Ultraviolet Sterilization of Sugar Solution Containing Spores of Obligate Anaerobes Causing Flat Sour Spoilage

(A New Type of Flat Sour Spoilage. V)

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A sugar solution inoculated with the spores causing O. A. flat sour spoilage was sterilized by the use of a commercial ultraviolet (UV) sterilizer.

The concentration of the spores in the sugar solution (45%) was decreased to 10^{-7} of the original level by treatment of the sugar solution in a continuous UV sterilizer at a flow rate of 500 L/hr, which corresponds to a UV dose of 10^4 ergs/mm². The UV dose required to reduce the number of survivors to one-tenth of the original population was 1500 ergs/mm² in the present practical experiment using a commercial UV sterilizer, and this is in reasonably good agreement with the result obtained in the laboratory.

This work confirms that prior UV irradiation of sugar solutions is an effective countermeasure to prevent O. A. flat sour spoilage of canned drinks kept hot in vending machines.

Key words: UV sterilization; sugar solution; flat sour spores; UV sterilizer; countermeasure to flat sour spoilage; canned drinks; hot vending

Introduction

In a previous paper¹⁾, the authors reported that the ultraviolet (UV) resistance of the spores causing O. A. flat sour spoilage²⁾ is not especially high, in contrast to the high heat resistance, and that UV irradiation decreases the heat resistance of the spores at least to one-half. On the basis of these results, it was suggested that prior UV irradiation of sugar solutions can be employed as a countermeasure to prevent O. A. flat sour spoilage of canned drinks kept hot in vending machines.

This paper deals with a commercial application of this principle to the sterilization of canned drinks, including UV pretreatment of sugar solutions prior to use.

Materials and Methods

Microorganisms

Strain No. 24-1, which was isolated from spoiled canned coffee²⁾, was used throughout

this work.

Preparation of Spore Suspension

The sporulation of the strain was achieved by the method described in the previous paper²⁾.

The spores in 10 L of culture were harvested by centrifugation, washed three times with 300 ml of sterilized deionized water, and suspended in 200 ml of sterilized deionized water. The spore suspension was homogenized by using a Universal Homogenizer (Nihon Seiki Seisakusho Co., Ltd.) for 1 min. These procedures were carried out at 0~4°C. The spore suspension was heated in a boiling water bath for 30 min to destroy vegetative cells. The spore suspension thus obtained was stored in a refrigerator.

Preparation of Sugar Solution Containing the Spores

Two batches of sugar solution were prepared by dissolving 120 kg of granulated sugar in 147 L of water that had been treated with activated charcoal. The volume of each sug-

ar solution was about 222 L and the Brix value was about 45%.

One of the batches was used as a "blank"; 10 ml of the spore suspension was inoculated into the other batch, to make a spore concentration of approximately 10^4 /ml.

UV Irradiation and Sampling

The UV sterilizer used was Steritron SF-1NSM, 90 W (Chiyodakohan Co., Ltd.). A diagram of the UV sterilization procedure is shown in Fig. 1. A variable flow metering pump was used to regulate the flow rate of the sugar solutions to 170, 250 or 500 L/hr, which, in the UV sterilizer, gave the sugar solutions UV doses of 3×10^4 , 2×10^4 and 1×10^4 ergs/mm², respectively.

Fig. 1. Schematic diagram of the UV sterilization of sugar solutions

First, the "blank" sugar solution was allowed to flow for 10 min at each flow rate. Then, the sugar solution containing the spores was treated in the same way. Approximately 2 L samples of the sugar solutions were taken in sterile containers at the

end of the flow rate periods as irradiated samples.

Before these treatments, samples of approximately 2 L of the "blank" sugar solution and the sugar solution containing the spores were taken in sterile containers as non-irradiated samples.

Determination of Survivors

After heat treatment at 100°C for 10 min, all the samples were inoculated into mTGC³⁾ as follows.

One hundred ml aliquots of each sample were inoculated into ten 1.5 L portions of mTGC, except for the non-irradiated sugar solution inoculated with the spores, for which the MPN method was used with mTGC.

Identification of the Survivors

To examine some principal characteristics of bacteria in the turbid mTGC media (except for those from the MPN method), 0.5 ml aliquots of the turbid mTGC media were inoculated into ISA⁴⁾, ISaA⁵⁾, TF²⁾, TSIF²⁾ and TSAF²⁾, and 1.0 ml aliquots of the turbid mTGC media were inoculated into DTA^{6),7)} and SMA⁸⁾ by the pour-plate method. All these media were incubated at 55°C for 10 days.

Results and Discussion

The numbers of the mTGC media which became turbid are shown in Table 1.

In the case of the "blank" sugar solution with no UV irradiation, seven media out of 10 became turbid, and in the "blank" with irradiation at flow rates of 500 and 170 L/hr

Table 1. Detection of Bacteria with 1.5 L mTGC Medium

Conditions of UV irradiation	Sugar solution (blank)					Sugar solution with the spores				
No irradiation (control)	+1*	+2*	+3*	+4*	+5*	**				
	+6*	+7*	—*	—*	—*					
Flow rate: 500 L/hr (1×10^4 ergs/mm ²)	+8*	—*	—*	—*	—*	+10*	+11*	—*	—*	—*
	—*	—*	—*	—*	—*	—*	—*	—*	—*	—*
Flow rate: 250 L/hr (2×10^4 ergs/mm ²)	—*	—*	—*	—*	—*	+12*	+13*	—*	—*	—*
	—*	—*	—*	—*	—*	—*	—*	—*	—*	—*
Flow rate: 170 L/hr (3×10^4 ergs/mm ²)	+9*	—*	—*	—*	—*	+14*	—*	—*	—*	—*
	—*	—*	—*	—*	—*	—*	—*	—*	—*	—*

* +, turbid; —, not turbid

The media which became turbid were numbered as in the table.

** Treated by the MPN method.

Table 2. Identification Tests with the Turbid mTGC Media

Medium No. ^{a)}	ISA ^{b)}	ISaA ^{b)}	TF ^{b)}	TSiF ^{b)}	TSaF ^{b)}	DTA ^{c)}	SMA ^{c)}
1	—	—	—	—	—	—	—
2	+	+	+	+	+	—	—
3	—	—	+	—	+	—	—
4	+	—	—	+	—	—	—
5	—	+	+	—	+	+	+
6	+	—	—	+	—	—	—
7	—	—	—	—	—	+	+
8	—	—	—	+	—	—	—
9	—	—	—	—	—	+	+
10	+	—	—	+	—	—	—
11	—	—	—	—	—	+	+
12	—	—	—	+	—	—	—
13	—	—	—	—	—	+	+
14	+	—	—	+	—	—	—

^{a)} See Table 1. ^{b)} +, blackening; —, no blackening ^{c)} +, colonies; —, no colonies

one turbid medium out of 10 media was found in each case. No turbid media were found in the case of the treatment at 250 L/hr.

In the case of the sugar solution inoculated with the spores, irradiation at flow rates of 500 and 250 L/hr gave two turbid media out of 10, and one turbid medium out of 10 was found with the treatment at 170 L/hr.

The results of identification tests are shown in Table 2. The media which showed blackening of ISA or TSiF, but no blackening of TF, TSaF and ISaA, and gave no colonies on DTA and SMA, were media Nos. 4, 6, 8, 10, 12 and 14. It is considered that Nos. 4, 6 and 8 became turbid due to the growth of contaminating microorganisms in the sugar that were similar to the bacteria used (strain No. 24-1), and that Nos. 10, 12 and 14 became turbid due to the growth of the bacteria used or similar microorganisms.

Table 3 shows the numbers of media in which turbidity was due to the growth of the bacteria used or similar microorganisms.

From the numbers of spores per ml estimated from Table 3 and by the MPN method, UV survival curves for these spores in the sugar solutions were obtained (Fig. 2). In the "blank" sugar solution used, the concentration of the spores of similar microorganisms was 2.23×10^{-8} /ml. At 500 L/hr flow rate, this value decreased to 1.05×10^{-8} /ml, and at

Table 3. Numbers of Turbid Media Containing the Bacteria Used or Similar Microorganisms out of Ten mTGC Media Tested

Conditions of UV irradiation	Sugar solution (blank)	Sugar solution with the spores
No irradiation (control)	2	/
Flow rate: 500 L/hr (1×10^4 ergs/mm ²)	1	1
Flow rate: 250 L/hr (2×10^4 ergs/mm ²)	0	1
Flow rate: 170 L/hr (3×10^4 ergs/mm ²)	0	1

the other two flow rates, the values were below 1.05×10^{-8} /ml.

On the other hand, after the treatment of the sugar solution containing the spores at all the flow rates (500, 250 and 170 L/hr) the concentration of surviving spores was 1.05×10^{-8} /ml, which is smaller than the original (2.30×10^4 /ml) by a factor of approximately 10^{-7} . The reason why the same values were obtained at all the flow rates is unknown. However, at the fastest flow rate (500 L/hr) the UV dose was 1×10^4 ergs/mm². Division of the latter by the D value for UV of this strain (1500 ergs/mm²)¹⁾ gives 6.7, which is consistent with the decrease of the concentration of the spores to 10^{-7} in the sugar solution.

It seems safe to say that at 500 L/hr flow

Fig. 2. UV survival curves for spores of strain No. 24-1 in sugar solution after treatment with the UV sterilizer
—△—, blank; —○—, strain No. 24-1

rate (UV dose of 1×10^4 ergs/mm²) the concentration of the spores of strain No. 24-1, one of the bacteria causing O. A. flat sour spoilage, can be reduced by a factor of 10^{-7} compared to the original level in 45% sugar solution by the use of a commercial UV sterilizer. Moreover, it has been established by the authors that UV irradiation decreases the heat resistance of the spores to one-half¹⁾.

Therefore, in view of the laboratory results¹⁾, it seems clear that prior UV sterilization of sugar solutions can be employed as

a countermeasure to prevent O. A. flat sour spoilage of canned drinks which are often kept hot in vending machines in Japan.

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