 10^{4\ cB}$	$5 \times 10^{5\ bB}$
Yeast (UFC/mL)	$101 \times 10^{6\ aC}$	$35 \times 10^{3\ cC}$	$65 \times 10^{4\ bC}$

[a, b, c]for each row, values with the same lowercase letter are not significantly different ($P > 0.05$).
[A, B, C]for each column, values with the same uppercase letter are not significantly different ($P > 0.05$).

average of 321 ± 104 mg/L phosphates, with a dominance in the Wakwa effluent. Ammonium (NH_4^+) represents overall the majority form of nitrogen in the three effluents, with an average content >1,000 mg/L. The effluent of Baladji 2 contains 5.8 times more NH_4^+ than the other two effluents and is globally identified as the effluent most loaded with chemical pollutants.

Microbiological characterization indicates that the seven microbial flora sought are present in all three samples with at least 102 CFU/mL. However, the effluent from Baladji 2 contains 102 CFU/mL in addition to each of the seven floras, compared to the other two effluents. This is in line with the other trends indicating that the effluent of Baladji 2 is the most chemically loaded of the three slaughterhouses. The microbiological characterization also reveals that *B. cereus*, *Pseudomonas aeruginosa* and yeasts flora constitute the microbial groups mainly represented in the three samples. But are these microbial groups also the most active during biological treatment?

3.2. Evolution of the different microbial flora during biological treatment of effluent

Figure 2 shows the evolution of the microbial flora in the slaughterhouse effluent during its treatment in the two reactors over 30 days.

Figure 2 shows that all seven microbial families are present in the effluent during the 30 days of treatment, whether or not the medium is supplied with oxygen. However, unlike *B. cereus*, *P. aeruginosa* and yeasts whose populations increase over time, all other flora decrease in the environment during the treatment. It would, therefore, be interesting to continue the work only with these three microbial families that grow during treatment. Using this premise, the results of Figure 2 show the existence of a treatment time effect on the growth of the three microbial families. This time effect is observable through the low growth of *Pseudomonas* and *Bacillus*, and a decrease in yeasts during the first 9 days. Indeed, with the exception of yeasts which have an average decrease rate (with or without feeding the medium in O_2) of 6.7%, *Pseudomonas* and *Bacillus* have, respectively, average growth rates of 0.6 and 6.15% on the ninth day of treatment. After this ninth day of treatment, the time

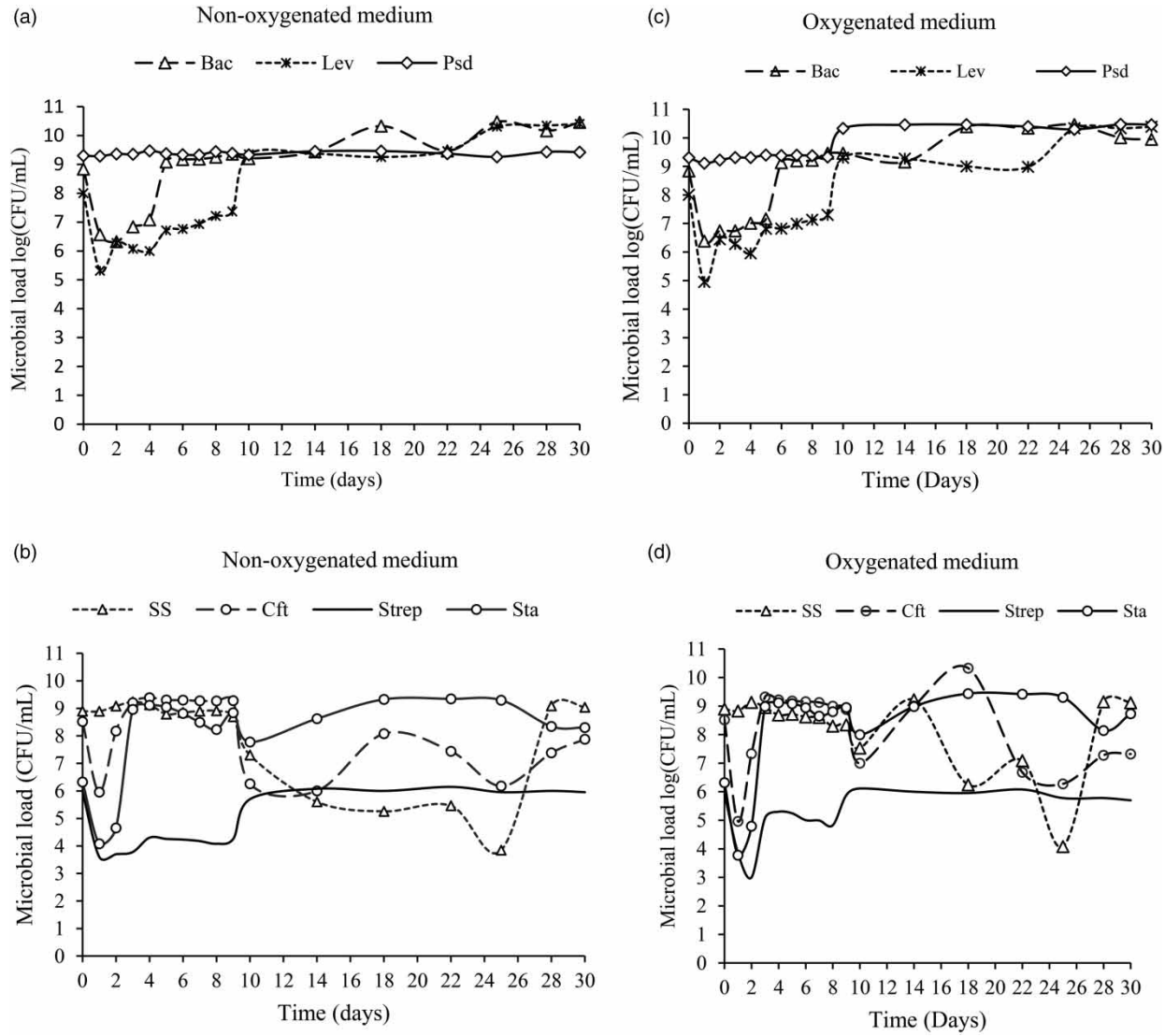

Figure 2 | Evolution of microbial flora in non-oxygenated (a,b) and oxygenated (c,d) medium during 30 days of biological treatment of effluent. SS, *Salmonella* spp; Cft, total coliforms; Strep, total streptococci; Sta, *Staphylococcus aureus*; Bac, *Bacillus cereus*; Lev, yeasts; Psd, *Pseudomonas aeruginosa*.

required to reach the maximum growth rate varies from species to species. Thus, the average maximum growth rate of *Pseudomonas* (12.47%) is reached on the 14th day of treatment. Those of *Bacillus* (18.38%) and Yeast (29%) are affected on the 25th day. It is important to note that, the average growth rates of *Bacillus* and Yeast are, respectively, 5.15 and 16.5% on the 14th day and 17.2 and 14% on the 18th day of treatment. This would mean that after the 18th day of treatment, only the yeast activity is really significant in the reactors.

However, the results of Figure 2 show a very low effect of the oxygenation environment on the growth of the three microbial flora. Indeed, on the 14th day, *Pseudomonas* reached its maximum growth rate. It had 10.46 CFU/mL in the oxygen-supplied reactor compared to 10.45 CFU/mL in the other reactor. On day 25 of treatment, *Bacillus* and Yeast each had 10.46 CFU/mL in the oxygen-supplied reactor, compared to 40.48 CFU/mL (*Bacillus*) and 10.31 CFU/mL (Yeast) in the other reactor. The differences observed in the two reactors were not significant.

3.3. Evolution of the pollutant load of slaughterhouse effluent during treatment

Figure 3 shows the evolution of the abatement of organic, nitrogen and phosphate loads during the treatment of slaughterhouse effluent, in the reactor not supplied with oxygen.

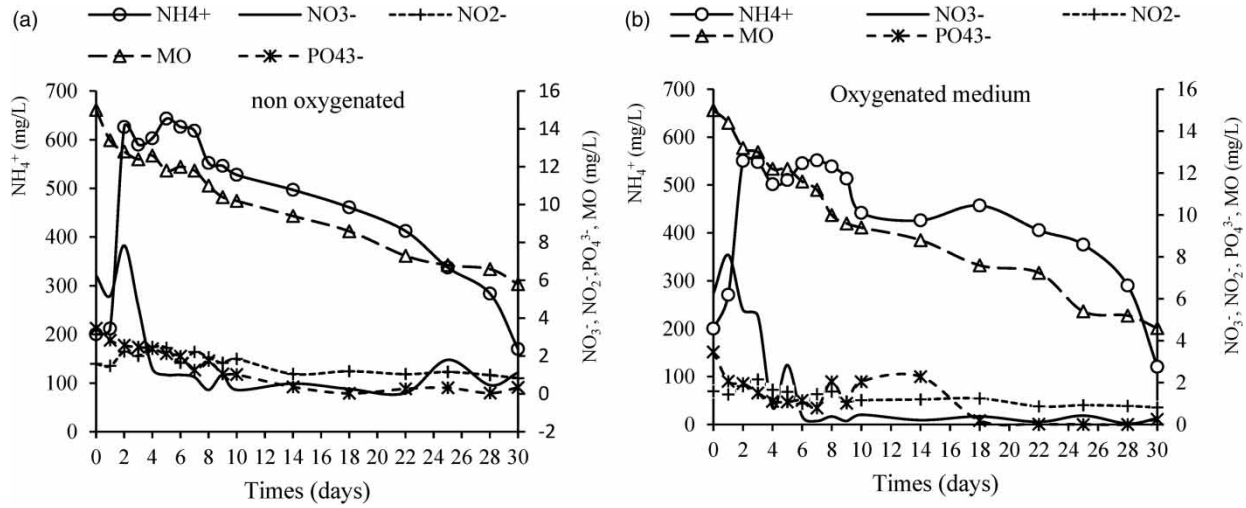

Figure 3 | Evolution of the reduction of polluting loads during the biological treatment of slaughterhouse effluent in a non-oxygenated (a) and oxygenated (b) medium.

Figure 3 shows two main types of evolution overall, concentrations of OM and phosphates (PO_4^{3-}), which decrease continuously over time. While the concentrations of ammonium (NH_4^+), nitrates (NO_3^-) and nitrites (NO_2^-) increased almost during 2 days of treatment before decreasing over time. Unlike MO and NH_4^+ whose decreasing speeds were almost regular, those of PO_4^{3-}, NO_3^- and NO_2^- had a phase of abrupt decrease (the first 8 days) and a phase of almost constant decrease (after 8 days).

Figure 3 also shows the existence of an oxygenation effect of the environment on the degradation of the pollutant load. This oxygenation effect is characterized by a greater reduction of MO, PO_4^{3-}, NH_4^+, NO_3^- and NO_2^- in the oxygen-supplied reactor than in the other reactor during treatment. After 30 days of treatment, the abatement rate in the oxygen supply reactor was 69.33% (MO), 92.51% (PO_4^{3-}), 39.82% (NH_4^+), 95.21% (NO_3^-) and 48.43% (NO_2^-); against 61.33% (MO), 90.2% (PO_4^{3-}), 15% (NH_4^+), 81.85% (NO_3^-) and 47.17% (NO_2^-) in the reactor not supplied with oxygen. The difference in abatement rate observed between the two operating conditions of the reactors on the 30th day of treatment was observed during the entire treatment. This oxygenation effect, which has an overall influence on the abatement rate of the pollutant load of the effluent during treatment, is an indicator of the choice of reactor for further work.

4. DISCUSSION

4.1. Influence of the sampling site on the characteristics of the slaughterhouse effluent

Table 1 shows the effect of the sampling site on the physicochemical and microbiological characteristics of the slaughterhouse effluent. This site effect is primarily due to the level of activity of the slaughterhouse. Indeed, the highest levels of pollutants recorded in the effluent of Baladji 2 were due to its high rate of daily slaughter of beef compared to that of the other two. This is because the more cattle slaughtered, the more polluted the effluent generated in the slaughterhouse. This effect on the site is also due to the cleaning method developed within the slaughterhouse. Since Baladji 2 slaughterhouse, which is less modern than the Wakwa slaughterhouse, uses untreated surface water (well, river) for cleaning, it is obvious that the effluent generated downstream is more loaded with OM than the effluent generated by the Wakwa slaughterhouse, which uses groundwater (borehole) that has undergone filtration operations, demineralization and neutralization. This site effect may finally be due to the diet of slaughtered cattle. Indeed, the ammonium content can vary from one wastewater sample to another, depending on the diet of the slaughtered animals, because 60–70% of nitrogen integrated by the beef is found in slaughterhouse wastewater through the rest of urine and fecal matter released during the slaughter operation (Peyraud *et al.* 2012).

4.2. Influence of microbial diversity on their growth during treatment

The microbial flora identified in both reactors can be classified into two categories. The first category consists of pathogenic microorganisms that are initially present in the effluent because they already exist in the animal's organism. These are

Salmonella, *Pseudomonas*, *Bacillus* and Yeasts (Sule *et al.* 2020). The second category consists of microorganisms that contaminate the effluent after slaughter. These are *Staphylococcus*, *Streptococcus* and Coliforms. Figure 2 shows that, with the exception of *Bacillus*, *Pseudomonas* and Yeasts, the other four microbial flora decrease in reactors with or without oxygen during treatment. Their decrease in the reactors is justified by the phenomenon of competition on the one hand and on the other hand, their difficulty to adapt to the physicochemical conditions of the unstable and unfavorable environment during the first 8 days of treatment.

Regarding the phenomenon of competition, the results of Table 1 show that the flora *Bacillus* spp, *Pseudomonas* spp, *Salmonella* spp and Coliforms represent at the beginning of treatment, the majority microbial populations in the effluent. The larger a microbial population, the faster it will attack the nutrient resources available in the environment at the expense of smaller populations. The more a microbial population feeds, the more it grows. The population that has difficulty feeding itself will decline (Zhang *et al.* 2014). This could justify the difficulty encountered by *Staphylococcus* spp and *Streptococci* spp during the first 8 days of treatment. On the other hand, the evolution is decreasing populations of *Salmonella* spp and Coliforms, which are nevertheless larger in the raw effluent, due to their difficulties in adapting to the physicochemical conditions of the environment imposed by the pollutant load of the effluent. These are indeed two bacteria of the class of pathogens that already exist in the animal's body. Their appearance in slaughterhouse effluent required an adaptation test (Vineeta *et al.* 2016), in an environment modified by aqueous solutions containing surfactants (detergent) and chlorinated substances, from the cleaning activity of the structure (Aderiye *et al.* 2017). This is what justifies their rather decreasing evolution during the first 8 days of treatment. Thus, whether the decreases are due to competition or the drastic condition of the environment, the four microbial floras that lose their populations during the so-called latency phase (or adaptation) will still have no more difficulties multiplying after the first 8 days. Because after 8 days of treatment, the environment is even more aged by populations that have successfully adapted to the latency phase, making it more difficult to access nutrient resources. This justifies the more or less decreasing evolution of *Staphylococcus* spp, *Streptococci* spp, *Salmonella* spp and Coliforms after 8 days of treatment.

Yeasts are the only microbial population that has the distinction of belonging to the category of pathogens and has a small population in the raw effluent. This is what justifies its difficulty in multiplying during the first 8 days of treatment. Indeed, during the latency phase, the Yeast population decreases drastically by 50% after 1 day of treatment, before gradually increasing until catching up with its starting population on the eighth day, and increasing during the rest of the treatment. The drastic drop in its population after only 1 day of treatment is due to the sudden change in its living environment, which was not facilitated by its small population (101×106 CFU/mL), compared to that of microorganisms called environmental contaminants, such as *Pseudomonas* spp (201×107 CFU/mL) and *Bacillus* spp (69×108 CFU/mL). The progressive growth of its population in the environment over time, despite the unstable physicochemical and microbiological conditions of the environment, is indicative of a microbial flora with particular characteristics.

Moreover, after the 18th day of treatment, only the Yeast activity is truly significant in both reactors. This demonstrates its usefulness or importance in the process of degradation of the pollutant load contained in the slaughterhouse effluent. This particular behavior is certainly due to its microbial nature (yeasts), which is different from that of the bacteria constituted by the rest of the microbial population presented earlier.

4.3. Influence of oxygenation on microbial growth during treatment

The results of Figure 2 showed an almost non-real absence of the oxygenation effect of the environment on the growth of the three microbial flora considered the most useful in the process of degradation of the pollutant load of slaughterhouse effluent. This already indicates that *Pseudomonas* spp, *Bacillus* spp and Yeasts are microorganisms of aerobic and anaerobic nature. In addition, several authors agree that *Pseudomonas* spp is a bacterium whose development in an environment (water) requires nutritional and physicochemical conditions that can be demanding (Iroha *et al.* 2016). This justifies in its case the real non-existence of an oxygenation effect of the environment on its growth over time. *Bacillus* spp is particularly a facultative anaerobic bacterium (Bagge *et al.* 2010) whose good development in an environment is favored by activators consisting mainly of silicon, iron and magnesium. The proportional contributions of each of these activators to the propagation rate of *Bacillus* spp in slaughterhouse effluent are not known, it is still important to indicate that this red effluent is particularly rich in iron (Djonga *et al.* 2019). This high presence of iron associated with the agitation of the medium is certainly at the origin of the easy growth of *Bacillus* spp in the reactor operating with or without an oxygen supply.

4.4. Relative influence of chemical composition on the evolution of microbial flora

The correlation that could exist between the evolution of the concentration of the pollutant load and that of the microbial flora is not really visible through the method developed in this work. However, it is possible to link the results of the two factors. Figure 3 shows that the concentrations of MO and PO_4^{3-} decrease continuously over time, while those of NH_4^+, NO_3^- and NO_2^- first increase during 2 days of treatment before decreasing in time. Each of the constituents of this pollutant load is dissolved in the treated effluent and is also a varied source of supply for the different microbial flora. Throughout the treatment, the evolution of the concentration of the pollutant and that of the microbial population are governed by the phenomena of adsorption of the pollutant on the microorganism and the metabolism of the latter. Indeed, at the beginning of the treatment, the microbial flora was an adsorbent support for pollutant loads that attach to it with the help of environmental agitation (Zhang *et al.* 2014). Flocs were formed and represented in the reactors by sludge. The main pollutant affected by this adsorption phenomenon was OM. The microorganism that was flagellated was also brought into contact with other pollutants in ionic forms through agitation. This contact of the microorganism with the pollutant did not promote the microbial growth. This justifies the absence of the increase of some microbial populations and even the fall in others observed in Figure 2. Moreover, the decrease in the concentration of MO during this contact phase was not due to its degradation but rather to the settling of the flocs formed (sludge).

It is only after some time of contact (2–3 days) with the adsorbed pollutant, that the microorganism begins its digestion and develops a metabolism that promotes its growth characterized by the multiplication of its population. As justified in Figure 2, there is a gradual increase in the population of certain microbial flora after 2 days of treatment. This also justifies the sudden increase in the concentration of NH_4^+, NO_2^- and NO_3^- observed in Figure 3. Indeed, NH_4^+ is a product of OM digestion. Once released, the concentration of NH_4^+ increases in the medium. NO_2^- and NO_3^- are the products of the respective oxidations of NH_4^+ and NO_2^- that occur in the environment and are caused by microbial activity (Gnowe *et al.* 2020). Hence the sudden increase in their concentrations in the effluent was observed on the second treatment. The different evolutions of microbial growth were not proportional to the concentration of the pollutant load contained in the effluent during treatment, but rather they were strongly dependent on its quality, which influenced the metabolism of the flora (Zhang *et al.* 2014). This is because after 11 days of treatment, the rate of increase of the microbial population is almost non-existent ($<1\%$), yet the concentrations of pollutants are still high in the effluent (nearly 40%), which lacks elements favorable to catalyze the biodegradation reaction. This is in agreement with one of the conclusions drawn by Gnowe *et al.* (2020).

5. CONCLUSION

This study sought to identify the different microbial flora that had a major contribution to the efficiency of the biodegradation of the pollutant load of slaughterhouse effluent. It showed that the genus Yeasts is the only microbial group whose activity remains significant during more than 18 days of treatment. The presence of oxygen in the reactor promotes the rate of elimination of the pollutant load without significantly influencing the microbial growth. *P. aeruginosa*, *B. cereus* and Yeasts represent the potentially effective microbial groups for biological treatment of slaughterhouse effluent.

ACKNOWLEDGEMENTS

The authors of this work address their gratitude to Dr Boukar HASSANA, Head of Department of Chemical Engineering of the University Institute of Technology (IUT) of Ngaoundéré. Thank you to the Department of Chemistry of the Faculty of Sciences of the University of Ngaoundéré in Cameroon.

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories https://ghenmimaurelle2012@gmail.com.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 6 March 2023; accepted in revised form 22 September 2023. Available online 9 October 2023