**“Entry to the Stockholm Junior Water Prize 2021”**

# Selection of Water-Purifying Bacteria and Development of a Dissemination System Using Vessels to Improve Water Quality of Rivers

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**ABSTRACT**

Accumulating river pollution caused by heavy metals and oil leaks from vessels has catastrophic effects on human and aquatic life. This research was conducted to determine the most effective microorganisms capable of purifying water and develop a model to safely disseminate the identified microorganisms into rivers. 16 species of yeasts and bacteria isolated from 2 streams were classified and their survival in an oil-contaminated environment was confirmed. Paramecium was used as a biomarker for water purification. Through analyzing the effectiveness of river microorganisms in decomposing oil, 4 species of bacteria were selected and their ability to purify water from other contaminants was tested—identifying the most effective bacteria to be *Phytobacter diazotrophicus*. Furthermore, a water purification system was designed to allow bioremediating microorganisms to be constantly disseminated from vessels passing through rivers, proposing a "continuous purification" system as a bioremediation method ensuring that contaminants do not accumulate in continuously polluted rivers.

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**KEY WORDS**

Water-purifying bacteria, Dissemination system, Ship, River, *Phytobacter diazotrophicus*

**ABBREVIATION AND ACRONYMS**

YPD; yeast extract peptone dextrose

NB; nutrient broth

NA; nutrient agar

DW; distilled water

OD; optical density

*E. coli; Escherichia coli*

*Paramecium; Paramecium aurelia*

Co; copper

Al; aluminum

*P. diazotrophicus; Phytobacter diazotrophicus*

UV; ultraviolet

**ACKNOWLEDGEMENTS**

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**INTRODUCTION**

The severe problem of river water contamination is becoming increasingly alarming due to the rise in the use of resources such as heavy metal, plastic, and oil, especially in developing countries [1]. These harmful substances usually enter rivers due to factory operation and human activities; in the case of oil contamination, ships also play a significant role as they tend to release oil into waters from small fuel leakages when the ship is operated or refueled. [2]. Heavy metals are normally released into rivers as a result of various human activities including mining and material processing, as well as from the weathering of soils and rocks. The most common heavy metal pollutants include arsenic, copper, lead, iron, zinc, cadmium, and chromium [3-5].

Spills and oil leaks into freshwater have devastating effects on nearby ecosystems as some organisms, especially fish and essential microorganisms, cannot prosper with oil in their habitats [6]. This is a growing concern as the growing consumption of oil is inevitably accompanied by more incidences of oil spills and leaks.

In addition to having direct consequences to aquatic life in rivers, oil can adsorb onto sediment particles which get moved by the river currents to backwaters, thus causing further contamination problems in those habitats.

Such oil leaks can severely endanger human life if the oil enters our drinking water supply. In 2016, a major oil spill in the Canadian province of Saskatchewan saw 66,000 gallons of heavy oil released into a major river [7]. As the central Saskatchewan town of North Battleford gets its drinking water from the river, it had to desperately switch its intake to groundwater with limited supply. The city of Prince Albert, with a population of 35,000 people, had to shut down its water treatment plant after the leak, leading to a shortage of water that limited water use for inhabitants for several days. Such cases of oil spills do not create life-threatening problems; however, if the oil spilled was less dense and thus less identifiable, or if it accumulated into waters over a longer period of time, eventually ending up into the drinking water supply, a number of severe health problems could arise.

Another severe concern is the accidental release of harmful chemicals from chemical plants into rivers, with devastating effects on nearby ecosystems [8]. The lowered pH of river waters as a result of this release means that metal solubility increases, and the mobility and toxicity of its particles increase in turn. These chemicals can persist at the bottom of the rivers for many years and thus cause environmental damage if not biologically degraded. One example is London during the Industrial Revolution of the 1800s, with pollution of the River Thames constantly worsening until in 1856, all the fish in the river died [9]. In 1878, 600 passengers out of 800 who crashed into the River Thames after a ship ride died due to the contaminated waters [10]. After this tragic event, the river was restored and cleaned with a significant effort. In South Korea, the Nakdong River and Taewha River have experienced a similar environmental disaster when the sewer water from nearby factories leaked into the rivers and caused significant environmental damage; the rivers have been restored since [11, 12].

As chemicals can accumulate in rivers with serious potential consequences, it is vital that not only do contaminated river waters get cleaned after major spills or leaks, but also that the amount of harmful chemicals released and present into the river water is reduced. In order to prevent harmful contaminants from becoming a long-term environmental problem as a result of ship crashes or oil leaked from ship fuel, it is important to immediately decompose or remove such contamination on a continuous basis, whether from oil or other harmful chemicals. Removing contaminants in rivers also has the benefit of preventing such harmful chemicals from entering and polluting the sea.

Ships that go through rivers are currently causes for contamination; however, they can also be used for the decomposition of contaminants in rivers. In contaminated rivers, there are microorganisms and aquatic life that thrive under such conditions by using contaminants as their source of energy [13]. Bacteria, yeast, and fungi produce biosurfactants, natural surface-active agents, with very different chemical structures and properties

[14, 15]. If such microorganisms are carefully selected and cultivated, and sprayed through moving ships into the river, they can have a significant effect on the continuous decomposition of contaminants.

Previously, a study was conducted to develop a soil remediation model where certain species of yeasts were utilized to effectively decompose oil in the soil of unused gas stations. Using knowledge gained from this previous study, in this research, the objective is to find certain types of microorganisms that can be used to remove oil, heavy metal, and other pollutants which cause environmental consequences in freshwater sources. Microorganisms were selected amongst the bacteria and yeasts which already dwell in rivers that are thus safe to humans and are nearby ecosystems, while also being effective in decomposing contaminants. By designing and implementing a system which can cultivate such microorganisms in ships, the objective is for the ships to continuously spray those microorganisms into rivers while moving to constantly decompose the contaminants.

## MATERIALS AND METHODS

### 1. Bacteria and yeast culture media

To make a YPD broth, 5 g of yeast extract, 10 g of peptone, and 10 g of dextrose were added to an Erlenmeyer Flask which contained 500 mL of distilled water. A nutrient broth (NB) was made by adding 4 g of NB powder to 500 mL of distilled water inside an Erlenmeyer Flask. The two solutions were then mixed by adding a magnet to each flask, then using a magnetic stirrer. The two flasks were then placed inside an autoclave at 121℃, 1.2 atm for 15 minutes. After having poured 40 mL of NB or YPD broth into 50 mL tubes, the 12 YPD containing tubes were centrifuged at 4,000 rpm for 10 minutes. The liquid parts of the tubes were transferred into 12 new tubes. The total of 26 tubes were placed inside a fridge at 5℃.

A nutrient agar (NA) media was made by adding 500 mL of distilled water into an Erlenmeyer flask, after which 4 g of Nutrient broth agar and 7.5 g of agar powder were added. An YPD agar base was made by adding 500 mL of distilled water into an Erlenmeyer flask, then adding 5 g of yeast extract, 10 g of peptone, 10 g of dextrose, and 7.5 g of agar. The media were autoclaved for sterilization. Then, 25 mL of each media was placed onto 90 mm petri dishes where they solidified. The petri dishes were then placed inside a container and then inside a refrigerator until use.

### 2. River water bacteria and yeast

River water was sampled from the Banpo side of the Han River twice at two different dates, and from freshwater in Okcheon, Chungbuk. While the experiments were being conducted, the river waters were stored at room temperature for use.

For one tube, 30 mL of NB and then 100 uL of the sampled river water were added by using a micropipette. For another tube, 30 mL of YPD broth and the same amount of river water was added. The microorganisms

inside the river water were cultivated at room temperature—a cell spreader was used to dip into the water, then spread across the two agar bases using the streaking method. This was done to 3 NA plates and 2 YPD agar plates. The 5 plates were sealed by using parafilm then were placed at room temperature.

Colony classification was then conducted; the colonies formed on NA or YPD agar were separated into different tubes by using a loop, according to their color, form, margin, elevation, transparency, or size. The colonies were then placed into tubes with either NB or YPD broth, according to the agars.

### 3. Oil degradation of river water bacteria and yeast

#### A. Oil dispersion analysis using petri dish

The tubes contained 3 mL of Nutrient Broth or YPD. Petri dishes with a diameter of 3 cm were prepared. To prepare the 10% diesel mixture, 900 uL of distilled water and 100 uL of diesel were transferred to a microtube and mixed. For the 1% diesel mixture, 900 uL of 10% diesel and 100 uL of distilled water were transferred to a new microtube and mixed. To confirm the dispersion of the oil solution, a 0.1x crystal violet solution was used. 1 mL of crystal violet and 9 mL of water were mixed to prepare a 0.1x crystal violet solution. 20 uL of the crystal violet solution was transferred onto each cap of the 5 microtubes, then 20 uL of the oil solutions with different concentrations was put onto each of the caps using a micropipette. The mixed solutions on the caps were then mixed using a micropipette, after which they were dropped onto each dish containing 3 mL distilled water. The dispersion of the crystal violet solution for different oil solutions was observed and recorded (Fig. 1).

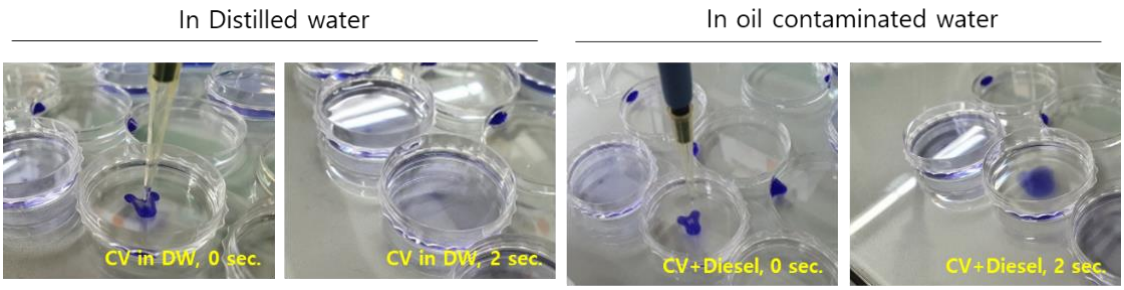


**Figure 1. Oil dispersion test using petri dish**

#### B. Oil dispersion test using parafilm

21 glass vials were prepared, with 10 mL of river water and 10 uL of diesel added to each vial. After the absorbance of the cultivated bacteria and yeast was measured, 0.1 A of bacteria and yeast was placed into each vial and then cultivated at room temperature for 48 hours.

Parafilm was cut to the proper size and labeled with the name of each microorganism to be placed over each petri dish (8 names for each petri dish, with 5 for the last one) (Fig. 2). On a separate long piece of parafilm,

10 uL of methylene blue solution was placed using a micropipette. 10 uL of each solution was placed onto each methylene blue solution and mixed. Then, the mixed solutions were placed onto the parafilm on the petri dish according to their names (eg. YPD A). The petri dishes were stored at room temperature and kept out of reach. When they dried, the diameter of the dispersion of oil solutions were measured.


**Figure 2. Oil dispersion test using parafilm**

**4. *Paramecium* in oil solution**

*Paramecium* were bought from Saeng Mul-Nara (Korea). After they were received, 100 uL of *Paramecium* was placed inside 10 holes in a 96 well plate using a micropipette, after which they were observed through an inverted microscope at 200x. The number of each organism was counted then noted. For the right half of the 96 well plate, 10 uL of diesel was added; for the left half, 10 uL of distilled water was added using a micropipette. The *Paramecium* that had been left to multiply were observed after 3 days using an inverted microscope. The images were captured and saved.

**5. Water metals and contaminant test**

To confirm whether the collected water contained heavy metals such as Cu, Co, Zn, Cd, or Hg etc., a semi-quantitative test was performed on the heavy metals. 16 tubes were prepared with 4 different waters—distilled water and 3 river waters—and 4 different conditions in each tube of water (control, copper strip, aluminum strip, and diesel). After 48 hours, the 16 different tubes were tested for heavy metals. The strips from the water metal testing kit (SenSafe, USA) were placed inside each bottle containing the river waters, Banpo #1, Banpo #2, Okcheon, or the 16 prepared waters, stirred for 30 seconds, then left for 2 minutes. The change in color of each strip was compared (Fig. 3).

The water quality test was conducted to determine the concentration of nitrate and nitrite, total hardness, total alkalinity, and pH in the waters using an Eco-check kit (SenSafe, USA). The strips were placed in the waters for 2 seconds, then immediately taken out to observe the colors after 25 or 60 seconds. (Fig. 3).


**Figure 3. Water metal and contamination test**

### 6. Water remediation model

2 polystyrene boxes were prepared, one for control and the other for the experiment; 400 mL of Banpo stream water and 1,600 mL of distilled water were placed into each. Then, 2 mL of oil, 5 copper sticks, and 5 aluminum sticks were placed into each box. 20 mL of *Paramecium* was then placed. Oxygen was also supplied using an oxygen supplier machine. After the solutions of YPD D, NA D, NA F, NA H were mixed and the volumes were determined with the ABS measurements, a 0.2 um filter and syringe were placed onto a new tube, after which the solution in the syringe was injected through the filter into the new tube. The filtrate solution was placed into the Experiment Polystyrene box using a micropipette. Afterwards, a water contamination test was conducted from the control and experiment solutions (Fig. 4).

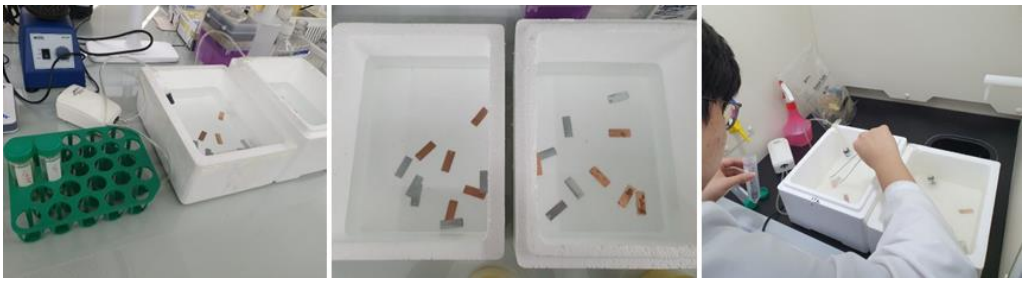

**Figure 4. Water remediation model**

### 7. Absorbance measurement and concentration adjustment

Using a UV Spectrophotometer, the absorbance (OD and optical density) of the bacteria and yeast solutions were measured. The spectrophotometer was set at 600 nm. 900 uL of NB or YPD broth and 100 uL of the bacteria solutions were added to cuvettes using a micropipette. Two blanks were used for zeroing: one cuvette with 1000 uL of YPD broth and the other with 1000 uL of NB. The absorbance was multiplied by 10. To obtain the amount of each solution needed per 1 mL, the desired amount (0.1 A) was divided by the actual absorbance of solutions, then multiplied by 1000. The optimal volume of each solution was placed into a tube micropipette and distilled water was further added.

**8. *E. coli* contamination**

The absorbance of *Escherichia coli* was 0.075, measured using a UV spectrophotometer set at 600 nm. To add 0.001 A to the water remediation model, 2666 uL of *E. coli* was put into each of the two polystyrene boxes containing 2 L of solutions each. 1 mL of a solution from the control box or the experiment polystyrene box was dropped onto an *E. coli* isolation media using a micropipette. After 48 hours, the culture was observed and the number of *E. coli* in 5 boxes for the cultures of the 2 solutions were measured (Fig. 5).


**Figure 5. *E. coli* isolation media packaging and contents; picture after *E. coli* was isolated.**

**9. DNA Sequencing and BlastN test**

Colonies from NA D, F, H, and YPD D were extracted using a loop then placed into tubes containing 10 mL of either NB or YPD broth accordingly. They were then spread onto petri dishes containing either NA or YPD agar using the streaking method. Then, the four petri dishes containing the colonies were wrapped in parafilm and sent to MACROGEN (Korea) for DNA sequencing. The results from the BlastN DNA sequencing were analyzed by inputting the results in Excel; 2 results, for example NA D Forward and NA D Reverse, were placed in alphabetical order to easily identify any overlaps in their sequences. Overlaps would indicate that the species have similar identities.

**10. Isolated bacterial solution preparation**

The 4 solutions YPD D, NA D, NA F, NA H were filtered and placed into new tubes. Then, the according volumes obtained from measuring the absorbance of YPD D, NA D, NA F, NA H were placed into 4 tubes. 1 new tube was filled with bacteria filtrate, and another was left as the control. River water was filtered using an injector and a disk filter into two 50 mL tubes. 5 mL from the tube was placed into 4 small tubes. For the 5 mL river water in 4 tubes, 54 uL of YPD D, 134 uL of NA D, 126 uL of NA F and 150 uL of NA H, for which the measurements were determined by an absorbance calculator, were added. The tubes were placed at room temperature for 48 hours, after which their absorbance was measured again and compared with the proliferation of bacteria.

### 11. Microorganism-spraying boat model

A model for releasing microorganisms into freshwater using boats was designed. An acryl box was first made by first taping 5 sides (no top cover) together, then adding 4 acryl sticks to each of the 4 sides to better hold the box in place. They were glued onto the sides horizontally using acrylic glue, which was deployed using an injector. A further 4 acryl sticks were glued onto the corners of the 4 sides vertically, to further ensure that the planes are in place. All the scotch tape was removed now that the glue held the 5 planes including the bottom together, then 2 holes were made on each of the longer sides of the box using a drill. Then, two straws were placed into the 2 holes and were stuck onto the holes using a glue gun, with 8 small holes on each of the two straws made using a pinset. The model was tested through floating on a water-filled polystyrene box, and a methylene blue solution was added to the acryl boat model. The dispersion of the solution into the polystyrene box through the holes in the 2 straws was observed.

## RESULTS

### 1. Isolation of river yeast and bacteria

The stream water sampled from the Han River (Banpo Stream) at two different dates and from Okcheon, Chungbuk was placed into NB and YPD broth in different tubes to be cultivated. The colonies formed from the 2 different media were classified and isolated for pure culture. 7 species of yeast were isolated from YPD media, and 9 species of bacteria were isolated from the NA base.

### 2. River microorganism growth in oil contaminated river water

To determine whether yeast and bacteria can survive in river waters containing oil, a mixture of yeast and bacteria at 0.01 A was placed into a base containing 0.1% diesel, an oil commonly used in ships. After being left to cultivate at room temperature for 48 hours, the mixture's absorbance was measured.

After 48 hours, the absorbance of yeast increased by 2850%, from 0.01 A to 0.285 A, and as the yeast proliferated in a similar manner in the river water contaminated with 0.1% diesel, it was determined that the survival of the yeast species was not affected by diesel contamination. Bacteria proliferated by 420% in the control water; however, in the 0.1% diesel contaminated water, it multiplied by 750%, 1.78 times that of the control (Table 1). Thus, it was concluded that diesel contamination in river waters caused the rapid proliferation of bacteria, which could lead to additional harm.

**Table 1. Growth in yeast and bacteria in diesel contaminated river water**

| | Control river water | 0.1% Diesel river water |
|---|---|---|

| | | |
|---|---|---|
| Total River Yeast | 0.285 | 0.282 |
| Total River Bacteria | 0.042 | 0.075 |

**3. Water quality of river samples**

The amount of heavy metal, nitrate, nitrite, total hardness, total alkalinity, and pH of the stream water sampled from Han River (Banpo Stream) or Okcheon (Chungbuk) was determined using a water metal check kit or an Eco-check kit. A higher level of total hardness represented a higher concentration of minerals such as magnesium and chalk, which can pose danger by facilitating the transfer of lead and other harmful substances in pipes to water, putting human health at risk. A higher alkalinity means a higher concentration of carbon-based mineral molecules suspended in the solution, which causes water to be 'harder'. Nitrites and nitrates come from fertilizers through run-off water, sewage, and mineral deposits. They can enter the body as nitrate then be converted into nitrite, which disrupts the oxygen-delivering ability of hemoglobin in the bloodstream. There were no signs of extreme contamination in the three waters, but in the two samples from the Han River, the total alkalinity was 40ppm. Han River water was slightly acidic with a pH of 6.5, and Okcheon Stream water was alkali with a pH of 8.5 (Table 2).

**Table 2. Water quality of different river waters**

| | Water metal (ppb (ug/L) | Nitrate (ppm) | Nitrite (ppm) | Total hardness (ppm) | Total alkalinity (ppm) | pH |
|---|---|---|---|---|---|---|
| Banpo #1 | < 10 | 0 | 0 | 0 | 40 | 6.5 |
| Banpo #2 | < 10 | 0 | 0 | 0 | 40 | 6.5 |
| Okcheon | < 10 | 0 | 0 | 0 | 0 | 8.5 |

**4. Water quality check using *Paramecium aurelia***

Various protists dwell in rivers. Protists are especially useful if the level of contamination of the water solutions is not able to be determined clearly using the water contamination kit. To find out whether water quality can be determined using *Paramecium* (*Paramecium aurelia*), the protist was placed inside stream water contaminated with diesel.

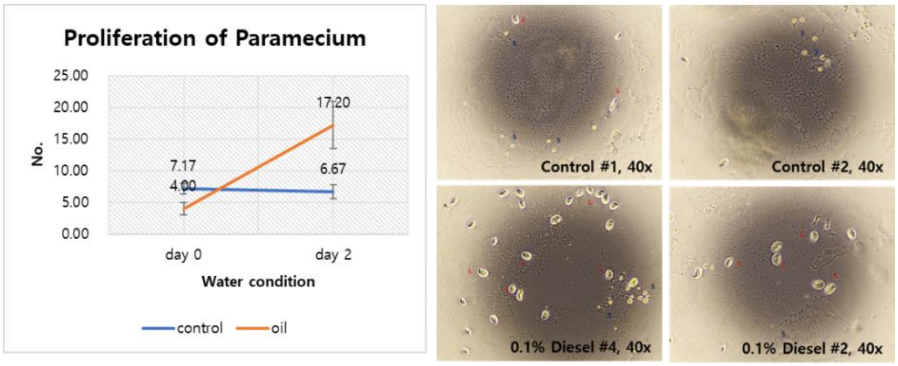


**Figure 6. Differences in number and size of paramecium in diesel contaminated river water**

After 48 hours, the number of *Paramecium* was counted under an inverted microscope (Fig. 6). In the control (no oil), on average, there was a 7% decrease in the number of *Paramecium*, from 7.17 to 6.67, and the size of the *Paramecium* was observed to be smaller. In the river water with 0.1% diesel, the number of *Paramecium* increased by 430%, from 4 to 17.2 on average, with their size observed to have increased and become rounder. The number of Paramecium on average was greater in the waters containing 0.1% diesel, and specimens were also bigger and rounder than the *Paramecium* found in distilled water. Therefore, *Paramecium* can be used as a biological indicator of the level of oil contamination in waters.

## 5. Contamination of river water with heavy metals and usage of *Paramecium* to detect level of contamination

*Paramecium*, as a living organism, will be able to proliferate better in river waters than in distilled water. As the proliferation of Paramecium is dependent on the various characteristics of river waters, its growth under different waters, with or without metal or oil, was observed. The level of heavy metal contamination in river water is originally low, so a copper stick and an iron stick were placed into tubes containing distilled water, Okcheon stream water, and Banpo stream water to encourage water contamination. After 2 days, it was observed that the heavy metal contamination level increased from 100 ppm to 400 ppm.

The number of *Paramecium* was determined in three waters containing either metals (copper and aluminum) or diesel. The number of *Paramecium* was greatest in the Okcheon Stream water, and its number was greater in presence of metals or diesel. For example, in the water containing diesel, the number of Paramecium was on average 19.00, while in the Okcheon Stream water without diesel, its number was 12.50 (Fig. 7). Through these results, it was determined that *Paramecium* is an effective biomarker of heavy metals and diesel in river waters with sufficient nutrients.

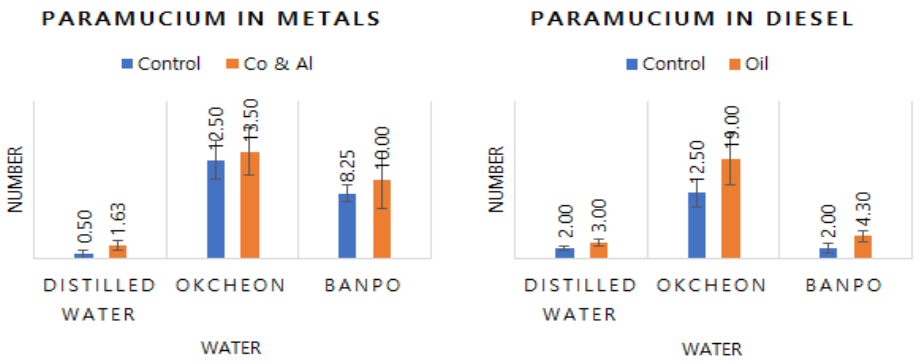


**Figure 7. Number of *Paramecium* in river waters contaminated with heavy metals and diesel**

### 6. Purifying effect of river microorganisms in contaminated river waters

2 sets of water remediation models were prepared by adding diesel, copper, aluminum, *Paramecium*, and *E. coli* to 2 L of water made from river water and distilled water at a ratio of 1 to 4. In the experiment model, river microorganism filtrate was added every day and the water quality was checked, and the total alkalinity, pH, or oil dispersion diameter of the control and experiment polystyrene boxes, which contained river water and diesel, was determined. The difference between the two boxes was that in the experiment box, a bacteria filtrate containing a mix of the selected bacteria was added regularly. On day 7, *E. coli* was added to both boxes. The graphs of total hardness and pH show that the levels were constant until day 7 when *E. coli* was added and the total hardness of the experiment box dropped to zero, while for the control, it increased to 50 ppm. The pH of the experiment box increased to 7 when *E. coli* was added, while the pH of the control remained the same, at 6.5. The total alkalinity did not change until the 7$^{th}$ day, but it rapidly increased to 300 ppm on the 9$^{th}$ day. This indicates an increase in the 'hardness' of water. Increased water hardness increases skin dryness, raising the risk of skin conditions such as eczema. The oil dispersion test shows that the oil diameter was the same with 4 mm for both boxes, but on day 2, the test conducted in the experiment box showed a decrease in the oil diameter to 2 mm, which indicated that oil was being decomposed. Nitrate and nitrite levels were also measured but no changes occurred during the measurement period (Fig. 8 & 9).

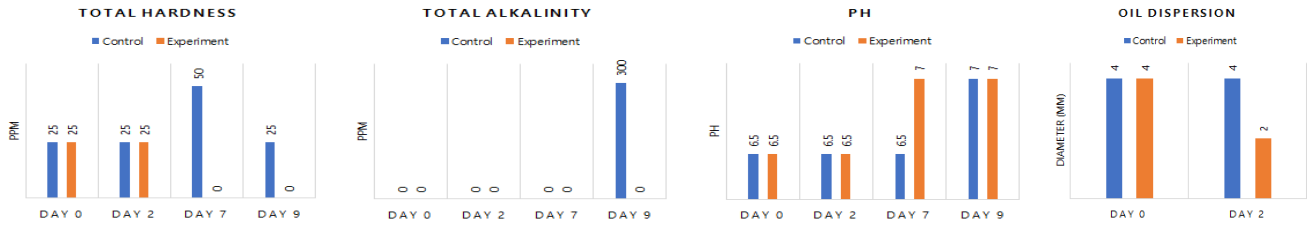


**Figure 8. Water quality check results from water remediation models**

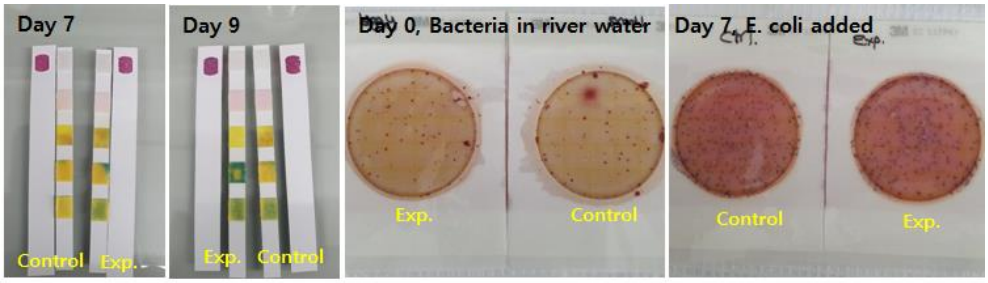


**Figure 9. Color changes in water quality kit and colony changes in *E. coli* culture media before and after the addition of *E. coli***

### 7. Selection of microorganisms effective in decomposing contaminants

#### a. Oil decomposing microorganism

The oil dispersion test was conducted to observe the microorganisms' ability to decompose oil. The negative control contained the NB or YPD media, and the positive control contained the media and oil. Both controls did not contain bacteria.

The bacteria grown in NB, NB D, NB F, or NB H showed the smallest solution diameter, with 4.35, 4.40, and 3.90 mm respectively, and the yeast grown in YPD D showed the smallest solution diameter at 4.00 mm (Fig. 10). This showed their effectiveness in decomposing oil.

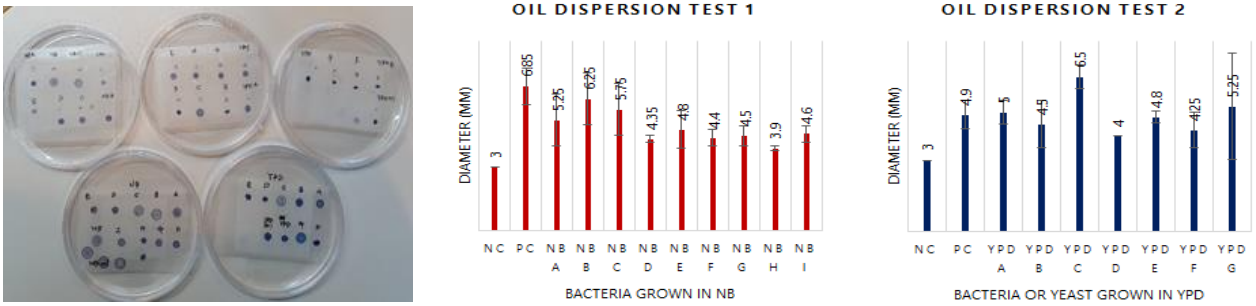


**Figure 10. Diameter of oil drops from oil dispersion test using parafilm after bacteria proliferation in diesel contaminated media**

#### b. Identification of species of selected bacteria using DNA sequence analysis

The bacteria grown in the selection media were isolated using the streaking method for pure culture towards conducting a genetic analysis (Fig. 11).

**Table 3. DNA sequencing of selected bacteria and their matches to species**

| Sample | YPD D | NA D | NA F | NA H |
|---|---|---|---|---|

| Species | *Phytobacter diazotrophicus* | *Comamonas testosteroni*, *Pseudoxanthomonas taiwanensis* | *Stenotrophomonas maltophilia*, *Uncultured gamma proteobacterium* | *Bacillus megaterium*, *Gamma proteobacterium* |
|---|---|---|---|---|
| Identities | 99% | 80%, 70% | 84%, 83% | 75%, 79% |

Table 4 shows the closest match of the species to each bacterium according to the percentage of similarity. In the case of YPD D, a match of 99% was found with *Phytobacter diazotrophicus*, while the matches for the other three species of bacteria ranged from 70% to 84%, which signified that the exact species could not be identified. Even though bacteria isolation and pure culture was conducted over 3 times, it seems that a mix of different bacteria was cultured. It was predicted that yeast would be identified from YPD D, as it was a YPD base, but the DNA sequence analysis identified *P. diazotrophicus*, a species of bacteria (Table 3).

### c. Ability of selected bacteria to purify contaminated water

In the water contamination test conducted on a control (no bacteria) and the 4 selected bacteria, it was observed that the level of nitrite for the 4 bacteria dropped to 0 (with the exception of NB D with 0.5 ppm on day 5) after day 2, while the control had a nitrite level of 1,1, or 0.5 ppm for day 0, day 2, and day 5, respectively. These results showed the bacteria's effectiveness in decreasing the nitrite concentration. The results from the total alkalinity test show that NB D was the most effective in decreasing the alkalinity, as it was the only bacteria out of the four with a level of 0 ppm (Fig. 11).

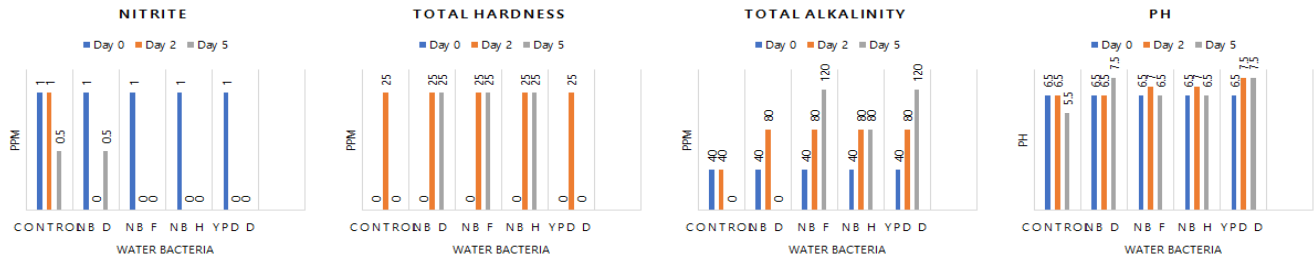


**Figure 11. Water quality check results after the addition of selected bacteria**

A water quality check was conducted for the 5 waters with the selected bacteria added. Using copper sticks, it was confirmed that the bacteria were most effective in decreasing the amount of water metal (ppb) in Banpo Stream water, as before they were added, the level was at 200 ppb; however, after the bacteria were added, the level of heavy metals in the water decreased to 10ppb. In addition, NB D and YPD D were the most effective in decreasing the level of heavy metals in Okchun AL, being the only species that brought the PPB down to 10 (Fig. 12).

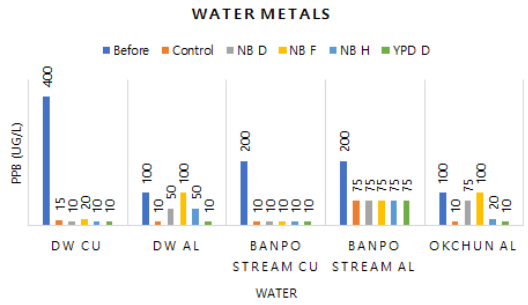


**Figure 12. Water metal check results after addition of selected bacteria**

### d. Selected bacteria proliferation in river water

The absorbance of the four bacteria in river waters were measured using a UV spectrophotometer, which showed that YPD D grew the best in river water as its absorbance was the highest with 0.146 (Fig. 13).

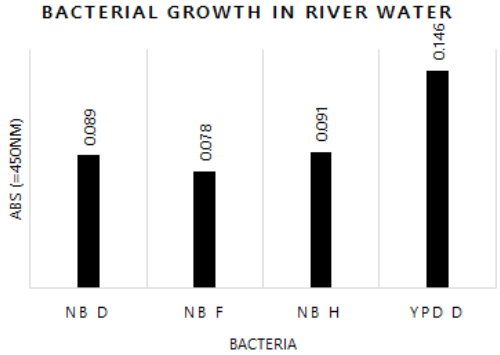


**Figure 13. Absorbance measured after the proliferation of selected bacteria in river water**

## 8. Development of bioremediation model for purifying bacteria in rivers

Bacteria need to be able to proliferate at temperatures below room temperature in order to survive in rivers. In this experiment, ink was used to represent the bacteria so that the spread in the water could be easily observed after it was deployed through straws with holes in the polystyrene box. In the beginning, the ship model floated too far underwater, making the deployment of ink difficult due to the increase in pressure. In order to solve this problem, styrofoam was attached onto the bottom of the ship model so that the ship could float more easily. After the change, the deployment and spread of ink could easily be observed as the ink was being sprayed from the air towards of water (Fig. 14).

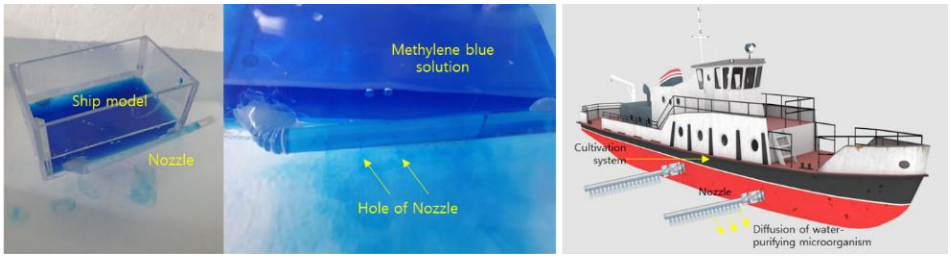

**Figure 14. Model of a microorganism diffusion system in ships and potential functioning of diffusion nozzles attached to the ship**

Additionally, through the water remediation model, the effectiveness of proliferated bacteria for water remediation even after continuous addition into the river was confirmed. With the attachment of a bacteria cultivation system and a percolation system inside the ship and an installment of tubes to the outside of the ship, microorganisms would be able to be sprayed into the river through a nozzle connected to the tubes. The current model includes a nozzle with a diameter of 20 mm, and a length between 2 m and 3 m. In addition, the model plans for an automatic mechanism enabling the nozzles to come out of the ship, spray the microorganisms, and then automatically return after the process is complete (Fig. 21). However, through further testing, the exact mechanisms may be corrected and changed.

## DISCUSSION

The purpose of the research was to select microorganisms effective in purifying water to design a bioremediation system where ships can continuously spray microorganisms into the river while traveling for constant water purification. Samples from two areas in the Banpo Stream and Okchun Stream were obtained to determine potential safe microorganisms effective in water purification. A total of 16 species were classified: 7 species of yeast and 9 species of bacteria. In the river water containing 0.1% diesel, the river yeast survived, but the numbers remained constant. However, in the case of river bacteria in the diesel-contaminated river water, the bacteria did not only survive, but their numbers also increased by 1.78 times. Additionally, in the case of *Paramecium*, the numbers of the protist increased by 4.3 times in diesel-contaminated water. *Paramecium* increased in size and amount in the presence of oil, as well as copper and aluminum, which signified that *Paramecium* could be effectively used as a biomarker for the purification of water in the bioremediation system.

A water quality test for water metal, nitrate, nitrite, total hardness, total alkalinity, and pH was conducted, after which it was detected that the total alkalinity was 40 ppm for the two areas in the Banpo Stream water. The research also sought to design a water remediation model in which stream water was added to 2 polystyrene boxes, after which each was contaminated with copper, aluminium, *Paramecium*, diesel, and *E. coli*. In one of the boxes, the ‘experiment’ box, a microorganism filtrate was added, and after 7 days, it was observed that the total hardness and total alkalinity of that box decreased to 0ppm, which demonstrated the filtrate’s effectiveness. 4 types of bacteria, YPD D, NA D, F, and H, were first selected from an oil dispersion test that was conducted in order to determine which bacteria species was the most effective in decomposing oil. After having observed the 4 bacteria’s ability to purify water and remove contaminants, and their ability to grow in river water, YPD

D bacteria, or *Phytobacter diazotrophicus*, was selected. Finally, a continuous dispersal system for the microorganisms was developed with nozzles attached to a ship model, through which the dissemination of water purifying bacteria into river waters could be observed and potentially applied to moving ships.

The addition of bacteria into rivers, however, may pressure the dissolved oxygen supply in the water. As bacteria respire aerobically, this may mean that, in the long term, the oxygen supply for other organisms may be restricted. This would depend on the rate of multiplication of the bacteria, but also such threat is limited as there is a constant inflow and outflow of freshwater in rivers.

## CONCLUSION

In conclusion, the study identified *Phytobacter diazotrophicus* as a species of bacteria highly effective in decomposing oil and purifying other harmful contaminants such as heavy metals in river waters. With this knowledge, a dissemination system which can be installed in vessels was developed to continuously release the selected bacteria into river waters as a continuous bioremediation method to combat the accumulation of harmful contaminants in rivers. Further studies can develop this system and design it to be applicable to real vessels traveling through rivers, posing an immediate solution to the growing danger of the accumulation of contaminants in freshwater sources. In addition, further research is required to deduce whether the existence of such bacteria in river water has an impact on downstream communities, especially on agriculture and human operations.

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