International Journal of Scholarly Research in
Multidisciplinary Studies
Journal homepage: https://srrjournals.com/ijsrms/
ISSN: 2961-3329 (Online)

(RESEARCH ARTICLE)

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Screening and identification of some selected fungi species from Abattior waste water

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International Journal of Scholarly Research in Multidisciplinary Studies, 2022, 01(01), 009–015

Publication history: Received on 22 June 2022; revised on 29 July 2022; accepted 03 August 2022

Article DOI: https://doi.org/10.56781/ijsrms.2022.1.1.0025

Abstract

This study was carried out to screen and identify some selected fungi species from abattoir waste water in Makurdi metropolis. Abattoirs Waste water samples were collected from different locations which include Modern Market, Wadata, Wurukum, North bank, new bridge. The samples were transported to the Microbiology laboratory, Federal University of Agriculture, Makurdi. Standard microbiological tests were carried out to identify the organisms from abattoir waste water. The fungi species identified were, *Aspergillus* spp, *Mucor*, *Absidia*, *Fusaruim*, *Rhizopus* spp, *Saccharomyces*. *Rhizopus* and *Mucor* spp had the highest prevalence accounting for 21.05%, while *Fusaruim* spp had the lowest prevalence accounting for 10.52%. From the statistical analysis using ANOVA, there was significant difference between the colony counts in the different locations. Wurukum had the highest fungi count (20.40± 3.20 CFU/ml), followed by Wadata market (18.40± 9.62 CFU/ml), New bridge had the lowest fungi count of (8.20 ± 8.49 CFU/ml). The Physicochemical characteristics of abattoir waste water. Physicochemical analysis of waste water from abattoir shows that the temperature of wastewater ranges from 28.9°C to 29.8°C. The temperature was within the limit for wastewater discharge of <40°. The pH of the wastewater was near neutral (8.0-9.4), which plays a major role in determining the qualitative and quantitative abundance of microorganisms in the wastewater. From the findings of this study, it is recommended that there is need for regulatory authorities to enforce strict compliance to environmental safety rules.

Keywords: Abattior; Waste Water; Fungi; Characterization; Identification

1. Introduction

An abattoir or slaughterhouse is a facility where animals are killed and prepared fresh for traders and consumers to buy for various types of food products [1]. They act as the starting point of the meat processing industry where stock comes from market or farms to enter the food chain. The abattoir industry is an important component of livestock industry in Nigeria, providing domestic meat supplies to over 150 million people and employment opportunities for the teeming population [2].They pollute the environment either directly or indirectly from their various processes, if not handled properly [3]. Wastewater from an abattoir is a concentrated source of oxygen-consuming waste. It contains high levels of organic matter due to the presence of faeces, manure, blood, fats, grease, hair, grit and undigested feeds. It can also contain high level of salts, phosphate and nitrates. Blood and fats contribute mostly to organic load [4]. Abattoirs generally use large quantities of water for washing meat and cleaning cutlasses used for cutting meat and they are usually located near water bodies in order to gain access to water for processing [5]. Contamination of river body and land from abattoir wastes could constitute a significant environmental and health hazard [6].

For food safety reasons, the layout of an abattoir must provide for the prevention of cross contamination and adequate separation of incompatible activities. The construction of the building and the equipment used in abattoirs must prevent proper slaughter and processing, allow for ease of cleaning and sanitation and be properly maintained [7].

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Sangodoyin *et al,* [8] reported that the ground water quality in vicinity of the abattoirs were adversely affected by seepage of abattoir effluents as well as water quality of receiving stream that was located away from the abattoirs. The health of the dweller is affected by the environment. In every neighourhood, there is a considerable range of biological and chemical pollutants that cause or contribute to diseases. Some may pose health risk for a particular group while others for the entire neighourhood. UNESCO [9] also reported that waste that is not properly managed especially excreta and other liquid and solid waste from abattoirs and communities are of serious health hazard and lead to the spread of infectious diseases.

An effluent of a major city abattoir in Nigeria was studied for possible pollutants and effects of such pollutants on the environment. Findings show that the various water samples were contaminated with *E. coli* and other enteric bacteria. The presence of Coliform, *Staphylococcus* spores indicated the presence of micro-organisms which are associated with water borne disease

An effluent of a major city abattoir in Nigeria was studied for possible pollutants and effects of such pollutants on the environment. Findings show that the various water samples were contaminated with *E. coli* and other enteric bacteria. The presence of Coliform Staphylococcus sores indicated the presence of micro-organisms which are associated with water borne disease [10].

Fungi can enter water bodies from various locations, especially through the adjoining soils. The filamentous fungi are group of organisms that can be found everywhere; they are present in virtually all ecological niches on earth. They are appraised to be accountable for the spoilage of up to 25% of all plant-derived foods produced annually [11]

Penicillium species have been frequently recovered from water in the various studies performed. Several of the species in genus Penicillium and Aspergillus are known to produce mycotoxins in other substrates, such as food and beverages [12]. Interestingly, detection of aflatoxins produced by *A. flavus* in water from a cold water storage tank was demonstrated by Paterson [13].

Predominant fungal genera and species in treated and untreated water are *Aspergillus, Cladosporium, Epicoccum, Penicillium, Trichoderma, Arthriniumphaeo spermum, A. flavus, C. cladosporioides, Fusarium culmorum, Mucorhiemalis and Trichodermaharzianum* [14].

The aim of this research is to Screen and identify some selected fungi species from Abattior wastewater and to determine the physicochemical parameters of abattoir wastewater.

2. Material and methods

2.1 Study Area

Makurdi the capital of Benue State is located along River Benue with coordinates as 7°.43'SO''N and 8°.32'10'E with an estimated population of over 260,000 as at 2007 and still growing. Average annual temperature 32°c with SW wind at 10km/hr and relative humidity of 66% annually.

2.2 Population of the Study

The population of this study comprises of three abattoirs which are identify and purposively selected for this study based on the criteria that they are operational and located in Makurdi Metropolis. The Abattoir includes New Bridge, North Bank Market, Wadata, Wurukum market and Modern Market located in Makurdi Metropolis. The abattoir operates at a medium scale; considering the population of people in the area and the number of animal slaughtered for public consumption.

2.3 Wastewater Sampling and Sample Size

Five abattoirs were identified and purposively selected for this study based on the criteria that they are operational and located close to in Makurdi Metropolis. One of them is located closed to River Benue (New Bridge) where water samples at point locations will be collected at distance of 20m before and after the abattoirs.

In North Bank Market and Wadata, water point sample was collected at a further distance of *50m* after the abattoir; the water is use for domestic purpose including cooking, laundry, irrigation, and bathing. This gave a total number of 3 water samples. Sterilized gloves were used to collect the water samples and sterilized (cleaned with dilute nitric acid

and rinsed with distilled water before use) IL glass bottles were used. The bottles were tightly close and placed in iced cold boxes to protect them from direct sunlight.

During water sample collection, each bottle was rinse with an appropriate amount of water from the point of collection before the actual water sample was collected. They were then transported to the laboratory for analysis. The samples were collected early in the morning between *5.30* am and 8.00 am which was during the period of slaughtering. All the wastewater samples were collected at the center open drainages untreated or flowing stream to obtain a representative sample in each three abattoir selected. This was done for three weeks. All the samples collected were analyzed within 24 hours of collection.

2.4 Physiochemical parameters

2.4.1 pH

About thirty milliliters (30 ml) of the water samples were placed in a 100 ml capacity beaker and HANNA multiparameter water tester model HI 98129 meter was dipped into it. The MODE key was used to select pH. The pH value which was displayed on the lcd were read and recorded after allowing it to stabilize.

2.4.2 Temperature

About thirty milliliters (30 ml) of the water samples were placed in a 100 ml capacity beaker and HANNA multiparameter water tester model HI 98129 meter was dipped into it. The MODE key was used to select temperature. The temperature value which was displayed on the lcd was read and recorded in degree Celsius after allowing it to stabilize.

2.4.3 Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) was determined by conventional methods described by APHA [15]. A sample of the solution *(50* ml) was poured in a 500 ml BOD bottle and filled to the mark with earlier prepared dilution water. A blank solution of the dilution water was equally prepared and placed in two BOD bottles. A control solution devoid of dilution water was also prepared and put in a BOD bottle. The bottles were stopped, airtight and incubated for five days at room temperature.

2.5 Culture media preparation

Culture media used were Nutrient agar (NA), Potato dextrose agar (PDA) and Eosin methylene blue (EMB) agar (LAB M) and were all prepared according to the manufacturer's specification. Serial dilution of the effluents were carry out and 1 ml each of the diluents were aseptically introduced into different plates after which sterile prepared medium were introduced using the pour plate technique and incubated at the 37°C for 24 hrs. Biochemical tests were carried out on pure bacterial cultures using standard methods.

2.6 Isolation of Microorganism

The microorganisms were isolated by serial dilution technique on Potato Dextrose Agar (PDA) and Nutrient Agar Media (NAM). In this technique, a sample suspension was prepared by adding 1.0 g sample to 10 ml distilled water and mixed well for 15 mm and vortexed. Each suspension was serially diluted. 0.1 ml was pipetted onto plates with PDA and NAM media, spread with a glass spreader and incubated at 28°C for fungal and 37°C for bacterial observation. Each colony that appeared on the plate was considered as one colony forming unit (CFU/ml)

2.6.1 Identification of Fungi

The fungal isolates were identified by morphological examination and its characteristics. Morphological characteristics were examined under microscope [13].

2.6.2 Optimization of culture condition for Fungi

For the determination of optimum condition of isolated fungi, three media were used (Potato dextrose agar media. Sabouraud dextrose agar). The media were adjusted to pH 4 to 7 for optimization of the incubation period, the culture plates were incubated at 28 °C for 4 to 7 days.

2.7 Data Analysis

Statistical analysis of data was done using SPSS (20). Comparisons of means were assessed statistically by subjecting data to one-way analysis of variance (ANOVA). A probability value (P-value) of less than 0.05 was considered as significant.

3. Results

The mean total fungi count of samples of Abattoir waste water collected from slaughter locations used for this study is presented in table 1:0. The result show that the most contaminated sample with respect to fungi load was Wurukum market with $20.40x10^1 \pm 3.20x10^1$Cfu/ml while the least was new bridge Abattoir with $8.20x10^1 \pm 0.49x10^1$Cfu/ml. there is no significant differences statistically across study locations ($P \geq 0.321$).

Table 2:0 shows the Cultural and Microscopic characteristics of fungi isolates from abattoir waste water in Makurdi Metropolis. The fungi were identified based on their distinct colonies appearance on the different culture plates and microscopically.

Table 3:0 present the prevalence of fungi species across study locations. Six genera of fungi where identified as contaminant of abattoir waste water; *Aspergillus Saccharomyces,* Rhizopus, Mucor, Absidia and *Fusarium* species. *Absidia*occured only in Wadata samples. In the study, *Rhizopus* and *Mucur*spp had the highest prevalence which account for 21.05%. While Fusaruimspp had the lowest prevalence accounting for 10.52%.

Table 4:0 shows the result of physicochemical analysis of abattoir waste water in this study. All the parameters except dissolved oxygen are higher than acceptable level for normal water.

Table 1 Total Mean Count of Fungi from the Slaughter Houses in Different Abattoir in Makurdi metropolis

Sample Locations	Mean Fungi Count (x101Cfu/ml)
Modern market	13.80±3.80
New Bridge	8.20±8.49
Wadata	18.40±9.62
Wurukum Market	20.40±3.20
North Bank	15.60±0.80
P. Value	0.321

Key: P≥0.05; df =1.

Table 2 Cultural and Microscopic Characteristics of Fungi Isolates from Abattoir Waste water in Makurdi Metropolis

Cultural characteristics	Microscopic characteristics	Suspected organisms
Media (Sabourad Dextrose Agar)		
Mold colonies with black Pigmentation.	Smooth colored conidiophores and conidia. The conidiophores are protrusions from septateHyphae. The conidiophores becomes dark at the apex nd terminating in a globose vesicle	*Aspergillus* spp
Wolly to cottony, flat white colonies.	Presence of septate hyphae, conidiophores and conidia	*Fusarium* spp
Grey to yellowish brown colonies. reverse pigmentation is white to pale.	Brown coloured branched sporangiophores occurring in clusters at the tip of the hyyhal tubes. The spores are rounded with flattened bases	*Rhiszopus* spp
They are flat, smooth, moist, glistening or dull and cream to tannish cream in color	Multilateral budding is typical pseudohyphe. Hyphae are present	*Saccharomyces* spp

Fluffy appreance, with cottony colonies	Unbranched sporangiosphores. Sporangia are dark brown	*Mucor*
cottony colonies .Gross colony morphology	Hyphae are septate .A few septa are present. The sporangiophores are branched and arise in groups at the internodes	*Absida spp*

Table 3 Prevalence of Fungi Isolate across Location

Location	Aspergillus	Saccharomyces	Rhizopus	Mucor	Fusarium	Absidia	Total
Modern market	2 (10.52)	0.00	1 (5.26)	2 (10.52)	0	1(5.26)	6 (31.57)
New Bridge	0.00	0.00	2 (10.52)	0.00	1(5.26)	0.00	3(15.79)
Wadata	0.00	0.00	1(5.26)	1(5.26)	0.00	0.00	2 (10.52)
Wurukum Market	0.00	1(5.26)	0.00	0.00	0.00	0.00	1 (5.26)
North Bank	1 (5.26)	2 (10.52)	0.00	1 (5.26)	1(5.26)	2(10.52)	7 (36.84)
Total	3 (15.79)	3 (15.79)	4 (21.05)	4 (21.05)	2 (10.52)	3 (15.79)	19 (100)

Table 4 Physicochemical Characteristics of Abattoir Waste water from Different Slaughter houses in Makurdi metropolis

Parameters / Locations	pH	TDS (mg/L)	EC µs/cm	Temperature (°C)	DO (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	BOD (mg/L)
WurukumMarket	8.67	831	1659	29.4	1.1	0.78	0.13	1.3
WadataAbattoir	9.14	704	1403	29.4	0.7	0.42	0.22	1.4
New Bridge	8.83	825	1640	29.6	0.8	0.81	1.12	1.0
North Bank Abattoir.	8.80	843	1690	29.8	0.6	0.92	0.11	1.1
Modern Market	8.27	732	1459	28.9	0.5	0.77	0.0	1.3
Mean ±	8.742±	785.000±	1570.200±	29.420 ±	0.740±	0.740 ±	0.316±	1.220±

Key: pH=Potential of hydrogen, TDS=Total dissolved solids, EC=Electrical conductivity, DO= Dissolved oxygen, Ammonia, BOD= Biochemical oxygen demand

4. Discussion

This study investigated the fungi associated with abatoir waste water. From the result of this study the fungi associated with Abattoir wastewater were identified as *Mucor* spp, *Aspergillus spp,* Rhizopus spp. *Fusarium* spp. and *Absidia spp*. This study agrees with the report of [16] who found out *Aspergillus spp, Fusarium spp* on Isolation and identification of microorganisms from abattoir. The presence of these fungi in water is of public health concern. *Aspergillus* species produces Aflatoxin that can cause Asthma [17]. When waste water from abattoir is released untreated into water body the waste water serves as a substrate for fungi proliferation.

In this study, the mean for the highest fungal count (20.40±3.20 CFU/ml), was moderate for the abattoir wastewater. Going by WHO standard, any water contaminated to this extent (20.40±3.20 CFU/ml), or level can to be released into the environment directly without treatment [18].

From the statistical analysis, there was significant difference between the colony counts in the different locations. Wurukum had the highest fungi count (20.40± 3.20 CFU/ml), followed by Wadata market (18.40± 9.62 CFU/ml), New bridge had the lowest fungi count of (8.20 ± 8.49 CFU/ml).

In the study *Rhizopus* and *Mucur* spp had the highest prevalence accounting for 21.05%, while fusaruim spp had the lowest prevalence accounting for 10.52%.

Physicochemical analysis of waste water from abattoir shows that the temperatures of waste water ranges from 28.9°C to 29.8°C. The temperature was within the limit for wastewater discharge <40°C [19]. The pH of the wastewater was near neutral (8-9.4), which plays a major role in determining the qualitative and quantitative abundance of microorganisms in the wastewater. This is in line with the limit for wastewater discharge of pH 6-9. This result is in agreement with that obtained by [20]. The anaerobic degradation of organic compounds releases ammonia, which reacts with carbon dioxide produced during the anaerobic process, resulting in ammonia bicarbonate, which may contribute to the increase in the pH values [21]. The highest DO of the samples was 1.1mg/ml and 1.4 mg/L BOD. This is in line with the limit from the FEPA for the discharge of wastewater from the Abattoir into the water body. The high concentration of nitrate can be attributed to high concentration of organic matter content of the wastewater samples resulted from the decomposition of protein and nitrogenous compound, which when broken down give rise to simpler substances including ammonia. This is in agreement with the finding of [21]. There are no significant differences in the fungi count across the study locations.

5. Conclusion

It has been concluded from the results of this findings that the fungi associated with abattoir are *Mucor* spp, *Aspergillus spp,* Rhizopus spp. *Fusarium* spp. and *Absidia spp*. and the most prevalent ones are *Mucor* and *Rhizopus* species accounting for 21.05%. Abattoir in wadata had the highest fungi load of 20.40±3.20 CFU/ml. The impact of waste in our environment cannot be overemphasized. Regulation meant to curb the environmental impacts, are not properly implemented, so there is the indiscriminate release of this waste to water bodies and to the earth surface without proper treatment of the waste water to make it environmentally friendly.

Recommendation

Based on the results of this study and conclusion drawn, the following are recommended.

- There is the need to draw public attention to the effect of this waste water to the environment and health of people exposed to it.
- There is the need for regulatory authorities to rise up to the challenge and enforce strict compliance to environmental safety rules.
- Butchers and other handlers should be trained and enlightened on small technologies that can help them handle waste properly.
- Further research is recommended to know the route of contamination and possible ways of avoiding it.

Compliance with ethical standards

Acknowledgments

We thank our institution, departments, and all laboratory Technologists for their support. We acknowledge Mr. Ogli Itolo of Department of microbiology, University of Agriculture Makurdi for his assistance.

Disclosure of conflict of interest

All authors report no conflicts of interest relevant to this article the conflicts that the editors consider relevant to this article are disclosed here.

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