#### BIOPRODUCTION OF MODIFIED ATMOSPHERES FOR INSECT CONTROL IN SMALL GRAIN BINS

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#### ABSTRACT

Waste plant materials were used to create modified atmospheres (MA's) in grain-containing bins. The substrates used were wheat bran (WB) at 35% moisture content (MC) and sugar cane (SC) at 75% MC. The MA's were produced in specially designed plastic containers (biogenerators). In the case of WB, biogenerators containing 500 or 1500 g were connected to bins containing 25 kg wheat of 11.6% MC, equipped with either narrow or wide connecting pipes (0.5 and 5.3 cm. diam. respectively). The effect of artificial inoculation of WB on MA production was also experimented. In the case of SC, amounts of 5, 10, and 15 kg were tested in biogenerators connected to metal drums containing 150 kg maize of 13.5% MC.

In storage bins connected to WB biogenerators containing either amount of substrate the  $O_2$  was lower when the wider connecting pipes were used. In all cases 100% control of the test insect (<u>Tribolium castaneum</u> adults) was obtained. The MA's produced by the artificially inoculated WB did not differ from these generated by the non inoculated ones. In the case of SC, the highest  $CO_2$  and lowest  $O_2$  levels were obtained by using 15 kg of the substrate.

#### INTRODUCTION

During the last decade, the search for nonchemical, effective methods for the prevention of losses in stored grain and seeds has been given major priority. Among the various techniques tested, the use of modified atmospheres (MA's) to create a deleterious or lethal environment for the insects found in the stored grain gave the most promising results, some of which are already in use commercially (1,3). Applying the same principle in a simple system, we made use of composting plant waste materials as substrata for biogenerating atmospheres of high  $CO_2$  and low  $O_2$  which could be transferred to a well sealed grain bin. This system was tested on a laboratory scale, with encouraging results (2). Further work was carried out in order to test the effect of additional parameters related to the generating gas mixture, more effective for insect control, and its transfer into the grain bin. The MA's obtained were tested also as to their efficacy in bringing about mortality of the stored grain insects.

# MATERIALS AND METHODS

# 1. Modified atmospheres generated in different types of biogenerators

Plastic containers of 2.0 and 10.0 l, containing 500 and 1500 g of substrate respectively, were used. The biogenerators, of a previously described design (2) but equipped with a connecting tube of 5.3 cm diam, were compared with the ones used formerly, having a connecting tube of 0.5 cm. The MA produced in the biogenerators was transferred through the connecting tube into plastic bins containing 25 kg of wheat (11.6% moisture content). The substrate used was wheat bran (WB) of 35% MC. The  $CO_2$  and  $O_2$  contents were measured during 10 days of incubation. These experiments were conducted in a control temperature room at  $28 \pm 1^{\circ}$ C. <u>Tribolium castaneum</u> adults were introduced into three cages (containing 50 insects each) to three depths in each wheat bin, and mortality was recorded at the end of the experiment. Each trial was repeated three times.

# 2. Effect of artificial inoculation of the substrate

The substrate used (WB) was artificially inoculated by using spore suspension of fungi isolated from moulded wheat bran. The dominant fungal species in the inoculum were: <u>Aspergillus flavus</u>, <u>Aspergillus</u> <u>niger</u> van Tiegem, <u>Hyphopichia burtonii</u> Boidin et al., and <u>Penicillium</u> <u>griseofulvum</u> Dierckx. The composting was done in the described biogenerators, using 500 g WB. The experiment included the following treatments:

- a. WB of 35% MC, sterilized by irradiating it with 1200 krad from a gamma source and artificially inoculated.
- b. WB of 35% MC, non sterilized by irradiation, but artificially inoculated.
- c. WB as in a, sterilized but without artificial inoculation.
- d. WB as in b, non sterilized but without artificial inoculation (= control).

Each treatment was repeated three times.

# 3.<u>MA production by composting sugar cane</u>

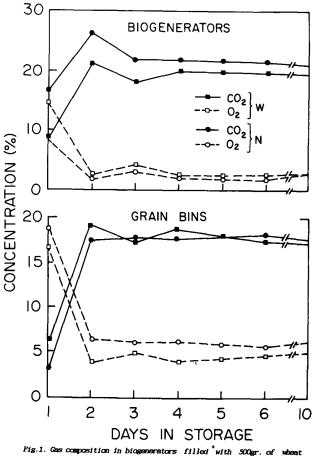
Plastic containers of 35 l were used as biogenerators in this study. The amounts of substrate (sugar cane, SC, of 75% MC) tested were 5, 10 and 15 kg. Each biogenerator was connected to a 200 l metal drum containing 150 kg maize of 13.5% MC. The mortality of insects naturally infesting the maize connected to biogenerators composting 10 kg. SC, was recorded after 10 days.

The experiment was conducted on the farm of the University of Costa Rica, at ambient temperature of 25±3°C.

RESULTS

1.<u>Effect of production and transfer of MA to the grain bins by</u> modifying the biogenerators

The following can be deducted from the results presented in Figurs 1 and 2. No marked difference in the production of  $CO_2$  was recorded in the various biogenerators or in the storage bins except for the first 2 days of incubation, when higher values were obtained in the large biogenerators, containing 1500 g WB.



bran and in grain bins connected to them either by a narrow (N) or a wide (W) pipe. However, the  $O_2$  contents recorded were markedly lower in the grain bins connected to both size biogenerators, when the latter were equipped with the wider connecting tube.

The mortality tests showed that in the bins connected to any of the above mentioned biogenerators, 100% control of the test insects was obtained.

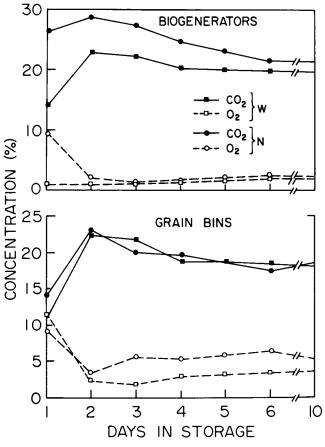


Fig.2. Gas composition in biogenerators filled with 1500gr. of wheat bran and in grain bins connected to them either by a narrow (N) or a wide (W) pipe.

# 2. Effect of artificial inoculation of the substrate.

The results presented in figure 3, indicate the following:

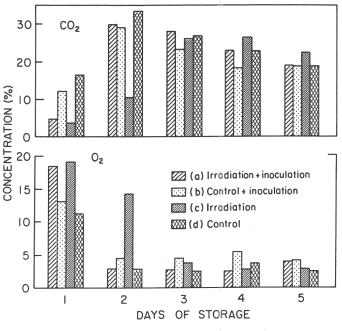


Fig.3. Effect of artificial inoculation of wheat bran on  $OO_2$ production and  $O_2$  consumption.

Differences in the  $CO_2$  and  $O_2$  contents were recorded only on the first day of incubation between the irradiated and the nonirradiated WB (a,c <u>vs</u> b,d). Following 3 days of incubation, the  $CO_2$  and  $O_2$  contents in all biogenarators were very similar.

# 3. <u>MA produced by composting sugar cane</u>

The CO<sub>2</sub> concentration increases with augmented amounts of substrate (Fig. 4). However, the differences in CO<sub>2</sub> content were not proportional to the amount of substrate. Eeven with the lowest quantity of SC <u>i.e.</u>, 5 kg, the O<sub>2</sub> concentration dropped to approx. 5%. No live insects were found in the maize samples taken from drums connected to biogenerators, after 22 and 36 days of storage.

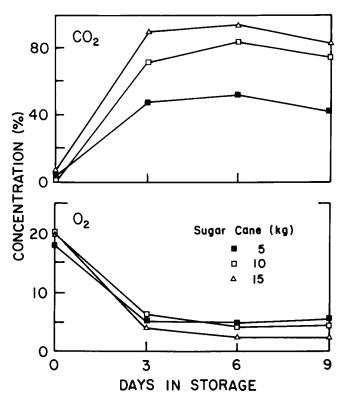


Fig.4. Effect of quantity (kg) of sugar cane on gas composition in 200 l. drums filled with 150 kg of maize.

# CONCLUSIONS

The use of the modified biogenerator (equipped with a wider connecting tube) resulted in obtaining of a more favorable gas mixture (for insect control) in the grain bin. This was expressed by the lower  $O_2$  concentrations, which persisted throughout the entire composting period.

The addition of inoculum (comprising mold species predominant in the substrate) did not result, under the given laboratory conditions, in the production of a better MA's (higher  $CO_2$  and lower  $O_2$  gas mixture) in the biogenerators. Further aspects of this subject, <u>e.g.</u> different inoculum levels, inoculation with single strains, etc., should be investigated. The use of plant waste material, commonly found in the tropics (like SC), seems to be very effective in creating MA's lethal to storage insects. The model of a biogenerator with waste plant material - storage bin, as a system for preventing insect damage in small grain bulks, can be easily adopted and further developed on the subsistence farmer level.

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# LA BIOPRODUCTION D'ATMOSPHERES MODIFIEES POUR LA DESINSECTISATION DE PETITES RESERVES A GRAIN

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#### RESUME

Des substrats végétaux humides, naturellement contaminés par la microflore, ont été utilisés pour créer des atmosphères modifiées (AM) pour les petites cellules de stockage de grain. Les substrats et leur degré d'humidité (TE) étaient : des gousses d'arachides (GA), 30 %, du son , 35 %, des pelures d'orange (PO), 80 %, de la canne à sucre (CS), 65 % et de la pulpe de banane (PB), 75 %. Les AM ont été créées dans des conteneurs en plastique spéciaux reliés, soit aux cellules de céréales contenant 25 kg de blé à 11,67 % TE pour GA, son et PO, soit aux silos contenant 200 kg de maïs à 13,5 % TE pour CS et PB. La composition de l'atmosphère (%  $CO_2/O_2$ ) mesurée dans les récipients à blé reliés aux GA, son ou PO étaient de 15/5,5, 16/6,5 et 19/6, respectivement. Ces niveaux se sont maintenus pendant 10 jours à 25° C. A ces AM, la mortalité des adultes de *Tribolium castaneum* s'est élevée à 80 - 100 %. Pour CS et PB, les AM étaient (à 30° C) (dans %  $CO_2/O_2$ ), 75/3,5 et 88/1,5, respectivement. L'utilisation de la canne à sucre comme substrat, dans les essais au niveau fermier (avec 200 kg de maïs), ont donné une mortalité de 100 % pour *Sitophilus zeamais*, après 10 jours de stockage.

Le transfert des gaz aux récipients à grains n'a pas augmenté la teneur en eau et ne leur a pas communiqué d'odeurs étrangères. On peut donc conclure que les AM créées pendant la formation du compost des différents restes végétaux mentionnés plus haut peuvent être utilisées en tant que méthodes alternatives de désinsectisation des stocks de grains.