### UFB Application to Agriculture, Animal husbandry, Fishery, General Industry.

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]

#### **@**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).

- ★ 1. TOSSLEC's proprietary fine bubble generation technology.
- $\star$  2. Empirical validation for establishment of high efficiency production technology.
- $\star$  3. Ultra fine bubble generator example.
- $\star$  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.
- $\star$  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.
- ★ 11. Verification and business process.





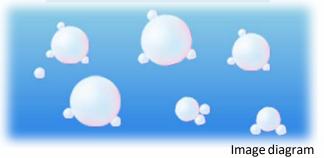
Aggregation and utilization of 'Knowledge 'Member of the Industry-Academia-Government Collaboration Association.

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

#### Conventional technology



#### Our new technology







\* Mixing FB and UFB

\* UFB is uniform (Homogeneousbubble)

FB: Bubble particle diameter less than 100μm.

UFB : Bubble particle diameter less than 1.0  $\mu m$ .

MB : Bubble particle diameter of  $\,$  equal or greater than 1  $\mu m$  to less than 100  $\,\mu m.$ 

\*Definition of bubble particle size: According to ISO 20480-1

- Pressure shearing melting method
- Swirling flow method
- Pressurized dissolution method
- Ultrasonic method
- Venturi method etc.



#### Proprietary new hybrid system

Japanese Patent No. 6123013

Name: Bubble-containing liquid manufacturing apparatus and Method

for producing bubble-containing liquid

Patent No. 6210917

Name: Nano bubble manufacturing equipment

12 above-mentioned other patent applications filed,

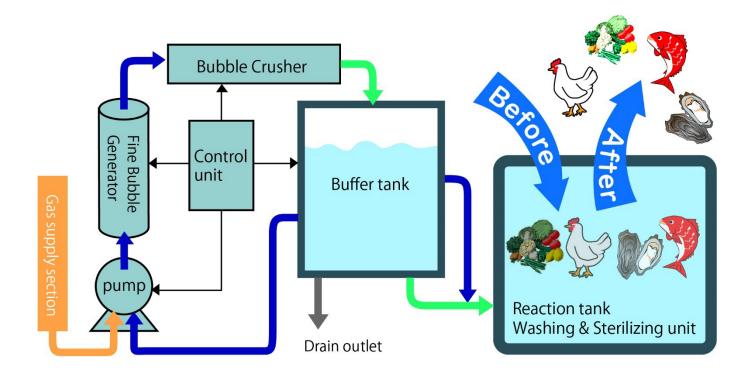
PCT application 3 cases

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"
- 2 30 billion cells / ml under low concentration alcohol "realized by NanoSight NS-300 measurement"
- **3** Bubble particle size / concentration control technology for target application
- No choice of liquid / gas.

## 2.Example of UFB generator Outline drawing of washing sterilizer

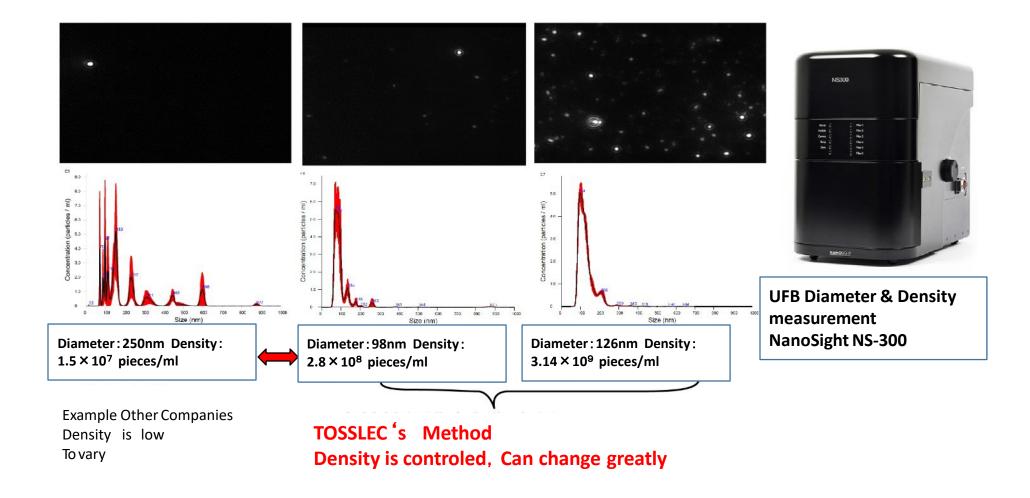
⇒Fine bubble is adapted to the object and cleaning and disinfection of foreign matter of foreign matter and virus / bacteria with a small amount of water current



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

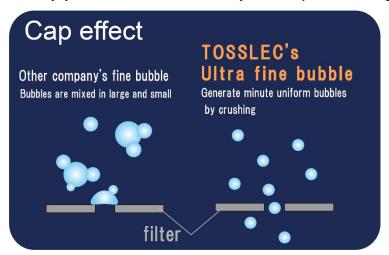
 $O_2$ ,  $O_3$ ,  $H_2$ ,  $N_2$ ,  $CO_2$ , NO,  $NH_3$ , Compressed air, CDA(Clean Dry Air), Hypochlorous acid, Hydrogen chlorine dioxide etc., Any type of pump can be used.

# 3. Characteristics of change in UFB particle by control of bubble particle size concentration



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

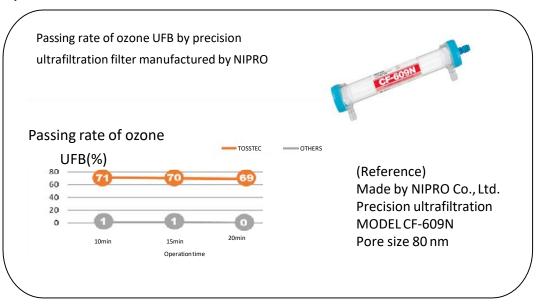
**UFB** trap phenomenon due to cap effect (act on the gills etc)



∴ 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)

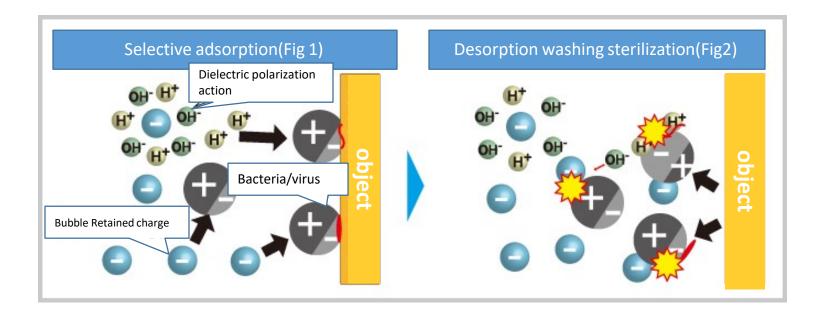


When microbubbles adsorb to fish and shellfish, the cap effect works, it becomes impossible to aspirate dissolved oxygen, the activity of fishes and shellfishes is drastically reduced, and in some cases it dies.

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.

## 5. 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.

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# 6.Oyster purification technology by selective adsorption / desorption washing sterilization principle

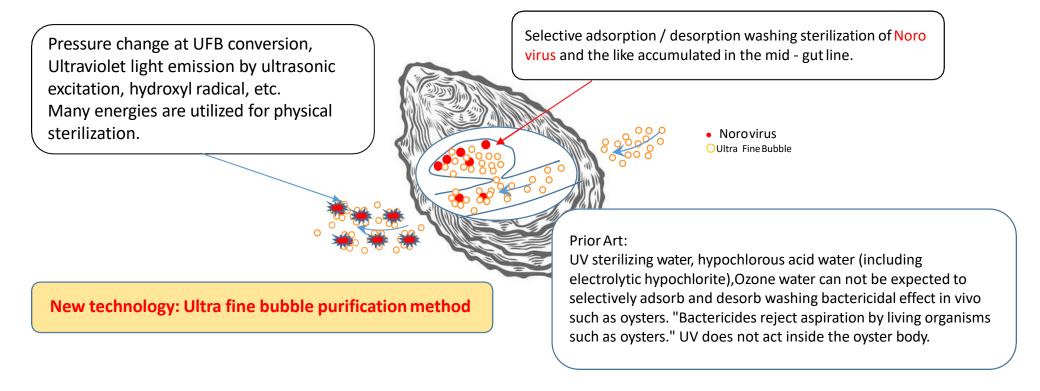
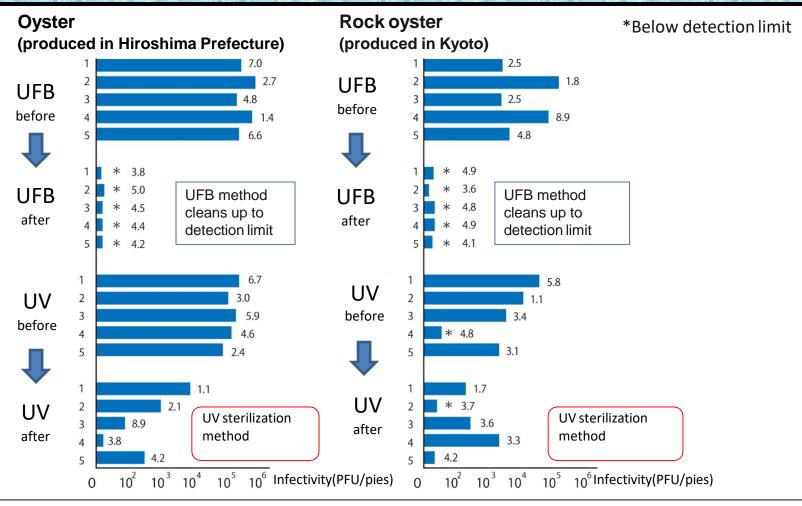


Illustration of selective adsorption / desorption washing sterilization principle

#### 7. 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 plague method.
- Experimental place: conducted at the Kyoto Prefectural Ocean High School BSL2 (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.

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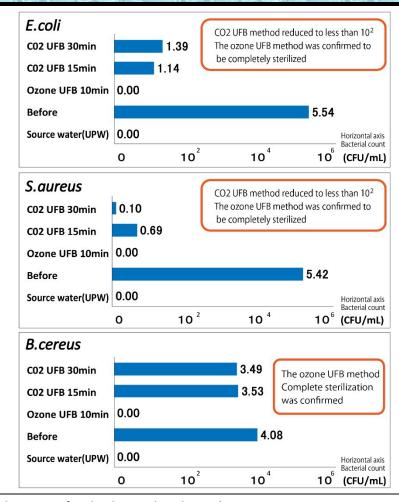
## 8.CO 2 / ozone UFB conversion result

### by model HMB-OZ02

1. *E. coli* NBRC NO.102203

2. *S. aureus* NBRC NO.13276

3. *B. cereus* NBRC NO.15305



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 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).

Test Inspection Association.

## 9. 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.









#### 10.Application example of fine bubble generation technology application equipment.



#### 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.

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