BIODEGRADATION OF ABATTOIR WASTEWATER USING INDIGENOUS BACTERIAL STRAINS

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Abstract

An abattoir is an approved and authorised area meant for the slaughtering, processing and preservation of meat products for human use. Bioremediation potentials of indigenous bacterial strains from abattoir wastewater located in Minna metropolis, Nigeria were examined. Wastewater was collected and serially diluted, plated on nutrient agar for bacterial isolation using pour plate isolation method. The isolates were identified based on their cultural, biochemical, and molecular characteristics. The isolates identified were Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Staphylococcus aureus. The isolates were cultured on Mineral Salt media for 7 days and were screened for their biodegradation potential using UV-VIS spectrophotometric analysis. Results revealed 94%, 90%, 82%, 75%, and 73% biodegradation rates for Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Staphylococcus aureus and Bacillus subtilis respectively. Bacillus subtilis and Pseudomonas aeruginosa were used singly and in combination for bioremediation of the wastewater. Changes in the physicochemical parameters were evaluated. Bacillus subtilis exhibited great potential in reduction of most parameters after 28 days of inoculation and treatment. The BOD of sterile wastewater was reduced from 259.00 mg/L to 75.12 mg/L, COD 173.08 mg/L to 64.59 mg/L, nitrate 16.09 mg/L to 5.39 mg/L, phosphate 1.73 mg/L to 0.61 mg/L, ammonia 4.86 mg/L to 2.43 mg/L and pH 6.41 to 5.22. Pseudomonas aeruginosa reduced BOD from 259.00 mg/L to 97.12 mg/L, COD 173.08 mg/L to 71.79 mg/L, nitrate 16.09 mg/L to 5.13 mg/L, phosphate 1.73 mg/L to 0.37 mg/L, ammonia 4.86 mg/L to 1.44 mg/L; pH reduced from 6.41 to 5.64. The combination efficacy was observed in the reduction of BOD from 259.00 mg/L to 58.82 mg/L, COD 173.08 mg/L to 56.39 mg/L, nitrate 16.09 mg/L to 2.49 mg/L, phosphate 1.73 mg/L to 0.24 mg/L ammonia 4.86 mg/L to 0.84 mg/L and pH 6.41 to 5.64. The results revealed significant difference ($p < 0.05$) amongst the parameters during the period of biodegradation. The results indicated that Bacillus subtilis and Pseudomonas aeruginosa are promising microorganisms for industrial applications such as bioremediation of abattoir wastewater.

Keywords: Abattoir wastewater, biodegradation, bioremediation, physicochemical parameters, spectrophotometry

Introduction

An abattoir is defined as an approved, authorised, registered and hygienic ground or area which is specifically meant for the slaughtering, processing and preservation of meat products for human utilization (Alonge, 2005). In Nigeria, the abattoir industry is a major supplier of a source of meat, income and contributes to fashion for the common man (Nafaranda et al., 2011). Protein serves as body builder and it is more prominent in meals via meat. Meat is obtained from slaughter houses and during the slaughtering of these animals, wastewater is released. The increase in the demand for meat therefore increases the release of wastewater from slaughter houses (Chukwu et al., 2011; Ogbomida et al., 2016).

Abattoirs are usually found near water bodies where different untreated waste products of the industry are released and this is of great concern to public health authorities (Adelegan, 2002). Abattoir wastewater can be regarded as waste or waste water produced from the activities of

an abattoir. The major components of this waste are condemned organs, bones, carcasses, animal faeces, blood, fat, hides, carcass trimmings, paunch content and urine (Adelegan, 2002; Adeyemi & Adeyemo, 2007). Furthermore, Amenu (2014) recorded that wastewater from slaughter houses are mainly the used water which is usually composed of 99% of liquid and 1% of solid particles. The quantity of water produced by slaughterhouses is large and contains organic matter, suspended solids, nitrogen and phosphorus compounds in high concentration (Merzouki et al., 2005).

Blood makes up the highest toxic waste of all the components of abattoir wastewaters, followed by fat (Chukwu et al., 2011). Blood is rich in nutrients, phosphorus and nitrates. Blood is one of the major dissolved component of abattoir wastewater and has the highest chemical oxygen demand (COD) of any wastewater from abattoir operations (Aniebo et al., 2009). It has biochemical oxygen demand (BOD) of about 450,000 mg/L and chemical oxygen demand of 375,000 mg/L (Amenu, 2014).

The disposal of untreated abattoir wastewater into water bodies would result to increase in BOD and COD, reduction of dissolved oxygen (DO) for aquatic life, methaemoglobinaemia, contamination of runoff, surface and underground water, threatening of natural habitat of organisms and increase in water borne diseases (Aniebo et al., 2009).

Studies have shown that the physicochemical parameters (BOD, COD, pH, nitrate, phosphate, total dissolved solid (TDS) and total suspended solid (TSS)) of abattoir wastewater are higher than the permissible standard set by regulatory bodies (Adeyinka et al., 2014). Measures have been made to treat the wastewater before disposal via different methods by treatment plants. Bioremediation, a means of treating the wastewater shows to be more cost effective and environmental friendly methods for wastewater treatments before discharge into water bodies. Microorganisms are the major tools used in the cleaning up process of bioremediation. This process is dependent on the growth and survival of the microbes in the polluted environment during the clean-up process (Strong and Burgess, 2008). Bioremediation is simply a technology specialised in the removal of pollutants from the environment thus restoring it to its original natural state that is free from contaminants (Sasikumar & Papinazath, 2003). It is a technology that has been used in the clean-up of chemicals in soils, groundwater, wastewater, sludge, industrial wastewater and gases (Divya et al., 2015). Therefore, this research examined the bioremediation of abattoir wastewater using indigenous bacterial isolates before the disposal of the waste into the environment.

Materials and Methods

Sample collection

Abattoir wastewater samples were collected aseptically using a 3 litres plastic container from an abattoir located at Kuta, Nigeria. A sterile syringe was used to introduce the wastewater into the sterile container. The sample was transported immediately to the Microbiology laboratory at Federal University of Technology, Minna, Nigeria for analysis.

Experimental design

The experiment design used in the treatment of the abattoir wastewater in the laboratory was Complete Randomized Design (CRD). This is the simplest experimental design and suitable for homogenous experimental materials (Umanu and Owoseni, 2013). In this experiment, the treatment with bacteria isolates is the only source of variation.

Experimental setup

The samples were collected randomly at different points and were mixed thoroughly. The experimental setup was labelled A, B, C and D. This was done in triplicates. The wastewater was dispensed into 250 mL Erlenmeyer flasks. Setup A was treated with no bacterial isolates and served as control. Setup B and C were treated with bacterial isolate B6 and B7 respectively. Setup D was treated with the combination of bacterial isolates B6 and B7 (consortium). The treatment and control designs were kept in the incubator shaker throughout the investigation period (28 days). Samples were taken at 7 days interval for analysis. The rate of degradation of the wastewater was compared amongst setups B, C and D.

Microbiological analysis of abattoir wastewater

A ten-fold serial dilution of the wastewater was carried out. The 10^{-7} dilution factor was plated using pour plate isolation technique. The plates were incubated at 37°C for 24 hours. The colonies that appear different in shape, size, texture and colour on the incubated plates were sub-cultured repeatedly on a nutrient agar plate to obtain pure isolates using streak plate method. The plates were incubated at 37°C for 24 - 48 hours. The pure isolates obtained from the streaked plates were inoculated into slant bottles containing nutrient agar for preservation at 4°C in a refridgerator, to stop bacterial growth (Cheesebrough, 2005).

Characterisation and identification of bacterial isolates

The bacterial isolates with percentage degradation potential of more than 70% were characterised based on their cultural and biochemical properties including the abilities of the organisms to utilize various carbohydrates. The identities of the isolates were confirmed by comparing their characteristics with already known taxa as outlined in Bergey's Manual of Systematic Bacteriology (Bergey & Holt, 1994).

Molecular characterisation of bacterial isolates

The molecular method employed was partial sequencing using 16S rRNA gene as target for the identification of bacteria and the sequence was analysed using BLAST (Nithya & Bhasar, 2013). The amplification and sequencing of the bacteria was outsourced to GeneOmbio Technology Pvt. Ltd.

Screening of bacterial isolates for biodegradation potential

Nutrient broth was prepared (1.3 g mixed with 100 mL of water). A syringe was used to transfer 9 mL of the broth into test tubes and autoclaved at 121°C for 15 minutes. The 10^6 cfu/ml colonies of the pure isolates were introduced into the test tubes containing the broth and incubated for 24 hours. Mineral Salt Media (MSM) was prepared according to the procedure described by Ijah et al. (2008). The composition of the mineral salt medium was 1.2 g KH_2PO_4, 1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl and 0.01 g $FeSO_4.7H_2O$. The prepared media (100 mL) was mixed with abattoir wastewaters (1 mL) and autoclaved at 121°C for 15 minutes. When cooled to room temperature (±28°C), 1 mL of the test organism in the broth (10^6 cfu/ml) was transferred into 9 mL of the sterile mixture (MSM and wastewaters) and incubated in shaker at 260 revolutions per minute for 7 days. At the end of incubation, a UV-VIS spectrophotometer, model 752 of wavelength 610 lambda was used to determine the optical density (OD). The best isolate was selected for the bioremediation of the wastewater.

Bioremediation of abattoir wastewater with screened bacterial isolates.

The setup consisted of 200 mL of sterile wastewater with mineral salt medium and 2 mL of the 24 hours old culture of each isolated organism contained in 250 mL Erlenmeyer flasks. The experimental setup was done in triplicates and had 12 flasks in total (i.e. 3 control flasks and 9 flasks each containing sterile wastewater with mineral salt medium for each individual isolates

(Bacillus subtilis or Pseudomonas aeruginosa) and consortium (Bacillus subtilis and Pseudomonas aeruginosa). The flasks were incubated in an incubator shaker at 260 revolutions/minutes. The incubation period was at intervals of one week (7 days) after which the physicochemical analysis were carried out.

Physicochemical analysis of water samples

The wastewater samples were analysed for the following physicochemical properties: pH, temperature, phosphate, fluoride, chloride, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), turbidity, total hardness, dissolved oxygen (DO) and total suspended solids (TSS) using the methods of American Public Health Association, APHA (2001).

Statistical analysis

Statistical analysis including mean, standard deviation, one way analysis of variance (ANOVA), as well as the significant evaluation were performed using the SPSS 17.0 statistics.

Results

Screening of bacterial isolates for bioremediation potentiality: The isolates were grown in mineral salt medium containing abattoir wastewater for 7 days. The optical density was measured to ascertain the level of growth of the indigenous bacterial isolates. Isolates B7 and B5 showed the highest and lowest levels of growth on the media as shown in Table 1.

Table 1: Growth of bacterial isolates in mineral salt media containing abattoir wastewater

Isolates	Optical Density (OD)	Percentage (%)
B1	0.731	73.1
B2	0.331	33.1
B3	0.823	82.3
B4	0.529	52.9
B5	0.257	25.7
B6	0.902	90.2
B7	0.943	94.3
B8	0.750	75.0
B9	0.435	43.5
B10	0.433	43.5
B11	0.377	37.7

B1 = Escherichia coli, **B3** = Bacillus cereus, **B6** = Pseudomonas aeruginosa, **B7** = Bacillus subtilis, **B8** = Staphylococcus aureus. **% = OD x 100**

Molecular characterisation of P. aeruginosa DM1 and B. subtilis RN40 strains isolated from abattoir wastewater

The molecular characterisation (gene sequencing) results illustrated that the test bacterial isolates gave similar sequence which has 99% identity to Pseudomonas aeruginosa strain DM1 16S ribosomal RNA gene and Bacillus subtilis strain RN40 16S ribosomal RNA gene.

Bioremediation of abattoir wastewater

The isolates used for bioremediation of the wastewater were used singly (Bacillus subtilis or Pseudomonas aeruginosa) and in consortium (Bacillus subtilis and Pseudomonas aeruginosa) as treatments. The results obtained are presented in Tables 2, 3 and 4. The values obtained

indicate high levels of the physicochemical parameters of the wastewater before remediation. Some of the parameters fell within the acceptable range of regulatory bodies after bioremediation. The results were compared with different regulatory standard limits to ascertain the efficacy of bioremediation capability of the indigenous isolates (Table 5)

Table 2: Physicochemical properties of sterile abattoir wastewater treated with B. subtilis Rn40

Parameters	Control	0	7	14	21	28
pH	6.41±0.04^{a}	6.36±0.06^{a}	5.76±0.14^{b}	5.76±0.05^{b}	5.82±0.15^{b}	5.22±0.1^{c}
Temperature	28.10±0.10^{a}	28.07±0.11^{a}	27.31±0.28^{b}	26.12±0.13^{d}	26.82±0.78^{bc}	26.40±0.13^{dc}
COD	173.08±2.27^{a}	172.48±2.22^{a}	141.25±2.06^{b}	114.36±4.18^{c}	95.53±2.17^{d}	64.59±5.87^{e}
BOD	259.00±2.65^{a}	258.85±2.39^{a}	231.10±10.73^{b}	180.25±14.74^{c}	137.21±7.57^{d}	75.12±1.53^{e}
TDS	7.69±0.27^{a}	7.71±0.26^{a}	5.19±0.68^{b}	4.15±0.233^{c}	3.15±0.15^{d}	1.81±0.10^{e}
TSS	182.33±2.08^{a}	182.33±2.08^{a}	214.00±15.09^{b}	253.00±2.65^{c}	326.00±10.00^{d}	397.33±6.43^{e}
Nitrate	16.09±0.05^{a}	16.12±0.09^{a}	13.04±0.25^{b}	10.75±0.11^{c}	8.52±0.11^{d}	5.39±0.06^{e}
Phosphate	1.73±0.06^{a}	1.72±0.06^{a}	1.34±0.06^{b}	1.12±0.07^{c}	0.78±0.12^{d}	0.61±0.02^{e}

Values are mean ± standard error of mean of triplicate determinations. Values with different subscripts down the rows are significantly different ($p<0.05$), values with same subscripts are not significantly different ($p>0.05$).

Table 3: Physicochemical properties of sterile abattoir wastewater treated with P. aeruginosa Dm1

Parameters	Control	0	7	14	21	28
pH	6.41±0.04^{a}	6.36±0.06^{a}	5.76±0.14^{b}	5.79±0.12^{b}	5.71±0.12^{b}	5.64±0.1^{b}
Temperature	28.10±0.10^{a}	28.07±0.11^{a}	27.31±0.28^{ab}	26.12±0.13^{c}	26.82±0.79^{bc}	28.03±0.68^{a}
COD	173.08±2.27^{a}	172.48±2.22^{a}	164.10±2.01^{b}	152.71±2.97^{c}	131.82±1.64^{d}	71.79±7.52^{e}
BOD	259.00±2.65^{a}	258.85±2.39^{a}	212.10±10.13^{b}	158.92±8.14^{c}	127.21±7.95^{d}	97.12±2.71^{e}
TDS	7.69±0.27^{a}	7.71±0.26^{a}	5.19±0.69^{b}	3.88±0.21^{c}	2.92±0.11^{d}	1.61±0.09^{e}
TSS	182.33±2.08^{e}	182.33±2.08^{a}	191.00±1.73^{b}	250.00±5.00^{c}	362.67±15.28^{d}	418.33±2.08^{e}
Nitrate	16.09±0.05^{a}	16.12±0.09^{a}	15.71±0.05^{a}	11.25±0.92^{b}	8.29±0.93^{c}	5.13±0.79^{d}
Phosphate	1.73±0.06^{a}	1.72±0.06^{a}	1.35±0.06^{b}	0.96±0.06^{c}	0.58±0.07^{d}	0.37±0.12^{e}

Values are mean ± standard error of mean of triplicate determinations. Values with different subscripts down the rows are significantly different ($p<0.05$), values with same subscripts are not significantly different ($p>0.05$).

Table 4: Physicochemical properties of sterile abattoir wastewater treated with consortium (B. subtilis RN40 + P. aeruginosa Dm1)

Parameters	Control	0	7	14	21	28
pH	6.41±0.04^{a}	6.36±0.06^{a}	5.76±0.14^{b}	5.79±0.12^{b}	5.71±0.12^{b}	5.64±0.12^{b}
Temperature	28.10±0.10^{a}	28.07±0.11^{a}	27.25±0.39^{b}	27.25±0.39^{b}	27.45±0.48^{b}	27.30±0.05^{b}
COD	173.08±2.27^{a}	172.48±2.22^{a}	166.30±3.59^{b}	152.71±2.97^{c}	131.82±1.64^{d}	56.39±1.09^{e}
BOD	259.00±2.65^{a}	258.85±2.39^{a}	220.60±8.27^{b}	185.09±10.04^{c}	124.37±4.02^{d}	58.82±1.65^{e}
TDS	7.69±0.27^{a}	7.71±0.26^{a}	6.51±0. 26^{b}	4.34±0.33^{c}	2.92±0.11^{d}	0.64±0.39^{e}
TSS	182.33±2.08^{e}	182.33±2.08^{a}	211.00±17.39^{b}	258.00±2.65^{c}	387.33±18.58^{d}	461.67±15.31^{e}
Nitrate	16.09±0.05^{a}	16.12±0.09^{a}	15.71±0.05^{b}	8.75±0.14^{c}	4.46±0.14^{d}	2.49±0.17^{e}
Phosphate	1.73±0.06^{a}	1.72±0.06^{a}	1.33±0.06^{b}	1.06±0.07^{c}	0.59±0.07^{d}	0.24±0.08^{e}

Values are mean ± standard error of mean of triplicate determinations. Values with different subscripts down the rows are significantly different ($p<0.05$), values with same subscripts are not significantly different ($p>0.05$).

Table 5: Physicochemical parameters of the sterile abattoir wastewater treated with bacterial isolates at the end of 28 days compared with standard limits

Parameters	Initial value	B. subtilis RN40	P. aeruginosa DM1	Consortium	FEPA	CPCB	NESREA
pH	6.41	5.22	5.64	5.64	6.0 - 9.0	5.5 - 9.0	6.8 - 7.2
COD (mg/L)	173.08	64.59	71.79	56.39	80	250	80
BOD (mg/L)	259.00	75.12	97.12	58.82	50	100	40
Nitrate (mg/L)	16.54	5.39	5.13	2.49	20	10	10
Phosphate (mg/L)	1.73	0.61	0.37	0.24	5	5	-

KEY: mg/L = milligramme per litre
BOD = Biochemical Oxygen Demand
FEPA = Federal Environmental Protection Agency (Nigeria)
NESREA = National Environmental Standard and Regulations Enforcement Agency
CPCB = Central Pollution Control Board (India)
NTU = NephelometricTurbidity Unit
COD = Chemical Oxygen demand Discussion

There was a gradual and steady reduction in the physicochemical properties of the sterile abattoir wastewater treated with bacterial isolates. The bacterial isolates were inoculated singly as well as consortium for twenty-eight (28) days. The overall study of the

physicochemical parameters in Tables 2, 3 and 4 showed that all individual isolates and consortium used as treatment of the wastewater was effective in reducing the pollutant of the wastewater. Also, the treatment by consortium showed more reduction when compared to the individual isolates which is in accordance with the work carried out by Sridevi et al. (2016).

The maximum reduction of BOD was observed after 28 days by the consortium (259.00 mg/L to 58.82 mg/L), followed by Bacillus subtilis (259.00 mg/L to 75.12 mg/L) and Pseudomonas aeruginosa (259.00 mg/L to 97.12 mg/L). Similar results were observed by Shrivastava et al. (2013) and Prasad and Manjunath (2011) where it was found that B. subtilis and Pseudomonas aeruginosa reduced BOD of Yamuna water and lipid rich wastewater.

The consortium showed the maximum reduction of COD (173.08 mg/L to 56.39 mg/L), followed by Bacillus subtilis (173.08 mg/L to 64.59 mg/L) and Pseudomonas aeruginosa (173.08 mg/L to 71.79 mg/L). In the biodegradation process, bacteria cellular metabolism, growth and development are feasible due to their utilization of organic compounds as substrate present in the wastewater (Sonune & Garode, 2015). The result in this study is agreement with Zhao et al. (2009) and Gaikwad et al. (2014) and that found reduction of COD carried out by Pseudomonas sp. and Bacillus sp. The nitrates concentration of the abattoir wastewater prior to treatment was higher than the limits set by regulatory bodies. In this study, nitrates maximum reduction was indicated by the consortium (16.09 mg/L to 2.49 mg/L), followed by Pseudomonas aeruginosa (16.09 mg/L to 5.13 mg/L) and Bacillus subtilis (16.09 mg/L to 5.39 mg/L). The result indicates that the process of denitrification took place during treatment (Sonune & Garode, 2015). Rajakumar et al. (2008) reported that Bacillus sp. and Pseudomonas sp. were most efficient for nitrate reduction which is similar to results obtained in this study.

Phosphate concentration in the wastewater before treatment was high. This can be attributed to the high faecal content, blood, stomach and intestine content present in the wastewater (Ogbomida et al., 2016). It is advisable to ensure that wastewater samples must have less than 50 mg/L of nitrates and 0.5 mg/L of phosphate before its discharge into aquatic environment (Rodier, 2009). The presence of phosphate in water bodies causes environmental problems such as eutrophication. In this study, there was maximum reduction in phosphate by consortium (1.73 mg/L to 0.24 mg/L), followed by Pseudomonas aeruginosa (1.73 mg/L to 0.37 mg/L) and Bacillus subtilis (from 1.73 mg/L to 0.61 mg/L). The results obtained in this study is in line with the reports of Krishnaswamy et al. (2011) who reported that the Bacillus sp. RS-1 and Pseudomonas sp. YLW-7 were found to be efficient in phosphate reduction.

The overall results obtained from this study revealed the consortium effectively biodegraded the sterile abattoir wastewater. This indicates that the consortium collaborated in the degradation of a wide range of substrate under a short period of time (Darshini & Sharpudin, 2016). The decreased level of the physicochemical parameters indicates the presence of synergy interactions in the consortium (Safitri et al., 2015). The efficacy in the reduction of chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solid (TDS), nitrates and phosphate as demonstrated by the test bacterial isolates indicates their ability to adapt and survive naturally in the presence of abattoir wastewaters and possess degradative enzymes for the degradation of the wastewater. Similarly, Bacillus and Pseudomonas sp have shown their capability to reduce physicochemical parameters of wastewater in several research works (Vijayakumar et al., 2005; Usman et al., 2012, Adeyinka et al., 2014).

Compared with the standards set by Federal Environmental Protection Agency (FEPA), National Environmental Standards and Enforcement Regulations Agency (NESREA) and

Central Pollution Control Board (CPCB) (Table 5), there were reductions in some of the physicochemical parameters of the wastewater bioremediated. The pH, COD, nitrate, and phosphate were reduced by the bacterial isolates and consortium within the ranges of the acceptable limits set by the regulatory bodies, but the BOD was not within the range even though there was a huge reduction from the initial value of the raw wastewater sample. This may be due to the length of time given for the isolates to act in this study (28 days).

Conclusion

In this study, five bacterial species were isolated from the abattoir wastewater. These organisms were Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli. The organisms were screened for the potential to degrade sterile abattoir wastewater. Pseudomonas aeruginosa DM1 and Bacillus subtilis RN40 showed greater potential in the degradation of the wastewater. The organisms were used in the treatment of the sterile abattoir wastewater and were found to reduce the physicochemical parameters of the wastewater within the acceptable range of regulatory bodies.

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