Microbial degradation of Pyrazine Compounds in Wastewater and cow dung Southwest Nigeria.

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ABSTRACT

Wastewater samples, industrial effluents from food industry and cow dung were obtained aseptically and the physico-chemical properties were determined following standard procedures. Serial dilution technique was used in isolating native pyrazine - degraders from composite wastewater and cow dung samples obtained from Agbara and Apapa wastewater treatment plant (WTP) as well as Agege and Agbara Abattoirs respectively. Nutrient agar and Mineral salt medium supplemented with (3%) Pyrazine-2-carboxamide were used for enrichment for the isolates. Standard and conventional methods were used for the characterization and identification of pyrazine degraders. Gas chromatography (GC) flame ionization detector (FID) was used to determine the degree of mineralization of pyrazine. The mean pyrazine-degrader count from wastewater samples was 1.1×10^6 cfu/ml while that of cow dung was 9.7×10^5 cfu/ml. The GC peaks revealed that the initial quantity of pyrazine (15mg/l) was mineralized leaving behind (0.175mg/l) residues after 21 days of its introduction. The bacterial genera found utilizing pyrazine as source of C and N energy includes; *Bacillus sp.,Flavobacterium sp.,Corynebacterium sp., Pseudomonas sp.,Streptococcus sp. and Staphylococcus sp.* The biodegradability of pyrazine by tropical indigenous bacterial consortium was established and the convincing evidence of its mineralization was with gas chromatography. The objectives of the current study was to isolate and identify indigenous pyrazine-degraders from tropical wastewater and cow dung.

Keywords: Biodegradation, consortium, microorganisms, pyrazine, sustainable development

1. INTRODUCTION

Pyrazines belong to the diazine groups of heterocyclic compounds which include pyridazine (1,2-diazine) and pyrimidine (1,3-diazine). They are a group of compounds that ubiquitous in nature, vegetables, and processed foods as well as in microorganisms (Muller and Rappert, 2010). Pyrazines are synthesized chemically or biologically. They serve as flavoring additives due to their odorous properties and are vital flavors in many food products. Pyrazines are mainly synthesized during heating of food (Grosch, 2001; Maarse, 1991; Rappert and Muller, 2010). They are categorized as major malodorous classes of compounds emitted by food industries (Ranau and Steinhart, 2004; Rappert and Muller, 2005). Dearth information has been published on degradation of pyrazines. In higher vertebrates, pyrazines are excreted as glucuronates or

bound to glutathione via the kidney through hydroxylation, leaving the pyrazine ring unbroken. They occur naturally and majority is anthropogenic. Pyrazines has been sought for at increased rates in the field of food security and health care. A few bacteria and fungi synthesize and degrade pyrazines. Microbial transformation of pyrazines has been published in previous literatures (Muller and Rappert, 2010; Rajini *et al*., 2011). Degradation of pyrazine is thus paramount for ecologically essential. Studies have been conducted on bioremediation of heteroaromatic compounds, with paucity of information about the microbial degradation of pyrazines. Rappert *et al*.,(2006) reported the discovery of a *Mycobacterium* sp. strain DM-11 that makes use of pyrazine as source of nitrogen, carbon, and energy in an aerobic process. This same strain metabolizes the waste gas in a food industry cometabolically.

The objectives of this study was to evaluate the presence of pyrazine-degraders in tropical natural environment as well as isolate and identify these microorganisms.

2. MATERIALS AND METHODS

2.1. Sample Collection

Wastewater samples were collected from Agbara and Apapa wastewater treatment plant (WTP), cow dung, domestic sewage and industrial effluents were collected in sterile bottles aseptically. Pyrazinamide (pyrazine-2-carboxamide) was purchased from CITO Pharmaceuticals Ltd. Lagos.

The unit composition of deionized water, of mineral salt mixture used for nourishment of the cultivation of the isolated bacteria and pyrazine-degrading bacteria was 1.0 g of KH_2PO_4, 2.0 g of $(NH_4)_2SO_4$, 2.5 g of K_2HPO_4 and 0.2 g of $MgSO_4 \cdot 7H2O$; supplemented with pyrazine-2-carboxamide(15mg/l), the pH was adjusted to 6.75. Nutrient agar was used to culture the heterotrophic bacteria population from natural environment The inoculated media were in duplicates and were incubated at room temperature 26 ± 2ºC (Rappert *et al*., 2006).

2.2. Physico-chemical properties of wastewater and cow dung

The proximate analysis of wastewater and cow dung samples were carried out to determine pH, total organic Carbon, N, e.t.c. (Smibert and Krieg, 1981).

2.3. Enrichment, isolation and identification of pyrazine-degraders

Composite wastewater samples from WTP, food industries and cow dung samples contaminated with odorous substances were collected and stored at 4ºC. Thereafter, analyzed using serial dilution technique and they were simultaneously inoculated onto Nutrient agar and Minimal salt medium supplemented with 3% pyrazinamide aseptically. Then, incubated at 26±2ºC for 24 – 48 hours. The mean heterotrophic bacterial population were determined and the isolates were sub-

cultured repeatedly to obtain pure isolates. The pyrazine-degraders were thereafter grown on the enrichment medium supplemented with 3mg/l pyrazinamide incubated on rotatory shaker at 26±2ºC for 21 days (Rappert *et al*., 2006). Residual hydrocarbon samples were taken at intervals Day 0 and 21 for gas chromatographic (GC) analysis.

2.4. Determination of residual hydrocarbons

The residual hydrocarbon concentrations in the pyrazine-degrader enrichment medium were extracted using dichloromethane and evaluated using Hewlett Packard 5890 Series gas chromatograph On - Column Injector type (column OV-101, thickness and width-80/100 mesh, stationary phase WHP 5%) with Flame Ionization Detector (FID). Temperatures of the injector and detector were kept at 200ºC and 260º C respectively. The column temperature was regulated to automatically increase to 230ºC. The GC was first set at 70^{o} C and allowed to remain for 2mins, then mixed at 10ºC /min to 230ºC and left for 10mins. The gas carrier was Nitrogen was of pressure 37psi while 1-2 µl of samples were respectively injected (Rappert *et al*., 2006; Adebusoye *et al*., 2007).

2.5. Microbiological and biochemical characterization of isolates

The Standard methods were employed in the microbial characterization, as described by Smibert and Krieg (1981) and Cowans and Steel (1990)

3. RESULTS

Proximate analysis of the composite wastewater and cow dung samples showed that they were acidic. The samples contained ample nutrients in relatively significant concentration to support the growth of the indigenous bacterial population in the samples (Table 1). The mean heterotrophic bacterial count on Nutrient agar was 1.2×10^5 and 16.0×10^5 for composite wastewater and cow dung respectively. The mean pyrazine-degraders from wastewater and cow dung were 11.0×10^5 and 9.7×10^5 respectively.

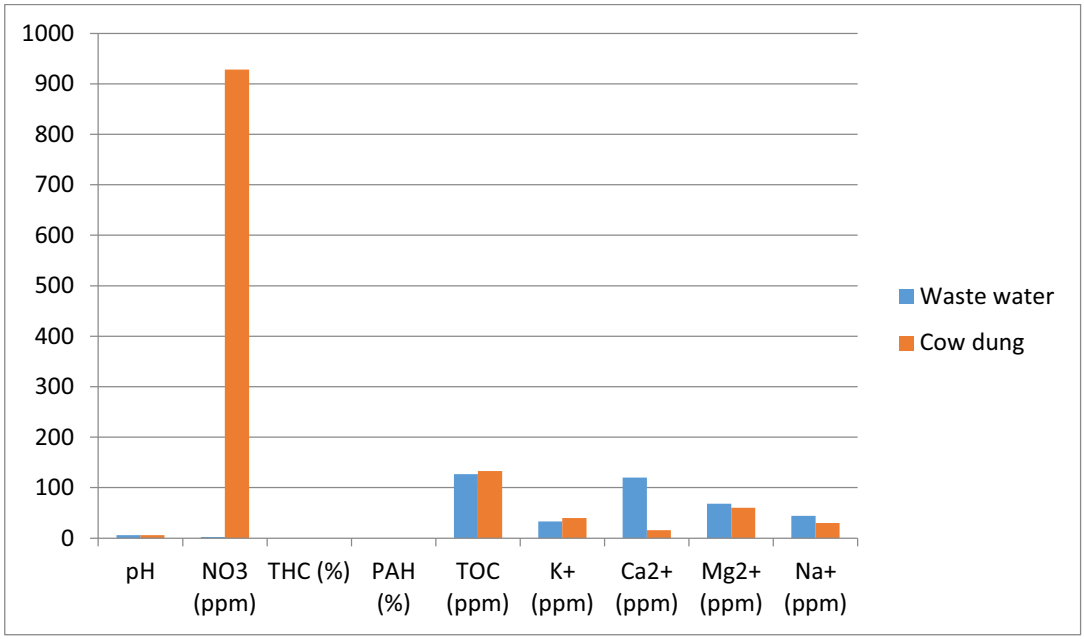

Figure 1: Physico-chemical properties of wastewater and cow dung samples

Table 1: Microscopic and Biochemical characterization of Pyrazine -degraders

Isolate code	Pigmentation	Gram's reation	Gram Reaction	Catalase test	Oxidase test	Indole test	Motility test	NO3 reaction	Citrate test	Urease test	MR test	VP test	Gelatin hydrolysis	Starch hydrolysis	Spore test	Probable Organism
A	Cream, mucoid	+	Rods	+	-	-	+	+	+	-	-	+	+	+	+	*Bacillus licheniformis*
B	Cream, rhiziod	+	Rods	+	+	-	+	-	-	-	+	-	+	+	+	*Bacillus firmus*
C	Cream, watery	+	Rods	+	+	-	+	+	-	-	-	-	+	-	+	*Bacillus laterosporus*
D	Cream, mucoid	+	Rods	+	-	-	+	+	+	-	-	+	+	+	+	*Bacillus licheniformis*
E	Cream, yellow	+	Rods	+	+	-	+	-	-	+	-	+	-	-	+	*Bacillus fastidiosus*

F	Pink	+	cocci	+	+	-	+	+	-	-	-	-	-	-	-	*Micrococcus roseus*
G	Cream	+	Rods	+	+	-	+	+	-	-	-	-	-	+	+	*Bacillus coagulans*
H	Cream, yellow	+	Cocci	+	+	-	-	+	+	-	-	-	+	-	-	*Micrococcus varians*
I	Orange	-	Rods	+	+	-	+	-	-	+	-	-	+	-	-	*Flavobacterium rigense*
J	Cream, yellow	+	Cocci d rods	+	-	-	-	+	-	+	-	-	-	+	-	*Corynebacterium pilosum*
K	Green	-	Rods	+	+	-	+	+	+	-	-	-	+	-	-	*Pseudomonas aeruginosa*
X	Cream, mucoid	+	Rods	+	-	-	+	+	+	-	-	+	+	+	+	*Bacillus licheniformis*
Y	Cream, watery	+	Cocci	-	-	-	-	-	-	-	-	-	+	-	-	*Streptococcus faecalis*
Z	Cream	+	Cocci	+	-	-	-	+	-	+	-	+	-	-	-	*Staphylococcus hominis*

4. DISCUSSION

There is scanty information on the biodegradation of pyrazine and pyrazine-derivatives in tropical environment. The presence of low organic carbon in composite samples of both the wastewater and cow dung encouraged the switch over to pyrazine compounds in the environment by the degraders. The enriched bacterial consortium requires the supply of Carbon and Nitrogen as source of energy for optimal growth in the wastewater ecosystem (Rappert *et al*., 2006; Muller and Rappert, 2010). The availability of ample nutrients for microbial growth in both the effluent and cow dung samples suggested that microbial consortium acclimated to the xenobiotic substance for growth and biomass accumulation (Girija *et al*., 2002; Rappert *et al*.,2006).

5. CONCLUSION

Pyrazine a monocyclic heteroaromatic substance is biodegradable by bacterial pure cultures and mixed cultures which is evident by the GC profiles obtained at intervals during the biodegradation process. This is in agreement with the processes of sustainable development and its incorporation or generation in food processing plants at industry stipulated concentrations is safe.

REFERENCES

[1] Adebusoye, S. A., Ilori, M. O., Amund, O. O., Teniola, O.D. and Olatope, S. O. (2007). Microbial degradation of Petroleum hydrocarbons in a polluted tropical stream.*World Journal of Microbiology & Biotechnology* 23: 1149-1159.
[2] Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments aroma models and omission. *Chem. Senses* 26:255-545.
[3] Maarse, H. 1991. Volatile compounds in food and beverages. Marcel Dekker, Inc., New York, N.Y. . Pp.
[4] Muller, R., and Rappert, S. (2010). Pyrazines: Occurrrence, formation and biodegradation. *Applied Microbiology & Biotechnology* 85(5): 1315-1320.
[5] Ranau, R., and H. Steinhart. (2004). Bewertung und Quantifizierung von Leitsubstanzen aus der geruchstragenden Abluft von Lebensmittelbetrieben und der Ferkelaufzucht. Erfassung und Minimierung von Gerüchen,. *In B. Niemeyer, A. Robers, and P. Thiesen (ed.), Messung und Minimierung von Gerüchen*. Hamburger Berichte 23, Verlag Abfall aktuell, Stuttgart, Germany. Pp. 147-165.
[6] Rappert, S., and R. Müller. (2005). Odor compounds in waste gas emissions from agricultural operations and food industries. *Waste Management* 25:887-907.
[7] Rappert, S.,Botsch, K.C.,Nagorny, W.F. and Muller,R. (2006). Degradation of 2,3-Diethyl-5-methylpyrazine by a newly discovered bacterium Mycobacterium sp. strain DM-11. *Applied & Environmental Microbiology* 72(2): 1437-1444.