#### Ministry of Economy, Trade and Industry Subsidies for management support for small and medium enterprises (strategic fundamental technology advancement support project)

The development of unheated sterilization equipment for beverages such as milk by Ultra High Concentration Ultra Fine Bubble (UFB). Specific R & D certification number: kinki1607030 [2016-2018]

#### **O**General Association of Mechanical Systems Promotion Association Grant Project

[Strategy formulation concerning sterilization treatment of oyster by using fine bubble. [2017-2018] Collaboration with the FBIA (Fine Bubble Industrial Association).

- $\star$  1. TOSSLEC's proprietary fine bubble generation technology .
- ★ 2. Empirical validation for establishment of high efficiency production technology.
- ★ 3. Ultra fine bubble generator example.
- ★ 4 UFB grain size variation characteristics by control of bubble particle diameter concentration.
- $\star$  5. Differences between our company UFB and other UFB.
- ★ 6. Selective adsorption / desorption washing sterilization principle.
- ★ 7. Oyster purification application of selective adsorption/desorption washing sterilization principle.
- ★ 8. Oyster norovirus substitute virus purification effect.
- ★ 9. Application to agriculture, animal husbandry, fishery industry, etc.
- ★ 10. Application of fine bubble generation technology Fish and shellfish animal feeder equipment.
- $\star$  11. Verification and business process.



・ の contract - 般財団法人 大阪科学技術センター Osaka Science & Technology Center Aggregation and utilization of 'Knowledge 'Member of the Industry-Academia-Government Collaboration Association.

## 1. TOSSLEC's proprietary fine bubble generation technology



Our proprietary new hybrid method generates micro uniform ultra high concentration bubbles (homogeneous bulls) by fine bubble continuous crushing technology. At the time of bubble generation, the coagulation effect can be minimized, and energy utilization at FB collapse and UFB ultra-high concentration result in non-heat sterilization effect.

- Under ultra pure water: 3 billion pieces / ml realization "NanoSight NS-300 measurement"
- 30 billion cells / ml under low concentration alcohol "realized by NanoSight NS-300 measurement"
- **Bubble particle size / concentration control technology for target application**
- A No choice of liquid / gas.

# 2. Establishment of high efficiency production technology by UFB

Purpose: By introducing UFB particle size / concentration control technology for Hatching and farming etc, we expect the following effects.

- Maintaining proper oxygen concentration by UFB particle size / concentration control and improvement of seedling and seedling activity.
  - i . Substantial reduction of oxygen gas consumption (reduction of levitation gas volume and improvement of dissolved rate)
  - ii . Prevention of gas diseases (maintenance of optimal dissolved oxygen concentration)
  - iii. Promotion of growth of seedlings (physiologically active effect by UFB, intestinal function etc.)
  - iv. Purification of viruses, bacteria and parasites (by selective adsorption / desorption washing sterilization principle)
- Suppression of collision between fish and prevention of injury by maintaining evenly dissolved oxygen concentration by multiplying discharge ports. (Prevention of seedling concentration in aeration port)
- Suppression of bacteria / algae generation in farm tanks and piping paths by UFB cleaning sterilization effect.

# **3.Example of UFB generator Outline drawing of washing sterilizer**

⇒Fine bubble is adapted to the object and cleaning and disinfection of foreign matter of foreign matter and virus / bacteria with a small amount of water current



The liquid bubble formation target is not limited in liquid type and gas type.

O<sub>2</sub>, O<sub>3</sub>, H<sub>2</sub>, N<sub>2</sub>, CO<sub>2</sub>, NO, NH<sub>3</sub>, Compressed air, CDA(Clean Dry Air), Hypochlorous acid, Hydrogen chlorine dioxide etc., Any type of pump can be used.



# 5.Differences between TOSSLEC's UFB and other UFBs (action on living fishes and shellfishes)

UFB trap phenomenon due to cap effect (act on the gills etc)



#### **※**1∶Cap effect

A phenomenon in which a large bubble blocks a passage in a narrow gap like a hat like a hat, and a small bubble that can pass through is trapped. **(TOSSLEC's named)** 



When microbubbles adsorb to fish and shellfish, the cap effect works, it becomes impossible to aspirate dissolved oxygen, the activity of fishes and shellfishes is drastically reduced, and in some cases it dies.

This phenomenon appears remarkably in aquaculture such as tuna, greater amberjack, yellowtail, etc. This phenomenon is also recognized in oysters etc. which aspirate a large amount of seawater which is said to be 100 nm in gills distance..

### 6.Principle of selective adsorption / desorption washing sterilization

(Bubble and virus · Bacteria (including spore and biofilm), Application to adherent pesticides, particles, radionuclides, etc.)



It is estimated that the surface of virus / bacteria has the property of bipolar electrolyte and negative charge UFB selectively adsorbs to virus and the like and is detached and washed from the object to be cleaned.

In addition, the hydroxyl group (OH -) and the hydrogen group (H +) around the bubble are subjected to dielectric polarization, and there is a cleaning action in a surface active state equivalent to that of soap. The anionic electrolyte is a factor to improve the bubble concentration.

Fig 1: Ultra fine bubble adheres, desorption and washing of the object by adsorption static elimination effect, at the same time bacteria loses bioactivity by electric charge disappearance.

Fig2: t of collapse of the bubble Physically destroy cell nuclei by hydroxyl radicals, cavitation etc. Do not create resistant bacteria.

Application the time examples: CIP cleaning, washing sterilization of drinks such as milk, disinfection of bivalves such as oysters, filter washing, wafer washing, intestinal flora control, etc.



Illustration of selective adsorption / desorption washing sterilization principle

#### 8.Oyster norovirus substitute virus purification result by model HMB-MAR015.



- Culture of feline calicivirus used in the experiment and extraction from oysters and evaluation of infectivity value are handled by Kitasato Center for Environmental Science, Virus Department.
- · The infectivity value is evaluated by the plaque method.
- Experimental place: conducted at the Kyoto Prefectural Ocean High School BSL 2 (Biosafety Level 2) compliant tent.
- Used seawater: Kyoto prefectural marine high school sand filter equipment, ultraviolet sterilized one used.
- Virus handling and suction, freezing transportation method etc are all handled under the guidance of Kitasato Environment Science Center.
- Feline calicivirus: FDA (United States Food and Drug Administration) has certified it as a Noro virus replacement virus.

### 9.CO2 / Ozone UFB conversion result by model HMB-OZ02

1. *E. coli* NBRC NO.102203

#### 2. *S. aureus* NBRC NO.13276

#### 3. *B. cereus* NBRC NO.15305

<b>E.coli</b> C02 UFB 30min C02 UFB 15min	1.	1.39	CO2 UFB metho The ozone UFB r be completely s	d reduced to les: nethod was con terilized	s than 10 <sup>2</sup> firmed to
Ozone UFB 10min	0.00				
Before				5.54	
Source water(UPW)	0.00				Horizontal axis
	0	<b>10</b> <sup>2</sup>	<b>10</b> <sup>4</sup>	10 <sup>6</sup>	(CFU/mL)
S.aureus CO2 UFB 30min CO2 UFB 15min	0.10		CO2 UFB metho The ozone UFB r be completely s	d reduced to les: nethod was con terilized	s than 10 <sup>2</sup> firmed to
Ozone UFB 10min	0.00				
Before				5.42	
Source water(UPW)	0.00				Horizontal axis
	0	10 <sup>2</sup>	10 4	10 <sup>6</sup>	Bacterial count (CFU/mL)
B.cereus					
C02 UFB 30min			3.49	The ozone Uf	B method
CO2 UFB 15min			3.53	Complete ste	rilization
Ozone UFB 10min	0.00			was commu	
Before			4.0	8	
Source water(UPW)	0.00				Horizontal axis
	0	10 <sup>2</sup>	<b>10</b> <sup>4</sup>	10	(CFU/mL)

NBRC number is a standard number managed by the Natural Resources Center of NITE (National Institute of Technology and Evaluation).

election of test bacteria is decided as representative indicator bacteria of beverages such as milk, such as beverage sterilization evaluation, in consultation with the Hokkaido Dairy Test Inspection Association.

Test water used in the experiment: Ultrapure water treated with tap water with ion exchange resin was used

UFB Water Production: Sample prepared under each condition using our company's food washing sterilizer (model HMB - OZ 02) which used ozone and carbon dioxide for UFB water production.

Measurement and handling of bacterial count: Associate Professor Food Processing Engineering Laboratory, Graduate School of Agriculture, Hokkaido University Associate Professor Shigenobu Koseki. Development aiming at sterilization of spore bacillus (Bacillus cereus).

TOSSLEC Co., Ltd. Fine Bubble Div.

# 10.Application to agriculture, animal husbandry, fishery etc. (Expected effect)

#### **Processed agricultural products**

- Promotion of growth and maintenance of health by bioactive effect.
- Long-term preservation by bactericidal effect (no preservative) by fruit juice bubble formation.

# Poultry

• Improvement of egg ratio.

### **Pig farming/Cow breeding etc**

• Reduce mortality from illness / stress.

# **Vegetables / fruits tree etc.**

• Growth acceleration, Improve immunity.

### **Marine products**

- Growth acceleration.
- Maintain freshness, wash bactericidal effect.
- Virus free.
- Biological non-heat sterilization.













**Model HMB**—**OZ02** Food cleaning and sterilizing apparatus.

#### **Model HMB — H201** Ultra high concentration hydrogen water production.

Characteristic: Ozone CIP, Drinking, hot bathing Purpose.



#### Model HMB-NHS01

Drinking suitable non-heated washing and sterilizing apparatus for food poisoning bacteria (Escherichia coli, salmonella, spore-forming bacteria etc.) and various gas UFB containing etc.

Characteristic: Spore fungi sterilization, drinking purpose non-heat sterilization.



Model HMB — MAR01 Fish and shellfish UFB generator.

#### Model HMB-MAR015

Fish and shellfish non-heated washing sterilizer.

#### Characteristic

Seedling and seed production utilizing physiological activity effect, purification of bivalves such as oysters etc.

Application of sterilization technology and sterilization technology by crushing fine bubble established by patent right. Undertaking efforts focusing on non-heated washing sterilization technology such as biological non-heated washing and sterilizing.



### We will answer your solution issue!!

#### TOSSLEC's

Development and provision of target devices.
 Performance guarantee.
 Considering mass production.
 Business model agreement.



2 Evaluation.
3 Task.
5 Verification of problem solving.
7 Business model agreement.





