



Evaluation of Improved Bioremediation Strategy for the Treatment of Abattoir Wastewater using *Bacillus licheniformis* ZUL012

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ABSTRACT: The abattoir generates a large volume and variety of biowastes, posing a high risk of environmental contamination, disease outbreaks, and contaminated food. The purpose of this research was to characterize and remediate abattoir wastewater (Aww). The physicochemical characterization of the Aww revealed high level of pollution which served as a baseline for monitoring treatment efficacy. The *B. licheniformis* ZUL012 isolated from textile wastewater was primed with H₂O₂ and used to remediate Aww waste water in the current study. This study revealed Aww high pollution level which necessitated a need for the Treatment with this bacterium resulted in a significant decrease in some of the waste water parameters tested. The induced cell reduced the parameters to 155 41 mg/L, 75 mg/L, 454 mg/L, 1750 mg/L, and 1000 mg/L, whereas the naive cell reduced them to 375 mg/L, 776 mg/L, and 3122 mg/L, respectively. This equates to an average reduction of 95 percent (COD), 95 percent (BOD₅), 77 percent (TOC), and 71% (TDS) compared to raw wastewater. These novel strategies show that H₂O₂-induced *B. licheniformis* ZUL012 could be a viable hybrid-bioremediation option for reducing or transforming the pollutants present in Aww, thereby contributing to compliance with wastewater discharge regulations into bodies of water.

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Abattoirs use a lot of water in their processing operations (slaughtering and cleaning), which results in a lot of wastes (Bustillo-Lecompte *et al.*, 2016). The slaughterhouse generates a large amount and a diverse range of biowastes, posing a high risk of contamination to the environment, disease outbreaks, and unsafe food safety (Al-Gheethi *et al.*, 2021). The wastewater and solid wastes from the abattoirs are directly disposed into bodies of water and lands adjacent to the abattoirs, posing a significant ecological biohazard (Fearon *et al.* 2014). This is because there are insufficient waste management and treatment facilities. However, the composition of abattoir wastewater (AWW) varies greatly depending on the various industrial processes and water requirements (Bustillo-Lecompte *et al.*, 2015). Meat processing effluents are recognized as harmful and threatening due to the complex composition of fibers, proteins, fats, and high organic content, as well as the

pathogenic risk from fecal bacteria and potentially infectious carcasses (Chang *et al.*, 2010; Bustillo-Lecompte *et al.*, 2015; Bustillo-Lecompte and Mehrvar, 2017). In addition, wastewater from slaughterhouses contains a number of toxic materials, including nitrate, detergents, surfactants, and chloric anions, which are classified as emerging contaminants (Latiffi *et al.* 2021). As a result, it necessitates specialized treatment for a safe and long-term release into the environment (Johns *et al.*, 1995; Bustillo-Lecompte and Mehrvar, 2015). At the same time, inadequate disposal of slaughterhouse biowastes (SH-biowastes) has resulted in harmful algal blooms in surface water (Arvanitoyannis and Kassaveti 2008) and groundwater contamination from antibiotics used in poultry production (Alam *et al.* 2019), raising safety concerns. Oller *et al.* (2011) contend that treating recalcitrant, toxic effluent with a single universally applicable end-of-pipe solution is unrealistic, and they

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advocate a combination of different techniques for economically feasible degradation. Increased oxidative stress improves environmental contaminant elimination and represents a promising strategy in environmental bioremediation by regulating microbial metabolism in oxidoreductase expression (Bains *et al.*, 2019; Liu *et al.*, 2020). Induced oxidative stress increased the production of highly active and efficient oxidoreductases and hydrolases (cytochrome P450, lignin, and horseradish peroxidase) (beta-glucosidase). This enzyme family, some of which may also degrade hazardous compounds, is a method for reducing organic micropollutants (Khatoon *et al.*, 2017). The advantages of the Chemically mediated bioremediation are the high efficiency, simplicity, the lack of residues and capacity to treat many different compounds (Hodaifa *et al.*, 2013), at short residence times (Martins and Quinta-Ferreira, 2011) and a cost effective process (Mert *et al.*, 2010). The ease of implementation, low cost, and potential to degrade a wide range of organic micropollutants and other pollutants of concern suggest that this mechanism should be researched and exploited for its treatment potential. The objective of this paper is to evaluate physicochemical and Bacteriological Characterization and Treatment of Abattoir Wastewater using *Bacillus licheniformis* ZUL012

MATERIALS AND METHODS

Study Area: The study site was Bukola Saraki Abattoir located along Sobi road in Ilorin west Local Government Area of Kwara State, Nigeria. The abattoir is a Large-scale business enterprises and it is managed by an Association of butchers and under the supervision of health workers from Ministry of Livestock and Fishery, Ilorin, Kwara State. The slaughtering area is fenced with sand concrete blocks while the floor is made of concrete slab. Normal abattoir operations are carried out from Monday to Sunday. The abattoir is provided with well water but has no slaughter gadgets, cold room and waste treatment facilities. However, there are drainages and channels through which the wastewater leaves the slaughter hall and finally empties into a river around the abattoir.

Collection of Wastewater Samples: Wastewater samples were collected from the abattoir with a sterilized 5-liter can. The Aww was collected aseptically from part of the wastewater running off the drainage system just as it was leaving the slaughter pavements. Samples were transported to the laboratory for the analysis.

Microorganisms: The bacterium *Bacillus licheniformis* ZUL012 used in the present study was

isolated previously from local textile effluent and soil contaminated with dye effluent obtained from local textile industries in Ilorin (Ajao and Awe, 2018). The Genbank accession is MH411110.

Physicochemical Analyses of Abattoir Waste: The method of Kumar *et al.* (2022) was adopted for the determination of the physicochemical parameters of the AWW, such as BOD₅, COD, TOC, TDS, EC, pH, phosphate, TN, and TSS as per standard protocol (APHA-AWWA-WPCF, 2017).

Bacteriological Examination of Abattoir Wastewater: The method of Ajao *et al.* (2021) was used for the isolation of bacterial contaminants in abattoir wastewater. Membrane Filtration Method The membrane filter with a 0.45 m pore size was used to filter 100 mL of water samples. The membranes were aseptically placed on the different agar media. Total coliforms and faecal coliforms counts were enumerated with the use of MarConkey agar and Eosin methylene blue (EMB) agar, incubated at 37 °C and 44.5 °C, respectively. The colonies were enumerated, characterized, and recorded. Their counts were expressed in cfu/100 mL

Preparation of Cells Extracts: The pretreated *Bacillus licheniformis* ZUL012 was grown in LB broth at 37 °C for 18 h. The bacterial cells were harvested by centrifugation (6000 g, 10 min, 4 °C), washed twice with deionised water and re-suspended in deionised water. The density of cell pellet was adjusted to OD₆₀₀ equal to 0.111 with the inoculum size of approximately 1.5×10^8 CFU/mL. Subsequently used as inoculums.

Priming of the *B. licheniformis* ZUL012 with 0.1 mM H₂O₂: *B. licheniformis* ZUL012 pretreated cells with a non-lethal concentration (100 µm of H₂O₂) to elicit oxidative stress was demonstrated using method of Rodriguez-Rojas *et al.* (2020) with modification. *Bacillus licheniformis* strain ZUL012 (1.5×10^8 CFU/ml) was exposed (stimulus) to 0.1 mM H₂O₂ for 30 minutes at 37°C in an orbital shaker at 100 rpm.

The H₂O₂ was removed by centrifugation at 4 000 x g for 10 minutes and cells were allowed to recover for 90 minutes, keeping the cell density constant by removing the required volume and replacing it with the appropriate amount of fresh pre-warmed-LB (37°C) every 30 minutes.

The trigger (1 mM H₂O₂) was added 90 minutes after removal of the stimulus. The challenge lasted for 30 minutes. The cultures was diluted and plated to

determine cell viability. Non-treated cells were used as control.

Coagulation Process: The method of Upadhyay and Mistry (2012) was followed for the coagulation and flocculation process as follows. About 500ml of abattoir wastewater was collected in the Erlenmeyer flasks 1litre size. Optimized dosage of Al_2SO_4 was added .pH of sample was adjusted to 8.5 ± 0.2 with use of lime. Mixing at 120 rpm for 30 mins using magnetic stirrer. Then the wastewater was allowed to stand still for 30mins to settle out flocs. After 30mins, supernatant was carefully transferred to the experimental flasks.

Bioremediation Process: The bioremediation process was performed in Erlenmeyer flasks (250 mL) containing 90 mL of autoclaved Abattoir wastewater amended with 0.05 % (v/v) as a source of nutrients. pH was adjusted to 6.8 ± 0.2 using NaOH and HCl 0.1 M solutions. About 1.5ml of PBS (0.02 mM), 1ml of $FESO_4$ (0.75mM) and 1ml of H_2O_2 (0.01%) was added. Ten percent of the bacterial suspension at the exponential growth phase with the cell density of ($OD_{600\text{ nm}}=1.11$ which correspond to 1.5×10^8 cfu/ml) were used as inoculums followed by incubation at 30 ± 1 °C and 120 rpm for 10 days. Physicochemical Parameters were determined at every 24 hours intervals to monitor the progress of bioremediation.

Statistical Analysis: The data obtained from the experiments were analyzed and expressed as mean and standard deviation. The percentage change was calculated between the controls and experimental for the various experiment.

RESULTS AND DISCUSSION

Table 1 shows the results of the physicochemical characteristics of the AWW, which indicate a high pollution load based on the values obtained in all of the measured parameters. This study revealed a high level of organic strength. After the coagulation process, the pH of abattoir wastewater rises from 6.9 to 8.6. All of the measured parameters are on the high side, which could have environmental and public health consequences. The high values obtained in this study for the measured parameters imply that abattoir wastes have a high pollution strength. BOD₅ values are widely used to assess the effects of pollution. It represents the amount of putrescible organic matter in water (Kumar *et al.*, 2011). A low BOD content indicates good quality water, whereas a high BOD content indicates polluted water. The BOD₅ and COD values obtained were perfectly consistent with the submission of Osuide *et al.* (2010), who proposed the correlation between BOD₅ and COD. In effluent

studies, BOD₅ and COD are well correlated parameters, and their relative amount depends on the nature of the effluent. COD roughly equals BOD₅ in effluents containing largely biodegradable pollutants, whereas COD exceeds BOD₅ in effluents containing largely non-biodegradable pollutants. This waste contains a high concentration of TDS. The COD, BOD₅, TOC, and TDS values indicate a high level of organic and inorganic matter pollution. The coagulation process reduced the mean values of the pollution indicators determined: BOD₅, COD, TOC, and TDS, while no noticeable changes occurred in total phosphorus or total nitrogen. There were high levels of coliforms and total heterotrophic bacteria counts. Their population was drastically reduced as a result of the coagulation process. Phosphate and nitrate are two of the most common compounds found in abattoir effluent. This effluent had higher levels of phosphate and nitrate compounds. This disparity could be attributed to the effluents' high fecal content. According to Rodier (2009), before being discharged into the aquatic environment, wastewater samples must contain less than 50 mg/l of nitrates and 0.5 mg/l of phosphate. The river will become eutrophic as a result of high phosphate levels. Blood also contributes significantly to the nitrogen content, while phosphorus is derived from stomach contents in the effluent. In the study of Ijah (2016); Elemile *et al.*, a similar pattern of results was observed (2019).

Table 1: Physicochemical Characteristics of Abattoir wastewater in Ilorin

Physicochemical parameters	Raw Wastewater	After Coagulation
pH	7.6 ± 0.17	6.5 ± 0.24
BOD ₅ (mg/L)	2487 ± 13.10	1420 ± 5.50
COD (mg/L)	4472 ± 12.36	3263 ± 7.15
TOC (mg/L)	2328 ± 7.81	2000 ± 10.89
TDS (mg/L)	6656 ± 31.22	5978 ± 14.78
TKN (mg/L)	1110 ± 8.45	1110 ± 0.22
Total Phosphorus (mg/L)	71.32 ± 2.10	71.32 ± 0.66
Conductivities (µs/cm)	7278 ± 15.70	6874 ± 32.67
Total Coliform (cfu/100 mL)	8.7×10^9	2.8×10^8
Faecal Coliform (cfu/100 mL)	4.0×10^7	2.2×10^5
Total Heterotrophic Count (cfu/100 mL)	126×10^9	53×10^7

BOD₅ Biochemical Oxygen Demand; COD, Chemical Oxygen Demand; TOC, Total Organic Carbon; TDS, Total Dissolve Solid; TKN, Kjeldhal Nitrogen

Table 2 summarizes some of the biochemical and physiological characteristics of the bacterial isolates from the abattoir wastewater. The bacterial isolates were tentatively identified as *Proteus mirabilis*, *Pseudomonas* sp, *Enterobacter* sp, *Bacillus* sp, *Klebsiella* sp, *E. coli* and *Staph* sp clustered into a total number of seven genera. These findings are very similar to what has been found previously (Seema *et al.*, 2009). Table 3 shows the result of treatment which

was done for five days at interval of 24 hours. The Initial values of organic Pollutant index, Biochemical oxygen demand (BOD₅), Chemical oxygen demand (COD), and Total Organic carbon (TOC) and Total Dissolve Solid were 1420 mg/L, 3263 mg/L, 2000 mg/L and 5978 mg/L respectively before the commencement of the bioremediation process. The efficiency of organic micropollutants biotransformation is linked to environmental conditions that enhance the expression of oxidoreductases by microbes (Alneyadi *et al.*, 2018). To improve the biodegradability of microorganisms is to induce microorganisms to produce degradative enzymes (Fischer and Majewsky, 2014). This study demonstrated an evidence that the increase of

oxidative stress artificially could improve the bioremediation of abattoir wastewater derived from *B. licheniformis* ZUL012. The hypothesis that can explain the high degradation observed in this study is that the priming of the bacterial cell induces oxidative stress which elicit gene expression that enhanced the bioremediation of the abattoir wastewater as measured in terms of the reduction in the organic pollutant strength of the abattoir wastewater such as COD, BOD₅, TOC and TDS by both the induced and naive cell. The induced cell reduced the parameters down to 155 ± 41 mg/L, 75 mg/L, 454mg/L and 1750 mg/L and 1000 mg/L respectively while naive cell reduced them to 375 mg/L, 776 mg/L and 3122 mg/L respectively

Table 2: Biochemical and Physiological Characterization of the Heterotrophic Consortium Isolated from Abattoir Wastewater

Test	G1	G2	G3	G4	G5	G6	G7
Form	Circular	Filamentous	Smooth	Irregular	Circular	Circular	Circular
Surface	Glistening	Glistening	Shiny	Rough	Moist	Smooth	Smooth
Colour	Cream	White	White	Creamy	Circular	Whitish	Yellow
Margin	Entire	Lobate	Entire	Undulate	Swarming	Entire	Entire
Elevation	Raised	Umbonate	Convex	Flat	Raised	Convex	Raised
Opacity	Transparent	Rough	Moist	Opaque	Opaque	Translucent	Opaque
Catalase	+	+	+	+	+	+	+
Oxidase	-	+	-	+	-	-	-
Citrate Utilization	+	+	+	+	+	-	-
Lactose fermentation test	-	+	+	-	+	+	+
H2S production	+	-	-	+	-	-	-
Methyl red	+	+	-	-	-	+	+
Voges Proskauer	+	-	+	+	+	-	-
Nitrate Reduction Test	+	+	+	+	+	+	+
Indole Test	-	-	-	-	+	+	-
Urease Test	+	-	+	-	+	-	+
Motility Test	+	+	+	+	-	+	-
	<i>Proteus mirabilis</i>	<i>Pseudomonas sp</i>	<i>Enterobacter sp</i>	<i>Bacillus sp</i>	<i>Klebsiella sp</i>	<i>E. Coli</i>	<i>Staph sp</i>

Table 3: Bioremediation of the abattoir waste water using chemically induced *Bacillus licheniformis* ZUL012

Duration (Hrs)	Treatment	Water Quality parameters of the treated Effluents (Mean ± SD)			
		COD	BOD ₅	TOC	TDS
24	a	2067 ± 12.44	1225 ± 5.77	1585 ± 5.90	5001 ± 6.00
	b	2873 ± 7.50	1395 ± 15.90	1755 ± 5.01	5463 ± 2.98
	c	3260 ± 22.33	1400 ± 1.43	2000 ± 9.12	5550 ± 3.56
48	a	1511 ± 3.21	1000 ± 2.45	1235 ± 11.50	4321 ± 6.34
	b	2550 ± 11.5	1167 ± 5.87	1680 ± 7.02	5111 ± 0.98
	c	3160 ± 2.45	1380 ± 4.07	1978 ± 4.55	5350 ± 1.57
72	a	836 ± 7.01	550 ± 3.21	995 ± 3.21	3768 ± 8.25
	b	1857 ± 3.98	880 ± 2.89	1140 ± 6.21	4963 ± 5.67
	c	3160 ± 4.44	1375 ± 1.99	1900 ± 13.00	5300 ± 11.44
144	a	323 ± 9.12	220 ± 2.22	720 ± 8.23	2233 ± 9.44
	b	1250 ± 2.04	656 ± 0.58	996 ± 0.43	4436 ± 5.66
	c	3000 ± 6.21	1375 ± 6.45	1800 ± 0.94	5300 ± 8.11
168	a	155 ± 1.22	75 ± 1.65	454 ± 1.77	1750 ± 4.90
	b	1000 ± 0.76	375 ± 0.76	776 ± 5.55	3122 ± 3.08
	c	2850 ± 2.31	1366 ± 12.78	1785 ± 7.25	5212 ± 3.46

*a*H₂O₂ induced cells; *b*: Non-Induced Cell; *c*: Control Cell; COD: Chemical Oxygen Demand; BOD₅, Biological Oxygen Demand; TOC: Total Organic Carbon; TDS: Total Dissolve Solid

This corresponds to an average reduction of 95 % (COD), 95 % (BOD₅), 77% (TOC) and 71% (TDS), relative to the raw wastewater while naive cell provides Average reductions of 69 % (COD), 74 % (BOD₅), 61 % (TOC) and 48 % (TDS). The reduction percentages reported in this study are higher than those previously reported in the literature. Overall, the combined process was sufficient to meet discharge requirements without further treatment for the measured parameters (COD, BOD₅, TOC, and TDS). This study confirmed the induction of the oxidative enzyme gene expression mediated by H₂O₂. This pattern of observation has been shown in previous works who reported that the regulation of microbial metabolism in oxidoreductase expression consequently synthesis antioxidative enzymes such as peroxidases and oxidoreductases to protect against such oxidative stress, these enzymes, some of which are also capable of degrading toxic substances and facilitates the removal of environmental pollutants (Bains *et al.*, 2019; Liu *et al.*, 2020).

Conclusion: This study showed that the Aww was grossly polluted with high organic strength and bacterial pathogens. Iron-H₂O₂-modified wastewater mediated with the *B. licheniformis* ZUL012. The results showed that approach was the most efficient to remove BOD₅, COD, TOC and TDS from slaughterhouse wastewater and also ensured dual advantages of a shorter reaction time and cost effective process.

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