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Experimental Verification of Fermentation Acceleration by Peristaltic Pump

-Initial Investigation of Fermentation Acceleration of Lactic Acid Bacteria by Fermentation Substrate made of Gel Material-*

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***Abstract*— In this study, we attempt to accelerate fermentation using a device that mimics the peristaltic movement of the intestine. This is based on the fact that the intestine promotes efficient digestion through peristalsis and fermentation in the intestine. In the intestine, good bacteria are conducting fermentation. Good bacteria have a role in regulating the intestinal environment through fermentation. And, there is a possibility that the acceleration of fermentation is effectively related to the movement of the intestines, such as peristalsis. Therefore, we aim to clarify the relationship between fermentation and peristalsis and further accelerate fermentation using a device that mimics peristalsis. In this paper, as an initial study, experiments were conducted to check the progress of fermentation of materials of different hardness using the device. The results showed that the crushing capacity of the device was low, but its ability to spread liquids was high. This results suggest that fermentation can be controlled by changing the physical characteristics of the fermentation substance and using a peristaltic pump to accelerate fermentation in combination.**

I. INTRODUCTION

The entire world is working toward the widespread use of renewable energy. There is growing interest in the use of bioenergy as one of these technologies. [1,2] One example of bioenergy is methane gas. Methane gas is produced from biomass through methane fermentation, and is considered to be an effective countermeasure against global warming in that it does not involve burning biomass. However, the production of bioenergy is time-consuming and difficult to spread. Therefore, there is an urgent need to accelerate the fermentation process to produce bioenergy efficiently. Accelerated fermentation is required not only in the field of bioenergy, but also in the production of fermented foods, known as health foods, and in the manufacture of pharmaceuticals, in order to produce them efficiently and in large quantities.

There are biological/chemical and physical methods to accelerate fermentation. [3,4] Biological/chemical acceleration methods are those that create an environment suitable for the microorganisms that conduct fermentation, such as the number of bacteria, temperature, and pH. Physical promotion methods accelerate fermentation by crushing, diffusing, and mixing materials to help the microorganisms break down the fermented material. Both methods are used in existing fermentation processes, but while biological and chemical promotion methods are used both before and during fermentation, physical promotion methods are used only before the start of fermentation in many fermentation processes, and there are few examples of physical promotion being used during fermentation. However, we believe that it is necessary to develop devices that focus on physical acceleration methods for efficient fermentation in order to meet the future demands for large-scale, high-quality, and diverse fermentations.

The intestines of humans and herbivorous animals contain many fermentative microorganisms such as lactic acid bacteria. These microorganisms constitute a bacterial layer called the intestinal flora, which promotes the decomposition of food masses and nutrient absorption through internal fermentation [5]. In addition, digestive organs such as the stomach and intestines induce fluid movement by peristaltic and segmental movements, which crush, diffuse, mix, and transport the food mass [6]. These movements promote efficient digestion of the food mass, which is the substrate for fermentation, through the interaction of physical action (decomposition and diffusion) and biological and chemical action (fermentation). Therefore, we consider that the movement of the food mass induced by peristaltic movement has crushing, diffusion, squeezing, and permeation effects on fermentation substrates with various mechanical characteristics.

The authors have developed a peristaltic mixing pump (hereafter, peristaltic pump) based on the peristaltic motion of the intestine shown in Fig. 1[7]. This device is able to mix and convey high-viscosity fluids and solid-liquid mixtures and has been applied to mixing and conveying solid rocket fuel and lifting earth and sand for construction [8]. Furthermore, it has a sealing function for anaerobic bacteria. Therefore, it is useful as a device for verifying the physical diffusion action of fermentation, and we consider that it can be easily introduced into industrial chemical processes. Based on the above, this study aims to develop a peristaltic bioreactor with structural and control modifications to the peristaltic pump.

In this paper, as an experimental study, we conduct experiments to verify the effect of the peristaltic pump on fermentation promotion of fermentation substrates with various physical characteristics as an initial study. In this paper, we verify whether there is a difference in the degree of fermentation when the driving conditions of the device are changed using three different hardnesses of the gel substrate.

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Figure 1. Peristaltic mixing pump [9]

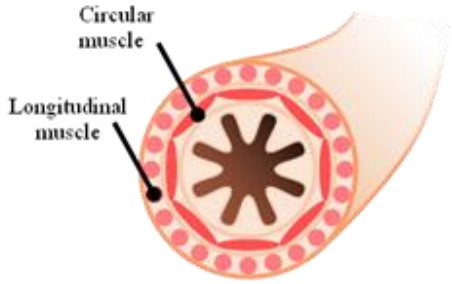


Figure 2. Structure of intestinal tract[10]

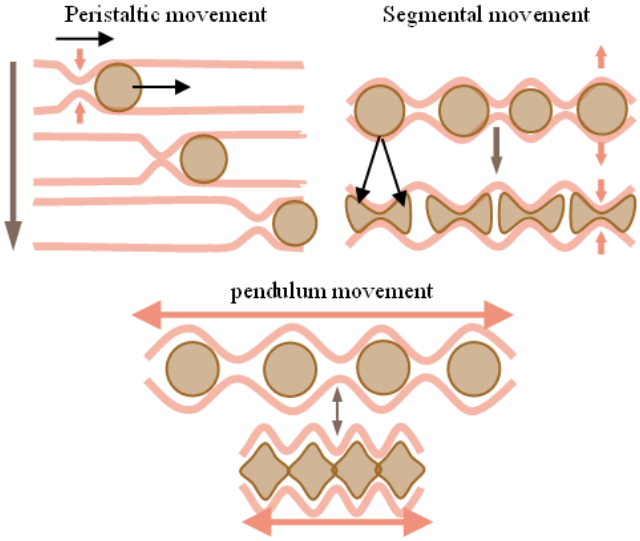


Figure 3. Types of intestinal movements [9]

The contributions of this paper are as follows.

- Fermentation can be slowed down by hardening the subject of fermentation.
- Dynamic mixing with a peristaltic pump can accelerate fermentation and restart fermentation.

II. Fermentation

Fermentation means that microorganisms break down substances to produce beneficial effects on humans. If it is not beneficial, it is called putrefaction. Fermentation is used not only for foods, but also for pharmaceuticals, dyes, and biomass utilization. There are three main types of bacteria that carry out fermentation: molds, yeasts, and bacteria. Molds include mold, blue mold, and cuttlebugs. Yeast is essential for the production of alcohol. In addition to beer yeast, wine yeast, sake yeast, and

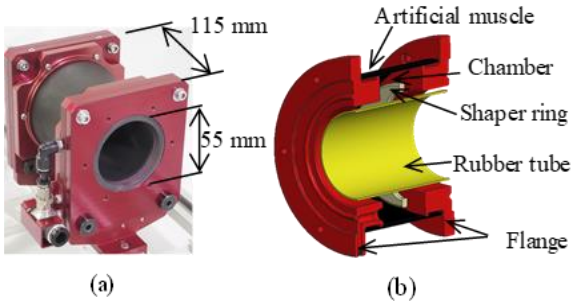


Figure 4. The single unit of Peristaltic Mixing Pump.(a) The unit appearance, and (b) The unit sectional view[11]

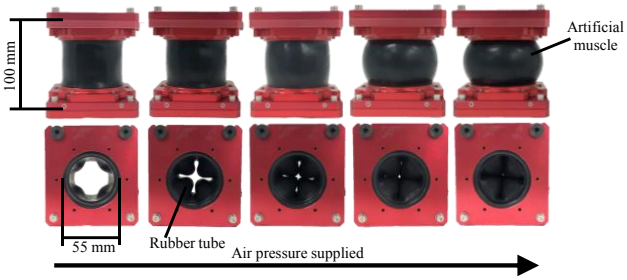


Figure 5. State of the driven conveyor unit. By supplying air pressure to the chamber, the rubber tube and artificial muscle expand respectively [9]

other yeasts used in sake brewing, yeast is also used to make bread. Bacteria are smaller microorganisms than molds and yeasts and increase in number through repeated cell division. Bacteria include lactic acid bacteria used in yogurt, cheese, and pickles, as well as acetic acid bacteria and natto (fermented soybeans).

Microorganisms that ferment can be classified as either aerobic or anaerobic, depending on their need for oxygen. Aerobic fermentation occurs only in the presence of oxygen. On the other hand, anaerobic fermenting bacteria can ferment without the presence of oxygen. Fermentation can also be classified according to the substances produced after the reaction. Alcoholic fermentation is a fermentation in which alcohol and carbon dioxide are produced from sugar by yeast, lactic acid fermentation in which lactic acid is produced by lactic acid bacteria, and methane fermentation by methanogenic bacteria. These are anaerobic fermentations, while acetic acid fermentation, in which acetic acid is produced from alcohol by the oxidizing ability of acetic acid bacteria, is a typical example of aerobic fermentation. This paper deals with lactic acid fermentation using yogurt. Fermentation of yogurt means that lactic acid bacteria in yogurt break down lactose contained in milk to produce lactic acid. The lactic acid bacteria are not self-running, and the milk and lactic acid bacteria must be mixed by self-diffusion of the liquid or artificial mixing.

III. Mixing and Transferring Device Based on Peristalsis of Intestines

A. Intestinal Structure and Movement Technique

The intestinal tube is composed of a muscle layer of annulus and longitudinal muscles as shown in Fig. 2 [12]. The annulus muscles are arranged in an annular pattern within the intestinal tube, and the longitudinal muscles are arranged along the axial

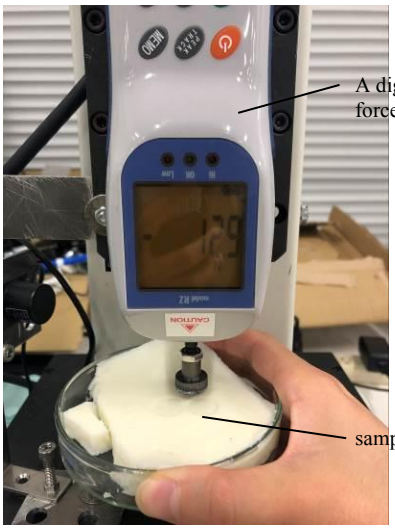


Figure 6. Measurement of agar hardness

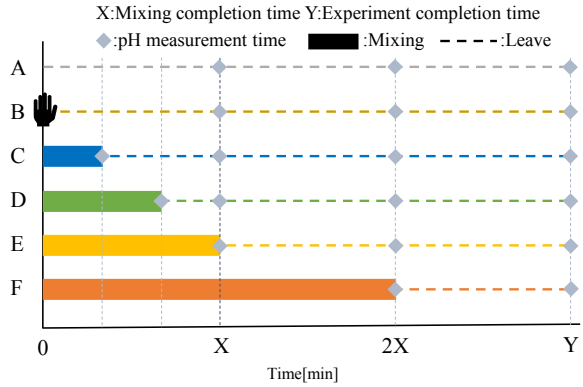


Figure 7. Mixing conditions and pH measuring time

direction. These muscles contract and relax to perform the opening and closing movements of the intestinal tube. As shown in Fig. 3, the intestinal tract is responsible for mixing and transporting food masses with digestive fluids by flexibly switching movement patterns such as peristalsis, segmentation, and pendulum motion.

B. *Peristaltic pumps*

This section introduces a mixing and conveying device based on the intestinal tube of an organism (hereafter referred to as a peristaltic pump). The appearance and cross-sectional view of a single unit are shown in Fig. 4. Fig. 5 shows the driving mechanism of the device. By applying compressed air to the chamber space between the rubber tube and the artificial muscle, the rubber tube is closed inside the tube. At the same time, the artificial muscle expands radially and contracts axially to close the tube and transport the fluid. The shaper ring contributes to the stable closure of the rubber tube. Several units can be connected and driven separately to simulate intestinal motion. By changing the unit to which compressed air is applied, peristalsis, which is the motion of transporting food masses in the intestine, segmentation, which is the motion of mixing food masses, and pendulum motion, which performs both functions, can be reproduced.

TABLE I. HARDNESS MEASUREMENT VALUE

Mass ratio (Milk:Agar[g])	100:1	100:1.5	100:2
Hardness measurement value[N]	3.3	5.6	8.8

TABLE II. MIXING CONDITIONS

A	Conditions for leaving the mixture unmixed
B	Conditions for mixing by hand for 1 minute and then leaving it alone
C	Condition to mix with the device for 1/3 of the mixing completion time.
D	Condition to mix with the device for 2/3 of the mixing completion time.
E	Conditions to be mixed by the device only for the mixing completion time.
F	Conditions for mixing in equipment for twice the mixing completion time

C. *Fermentation acceleration method using a peristaltic pump*

Fermentation is accelerated by placing a substance to be fermented (fermentation substrate) in a peristaltic pump and driving the pump to adapt a physical diffusion action that mimics the movement of the intestines. The effective driving method is expected to vary depending on the physical characteristics of the fermentation substrate, such as viscosity and hardness, and the type of microorganisms contributing to fermentation. In particular, lactic acid bacteria, including yogurt bacteria, do not have a self-propelled function, so physical diffusion action is necessary to break down fermentation substrates with high hardness. Compared to a stirring-blade type mixing device, the advantages of this device are that it can continuously produce fermentation products and that it can both mix and convey, which saves space.

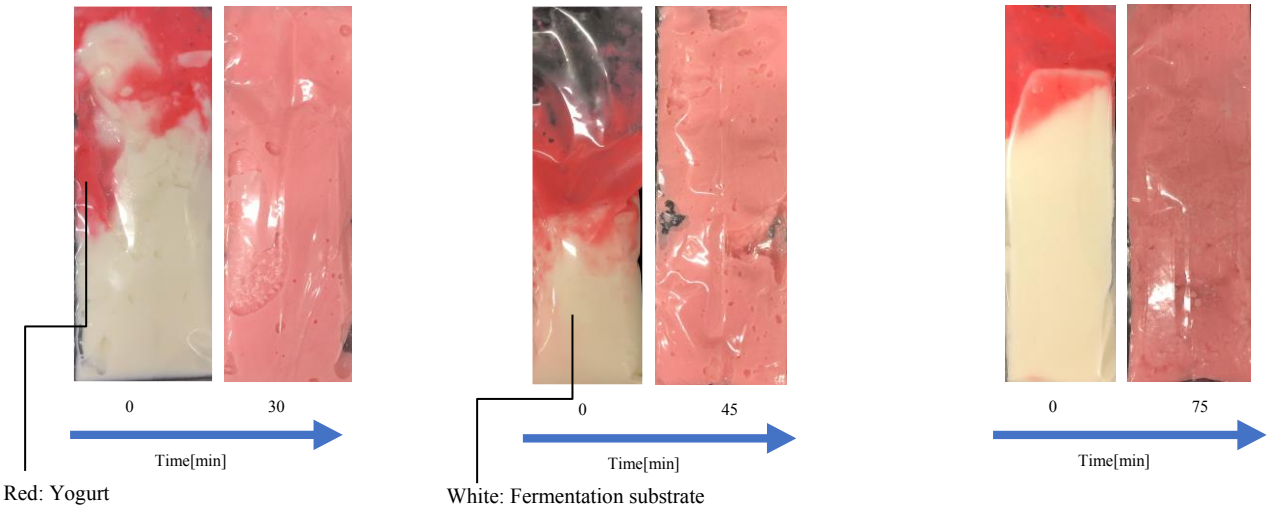
IV. EXPERIMENTS TO ACCELERATE FERMENTATION OF HIGH-HARDNESS SUBSTANCES

The purpose of this experiment was to verify whether the physical diffusion action of a peristaltic pump can accelerate fermentation of hard substances while mixing them at the same time. The target substance was powdered agar dissolved in milk. As mentioned in Chapter 2, the degree of fermentation can be estimated from the pH value because the pH decreases with the formation of lactic acid in lactic acid fermentation.

A. *Experimental Methods*

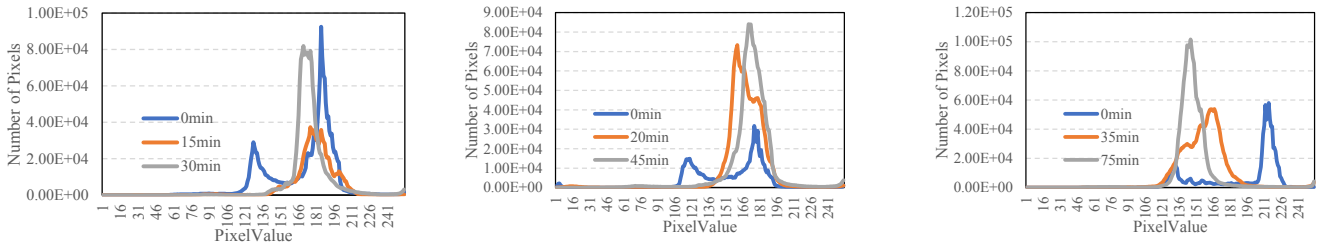
A. I *Fermentation substrate*

In this experiment, a mixture of milk and agar powder was used as the object to be mixed. The mixture of milk and agar powder was heated in a microwave oven until it was just before boiling, the agar powder was completely dissolved, and then the mixture was placed in a metal pad and cooled at room temperature for 1 hour. This cooled mixture is hereafter described as the fermentation substrate. In this experiment, three different hardness ratios of milk and agar powder were tested: 100:1 (low hardness), 100:1.5 (medium hardness), and 100:2(high hardness). The number of trials was three times for each.



(a) mass ratio of 100:1 (b) mass ratio of 100:1.5 (c) mass ratio of 100:2

Figure 8. Mixing completion time measurement



(a) mass ratio of 100:1 (b) mass ratio of 100:1.5 (c) mass ratio of 100:2

Figure 9. Changes in the histogram when measuring mixing completion times

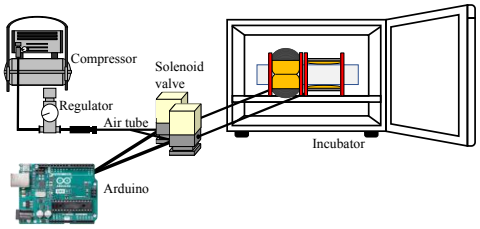


Figure 10. Experiment Environment

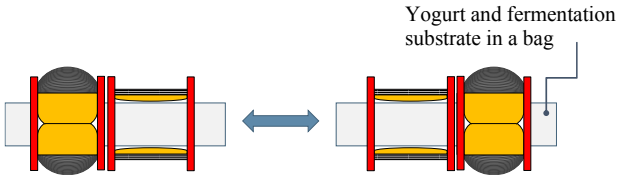


Figure 11. Mixing method

TABLE III. VISCOSITY OF FERMENTATION SUBSTRATE TABLE TYPE STYLES

Mass ratio (Milk:Agar[g])	100:1	100:1.5	100:2
Mixing completion time[min]	30	45	75

A. II Mixing conditions

Experimental samples were mixed under the six mixing conditions shown in Table 2. A schematic diagram of the experimental conditions is shown in Fig. 7.

The mixing completion time (X in Fig. 7) shows the time when the samples were completely mixed by the apparatus. The mixing completion time was measured in a preliminary experiment. The measurement method used in the preliminary experiment was as follows.

(1) Take appropriate amounts of yogurt and food coloring into a container and mix them to make the color uniform.

(2) Place 80 g of fermentation substrate in a polyethylene bag.

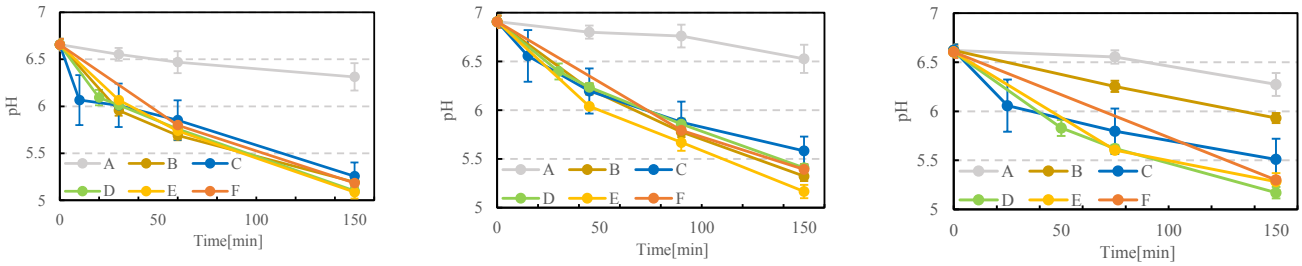
(3) Add 8 g of the colored yogurt on top of the fermentation substrate.

(4) Remove as much air as possible and close the mouth of the bag with a heat sealer.

(5) Place the bag in the pump and activate the pump.

(6) Remove the bag every 5 minutes to check the colored area.

(7) The time when the entire bag is uniformly colored is the time when the mixing is complete.



(a) mass ratio of 100:1 (b) mass ratio of 100:1.5 (c) mass ratio of 100:2

Figure 12. Time variation of the pH values of the fermentation substrates

Table 3 shows the mixing completion time for each viscosity, and Fig. 8 shows how it looked at this time. The same amount of yogurt was placed in the bag, although the amount of yogurt appears to be different because more colored yogurt went behind the agar. The histogram at this time is shown in Figure 9. The histograms are concentrated in one place over time, indicating that the colors are uniform within the angle of view. Therefore, it was checked that the yogurt and the fermentation substrate were mixed over time. In this study, mixing was considered complete when the standard deviation of the histogram was less than 18.

A. Ⅲ Mixing experiment

Changes in pH were measured after the fermentation substrates were mixed under the mixing conditions described above. 80 g of fermentation substrate and 8 g of yogurt were put into polyethylene bags in turn, and after removing as much air as possible, the bags were closed with a heat sealer. Six of these bags were used as experimental samples.

The experimental environment for the mixing test is shown in Fig. 10. The experiment was done in an incubator set at 40°C. The device was left in the incubator for about 20 minutes in advance, and the temperature inside the device was set to the same condition as that in the incubator. The sample was left on the metal plate for the leaving time, and the experimental sample was inserted inside the device for the mixing time using the device, as shown in Fig. 11. The peristaltic pump was driven with an applied pressure of 0.04 MPa and a drive cycle of 5 s. The two units were opened and closed in turn.

A. Ⅳ pH Measurement

pH Measurement

The relationship between mixing conditions and pH measurement time is shown in Fig. 7. The bottom of the bag was cut about 5 mm, and 2 g of the contents were squeezed out and measured with a pH meter (pH-33B, HORIBA).

The pH values for conditions A and B were measured at the moment the bag was allowed to stand still, which was the 0-minute data period. The data were measured when 150 minutes had elapsed, which is the time when mixing was completed by the device and the time when condition F was finished.

For conditions C-F, the start of the apparatus drive was set to 0 min. Conditions C and D, in which the drive ends before the mixing completion incident, were measured four times: at the respective drive completion times, the mixing completion time, the time at which condition F ends, and 150 minutes. Condition E was measured three times: the time when the drive was completed and the time when condition F ended, 150 minutes. Condition F was measured at the time the device drive was completed and 150 min.

B. Results and Discussion

The pH measurement results are shown in Fig. 12(a)-(c) in order of decreasing hardness. The trial-to-trial variation was large for all hardness levels. Especially for the low and medium hardness, there was no relationship between the time of mixing in the device and the decrease in pH. This may be due to the low crushing capacity of the peristaltic pump and the easy flowability, which caused only the highly fluid yogurt to spread throughout the bag before breaking the agar. The pH may have been low because the yogurt spread throughout the entire bag even when the mixing time was short.

It turned out to be a disadvantage that it takes time to spread the bacteria to the inside of the agar. On the other hand, the ability to spread the bacteria widely inside the bag around the outside of the agar was found to be high, which may have the effect of speeding up the initial speed of fermentation. We suggest that fermentation can be controlled by using agar as the fermentation substrate, slowing down fermentation by making it less fluid, and then using a peristaltic pump to accelerate fermentation in combination with the agar.

I. Conclusion

In this paper, we proposed the use of a peristaltic pump to accelerate fermentation, focusing on the physical diffusion effect of fermentation. Fermentation using yogurt as a fermentation substrate was tried on samples with three different hardness conditions by driving the pump alternately. As a result, it was found that the time to complete mixing was long due to the low crushing capacity, but the time to spread the bacteria throughout the whole mixture was short. Therefore, we consider that this device is effective for

efficient fermentation by physical diffusion. It was also suggested that fermentation could be controlled by using this device in combination with a fermentation substrate with low flowability. Future experiments will be conducted on porous fermentation substrates to confirm how the device acts on physical characteristics other than viscosity and hardness. We will also improve the mechanism to increase the crushing capacity. In terms of control, a feedback control system will be installed. Also, compare crushing capacity, etc. with agitating blade type mixing equipment.

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