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

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INTRODUCTION

In a previous paper[1], the authors reported that the ultraviolet (UV) resistance of the spores causing O.A. flat sour spoilage[2] 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

Microoraganisms

Strain No. 24-1, which was isolated from spoiled canned coffee[2], was used throughout this work.

Preparation of spore suspension

The sporulation of the strain was achieved by the method described in the previous paper[2].

The spores in 10 l of culture were harvested by centrifugation, washed three times with 300ml of sterilized deionized water, and suspended in 200ml 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 sugar solution was about 222 l and the Brix value was about 45%.

One of the batches was used as a "black"; 10ml 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, 90W (CHIYODAKOHAN Co., Ltd.). A diagram of the UV sterilization procedure is shown in Fig. 1. A variable flow metering pump was used to

* A New Type of Flat Sour Spoilage-V.

注　本論文は日本食品衛生学会の好意により，食品衛生学雑誌・第22巻第5号掲載論文を転載したものである。

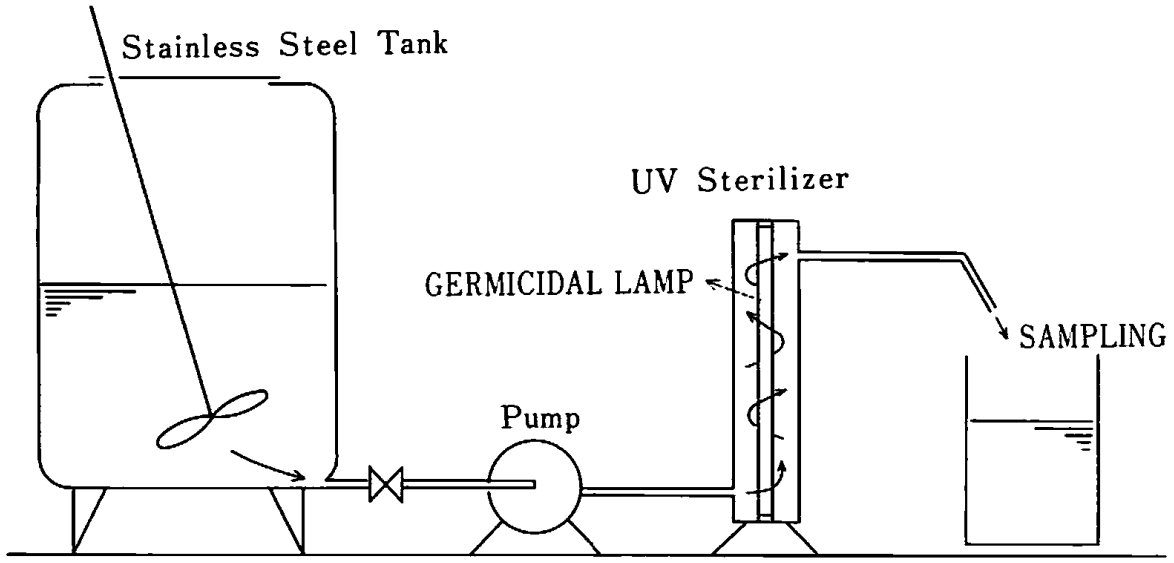

Fig. 1. Schematic Diagram of the UV Sterilization of Sugar Solutions

regulate the flow rate of the sugar solutions to 170, 250 or 500l/hr, which, in the UV sterilizer, gave the sugar solutions UV doses of 3×10^4, 2×10^4 and 1×10^4 ergs/mm^2, respectively.

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 mTGC[3] 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), 0.5ml aliquots of the turbid mTGC media were inoculated into ISA[4], ISaA[5], TF[2], TSiF[2] and TSaF[2], and 1.0ml aliquots of the turbid mTGC media were inoculated into DTA[6,7] and SMA[8] 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 170l/hr one turbid medium out of 10 media was found in each case. No media were found in the case of the treatment at 250 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.*1	ISA*2	ISaA*2	TF*2	TSiF*2	TSaF*2	DTA*3	SMA*3
1	−	−	−	−	−	−	−
2	+	+	+	+	+	−	−
3	−	−	+	−	+	−	−
4	+	−	−	+	−	−	−
5	−	+	+	−	+	+	+
6	+	−	−	+	−	−	−
7	−	−	−	−	−	+	+
8	−	−	−	+	−	−	−
9	−	−	−	−	−	+	+
10	+	−	−	+	−	−	−
11	−	−	−	−	−	+	+
12	−	−	−	+	−	−	−
13	−	−	−	−	−	+	+
14	+	−	−	+	−	−	−

*1 See Table 1.
*2 +: blackening, −: no blackening.
*3 +: colonies, −: no colonies.

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

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

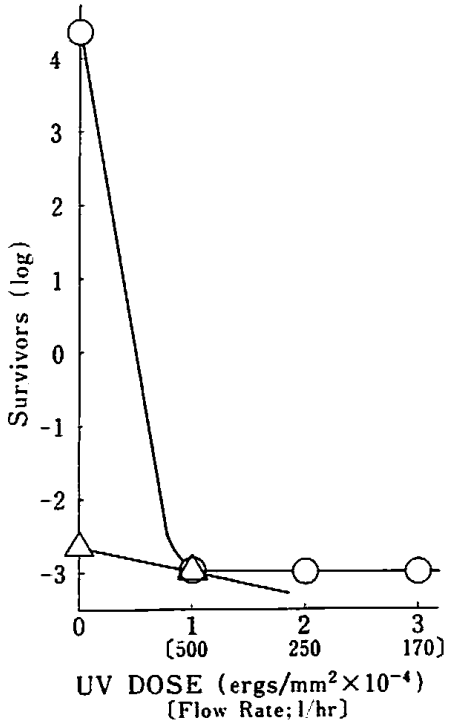

Fig. 2. UV Survival Curves for Spores of Strain No. 24-1 in Sugar Solution after Treatment with the UV Sterilizer

—△— : blank, —○— : Strain No. 24-1.

solution used, the concentration of the spores of similar microorganisms was 2.23×10^{-3}/ml. At 500l/hr flow rate, this value decreased to 1.05×10^{-3}/ml, and at the other two flow rates, the values were below 1.05×10^{-3}/ml.

On the other hand, after the treatment of the sugar solution containing the spores at all the flow rates (500, 250 and 170l/hr) the concentration of surviving spores was 1.05×10^{-3}/ml, which is smaller than the original (2.30 x 104/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 (500l/hr) the UV dose was 1×10^{4} ergs/mm^2. Division of the latter by the D value for UV of this strain (1500 ergs/mm^2)[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 500l/hr flow rate (UV dose of 1×10^4 ergs/mm^2) 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 the UV irradiation decreases the heat resistance of the spores to one-half[1].

Therefore, in view of the laboratory results[1], it is 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.

ACKNOWLEDGEMENT

The authors wish to express their thanks of Prof. Hajime Kadota, Faculty of Agriculture, Kyoto University, for many helpful discussions and suggestions throughout this study. Thanks are also due to Prof. Shigeru Otsuka, Toyo Junior College of Food Technology, for many discussions.

This study was supported in part by a scientific research grant from the Ministry of Health and Welfare.

A part of this paper was presented at the annual meeting of the Japan. Soc. Sci. Fish. on Oct. 5, 1979, in Hakodate.

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