Navarro, S. and Donahaye, E. [Eds.](1993) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, Canada, June 1992, Caspit Press Ltd., Jerusalem, pp. 533-539.

GRAIN PRESERVATION BY REDUCTION OF OXYGEN CONCENTRATIONS USING MICROORGANISMS

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ABSTRACT

An "oxygen converter cart" was designed, consisting of a "culture box", a dehumidifying device, a circulation fan, and a mobile frame. The fungus *Aspergillus niger* (strain 3758) which is a non-toxic fungus that can be propagated rapidly on agricultural by-products, was artificially cultured, and used to consume the oxygen and generate carbon dioxide in the inter-granular air of hermetically sealed stacks of grain. This was done by mixing the air in the culture box with that of the stack in a sealed recirculatory system. Wheat bran (the culture material) was inoculated with *A. niger* spores. After careful culture for a week, the oxygen content of a 500-tonne experimental rice stack was reduced to 2% and all insects were killed. This procedure has been shown to be effective in controlling insects and maintaining grain quality. It is non-pollutive and inexpensive.

MATERIALS AND METHODS

In this experiment, a "culture box" of microorganisms was placed next to a grain stack and connected to it to form a sealed recirculatory system. The function of the microorganisms was to consume the oxygen (O_2) within the stack and produce carbon dioxide (CO_2).

Choice of microorganisms

The microorganism was chosen to conform with the following requirements:

1) It should be non-toxic to humans and animals and should not contaminate the grain.
2) It should be capable of rapid propagation and possess a high level of aerobic metabolism even at low O_2 concentrations.
3) It should be available and easily cultured on by-products of the grain industry.

In accordance with the above requirements, seven different microorganisms were examined including *Aspergillus niger* (strain 3758), Yeast Spirits Number 1 (Variety of 3798) and beer yeast, all of which are non-toxic and easily obtainable from grain distilleries. Among those tested, the above strain of *A. niger* was the most effective in reducing O_2 concentrations and remaining propagative at high temperatures.

"Oxygen converter cart"

The cart consisted of a culture box, a cooling device, a circulation fan, and frame (Fig. 1). The main component - the culture box - was a sealed container in which conditions were provided to suit the ecological requirements of *A. niger* (strain 3758), namely, a temperature range of 33 - 37°C, a relative humidity range of 90 - 100%, a supply of oxygen and a specified quantity of culture medium.

The experimental requisite was that the O_2 level be reduced to below 2%. We used wheat bran as a culture medium in a bran/stored grain ratio of of 2/10,000. To facilitate fungal growth, the wheat bran was separated into layers on mats of coarsely woven bamboo. Each layer of bran was 1.5 - 2.0 cm thick, and the vertical distance between mats was 7.5 cm. The total volume of the culture box was 3.6 m^3 (2 m long x 1.2 m wide x 1.5 m high).

Culture medium and culture method

The wheat bran was mixed with rice husks to keep the material loose and permit good ventilation. Data obtained from previous trials indicated that the wheat bran and rice husks should first be mixed thoroughly at a ratio of 100:10. Water was then added to the medium at a ratio of 1.5 :1, and the material was mixed thoroughly. Then, the mixture was placed in a steamer with boiling water, taking care not to compress the material, and the medium was steamed for 40 min. All utensils to be used were placed in the steamer together with the culture material for sterilization. Upon removal from the steamer, the culture medium was taken to the storehouse and spread out evenly on the bamboo mats. When it had cooled to 35°C, the *A. niger* spores were added and mixed gently into the medium.

Monitoring and management of the generator

The time required for fungal development in the "culture box" depended on the season. In the autumn, the process of germination and rise in temperature of the culture took 5 - 6 days, followed by decay and a decrease in temperature. In the summer this process took 4 - 5 days, and in winter more than 7 days. In order to control the growth period, propagation of the mold, and efficient reduction in O_2 concentration, it was necessary to regulate the temperature in the culture box. The box temperature had to be monitored every 30 min and the circulation fan run frequently to keep the box temperature below 40°C.

When the cart was in operation, care was taken to prevent air-leaks. A leak test was conducted using a flame or soapsuds for the circulation-fan, joints between ducts, and welded seams. Rubberized fabric was used to seal over places where leaks were detected.

Grain stacks

Three stacks were set up. The experimental stack, connected to the "culture" cart, was a sealed stack. Another sealed stack was for storage under "oxygen-low"# conditions, and an uncovered stack served as control. Each stack contained 500-tonnes of long-grain non-glutinous rice of "more-than-moderate" quality, and averaging 13.5%, 13.3%, and 12.7% moisture content (m.c.) respectively.

RESULTS

Oxygen reduction

O_2 reduction by the "oxygen converter cart" was analyzed frequently with very satisfactory results. Winter and summer trials are comparable (see Table 1) as in these two seasons, temperatures are most difficult to regulate and control. The Table shows that O_2 concentrations were reduced to 2% in 8 days in the winter and in 6 days in summer whereas under the "oxygen-low" technique, O_2 concentrations did not decrease in winter and only decreased to 17.4% in summer within the same time periods.

Insect control

Results of insect control are given in Fig. 2. The figure shows that the tolerance to low-oxygen concentrations of the adult stages of four stored-product insect species is according to the following decreasing order: *Tribolium castaneum* (Herbst) > *Oryzaephilus surinamensis* (L.), > *Rhyzopertha dominica* (F.), > *Sitophilus zeamais* (Motschulsky).

Inhibition of grain microflora

Results on the inhibition of grain microflora are given in Table 2. The experiment to evaluate the inhibiting effect of the "oxygen converter cart" method on grain microflora was carried out over a 3 month period. During the first month, O_2 was kept at below 5%, while for the second and third months, it was kept at 8%. The table shows that due to the low m.c. of the stacks, the microorganisms in both the experimental stack and in the "low oxygen" technique stack were basically controlled. The process of O_2 reduction using the "oxygen converter cart" inhibited the development of

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term used to denote hermetic stack storage used in the "Triple-Low" technology developed in China - Eds.

several of the molds recorded at the beginning of storage, while after three months, the molds *A. ochraceus, A. fumigatus* and *A. terreus* had vanished. The very high infestation of *A. niger* was due to the propagation of this species in the culture box; however, if this is well-controlled in the oxygen exchange process, then there should be no harmful effect on the stored grain.

Table 1: Changes in air composition of rice stacks stored under the "microorganism culture box method" and the "oxygen-low" technique.

		Microorganism culture box		"Oxygen-Low" technique	
season	date	CO_2	O_2	CO_2	O_2
winter	2/12		20.5	0.5	20.3
	3/12	2.6	19.6	0.4	20.2
	4/12	2.8	19.0	0.4	20.2
	5/12	3.6	17.2	0.5	20.0
	6/12	6.1	13.4	0.5	20.3
	7/12	7.1	10.1	0.6	20.1
	8/12	10.2	4.8	0.6	20.1
	9/12	12.4	2.0	0.6	20.0
summer	12/7	1.1	19.2	1.2	18.9
	13/7	3.7	15.4	1.4	18.0
	14/7	7.8	10.6	1.5	17.8
	15/7	5.4	12.3	1.5	17.7
	16/7	12.6	2.1	1.6	17.6
	17/7	14.0	2.0	1.7	17.4
	18/7	14.0	1.8	1.9	17.0

Grain quality

As can be seen from Table 3, there were no salient differences in rice quality among the stacks stored using the "oxygen converter cart" method of O_2 depletion by microflora, the conventional "oxygen-low" technique and normal storage; however, the results do show that the experimental procedure had no harmful effect on grain quality.

CONCLUSIONS

At present, chemical insecticides are still widely used to control stored-product insect pests and reduce losses. Doubtlessly they will still be used in the future, though reliance upon one method tends to enable the insects to

develop resistance to the insecticide and results in increased amounts of residues in the grain. The method of using microorganisms to deplete the O_2 content of the grain bulk is an innovative and efficient way of controlling insect pests.

Table 2: Influence of "culture-box" treatment on grain microfloral composition.

	Experimental stack		Conventional "oxygen-low"stack	
Species	Initial Quantity	After 3 months	Initial Quantity	After 3 months
A. candidus	2.64*	1.76	2.12	1.78
A. glaucuss	0.84	0.67	1.13	1.45
A. nidula	0.21	0.05	0.10	0.02
A. vidulans	0.10	0.06	0.12	0.02
A. flavus	1.80	0.98	2.10	1.84
A. niger	3.12	1.65	0.30	0.32
Mucor sp.	0.05	0.20	0.06	0.06
A. ochraceus	0.30		0.24	0.04
A. fumigatus	0.15		0.13	0.28
A. terreus	0.08		0.10	0.62
Penicillium sp.	0.50	0.41	0.61	0.41
Total count	9.79	5.66	6.89	6.84

* fungal count $x10^4/g$

Table 3: Changes in grain quality under the three storage methods.

						Cooking quality			
	Storage period (months)	Moisture content (%)	Fatty acid value	Visco-sity	Germi-nation (%)	Expan-sion percen-tage of rice	Water absorp-tion	pH value rice-water	Dry matter in rice-water*
Culture Box	Initial	13.5	14.53	3.2	92	212.0	140	7.3	0.30
	10	13.5	26.81	3.0	74	222.5	149	7	0.25
Oxygen -Low	Initial	13.3	13.98	3.1	94	218.0	138	7	0.28
	10	13.1	34.21	2.9	71	251.0	149	6.8	0.25
Conven-tional	Initial	12.7	13.61	3.3	93	221.3	141	7.2	0.31
	10	13.6	49.14	2.8	64	241.7	146	6.6	0.21

* Rice-water from 10 g of rice

Compared with grain preservation using the "oxygen-low" method employed in China, this method has the advantage of expediency in reducing the O_2 content and controlling insect pests. It is a suitable treatment for rice stored at low m.c.s, such as early and semi-late rice grown in areas south of the lower reaches of the Yangtze river, which lack the natural O_2 depletion capacity of other grains subjected to "oxygen-low" storage. The system can be popularized and constructed for use in medium-sized and small-sized storehouses.

The "oxygen converter cart" is mobile and convenient to use. However, in order to achieve satisfactory results, temperature and humidity in the culture box must be carefully regulated as should the m.c. of the culture medium.

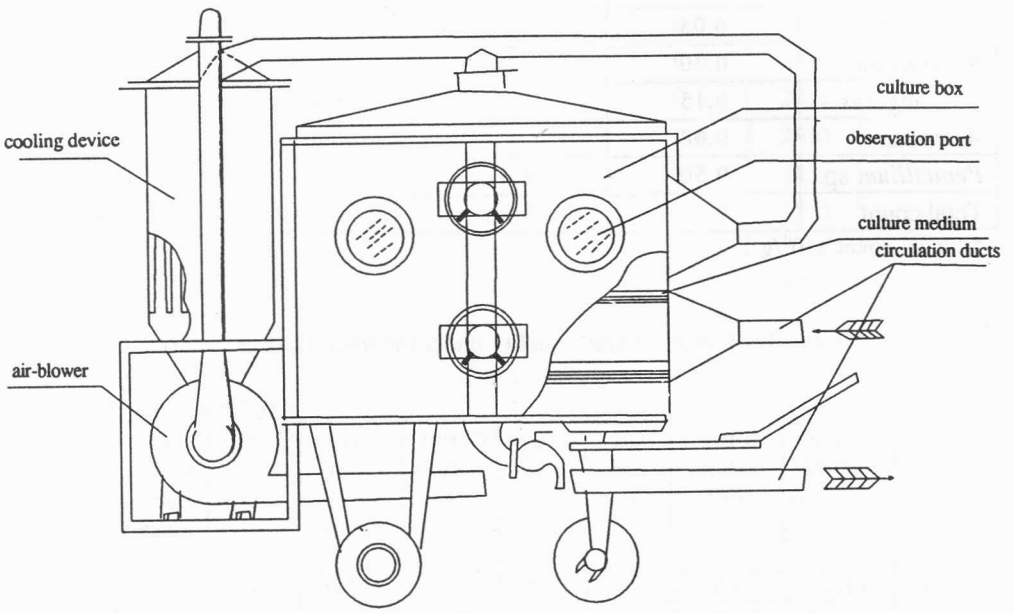

Fig. 1: The "Oxygen Converter Cart".

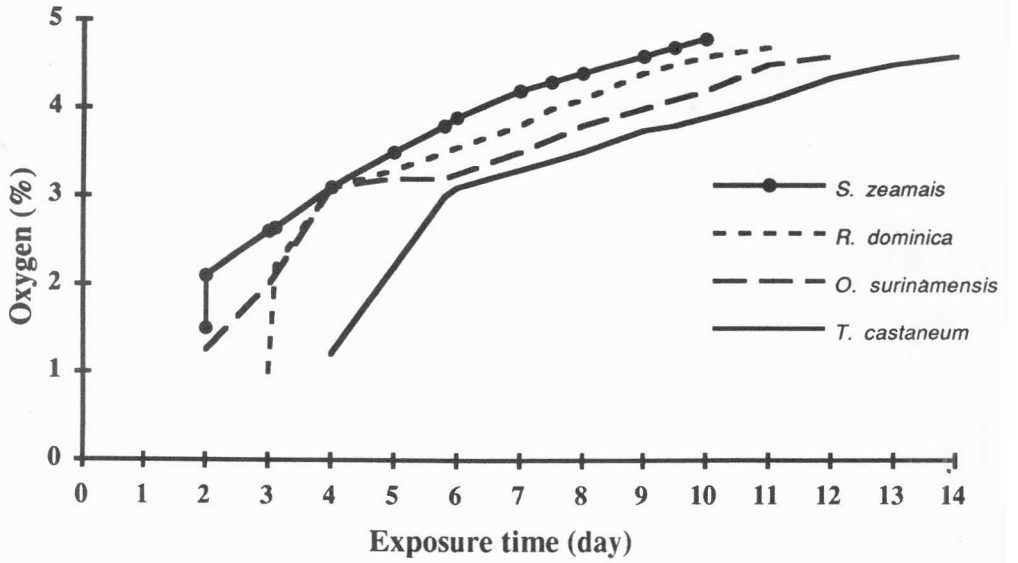

Fig. 2: Relationship between oxygen concentration and exposure time for mortalities of four species of stored-product insects at 25°C, and 75% r.h.