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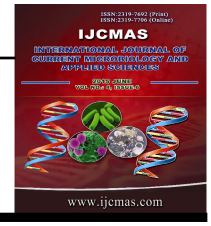

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Original Research Article

Some Physico-Chemical Parameters and Major Microorganisms Isolated from Cassava Wastewater and a Receiving nearby Stream

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A B S T R A C T

In this study, the effect of two cassava wastewater (C1 & C2) from a local cassava processing mill located in Saw-mill area of Ibadan, South-west, Nigeria, on a nearby receiving stream water (C3) was investigated. Physico-chemical parameters measured included pH, turbidity and biological oxygen demand (BOD). An estimation of the microbial load and isolation of major microorganisms present was also carried out. Values of pH showed that the water samples were acidic with C1 ranging from 5.49 to 6.68 (mean of 6.41); sample C2 ranged from 5.55 to 6.68 (mean of 6.42) and C3 ranged from 6.31 to 6.64 (mean of 6.47). There was significant difference ($P < 0.05$) in mean turbidity values of C1 (1.43 NTU) & C2 (1.41 NTU) compared to the mean value of C3 (1.81 NTU). There was also a significant difference ($P<0.05$) in the mean BOD values obtained for C1 (270 mg/L) & C2 (264 mg/L) compared to C3 (251 mg/L). An estimation of the microbial load of the wastewater samples shows that they were heavily polluted. Sample C1 had mean bacterial counts ranging from 3.2 to 3.9 x 10^6 cfu/ml and mean fungal counts ranging from 4.1 to 6.0 x 10^4 cfu/ml; sample C2 had mean bacterial counts ranging from 2.8 to 3.8 x 10^6 cfu/ml and fungal counts ranging from 3.2 to 4.5 x 10^4 cfu/ml; while the receiving shallow stream (C3) recorded mean bacterial counts ranging from 5.2 to 6.4 x 10^6 cfu/ml with mean fungal counts ranging from 6.0 to 8.2 x 10^4 cfu/ml. Major bacterial isolates included *Lactobacillus* sp. (48%), *Streptococcus* sp. (18%) and *Proteus* sp. (28.1%).While major fungal isolates included the yeast *Saccharomyces* sp. (75%) and *Aspergillus niger* (25%). The implication of the findings was discussed.

K e y w o r d s

Physico-chemical, Cassava, Wastewater, Bacteria, Fungi

Introduction

Wastewater, also known as sewage, originates from household wastes, human and animal wastes, industrial wastewaters, storm run-off and groundwater infiltration. It is largely the water supply of a community after it has been fouled by various uses. Basically, it is used water from a community. It is 99.94% water by weight with the remaining 0.06% consisting of dissolved materials or suspended (Ubalua, 1998). The chemical and biological nature of wastewater includes physical, chemical and biological characteristics which depend on water usage in the community, the industrial and commercial contributions weather and infiltration/inflow. An understanding of the physical, chemical and biological characteristics of wastewater is very important in the design, operation and management of collection, treatment and disposal of wastewater.

A typical example of waste water is the cassava wastewater that is obtained during the processing of cassava into various products. It is composed mainly of lactic acid bacteria with pre-dominance of the genera *lactobacillus* (especially *L. plantarum, L. fermentation, by Streptococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus* and *Lactococcus* spp) as reported by Molon *et al.*, 1998 and Lacerda *et al.*, 2005. Additionally, yeasts such *Galactonyces geothricum*, *Candida ethanolica* and species of *Saccharomyces* have also been reported (Avancini *et al.*, 2007). Oyewole (2001) had reported that yeasts (especially *Saccharomyces* sp.) seems to have an important roles in the survival and activity of lactic acid bacteria during fermentation process, as they are involved in the cassava starch hydrolysis into simple sugars, which are ultimately converted into organic acids by the lactic acid bacteria.

Cassava mill wastewater has a high organic and cyanide contents and is an important economic product of traditional and rural low technology agro-industry in many parts of the world. However, wastewaters could be toxic and can pose serious threat to the environment and aquatic life in the receiving waters (Kaewkannetra *et al.*, 2009). Water bodies receiving untreated cassava wastewater have been reported to be highly acidic sometimes with pH as low as 2.6 (Arimoro *et al.*, 2008). In this study we analysed some major physico-chemical parameters and isolated major microorganisms present in cassava wastewater.

Materials and Methods

Sampling

Cassava wastewater was collected from a cassava processing local factory in Saw-mill area of Ibadan, Oyo State, Nigeria and taken to the laboratory for analysis. Two separate samples were collected three times on a forth night basis. The samples were labeled as C1 (which was collected near the source-15m away) and C2 (which was collected-100m away from the source) and C3 (which was collected from a receiving shallow stream located within the premises of the processing mill). Wastewater samples were placed in ice-pack and analysed within 24 hours after collection.

Physico-chemical parameters

pH

The pH of the wastewater samples was determined using the Jenway pH meter (model 3150). The meter was first deepened into sterile de-ionised water, so as to read a neutral pH of about 7.0. Thereafter, it was deepened into the wastewater samples and read-off appropriately.

Turbidity

Turbidity of the wastewater was determined using a colorimeter (Jenway model 6501). A cuvette containing de-ionised water as control and is first inserted into the machine. This was done to first standardize the machine to zero value. Another cuvette containing the wastewater sample was inserted into the colorimeter. It was closed and the reading was taken using the instrument's scale caked absorbance at a wavelength at 540nm.

Biochemical oxygen demand (BOD)

This was done using a special BOD bottles with air-tight stoppers. The wastewater was initially aerated to provide a relatively high level of dissolved oxygen. The wastewater samples were each diluted to a total of 157ml at the dilution inside the BOD bottles. Four drops of 40% KOH was put in the air-tight stoppers to aid absorption of oxygen from the sample. The bottles were then placed on the BOD machine and closed. The bottles were incubated in the dark for 5 days. The amount of oxygen required by microbes was determined by measuring the rise of the mercury in the machine.

Microbial counts and isolation

Wastewater samples were first diluted serially by first pipetting 1.0ml of cassava wastewater into sterile 9ml de-ionised water in a test tube to give 10^{-1} dilution. From the 10^{-1} dilution 1.0ml was transferred into a fresh 9.0ml sterile de-ionised water to give 10^{-2}. This was continued until 10^{-6} dilution was reached. One milliliter from each dilution was transferred a sterile Petri dish and 20ml sterile nutrient agar was added; swirled gentle and allowed to cool, incubation was done at 37 °C for 24hrs. Discrete colonies were counted and transferred onto fresh Nutrient agar plates until repeated until pure cultures were obtained. Identification of the bacterial isolates was done using various morphological, physiological and biochemical characteristics.

For fungal isolates, potato dextrose agar (PDA) was used as the growth medium. Incubation was done at 25^0C. Fungal isolates were identified by morphological and microscopic observations compared with Barnett and Barry (2010).

Statistical analysis

Data generated were analysed by the Duncan's multiple range test of significance at $P< 0.05$.

Results and Discussion

Values of physico-chemical parameters measured is presented Table 1. Sample C1 had pH values ranging 5.49 to 6.85 with a mean value of 6.41 that of sample C2 ranges from 5.55 to 6.88 with a mean value of 6.42. While sample C3 had pH values ranging from 6.31 to 6.64 with a mean of 6.47. There was no significant difference in the pH values of the samples ($P < 0.05$). Turbidometry values for the samples ranged between 1.30 and 1.55 NTU (mean of 1.43 NTU) for sample C1; 1.35 and 1.53 (mean of 1.41) for sample C2 and 1.10 and 1.25 NTU (mean of 1.18 NTU) for sample C3. There was a significant difference between the mean turbidity values of samples C I and C2 compared to C3 ($P < 0.05$). Biological oxygen demand (BOD) values for sample C1 ranged between 260 and 280 mg/L (mean of 270 mg/L); 255-270mg/L (mean of 264 mg/L) for sample C2; while sample C3 ranged between 245-260mg/L (mean of 251 mg/L). There was also a significant difference between samples C1and C2 compared to C3 ($P< 0.05$).

An estimation of the microbial load of the wastewater samples in presented in figures 1 and 2. Sample C3 recorded the highest bacterial counts ranging from 4.8 and 6.9 X 10 cfu/ml. and sample C2 ranging between 2.5 and 4.1 $X10^6$ cfu/ml. A similar pattern was observed for fungal load. Sample C3 recorded the highest fungal counts ranging between 5.9 and 8.7 $X10^4$ cfu/ml. Sample C1 recorded mean fungal counts ranging between 3.9and 6.4 $x10^4$ cfu/ml; while sample C2 recorded mean fungal account ranging between 3.1 and 4.8 $x10^4$ cfu/ml.

Table 2 shows the major microbial isolations from the cassava wastewater samples. A total of 89 bacteria were isolated and this comprises 43 *Lactobacillus* sp. (48%), 16 *Streptococcus* sp. (18%) and *Proteus* sp. (28.1%). Major fungal isolates were *Aspergillus niger* (25%) and *Saccharomyces* sp. (75%).

Cassava wastewater is an industrial residue obtained during the processing of cassava into various fermented products such as *gari*, *fufu*, *lafun* etc. The result of the measure of acidity or alkalinity of the cassava wastewater indicates the samples tended to be acidic. Similar result has been reported by Kaewkanetral *et al.* (2009) and that this probably arises from the acidification processes of starch due to organic acid production. The acidity of the wastewater samples may be as a result of high populations of lactic acid bacteria that convert simple sugar into organic acids which included basically lactic acids but small amounts of butyric, acetic and propionic acids (Okafor and Opuene, 2007).

The wastewater samples tended to be heavily contaminated as revealed by the high values of BOD which ranged between 245-280 mg/L. The high BOD values is similar to that reported by Avancini *et al.* (2007) who showed that cassava wastewater is an effluent with high BOD values. However, mean BOD values for raw cassava wastewater was higher (C1=270mg/l and C2 260mg/l) while BOD mean value for the shallow stream water was significantly lower (251mg/L at $P<0.05$). Also, from the high turbidomety values obtained (ranged from 1.10 to 1.55 NTU) it can be deduced that cassava wastewater is highly turbid and polluted. However, mean turbidometry values of C1 and C2 tend to be significantly different from C3.

An estimation of the microbial load water samples shows that they are heavily polluted (figures 1 & 2). Mean bacterial counts for C1 ranged from 3.2 to 3.9 x 10^6 cfu/mL; C2 range for 2.8 to 3.8 x 10^6 cfu/mL while C3 rents recorded the highest bacterial counts (5.2-6.4 x 10^6 cfu/mL). A similar pattern was observed for fungal counts. It can be deduced from this that waste water from C1 and C2 must have resulted into higher microbial observed in the receiving nearby shallow water (C3).

Three major bacterial genera were isolated and these are *Lactobacillus* species (48%), *Streptococcus* species (18%) and *Proteus* species (28.1%). Higher occurrence of *Lactobacillus* sp. in the water samples is in agreement with the report of Lacerda *et al.* (2005) which showed that cassava starch fermentation wastewater composed mainly of lactic acid bacteria with the predominance of the genera *Lactobacillus.* Also, the presence of *Lactobacillus* sp. and *Proteus* species corresponds with the reports of Westby and Twiddy (1992) which showed the ability of these microorganisms to hydrolyse linamarin present in a cassava wastewater.

Table.1 Some physico-chemical values measured from cassava wastewater samples

Sample Code	Sampling regime	pH	Turibidity (NTU)	BOD (mg/L
C1	1st	5.49	1.54	280
	2nd	6.83	1.30	260
	3rd	6.85	1.33	265
	4th	6.45	1.55	275
	Mean	6.41^a	1.43^b	270^b
C2	1st	5.55	1.53	270
	2nd	6.51	1.35	255
	3rd	6.88	1.37	269
	4th	6.73	1.40	265
	Mean	6.42^a	1.41^b	264^b
C3	1st	6.51	1.25	260
	2nd	6.42	1.15	255
	3rd	6.31	1.10	245
	4th	6.64	1.23	245
	Mean	6.47^a	1.18^a	251^a

note: means with the same alphabets are not significantly different ($P < 0.05$)- within the same column

Table.2 Microbial isolations and frequency of isolation from cassava wastewater

Isolate	No: of Isolates	Frequency of Isolation
Bacterial		
Lactobacillus sp	43	48
Streptococcus sp	16	18
Proteus sp	25	28.1
Unidentified	5	5.6
Total	89	
Fungal		
A. niger	17	25%
Saccharomyces sp	51	75%
Total	68	100%

Figure.1 Comparison of mean bacterial counts of cassava wastewater (C1 and C2) and receiving stream (C3)

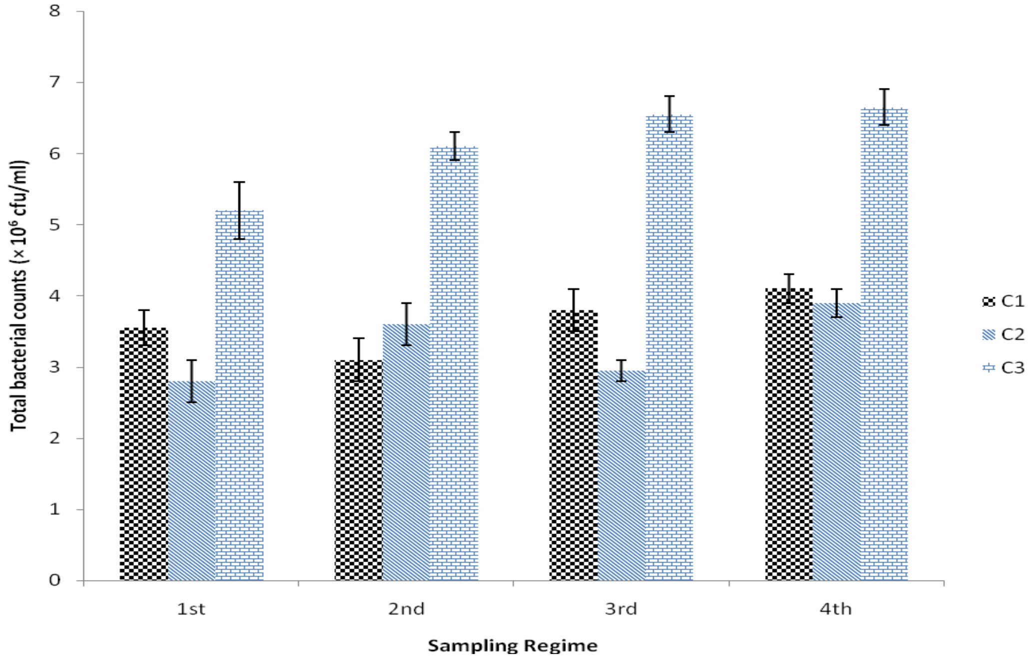
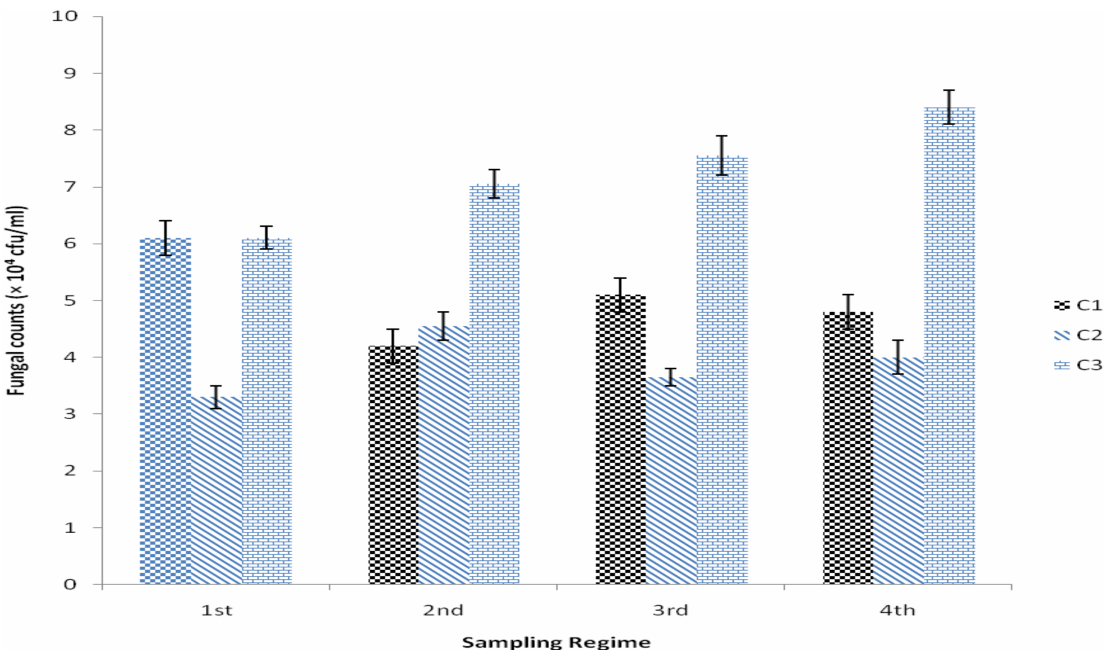


Figure.2 Comparison of mean fungal counts of cassava wastewater (C1 and C2) and receiving stream (C3)



Fungal isolates were primarily yeast (*Saccharomyces* sp.) and *Aspergillus* sp. The occurrence of the yeast correspond with the report of Oyewole (2001) that showed that yeast plays important roles in the fermentation of cassava and survival of lactic acid bacteria.

Conclusively, high organic acid and cyanide content of cassava wastewater makes it toxic and therefore pose serious threats to the environment and aquatic life in the receiving waters. Cassava mills are often small scale industries that discharge their effluents directly into the environment, therefore pre-treatment in holding tanks before discharging into the environment would be helpful (Onyedinwe *et al.*, 2010).

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