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Full Length Research Paper

Assessment of the efficiency of a yeast biofilter in the treatment of abattoir wastewater

Abdullahi Bako Rabah[1]*, Maryam Lami Ibrahim[1], Udeme Joshua Josiah Ijah[2] and Shu'aibu Bala Manga[1]

[1]Department of Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Microbiology, Federal University of Technology, Minna, Nigeria.

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A yeast biofilter consisting of *Candida krusei*, *Candida morbosa, Torulopsis dattila, Torulopsis glabrata,* and *Saccharomyces chevalieri* was constructed to bioremediate abattoir wastewater. Potato peels were used as filter bed for the growth of the yeasts. Wastewater samples were collected from three different points in Sokoto (Nigeria) abattoir and the physiochemical as well as the microbiological qualities of the wastewater were determined before and after biofiltration. The results revealed that after the biofiltration process, the pH, biochemical oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD), and the nitrate content of the wastewater were drastically reduced. Similarly, there was a decrease in the variety of microorganisms isolated as well as in microbial counts after the biofiltration process. *Salmonella typhi, Neisseria lactamica, Serratia marcescens, Branhamella catarrhalis, Shigella* sp, *Penicillium* sp, *Curvularia* sp, and *Trichophyton rubrum* were completely eliminated after the biofiltration process. The reduction in the pH, BOD, DO, COD, nitrate as well as in the variety and total counts of bacteria and fungi for the wastewater after the biofiltration process indicated that the biofilter was effective in bioremediation of the wastewater. The percentage efficiency of the biofilter was found to be 42.5%.

Key words: Assessment, efficiency, yeast, biofilter, abattoir, wastewater.

INTRODUCTION

In abattoir operation, opportunities exist for the release into the ecosystem of potentially hazardous materials. Abattoir effluents can seep into the aquifer and pollute the underground water, or where it is discharged without proper treatment into water bodies, the pollutants cannot be confined within specific boundaries. They can therefore affect aquatic life in enormous ways (Asamudo et al., 2005). Most abattoirs in Nigeria do not have wastewater treatment facilities. The implication of this is that the wastewater is discharged directly into water bodies or land thereby polluting the environment. Wastewaters are mostly bioremediated in a process generally referred to as biofiltration, and the microorganisms used are generally referred to as biofilters (Ashoka et al., 2002). In this study the microorganisms used were isolated from locally fermented yoghurt (Nono) and a beverage called Kununzaki that is consumed in Nigeria as refreshing drinks.

A number of researches have been conducted on the application of microorganisms as biofilters in wastewater treatment. Sakano and Kerkhof (1998) identified a bacterium, *Proteobacterium* that served as a biofilter in removing ammonia through the process of nitrification in a laboratory-scale ammonia biofilter. Similarly, Woertz et al. (2001) reported the performance of a biofilter containing the dimorphic black yeast *Exophiala lecanii-cornii* with an average toluene elimination capacity of around 80g/m^3 of biofilter/h and removal efficiencies greater than 95%. In a related study, a biofiltration system inoculated with the mould *Paecilomyces variotti* CBS115145 showed

*Correspondence author. E-mail: abrabah2009@gmail.com. Tel: +2348065631286.

Abbreviations: BOD, Biochemical oxygen demand; **DO,** dissolved oxygen; **COD,** chemical oxygen demand; **PAH,** polycyclic aromatic hydrocarbons; **PA,** point A; **PB,** point B; PC, point C; **MEA,** malt extract agar; **YEA,** yeast extract agar; **NA,** nutrient agar; **MCA,** macConkey agar; **SDA,** saboraud dextrose agar; **TVC,** total viable counts.

a toluene elimination capacity of around 250 g/m^3 of biofilter/h (Garcia et al., 2005).

Asamudo et al. (2005) demonstrated the effectiveness of using the fungus *Phaerochaete chrysosporium* in the biofiltration of textile effluent, polycyclic aromatic hydrocarbons (PAH), and pulp and paper effluents. The microorganism was capable of producing extracellular enzymes such as manganese peroxidase, cellulases, and lignin peroxidases, in achieving total remediation of these effluents. Also, a study carried out by Ezeronye and Okerentugba (1999) demonstrated that the effectiveness of a yeast biofilter composed of a mixed culture of *Saccharomyces* spp., *Candida* spp, *Schizosaccharomyces* spp. and *Geotrichium candidum* in the treatment of fertilizer factory effluents was 98%. The biochemical oxygen demand (BOD) of the effluent was reduced from a range of 1200-1400 to 135-404mg/l. Besides, ammonia-nitrogen ($NH_{3-}N$) and nitrate-nitrogen (NO_3-N) were reduced from 1000-10 mg/l and 100-17.6 mg/l, respectively. Little or no study has been conducted on the reclamation of abattoir wastewater despite the fact that abattoirs are located in close proximity to human settlements in developing countries and generate enormous wastewater. Therefore, the objective of this study was to isolate yeasts from natural environments and use them as biofilter to bioremediate abattoir waste-water.

MATERIALS AND METHODS

Sample collection

Nono (locally fermented milk product) and kunun-zaki (a refreshing drink made from millet) samples were obtained at the Mini market of the main campus of the Usmanu Danfodiyo University, Sokoto, Nigeria in sterile sample bottles and transported in an icebox to the laboratory for the isolation of yeasts. Wastewater was collected from Sokoto abattoir in sterile two litre capacity sample bottles and transported in an icebox to the laboratory. The wastewater was collected from three points in the abattoir: At the point where the wastewater leaves the slaughter hall (Point A, PA), midway through the drainage channel (Point B, PB), and the point where the wastewater drained to the surrounding soil (Point C, PC). A total of three samples were collected from each point at different times.

Isolation and identification of yeasts

This was carried out according to the method described by Ijah (1998). Serially diluted samples of the nono and kunun-zaki were plated in triplicates on malt extract agar (MEA) and yeast extract agar (YEA) and incubated at ambient laboratory temperature (28 ± 2°C) for 24 h. Colonies that appeared on the plates were further subcultured on fresh YEA to obtain pure cultures. The pure cultures were characterized according to the methods of Robert and Ellen (1988) based on their morphological characteristics, cell shape, pseudomycelium, blastospore formation and their ability to ferment various sugars.

Physico-chemical analysis of raw and bioremediated abattoir wastewater

The physico-chemical qualities of the abattoir wastewater determined were pH, electrical conductivity, sodium, potassium, calcium, magnesium, carbonate, bicarbonate, nitrate, chloride, dissolved oxygen (DO), BOD and chemical oxygen demand (COD) using the methods of Ademoroti (1996).

Microbiological analysis of raw and bioremediated abattoir wastewater

The microbiological qualities were determined using the methods of Adesemoye et al. (2006) as follows: 0.1 ml aliquots of serially diluted samples were plated in triplicate plates of nutrient agar (NA), macConkey agar (MCA), MEA, and saboraud dextrose agar (SDA) for the enumeration of total aerobic heterotrophic bacteria, coliforms, yeasts and moulds, respectively. The NA and MCA plates were incubated at 37°C for 24 h while the MEA and SDA plates were incubated at ambient laboratory temperature (28 ± 2°C) for 24 - 72 h. Colonies, which developed on the plates, were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also subcultured repeatedly on fresh NA, MEA and SDA media to obtain pure isolates. The pure bacterial isolates were gram-stained and subjected to different biochemical tests which included production of coagulase, catalase, urease, oxidase, methyl red, voges-proskaeur, citrate utilization test, H_2S production and carbohydrate fermentation as described by Cheesebrough (2006). The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of Cowan and Steel (Barrow and Feltham, 1993). The yeasts were identified using the schemes of Robert and Ellen (1988) while the moulds were identified using the schemes of Bernand and Hartmann and Bernard, (1983).

Design of biofilter and biofiltration of wastewater

A biofilter made up of perspex glass with a length of 18.0 cm, width of 10.8 cm and a depth of 10.5 cm was constructed. The filter has upper and lower compartments separated by a perforated partition made up of the same perspex glass. It also has a tap for the collection of filtered wastewater. The potato peels were ground to smaller particles, wetted and placed on the perforated partition. The yeast biomass was inoculated on the peels and left for one week at ambient laboratory temperature (28 ± 2°C) to allow the cells to grow. Then the abattoir wastewater was introduced into the filter bed and left to stand for a minimum period of 14 days. The filtered wastewater was collected from the lower chamber of the filter through a tap fitted to the chamber. The percentage efficiency of the biofilter was calculated according to the method of Garcia et al. (2005).

Statistical analysis of data

Data generated was subjected to statistical analysis using one-way analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS) (version 14.0) to establish significant differences at 95% confidence limit of various parameters determined.

RESULTS AND DISCUSSION

The yeast species isolated from nono and kunun-zaki and identified for use as biofilters in the biofiltration process were identified as *Candida krusei, Candida morbosa, Torulopsis dattila, Torulopsis glabrata, and Saccharomyces chevalieri.* These organisms constituted the

Table 1. Total viable counts* of bacteria and fungi in abattoir wastewater before and after biofiltration process.

Points of collection of wastewater	Bacteria (x10^7 cfu/ml)		Fungi (x10^4 cfu/ml)		Coliforms (x10^5 cfu/ml)	
	Before	After	Before	After	Before	After
PA	7.0	2.2	13.9	7.4	2.0	1.8
PB	4.9	1.8	14.0	9.6	1.3	1.1
PC	7.3	2.4	7.2	7.0	2.2	1.8

*Counts represents mean of triplicate samples; cfu/ml: colony forming unit per milliliter: PA: point where the wastewater leaves the slaughter hall; PB: midway through the drainage; PC: point where the wastewater drained to the surrounding soil.

Table 2. Occurrence of bacteria and fungi in abattoir wastewater before and after bioremediation process.

Bacteria	Before	After	Fungi	Before	After
Escherichia coli	+	+	*Penicillium* sp.	+	-
Salmonella typhi	+	-	*Aspergillus clavatus*	+	+
Neisseria lactamica	+	-	*Aspergillus flavus*	+	+
Klebsiella pneumoniae	+	+	*Aspergillus niger*	+	+
Serratia marcences	+	-	*Curvularia* sp.	+	-
Brahamella catarrhalis	+	-	*Trichophyton rubrum*	+	-
Shigella sp	+	-	*Penicillium echinulatum*	+	+
Aerococcus viridians	-	+	*Mucor* sp.	-	+
Lactobacillus brevis	-	+			

+: Present,-: absent.

yeast consortium used in the biofiltration of wastewater. The two beverages (Kunun-zaki and nono) are locally available in Sokoto and constitute the staple foods of the poor man. Isolating yeasts from such natural environments could make their usage in the bioremediation of different wastewaters less expensive, thus making the operation less capital intensive. Besides, the fear for pathogenicity is erased because the yeasts are usually consumed with the beverages. Chandrasena et al. (2006) reported the isolation of *Saccharomyces, Hansenula, Debaromyces, Schizosaccharomyces, Trichosporon, Pichia, Candida,* and *Torulopsis* from natural environments and their efficiencies in high alcohol production and waste management.

The results of the total viable counts (TVC) of bacteria, fungi and coliforms in the abattoir wastewater before and after the biofiltration process are presented in Table 1. The wastewater samples collected at PC that is, as it drained onto the surrounding soil) had the highest counts of 7.3 x 10^7cfu/ml while the lowest counts of 4.9 x 10^7cfu/ml were recorded at PB. The bacterial counts at PA were 7.0 x 10^7cfu/ml. After passing the wastewater through the yeast filter, the TVC decreased in all the three points proportionately (Table 1). Similarly, the fungal counts decreased but more appreciably at PA (Table 1). In terms of bacterial and coliforms counts, statistical analysis (ANOVA) indicated that there is significant difference between the counts in PA and PC to that of PB ($p \leq 0.05$) whereas in terms of fungal counts there is significant difference between PA and PB to that of PC ($p \leq 0.05$). The high count of these organisms in the wastewater is due to the fact that the wastewater has a high content of whole blood which served as a rich protein medium for microbial growth. Similar findings were reported by Asamudo et al. (2005) who found a mean bacterial count of 3.32×10^7 cfu/ml and fungal population of 1.60×10^5 cfu/ml in wastewater collected from Agege (Nigeria) abattoir.

The bacteria isolated from the abattoir wastewater before the biofiltration process were identified as *Escherichia coli, Salmonella typhi, Neisseria lactamica, Klebsiella pneumoniae, Serratia marcescens, Branhamella catarrhalis,* and *Shigella* sp. After the biofiltration of the wastewater, only *E. coli,* and *K. pneumoniae* were still present. *S. typhi, N. lactamica, S. marcences, B. catarrhalis, and Shigella* sp had disappeared. However, *Aerococcus viridans* and *Lactobacillus brevis,* which were not detected before the biofiltration process, emerged (Table 2), possibly because they are slow growers. It is also possible that the growth factors that encouraged their growth were not present at the initial stage.

The fungal isolates were identified as *Aspergillus clavatus, Aspergillus flavus, Aspergillus niger, Curvularia* sp*, Trichophyton rubrum, and Penicillium echinulatum*. After the biofiltration process *A. flavus, A. niger, Mucor* sp*, P. echinulatum, and A. clavatus* were identified. It was observed that *Penicillium* sp, *Curvularia* sp, and *T. rubrum* disappeared after the biofiltration process leaving *A. clavatus, A. flavus, A. niger,* and *P. echinulatum.* However, *Mucor* sp that was not detected before the biofiltration

Table 3. Physico-chemical qualities of abattoir wastewater before and after the bioremediation process.

Parameter	PA		PB		PC	
	Before	After	Before	After	Before	After
Colour	Oxblood	Red	Oxblood	Red	Oxblood	Red
Appearance	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
pH	7.22	5.61	7.47	7.38	7.24	6.49
Electricalconductivity (µS/cm)	1342	6.40	4448	6.4	5.97	6.40
Sodium (mg/l)	13.20	40.0	58.00	19.00	70.00	32.00
Potassium (mg/l)	3.30	210	80.00	2.90	89.00	4.60
Calcium (mg/l)	0.60	0.55	1.10	0.75	0.60	1.35
Magnesium (mg/l)	1.50	0.80	1.20	1.00	1.25	2.55
Phosphorus (mg/l)	0.155	0.22	0.21	0.18	0.41	0.47
Carbonate (mg/l)	0.00	0.10	0.00	0.00	0.00	0.00
Bicarbonate (mg/l)	0.00	0.55	0.40	0.75	0.55	1.55
Chloride (mg/l)	0.35	1.00	0.08	0.60	1.20	1.90
Nitrate (mg/l)	16.00	0.14	56.00	44.00	0.88	0.64
DO (mg/l)	1.40	0.65	1.80	0.54	1.70	0.62
BOD (mg/l)	148.00	33.50	142.00	32.16	140.00	33.40
COD (mg/l)	280.00	170.50	265.00	139.30	265.00	145.26

PA: Point where the wastewater leaves the slaughter hall; PB: midway through the drainage; PC: point where the wastewater drained to the surrounding soil; mg/l: milligramme per litre, DO: dissolved oxygen, BOD: biochemical oxygen demand, COD: chemical oxygen demand; µS/cm: microSiemenspercentimeter.

process appeared after passing the wastewater through the biofilter (Table 2).

The results of the physico-chemical qualities of the abattoir wastewater before and after biofiltration process from the three sampling points (PA, PB and PC) are presented in Table 3. It was observed that there was a considerable reduction in pH, nitrate (NO_3), DO, BOD, and COD after the biofiltration of the wastewater collected from the three sampling points. It was also observed that the concentrations of other compounds in the wastewater varied with the sampling points probably due to contamination from human activities in the abattoir such as dumpimg of cow dung and pieces of bones in the wastewater channels. According to Ezeronye and Okerentugba (1999) the combination of yeasts and cassava peels acting as carbonaceous substrate for microbial nutrition greatly enhanced BOD reduction by increasing the C/N ratio of the effluent. The investigators reported a BOD reduction from initial concentrations of 1200 and 1440 mg/l - 135 and 404 mg/l, respectively. Similarly, Melamene et al. (2007) reported an initial COD reduction of 53.3% and a total COD removal of 99.5% from wine distillery wastewater. The low BOD values obtained may be due to the fact that the microorganisms are not acclimated to the wastewater due to the existence of certain inhibitory compounds (Ademoroti, 1996). There was significant reduction of nitrate in all the samoling sites and this could be as a result of biological stabilization in the effluents. The pH of the effluents as well as the treated wastewater was within the permissible level of 6.0 - 9.5 and this significantly affects the growth of microorganisms. Also, the chloride level was within the acceptable limit of 0.50 mg/l and its presence in the wastewater could be attributed to sources of pollution which might result from introduction of mineral salts into the wastewater (Adeyemo et al., 2002).

Generally, the results indicated that the yeast biofilter was fairly effective in the bioremediation process. The biofilter had a percentage efficiency of 42.5%. Ezeronye and Okerentugba (1999) reported a percentage efficiency of a biofilter of 98%. Similarly, Jang et al. (2005) reported the use of a laboratory-scale biofilter packed with a mixture of peat and ceramic and inoculated with *Pseudomonas* sp SR-5 which proved to have more than 90% removal efficiency and an elimination capacity of 290 /m^3/h. In a similar research Xu et al. (2005) reported high efficiency of a self-made biofilter in reducing COD_{cr} of wastewater from 60 mg/l to less than 0.5 mg/l.

Conclusion

The abattoir wastewater analyzed had high counts and various species of fungi and bacteria. It also had some physicochemical properties in amounts that indicate that the wastewater was highly polluted. However, after passing the wastewater through the yeast biofilter, both the microbial counts and high amounts of these chemicals in the wastewater were reduced. The biofilter had an elimination capacity of 42.5%. It is possible to treat abattoir wastewater by biofiltration. Therefore, it is highly recommended that the yeast biofilter should be optimized

so that greater efficiency can be achieved.

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