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FINAL REPORT - CDR

Covering Period: July 30, 1987 - March 31, 1991.

Submitted to the Office of the Science Advisor, U.S. Agency for International Development.

Title of Project: Bioproduction of Modified Atmospheres in Small Grain Storage Containers for Insect Control.

Principal Investigators: Prof. M. Calderon and Dr. N. Paster, in collaboration with Mrs. M. Menasherov.

Grantee Institution: Department of Stored Products, ARO, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel.
Fax: 972-3-9683659

Collaborator: Dr. Miguel Mora, CIGRAS, University of Costa Rica, San Jose, Costa Rica.
Fax: 506533762.

Project Number: C7-052
Grant Number: DPE-5544-G-SS-7024-00
A.I.D. Grant Project Officer: Gary V. Kinney
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3. EXECUTIVE SUMMARY

The main objective of the present project was to bioproduce high CO_2/low O_2 atmospheres for insect control in the rural storage of grain. After a series of trials using different types of substrates (waste or low value agricultural materials) and various designs of biogenerators (containers in which the biodegradation of the substrate took place), we were able to establish an operational system of "biogenerator-substrate-grain bin". The modified atmospheres (MAs) are generated by the microorganismal degradation of the substrate (wheat bran, peanut shells, sugar cane, etc.) in the biogenerator, which is placed above a grain bin (containing wheat or corn). The gas mixtures obtained are easily transferred into the bin, causing up to 100% mortality of the insects present in the stored grain. Data on the field studies, carried out at the last stages of the project in Israel and in different regions of Costa Rica (in bins commonly used by the farmer), demonstrated clearly the effectiveness of the system in achieving insect control in the bins. The project objectives were fully accomplished, and a low-cost, safe and immediately applicable system was developed. The system can be easily adopted by farmers in LDC countries, thus contributing to the prevention of storage losses in subsistence farming management.

However, more work is needed for further development and optimization of the system's elements: the biogenerator, the selection of the substrate, and the ease of construction of properly sealed grain bins, for the successful and efficient application of the method in the various LDC countries.

4. RESEARCH OBJECTIVES

Stored grains are subjected all over the world to heavy losses caused by insects. Therefore, the search for new and effective methods for insect control continues unceasingly. These

efforts attract the utmost interest, particularly in developing countries, because of the considerable losses in quantity and quality of the stored grain due to the warm, humid climate prevailing in the regions of the LDCs. The application of chemicals for insect control is still widespread. However, the application of insecticides (in solid, liquid or gaseous form) does not solve the problem but rather creates new ones, e.g. insect resistance and toxic residues.

Hence, the main objective of our project was to provide the farmer with a safe and easy-to-handle technique for prevention of grain losses at the on-farm storage level. The project offers the environmental approach, according to which are created ambient conditions noxious for the development of the storage insects present in the "grain bulk ecosystem" (Calderon, 1981). This can be done by changing the atmospheric gas composition in the intergranular air. Thus, gas mixtures of high CO_2 and low O_2, lethal to insects, could be produced and insect control achieved (Bailey and Banks, 1975; Banks and Annis, 1977; Annis, 1987).

Our proposal was to simplify the MAs method, while using modern and efficient techniques in utilizing the biological processes for creating atmospheres lethal to insects. In fact, we employed the process of composting, which is defined as "the controlled aerobic biodegradation of a mass of organic material accompanied by temperature increase as a sequence of self heating and insulation by exterior layers" (Levanon et al., 1986). We put into use a closed composting system, which produced the needed gas mixture of high CO_2 and low O_2 to be transferred into the grain bin.

The use of biological sources to obtain a high CO_2 and low O_2 atmosphere to be introduced into a grain bin is a relatively new idea (Calderon and Pushinsky, 1983), and employing the composting process for the setup of a "biogenerator-substrate - grain bin" system, is an innovative concept which was developed and tested in this project. The further development and application of this system offers the subsistence farmer in the

LDC a new, nonchemical, safe and inexpensive alternative technique for the protection of his stored grain.

5. METHODS AND RESULTS

In this section the results of the field studies which comprise the main goal of the project are presented.

A. Studies with Bins Holding 200 kg of Wheat

Materials and Methods

Experimental design

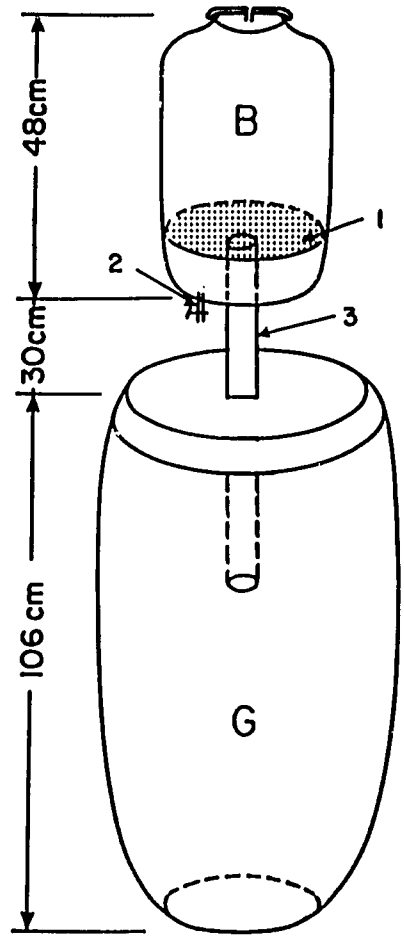
Plastic containers of 25-l capacity were used as biogenerators of the type which was described previously (Paster et al., 1990). The biogenerators were equipped with a 5.3-cm-diam connecting tube and the substrate used was wheat bran (5 kg in each biogenerator) at 35% moisture content (MC). The MA produced in the biogenerators was transferred through the connecting tube into plastic bins containing 200 kg wheat at 12.8% MC (Fig. 1). Three cages each with 50 adults of *Tribolium castaneum*, aged 7 to 10 days, were introduced into three depths of the grain bins and the mortality of insects in the treated and untreated bins was recorded at the end of the experiment. The ambient conditions (temperature and humidity) were recorded on a thermohygrograph placed close to the storage bins. The experiment was carried out in three replicates.

Measuring the CO_2 and O_2 levels

CO_2 levels were measured using a Riken Infrared Gas Analyser, model RI-550A, and O_2 content was measured by a portable paramagnetic O_2 detector (Herman – Moritz Oxymeter, type HM 18).

Air samples were withdrawn from the bins through the sampling tubes, which had been installed previouly at three different depths in the grain bins. As the two instruments were connected, the same air sample was analyzed by passing it through

Fig. 1. The biogenerator (B)- grain bin (G) system.
1) Wire mesh. 2) Tube for removing excess water.
3) Connecting tube.



the two meters. The air composition in the biogenerator was analzsed in the same manner.

Results

Following 24 h of composting, the microflora activity in the wheat bran in the biogenerator produced gas composition of 25.3% CO_2 and 1.5% O_2, while the MA transferred into the grain bin was 18.8%/4.7% (CO_2/O_2) (Fig. 2). These gas levels persisted in the grain bin for an additional 2 days, after which the CO_2 content in the bins decreased gradually, concomitant with a slow increase in the O_2 level. After 9 days of the treatment, the CO_2/O_2 gas levels in the biogenerator were 19.7%/2.7%, while those in the grain bin were 13.8%/8.3%. A normal air composition was recorded in the untreated bin during the trial period.

According to calculated levels of exposure times and gas concentrations causing mortality to T. castaneum (Calderon and Navarro, 1979), a ratio of approximately 22% CO_2: 5% O_2 is required in order to cause 95% mortality after 5 days of exposure in that species. The CO_2/O_2 combinations obtained in our study were very close to that ratio and, combined with the longer exposure time (9 days), may well explain the 100% mortality obtained in the test insects. The mortality level recorded in the control bins was 4%. The range of ambient temperatures during the trial was 26 - 41°C (Fig. 3) and the wheat MC recorded at the end of the trial was 12.8%. No objectionable odor was detected in the treated grain. The data presented in this study as well as in our previous work (Paster et al., 1990), indicate the usefulness of the above described system of biogenerating MAs lethal to stored product insects by the microbial degradation of plant waste materials.

Fig. 2. Gas composition in the biogenerators and the grain bins.

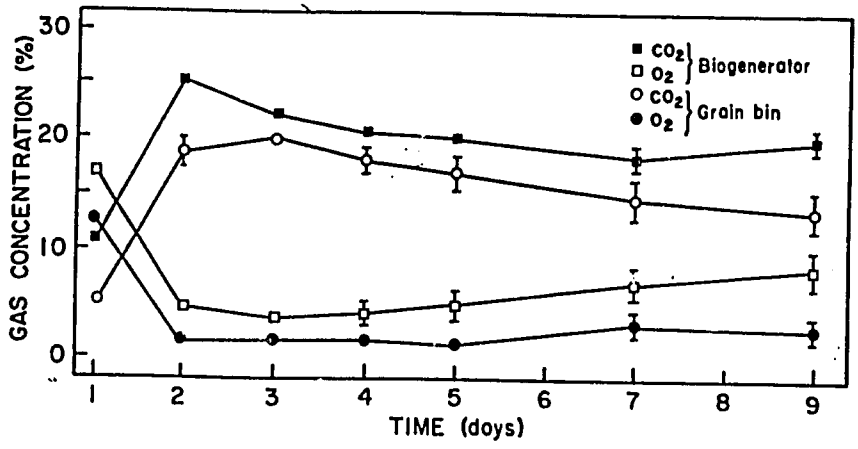
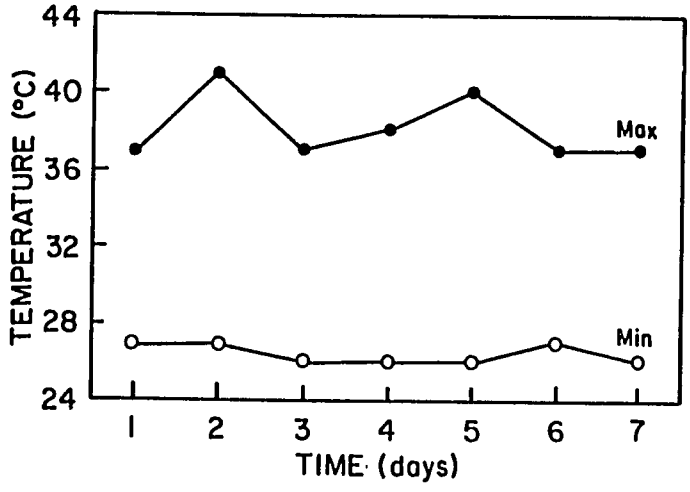


Fig. 3. Ambient air temperatures fluctuations during the study.



B. Trials on Insect Control at the Farm Level (Costa Rica) Reported by Dr. M. Mora:

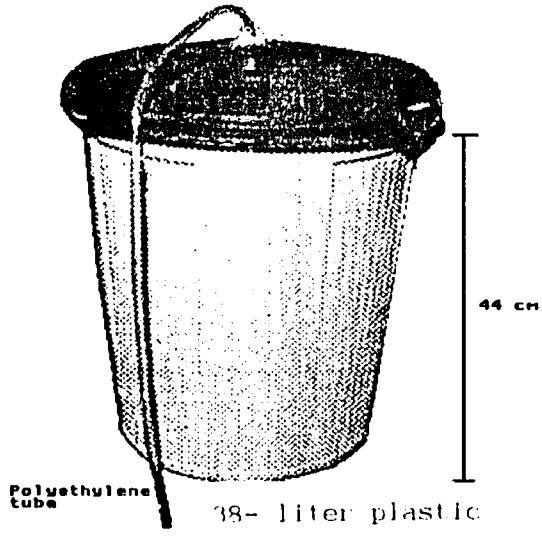
Materials and Methods

The field trials included tests at the following sites: in a CNP installation near the University of Costa Rica; on a farm in Rio Frio; and at two locations in each of the regions of Brunca (farms in Brisas and Volcan) and Huetar (farms in Guacimo and Jimenes). Two types of substrates (at 10 kg each) were used: sugar cane - cut into small pieces and covered with water, and mashed banana mixed with 10 l of water. Yeast was added at a concentration of 0.5% of the substrate's weight.

Biogenerators and grain bins

A plastic container of 38 l volume served as the biogenerator in the studies (Fig.4). A polyethylene tube was

Fig. 4. The type of biogenerator used in Costa Rica.



38- liter plastic fermentation container.

connected to a hole in the lid of the biogenerator, and through this tube the MAs flowed into the grain bins.

Metal drums (200 l) were used in the studies. In addition, we used on the UCR farm also silos of 330 l containing 200 kg of corn.

Gas analyses

Gas concentrations were measured using a Riken Infrared Gas Analyser model RI 550 A for CO_2, and a Fyritte II Analyser (Bachrach) for O_2 readings.

Insect mortality

In one study, small cloth bags with 20 g of corn from insect cultures and 50 adults of Sitophilus zeamais were placed at the bottom and the center of the drums and the silos were filled with corn.

Results

1. Trials in the CNP instalation

The CO_2 concentrations in the drums and the silos are shown in Fig. 5.

Gas generation was very rapid and CO_2 concentration increased to 50% of more within one day. The concentration continued to increase, reaching its maximum in 5 days. From that point the concentration began to decrease during the rest of the experiment, but even after 10 days the CO_2 concentration was above 72%. The highest CO_2 concentration obtained was 92% in drums and 84% in silos. CO_2 concentration was always below 4% in all control containers without a biogenerator attached. Complete mortality of the test insects in the drums and the silos was obtained (Table 1).

Fig. 5. CO_2 concentrations in drums and bins filled with maize and connected to biogenerators.

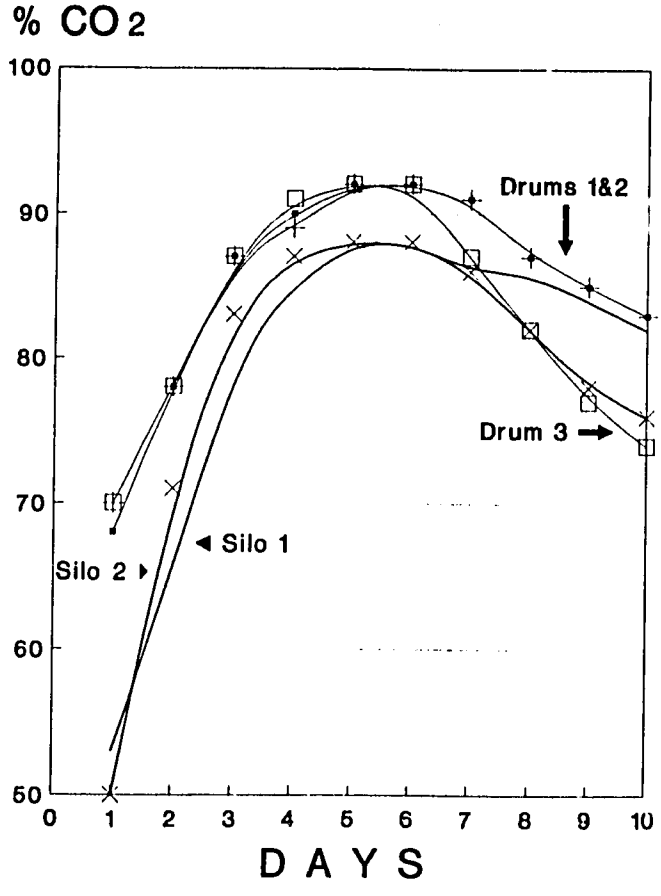


Table 1: Sitophilus zeamais survival in two parts of containers treated with gas from a biogenerator (means of three replicates).

Treatment	% Survival		Moisture
	center	bottom	content (%)
Drums			
With biogenerators	0.0	0.0	13.3
Control	7.0	8.0	13.6
Silos			
With biogenerators	0.0	0.0	14.05
Control	92.5	80.0	14.0

Trials in Rio Frio

Results from a test carried out on a farm in Rio Frio are presented in Table 2. Since the first readings of CO_2 and O_2 concentrations were made one week after the maize was placed in the drums, the initial CO_2 concentration without connecting biogenerators was fairly high (14-16%) and O_2 was below 5% due to natural grain insects' and microorganisms' activity. Concentrations of CO_2 were between 15% and 19% and of O_2 between 2.2% and 5.9% in the drums without biogenerator during the rest of the experiment. CO_2 concentration in the drums with the biogenerators was much higher, starting at 88% after the treatment and remaining at 40-49% even after 36 days.

It should be noted taht although insect mortality in the control bins was recorded after 22 days, a high level of damaged grains could be detected in those bins, since the insects infestation was continuing in these bins.

Table 2 Insect control in stored maize with CO_2 from biogenerators (Rio Frio).

TREATMENT	SAMPLING TIME	Rep. / LEVEL	% CO_2 1	% CO_2 2	% O_2 1	% O_2 2	% GERMINATION 1	% GERMINATION 2	Sitophilus LIVE 1	Sitophilus LIVE 1*	Sitophilus LIVE 2	Sitophilus LIVE 2*	Sitophilus DEAD 1	Sitophilus DEAD 1*	Sitophilus DEAD 2	Sitophilus DEAD 2*	Tribolium LIVE 1	Tribolium LIVE 1*	Tribolium LIVE 2	Tribolium LIVE 2*	Tribolium DEAD 1	Tribolium DEAD 1*	Tribolium DEAD 2	Tribolium DEAD 2*	Cryptolestes LIVE 1	Cryptolestes LIVE 1*	Cryptolestes LIVE 2	Cryptolestes LIVE 2*	Cryptolestes DEAD 1	Cryptolestes DEAD 1*	Cryptolestes DEAD 2	Cryptolestes DEAD 2*
DRUM WITH GENERATOR	INITIAL		16.0	14.0	4.0	3.0	11.0	11.0	2	25	1	20	2	1	4	2	0	0	0	0	2	0	4	0	0	0	0	0	2	0	2	0
	6 DAYS		88.0	88.0	0.9	0.9																										
	22 DAYS	SUP	72.0	74.0	1.2	1.6	9.0	9.0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		INF	60.0	60.0	4.5	5.2																										
	36 DAYS	SUP	46.0	49.0	5.8	6.1			0	—	0	—	11	—	4	—	0	—	0	—	0	—	1	—	0	—	0	—	0	—	0	—
		INF	40.0	40.0	7.8	9.4																										
DRUM WITHOUT GENERATOR	INITIAL		15.0	15.0	3.3	4.1	8.0	9.0	1	26	1	47	1	1	12	4	0	0	0	0	2	0	15	0	1	0	0	0	0	0	10	0
	6 DAYS		16.5	17.0	4.0	4.1																										
	22 DAYS	SUP	18.0	19.0	2.6	3.0	7.0	10.0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		INF	16.0	16.5	4.4	5.5																										
	36 DAYS	SUP	17.0	19.0	3.2	2.2			0	—	0	—	0	—	2	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—	0	—
		INF	15.0	16.0	5.7	5.9																										

* After 30 days sample incubation.

Trials in the Brunca and Hueter regions

Data on CO_2/O_2 concentrations are presented in Figure 6. Both kinds of substrate worked very well. After 6 days the biogenerators had produced enough CO_2 and initially elevated the concentration in the drums to approximately 80% CO_2, a level which persisted also after 4 weeks of storage. The O_2 level in the drums was at that time less than 4%. Although drums were tightly closed after the treatment with the biogenerator, CO_2 concentrations decreased during the 24 weeks of storage. However, except in one case, CO_2 concentration remained above 25% for at least 6 weeks. The data on insect mortality (Table 3) revealed 100% control of insects found naturally in the stored corn.

6. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The most significant achievement was conducting the field trials in both Israel and Costa Rica. The system of "biogenerator-substrate-grain bin" developed during the 3-year project was implemented on farms in Costa Rica in the Brunca and Hueter regions and encouraging results were obtained. There is a markedly increasing trend to use this method in other parts of Costa Rica. The successful termination of the project is the result of a fruitful and close cooperation between the collaborating countries. Technologies, especially of analyzing gas concentrations in grain bins, were brought to Costa Rica and the equipment purchased for the project remains there and will be used for further studies of grain storage.

Fig. 6. CO_2 and O_2 concentrations during the trial period in bins filled with corn (Field studies, Costa Rica).

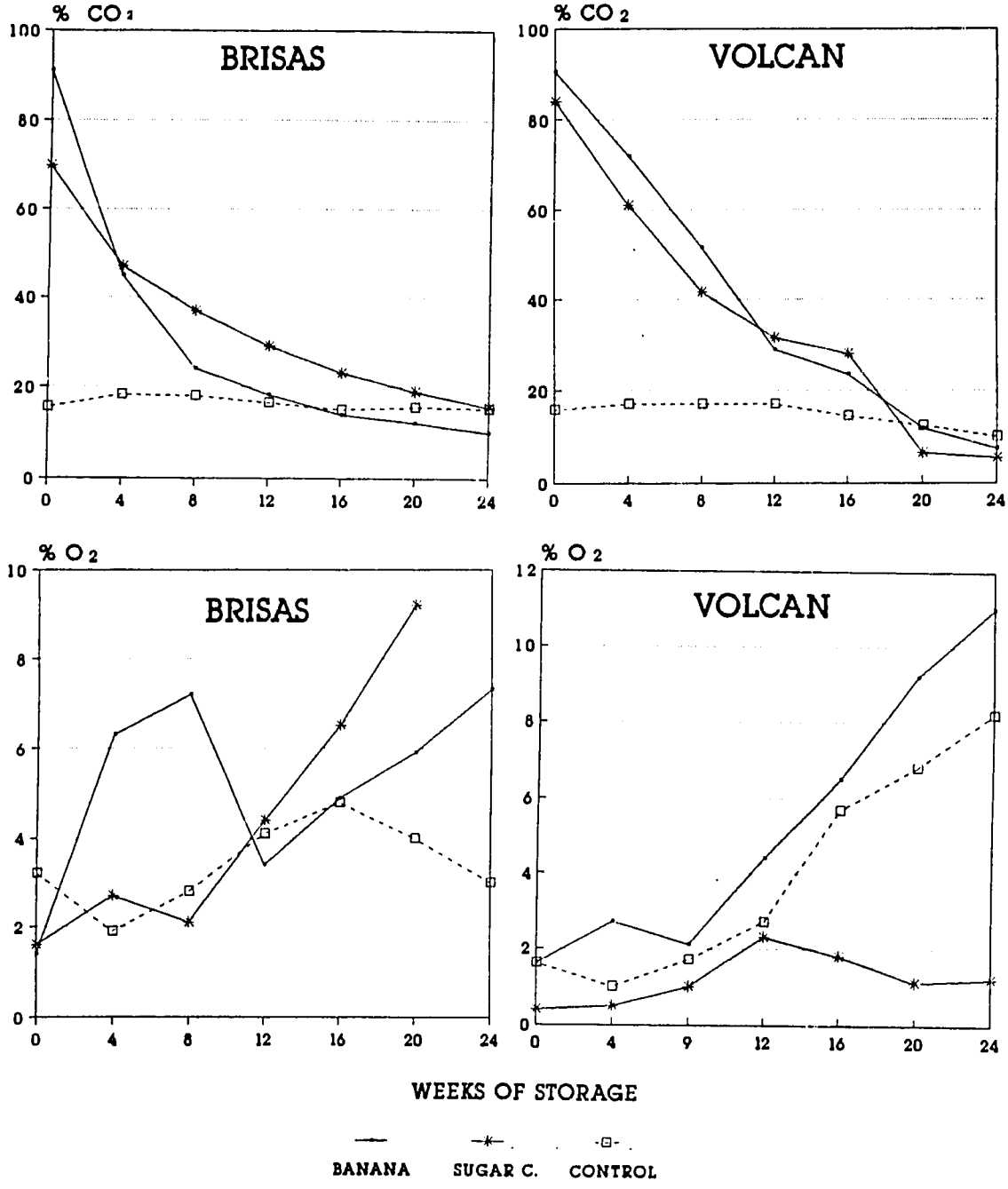


Table 3 - Gas concentrations and insect mortality (from natural infestation) following 4 weeks' storage (field trials).

Insect species				Place and type of substrate													
				Brisas			Volcan				Guacimo				Jimenes		
				A*	B	C	A	B	C		A	B	C		A	B	C
		Inf.*	CO_2	45	70	18	72	61	17	Inf.	63	60	20	Inf.	50	50	19
			O_2	6.3	2.5	3.2	2.7	2.0	1.0		1.0	1.6	0.8		0.3	0.5	0
Sitophilus	L[Y]	13		0	0	0	0	0	0	>100	0	0	0	65	0	0	0
	D	0		0	0	0	0	0	0	0	140	180	105	0	13	13	60
Tribolium	L	1		0	0	0	0	0	0	3	0	0	0	2	0	0	0
	D	0		0	0	0	0	0	0	0	16	14	6	0	2	0	2
Cryptolestes	L	3		0	0	0	0	0	0	6	0	0	0	3	0	0	0
	D	0		0	0	0	0	0	0	0	11	13	3	0	2	0	0

*A, banana + 0.5% yeast;
B, sugar cane + 0.5% yeast;
C, Control without biogenerators.
[Y]L = Live; D = Dead.
* Initial infestation.

7. PROJECT ACTIVITIES

A. Meetings

February 1988: Dr. N. Paster visited CIGRAS to plan the start of the project and prepare the general time table for progress of the work.

February 1990: Dr. M. Mora visited The Volcani Center to gain experience in the methodology of gas analysis and to become familiar with the model developed in Israel.

May 1990: Dr. Paster visited Costa Rica (the trip was not financed by the AID-CDR) and participated in the two field trials carried out in there and carried out discussions with Dr. Mora concerning the last phase of the project and further development for the implementation of the system after the end of the project.

May 1991: Dr. Mora came to Israel to summarize the project and to prepare the final report. Also, a manuscript was written jointly for publication in a refereed journal.

B. Publications

1. Paster, N., Calderon, M., Menasherov, M. and Mora, M. (1990). Biogeneration of modified atmospheres in small storage containers using plant waste. Crop Protection 9: 235-238.
2. Paster, N., Calderon, M., Menasherov, M. and Mora, M. (1990). Bioproduction of modified atmospheres for insect control in small grain bins. 5th Int. Working Conf. on Stored Product Protection. (Bordeaux, France).
3. Paster, N., Calderon, M., Menasherov, M. and Mora, M. Application of biogenerated modified atmospheres for insect control in small grain bins. Tropical Science. (Accepted for publication)

8. PROJECT PRODUCTIVITY

After 3 years, it can be stated that the project fully accomplished the goals proposed at the start of the laboratory studies (see section 5). We have learned the dynamics of MA production by several substrates and developed the appropriate model of biogenerators based on the principle of MA transfer to the grain bin. In small-scale studies we have learned the rate of the build up of MA in the grain bin and adapted the suitable substrates to the types of grains being protected (wheat and corn). Following the results of the second phase, field trials were conducted, in which farmers in Costa Rica were involved. In this terminating phase, the applicability of the system under field conditions has been proved, leading to the conclusion that the initial objectives of the project were, indeed, achieved.

9. FUTURE WORK

Based on the fruitful cooperation which developed between the teams of Costa Rica and Israel, it was decided by the two sides to plan more field trials and to locate systems in additional zones in Costa Rica. Efforts will be devoted to include more countries among the users of the system and to establish a network of farmers using the biogenerator grain bin model.

A brochure to promote dissemination of the method in developing countries will be prepared and will include a description of the system.

10. LITERATURE CITED

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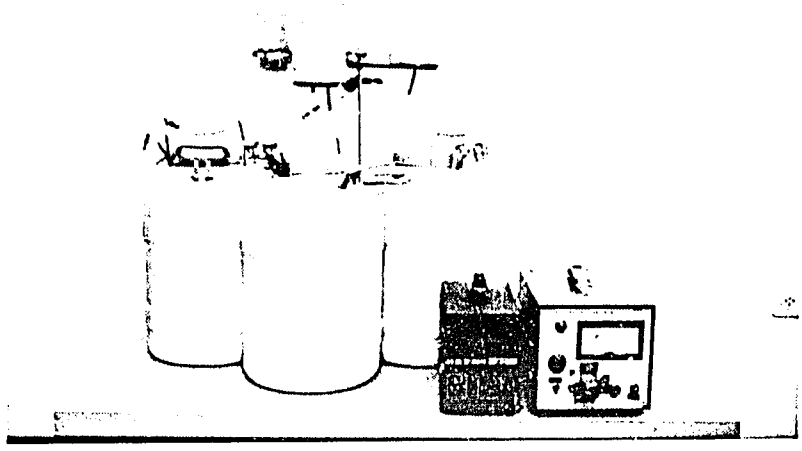
Paster, N., Calderon, M., Menasherov, M. and Mora, M. (1990) Biogeneration of modified atmospheres in small storage containers using plant wastes. Crop Protection 9: 235-238.

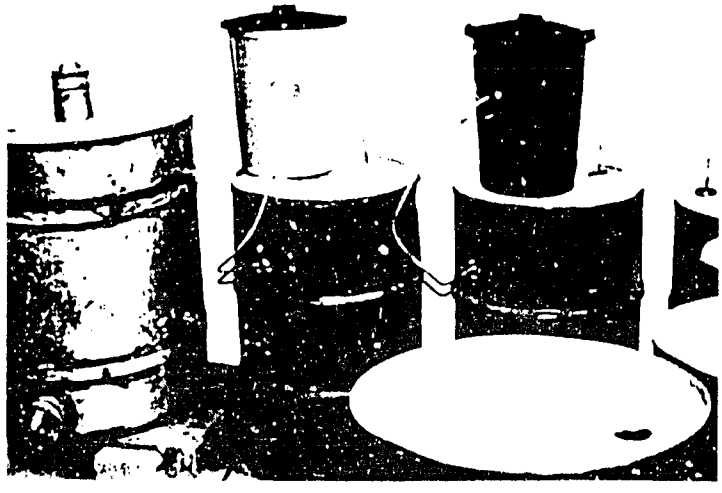
PHOTOGRAPHS

A general view of the "Biogenerator- Grain bin" model.



Field trials with wheat (Israel)

Field trials with maize (Costa Rica)



PUBLICATIONS

Biogeneration of modified atmospheres in small storage containers using plant wastes

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Department of Stored Products, ARO, The Volcani Center, POB 6, Bet Dagan 50-250, Israel and
**CIGRAS, University of Costa Rica, San Jose, Costa Rica*

Abstract. The production of modified atmospheres (MAs) by composting peanut shells (PS) and wheat bran (WB), was studied at moisture contents (MCs) of 25, 30, 35 and 40%. The MAs were produced in specially designed plastic cans (biogenerators). The transfer of the atmosphere obtained in biogenerators, by composting PS (30% MC), WB (35% MC) and orange peel (OP) at 80% MC, to bins containing 20 kg corn, was also investigated. WB wetted to 40% MC yielded the highest CO_2 levels (25%) following 48 h incubation. However, the same CO_2 levels were recorded after 4 additional days at all MCs tested. High CO_2 concentrations were produced in biogenerators containing PS at all MCs tested within 48 h, but the CO_2 content then decreased gradually. The amount of CO_2 transferred to the corn bins from biogenerators containing OP was significantly higher and the O_2 content of the bins significantly lower than for the other substrates after 4 and 7 days, whereas after 10 days the differences in CO_2 content were not significant. This study and its findings may serve as a model for further investigation in this field.

Keywords: Biogenerators; carbon dioxide; wheat; modified atmosphere

Introduction

Much effort has been devoted recently in developing countries to raise grain yields in order to meet the food requirements of the population. Success in increasing the production of grain in the field was recorded in many of these regions. However, preserving grain quantity and quality by prevention of losses in the postharvest phase is still the bottleneck in the food supply chain. There is an urgent need to develop and introduce new strategies and techniques for the protection of grain in storage. This is especially so in rural areas of the less-developed countries, where the farmers store only small amounts of grain for their subsistence. Insect pests are still the major problem at this storage level and the search for effective and less hazardous methods for control is continuing. Among these methods, the use of modified atmospheres (MAs) in the storage container has lately attracted considerable interest. It has long been known that decreasing the oxygen (O_2) content in the storage bin to below a certain level ($< 1.5\%$) may become lethal for most storage insect species (Bailey and Banks, 1975). More recently, it was proposed to raise the carbon dioxide (CO_2) concentration to 60% to achieve mortality of all grain pests in airtight containers (Banks and Annis, 1977). It was also reported (Calderon and Navarro, 1979) that a synergistic effect on insect mortality is exerted by exposure to a low-oxygen atmosphere when CO_2 is added. There is an indication that such gas mixtures can be obtained by composting, i.e. 'controlled aerobic biodegradation of a mass of organic materials...' (Levanon, Motro and Marchaim, 1986). The gas mixture produced could be transferred to a small grain bin (in use in less-developed countries) and maintained until the mortality of the insects in the stored grain is achieved. A preliminary study of this principle gave encouraging results (Calderon and Pushinsky, 1984).

The aim of this study was to evaluate quantitatively the CO_2 production and O_2 consumption by composting plant waste material (substrate), the amount of the generated gas mixture transferred to and maintained in the grain bin, and to determine by experiment the most suitable design of CO_2 biogenerator for this purpose.

Materials and methods

Wheat bran (WB) and peanut shells (PS) were used as the substrates in the first series of trials for producing CO_2 in the biogenerators. Lots of 300 g of each substrate were wetted to 25, 30, 35 and 40% MC and allowed to equilibrate for 14 days at 5°C before use. After these trials, experiments related to transfer of the gas mixture produced in the biogenerators to a grain bin containing 20 kg corn were carried out using WB of 35% MC, PS of 30% MC and an additional substrate, namely orange peel (OP), at 80% MC. For this purpose, lots of 300 g of each substrate were placed

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separately in plastic cans (which served as biogenerators) and then each biogenerator was connected with plastic tubing (5 mm inner diameter) to a separate grain bin.

Biogenerator design

After preliminary tests the following design of model biogenerator (*Figure 1*) was developed. In a plastic container (volume ≈ 2.0 litres, height 21 cm and inner diameter 12 cm) a false floor of wire mesh was installed, 5 cm above the bottom, to allow drainage of excess water through a tube fixed at the bottom. Another tube connected the biogenerator with the corn bin, to allow the transfer of the gas mixture produced to the grain bin. In addition, a hole 5 mm in diameter was made in the top to allow access of oxygen from the ambient air in the biogenerator, thus preventing anaerobic conditions.

Grain bin

Plastic bins holding 20 kg corn were used. Three plastic tubes (5 mm diameter) for gas sampling were inserted at different heights into the grain in each bin.

In order to avoid CO_2 accumulation due to insect activity, the grain was treated, before each trial, by fumigation with phosphine and aeration after treatment. The initial MC of the corn was 13.5%.

Measuring the CO_2 and O_2 levels

CO_2 levels were measured using the Riken Infrared Gas Analyzer, Model RI-550A, and O_2 content by a portable paramagnetic O_2 detector (Herman-Morits Oxymeter, type HM 18). Air samples were withdrawn from the bins through the air sampling tubes. As the two instruments were connected, the same air sample was analysed, first for CO_2 and then for O_2, by passing it through the two meters assisted by their built-in pumps. The air composition of the biogenerators was analysed in the same manner.

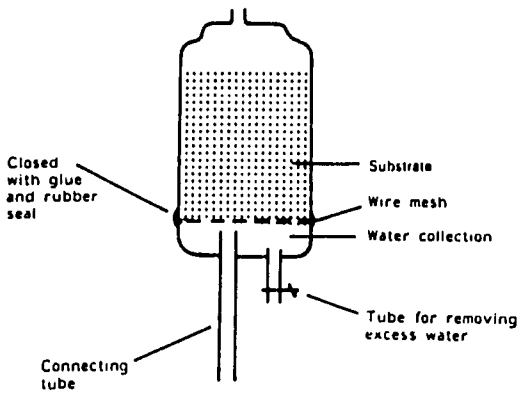

FIGURE 1. Schematic drawing of the biogenerators used in this study

The intergranular relative humidity (r.h.) was measured using a Hygrotest 6400 (Testoterm, W. Germany), equipped with a probe inserted into the centre of each bin. The MC of the corn was determined from the r.h. values. The MC of the substrate was measured by drying a sample using the Air-Oven method (130°C for 4 h). In order to measure the MC values without opening the biogenerators, a separate set of these containers was placed alongside the test bins and samples of each substrate were taken periodically and their MCs determined.

All trials were carried out in triplicate, in a constant temperature room (26 + 1°C). Analysis of variance of the data used the programs of the SAS Institute (Barr *et al.*, 1976).

Results and discussion

CO_2 and O_2 contents of biogenerators containing WB and PS at four moisture contents

The results are shown as histograms (*Figure 2*). Following 2 days' incubation, the CO_2 output of WB of 35% and 40% MC was significantly higher than that recorded in the biogenerators containing WB of 25% MC. Correspondingly, the O_2 level for the two highest MCs was significantly lower than in the air mixtures produced by WB of 20% and 25% MC. However, after the sixth day the CO_2/O_2 levels recorded in the WB biogenerators at all four MCs tested did not differ significantly and the same $CO_2 : O_2$ ratio (18 : 5) persisted until the end of the incubation period.

In the case of the PS substrate, high CO_2 concentrations were obtained at all four MCs during the first 4 days of the composting period. However, these CO_2 levels dropped during the remaining 8 days of this period. Analogously, the O_2 consumption was quite low and similar at all four different MCs during the earlier stages of incubation. The O_2 content of the PS biogenerators increased during the remaining composting period.

Summing up, *Figure 2* shows that there was better and more consistent CO_2 production and O_2 consumption in the WB compared with the PS biogenerators, at all MC levels, whereas the differences due to the MCs were temporary and not significant towards the end of the composting period.

Modified atmospheres obtained in biogenerators and transferred into grain bins

Figure 3 shows the levels of the CO_2 and O_2 in biogenerators composting three different substrates and the transfer of these gases into the connected experimental bin containing corn. The histograms show that, during the experiment, significantly higher CO_2 and lower O_2 levels were maintained in the

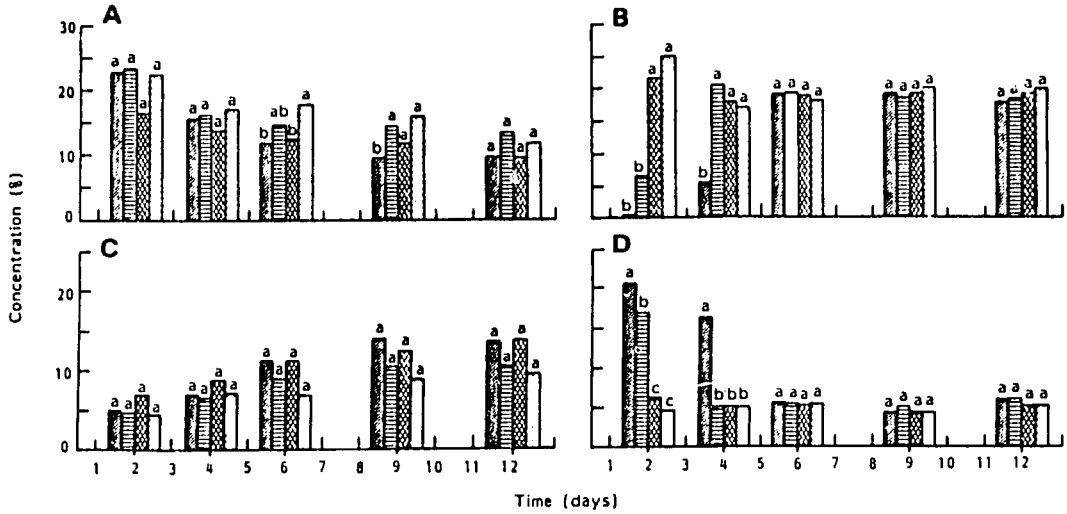

FIGURE 2. CO_2 and O_2 content of biogenerators, obtained by composting two different substrates at four moisture contents: ▨, 25%; ▤, 30%; ▩, 35%; ▥, 40%. (Within the same group, values with a common letter do not differ significantly, $p < 0.05$). A, CO_2 content, peanut shell substrate; B, CO_2 content, wheat bran substrate; C, O_2 content, peanut shell substrate; D, O_2 content, wheat bran substrate

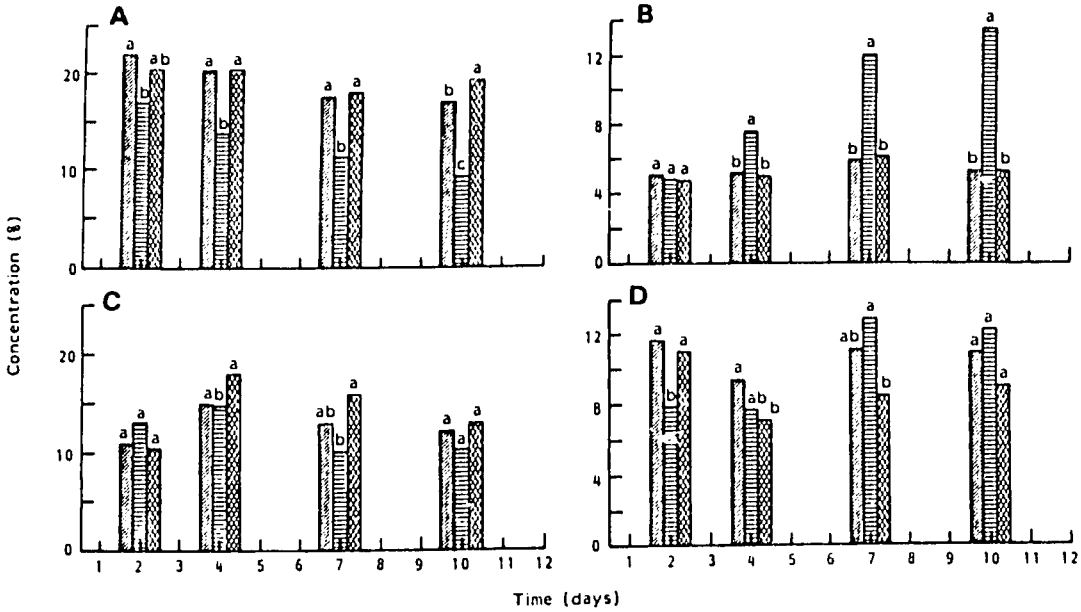

FIGURE 3. A, CO_2 content of biogenerator, B, O_2 content of biogenerator; C, CO_2 content of corn bin; D, O_2 content of corn bin. (Within the same group, values with a common letter do not differ significantly, $p < 0.05$) ■, wheat bran; ▤, peanut shells; ▩, orange peel

biogenerators containing WB and OP compared with those recorded in the PS biogenerators (except for the O_2 level recorded on the second day). However, considerably lower levels of CO_2 and higher O_2 levels were recorded when the modified atmospheres produced in the biogenerators were transferred into the grain bins. In all cases, in the control grain bins the air mixtures contained 20.8% O_2 and <0.1% CO_2. It should be noted that the transfer of gases neither increased the MC of the grain nor did it add any foreign odour: the corn remained in its original condition.

The lethality of the atmospheres obtained in the grain bins against storage insects has still to be tested. According to the graph of calculated 95% mortality levels of the flour beetle (*Tribolium castaneum*) produced

by CO_2 at four O_2 concentrations (Calderon and Navarro, 1979), about 22% CO_2 is needed to cause this mortality at 5% O_2 content. However, these calculations are adjusted for 5 days of exposure only. As, in our experiments, the O_2 levels were maintained for 10 days, it may well be that the lower CO_2 and higher O_2 levels obtained could be lethal to the above-mentioned insects. Further work is needed to clarify this issue, as well as to improve gas transfer from the biogenerators to the grain containers and to obtain gas mixtures of higher CO_2 and lower O_2 content by taking into account the amount of CO_2 absorbed by the grain. To this end, the biogenerator design, the type or the quantity of substrate, or the conditions in the biogenerators during composting, should be thoroughly investigated. The system used in this study may serve as a model in future investigations of these parameters.

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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 (Tribolium castaneum 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±1°C. *Tribolium castaneum* 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: *Aspergillus flavus*, *Aspergillus niger* van Tiegem, *Hyphopichia burtonii* Boidin et al., and *Penicillium griseofulvum* 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. MA production by composting sugar cane

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. Effect of production and transfer of MA to the grain bins by 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.

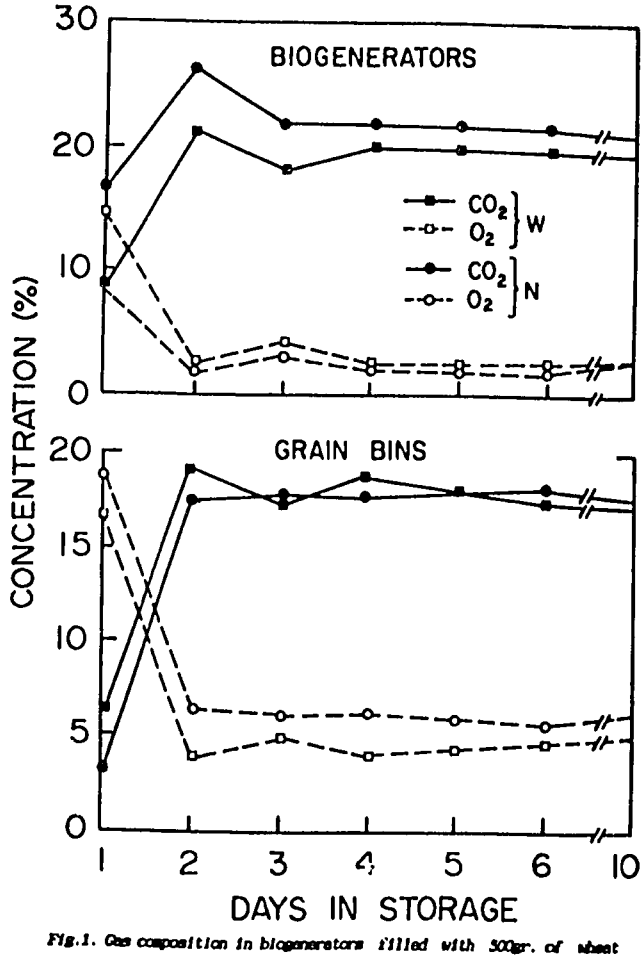

Fig.1. Gas composition in biogenerators filled with 500gr. of wheat bran and in grain bins connected to thrs either by a narrow (N) or a wide (W) pipe.

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.

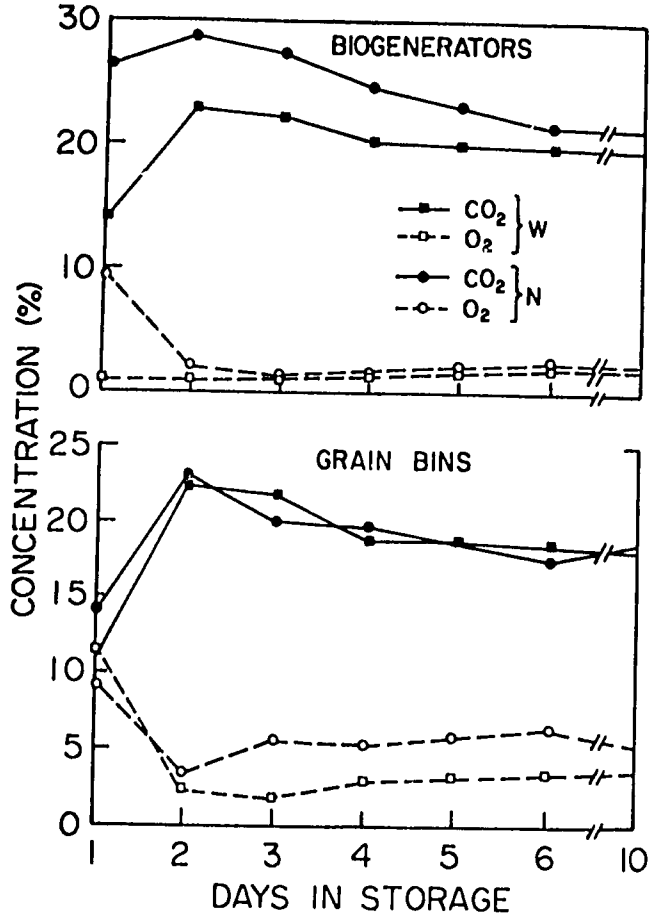

Fig.2. Gas composition in biogenerators filled with 1500gr. of wheat bran and in grain bins connected to them either by a narrow (N) or a wide (W) pipe.

2. Effect of artificial inoculation of the substrate.

The results presented in figure 3, indicate the following:

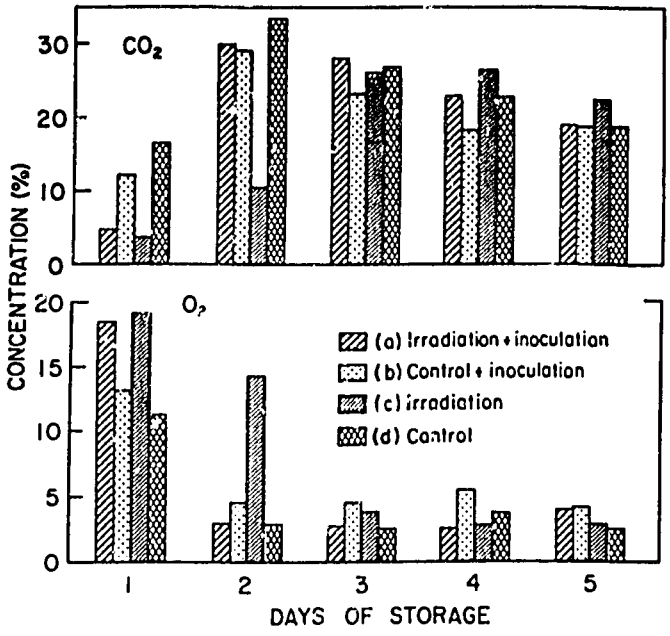

Fig.3. Effect of artificial inoculation of wheat bran on CO_2 production and O_2 consumption.

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 vs b,d). Following 3 days of incubation, the CO_2 and O_2 contents in all biogenarators were very similar.

3. MA produced by composting sugar cane

The CO_2 concentration increases with augmented amounts of substrate (Fig. 4). However, the differences in CO_2 content were not proportional to the amount of substrate. Eeven with the lowest quantity of SC i.e., 5 kg, the O_2 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.

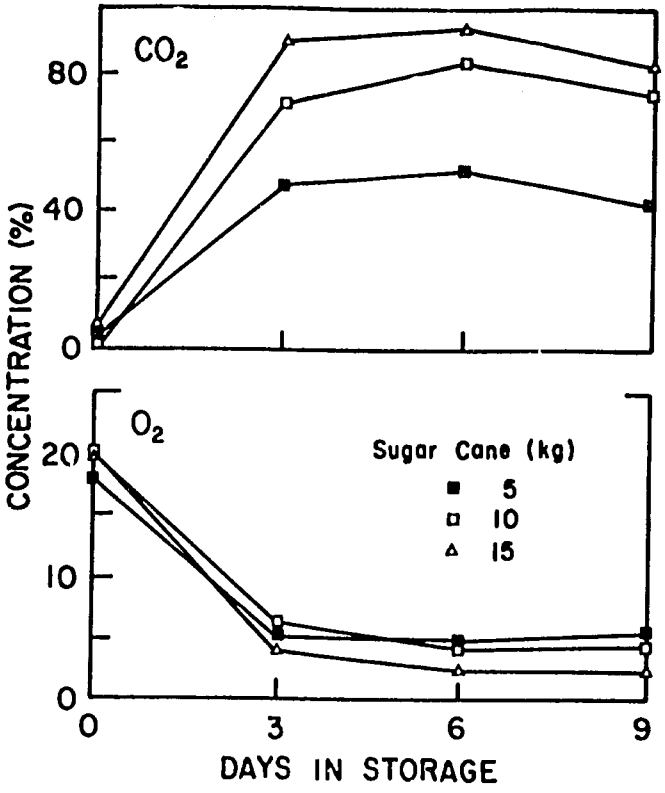

Fig.4. Effect of quantity (kg) of sugar cane on gas composition in 200 l. drums filled with 150 kg of maize.

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, e.g. 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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