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Treatment of Grey Water from Different Restaurants in Futa Using Fungi

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Abstract - Greywater samples were obtained from three restaurants in the Federal University of Technology; Akure coded SSR, MGR and GGR. Fungi isolates obtained include *Rhizopus stolonifer, Aspergillus niger, Mucor mucedo, Aspergillus flavus, Saccharomyces cerevisiae*. Of these fungi isolates obtained, *R. stolonifer, A. niger and A. flavus* showed significant degradation ability on grey water and was used for this research. A simple bioreactor was constructed using biodegradation process in purification of waste water samples. Waste water undergoes primary treatment; secondary treatment involves the introduction of the isolated organisms into the waste water sample and the tertiary treatment which involved the use of filter candle and the sand bed filtration process to achieve the end product without the use of chemicals. *A. niger* brought about significant reduction in both the bacterial load and the fungi load of the greywater samples of the three respective restaurants with a reduction of (1.29×10^8 to 1.57×10^2 cfu/ml; 1.04×10^8 to 1.12×10^2 cfu/ml and 1.72×10^8 to 1.60×10^2 cfu/ml) for bacterial load in SSR, MGR and GGR respectively. Reduction of 2.01×10^4 to 1.2×10^1; 1.72×10^4 to 1.1×10^1 and 2.50×10^4 to 1.5×10^1 in fungi load from SSR, MGR and GGR respectively. Result of degradation of these selected waste water by the fungi showed that *A. niger* was probably more potent in the degradation of organic matter and hence, *A. niger* could be used in the treatment of wastewater

Keywords – *Aspergillus niger, greywater, bacterial, fungi, microbial load, bioreactor, biodegradation, purification, organic matter and filtration.*

INTRODUCTION

Grey-water is the waste water from wash hand basins, showers and baths. Often called as sullage, it has been observed that Grey water also contains pathogens and there is potential for spreading illness. They can cause a range of diseases, lung infections, ingestion, gastroenteritis, eye and skin infections, pneumonia, pulmonary disease and toxic shock syndrome [25]. Electrocoagulation method has been in use in advanced countries which is a simple and efficient method for the treatment of many water and wastewaters. It has been tested successfully to treat textile wastewater potable water [24] tar sand and oil shale wastewater [16], carpet wastewater,[26] urban wastewater [14] chemical fiber wastewater and oil–water emulsion [13]. It has also been used to remove clay suspensions, bentonite, and dye stuff [27] from wastewaters. This method despite been effective is rather too expensive for small scale fast-food, restaurant and cafeterias. However, other methods have been reportedly been in use. Various treatment technologies have been employed to store grey water for irrigation purposes. The common treatment technologies are physical treatment (sand filtration, membrane filtration) and biological process followed by chemical treatment (disinfectants). Different disinfectants have been employed to store the grey water for a long time and they are chloramine, chlorine, hydrogen peroxide, UV light and oils. The chemical and physical quality of the grey water will heavily influence what type of disinfection method is most suitable [25]. For example, presence of organic matter and suspended solids in grey water can affect efficiency of disinfection and disinfectant demand. Organic material generally reacts with disinfectant and therefore a greater initial dose is needed to achieve total inactivation of bacteria [2]. Asano *et al.* [2] also found that a greater initial dose of chlorine is needed to overcome its interaction with organic matter. It was also found that larger particles can help shield bacteria from disinfection [26]. Another means of waste treatment that eliminates the limitation posed by the use of chemicals is the use of biodegradation using microorganisms. The use of microbial treatment systems has the advantage of being simple in design and low in cost and as well effective [5]. The biology, economic value and pathogenic capabilities of fungi are not new; they have been used from fermentation of foods to production of pharmaceuticals, they thrive well in inhospitable habitats with environmental extremes because of their enzyme systems [9]. Fungi are involved in biodegradation of undesirable materials or compounds and convert them into harmless, tolerable or useful products; they are recognized for their outstanding ability to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints. Fungi have both biochemical

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pathways for nitrification and denitrification, higher rates of nitrification-denitrification, greater resistance to toxic-inhibitory compounds, lower oxygen and carbon source concentration requirements. Hence, this study determines the:

1. Microbial population and identification of microorganisms present in grey-water samples collected from selected restaurants in Federal University of Technology, Akure and
2. Effect of biodegradation using a simple bioreactor developed in purifying wastewater samples collected from selected restaurants in Federal University of Technology, Akure.

MATERIALS AND METHODS

Sources of Greywater

Three liters of grey water was obtained separately from 3 different restaurant located at Obanla of The Federal University of Technology, Akure.

Sample preparation

One ml was pipetted from each grey-water samples (for SSR, MGR and GGR restaurant) using different sterile pipettes aseptically and it was individually dispensed into different sterile test tubes containing 9mls of sterile distilled water. Serial dilution of the samples was obtained.

Isolation of organisms

This was done according to the method of [23]. An aliquot (0.1 ml) of each diluent was pipetted into sterile Petri dishes and overlaid with about 15 ml of molten agar using the pour plate method. The plates were swirled for homogeneity of the diluents and the medium. They were allowed to solidify and incubate in inverted position. For Nutrient agar, MacConkey agar, Eosine Methylene Blue agar, *Salmonella-Shigella* agar incubation was at 37 ^{0}C for 24 h while for Yeast extract agar, Potato Dextrose Agar were incubated at 28 ^{0}C for 72 h.

Preparation of pure culture

This was done according to the method of [23]. The 24 h bacteria isolates from for Sweet savour restaurant, Mega restaurant and Great grace restaurant grey-water sample were sub- cultured on sterile nutrient agar plates to obtain pure cultures. This was done aseptically by taking a loopful of a colony that is separated from other colonies onto a solidified nutrient agar plate and it was streaked over the plate using a sterile inoculating loop, this relatively reduce the density of the microbial cells of the given organism from the plate. The plates were incubated at 37 ^{0}C for 24 h in an inverted position.

Identification of fungi

On a clean grease free microscopic slide, a little mycelium was transferred from the sub cultured plate and a drop of cotton blue in lacto phenol was dropped on the mycelium and viewed under the x40 objective lens of the binocular light microscope. Their cellular and morphological characteristics were observed and it was based on references from [6].

Identification of bacteria

The identification of bacteria isolates was based on cultural characteristics, biochemical test and staining reaction according to Paul [14], Cowan and Steel [10].

Construction of bioreactor

The bioreactor used for the research was constructed personally using plastic buckets with covers joined together to prevent contaminations. It has an inlet orifice, a treatment candle, filter paper, and funnel, sterile sharp sand and connecting channels (plate 1).

RESULTS

The results of the microbiological analysis of wastewater from the various restaurants before treatment are shown in Table 1. GGR had the highest count of both fungi and bacteria with 2.50×10^4 sfu/ml for total fungi count and 1.72×10^8 cfu/ml for total bacteria count. MGR had the lowest count for both bacteria and fungi with 1.04×10^8 cfu/ml for bacteria count and 1.72×10^4 sfu/ml for the fungi count. SSR had 1.29×10^8 cfu/ml for bacteria count and 2.01×10^4 sfu/ml for fungi count. After treatment, separately with pure cultures of *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*. The bacteria count after the treatment process was determined and results shown in Table 6, the samples treated with *Aspergillus niger* had the lowest bacteria count after treatment with SSR reducing from 1.29×10^8cfu/ml to 1.57×10^2cfu/ml, MGR reducing from 1.04×10^8 cfu/ml to 1.12×10^2 cfu/ml and GGR reducing from 1.72×10^8 cfu/ml to 1.60×10^2 cfu/ml while the samples treated with *Rhizopus stolonifer* had the highest bacteria count after treatment with SSR reducing from 1.29×10^8 cfu/ml to 2.28×10^3 cfu/ml, MGR reducing from 1.04×10^8 cfu/ml to 1.74×10^3 cfu/ml and GGR reducing from 1.72×10^8cfu/ml to 1.98×10^3 cfu/ml. The fungi count after treatment process was determined and results shown in Table 7, the samples treated with *Aspergillus niger* had the lowest fungi count after treatment with SSR reducing from 2.01×10^4 sfu/ml to 1.20×10^1sfu/ml, MGR reducing from 1.72×10^4 sfu/ml to 1.10×10^1 and GGR reducing from 2.50×10^4sfu/ml to 1.50×10^1sfu/ml while the samples treated with *Rhizopus stolonifer* had the highest fungi count after treatment with SSR reducing from 2.01×10^4 sfu/ml to 2.20×10^1sfu/ml, MGR reducing from 1.72×10^4 sfu/ml to 1.70×10^1sfu/ml and GGR reducing from 2.50×10^4 sfu/ml to 2.0×10^1 sfu/ml.

Table 1: Microbial count of wastewater before treatment

Wastewater	Bacteria (cfu/ml)	Fungi (sfu/ml)
SSR	1.29×10^8	2.01×10^4
MGR	1.04×10^8	1.72×10^4
GGR	1.72×10^8	2.50×10^4

Key: cfu/ml – colony forming unit/milliliter; sfu/ml – spore forming unit/milliliter

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Table 2: Microbial count of wastewater on Selective agar before treatment

Wastewater	Yeast extract agar (cfu/ml)	SSA (cfu/ml)	MAC (cfu/ml)	MRS (cfu/ml)	EMB (cfu/ml)
SSR	3.2×10^6	3.4×10^3	4.4×10^4	ND	3.5×10^4
MGR	2.7×10^6	6.6×10^2	4.2×10^4	ND	3.2×10^4
GGR	3.4×10^6	4.0×10^3	5.2×10^4	ND	4.8×10^4

Key: SSA – *Salmonella-Shigella* Agar**,** MAC – MacConkey Agar, MRS – *Lactobacilli* Agar, EMB – Eosine Methylene Blue Agar and ND – Not detected

Table 3: Morphological characteristic of organisms isolated

Isolates	Colour	Elevation	Edge	Shape	Surface
A	Cream	Raised	Dentate	Irregular	Smooth
B	Cream	Raised	Smooth	Regular	Smooth
C	Pink	Flat	Lobate	Irregular	Smooth
D	Pink	Flat	Entire	Irregular	Rough
E	Pink	Raised	Crenated	Irregular	Smooth
F	Shiny white	Flat	Entire	Round	Smooth
G	Blue-green	Raised	Entire	Round	Smooth

Table 4: Characterization of Isolated Bacteria

Isolates	A	B	C	D	E	F	G
Gram reaction	+	+	-	-	-	+	-
Motility	+	-	+	-	-	-	+
Spore formation	-	-	-	-	-	-	-
Catalase reaction	+	+	+	+	+	+	+
Coagulase reaction	-	+	-		-	+	-
Sucrose	AG	-	AG	G	AG	AG	AG
Galactose	AG	AG	AG	G	AG	-	AG
Lactose	AG	AG	AG	AG	A	-	-
Mannitol	AG	AG	AG	AG	AG	AG	AG
Glucose	AG	AG	AG	-	AG	AG	AG
Probable organism	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Shigella* sp.	*Klebsiella pneumoniae*	*Salmonella* sp.	*Pseudomonas aeruginosa*

Key: AG – Acid and gas production (+ positive) G – Gas production (- negative) and A – Acid production

Table 5: Morphology and Identification of Fungal isolates

Isolates	Physical appearance	Microscopic appearance	Probable microorganisms
1	Cotton-like mycelia at 24 hours forming dirty with development of black spores on mycelium.	Non-septate hypha, thin sporangiophore with a sporangium in an umbrella-like form.	*Rhizopus stolonifer*
2	White colonies becoming black.	Single celled (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicles, long conidiophores arise from a septate mycelium.	*Aspergillus niger*
3	White fluffy growth	It possesses septate hypae, possess no stolons, the sporangiophores arise at any point on the mycelium and around columella.	*Mucor mucedo*
4	Yellow mycelia growth	Single celled (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiospore, the vesicle, long conidiophores arise from a septate mycelium.	*Aspergillus flavus*
5	Milky colonies on agar	The cells stained blue, with spherical columella which bore sporangia on sporangiophores, arranged in rows.	*Saccharomyces cerevisiae*

The grey water sample was cultured on different selective agar to determine the presence of some particular organisms; Table 2 reported the microbial load of SSR, MGR and GGR on respective agars of *Salmonella-Shigella* agar (SSA), MacConkey agar (MAC), *Lactobacilli* agar (MRS), Eosine Methylene Blue agar (EMB) with no growth detected on the *Lactobacilli* agar. The agar used were SSA for isolation

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of *Salmonella*, *Shigella* and *E. coli*; MacConkey used for the isolation of coliform and enteric pathogens; *Lactobacillus* agar for the isolation of *Lactobacillus* spp; Eosine methylene blue agar for the isolation of *E. coli* and Yeast extract for the isolation of yeast. *Lactobacillus* spp. was not detected at all in all the three restaurants but they were found to have the indicator organism *E.coli* which means they all had faecal contaminations. GGR had the highest number of yeast count as 3.4×10^6 cfu/ml and MGR had the lowest yeast count as 2.7×10^6. *Salmonella* and *Shigella* were also present in all three samples with the result of table 8 reported the result of the treatment using A. *niger*. Table 3 shows the morphological characteristics of organisms isolated from the greywater samples. The organisms that were isolated include: *Bacillus subtilis, Stapjylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Shigella* sp., *Salmonella* sp., *Pseudomonas aeruginosa*. They were identified using different biochemical tests and the results are shown in Table 4.

Table 6: Bacteria count of water after the treatment process using different fungal isolates

Fungal Isolates	SSR (cfu/ml)	MGR (cfu/ml)	GGR (cfu/ml)
Aspergillus niger	1.57×10^2	1.12×10^2	1.60×10^2
Aspergillus flavus	2.01×10^3	1.50×10^3	1.82×10^3
Rhizopus stolonifer	2.28×10^3	1.74×10^3	1.98×10^3

Table 7: Fungal count of water after the purification process using the different fungal isolates

Fungal Isolates	SSR (sfu/ml)	MGR (sfu/ml)	GGR (sfu/ml)
Aspergillus niger	1.2×10^1	1.1×10^1	1.5×10^1
Aspergillus flavus	1.9×10^1	1.5×10^1	1.7×10^1
Rhizopus stolonifer	2.2×10^1	1.7×10^1	2.0×10^1

Table 8: Microbial count of wastewater on Selective agar after treatment

Wastewater	Yeast extract agar (cfu/ml)	SSA (cfu/ml)	MAC (cfu/ml)	MRS (cfu/ml)	EMB (cfu/ml)
SSR	0.5×10^3	2.3×10^1	1.2×10^2	ND	1.5×10^2
MGR	1.5×10^4	4.3×10^2	1.6×10^2	ND	1.2×10^3
GGR	2.3×10^4	2.0×10^3	2.1×10^2	ND	2.3×10^2

Key: SSA – *Salmonella-Shigella* Agar, MAC – MacConkey Agar, MRS – *Lactobacilli* Agar, EMB – Eosine Methylene Blue Agar and ND – Not detected

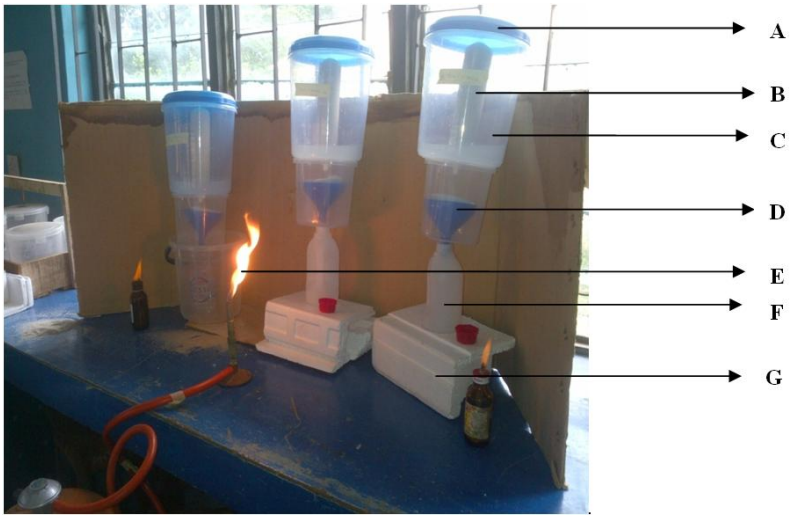

Plate 1: Set-up of the treatment process
A-*Lid;* **B**-*Ceramic filter candle;* **C**-*Plastic bowl;* **D**-*Funnel containing filter paper and sterile sand* **E**-*Flame;* **F**-*Reservoir container;* **G**-*Stand*

DISCUSSION

From the results obtained, Table 5 reported the fungi isolates observed in the wastewater under study of which *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus stolonifer* are the three fungi isolates that shows the ability to reduce microbial load after preliminary experiment out of the three fungi species used in this study, *Aspergillus niger* was observed to be most effective in reducing the microbial load of fungi in table 1 to that observed in table 7 with the wastewater from SSR (2.01×10^4 to 1.2×10^1 sfu/ml); MGR (1.72×10^4 to

$1.1x10^1$ sfu/ml) and GGR ($2.50x10^4$ to $1.5x10^1$ sfu/ml) while for the bacteria load was observed to be most reduced using the fungi isolate A. niger too with SSR reducing from (1.29×10^8 to1.57×10^2 cfu/ml), MGR from (1.04×10^8 to 1.12×10^2 cfu/ml) and GGR (1.72×10^8 to 1.60×10^2 cfu/ml). This shows that A. niger is a better microbe that can be used for the treatment of wastewater as it degrades organic matter more. While the comparison of tables 2 and 8 show the significant decrease in the microbial load of the wastewater after treatment using selective agars. When using the selective agar *Lactobaccili* agar, MRS it shows that there is no growth detected. The focus of most microbiological analysis of greywater has been on faecal pollution and enteric pathogens. Rose *et al.*, [18] reported total coliform concentrations in greywater ranged from 10^4 – 10^6 cfu/ml. Cassanova *et al.* [8] found higher concentrations and reported a mean of 8.03×10^7 cfu/ml in their study of greywater. In addition to total coliform organisms, enteric pathogens such as *Salmonella*, *Shigella* and Poliovirus Type 1 have all been reported to be present in greywater and concerns about the potential for re-growth or persistence of these organisms has been raised [18]. In addition to enteric pathogens, there are several human-associated opportunistic pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in the greywater as illustrated by table 4. In recent years, *S. aureus* has been recognized as the cause of community-associated infections. Outbreaks of *S. aureus* have been associated with a variety of contact sports such as football [7], wrestling [12], and rugby [22]. In addition, swimmers and others involved in water contact sports are at increased risk of *S. aureus* infection. *P. aeruginosa* is ubiquitous being widely distributed in aquatic and terrestrial habitats; it is even able to proliferate in distilled water [11]. While usually not a significant risk to healthy individuals, *P. aeruginosa* has been associated with cases of folliculitis, dermatitis, and ear and urinary infections. In addition, it causes 10–11% of all nosocomial infections in hospitals and has the highest fatality rate of all hospital-acquired bacteremias [4]. *Aspergillus niger* is known to causes a disease called black mould on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and also commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould") which could be one major purpose for its presence in the waste water [19].Therefore the presence of *Aspergillus niger* could be attributed to the soil environment in which the waste water is released into and also because the flushed machines are usually kept [1]. *Rhizopus stolonifer* (black bread mold) is a widely distributed thread-like Mucoralean mold commonly found on bread surfaces [3]. It takes food and nutrients from the bread and causes damage to the surface where it lives. It has a cosmopolitan distribution and is capable of causing opportunistic infections of humans (zygomycosis). It is most commonly found growing on bread and soft fruits such as bananas and grapes. Because its spores are common in the air, it can often be grown within a few days by keeping moistened pieces of bread in an enclosed, humid environment [21]. The presence of *Rhizopus stolonifer* which is commonly found growing on bread and soft fruits such as bananas and grapes are capable of causing opportunistic infections of humans (zygomycosis) could be ascribed to the presence of its spores in the air [21]

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