

Research Article

Bactericidal Activity Evaluation of a Novel Reactive Oxygen Radicals Generating Machine for Drinking Water

Prasit Chanarat*, Marasri Krairojananan**, Fumiaki Taguchi***

Abstract

Background : Reactive oxygen radicals are used in water treatment for drinking. A novel reactive oxygen radicals generating machine, Kikuchi prototype, for inactivate microorganism contaminants in drinking water was evaluated.

Methods :The machine consists of water pump, water filtration unit, water activation and microbe inactivation unit and a water container. Oxygen was irradiated to generate ozone by a UV lamp and then break the ozone down to oxygen which was activated by exposing to another UV lamp with a specific wavelength. Bactericidal activity was evaluated by passing tap water of Chiang Mai city adding suspensions of local strain microorganisms : *E. coli*, *Klebsiella*, *Shigella*, *Salmonella*, *Enterobacter* and *Proteus* through the machine.

Results : The results revealed that at the flow rate of 1 L/15 seconds the microorganisms (3.0×10^6 – 8.3×10^6 CFU/mL) could be killed to 0 – 3 CFU/mL and MPN (> 1600 before treatment) was significantly reduced to be less than 2. It means that all of water specimen contaminated with microorganisms become suitable as drinking water after passing through the machine.

Conclusion : This paper documented that the Kikuchi ozone generating machine could effectively treat bacterial contaminated water for drinking. *Bull Chiang Mai Assoc Med Sci* 1999; 32: 180-5.

Key words : Bactericidal activity, ozone generating machine

* Department of Clinical Microscopy,

** Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Chiang Mai University

*** School of Hygienic Science, Kitasato University, Sagamihara, Japan

บทคัดย่อ : ประสิทธิภาพการทำลายเชื้อแบคทีเรียในน้ำสำหรับดื่ม โดยเครื่องกำเนิดอนุมูลออกซิเจนอิสระแบบใหม่

ประสิทธิ์ ชนะรัตน์*, มารศรี ไกรโรจนานันท์**, Fumiaki Taguchi***

ความเป็นมา: มีการใช้ออนุมูลออกซิเจนอิสระ (Reactive oxygen radicals) เพื่อเตรียมน้ำสำหรับดื่ม รายงานนี้จึงต้องการประเมินเครื่องมือแบบใหม่สำหรับเตรียมอนุมูลออกซิเจนอิสระของ Kikuchi ที่ประดิษฐ์ขึ้นว่าสามารถทำลายเชื้อแบคทีเรียในน้ำได้ดีพอสำหรับใช้ดื่มได้หรือไม่

วิธีการ: เครื่องมือประกอบด้วยปั้มน้ำ ส่วนที่กรองน้ำ ส่วนที่ทำลายเชื้อแบคทีเรีย และภาชนะใส่น้ำ เมื่อออกซิเจนถูกแสงอุลตราไวโอเล็ตจะเปลี่ยนไปเป็นโอโซน และแตกตัวเป็นออกซิเจน ซึ่งจะถูกระตุ้นโดยแสงอุลตราไวโอเล็ตที่ความยาวคลื่นจำเพาะอีกครั้งหนึ่ง ทำให้เป็นอนุมูลอิสระ เมื่อทดลองผ่านน้ำประปาที่เดิมเชื้อแบคทีเรียชนิดต่างๆที่พบได้ทั่วไปในจังหวัดเชียงใหม่ (*E. coli*, *Klebsiella*, *Shigella*, *Salmonella*, *Enterobacter* และ *Proteus*) ลงไป นำน้ำที่ผ่านออกมาจากเครื่องมาเพาะเชื้อต่อไป

ผลการทดลอง: เมื่อผ่านน้ำในอัตราเร็ว 1 ลิตรต่อ 15 วินาที สามารถทำลายเชื้อแบคทีเรียจาก 3.0×10^6 - 8.3×10^6 CFU/มล. ให้เหลือเพียง 0 - 3 CFU/mL และจำนวนเชื้อที่น่าจะเป็นไปได้ มากกว่า 1600 ก่อนผ่านเข้าเครื่อง จะลดลงอย่างชัดเจนเหลือเพียงไม่เกิน 2 แสดงว่าน้ำทุกชนิดที่ปนเปื้อนเชื้อแบคทีเรียเมื่อผ่านเข้าเครื่องมือนี้แล้ว สามารถนำไปใช้ดื่มได้

สรุป : รายงานนี้เสนอแนะว่า เครื่องกำเนิดอนุมูลอิสระที่แนะนำโดย Kikuchi สามารถใช้ทำลายเชื้อแบคทีเรียในน้ำเพื่อใช้ดื่มได้ วารสารเทคนิคการแพทย์เชียงใหม่ 2542; 32: 180-5.

คำรหัส : Bactericidal activity, ozone generating machine

* ภาควิชาจุลทรรศน์ศาสตร์คลินิก

** ภาควิชาจุลชีววิทยาคลินิก, คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่

*** School of Hygienic Science, Kitasato University, Sagamihara, Japan

Introduction

Ozone is popular used for improving air and water quality in daily life. First report of ozonization was from Europe which use ozone

treatment for drinking water purification. The outlet water was assured as clean and safe for drinking without addition of any chemical. Ozone destroys the odor, allergens and also kills

bacteria, molds, fungi, and viruses. Ozone has quickly become the “purifier of choice” because ozone pierces the cell wall of bacteria and virus microorganism on contact, destroying them 3,000 times faster than chlorine does¹. Once ozone performs its oxidizing and purifying tasks, it then was reversed to oxygen. Since there is no residue left, ozone is attractive as an alternative to chemical sterilants according to the Environmental Protection Agency². The ozone generation technologies available today are based on ultraviolet (UV), corona discharge (CD) and low, medium or high frequency generation³. This paper aims to evaluate bactericidal activity of a reactive radical generating machine prototype for drinking water in Thailand.

Materials and Methods

Specimens : Tap water of Chiang Mai City was collected using steriled technique⁴. Local strains of microorganisms, *E. coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Enterobacter* and *Proteus* were suspended in tap water, porcelain pre-filtered water and distilled water.

Culture techniques : Bacteria in water, before and after treatment with the proposed prototype of ozone generating machine, was cultivated for total bacterial count (CFU/mL) and most probable number (MPN) was studied using standard method⁵.

Prototype machine : A Kikuchi novel prototype machine was assembled. The machine consists of water pump, a water filtration unit,

water activation and microbe (bacteria and viruses) inactivation unit and a water container. The microbe inactivation system has a new rationale, first O_2 was irradiated to generate ozone (O_3) by a UV lamp and then break the ozone down to O_2 which was activated by exposing to another UV lamp with a specific wavelength. The ozone and the radical oxygen has extremely strong energy for killing microbes.

Results

Bactericidal activity determined by total bacterial count (CFU/mL) and MPN of a Kikuchi novel machine (reactive oxygen radical generator) on Chiang Mai tap water at flow rate of 1 L/50 and 1 L/15 seconds were shown in Tables 1, 2 and 3.

The results showed that after addition of microorganisms to make a contaminated drinking water, all of water specimens became suitable as drinking water after they passed through the machine.

Discussion

Hydrogen peroxide is a germicidal agent composed only of water and oxygen. Like ozone, it kills microorganisms : bacteria, virus, fungus and also parasites by oxidation. Ozone molecules are composed of 3 oxygen atoms, that is, ozone is triatomic at the same state. The formation of ozone from oxygen is an endothermic reaction in which the energy is

Table 1 Bactericidal activity as total bacterial count of the Kikuchi novel machine on Chiang Mai tap water at flow rate 1 L/50 seconds.

Type of water	Organism added	Total bacteria count (CFU/mL)	
		Before	After
Distilled water	<i>E. coli</i>	2.3×10^6	1
		5.0×10^6	0
		4.0×10^6	0
Tap water	<i>E. coli</i>	9.3×10^6	3
		8.6×10^6	4
		9.0×10^6	3
Tap with pre-filtered water	<i>E. coli</i>	8.8×10^6	2
		3.8×10^6	1
		5.0×10^6	0
	<i>Klebsiella</i>	1.1×10^6	2
	<i>Salmonella</i>	4.9×10^5	1
	<i>Shigella</i>	3.3×10^5	0
	<i>Enterobacter</i>	3.8×10^5	1

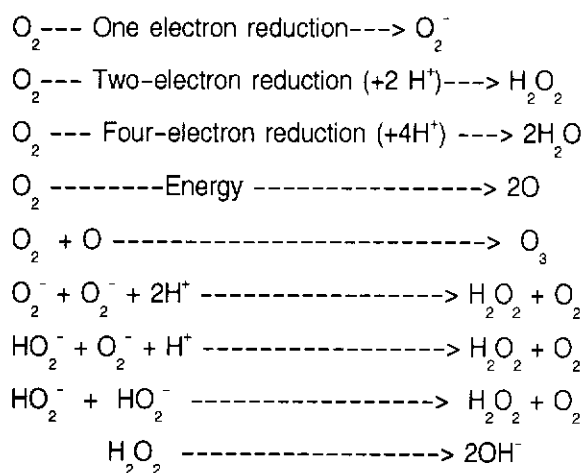
Table 2 Bactericidal activity as total bacterial count of the Kikuchi novel machine on Chiang Mai tap water at flow rate 1 L/15 seconds.

Type of water	Organism added	Total bacteria count (CFU/mL)	
		Before	After
Tap water	<i>E. coli</i>	8.3×10^6	3
	<i>Klebsiella</i>	8.3×10^5	0
	<i>Salmonella</i>	4.8×10^6	1
	<i>Shigella</i>	4.6×10^5	1
	<i>Enterobacter</i>	7.6×10^5	1
	<i>Proteus</i>	3.0×10^6	2

Table 3 Bactericidal activity as MPN of the Kikuchi novel machine on Chiang Mai tap water at flow rate 1 L/50 seconds.

Type of water	Organism added	MPN	
		Before	After
Distilled water	<i>E. coli</i>	> 1600	< 2
		> 1600	< 2
		> 1600	< 2
Tap water	<i>E. coli</i>	> 1600	< 2
		1600	< 2
		> 1600	< 2
		> 1600	< 2
		> 1600	< 2

furnished in the form of an electrical discharge, heat or UV. Substances formed by endothermic reactions tend to be less stable than those formed by exothermic reactions, so ozone is more chemically active than oxygen and decomposes readily to oxygen by exothermic reaction. Ozone is a powerful oxidizing agent⁶. The oxygen can be converted to ozone and reactive oxygen radical (SOR): superoxide, singlet oxygen, hydroxyl radical and hydrogen peroxide as followed⁷:



Water is passed by the 254 nm lamp between a quartz glass sleeve and an outer chamber. It is the frequency of the light that impacts the organism. Proper equipment sizing is essential to be effective in treating water; the output of the ampere must be matched the flow rate of water being treated. The Kikuchi prototype for bactericidal activities can demonstrate that passing water at flow rates of both 1 L/50 seconds and 1 L/15 seconds through 185 nm lamp which produces ozone. Air (usually ambient) is passed over a UV lamp which split oxygen molecules into the gas resulting oxygen atom (O_1), seeking stability, attach to other oxygen molecules, forming ozone (O_3) which then inactivates the contaminants by actually rupturing the organism cell wall.

Which technology is better for ozone generating, UV or CD ? Which frequency: low (50 to 100 Hz), medium (100 to 1000 Hz) or

high frequency (>1000 Hz) ? There are some key factors to be considered : First, the concentration of ozone output that is enough for treating contaminant or not. UV ozone generators generally produce ozone concentration between 0.1% and 0.001% by weight while CD system can produce between 1% and 6% by weight. Concentration significantly impacts the effectiveness of ozone because it must be dissolved before reacting with water-borne contaminants and the lighter the ozone concentration, the more soluble ozone is in water. Anyhow, since 85% to 95% of the electrical energy supplied to a corona ozone generator produces heat, so the method for heat removal is required and it is necessary to make sure that some type of cooling system is included. Also, because low, medium and high frequency ozone generators have no lamp, they could be durable and less maintenance. However, UV lamp (12 V 6 W) in this prototype is about 20,000 hours lifelong. UV lamp ozone could cost effective. Since ambient air is used as the feed gas and do not require air preparation equipment. However, ozone

output depends on humidity, and UV lamps gradually loss their intensity over time.

Reference

1. Rich T. Water Technology Magazine, October 1994, Clear Water Tech, Inc, USA.
2. Milton M, Olson R. The Ozone Story, 1999, <http://www.enviro.org/ozone.html>
3. Milton M, Olson R. The O₃ Zone : About water, 1999, <http://www.enviro.org/ozone.html>
4. Collins CH, Lyne PM, Grange JM. In Microbiological Methods, 6th ed, Butterworth & Co, Ltd, 1989, pp 231-9.
5. Finegold SM, Martin WJ, Scott EG. In Bailey and Scott's Diagnostic Microbiology, 1978, The CV Mosby Co, USA.
6. Halliwell B, Gutteridge JMC. In Free Radicals in Biology and Medicine, Clarendon Press, Oxford 1989, pp 1-21.
7. Ahmad D, ed, In Oxidative Stress and Antioxidant Defenses in Biology, Chapman & Hall, NY, USA, 1995, pp 1-61.