Microbiological and Physico-Chemical Assessment of Waste Water from Selected Food Industries in Port Harcourt and Snail Shell Treatment Potentials

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Abstract: Composite wastewater from 3 food industries (Pabod Breweries, Courdeau Catering Industry and Coca-cola bottling Company) were characterized microbiologically and physico-chemically before and after treatment using snail shell. The aim was to determine the microbial load and physico-chemical parameters of wastewater and also the effectiveness of snail shell in the treatment of the samples obtained. The samples were cultured on nutrient. Macconkey and Sabourad dextrose agar before and after treatment. Snail shells were collected from Choba market, Port Harcourt and washed properly. They were dried and grind to fine powder. Aseptic procedures were duly followed. For treatment, 5g, 15g, and 25g of ground snail shell were added to different test-tubes of wastewater samples of the same volume (20mls). The results of the parameters studied before and after treatment show a change in colour from cloudy to a colourless liquid. There was a reduction in the turbidity values which ranged from 423NTU to 158NTU, there was a reduction in the total solid and total suspended solid values from 16000mg/ml and 0.91mg/ml to 1320mg/ml and 0.61mg/ml respectively. There was an insignificant reduction in the total heterotrophic bacteria and fungi count from 13.7 $x10^8$cfu/ml to $7.7x10^{8cfu/ml,}$ $7.9x10^8$cfu/ml and $7.1x10^8$cfu/ml in response to the different masses of snail shell added. Some of the microorganisms identified includes, Escherichia coli, staphylococcus aureus, Bacillus cereus, Pseudomonas species, Fusarium, Aspergillus, Yeast. The research shows that snail shell can be used to rid wastewaters of solids but cannot be used alone in the removal of microorganisms since some microorganisms also use it as source of nutrient.
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1. INTRODUCTION

Wastewater comprises liquid waste discharge by domestic residence, commercial properties, industries and agriculture. These wastewaters contain a wide range of potential contaminants and of high concentration. Wastewater contains offensive and potentially dangerous substances which cause pollution and contamination of receiving water bodies (Shaw and Schrudam, 2000). One of the most important factors of water pollution is the microbial contamination, especially with pathogenic microorganisms. Enteric pathogens are typically responsible for several waterborne sicknesses (Johnson et al, 1985). Contamination of water is a serious environmental problem as it adversely affects the human health and the bio-diversity in the aquatic ecosystem. The use of indicator bacteria such as feacal coliforms (FC) in water quality determination on fresh water source is widely used (You-Joe et al, 2003). Currently, coliforms and *Escherichia coli* are of great importance among bacterial indicators used in water quality definitions and health risk (Sasikala and Kamana, 1995). However, operational evaluation of microbial load of wastewater (biologically) is often complicated because of variation in raw waste water composition, strength and flow rate owing to the changing and complex nature of the treatment processes (Boscar et al, 1992). Pathogens are a serious concern for managers of wastewater because excessive amount of faecal bacteria in sewage and urban run-off have been known to indicate risk of pathogen induced illness in humans (Hajji et al, 2000). Thus, identification of these pathogenic agents in water resources is beneficial for controlling and preventing planning of the infectious diseases.

In recent years, the efficacy of industrial wastewater evaluation has focused on new technology rather than conventional method such as ion-exchange, chemical precipitation and solvent extraction amongst others which are prohibitatively expensive and inefficient. Thus, the need for the use of potential microorganisms in their treatment or in some cases, recycling is done (Orsini et al, 2002).

The average daily amount of waster water in the sewage produced by individual industry is customarily expressed by a related Biochemical Oxygen Demand (BOD) (Yang, 1996). Effluents generally have an adverse effect on water bodies such as lakes, rivers, oceans and groundwater, and this is caused by human activities. (Abel, 1996). Pollutants in water include a wide spectrum of chemicals,

pathogens and physical chemistry or sensory changes. Alterations of water's physical chemistry include acidity, conductivity, temperature and eutrophication (Novak, J.T. 2001). Alterations are often caused by changes in characteristic and operation conditions. Many problems found in wastewater treatment that perform biological removal of pollutants are due to alteration in the microbial communities involved.

Plate counting and most probable number (MPN) techniques, have been used for the study of microbial communities in mixed culture systems. However, less than 1% of microorganisms in the environment can be cultivated by standard techniques because culture techniques fail to reproduce in artificial media.

The development of aqueducts in Rome around 300 B.C meant waste could be diverted away from population centers. These aqueducts were a form of combined sewer overflow system that is still common today, except sewage was simply sent to the nearest point of a stream or river. As cities grew and water demands increased, so did the occurrence of bacterial related illness from human contact and consumption of untreated water. This ushered in the need to actually clean the effluent before it reached waterways.

Though, the use of snail shell is not a very reliable method of treating wastewater compared to other formal design treatment facilities, like the use of Septic tanks and wastewater treatment tanks, snail shell helps to neutralize fine particles of suspended and dissolve matter in a water sample to form flocs that settles and can be filtered out.

Snail belongs to the phylum mulluscs and to the class gastropods. The shell has a brownish color with a characteristic stripe pattern. The main constituent of the shell is calcium carbonates which are either of two crystalline forms, calcite and aragonite. The remainder is organic matrixes which constitute a protein known as conchiolin that usually make up 5% of the shell.

2. MATERIALS AND METHODS

Wastewater samples were collected on the 2nd of November, 2010 from three food industries for physico-chemical and microbiological analysis. Pabod Breweries Trans-Amadi Port Harcourt, Courdeau Catering Industry RIVOC Road Trans-Amadi Port Harcourt and Coca-cola Trans-Amadi Port Harcourt. Sterile containers were used to collect water sample and were immediately transported to the laboratory for immediate analysis. Serial dilution (using saline water in sterile test tube) were carried out for each of the samples and cultured on Nutrient agar (NA). Macconkey agar (MA) and Sabourad Dextrose agar (SDA) (on Petri dishes). The nutrient agar plates were incubated at 35^{o}C for 24 hours while the SDA plates were incubated at 25^{0}C for 48-72hours. For each sample cultured, observation was made on at least one plate of the series whose bacteria or fungi numbers were sufficiently low which allowed the development of well separated colonies. The colonies were sub-cultured until pure cultures of the isolates were obtained. These were then stocked for further biochemical analysis.

After the physico-chemical and microbiological assessment, the various wastewaters were subjected to treatment using snail shell.

The snail shells were collected at Choba Market in Port Harcourt and washed with water. They were dried and ground to fine powder. 5g, 15g, and 25g were added to different test tubes containing 20mls of wastewater samples. They were allowed to stay for a period of 3 hours so that the treatment can take place. This process was carried out for 3 samples and all samples were cultured on the NA, MA and SDA as it was before treatment.

For the physico-chemmical assessment after treatment, 15g of mass of snail shell (i.e. the average of the mass of the snail shell used to treat the water) was added to 20ml of each water sample. The samples were allowed to stay for 3hours and the physico-chemical parameters were observed.

The isolates were subjected to biochemical reactions such as methyl red, voges proskauer, hydrogen sulphide production, indole test, urea hydrolysis, citrate, catalase, sugar fermentation, wet mount and motility. Physico-chemical tests such as pH, turbidity, total solids, total dissolved solids, odor and color determination were also carried out.

3. RESULTS ANALYSIS

3.1. RESULTS FOR PHYSICO-CHEMICAL TEST CARRIED OUT ON WASTEWATER

Table 1: Showing Result Before Treatment with Snail Shell

Sample code	pH	Turbidity	TS(Mg/L)	TSS(Mg/L)	TDS(MG/L	color	Odour
A	4.80	380	14000	0.722	13.990	White	Less offensive
B	6.01	423	16030	0.91	15.716	Cream	Less offensive
C	5.05	392	14220.5	0.80	14.165	White	Less offensive

Table 2: Showing Result after Treatment with Snail Shell

Sample code	pH	Turbidity	TS(Mg/L)	TSS(Mg/L)	TDS(MG/L	color	Odour
A	5.72	250	13200	0.22	13.100	Colourless	Less offensive
B	6.62	273	15285.6	0.29	15.111	Colourless	Less offensive
C	5.92	158	13922.5	0.16	13.700	Colourless	Less offensive

KEY: TS =TOTAL SOLIDS; TSS =TOTAL SUSPENDED SOLIDS; TDS=TOTAL DISSOLVED SOLIDS; A=Wastewater Sample collected from Pabod Breweries; B=Wastewater sample collected from courdeau catering industry; C=wastewater sample collected from coca-cola bottling company

Table 3: Total Heterotrophic Bacteria Count Before Treatment Using Nutrient Agar

Sample code	Total viable bacteria count (Cfu/ml)
A	11.5×10^8
B	13.7×10^8
C	11.3×10^8

Table 4: Total Heterotrophic Bacteria Count After Treatment Using Nutrient Agar Medium

Sample code	Total viable bacteria count (Cfu/ml)
A_5	11.7×10^8
A_{15}	11.9×10^8
A_{25}	11.4×10^8
B_5	12.2×10^8
B_{15}	11.8×10^8
B_{25}	11.8×10^8
C_5	10.1×10^8
C_{15}	10.3×10^8
B_{25}	10.9×10^8

Table 5: Total Heterotrophic Bacteria Count Before Treatment Using Mac Conkey Agar Medium

Sample code	Total viable bacteria count (Cfu/ml)
A	10.5×10^8
B	11.5×10^8
C	8.0×10^8

Table 6: Total Heterotrophic Bacteria Count After Treatment Using Mac Conkey

Sample code	Total Heterotrophic count (Cfu/ml)
A_5	9.4×10^8
A_{15}	10.0×10^8
A_{25}	9.1×10^8
B_5	10.9×10^8
B_{15}	9.1×10^8
B_{25}	9.6×10^8
C_5	7.7×10^8
C_{15}	7.9×10^8
B_{25}	7.1×10^8

Table 7: Total Heterotrophic Fungi Count Before Treatment Using Sabourad Agar

Sample code	Total viable Fungi count (Cfu/ml)
A	2.22×10^8
B	27×10^8
C	2.1×10^8

Table 8: Total Heterotrophic Fungi Count After Treatment Using Saboured Agar

Sample code	Total Fungi count (Cfu/ml)
A_5	2.1×10^8
A_{15}	1.9×10^8
A_{25}	1.9×10^8
B_5	2.5×10^8
B_{15}	2.3×10^8
B_{25}	2.2×10^8
C_5	2.1×10^8
C_{15}	1.8×10^8
B_{25}	1.7×10^8

KEY: A, B, C = Stands for various samples; 5, 15, 25= Stands for the mass in gram of snail shell used in treatment; Subscript figures represents the different isolates (1, 2, 4, 4 etc)

Table 9: Biochemical Results for the different isolates from the Microbiological Assessment of Wastewater from Selected Food Industries

Genus and species	Oxidase	Glucose	Lactose	TSI slant	Agar salt	Gas	H_2S	Indole	MR	VP	Criteria	Urease	Motility
E.coli	-	+	+	A	A	+	-	+	+	-	-	-	+
Klebsiella	-	+	+	A	A	+	-	-	-	+	+	-	+
Serratia	-	+	-	B	A	-	-	-	-	+	+	-	+
Citrobacter	-	+	+	B	A	+	+	-	+	-	+	-	+
Proteus	-	+	-	A	A	+	+	+	+	-	-	+	+
Pseudomonas	-	+	-	A	A	+	-	-	-	-	+	-	+
Salmonella	-	+	+	B	A	+	+	-	+	-	+	-	+
Shigella	-	+	-	B	A	-	-	-	+	-	-	-	-
Staphylococcus	-	+	+	A	A	-	-	-	-	+	+	-	-
Enterobacter	-	+	+	A	A	+	-	+	+	-	-	-	+
Bacillus cereus	-	+	-	B	A	-	-	-	-	+	+	-	+
Acetobacter	-	+	+/-	A	A	+	NA	-	NA	+o r-	NA	NA	-

KEY: MR=Methyl red; VP=Voges –proskaves; A=acid; B=Base; NA = Not applicable

4. DISCUSSION

Wastewater samples were collected from 3 food industries and immediately taken to the laboratory for analysis. From the microbiological analysis before treatment with snail shell, it was observed that the wastewaters from the Pabod and coca-cola companies showed fewer bacteria and fungi as compared to the Courdeau Catering industry water sample. From the result, it can be said that Courdeau Catering Industry produces wastewater very rich in nutrient as to support a high population of microorganism than Pabod Breweries and Coca-cola bottling company. In a similar research carried out at the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria, using effluent samples from two top Nigerian food and beverage industries (Swords food industry and 7-Up Bottling Company), Ibadan, Oyo State, there was high concentration of microbial load in the food industry water sample than the bottling company water sample. This is as a result of the activities carried out to achieve their final desired products. Such activities can include the washing of food raw materials like meat, fish, rice, washing of cooking utensils, remains of cooked foods which are thrown into the water channels, used oils etc. Accumulation of industrial food processing effluent in Courdeau Catering Industry supported the growth of bacteria and fungi which some are pathogenic to man. Take for example, *Bacillus cereus* which causes two distinct illness (anametic and diarrhea types). To this end, significant progress should be made in ensuring proper treatment of this effluent in order to safe guard the health of those within and outside the industry. It was also observed that *Escherichia coli,* a coliform organism was present in all three samples but dominant in the courdeau catering water sample. *Salmonella* and *shigella* were also present in this sample. *E.coli* is an enteric bacterium which is commonly found in lower intestine of warm blooded organisms. It was dominant in the wastewater of the catering industry since fishes and other aquatic organisms carry this bacterium from faecally polluted water. *E.coli* is not only confined to the intestine and their ability to survive easily makes them present anywhere including bottling companies and other food industries. *E.coli* is harmless but some serotype can cause serious food poisoning in humans, so the need to rid it of these organisms should be of utmost concern.

From the values of total solids suspended and total dissolved solid tests of the wastewater samples;

it showed that the sample contains high solid concentration. But when these samples were subjected to snail shell treatment, it was observed that snail shell acted as a coagulant since it caused the solids present in the wastewater to form flocs and settles and this facilitates filtration. After the addition of ground snail shell, it was observed that the color of all samples changed from its initial colour (before treatment) into a colourless liquid (after treatment). This indicates that the addition of the snail shell to the wastewater samples caused clarification in the water through the removal of solids. On addition of increasing masses of snail shell (5g, 15g, and 25g) to different glasses of water sample of the same volume (20mls), the wastewater showed corresponding response to different masses of snail shell. This was not same for the microbiological analysis. In this case, there was an insignificant reduction in the total heterotrophic bacteria and fungi count in the wastewater from the 3 industries (Pabod Breweries, Courdeau Catering industry and Coca-cola bottling company).

Since snail shell caused the suspended liquid to form flocs and settles, some microorganisms will attach to these flocs thereby causing the decrease in microbial population observed. However, in response (growth) to the addition of snail shell to the water samples, it was observed that sample A (Pabod Breweries) experienced an increase in the microbial load. The initial bacterial count was 11.5 x 10^8cfu/ml. after treatment with 5g, 15g and 25g of ground snail shell, the count increased to 11.7 x 10^8, 11.9 x 10^8 and 11.4 x 10^8cfu/ml respectively. It can be explained that some of the organisms could have utilized the ground snail shell as a source of nutrient. The organism, Acetobacter was observed to have increased geometrically. This is an indication that the ground snail shell was utilized by this organism as source of nutrient. On the addition of 25g of snail shell to sample A (Pabod Breweries), there was a decline in the microbial population. It is likely that the water was not enough to dissolve the snail shell added thereby, the nutrient were not circulated properly for those organisms that make use of the ground snail shell as nutrient. The odour of the water samples were also observed before and after treatment. It was discovered that snail shell had no effect on the odour of the wastewater since the offensive odour did not reduce.

5. CONCLUSION

It can be concluded that snail shell has a more significant effect on the physico-chemical characteristics of industrial wastewaters than its microbiological characteristics in terms of treatment aimed at reduction of microbial population.

Snail shell can reliably be used in the treatment of industrial wastewater. In treatment generally, it can be used to remove solids and suspended solids but its effect on the microbial load to wastewaters is not outstanding so, further research should be encouraged towards the use of snail shell in combination with other substances in the treatment of wastewater. From this research, it can also be concluded that some organisms use the ground snail shell as a source of nutrient. To this end, I recommend that snail shell be incorporated in media composition since it supports the growth of microorganisms.

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