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SOME QUESTIONS ABOUT USING SUGARCANE RUM DISTILLERY EFFLUENT TO FERTILIZE LEGUMINOUS CROPS

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ABSTRACT. Large volumes of sugarcane molasses distillery effluent are produced by refineries in the Caribbean region throughout the year. This has to be disposed and may have negative effects on those ecosystems on which it impacts. An alternative to disposal is to use the raw or digested effluent as a crop fertilizer. Preliminary experiments showed that *Phaseolus vulgaris* plants fertilized with raw digested, neutral digested and undigested effluent, inoculated with *Rhizobium* sp. NGR 234, had few root nodules. *Rhizobium* sp. NGR 234, is a species known to effect nodulation in 35 different genera of legumes. The effects of digested and raw effluent on the growth and production of this *Rhizobium* species were examined. The growth of the bacterium was negatively affected as the effluent concentration increased. Estimated LD_{50} values were higher for undigested effluent. The results are discussed in the context of using sugarcane distillery effluent as a crop fertilizer.

INTRODUCTION

Sugarcane molasses distillery effluent is produced in large volumes by refineries in Barbados and other territories in the region throughout the year. Investigations on the use of sugarcane rum distillery effluent as a crop fertilizer were carried out as early as 1918 (Sheehan and Greenfield, 1980) and have continued into the 1990's, e.g. Pandey and Soni (1994).

Rhizobia in the soil form mutualistic relationships with the host plant to which they are chemotactically attracted and there is mutual recognition by the symbiotic partners (Currier and Stroebel, 1976, in Verma and Delauney, 1988). The relationship is usually very specific and a particular *Rhizobium* species will effect nodulation in only one specific host plant. However, there are some species termed broad spectrum rhizobia, which can nodulate several species of host plants, e.g. *Rhizobium* sp. NGR 234, used in our experiments can nodulate 35 different genera of legumes (Hirsch, 1992). Some researchers have found that nodule production is repressed when adequate amounts of nitrogen compounds are available to the plant, even though the rhizobium and its host plant were in intimate contact, and only occurred when the soil was nitrogen deficient (Hirsch, 1992). In an experiment to investigate the use of effluent to fertilize bean plants, it was found that nodule production was repressed as effluent concentration increased (Rameshwar, 1996), perhaps due to the fact that nitrogen availability increased with increasing effluent concentration. To determine whether the effects of rum refinery effluent on the growth and development of rhizobia were similar to those observed with nodule production, the effects of raw digested, neutral digested (Hiatt *et al.*, 1973) and undigested effluent on the growth and production of *Rhizobium* were investigated.

METHODS

A culture of *Rhizobium* sp. NGR 234 was prepared by adding one loopful of a culture, growing on agar, to 100 ml of yeast mannitol broth (YMB) in a conical flask, stoppered with a plug of sterile cotton wool, and covered with aluminium foil. This flask was agitated in a mechanical shaker for 48 hr at room temperature.

Determination of the optimal concentration of rhizobia:

Dilutions of the parent culture were made by addition of sterile distilled water to achieve concentrations from 10^{-1} to 10^{-10}. Portions were spread on plates, previously prepared by mixing YMB at the rate of 100 µl per agar plate. The dishes were covered, sealed with parafilm and incubated at room temperature for one week. They were observed at 15 hr intervals. It was observed that with dilutions lower than 10^{-6} the number of colonies was small while with concentrations greater than 10^{-5} confluent growth occurred.

Effects of effluent on rhizobial growth:

The effluent was collected from West Indies Rum Distilleries, Brandons, St.Michael - one of three rum distilleries operating in Barbados.

100 µl portions of a 10^{-6} dilution of rhizobia were spread on five replicate plates, previously prepared by mixing YMB and agar with specific volumes of autoclaved effluent to obtain 1.25, 2.5, 5, 10 and 20% concentrations of raw, two week raw digested or two week neutral digested effluent (Rameshwar, 1996). The above procedure was repeated with a 10^{-5} dilution. Controls were prepared by spreading 100 :l of the rhizobia cultures on plates containing only YMB and agar. The dishes were covered, sealed with parafilm and incubated at 28.5°C for 45 hr. The number of colonies was determined at 15 hr intervals by dividing each dish into four equal sections and counting the number of colonies in one section and multiplying this by four.

Estimating LD_{50} values:

Probit analysis was conducted according to the method of Finney (1952) using a sub-program of MSTAT-C.

RESULTS

At the lower dilution of the rhizobia culture (10^{-6}), where colonies were apparent, it was observed that the number of colonies with the raw digested effluent, was generally higher (Fig. 1H and K) than the number of colonies for the neutral digested effluent (Fig. 1I and L).

It was also found that while with 5% effluent no colony was apparent with the neutral digested effluent after 30 hr (Fig. 1I) and likewise in some of the five replicates of the raw digested effluent (Fig. 1H). Similar results were obtained with the 10^{-5} dilution (Fig. 1B and C). There was no growth of *Rhizobium* with 10 or 20% effluent.

With 5% undigested effluent at the 10^{-6} dilution, an average of approximately 550 colonies was found after 30 hr (Fig. 1G), and confluent growth was observed after 45 hr (Fig. 1J).

Further it was observed that with increasing effluent concentration from 1.25 to 5% in the two digested effluents, the number of colonies dropped. For the same concentration range, the number of colonies with the undigested effluent also decreased as concentration was increased from 1.25 to 5%, though with the 2.5 and 5% the average number of colonies after 30 hrs was close (537 and 554 respectively).

The trends were still present after 45 hr for both dilutions (Fig. 1D-F and J-L). The LD_{50} values obtained are presented in Table 1.

Table 1. Estimated LD_{50} values (%) for digested and raw rum refinery effluent after 30 hours.

Intial Concentration of Rhizobia	Undigested	Raw digested	Neutral digested
10^{-5}	1.78	0.83	0.95
10^{-6}	2.18	1.79	0.96

DISCUSSION

The differences observed with the digested and undigested effluents may be attributed to more simple nitrogen compounds being released in the digested effluent therefore making the concentration of available nitrogen compounds greater than that in the undigested effluent where it is bonded and thus less readily available.

As mentioned earlier, at high concentrations of nitrogen, the activity of rhizobia is low, while when the soil is deficient in plant available nitrogen, the activity of rhizobia is high. Though Hirsch (1992) discussed this in relation to the availability of nitrogen compounds to the host plant, the growth of *Rhizobium* sp. NGR 234 in relation to nitrogen availability appears to follow the same trend as observed when the host plant is present. Thus it was observed that with increasing effluent concentration from 1.25 to 5% in the two digested effluents, the number of colonies dropped with increasing concentration (Fig. 1). For the same concentration range, the number of colonies with the undigested effluent decreased as concentration was increased from 1.25 to 5% (Fig. 1A and D), but not as rapidly.

That high concentrations of effluent negatively affect the bacterial growth is significant if rum refinery effluent is to be used to fertilize leguminous crops. Also, the consequence of first digesting the effluent to obtain biogas is to increase the negative effects on this species of *Rhizobium*. Boyce (1988), however, found that biogas digested effluent had a positive effect on the growth of corn (*Zea mays* L.).

Experiments need to be conducted to determine precisely the concentration of digested and undigested effluent below which negative effects on rhizobial growth are negligible and at which there are positive fertilizer effects on crop growth. The LD_{50} values (Table 1) suggest

that concentrations of one or two percent can be detrimental. This however does not take into the dilution effects of the soil to which the effluent is applied or the quantity of effluent that would be applied.

Besides the effect of increasing nitrogen availability the reduced growth observed with increasing effluent concentration could possibly be due to the toxic effects of either the inorganic constituents of the effluent (such as metal ions and chloride ions), or the organic components (such as carboxylic acids). In the case of the latter the acid concentration will be directly dependent on the effluent concentration, being high when the latter is high, and low when the latter is low. With greater acidity the overall pH of the growth media will be lowered, thereby possibly adversely affecting the growth of rhizobia (Glenn and Dilworth, 1994). Suitably designed experiments should be carried out to evaluate the significance of the contributions to toxicity to rhizobial growth and development of parameters such as pH, acidity, metal ion concentration and chloride concentration all of which have the potential to affect the growth and development of rhizobia.

The economics of preparing, and applying, diluted rum refinery effluent for use as an alternative fertilizer must be determined. But, this should take into consideration the advantages of this method of disposal over those now employed.

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REFERENCES

Boyce, I.A. 1988. The production of biogas and its environmental consequences. C.E.R.M.E.S., University of the West Indies, Barbados (Unpubl.).

Finney, D.J. 1952. Probit Analysis. 2nd Edn. Cambridge University Press, UK.

Glenn, A.R. and Dilworth, M.J. 1994. The life of root nodule bacteria in the acidic underground. FEMS Microbio. Lett 123:1-10.

Hiatt, W.C., Carr, A.D. and Andrews, J.F. 1973. Anaerobic digestion of rum distillery wastes.

Eng. Bull., Purdue Univ. Eng. Exten. Ser. 142:916-976.

Hirsch, A.M. 1992. Developmental biology of legume nodulation. New Phytol. 122:211-237.

Pandey, D.K. and Soni, P. 1994. Distillery effluent - a potential resource for irrigating forest seed beds. Ambio 23:267-268.

Rameshwar, G. 1996. Effects of rum refinery effluent on seed germination and plant growth. Unpubl. M.Sc. Thesis, University of the West Indies, Barbados.

Sheehan, G.J. and Greenfield, P.F. 1980. Utilisation, treatment and disposal of distillery wastewater. Water Res. 14:257-277.

Verma, D.P.S. and Delauney, A.J. 1988. Root nodule symbiosis: nodulins and nodulin genes. Pp. 169-199 in *Plant Gene Research* (Verma, D.P.S. and Golberg, R.P., eds.). New York: Springer Verlag.

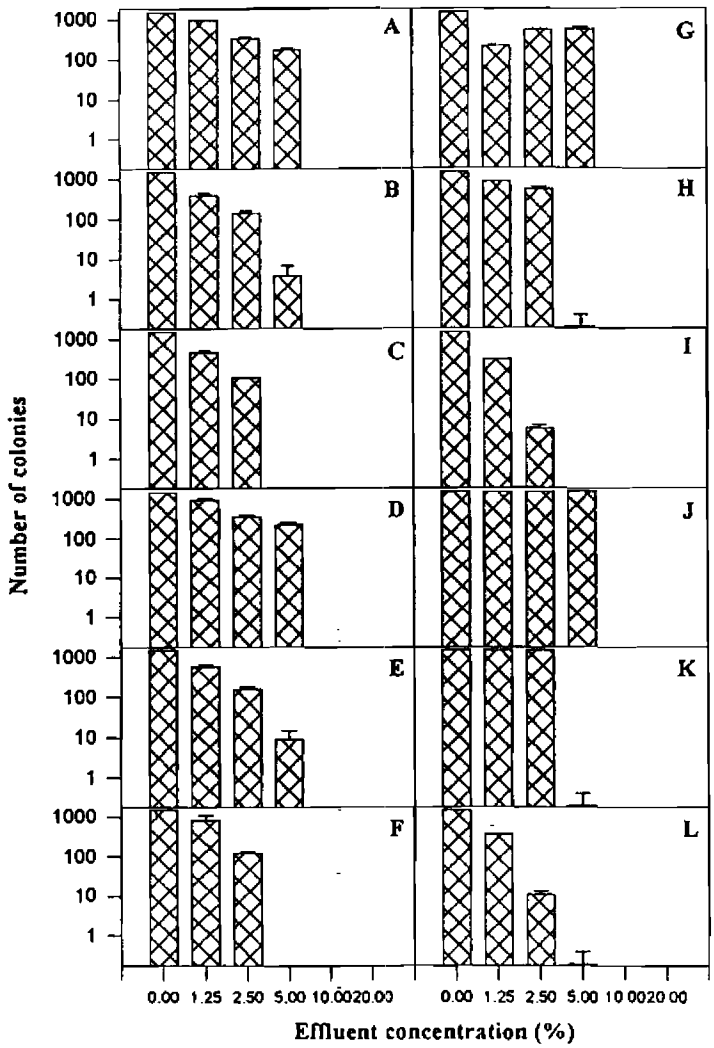

Fig. 1. Effects of raw (A, D, G, J), raw digested (B, E, H, K) and neutral digested (C, F, I, L) effluent on the growth of *Rhizobia*. (A-F 10^{-5} and G-L 10^{-6} dilutions of *Rhizobia*; A-C and G-I after 30hr and D-F and J-L after 45hr) (Data are means and stadard errors).