ADVANCES IN GRAIN STORAGE IN A CO_2 ATMOSPHERE IN JAPAN.

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INTRODUCTION

The reduction of post-harvest losses is now a common food strategy throughout the world. A considerable amount of work has been carried out by several groups in each country on loss assessment and reduction studies. Storage must be one of the most important stages for the reduction of post-harvest losses.

In Japan rice is the major staple food grain and one of the few self-sufficient foods. The storage and distribution system controlled by the government is well established for the annual consumption of rice and post-harvest losses are assumed to be kept at an acceptable level. Reserves of surplus rice for food security purposes, however, are still hindered by storage problems. The Japanese people usually cook polished rice grains in water without adding seasoning, so the eating quality of the cooked rice directly reflects the properties of the grain. The Japanese are very sensitive to the taste of cooked rice, so that even the very slight changes in the physical and chemical nature of rice after it has been stored for a year are detected and cause dislike. The loss of quality or acceptability in rice during storage is serious because rice is stored in ambient temperature warehouses where it is hot and humid in summer. In addition to this, the rice has been transported and stored non-hermetically in small bags made of jute or kraft paper. The longer the storage period, the greater the quality loss. Grain storage in a CO_2 atmosphere has been advancing step by step since 1967 in Japan. Few scientific studies on controlled atmosphere storage of grains have been reported, so advances in grain storage in a CO_2 atmosphere can be summarized in the results obtained from the experiments of the author's group.

GRAIN STORAGE IN A CO_2 ATMOSPHERE

It is favorable for the safe storage of grain to eliminate the oxygen from the structures where grains are stored, in addition to controlling the stored temperature and the moisture content of the grains. Although oxygen concentration in small pouches of dried foods can be reduced easily by evacuation or the use of oxygen scavenger, it does not seem to be practical to apply these techniques to grain storage. In the hermetic storage of grains, it is assumed that the oxygen will be exhausted and that CO_2 will be produced within a few days due to the respiration both of the grains and of contaminated organisms. The rate at which the oxygen is exhausted depends mainly on the moisture content of the grain. The higher the moisture content of the grain, the higher the rate of exhaustion. During the period of oxygen exhaustion, however, chemical changes inevitably take place affecting the quality of the grain. Grain storage in an inert gas atmosphere seeks to avoid or lower the changes which must be caused by the presence of oxygen. Fundamentally, what is involved is the retardation or suppression of the respiration of the grain itself, the insects, microorganisms and so on. Normally, the gases used for this purpose are either carbon dioxide or nitrogen, both readily available commercial products. Results obtained in the controlled atmosphere storage of various fruit and in the gas storage of processed foods show some differences with regard to these gases. The authors are interested in the following two distinctive features of CO_2.

(1) Carbon dioxide is the end product of the aerobic combustion of organic materials in living organisms. Thus, the accumulation of CO_2 beyond a certain critical concentration acts as a signal that can slow down the rate of the reactions by which it was formed, a type of control known as "feed-back inhibition". In fact, inhibition of succinate dehydrogenase is well known in connection with the CO_2 disease of some fruit. This enzyme is one of the members in the tricarboxylic acid cycle, which takes place in the mitochondria and consists of a cycle series of reactions in respiration. The authors also reported the inhibition of catalase by higher partial pressure of CO_2. This enzyme is often noticed as a good index of viability of the grain. In addition, it is proved that the activity of ribulose-1,5-diphosphate carboxylase, which is the key enzyme in the Calvin cycle, is controlled by CO_2 and O_2 , similar to the manner of hemoglobin in animal blood. The reversible nature of the inhibition by CO_2 has been reported in the study on the activity and metabolism of goat sperms stored in physiological liquid under various partial pressures of CO_2. These biological results indicate the distinctive feature of CO_2 in the metabolism of stored grains.

(2) Another distinctive feature of the action of CO_2 is its sterilisation effect on the growth of fungi and other microorganisms. Even when there is a high level of oxygen remaining in the vessel, a higher concentration of CO_2 (more than 80-90%) effectively prevents the fungal growth, while N_2 cannot do so. This is a favorable characteristic for the practical use of CO_2 in the prolonged storage of grain, because the risk of contamination of oxygen into a stored vessel cannot be eliminated.

RESULTS OF SEVERAL EXPERIMENTS

1. Fundamental experiments on a laboratory scale (Mitsuda and Yasumatsu, 1955, Mitsuda et al., 1958, 1969, Mitsuda, 1969a, 1969b)

On the basis of hints obtained from studies on the respiration of cold-blooded animals in hibernation, which were conducted in the laboratory at Kyoto University around 1952, the authors introduced the hermetic storage of cereal grain in naturally cold places. This is based on lowering the respiration in grain by using both CO_2 and low temperatures, thus retaining the original freshness of the grain even after prolonged storage. The most desirable temperature for maintaining rice grain quality is around 13^{o}C. The less the variation in temperature throughout the year, the better will quality be retained. Appropriate places satisfying these conditions are spaces under the water of lakes, ponds or man-made pools at the seacoast or spaces under the ground. Laboratory-scale storage experiments were carried out from 1967 to 1969. The results obtained in these experiments are:

(1) Packaging materials for underwater or underground storage of grains must be:
i) Resistant to structural attack by water, ii) Moistureproof,
iii) Resistant to physical shock, and iv) Airproof.

A flexible bag made of plastic laminated films with a biaxially-oriented polyamide film (nylon type), a polyvinylidene chloride film and a polyethylene film, was found to meet all of the above requirements very successfully.

(2) The moistureproof quality of the bags was confirmed in storage experiments which were carried out in water baths, ponds and man-made pools.

(3) Carbon dioxide was used for grain storage and the airproof quality of the bags was confirmed. An interesting phenomenon was also found that grain in flexible bags containing CO_2 was tightly packed as if packed in vacuo.

(4) Various factors for large-scale storage in water were examined and suitable forms of storage unit and equipment were designed. The annual data of the weather conditions in Lake Biwa were collected and examined in order to select a storage place in the lake.

2. Underwater and underground storage experiments (Mitsuda and Kawai, 1970, Mitsuda et al., 1971a, 1971b, 1972, 1973).

An experiment in underwater storage of cereal grains was carried out from 1969 to 1972. Paddy, brown and polished rice were packaged in the plastic bags with CO_2 and stored for 3 years in the water of Lake Biwa. Wheat, barley and soybeans were stored in the same way as the rice grains. In parallel with the underwater storage experiment, the underground storage of brown rice was also carried out at an abandoned mine on Shikoku Island. These experiments were carried out to investigate the possibility of storing grain on a practical-scale in naturally cold places,i.e. with a temperature below 13^{o}C. Results obtained are summarized as follows:

(1) The waterproof quality of the laminated film in such grain storage conditions proved to be highly satisfactory over a relatively long period.

(2) Underwater storage prolonged the storage life of the grains. The original freshness of the grains even after prolonged underwater storage could be shown by the fact that considerably higher biological activities such as germinative capacity, and catalase and peroxidase activities were detected for the grains stored in the

water than those kept in atmospheric conditions. In particular the germinative capacity of the paddy rice retained its original value even after a storage period of 3 years. Changes in the contents of vitamin B , reducing sugar, water-soluble nitrogen, and other substances were also found to be less for the rice stored underwater. Palatability of the grains stored underwater was found to be satisfactory by panel tests. The strength of stale-flavor as determined by the amount of volatile carbonyl compounds, rheological characteristics as measured by texturometric indices and cooking qualities as revealed on boiling, showed that the deterioration proceeded at a considerably lower rate for the grains stored in the water than for those stored in atmospheric storage.

(3) As to the atmosphere within the bags of laminated film, the bag containing CO_2 gas was found to be more effective than that containing air in preventing changes in chemical composition, in lowering the diminution of peroxidase activity, and in retarding the development of stale-flavor.

(4) A total system on a 10 thousand metric ton-scale has been drafted for underwater storage. The cost for this system amounts to about $34.00 or less per ton of rice, as calculated by the supporting committee for grain storage research at the Research Institute for Production Development in March, 1970. The committee was composed of five companies in Japan; UNITIKA Ltd., Sumitomo Chemical Co., Sekisui Chemical Co., Ltd., Asahi-Dow Co., Ltd., and Taiyo Kogyo Co., Ltd. The cost appeared fairly low compared with that for temperature and humidity controlled storage systems.

(5) Various kinds of plastic laminated films were compared with each other as to their abilities to maintain grain qualities. Experimental results suggested that the composition of the laminated film could be simplified in accordance with the storage conditions. The usefulness of polyvinyl chloride bottles was also tested in underground storage.

3. <u>Transportation and storage experiments in ambient temperatures (Mitsuda et al., 1974).</u>

The rice stored in low-temperature warehouses (below 20^{o}C) or in country elevators (12-13^{o}C) located in granary areas had often suffered significant deterioration when polished in a large-scale central mill and transported from there to urban areas. This kind of trouble was particularly noticeable in the rainy season or summer. The biggest cause of this trouble was the packaging method. Open packages such as straw, jute or kraft paper could not prevent the permeation of vapor and/or gases in the atmosphere. Polyethylene bags were also unsuitable in the same way because they contained a few tiny holes to add flexibility and prevent slipping in loading. Another problem was the storage of rice in polished form. In Japan, storage of rice in polished form had been avoided because the rice deteriorated much more rapidly than brown or paddy rice. The recent development of a large-scale central polishing system was, however, increasing the necessity to store and transport rice in polished form and thus causing deterioration of the qualities of rice as consumed. The need for new methods of storing and transporting the polished rice was the major impetus for this experiment. A transpotation and storage experiment of grain packed by the CEM (Carbon dioxide Exchange Method) technique was carried out from August 1971 to March 1973 in order to develop a new system for transportation and storage of grains with the least loss of quality. Two varieties of japonica rice which had been stored in a low-temperature warehouse (below 20^{o}C) and in a country elevator (12-13^{o}C) were polished at a large-scale central polishing factory in Iwate Prefecture, one of Japan's granary areas. About one metric ton of polished rice was packed by the CEM and the conventional methods using kraft paper bags and polyethylene bags. The rice was transported from Iwate to an urban area in a 5 ton container by rail and the journey took 5 days during August, the hottest month in Japan. The ambient temperature in the container fluctuated between 20^{o}C and 47^{o}C during transportation. Biochemical and palatability changes were examined in the rice just after arrival at destination and after 3 and 8 months storage at a rice dealer's. Some of the rice was stored for another 11 months and then examined again. Sample analysis included:

moisture content, water-soluble acidity, free fatty acid content, TBA value, amylography, cooking qualities, microbiological tests, gas chromatography of volatile carbonyls from cooked rice, texture evaluation of cooked rice by using a texturometer, and sensory analysis of cooked rice by 24 panels.

The results obtained in these analyses demonstrated that rice packed by the CEM technique could be maintained in better condition than that packed by conventional methods, particularly in terms of the analyses of water-soluble acidity, free fatty acid content, amylography and gas chromatography of volatile carbonyls from cooked rice. The development of mould and insects was perfectly retarded in the skin-packages in the CEM method without using fumigation, but this was not possible in the rice stored in conventional bags.

A new type fully automatic packaging machine was constructed by the Japan Steel Works, Co., Ltd. which made it possible to carry out the large-scale packaging and transportation test in January 1973 needed to obtain the basic data for the practical feasibility of CEM. These experiments were performed in cooperation with agricultural government offices, agricultural auxiliary organisations and some private companies. In this experiment, about 3,500 packages of polished rice weighing 5kg each were prepared by using the packaging machine at Niigata Prefecture, also one of Japan's granary areas. The packages were palletised and transported by train from Niigata to Osaka, the second biggest city of Japan. Polishing conditions, the amount of CO_2 gas required for the process with the packaging machine, the temperature changes of the rice, the correlation between breakage incidents and the thickness of bags and so on were all observed. Opinions of retailer on the process were also gathered. Examination of all the packages carried out after their arrival at Osaka revealed that only two packages were broken and only 1.4% of them had suffered pin-holing and failed to remain airtight. The data obtained in this experiment together with the relatively low cost suggested that this technique could be suitable for use on an industrial scale.

4. Development of CEM skin-packages in Japan (Mitsuda et al., 1975, Mitsuda, 1979).

After accumulating the basic data for the practical feasibility of the CEM technique in cooperation with agricultural government offices, the technique has been adopted by the industry for the preservation of polished rice since July 1973. The merits of CEM skin-packaging are:

(1) The packages are easy to produce;
(2) Permeation of moisture and gas is almost completely prevented;
(3) Deterioration caused by aerobic microorganisms, insects and oxygen is effectively prevented;
(4) There is no breakage or slipping of bags in loading;
(5) Packages are reshapable even after sealing;
(6) This technique can be widely used in food packaging and safe storage because it eliminates the need for chemical food additives.

The skin-packaging of polished rice has also been accepted by various types of consumer. For example, many Japanese who live abroad as well as people engaged in deep sea fishing and mountain-climbing have reported the successful retention of flavor in rice stored in CEM skin-packages. CEM skin-packages have also made it possible to develop a new type of polished rice which retains the germ portion, because the rapid deterioration of this type of polished rice is effectively prevented by this packaging and storage method. At present, CEM skin-packages are produced at more than 30 polishing factories in Japan. Financial support for promoting the CEM packaging of polished rice is offered by the National Rice Distribution Association to rice retailers under the direction of the government.

5. The seed storage experiment (Mitsuda et al., 1979).

A long-term storage experiment was carried out from 1972 to 1977 with the cooperation of the Chiba-Ken Foundation Seed and Stock Farm in order to develop a convenient system for long-term storage in place of the annual reproduction of seed grain. Paddy rice from the harvest of 1971 with moisture contents of 5.9, 8.7, 11.3, 14.0 and 16.3% respectively were packed in CEM skin-packages and stored at temperatures of 0, 10, 20, 30°C and ambient temperatures respectively.

Table 1. shows one of the results obtained by germination tests of the storage of paddy rice in a CO_2 atmosphere. Significant viability reduction was observed in the paddy rice stored with higher moisture content and higher storage temperatures. Compared with the data obtained from non-airtight storage, the rate of viability reduction was found to be 2 to 4 times slower in the high moisture

content of rice packed by CEM technique. Actual germination ability in the field was proved to agree well with germination capacity in Petri dishes in the laboratory. In addition to this, growth rate in the field and various characteristics of plant and frequency of abnormal growth were also examined on the Farm. Results obtained showed any abnormality in growth of paddy rice stored in a CO_2 atmosphere for 5 years. Peanuts and wheat have also been tested in the same way.

TABLE 1.
Storage periods before the germination of paddy rice decrease below 90% at various moisture contents and temperatures

Moisture content (wet basis)	Storage temperature				
	30^{o}C	20^{o}C	10^{o}C	0^{o}C	ambient temperature[a]
16.3 %	1.6	6.9	5.8	32.6	3.8 months
14.0	3.1	11.1	49.4	>60	6.1
11.3	16.0	52.9	>60	>60	37.6
8.7	37.8	>60	>60	>60	>60
5.9	>60	>60	>60	>60	>60

a) 4.0-34.2^{o}C.

6. Experiments on grain storage in a CO_2 atmosphere in steel cans.

With the discovery of the usefulness of CEM skin-packages for safe storage of rice, needs and feasibilities for famine reserves of rice have been one of the serious concerns in local governments and governmental offices. The development of storage systems on larger scales of one metric ton or more in steel cans and containers has been required, in addition to the system using small bags of CEM skin-packages. To meet these social requirements, a project team was organized on May, 1976 for the development of a new system of CO_2 gas storage on larger scales with the cooperation of the university, companies and corporations under the leadership of the author's group. The Nippon Steel Corporation, one of the project members and the biggest company in Japan, had been conducting some basic experiments on grain storage in its Fundamental Research Laboratory since 1971 in order to find wider application of the use of steel cans. In this project team, the artificial gas storage using CO_2 gas had been adopted in a steel can storage system. Experimental results obtained for the CO_2 gas storage of grain in steel cans can be summarized as follows:

(1) Grain quality analysis: Polished rice has been packed in more than 40 drum cans 200 l in volume and stored at ambient temperature (below 30^{o}C) for 2 years in a CO_2 atmosphere. The chemical and sensory evaluation tests of the stored grain have been carried out mainly by the Japan Grain Inspection Association and the quality of the stored polished rice has proved to be of a satisfactory level for

conventional consumers' use. These results using steel cans have confirmed the previous data obtained using CEM skin-packages.

(2) The characteristics of CO_2 gas adsorption by canned grain: The characteristics of CO_2 gas adsorption by the canned grain proved to be in satisfactory agreement with the results obtained by Warburg's manometry in our laboratory. These results, some of which were shown in Figs. 1 to 3, provided the fundamental knowledge for the development of automatic filling equipment and a pressure-controlling method for storage cans.

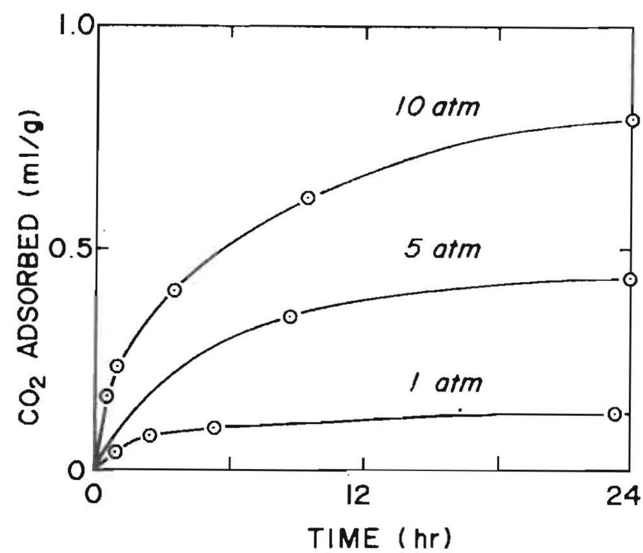

Fig. 1. CO_2 adsorption rate in various pressures.
A small autoclave 0.8 liter in volume with a pressure gauge was used as a test vessel in the experiment at various pressures. The amount of CO_2 gas adsorbed by grains was determined by the titration method and calculated with the amount of acid required to neutralize the sodium hydroxide solution in which the desorbed CO_2 gas was trapped by the grains in a N_2 gas stream. Although the adsorption rate did not increase even at higher CO_2 pressures, the amount of CO_2 gas adsorbed by grains increased with the increase of CO_2 gas pressure. The amount of CO_2 gas adsorbed by grains and CO_2 gas pressure agree with the sorption isoterm of Freundlich as in the case of various partial pressures of CO_2 gas determined previously (Mitsuda et al., 1973b).

(3) The development of counter-current type filling equipment: In order to get effective purge of intergranular air and maximum adsorption of CO_2 gas, filling equipment of counter-current type was newly developed in the Nippon Steel Corporation. It has a filling capacity of 150-450kg grain per hour into steel cans. Operation of this equipment was stable and smooth for the production of grain cans

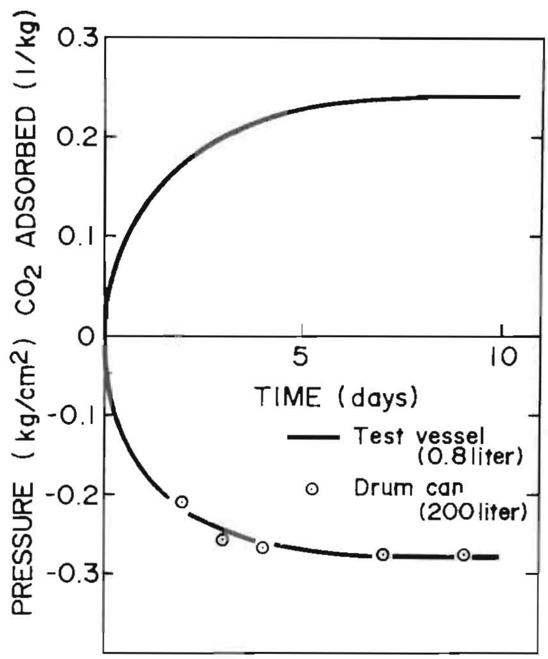

Fig. 2(a). Adsorption equilibrium in the test vessel and drum can. Changes in the amount of CO_2 gas adsorbed by grains and changes in the inner pressure of the test vessel were determined correspondingly. Adsorption equilibrium was obtained after 7 days. Changes in the inner pressure of the drum can also followed the same curve obtained with the test vessel. Initial high-pressure treatment was carried out by pressing the grain to 10 atm with CO_2 gas for several minutes as shown in Fig. 2(b). Changes in the inner pressures followed the same procedure after the inner pressure had been reduced to atmospheric pressure. When the rice grain had been pressed to 10 atm and reduced to atmospheric pressure, temporary increase of inner pressure was observed as shown in the above three curves, while gradual decrease was observed without an initial high-pressure treatment as shown in the lowest curve. This behavior may be caused by two opposite factors of increasing and decreasing the pressure. The relationship between equilibrated pressure and the required time for initial high-pressure treatment was plotted in Fig. 2(c). This indicates that the equilibrated pressure in the vessel can be set at positive when the high-pressure treatment is carried out for more than 60 min. (as shown in Fig. 2(c)).

with an adequate pressure of CO_2 gas. This equipment has made it possible even at present to start making large-scale famine reserves of polished rice in steel cans and containers below the one-metric ton unit.

For the practical application of these systems to the prolonged storage of rice in Japan, further studies are also necessary concerning prolonged experiments on the strict determination of shelf life for canned rice, selection of suitable varieties of rice for prolonged storage, and finding the suitable conditions for the storage of rice in brown and paddy forms.

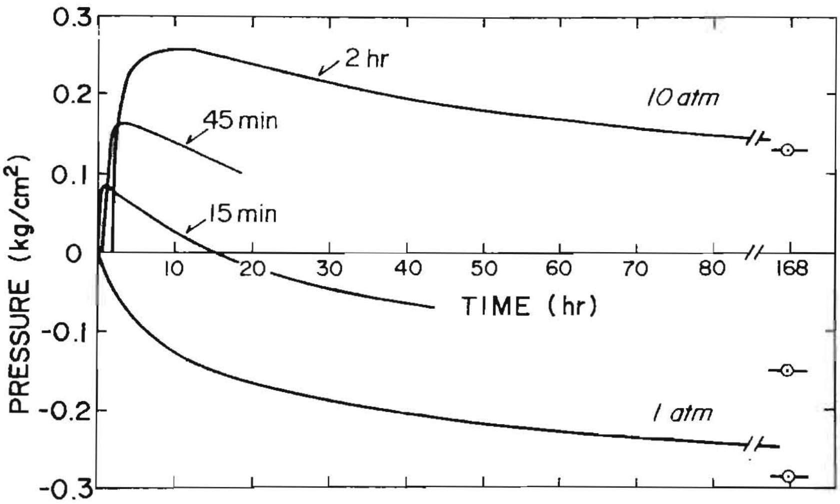

Fig. 2(b). Changes in the inner pressures of a test vessel with or without initial high-pressure treatment.

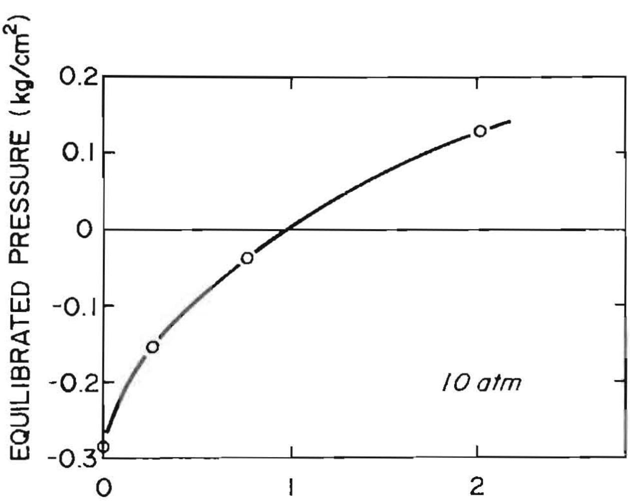

Fig. 2(c). The relationship between equilibrated pressure and the required time of initial high-pressure treatment.

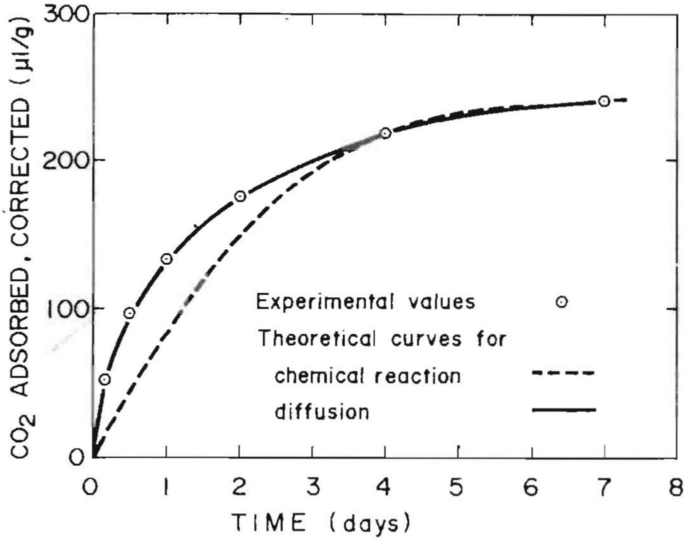

Fig. 3. Rate-limiting curves of CO_2 adsorption by grains.
Equation for the rate-limiting curves were as follows:

$$1 - (1-f)^{1/3} = \theta/\theta_B \quad \text{(reaction rate-limiting)}$$

$$3\,[1-(1-f)^{2/3}] - 2f = \theta/\theta_B \quad \text{(diffusion rate-limiting)}$$

Experimental values were proved to agree well with the theoretical curve for diffusion rate-limiting into the granule.

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