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A Newly Developed Wastewater Treatment Using Solidification Reaction of Milk Fats and Proteins Through Ozonation

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Environmental regulations for dairy farms and milk plants tend to be tight in Asian countries, particularly in Japan, because if the wastewater discharged from them contains milk, it can cause serious regional environmental problems. For small and medium-sized dairy farms and milk plants, we propose a novel wastewater process using ozone, which has very strong oxidative power. The proposed process is different from conventional treatments with ozonation, and rapidly reduces BOD, TOC, total nitrogen (T-N) and chromaticity of the wastewater containing milk. This new ozonation process imposed partial polymerization of unsaturated fatty acids, including carbon-double bonds, as well as simple decomposition of the acids into small molecules. Whitish, solid-appearing products formed from the polymerized fatty acids (i.e., ozonide), and insoluble proteins were adsorbed into the ozone bubbles. The solidification and purification efficiency of this process depended on the initial condition of the milk-containing wastewater. Thus, we propose a two-stage process for this wastewater treatment method. Under the initial acidic condition, the ozonation increased yield of the solid-like products compounded from the polymerized fatty acids and insoluble proteins. When these products were removed from the wastewater, pollutants (i.e., BOD, TOC, T-N and chromaticity) were simultaneously decreased. Accordingly, water quality was improved in a short period of time. Ozonation under an alkaline initial condition with a low temperature also improved water quality, despite the fact that the yield of solid-like products was small. In this condition, the solubility of the ozone was higher than under the high-temperature condition, hence the OH-radicals generated from the ozone were enhanced and decomposed the pollutants powerfully. These two reaction pathways, depending on pH and temperature, were strongly related to the decomposition of the pollutants by ozone. Thus, the ozonation under an acid condition is more useful for small and medium-sized dairy farms and milk plants, because it is not necessary to control the wastewater temperature under this condition. Although the ozonation for the wastewater treatment does require energy, the conventional floatation method consumes a much larger amount of energy.

1. Introduction

Wastewater from dairy farms and milk plants generally includes some milk, and because of this is very difficult to treat by means of conventional wastewater treatment methods. Historically, this type of wastewater has been discharged directly into rivers, particularly in many Asian countries, due to undeveloped environmental legal systems and poor wastewater treatment facilities. Rapid increase in milk production (e.g., in India and China) has caused serious local environmental impacts. In Japan, environmental problems such as stench, chromaticity and antibiotics are so serious in some suburban areas that milk farmers have been forced to shut down. The pollutant level of dairy wastewater is very high in relation to the small amount of milk produced (Omil et al., 2003).

In response to this situation, the worldwide trend is toward stricter environmental regulations for dairy farms and milk plants (e.g., EPA, 2011; EC government, 2011). Traditional microbial oxidation and anaerobic bio-

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decomposition are often used to treat wastewater including milk (WIM), (Demirel et al., 2005). Recently, artificial wetland systems have been used for wastewater treatment (Vymazal, 2009). Such conventional treatment methods, however, require additional processes (e.g., enzymatic hydrolysis of fatty acids [Jung et al., 2002]) in order to completely decompose the fats, proteins and antibiotics in the milk. As a result, the treatment of WIM by conventional methods requires a large-scale system, long treatment time, and substantial outlay of energy (which increases treatment costs and difficulty).

Small milk factories and milk farmers have thus been yearning for a new, reliable, economical and low-maintenance wastewater treatment process. The most promising option is an ozonation treatment that is already being applied in the field of urban drinking water treatment. There are few previous studies on the use of ozonation for the treatment of WIM. Only two papers have reported on the combined use of ozonation and nanofiltration for dairy wastewater (Laszlo et al., 2007, 2009), and these did not explain the purification effects on issues such as chromaticity and BOD.

The ozonation process has rarely been applied to practical wastewater treatment. One of the few examples is the treatment of olive-mill wastewater, because ozonation is highly effective in treating saturated fatty acids (Lafi et al., 2009). Since the ozonation process is also very useful for decolorization, it has been used to bleach pulp in the paper industry (Prat et al., 1990) and to decolorize the azo dyes of textile wastewater (Tokumura et al., 2009). However, the white turbidity (chromaticity) of milk is different from these substances, due to the total reflection of visible light by casein micelles and milk fat globules.

Our experimental results suggest that the expected decolorization of WIM by ozonation was due to the destruction of the hydrophobic structure of the casein micelles and milk fat globules, which were the immediate pollutants. Consequently, ozonation was found to effectively bleach and purify WIM in one treatment action. Small and medium-sized farms and milk plants will find the concept of simultaneously treating for chromaticity and pollutants in WIM both useful and attractive.

2. Experiments

2.1 Experimental setup and ozonation procedure

The experimental setup used in the present study is outlined in Figure 1. The setup consisted of a cylindrical plastic reactor vessel (a) 550 mm in diameter and 900 mm in depth, a vortex pump (b) (Nikuni, 20NPD), and an ozone generator (c) (Hamanetsu, OG-R6). The ozone generator had a type of silent electric discharge, and was provided with a discharge tube (d) and gas flow meter (e). O_2 gas was supplied to the ozone generator from an O_2 gas cylinder (f). A 250mm-diameter inner cylinder for fixing the pipe (g) was set in the reactor vessel.

The reactor vessel was filled with simulated wastewater of 0.1 m^3 (a mixture of 1 kg/m^3 raw milk and 999 kg/m^3 tap water). The properties of the initial simulated wastewater are listed in Table 1. The temperature of the wastewater was adjusted to an initial target temperature using either ice or heated water, and was maintained at this temperature ±5°C during the experiments. Initial pH of the wastewater was measured with a type of glass electrode pH meter (h) (DKK-TOA, IM-32P). The pH of the wastewater was then adjusted with the addition of HCl (if the initial pH was acid) or KOH (if the initial pH was alkaline). A type of alternating double-electrode conductivity meter (i) (DKK-TOA, CM-31P) was used to adjust the conductivity in 0.5 dS/m by the addition of KCl.

Table 1: Properties of the simulated wastewater

Chromaticity	COD_{Mn}	BOD_5	TOC	Total Nitrogen
549±131	349±26 mg /L	1,110±177 mg/L	453±105 mg/L	35.9±6.9 mg/L

The simulated wastewater was circulated through the vortex pump at a flow rate of 0.02 m^3/minute, adjusted using a Karman's vortex flow meter (j). Ozone gas from the generator was injected into the simulated wastewater through an injector (k) (Mazzei, Model 648) inserted into a downcomer set in the vessel. This deviced generated a stable supply of O_3 gas of 0.135 mol/h from O_2 gas of 23.7 mol/h. The ozone concentration of the injected gas mixture was 0.24 mol/m^3 (in mass). Power consumed by the ozone generator and vortex pump was measured with an electrical energy meter (l) (Mitsubishi M7UM-S33R).

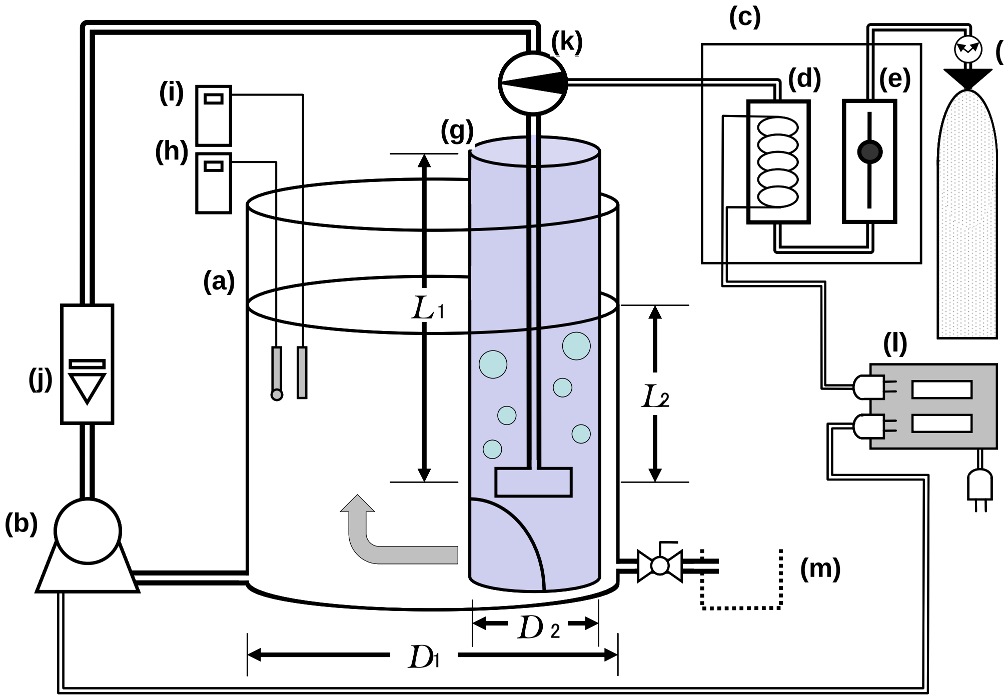


Figure 1: Diagram of the experimental setup. Pipe length of ejector to discharge position (L_1) = 105 cm, Water depth of discharge position(L_2) = 38 cm

2.2 Capture and separation of the solid-like products from the ozonized wastewater

The drain outlet of the reactor was equipped with a solid-like products catcher (nylon 40 mesh) (Figure1 (m)). After 3 hours of ozonation, the ozonized wastewater (including foam) in the reactor was discharged through the drain outlet. The solid-like products in the ozonized wastewater were separated and captured by the mesh. The recovered solid-like products were inserted into a circulation dryer (60°C) for more than 12 hours. The dried solid-like products were then weighed with an analytical balance.

A part of solid-like products were analyzed by Fourier transform infrared spectorometer (Horiba, FT-710), its transmittance was measured scanning the wavelength between 1,000cm^{-1} to 3,500cm^{-1}, using 2.0 cm^{-1} spectral resolution. In addition, infrared transmittance spectrum were recorded and searched some peaks characterized by the molecular structure. The characteristic peaks analyzed by spectral data (Badertscher et al. 2009)

2.3 Analysis of pollutant levels

The biological oxygen demand (BOD) was measured by determining oxygen consumption (concentration of residual dissolved oxygen via Winkler's method), during 5 days incubation at 20 °C with microorganisms obtained from a liquid compost at a dairy farm. Chemical oxygen demand (COD) was measured from consumption of residual potassium permanganate titrated by sodium oxalate after 5 min oxidation at 105 °C. These two methods (BOD, COD) conformed to ISO 5815 and 6060. Total organic carbon (TOC) was measured using a TOC analyzer (Shimadzu, TOC-5000). Total nitrogen was measured through a steam distillation method of thermally decomposed organic nitrogen in sulphuric acid, according to ISO 8425. The chromaticity was measured through a chromaticity sensor (Kasahara-Rika, TCE-5Z).

2.4 Floatation by O_2 gas

O_2-gas-floatation was carried out using the same setup and procedure as the ozonation experiment in order to compare the ozonation wastewater treatment with the floatation method. The pollutant levels of the floatation experiments were measured in the same way as in the ozonation experiment.

3. Results

3.1 Solid-like products by the ozonation and floatation processes

Small flocculated particles were generated in the simulated wastewater after injection of the ozone-including bubbles. Most of these particles were gradually adsorbed on the bubble surfaces, and the rest settled on the bottom of the vessel. Most of the contaminated bubbles ascended to the wastewater surface, and some of them circulated in the wastewater. Thus, the bubbles reaching the wastewater surface gradually grew into break-resistant and flexible foam (Figure 2). At the end of the ozonation process, the solid-like products were left on the degassed foam or in the aqueous solution. The products caught on the mesh were like whitish clay in appearence (Figure 3), and were 60 ~ 70 % water.

Figure 2: Foam at the upper part of reactor

Figure 3: Features of collected solid-like products

3.2 Yields of solid-like products under two pH and temperature conditions

Yields of the solid-like products changed with the pH level of the wastewater; a pH of 5.5 was a border value against the yields (Figure 4). In an acid condition with a pH lower than 5.5, the yield of solid-like products increased rapidly as pH level decreased. In contrast, yield of the solid-like products was less than 10 g under a natural-alkaline condition. This suggested that the yield of solid-like products depended on the pH level of the wastewater. On the other hand, the temperature of the wastewater did not influence the yield of the solid-like products under any pH conditions. In addition, the yield of solid-like products varied widely, even under the acid condition. These variations were thought to be due to differences in the raw milk used; i.e. the initial pollutant levels ranged from 800 mg/L to 1,450 mg/L (BOD). Figure 5 shows the close correlation between the yield of the solid-like products and the initial pollutant level (BOD).

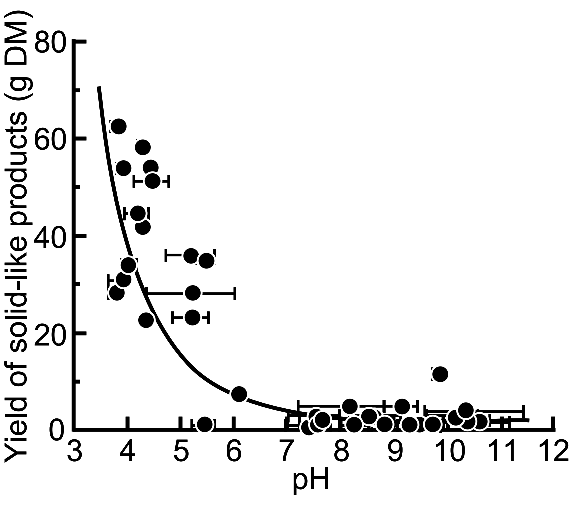


Figure 4: Effects of pH against yield of solid-like products

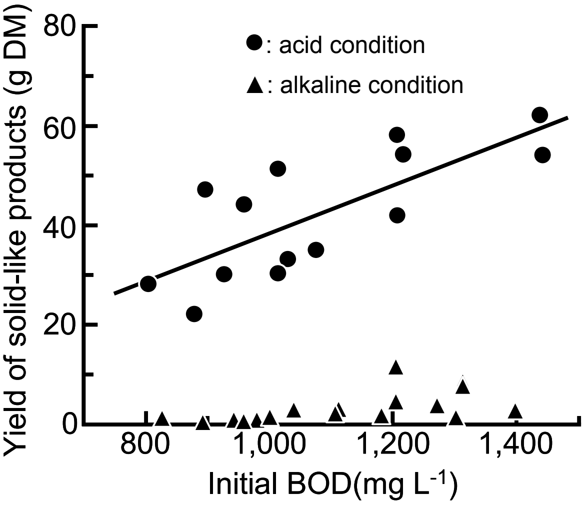


Figure 5: Effects of initial BOD against yield of solid-like products in acid or alkaline conditions

3.3 The comparison analyses of solid-like products by FT-IR

Figure 6 shows FT-IR spectrum of solid-like products (pH4.0 and pH10.0), oleic acid and whole milk powder. The spectrum of solid-like products were similar to the spectrum of whole milk powder. It had the same peaks at 1,700cm^{-1} (depends on carboxylic acid), 2,800cm^{-1} and 2,900cm^{-1} (depends on methyl group). It is indicated that the solid-like products included some fatty acids, because these peaks appeared in oleic acid. In addition, the spectrum of solid-like products had two peaks in 1,630cm^{-1}and 3,270cm^{-1} ,these peaks were derived from nitrogen compounds of casein. It shows that the solid-like products included some proteins.

Figure 7 is a detailed spectrum under 1,250cm^{-1}. The solid-like products had two peaks 1,100cm^{-1}(depends on ester bond) and 1,175cm^{-1} (depends on peroxide group). It characterize the structure of ozonide, these peaks did not appear in oleic acid and whole milk powder. In addition, the spectrum of solid-like products were disappeared the peak in 3,010cm^{-1} (depends on carbon double bond), but it was barely visible in the spectrum of whole milk powder(Fig.8).

Figure 9 shows the effect of the ozonation time against ozonide structures. The characteristic peaks in 1,100cm^{-1} and 1,175cm^{-1}, in fewer than 30 minute are appeared. It is indicated that the reactions of forming into ozonide happened at short times. These reactions did not depend on pH and temperature of wastewater, and were not changed against FT-IR spectrum in any conditions.

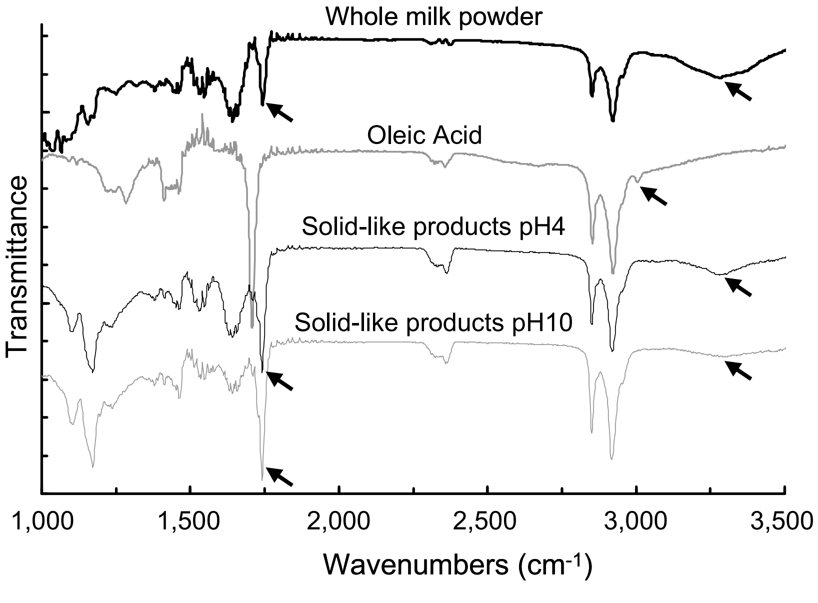
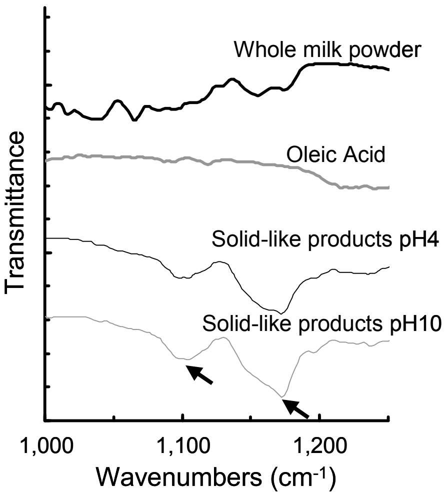


Figure 6: FT-IR spectrum analysis of solid-like products from milk ozonation



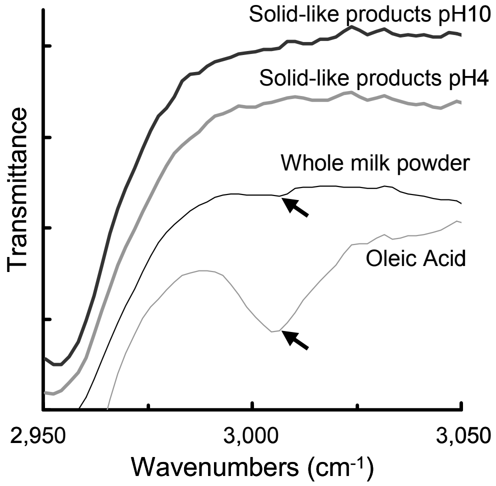


Figure 7: The detailed FT-IR spectrum under 1,250cm^{-1}

Figure 8: The detailed FT-IR spectrum over 2,950cm^{-1}

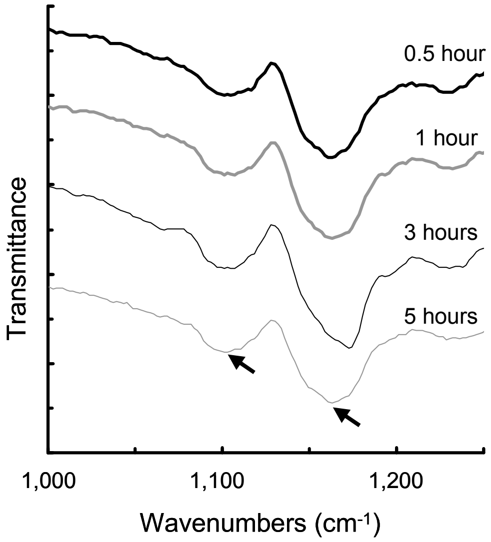


Figure 9: The effect of ozonation time against FT-IR spectrum of solid-like products

3.4 The relationship between water quality and ozone consumption under tested conditions of pH and temperature

T Water quality depended on the initial pH level and temperature of the wastewater. For chromaticity, a higher color removal ratio (CRR) of 0.88 ~ 0.99 was obtained under the acid condition at any temperature. Under the alkaline condition, the CRR took 4/5 levels of what it had under the acid condition, and only below 10 °C. However, the CRR rapidly dropped as the temperature increased. Over 25 °C, chromaticity was actually higher than the initial level (Figure 10).

The BOD removal ratio (BODRR) took higher levels of 0.65 ~ 0.83 in an acid condition. However, the BODRR decreased slightly as the temperature increased. Remarkably, in an alkaline condition, BODRR decreased as the temperature increased. At 35 °C, the BODRR took 1/4 levels of what it had at 10 °C, as shown in Figure 11. The TOC removal ratio (TOCRR) indicated a tendency similar to the BODRR in the alkaline condition. However, the TOCRR increased with an increase in temperature in the acid condition (Figure 12). Although the TNRR under the acid condition was higher than 0.8 at any temperature range, the TNRR under the alkaline condition was less than 0.4 at a low temperature range, in which the ozone gas was easily dissolved in water (Figure 13). The COD removal ratio (CODRR) was less than 0.3 under any condition. This value was lower than the CRR, BODRR, TOCRR and TNRR, as shown in Figure 14.

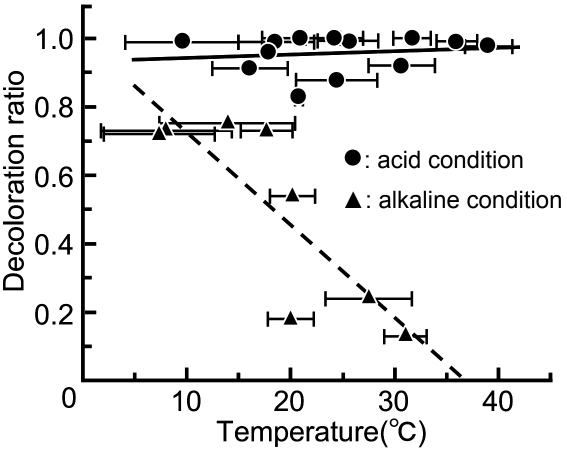


Figure 10: Decoloration ratio by ozonation in temperature and acid or alkaline conditions

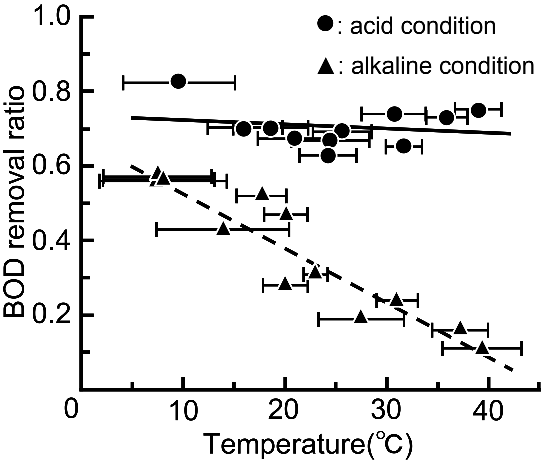


Figure 11: BOD removal ratio by ozonation in temperature and acid or alkaline conditions

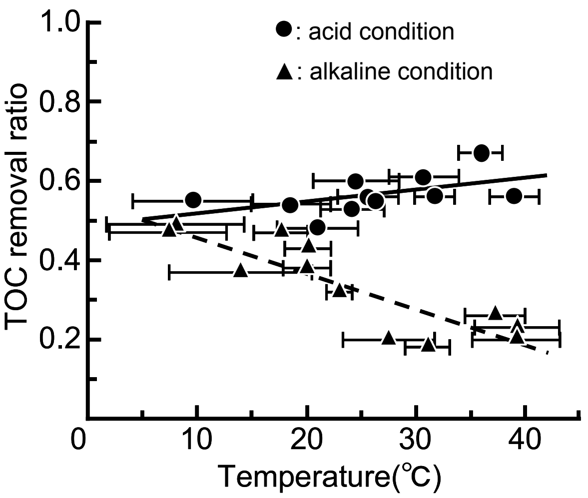


Figure 12: TOC removal ratio by ozonation in temperature and acid or alkaline conditions

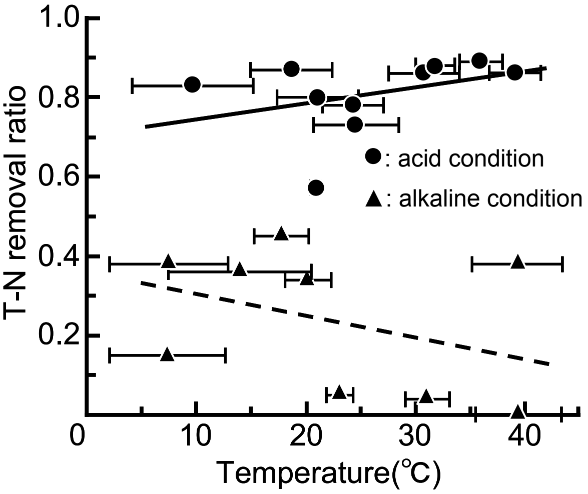


Figure 13: Total nitrogen removal ratio by ozonation in temperature and acid or alkaline conditions

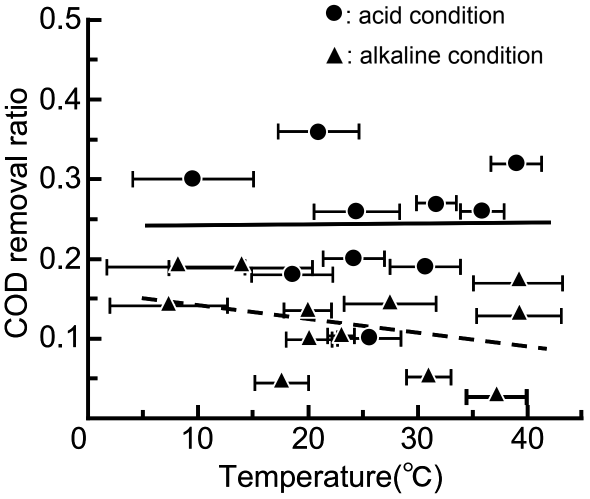


Figure 14: COD removal ratio by ozonation in temperature and acid or alkaline conditions

3.5 Comparisons of water quality and energy consumption between ozonation and O_2-floatation

The purification effects of ozonation were different from those of O_2-floatation in several respects (Table 2). Although the difference in the yield of solid-substances between them was almost nonexistent, the pollutant level of the processed wastewater with ozonation was lower than that of O_2-floatation. Chromaticity level by ozonation treatment under the acid condition was one fifth that of the O_2-floatation. Similarly, BOD level by ozonation treatment was one fourth that of O_2-floatation. These results indicate that the structures of solid-like products by ozonation were different from those by O_2-floatation.

Power consumption of ozonation and O_2-floatation was measured and compared. Although the energy required to generate ozone was higher than needed for O_2-floatation, a comparison of the power necessary to decrease 1kg of BOD (i.e. purification efficiency) in the ozonation was lower than that needed for the same reduction with O_2-floatation. Thus, using ozonation, we used 23% less energy to achieve the same purification level.

Table 2: Pollutant levels of a processed wastewater after 3 hours by the ozonation and the O_2-floatation

Content of process		Yield of solid - like products (g FM)	Chromaticity	BOD_5 (mg/L)	Power consumption (kWh)	Purification efficiency[1)] (kWh/kg)
Initial pollution		-	653	1,150	-	-
Ozonation	pH 4	179	34	158	1.32	13
	pH 7	32	185	553	1.32	22
O_2 floatation	pH 4	124	171	631	0.89	17
	pH 7	34	544	1,131	0.88	469

1) Purification efficiency = Power necessary to decrease 1kg of BOD

4. Discussion

The treatment process discussed in the present study is different from conventional ones that use the oxidative decomposition effects of ozone. The effects of pollutant decomposition by ozone are very low in conventional treatment processes; hence, they require large amounts of energy to treat wastewater including milk. Conventional treatment processes using oxidative decomposition are impractical for treating wastewater that includes high concentration pollutants such as wastewater including milk.

We have developed a new wastewater-treatment technique using a different approach. We utilized ozone in order to solidify the pollutants of the wastewater including milk, and then removed the pollutants as solid-like products. In this technique, ozone-containing bubbles were injected into the WIM and the solid-like products were adsorbed on the bubble surfaces and captured/separated from the WIM. For the practical use of this technique, we still need to investigate the most suitable conditions for increasjng the removal efficiency of the pollutants.

The most important factor is the dissolution rate of ozone into the wastewater including milk, which depends on wastewater including milk temperature. The dissolution rate of ozone is decreased with increases in temperature; for instance, the Henry's constant increases from 5.10 H_0/kPam^{3}mol^{-1}(at 5 °C) to 9.19 H_0/kPam^{3}mol^{-1}(at 20 °C) (Rischbieter et al., 2000). However, the present results showed that wastewater including milk temperature had no influence on yield of the solid-like products. The wastewater including milk pH level influenced the yield of solid-like products more remarkably than the temperature. The yield of solid-like products increased rapidly in an acid condition where pH was less than 5.5.

We speculated that three factors affected the increase in the yield of the solid-like products in the acid conditions. The first was that the milk protein (mainly casein) depressed its own solubility due to higher-order structural changes brought about by ozone attack.
The second was that the casein was likely coagulated as a “curd” in the acid condition, because the isoelectric point of casein is pH 4.6 (Schmidt and Poll, 1986).

Third, considering that this reaction was oxidation by direct reaction of ozone and unsaturated fatty acids (Criegee, 1975), in the acid conditions, the yield of the ozonide formed or polymerized by the reaction of unsaturated fatty acid and ozone must have increased. The direct oxidation of ozone and unsaturated fatty acid was active only in the acid condition, which decreased the self-decomposition of ozone.
These reactions, which produced distinctive structures by ozonation, occurred in a short time period. And we indeed found that the pollutant levels (i.e., chromaticity, BOD, TOC and T-N) of the WIM rapidly decreased with the generation of solid-like products. This fact suggested that purification through this technique was more effective than complete decomposition, because it consumed less energy than the other techniques.

Interestingly, in this study, purification through ozonation in the alkaline conditions was also effective. The alkaline conditions were considered to enhance the self-decomposition of ozone and generate many OH radicals (Ku et al., 1996). The OH radicals then immediately attacked the pollutants, degrading them into low-molecular compounds.

In the alkaline conditions, the purification ratio was depressed rapidly with increases in the wastewater temperature, however. This suggested that wastewater temperature is a limiting factor of purification under alkaline conditions; similarly, it was a limiting factor of purification in conventional techniques by ozonation process. This means that it is disadvantageous for dairy farms, because farmers discharge heated water after washing a milking machine or storage tank. On the other hand, the reaction under an acid condition is more advantageous for dairy farms, because wastewater temperature does not affect purification through the solidification process.

Thus, the pH level significantly influenced the reaction that produced solid-like products (including ozonide) and the process performance in terms of removing pollutants from wastewater including milk. In particular, pH 4.6 (isoelectric point of casein) and pH 5.0 (inflection point of the self-decomposition speed of ozone) represented the boundaries of the two types of purification reactions, namely solidification and decomposition. The solidification was polymerization and coagulation. Our study represents an innovative technique to take advantage of solidification by ozonation.

In addition, we found that ozonation was more efficient than floatation under all conditions. This fact overturns the conventional belief that ozonation requires more energy than usual wastewater treatment methods. We tentatively suggest that the ozone bubble size may be smaller than the O_2 bubble size; as a result, increased surface area might explain the more efficient purification. We will pursue this issue further in future studies.

5. Conclusion

In acid conditions below pH 5.5, the pollutants in WIM were solidified into pure ozonide, formed by reacting unsaturated fatty acid and ozone, and the proteins coagulated as “curd” due to decreased solubility. In this ozonation technique, the pollutants were removed as the solid-like products immediately, improving water quality quickly and with low input of energy. These characteristics of the new technique make it especially suitable for small and medium-sized dairy farmers.

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