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EFFECTS OF CASSAVA EFFLUENTS ON THE PHYSICOCHEMICAL PARAMETERS AND MICROBIAL FLORA OF NKISSA RIVER, EGBEMA, RIVERS

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ABSTRACT: The effects of cassava effluent in the physicochemical parameters and microbial flora of Nkissa River Egbema was investigated. Samples were collected from the upstream (US) which served as control, the Discharge/Fallout point(DFP) , Down Stream I (DS I) and Down Stream II (DS II) each 120m away form the preceeding point. The DO, BOD, TSS, and TDS were adversely affected by the cassava effluent but the effect waned with distance and flow away from the DFP to the DS. The metallic ions were not significantly affected. The cyanogenic potentials of the water samples were quite low (1.03-0.42mg/l). The cassava effluent was concluded to have supplied microbial nutrients which increased microbial prevalence and bioloads. *Klebsiella, Corynebacterium, Acinetobacter,* and *Morexalia* species which were absent from the US were found from the DFP to the DS samples. *Saccharomyces, Enterobacter, Staphylococcus Escherichia, Lactobacillus, Bacilius*, and *Micrococcus* species were found in all the samples analyzed. The cassava effluent utilization test showed that *Alcaligenes, Xanthomonas, Lactobacillus, Coryneabacterium and Micrococcus species* are good metabolizers of the effluents indicated by change in pH and turbidity. Only *Escherichia* and *Enterobacter* species did not utilize the effluent at all.

Keywords. Cassava effluent, physicochemical parameters, bacteria, water flow.
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INTRODUCTION

Cassava (*Manihot esculanta (*Crutz*)* synonymous with *Manihot utilissima* Rohl*)* belongs to the family Euphorbiaceae (Onwueme, 1978). The tubers are often harvested between 7-13 months depending on the variety or cultiver platned (Cook 1985, Taye 1994). The edible fleshy position makes up to 85-90% of the tuber and contains up to 35-40% carbohydrate, 50-60% water and perhaps, 1-3% protein in addition to small quantity of cyanogenic glucosides (linamarin and lotaustialin), Onwumeme 1978. Nwabueze & Odunsi, 2007 Oyewole & Afolami, 2001).

The high Carbohydrate contents therefore makes cassava a major source of Carbohydrate particularly for the low income earners in most tropical countries. African in particular (Desse & Taye, 2001; Nweke, 1996; Adeniye & Laleye 2003). Cassava can be processed in various forms for which include flour, cakes, biscuits, chips or pellets. The floor could be fried to produce garri or cooked as foofoo (Onwueme, 1978; Cook, 1985 Nweke, 1996, Adeniye & Laleye, 2003). The consumption of cassava and cassava products have increased extensively in

Africa and Asia especially Nigeria where the Government is now offering some incentives for increased production. This increased production has equally resulted in increased processing coupled with an equally increased environmental pollution with the resultant wastes (Adeyemo, 2005; Adewoye *et al.*, 2005 Nwabueze & Odunsi, 2007). The land disposal of the effluent emanating from the grating and subsequent (processing) of the cassava tubers/paste gives very offensive stench with time (Geshe, 1985; Ashanafi 1994 Aderiye *et al.*, 2005). This has resulted in establishment of cassava processing plants near some natural flowing water bodies which act as sinks to this effluent.

This work examines the effects of cassava tuber effluents from two of such cassava processing plants (located at the same vicinity) on the aquatic bacterial flora of Nkissa River, Egbema a community in the Niger Delta Area of Nigeria.

MATERIALS AND METHODS

Nkissa River empties into the Orashi River in Egbema. The river is an all season one. Two large cassava-processing plants were established in 2002 on either side of the river and process between 2-3 tones of cassava tubers daily.

Sampling of the River was done at four different points- one was up stream (US), 120m before the Discharge/Fallout Point (DFP), and then two samples down stream (DS I and DS II). Down stream 1 was 120m from the DFP, while the second DS (DS II) was 120m from DS I. At each sampling point, two sample types were collected, water column and sediments which were pooled to give one sample. Three of such pooled samples were then bulked to one composite sample for each sampling point. Sampling was done ten (10) times at two weeks intervals for microbiological analyses while five (5) of such samples were analyzed for physicochemical properties.

The physicochemical properties of the water studied were done using different methods. The temperature and pH were determined at the site using multipurpose tester of Jenway (Model HANNA 1910). The DO and BOD were measured using the Winkler's titration method as described in APHA (1992). The SO_4, NO_3, PO_4, TDS and TSS were determined spectrophotometrically using HACH/D2/2010 spectrophotometer. The metallic ions examined were Ca^{2+}, K^{+}, Na^{+} and Mg. which were measured using the Atomic Absorption Spectrophotometric (AAS) method as described by APHA (1992).

Cynogenic potentials of the impacted water.

Cyanogenic poyentials of the impacted water was determined using modified pricate paper kits method as described by Bradburg *et al.*, (1999). 2mls of the water sample was put into 250ml conical flask containing 25ml distilled water. A strip of the test paper was soaked in the alkaline sodium picrate solution and fixed into the conical flask with the cork (stopper). The flask was allowed to stand at room temperature for 18-24 hours. The paper was removed and eluted in 60ml water. The absorbance of the water was read at 540nm using spectrophotometer (Unicam Heλ105y England). The results were recorded as mg HCN/L.

Microbiological Analysis

Ten water samples of each sampling point were analyzed for microbial prevalence using t culture techniques involving various culture media – Nutrient Agar, MaConkey Agar, Mine Salt Agar modified with the cassava effluent as described by Okpokwasili & Okorie (1988). T

fold serial dilution of each sample was done before inoculation using sterile peptone water as diluent. Inoculation was by the spread plate technique as described by Cheesbrough (2001) while pure isolates were obtained by the streaking techniques. The observed pure isolates were subjected to macroscopy, microscopy (after staining) and some biochemical tests for characterization and identification according to Cowan and Steel, (1976), Cheesbrough (2001) and Buchanan & Gibbons (1976). While the spread plate culture on Nutrient Agar yielded the Total Heterotrophic Count (THBC), that on McConkey Agar gave coliforms (CBC) and Cassava Effluent Utilizing Bacteria Count (CEUBC) was obtained using in the modified Mineral salt Agar, with fresh cassava effluent.

Screening for Cassava effluent utilization

This was investigated using the mineral salt agar of Mills *et al* (1976) as modified by Okpokwasili & Okorie (1988). To 9.5ml of the mineral salt medium was added 0.5ml of the fresh Cassava effluent. Each Isolate was inoculated in duplicates and incubated at room temperature for five days. Growth was indicated by turbidity and change in pH.

RESULT

The pH of the Nkissa River changed from nearly neutral (6.8) to alkaline but this decreased with distance away from the plants. TDS, TSS, PO_4 and SO_4 which had their lowest values in the US, had the highest values in the DFP. The DS values are shown in Table 1. The DO was highest in the US but decreased extremely in DFP with value in the DS I and DS II, being less than the DFP. The reverse was the case for the BOD. (Table 1)

Among the metallic ions only Na showed some increase from their DFP but decreased in the DS I and DS II. There was no significant change in the value of Mg^{2+}, Ca^{2+} and K^{t}. All other values of the parameter increased are shown in Table 1.

The cyanogenic potentials of the impacted water showed a reducing gradient. The highest value was at the DFP. (1.03mg/l HCN) followed by the DS1 point (0.42mg/l HCN). Only traces were seen in DSII. No traces were seen in the US (Table 1).

Table 2 shows the prevalence of the isolated organisms from the various samples screened. Twelve microbial species were observed. These organisms and their prevalence rates are shown in Table 2. The organisms include *Escherichia, Enterobacter, Staphylococcus, Xanthomonas, Lactobacillus, Micrococcus* and *Bacillus* species. Others were *Alcaligenes, Klebsiella, Corynebacterium, Acinetobacter* and *Saccharomyces* species. *Alcaligenes, Kelebsiella* and *Corynebacterium* species were not isolated from the freshly produced cassava effluent. These three organisms along with *Xanthomonas, Acinetobacter* and *Saccharomyces* species were absent in the US. However, all the twelve organisms were observed in the DFP and DS samples with highest prevalence in the DS I, then DFP and DS II had the least rates (Table 2).

In Table 3, the biolaods of the various groups of organisms investigated are shown. Highest bioloads were seen in the DS I while there was no significant difference between the values in DFP and DSS II. The highest occurring group was the THB, while the least was the coliform. The CEUB were more than the CB, except in the US (Table 3).

Screening for cassava effluent utilization showed that *Lactobacillus, Alcaligenes, Xanthomonas* and *Micrococcus* species are the major ultilizers of the effluent. This group was followed by *Staphylococcus, Klebsiella, Corynebacterium* and *Saccharomyces* species. *Eschericha, Morexeall* and *Enterobacter* species did not utilize the effluent. The utilization of the cassava effluent was indicated by the changes in pH and turbidity i.e. more change in pH and turbidity indicated higher utilization of the wastes (Table 4).

Table 1. Values of the physiochemical properties analyzed according to the sampling points

PARAMETER	US	DFP	DSI	DSII
pH	6.8	7.1	7.8	6.4
Temperature	28	28.9	29.1	28.8
TDS mg/l	540	1750	1320	920
TSS mg/l	20	60	40	31
DO mg/l	3.0	4.0	25	30
BOD mg/l	20	60	50	30
SO4 mg/l	24.3	30.1	27	25
PO4 mg/l	0.06	0.087	0.07	0.06
NO3 mg/l	10.3	15.1	13.1	10.2
Ca mg/l	24.3	29.1	27.1	24.4
Na mg/l	0.37	0.42	0.40	0.38
Mg mg/l	1.2	1.6	1.4	1.2
K mg/l	0.91	1.8	1.11	0.92
Oil coul	ND	1.01	1.06	1.01
Cyanogenic potential mg/l	ND	1.03	0.42	traces

Key: ND - Not detected

Table 2: Relevance of organisms observed in various samples analyzed (10 samples were analyzed).

	US	DFP	DSI	DSII
Escherichia sp	5 (50%)	2(20%)	3(30%)	2(20%)
Enterobacter sp	4 (40%)	2(20%)	4(40%)	2(20%)
Staphylococcus sp	4(40%)	6(60%)	7(70%)	5(50%)
Lactobacillus sp	2(20%)	5(50%)	7(70%)	5(50%)
Micrococcus sp	2(20%)	5(50%)	7(70%)	5(50%)
Bacillus sp	6(60%)	8(80%)	10(10%)	7(70%)
Alcaligenes sp	1(10%)	3(30%)	5(50%)	5(50%)
Klebsiella sp	-	2(20%)	4(40%)	4(40%)
Corynebacterium sp.	-	3(30%)	5(50%)	5(50%)
Saccharomyces sp	-	4(40%)	6(60%)	4(40%)
Morexella sp.	-	2(20%)	2(20%)	2(20%)
Aemietobacter sp	-	2(20%)	4(40%)	2(20%)

KEY: - = Not Seen Figures in bracket represent percentage occurrence

Table 3: Bioload of various groups of organisms in the samples examined

SAMPLE	US	FB	DSI	DSII
CBC	1.5×10^2	1.7×10^2	1.9×10^2	2.4×10^2
THBC	4.5×10^4	4.6×10^4	6.7×10^4	5.1×10^4
CEUBC	2.1×10^2	3.7×10^3	4.2×10^4	3.9×10^4

*Figures are average of five tests carried out.
KEY: Us = Upstream; DFP = Discharge/Fallout Point; DSI = Downstream I; DSII Downstream II
CBC = Coliform Bacterial Count; THBC = Total Heterotrophic Bacterial Count
CEUBC = Cassava Effluent Utilizing Bacterial Count

Table 4: Utilization of cassava effluent by isolates

SAMPLE	TURBIDITY	INITIAL PH	FINAL PH
Escherichia sp	-	6.7	6.7
Enterobacter sp	-	6.7	6.7
Staphylococcus sp	+	6.7	6.9
Lactobacillus sp	+++	6.7	8.2
Micrococcus sp	++	6.7	7.9
Alcaligenes sp	+++	6.7	8.10
Bacillus sp	++	6.7	8.0
Klebsiella sp	++	6.7	7.9
Corynebacterium sp	++	6.7	8.0
Saccharomyces sp	++	6.7	8.0
Morexella sp	+	6.7	6.9
Acinebacter sp	++	6.7	7.8

KEY: + = Scanty Growth; ++ = Moderate Growth; +++ = Heavy Growth; - = No Growth

DISCUSSION

In this work twelve microbial species were isolated. Most of them had been reported earlier in similar work. Desse and Taye (1996) and Akani *et al* (2006). Geopfort (1980), Oyewole and Odufa (1992) and Aderiye *et al.*, (2003) had observed some of them in fermenting tubers and vegetables. While some are natural saprophytes like *Corynebacterium, Bacillus, Micrococcus, Alcaligenes, Acinetobacterium* species, others like *Staphylococcus, Escherichia, Enterobacter* and *Sacharomyces* species could occur due to human activities. Some of the organisms in the water body could have come from the processing plants or effluent as some of them which were not in the US, were found in the DFP and DS samples.

Some of the other organisms increased in prevalence in the presence of the cassava effluent indicating that the effluent was utilized as nutrient. This was the case of *Alcaligenes, Lactobacillus, Micrococcus* and *Bacillus* species. These organisms were those, which could utilize the effluent. Abiona *et al*, (2005) and Oyewole & Odunfa (1992) stated that effluent from cassava was nutritive enough to support microbial growth, which agree with this work.

Prevalence of organisms was highest in DSI, followed by DFP and then DS II with the least in US. This meant that the cassava effluent supplied both nutrients and organisms to the Nkissa River from the DFP, but was too harsh or in a state not very appropriate for immediate microbial utilization. The conditions become conducive with distance away from the DFP and water flow, which summed up to dilution of the effluent and conversion to utilizable state. However, by the DSII, some of the essential nutrients had been exhausted resulting in low prevalence of the organisms. A similar situation had been reported by Nwaugo *et al.*, (2004) and Chinyere (2001). In the same vein Nwaugo *et al* (2006) working on Petroleum Produced Water on a tributary of the some Nkissa River reported the diluting effect of the water flow (current) which agrees perfectly well with this work.

The above observation was buttressed by the values in the bioloads recorded. Highest bioload occurred in the DS I but decreased in DS II and DFP was higher than US values. The THB was the highest, followed by CEUB before CB, which was very low. This means that survival of the CB in the down stream portion of the river was due to the metabolism of the intermediates of the effluent. From the results CB were not utilizers of the cassava effluents. Again, CEUB occurred in the US indicating that the cassava effluent degraders could be found anywhere without the presence of the effluents. However, the presence of the effluent ensured better adaptation to its metabolism hence the increase in number and prevalence.

These observations agree with the results in the screening for cassava effluent utilization. *Escherichia, Morexalla* and *Enterobacter* species could not utilize the effluent and so could not grow. On the other hand, *Alicaligenes, Lactobacillus, Xanthomonas* and *Micrococcus* species which were the greatest utilizers of the effluent could be introduced into the effluent channels to metabolize it before the effluents enter the water body. This will reduce the modification of the aquatic habitat parameters seen in the physicochemical parameters measured. Abiona *et al.*, (2005) and Arimoro *et al.*, (2007) reported that high microbial growth in any system encourages biofilm formation. If the effluent is first treated, the amount of microbial nutrients entering the river will be extensively reduced which will also reduce microbial growth in the river. Jonnalagadda and Mhere (2001), Akpan (2004) and Aderinye *et al.*, (2007) also agree with this assertion

The physicochemical parameters showed a modification of Nkissa River values by the cassava effluent. The most affected were, BOD, DO, pH, TDS, and TSS. These values were highest at the DFP but decreased from the DS I. A similar situation had been reported by Chinyere (2001), Nwaugo *et al*, (2004 and 2006) with Adewoye *et al.*, (2005). These researchers agreed that the concentration of any pollutant is highest at the Discharge/Fallout Point but decreases with distance and time from the pollution source due to dilution and microbial utilization.

Analysis of the cyanogenic potentials of the impacted water showed very low values (0.42 - 1.03 HCN mg/l). Aderiye *et al*, (2005) had reputed values between 2.91-4.11 HCN mg/100g, while Nwabueze & Odunsi (2007) reported 2.17 - 5.00mg HCN/kg. This could be expected as Aderiye *et al.*, (2005) and Nwabuze and Odunsi (2007) worked on fermenting cassava tubers and paste directory. This work only examined impacted water body which means that the cyanide contents had been extensively diluted. Results obtained in this work showed that though the cyanide content of the water changed, it was not above tolerable level (Aderiye *et al.*, 2005, Muhungu *et al.*, 1987). The micro-organisms in the water especially the CEUB might have metabolized the cyanide discharged into the water.

The effluent is very rich in carbohydrate but with little protein and other substances. The fermentation of these organic materials requires oxygen, which caused the increase in BOD and low DO observed from the DFP to the DSS II. The breakdown of protein releases Ammonia (Onwueme, 1978, Aderiye *et al*, 2003) which caused the pH charge towards alkalinity. The increase in TSS and TDS occurred because the effluent contained both water soluble and insoluble components that changed the initial situation. This concurs with Asonye *et al.*, (2007) and Chidah and Braide (2004).

In conclusion therefore, this work shows that the cassava effluent affected both the physicochemical and microbial parameters of the Nkissa River. However, this effect decreases away from the discharge point.

The work suggests that the adverse effects of the cassava effluents could be avoided by treating with CEUB before discharge.

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