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The Effect of Alternating Magnetic Field on the Magnetic Anisotropic Gel Beads Immobilized Catalase

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It has been reported that magnetic anisotropic gel beads (MA gel beads) prepared by the gelation of a ferrite suspension in a static magnetic field have a property of oscillating in an alternating magnetic field. This paper presents the results concerning the application of MA gel beads to a study of immobilized enzymes. MA gel beads were prepared by the gelation of a suspension containing 5% catalase, 3.5% κ-carrageenan and 0—17% Sr-ferrite in a static magnetic field of 500—2500 Oe (1 Oe=$1/4\pi\times10^3$ A m^{-1}). The enzyme reaction was carried out in a column, which was filled with MA gel beads, by continueously flowing a solution containing H_2O_2 as a substrate. It was found that ① MA gel beads oscillated intensely upon applying an alternating magnetic field of 100—600 Oe, 50 Hz in the column, ② the concentration of O_2 in the elution that passed through the column increased by 10—60% compared with that in the absence of a magnetic field, and ③ the concentration of O_2 in the elution was influenced by the strength of the alternating magnetic field, the strength of the static magnetic field and the concentration of ferrite in the gel.

The ferrite-containing gel beads prepared in a static magnetic field were found to exhibit a property of magnetic anisotropy. These gel beads (abbreviated to MA gel beads) oscillated with turning and spinning in an alternating magnetic field. The diffusion layer on the surface between the MA gel beads and the solution could, thus, be thinner, owing to this oscillation; in turn, the transport of molecules through the surface could be facilitated. The above-mentioned suggestions were confirmed in a previous paper by a measurement of velocity of pigment ejection from MA gel beads into a solution with a magnetic field that was turned off and on.[1)]

Enzyme immobilized gel beads filled in a column might be hardly stirred by a mechanical method. In that case, the process of molecule transport through the diffusion layer is deduced to be the rate-determining step of the enzyme reaction. On the other hand, there are many papers concerning fluidized beds using magnetic fields for both stabilization[2)] and stirring,[3)] and for prevention of particles flowing out.[4)] Magnetic gel beads (not exhibiting an anisotropic property) in a fluidized bed can be stirred by applying a magnetic field from outside in order to facilitate the enzyme reaction.[5,6)] In this connection, there have been application reports regarding the recovery of enzymes immobilized to magnetite particles[7,8)] and magnetosomes isolated from magnetotactic bacteria.[9,10)]

The purpose of this study regarding applications to immobilized enzymes was to expand the utilization of MA gel beads. The promotion of enzyme reactions was expected by applying an alternating magnetic field in a column filled with MA gel beads, which might play the role of a stirrer in the column under a magnetic effect. MA gel beads were prepared with catalase, κ-carrageenan and Sr-ferrite. The following effects were surveyed regarding the properties of MA gel beads by measuring decomposition (%) of H_2O_2: The effect of the strength of the static magnetic field on the preparation of MA gel beads, the effect of the ferrite concentration in MA gel beads and the effect of the strength of an alternating magnetic field. This paper presents the result that the decomposition (%) of H_2O_2 increased under the influence of an alternating magnetic field by around 60%, as compared with no magnetic field by using MA gel beads of κ-carrageenan, like those of alginic acid.

Experimental

Materials. The Sr-ferrite used was the same as that mentioned in a previous paper.[1)] The catalase used was a product of Boeringer Mannheim Co. made from bovine lever (26×10^4 U cm^{-3}). κ-Carrageenan and the other chemicals used were of guaranteed grade or the best commercially available.

Preparation of MA Gel Beads. A mixture containing 3.5% κ-carrageenan and 0—17% Sr-ferrite in 0.5% NaCl was warmed to more than 60 °C. κ-Carrageenan was used as a carrier of enzyme.[11)] Into 19 parts of the mixture after dissolving κ-carrageenan, 1 part of the catalase solution was added. This mixture (kept at 55 °C) was dripped into a 0.1 mol dm^{-3} KCl solution at 25 °C by a syringe. MA gel beads (about 3 mm in diameter) were formed immediately after each droplet had fallen into the KCl solution in a vessel, which was placed in various static magnetic fields of 0—2500 Oe (lines of magnetic flux were parallel to horizontal). κ-Carrageenan made gel much easier than did alginic acid. MA gel beads were allowed to stand for half an hour in the vessel. Figure 1 shows a picture of MA gel beads aligned in a static magnetic field.

Equipment and Analysis. The same equipment, including a coil which generates an alternating magnetic field (50 Hz) introduced along the axis of the column, and a column (1 cm in inside diameter, 8 cm in bed height of gel beads) were used, as in a previous paper.[1)]

The concentration of O_2 dissolved in the elution passed

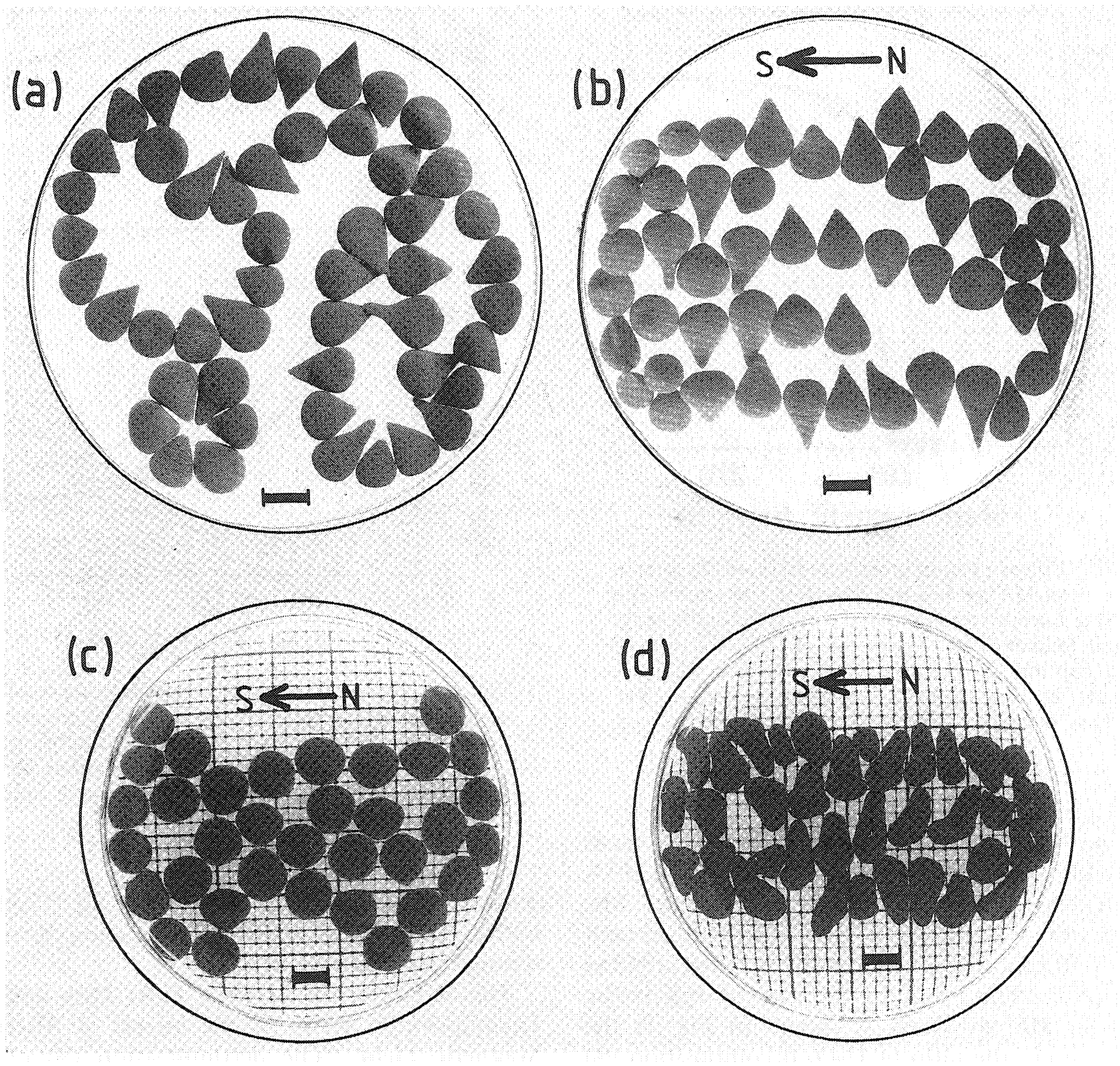

Fig. 1. A picture of MA gel beads. MA gel beads, which were prepared in static magnetic field of 1600 Oe, contained 3.5% κ-carrageenan, the fixed amount of catalase and 2.5—15% Sr-ferrite. (a) Ten % ferrite containing MA gel beads were placed in no magnetic field. (b) The same MA gel beads aligned in static magnetic field of 150 Oe. MA gel beads contained 2.5% (c) and 15% (d) ferrite aligned in static magnetic field of 150 Oe. In the figure, the direction of magnetic flux is shown by arrows, and the bar, ——, is expressed as 3 mm long. ($Oe=10^3(4\pi)^{-1}\,A\,m^{-1}$).

through the column was measured by an O_2 electrode (Able, DG-5G) connected to a voltammeter (Kikusui Electronics, 116A) and a vertical pen recorder (Yokogawa, type 3056).

Results and Discussion

Effects of Static Magnetic Field on the Preparation of MA Gel Beads. When a suspension containing κ-carrageenan, catalase and Sr-ferrite in a syringe was dripped into a 0.1 mol dm^{-3} KCl solution, each droplet was found to make a gel bead with a tear-like shape. This was different from alginic acid MA gel beads which formed with a spherical shape, as reported in the previous paper.[1] The different shape of κ-carrageenan MA gel beads resulted from the properties of κ-carrageenan, which was more viscous in solution and made gel easier than did alginic acid.

The MA gel beads which were prepared in the static magnetic field were transfered into a region with no magnetic field. It was found that MA gel beads exhibited a magnetized property; MA gel beads made a chain caused by pulling against each other (Fig. 1a). The same MA gel beads were again placed in a static magnetic field. The beads were then found to align parallel to the lines of the magnetic flux (Fig. 1b). It seems that the shape of the MA gel beads depends on the fluidity of the suspension, the fluidity of which is reduced by increasing the ferrite concentration. The shape transformed to round for MA gel beads containing less than 10% ferrite, and to a rod shape for MA gel beads containing more than 15% ferrite (Fig. 1c, 1d). In the case that the strength of static magnetic field was more than 2 000 Oe, the shape of the MA gel beads

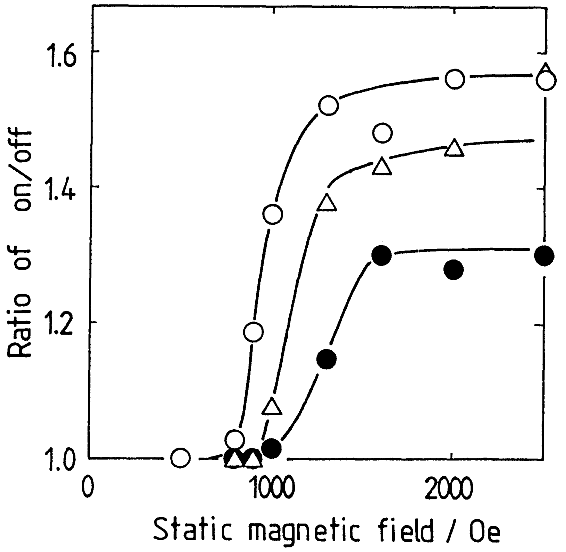

Fig. 2. Effects of static magnetic field on the preparation of MA gel beads. Ten % ferrite containing MA gel beads were prepared in various static magnetic field at 55 °C. Flow rate of H_2O_2 solution in the column: 25 $cm^3 min^{-1}$. Alternating magnetic field (50 Hz) applied; —●—: 100, —△—: 300, —○—: 600 Oe.

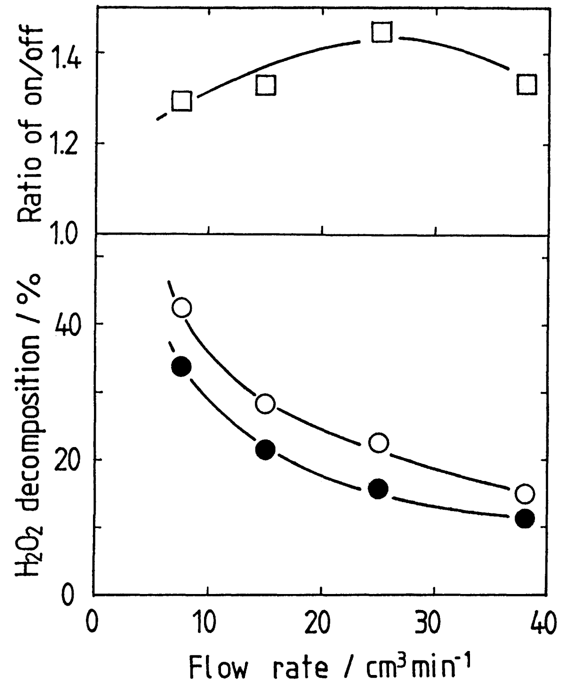

Fig. 3. The relationship between flow rate and H_2O_2 decomposition ratio in the on/off of alternating magnetic field. Ten % Sr-ferrite containing MA gel beads were prepared in static magnetic field of 1600 Oe at 55 °C. Alternating magnetic field (50 Hz) applied: —●—: 0, —○—: 300 Oe.

transformed to conic form.

MA gel beads prepared under various static magnetic fields were filled in a column, and a H_2O_2 solution was flowed through the column. The decomposition (%) of H_2O_2 to O_2 was determined with and without exposing an alternating magnetic field to the column. The result in the case of 10% ferrite containing MA gel beads is shown in Fig. 2; the ordinate shows the ratio of H_2O_2 decomposition (%) between on-and-off states in the alternating magnetic field, and the abscissa shows the strength of the static magnetic field for the preparation of MA gel beads. The effect of an alternating magnetic field on the H_2O_2 decomposition (%) appeared in the case that MA gel beads were prepared in a static magnetic field of more than 800 Oe. It therefore looks like there is a threshold value of static magnetic strength, as described in the previous paper concerning of alginic acid MA gel beads.[1] The stronger the static magnetic field above the threshold value was, the more the alternating magnetic field caused H_2O_2 decomposition (%). However, ferrite particles were found to leak from the MA gel beads in the process of gelling under exposure of a static magnetic field of more than 2000 Oe.

The Movement of MA Gel Beads in the Alternating Magnetic Field. In the case of alginic acid MA gel beads, ferrite particles align along the long axis of gel beads, as described in the previous paper.[1] On the contrary, κ-carrageenan MA gel beads indicate the direction of the NS polarity to be perpendicular to the long axis of gel beads as shown in Fig. 1. It was observed by slow-motion video that MA gel beads were both swinging with spinning (or twisting) in an alternating magnetic field of 50 Hz.

The Relationship Between Flow Rate and H_2O_2 Decomposition Ratio in the on/off of Alternating Magnetic Field. The decomposition (%) of H_2O_2 with or without an alternating magnetic field was compared by changing the flow rate of a 15 ppm H_2O_2 solution containing 0.1 mol dm^{-3} KCl and 20 mmol dm^{-3} tris-HCl buffer (pH 7.3, 25 °C) in the column. The result is shown in Fig. 3. The decomposition (%) of H_2O_2 increased, reaching 100% by decreasing the flow rate of the H_2O_2 solution in the column. The curve for the case without a magnetic field in Fig. 3 (bottom) corresponds to that of the H_2O_2 decomposition (%) vs. the reciprocal of the time passed through the column. The H_2O_2 solution in the column was mixed well by the intense oscillation of MA gel beads under an alternating magnetic field. Figure 3 (top) shows the ratio of H_2O_2 decomposition (%) between the on-and-off states of the alternating magnetic field against the flow rate of the H_2O_2 solution in the column. As the optimum flow rate, which results in a maximum on/off ratio of H_2O_2 decomposition (%), 25 $cm^3 min^{-1}$ was used for the experiments thereafter.

In this connection, this experiment under the optimum conditions (Fig. 3) was continued for 58 hrs in order to determine whether or not enzymes might leak

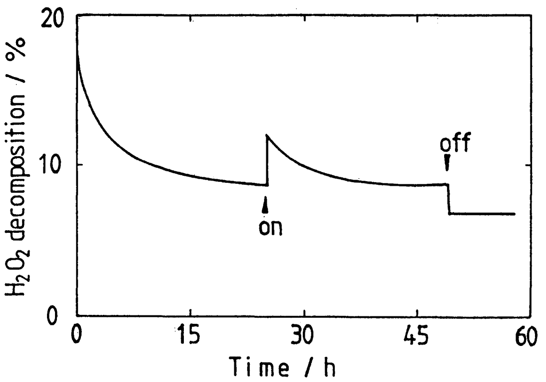

Fig. 4. The curve of H_2O_2 decomposition (%) with the lapse of time in off and on of alternating magnetic field. Ten % Sr-ferrite containing MA gel beads were prepared in static magnetic field of 1600 Oe at 55 °C. Alternating magnetic field (50 Hz) applied: 300 Oe. Flow rate of H_2O_2 solution in the column: 25 $cm^3 min^{-1}$.

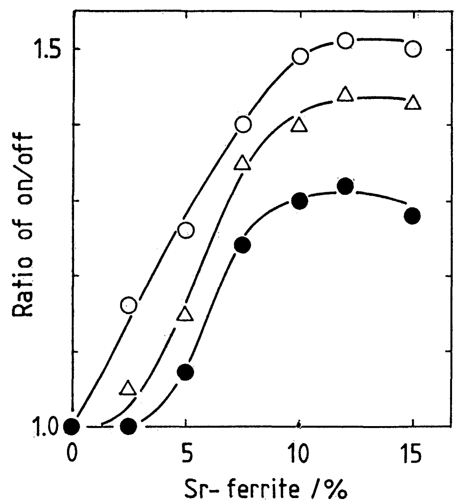

Fig. 5. Effects of alternating magnetic field and Sr-ferrite concentration on H_2O_2 decomposition ratio. MA gel beads were prepared with various concentration of Sr-ferrite in static magnetic field of 1600 Oe at 55 °C. Alternating magnetic field (50 Hz) applied: —●—: 100, —△—: 200, —○—: 400 Oe. Flow rate of H_2O_2 solution in the column: 25 $cm^3 min^{-1}$.

from the MA gel beads. The results are shown in Fig. 4. The H_2O_2 decomposition (%) was found to decrease with the lapse of time under no magnetic field. The trend of decreasing H_2O_2 decomposition (%) did not change, even though H_2O_2 decomposition (%) increased after turning the magnetic field (300 Oe) on. It was thought that catalase in MA gel beads was leaking steadily. The leakage of catalase was confirmed by measuring the H_2O_2 concentration in the H_2O_2 solution passed through the column. The H_2O_2 concentration was found to decrease after allowing it to stand for several hours, due to the presence of catalase in the eluted solution. However, the concentration ratio of catalase in the eluted solution was estimated to be less than 10^{-5}, as compared with that inside the MA gel beads. For a further investigation, prevention against the leakage of catalase must be devised.

Effects of Alternating Magnetic field and Sr-ferrite Concentration on H_2O_2 Decomposition Ratio. The effect of an off and on alternating magnetic field on the ratio of H_2O_2 decomposition (%) was surveyed by varying the strength of the alternating magnetic field as well as the ferrite concentration in the MA gel beads. A catalase reaction was carried out in the column under the same flow-rate and temperature conditions as described above. MA gel beads were prepared in a static magnetic field of 1600 Oe. The result in Fig. 5 shows that a higher ratio of H_2O_2 decomposition (%) between the on-and-off states of the alternating magnetic field was obtained by increasing the field strength and increasing ferrite concentration in MA gel beads. We obtained the result (not shown) that the curve produced upon applying 600 Oe of alternating magnetic field was almost superposed on that of 400 Oe (Fig. 5).

Ferrite powder was found to leak from MA gel beads, which contained more than 15% ferrite as the strength of alternating magnetic field was increased. The higher concentration of ferrite made beads so fragile and oscillation so strong that beads were crashed through collisions with each other. For more than a 10% ferrite content in the MA gel beads, the ratio of the H_2O_2 decomposition (%) between the on-and-off states of the alternating magnetic field was not linearly related to the ferrite concentration and field strength. As the ferrite concentration increased, the MA gel beads changed from spherical to rod shape

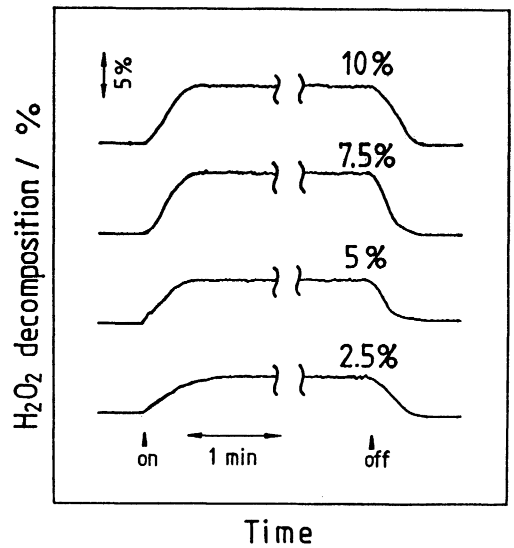

Fig. 6. Magnetic response after on and off of alternating magnetic field. MA gel beads were prepared with 2.5—10% Sr-ferrite in static magnetic field of 1600 Oe at 55 °C. Alternating magnetic field (50 Hz): 400 Oe.

(Fig. 1). The magnetic moment of the MA gel beads with a rod shape had to be reduced, because the distance between both magnetic poles of north and south is shorter than that in spherical shape. Rod MA gel beads were observed to make spinning about the NS-axis more difficult than spherical MA gel beads. Therefore, the ratio of the H_2O_2 decomposition (%) between the on-and-off states of the alternating magnetic field can decrease under higher ferrite concentrations. If spherical MA gel beads were used, the H_2O_2 decomposition (%) might increase upon increasing the ferrite concentration. It is suggested that the curves in Fig. 5 show both the effects of acceleration and decceleration of H_2O_2 decomposition.

The response on the H_2O_2 decomposition (%) immediately after turning the alternating magnetic field on was found to quicken upon increasing the ferrite concentration in the MA gel beads and upon increasing the strength of the alternating magnetic field (Fig. 6). The time for the H_2O_2 solution to pass through the column was estimated to be around 10 seconds. It therefore takes more than 10 seconds to reach a steady state in the column after turning the magnetic field on, even though a plug flow of the H_2O_2 solution is assumed to occur. Because the height of the column bed of the MA gel beads was observed to increase due to the movement of MA gel beads in an alternating magnetic field, it is reasonable that the response time to reach a steady state takes 20—30 seconds for MA gel beads containing more than 5% ferrite (Fig. 6). It is speculated that 2.5% ferrite containing MA gel beads, which have a smaller magnetic moment, may require a time-lag in order to move by overcoming static friction.

On the other hand, no effect was observed in the process of relaxation after turning magnetic field off.

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