Stockholm Junior Water Prize 2023

Groundwater resilience: A sustainable approach to utilize nitrate contaminated water for agricultural use

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I. Preliminary Sources

A. Research Summary

High levels of nitrate pollution in groundwater threatens the quality of water resources, especially contaminating agricultural water and infecting people living on developing countries where there is lack of water treatment facilities. Also, the pollution and scarcity of agricultural water involves implementing water management practices, crop harvesting methods, and purifying the water resources in use. Addressing the problems above, the research mainly studied the ion effect of nitrate-contaminated groundwater on the growth of *B. subtilis,* interaction of *B. subtilis* with nitrate in groundwater, and further impact of this interaction on the whole growth of the plant.

Using analysis of ion chromatography, the ion composition of the nitrate-contaminated groundwater sample was analyzed. Firstly, after the incubation of bacterial suspension (i.e., *B. subtilis)*, the supernatant liquid of TSB media was removed and the media containing different ion compositions were tested to examine whether it has an ability to sustain *B. subtilis* growth. The nitrate uptake efficiency was tested with the ion chromatography analysis by comparing the difference between initial amount of nitrate on sample ion media that contained nitrate and the final amount after *B. subtilis* was inoculated. Secondly, the treated groundwater by *B. subtilis* was inoculated to the kidney bean in order to determine the effect of the biologically treated groundwater on promoting plant growth. The experimental results showed that the nitrate ion in groundwater genuinely affects the microbial growth and revealed that the content of nitrate was reduced as their microbial growth. Furthermore, the group that included the nitrate substance positively contributed to the plant growth.

This study is significant in that it can help to secure available water resources, which is a global issue, and thus ensure a smooth supply of future human food resources. In future studies, we would like to investigate the secondary effects of growing microorganisms in groundwater over a longer period of time to see if there are any factors that can have a positive effect not only for growth of plants but also other matters such as production of crops, water saving, and surrounding ecosystem.

B. Key Words

water pollution, cyanosis, groundwater resilience, livestock operation, biomanipulation, water contamination, bioremediation, plant growth, nitrate, contaminant, livestock, usable agricultural water, Plant Growth Promoting Rhizobacterium (PGPR)

C. List of Abbreviations and Terminologies

DW (Distilled water), GW (Groundwater), NO_3^-- GW (Nitrate-contaminated groundwater), PGPR *(*Plant Growth Promoting Rhizobacteria)*, B. subtilis* (*Bacillus subtilis*), UV (Ultra-violet), IC (Ion Chromatography), OD (Optical Density)

D. Acknowledgements

First of all, our team expresses a sincere appreciation to all Korean Minjok Leadership Academy teachers and students who taught and supported us to successfully lead our project. We would like to give a special appreciation to Jong-Uk Na, high school teacher who led us on Korea Junior Water Prize. We also would like to give a special thanks to Korea Water Forum (KWF) for providing an

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E. Researcher Biography

Seungik Cho (18) is an incoming undergraduate student at Rice University who pursues to study biological sciences and water sustainability at Wiess College of Natural Sciences. He is passionate about engaging research projects related to natural resources, microbiology, and sustainable development. As a national representative team, he previously won silver medal on Korean Science & Engineering Fair ('21), gold medal on Youth International Science Fair ('22), and bronze medal on GENIUS Olympiad Environment Project Fair ('22).

Jungwoo Kim (18) is a junior student attending Korean Minjok Leadership Academy, an independent boarding school which is a member of G20 school group. He is a big fan of nature and passionate explorer on the field of biochemistry. As a finalist, he awarded the power pitch winner of the Conrad Challenge 2023 by presenting new method of eliminating space debris for sustainable future of space exploration. He also served as an intern at department of nuclear medicine in Seoul ST. MARY's Hospital since 2019.

II. Introduction

A. Problem Statement

(1) Groundwater contamination

Groundwater, a valuable natural resource with significant economic and social importance, plays a crucial role in supplying drinking water and supporting agricultural irrigation globally. However, in recent decades, water resources have confronted great threat due to unsustainable extraction and increased pollution levels. Factors such as climate change, land use changes, and population growth pose various threats to the quantity and quality of groundwater. [1] Among these threats, agricultural activities contribute the most to diffuse pollution, primarily through the application of fertilizers and spreading of animal manure. Consequently, nitrate pollution has emerged as a major concern, with elevated nitrate concentrations being a common problem in many regions worldwide. [2]

(2) Public health risks

High nitrate levels in groundwater pose both public health risks and environmental hazards. Immediate effects, known as acute toxicity, can occur when individuals consume water with high nitrate concentrations, potentially leading to methemoglobinemia or "blue baby syndrome," where blood cannot adequately transport oxygen to the body's cells. [3] Long-term exposure to elevated nitrate levels in drinking water has also been linked to increased risks of various types of cancer, including gastric, colorectal, bladder, urothelial, and brain tumors. Therefore, regulating nitrate concentrations in drinking water is crucial to minimize public health risks. The presence of excessive nitrate levels in groundwater poses a significant environmental and public health concern.

(3) Biomanipulation occurring on ecosystem

Moreover, the contamination of groundwater with nitrates yields detrimental consequences for ecosystems. When nitrates infiltrate surface water bodies, they instigate eutrophication, an excessive proliferation of algae and other aquatic plants. [4] This disrupts the delicate balance of the aquatic ecosystem, depletes oxygen levels, and ultimately results in the demise of fish and other aquatic organisms. Additionally, nitrate pollution has the potential to taint drinking water sources, necessitating costly treatment procedures to ensure its safety for human consumption. Resolving the issue of nitrate contamination in groundwater necessitates a comprehensive approach encompassing effective regulations, sustainable agricultural practices, improved waste management systems, and heightened public awareness.

(4) Agricultural water scarcity

The global challenge of agricultural water scarcity and food shortage is intensifying, jeopardizing the livelihoods of millions and exacerbating hunger and malnutrition. Factors such as population growth, urbanization, climate change, and unsustainable water management practices contribute to the scarcity of water in agriculture. Insufficient access to water for irrigation leads to reduced crop yields, lower agricultural productivity, and food shortages. This problem is further compounded by climate change, which disrupts farming practices and exacerbates water scarcity. Addressing these challenges requires sustainable water management, efficient irrigation techniques, and investments in agricultural technologies.

(5) Water resilience matter

Johan Rockström, an authority on water research, mentioned that when nature, which was previously in A state, reached a more negative state, B, transitioning to exceeding state C must be needed rather than recovering it back to A state. [5] The strategy for improved state is defined as "water resilience." To reach out to the state of water resilience, it requires an alternative method of water management regarding water sanitation, agricultural water resources, and water sustainability issues. Since it requires energy resources to recover the state of purified water from contaminated water, the sustainable approach of the problem is required in modern water management system.

B. Theoretical Background

The research conducted by C. de Fraiture (2010) stated that increasing global demand for water in agriculture is becoming huge issue due to population growth, rising incomes, and changing dietary preferences. The study highlighted the intensifying competition for water among industrial, urban, and environmental users. [6]

From the previous research conducted by S. Zaidi (2006), it has been known that Bacillus subtilis had the role of facilitating nickel accumulation on mustard plants, revealing the data that the inoculated *Bacillus subtilis* helped the plant growth promotion and increased the capacity of bioaccumulation. [7] According to the study of Jorge Olmos (2019), it has been revealed that the utilization of probiotic bacteria, particularly *B. subtilis*, offers the ability to suppress pathogenic growth, improve nutrient assimilation, and enhance environmental conditions in aquaculture settings. [8]

Also, the study conducted by Usha Bishnoi (2015) states that soil, as a dynamic and valuable natural resource, houses a vast array of microorganisms and plays a crucial role in food and fiber production. Moreover, it is responsible for maintaining global nutrient balance and supporting ecosystem function. [9] Within the soil-plant system, plant growth promoting rhizobacteria (PGPR), which are a diverse group of soil bacteria, engage in complex interactions in the rhizosphere, thereby influencing plant growth and yield. In recent years, PGPR have emerged as a significant and promising tool for sustainable agriculture. The research highlights that PGPR directly or indirectly promote plant growth and development through various means. This includes the release of plant growth regulators/phytohormones and other biologically active substances, manipulation of endogenous phytohormone levels, enhancement of nutrient availability and uptake through fixation and mobilization, and mitigation of the harmful effects of plant pathogens. The research emphasizes that by harnessing these mechanisms, PGPR offer an economically attractive and ecologically sound approach to augmenting nutrient supply and preserving soil fertility. Therefore, it underscores the need for the development and commercialization of PGPR to achieve sustainable agricultural practices.

Since the groundwater nitrate pollution has been a severe global problem, the research of A. H. Mahvi (2013) studied the significant effects of high nitrogen fertilizer application rates on groundwater pollution. [10] The results of the study have shown that the excessive usage of nitrogen fertilizer might cause severe damages on farmland pollution.

C. Objectives of Study

Groundwater contamination poses an escalating threat as nitrates from factory wastewater and other sources infiltrate this vital resource. The repercussions of such contamination are already evident, with reduced availability of agricultural water, thus exacerbating the challenge of nourishing an ever-expanding global population. Consequently, the research mainly studies the ability of plant growth promoting rhizobacterium on groundwater condition which can not only hold potential for remediating contaminated groundwater but also have the capacity to revolutionize food production methods by contributing to plant growth. Through an investigation into the growth patterns of *B. subtilis* and its ability to mitigate nitrate concentration, an indicator of water pollution, we aim to establish a viable solution to address the dire water quality conditions in developing nations while simultaneously addressing the global food supply imbalance.

D. Hypothesis

Under groundwater condition, *B.subtilis,* plant growth promoting rhizobacterium, should show significant microbial growth performance since the ions will genuinely contribute to the microbial growth. Not only the growth of bacteria, but with a nitrate-contaminated groundwater, *B. subtilis* would show an efficient nitrate uptake rate by using nitrate as the metabolic regulators. Inoculating *B. subtilis*, which is grown in nitrate solution and groundwater, to kidney beans under growth in soil would promote plant growth while plants with nitrate-contaminated groundwater and distilled water added would not show less superiority on plant growth.

III. Materials and Methods

A. Media and Reagents

Ground water was sampled from a well in Hongseong City, South Korea (36˚35’54.3”N, 126˚40’3.4”E). As shown on the figure, the livestock industry in the city of Hongseong is causing significant pollution to the local water resources, resulting in environmental degradation and posing a severe problem due to the excessive amounts of fertilizers and excreta produced by livestock (Fig 1). The presence of high levels of nitrate substances in the water has further exacerbated the environmental issues and sustainability of the city's water supply. [11]

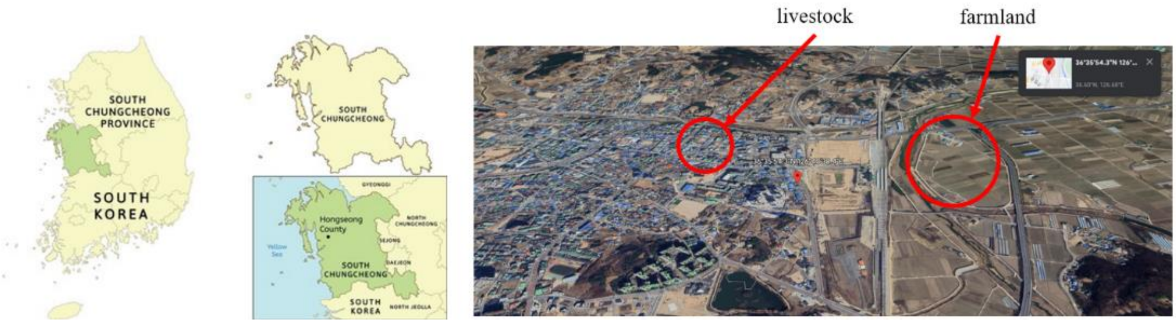

Fig 1. The location and field map of Hongseong City in South Korea

Sodium nitrate ($NaNO_3$, ≥98%), sodium sulfate anhydrous (Na_2SO_4, ≥99%), magnesium chloride hexahydrate ($MgCl_2$ $6H_2O$, ≥99%) were purchased from Samchun Chemicals, South Korea. Sodium hydroxide (NaOH, ≥97%) and hydrochloric acid (HCl, 35%) were purchased from Daejung Chemicals & Metals, South Korea. Sodium chloride (NaCl, 99.5%) and calcium chloride ($CaCl_2$, ≥95%) were purchased from Showa, and Junsei, Japan respectively. D-(+)-Glucose (≥ 99.5%) was purchased from Sigma-Aldrich, USA. All solutions were prepared using deionized water (DIW, 18.2 MΩ) which was purified by ultrapure filtration system (HUMAN POWER I+ Water purification, Korea).

The bacterial strain resources of *Bacillus subtilis* (KACC 13751, Source: plant growth promotion) used in this research were distributed from Korean Agricultural Culture Collection (KACC) in a freeze-dried ampoule form. The media used for the growth of bacteria was BD Bacto Tryptic Soy Broth (Soybean-Casein Digest Medium) for *Bacillus subtilis*.

B. Analytical Method

(1) Ion Chromatography Analysis

All the aqueous samples were filtered using 0.2µm polyvinylidene fluoride syringe filters (Whatman) before the analysis. The concentrations of the dissolved cations (i.e., Na^+, K^+, NH_4^+, Ca^{2+} and Mg^{2+}) and anions (i.e., F^-, Cl^-, Br^-, NO_2^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}) were measured using ion chromatography (IC) (Metrohm, 883 Basic IC plus) with a cation column (Metrosep c4-150/4.0, Metrohm AG) and anion column (Shodex IC Anion sep No. 82504A). A mixture of nitric acid (34 mM) and dipicolinic acid (14 mM) was used as the cation IC eluent with a flow rate of 0.9 mL/min, and a mixture of Na_2CO_3 (3.2 mM) and $NaHCO_3$ (1 mM) was used as the anion eluent with a flow rate of 0.7

mL/min. We prepared the calibration curves for 1, 5, and 10 ppm using standard solutions for various ions, and the peaks were identified according to their retention times. Fig 2(a) and (b) show the peak results for the standard solution of 10 ppm of anions and cations, respectively. Anions were detected in the order of F^- (RT = 5.35 min), Cl (RT = 7.92 min), NO_2 (RT = 9.3 min), PO_4 (RT= 18.77 min), and SO_4 (RT = 21.6 min), and cations were detected in the order of Na (RT = 5.07 min), NH_4 (RT= 5.64 min), K (RT = 7.60 min), Ca (RT = 14.69 min), and Mg (RT = 18.09 min). The prepared calibration curve confirmed high correlation coefficients ($R^2 > 0.99$) for all ions as a result. Fig 3(c) and (d) show the IC results of sampled groundwater. For anions, strong peaks were observed at 7.89, 13.22, and 21.69 min, corresponding to Cl, NO_3, and SO_4, and in the case of cations, strong peaks appeared at 4.96, 15.11, and 18.95 min corresponding to Na, Ca, and Mg. After confirming the accuracy of IC analysis, we measured the ion concentration in nitrate-contaminated groundwater (i.e., Cl^-, NO_3^-, SO_4^{2-}, Na^+, Ca^{2+}, and Mg^{2+}) (Table 1).

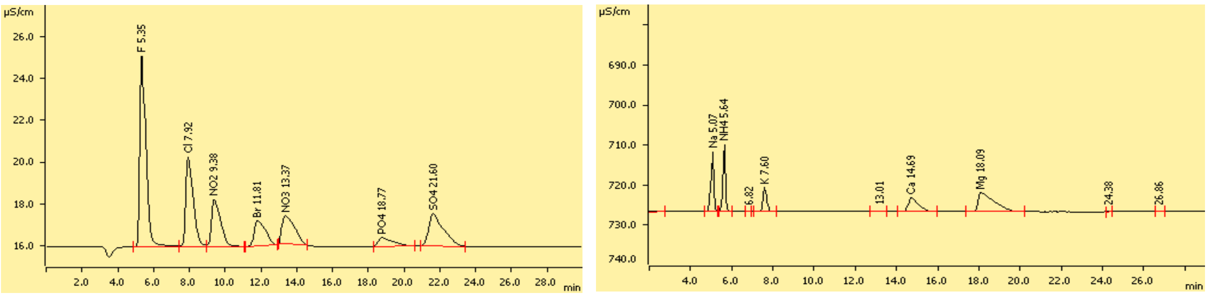

Fig 2. The IC results for the standard solution [(a): left / (b): right]

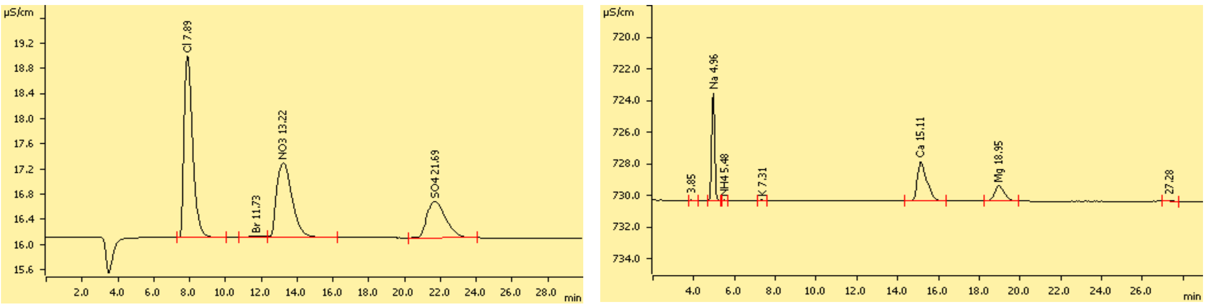

Fig 3. The IC results for the groundwater sample [(c): left / (d): right]

Table 1. Concentration of each ion on groundwater sample

Ion type	Concentration (mg/L)
Cl^-	77.74
NO_3^-	103.13
SO_4^{2-}	48.83
Na^+	40.32
Ca^{2+}	66.89
Mg^{2+}	11.51

(2) UV-vis spectrophotometer Analysis

In order to confirm the quantity of microbial populations (i.e., *B. subtilis*) at each batch culture medium, we carried out analysis by indirect methods. UV-vis was used to decide the absorbance under 600 nm (i.e., OD 600). Before the analysis, a baseline for the wavelength assigned to 600 nm was set using a polystyrene cuvette (path length = 10 mm) containing DW, and it was confirmed that the absorbance value using DW was 0. At each sampling time, 3 mL of microbial suspension was aliquoted using a sterilized micropipette and transferred to a cuvette. The analysis was performed after confirming that there were no bubbles and foreign substances in the cuvette wall.

(3) Plant growth Analysis

After the germination of kidney bean, the size of each structure of plant were measured. The germination rate was calculated by counting how many kidney bean seeds germinated compared to the total number of kidney bean seeds initially planted on the soil. For each germinated kidney bean, the length of root, stem, and the size of the leaves (horizontal, vertical length of each point), biomass of each structure were measured (Fig 4).

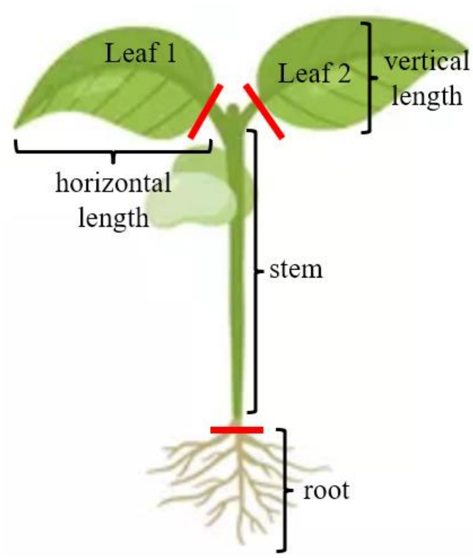

Fig 4. The structural diagram of kidney bean for analysis

C. Effect of nitrate-contaminated groundwater (NO_3^--GW) on *B. subtilis* growth

(1) Incubation of *B. subtilis*

The freeze-dried ampoule of *B. subtilis* were incubated on lysogeny broth condition. For the bacterial suspension preparation, the *B. subtilis* ampoule was suspended with 1 mL of distilled water. For the bacterial medium, 3 g of tryptic soy broth (TSB) powder was dissolved on 100 mL of distilled water with 30 g/L concentration. After the sterilization with autoclave, the bacterial suspension was inoculated on liquid TSB medium and incubated for 24 hours in 37˚C, 100 rpm condition. For even time intervals, the absorbance of bacterial suspension was measured using 600 nm of UV-vis spectrum spectrophotometer and the growth curve was sketched (Fig 5).

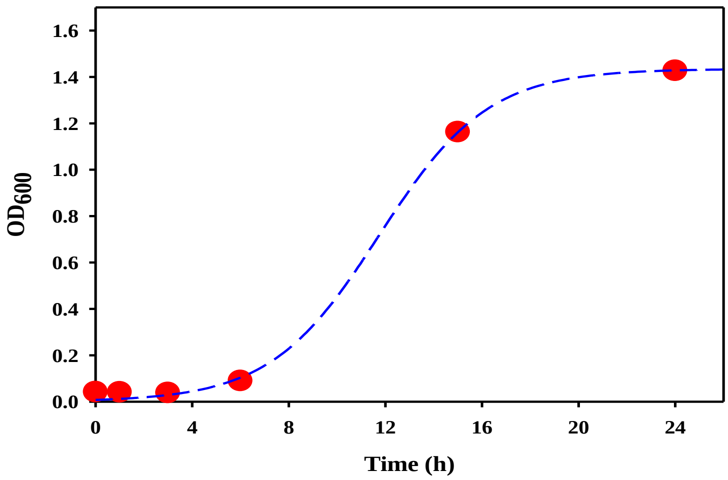

Fig 5. The growth curve of *B. subtilis* (KACC 13751) on TSB medium

(2) Preparation of ion sample medium

Based on the ion composition of real groundwater measured by IC, the ion sample media for bacterial suspension was made. Based on the result (Table 1) of ion concentration of groundwater, the ion sample medium was made with same composition of groundwater. The concentration of each ion was made with 1.7mM of nitrate, 0.5mM of sulfate, 1.7mM of sodium, 0.5mM of magnesium ion. For the initial experiment, all of ion sample medium had 1% of sodium chloride due to controlling osmotic regulation of microbial cell. Also, the 0.1% of glucose solution, filtered using 0.45μm polyvinylidene fluoride syringe filters (Whatman), was added on the sample ion sample medium.

(3) Inoculation of *B. subtilis* to ion sample medium

The seven groups of sample medium (NaCl + DW, NaCl + NO_3^-- GW, NaCl + $MgCl_2$, NaCl + $CaCl_2$, NaCl + Na_2So_4, NaCl + $NaNO_3$, NO_3^-- GW) were made to incubate *B. subtilis* on the condition with selective ions. 100 mL of each samples containing 0.1% of filtered glucose solution were duplicated and evenly distributed to the autoclaved 250 mL-bottles. To change the TSB liquid medium of bacteria, the supernatant solution of bacteria was removed after the centrifugation on 600 rpm for 10 minutes. The washing process of growth medium was done twice by inoculating 1 mL of sample ion sample medium on the tube containing bacterial suspension. After substituting the media of the bacterial suspension, *B. subtilis* was inoculated on each bottle and incubated for each specific time of UV sampling in 37˚C, 100 rpm condition.

(4) Evaluation of nitrate uptake ability

After measuring the absorbance of bacterial suspension that contained nitrate ion, the bacterial suspension was filtered using 0.2μm polyvinylidene fluoride syringe filters (Whatman). The initial and final amount of nitrate ion of filtered solution was measured using IC analysis and the nitrate uptake efficiency was evaluated.

D. Examination of plant growth promotion

For testing the synergy effect between groundwater and plant growth promotion rhizobacterium, the five groups of kidney beans with three grounds each were planted on the soil. The five kidney bean groups containing DW, NO_3^-- GW, NO_3 (DW 90% + NO_3 10%), NO_3+*B.subtilis,* NO_3^-- GW+*B. subtilis* were triplicated. The plant was watered with these five different waters every day and observed for a week. After the germination, the growth of germinated kidney beans was analyzed and dried on the dry oven for 24 hours in 105˚C condition to compare the biomass of each group.

IV. Results and Discussions

A. Microbial growth in NO_3^--GW and DW

(1) Comparison of groundwater and distilled water

As the absorbance was measured on 600 nm of UV-vis spectrophotometer, the groundwater and distilled water showed the significantly different trend on the microbial growth. The *B. subtilis* incubated in distilled water showed the low growth rate and the absorbance finally decreased in the time interval of 40 h to 80 h. The group of groundwater showed the increasing rate of the absorbance finally reaching over 0.04 compared to the initial absorbance, indicating that the groundwater has significant effect on microbial growth (Fig 6).

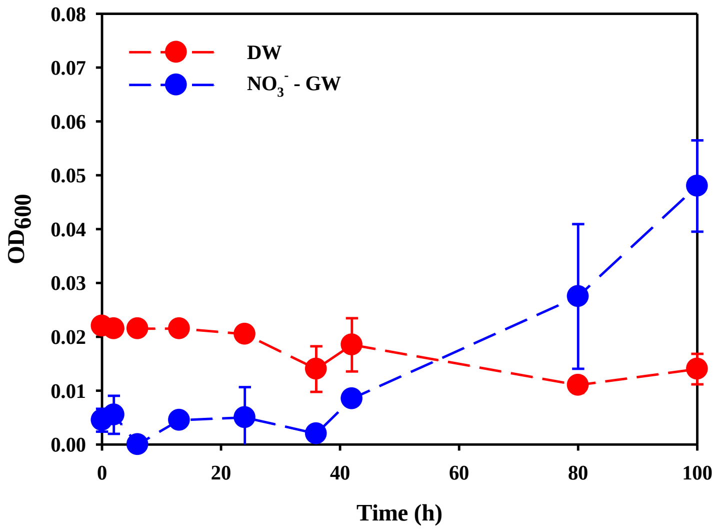

Fig 6. The microbial growth of *B. subtilis* in distilled water and groundwater

B. Effect of NO_3^- - GW ions on microbial growth

(1) Effect of cation and anion on microbial growth

After the ion composition of groundwater was analyzed, the effect of cation and anion to the growth of *B. subtilis* (when sodium chloride present) was measured. It has been shown that there was no cation that showed significant growth as groundwater group and the absorbance finally reached around 0.01 with no increase (Fig 7). However, the nitrate ion showed the significant growth compared to other anion groups, outdoing the final absorbance of groundwater group with 0.07 of absorbance after 100 hours passed (Fig 8). Since it has been known that *B. subtilis* can utilize nitrate as an alternative electron acceptor for its growth, allowing it to carry out respiration even the oxygen is limited. It shows the metabolic advantages that *B. subtilis* can take up when the sample media contained the substantial amount of nitrate ion.

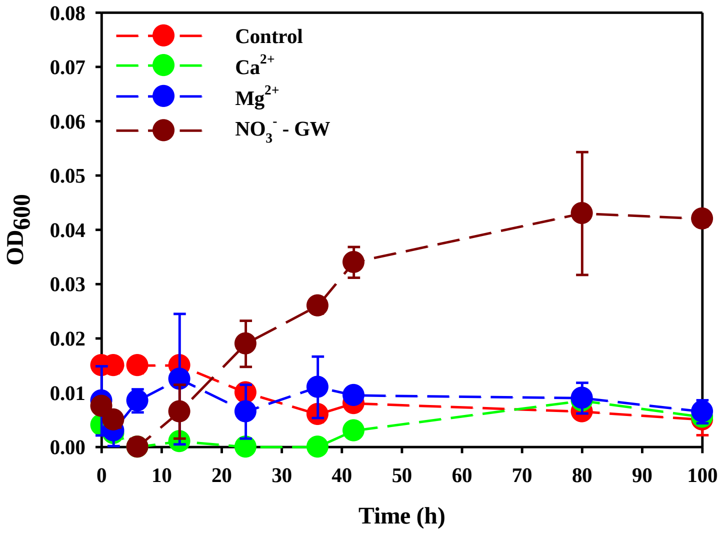

Fig 7. The microbial growth on the cations of groundwater

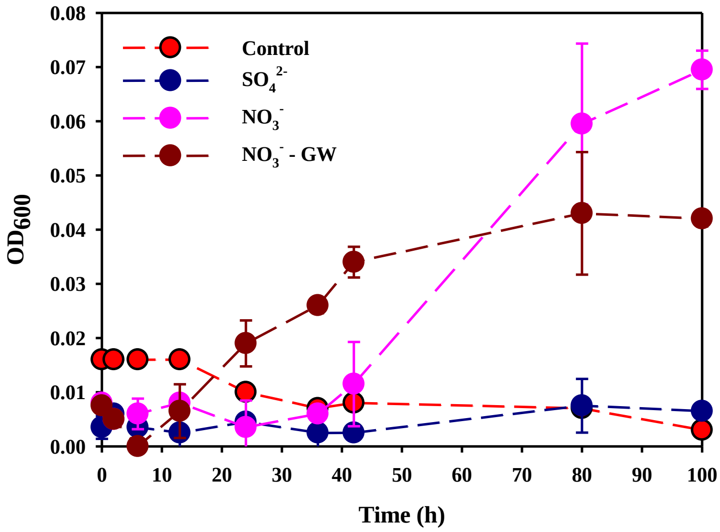

Fig 8. The microbial growth on the anions of groundwater

(2) Effect of nitrate on microbial growth

To prove that the nitrate purely contributes to the microbial growth on the groundwater condition, the sodium chloride was removed in the second experiment and the absorbance was measured. After 120 hours, the final absorbance of the bacterial suspension that contained nitrate showed the significant amount of increase compared to other groups with distilled water, calcium, magnesium, sulfate ion in both NaCl, without NaCl conditions (Fig 9). Since it showed an alignment with the groundwater group, it could be concluded that the nitrate has a central role for contributing to microbial growth in groundwater condition. Since the real groundwater condition doesn't have 1% amount of sodium chloride, the experiment proved the ability of nitrate on supporting microbial growth on groundwater (Fig 10).

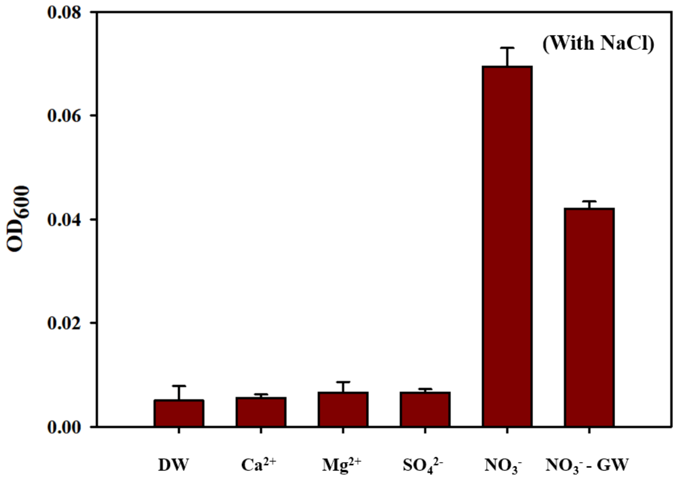

Fig 9. The microbial concentration of when sodium chloride present

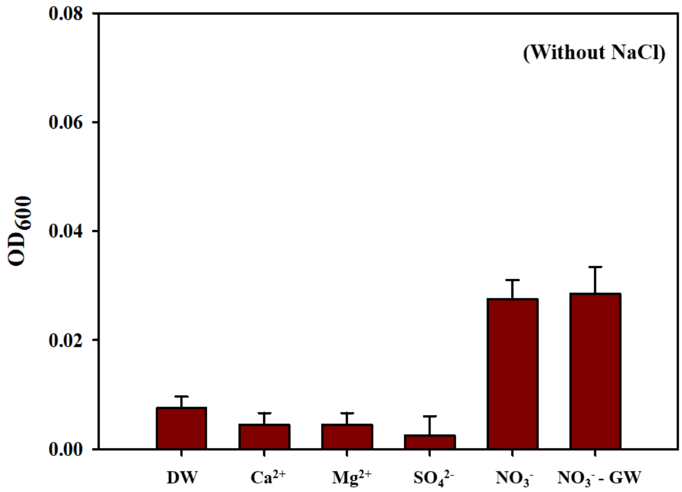

Fig 10. The microbial concentration of when sodium chloride not present

(3) Optimal range of nitrate effect on microbial growth

After confirming the effect of nitrate on microbial growth of groundwater, the microbial growth on different concentration of nitrate was measured. The data shows that the bacterial concentration slightly decreased on 100 ppm of nitrate condition compared to 10 ppm of nitrate condition. It shows that the optimal point of contributing to microbial growth lies between 10 ppm and 100 ppm. Since it shows that the absorbance of NO_3^-- GW showed the similar trend with NO_3^- 100, it can be shown that the *B. subtilis* can have an ability to survive on same condition (Fig 11).

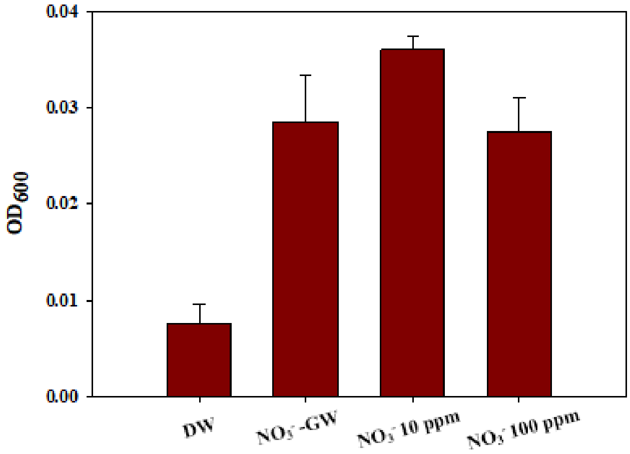

Fig 11. The microbial concentration on different conditions

(4) Nitrate uptake amount on each condition

For the same groups for examining optimal range of nitrate effect on microbial growth, the high amount of nitrate was uptake on the group where the nitrate contamination was in 100 ppm level compared to 10 ppm level. It shows that the amount of nitrate being in uptake is well activated in high concentration of nitrate, indicating the further use on water purification. Compared to the Fig 11, the microbial concentration is higher on the concentration of 10 ppm while it has less amount of uptake on nitrate. The result could be confirmed that the higher concentration of nitrate on groundwater could induce the high amount of nitrate uptake since the results shown on the graph is identical for NO_3^--GW and NO_3^- 100 ppm (Fig 12).

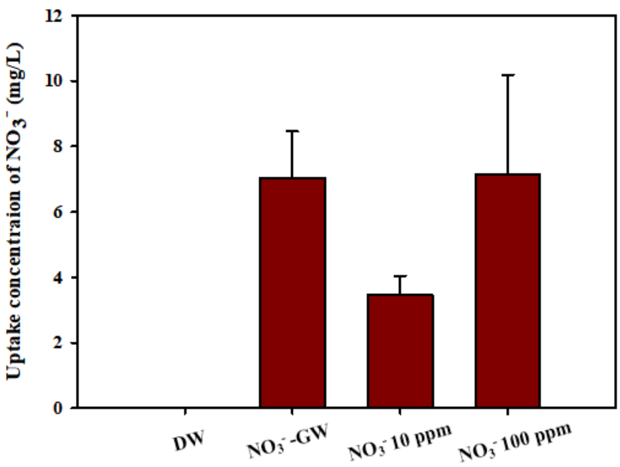

Fig 12. The uptake concentration of nitrate on different conditions

C. Comparison of plant growth promotion

(1) The germination and growth of kidney bean

To examine the rate of plant growth, the triplicated soils for the kidney beans were observed in a daily measure. It has been shown that the kidney where distilled water was used had its first germination on fourth day after planting. However, the group that contained nitrate contaminated groundwater had its first germination on sixth day, which was the latest among the other four groups. It could support the fact that nitrate contaminated water delays the germination of the plants and finally deteriorates the growth of kidney beans. With the similar trend of nitrate contaminated groundwater, nitrate sample ion medium showed the late germination on the fifth day after planting. However, the groups where PGPR (*B. subtilis*) was contained with nitrate had the early germination period after three or fourth days of planting. Also, it performed the superior growth on the last day before cultivating. The daily growth data of kidney bean shows that the interaction between PGPR and nitrate contaminated groundwater interacts each other by producing positive effect on plant growth acceleration (Fig 13).

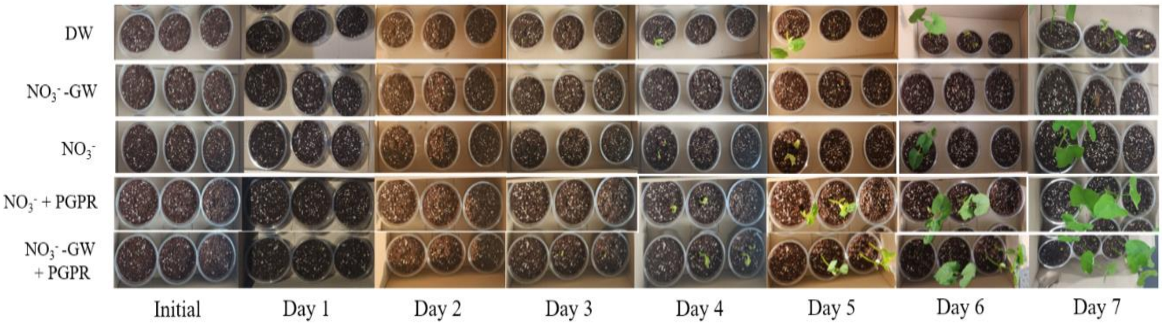

Fig 13. The daily growth of kidney bean on different conditions

Also, the plants were analyzed in a detailed measure after cultivating each group of kidney beans. It has shown that the size of the kidney bean was superior where PGPR and nitrate contaminated water was interacting together. The group where nitrate contaminated groundwater was included significantly has the lowest stem length compared to other groups (Fig 14).

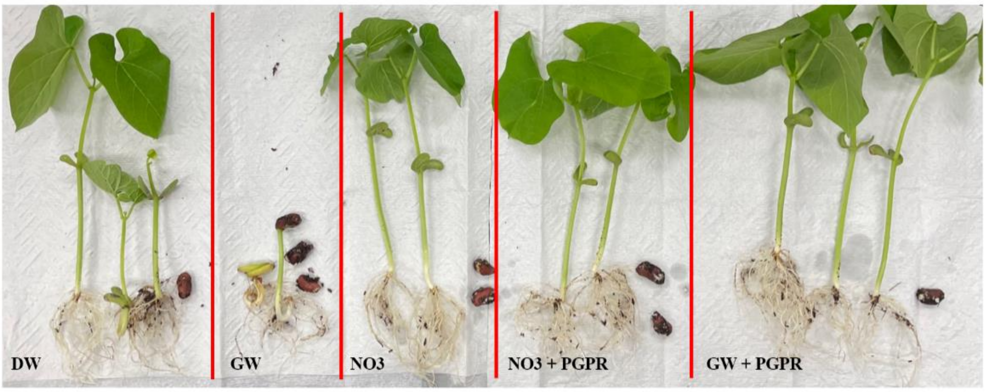

Fig 14. The structural development of kidney bean on different conditions

(2) Structural comparison of germinated kidney bean

For the length of roots, the group of groundwater + PGPR showed the longest length exceeding the longest length of distilled water group. The stem length also follows the trend that the groundwater + PGPR had the longest length. The size of leaf determined by two factors, vertical and horizontal length, shows that the group where PGPR is present have the highest peak. Overall, the group with PGPR where nitrate was present (groundwater + PGPR, nitrate + PGPR) showed the highest development on structures of kidney beans. It has been shown that the group of nitrate-contaminated groundwater showed much less growth rates compared to the other groups in whole structures of plant (Fig 15).

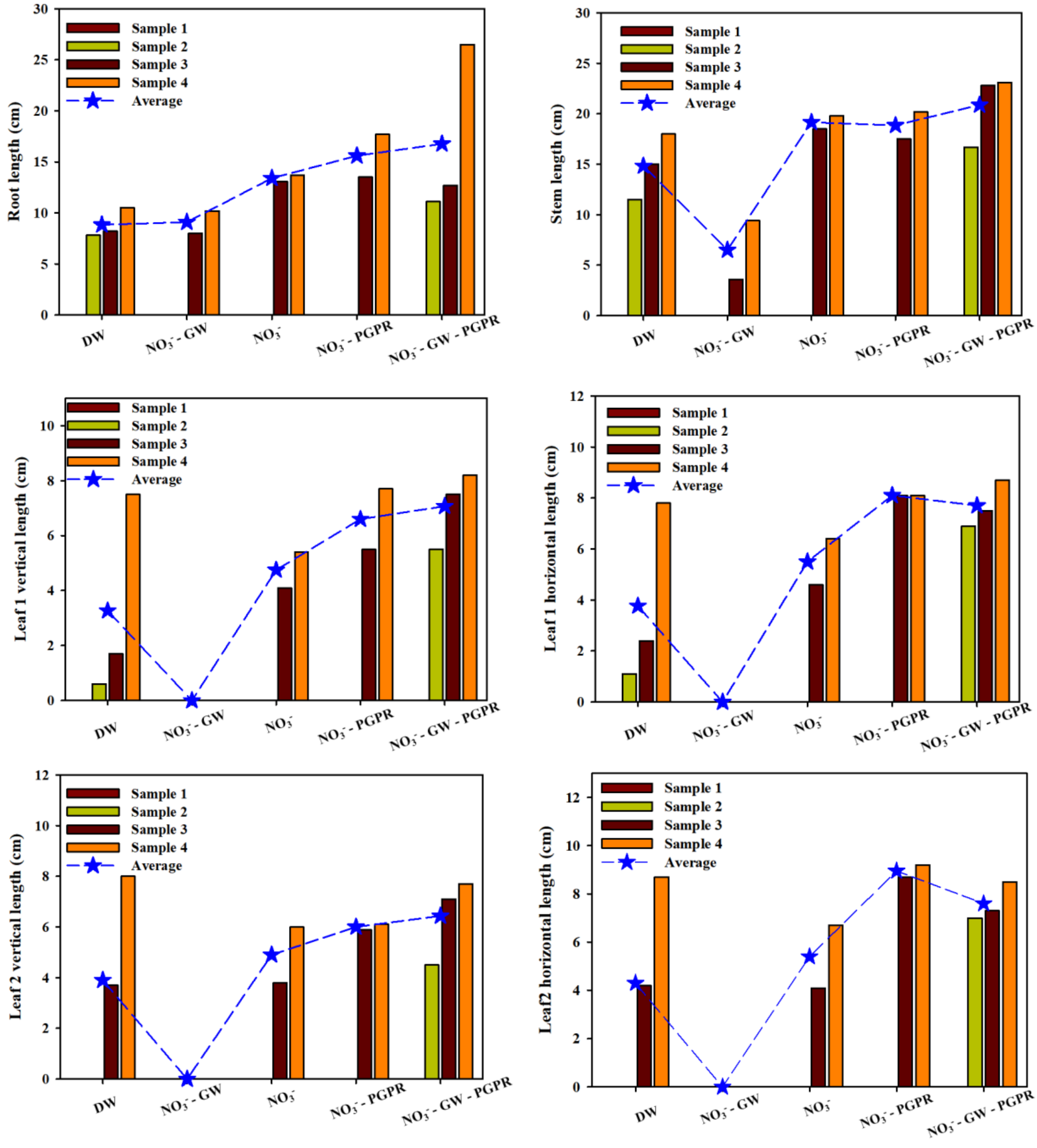

Fig 15. The structural development of kidney bean on different conditions

(3) Biomass of structures on kidney bean

As shown on the graph (Fig 16), the NO_3^- - GW with PGPR included showed the highest total biomass (dry weight) compared to other groups. Since PGPR impacts the growth of leaf by producing such plant growth regulator and hormones, the group where PGPR were distributed had the highest mass of leaf. Also, the group where there was no bacterial solution showed the low biomass of leaf. The experimental results of this study indicate that PGPR treatment of NO_3^- - GW could be applied to the improvement of plant growth, potentially resulting in the higher food production.

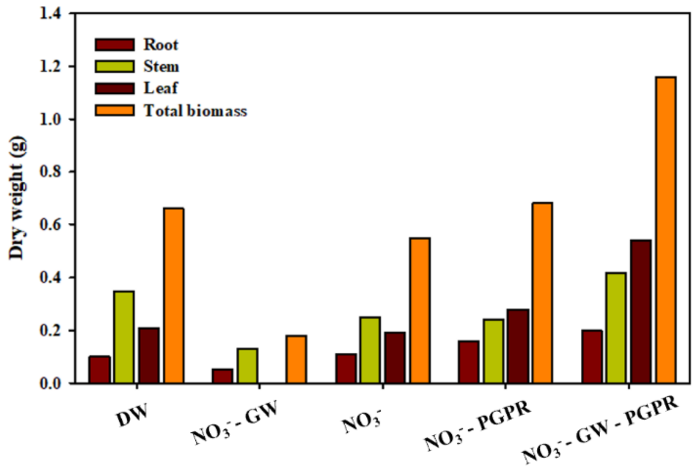

Fig 16. The biomass comparison of each structure on kidney bean

V. Conclusion

(1) Significance of the study

In this study, the significance lies in its potential to address the pressing issue of high levels of nitrate pollution in groundwater, which poses a threat to water quality and safety. Particularly in developing countries with inadequate water sanitation facilities, this pollution can contaminate agricultural water and endanger the health of local communities. Moreover, the pollution and scarcity of agricultural water necessitate the implementation of effective water management practices, crop harvesting methods, and water purification techniques. In light of these challenges, the research focused on investigating the impact of ions effect on the growth of *B. subtilis,* the ability of *B. subtilis* to purify the nitrate-contaminated groundwater, and how these interactions influence overall plant growth.

The significance of this study stems from its potential contribution to securing available water resources, which is a global concern, and ensuring a sustainable supply of future food resources for humanity. Future research endeavors could explore the secondary effects of growing microorganisms in groundwater over an extended period to identify additional factors that may positively affect plant growth. By establishing systematic approaches for treating contaminated water using microorganisms, the study aims to develop plans for the systematic and gradual distribution of agricultural water to farmers in different regions.

Overall, this study has implications for addressing the critical issue of nitrate pollution in groundwater, preserving water resources, and establishing sustainable agricultural practices. By elucidating the role of *B. subtilis* in purifying water and promoting plant growth, the research opens avenues for innovative solutions to mitigate water pollution and ensure food security in the future.

(2) Application and suggestions

Considering the fact that Hongseong city of South Korea has a significant amount of nitrate since it is known for livestock operations, the nitrate-contaminated groundwater could be an indicator for the polluted groundwater of developing countries where the livestock operations are the base industries of the nation. As the objective of the study centers on making a sustainable system that the nitrate-contaminated water could be used as a water resource for the food production, the study could be applied to architecting the flow of contaminated water converted to the usable water for agricultural use.

A diagram below (Fig 17) shows that the nitrate pollutants originated from livestock operations, excessive use of nitrogen fertilizers, and industrial wastes will be the main source of pollutions that the application of research is tackling. Since these nitrate contaminants are dissolved in the water with the ion state, this contaminated water will be piped to container where the well grown PGPR (concentration of PGPR that reached the static phase of bacteria) are incubated. After injecting the substantial amount of water, the incubated PGPR will be grown for about 7 days and these converted agricultural water will be secondly piped to the farmlands where the crops are being grown.

Compared to the present system, the water resources contaminated with nitrate substances are being moved to filtration system where the additional energy sources are utilized to convert this water to original state. The sustainable system of utilizing nitrate contaminated water shows how the additional PGPR on present groundwater could bring synergy effects on agricultural development.

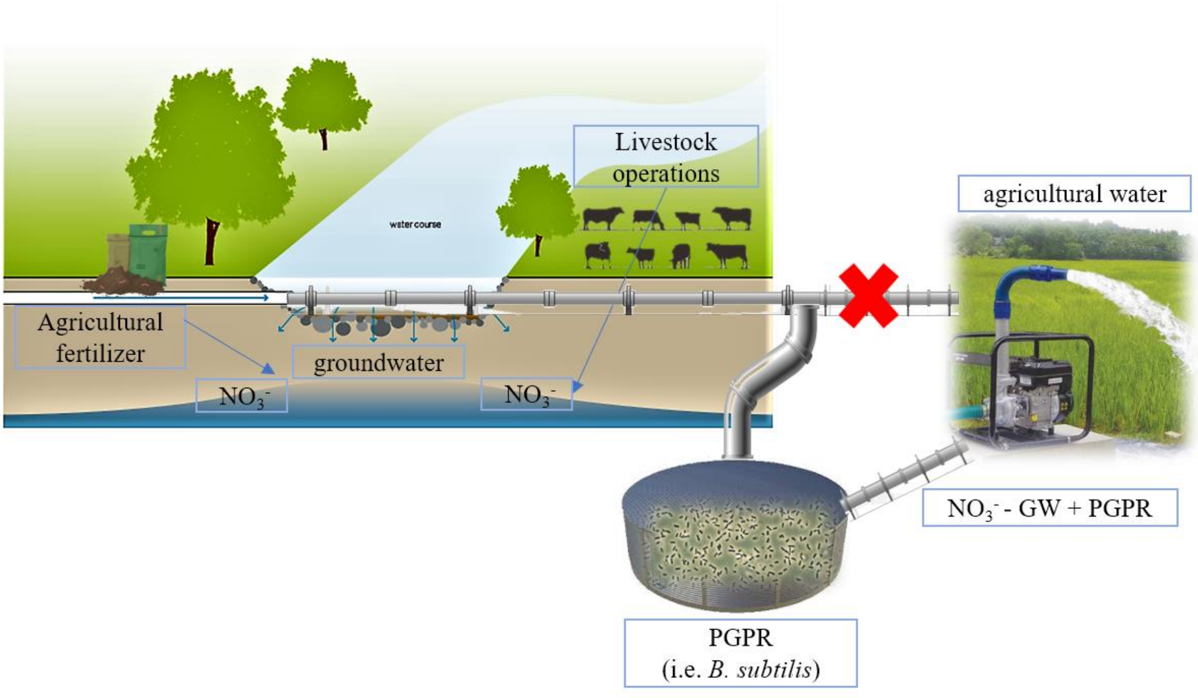

Fig 17. Schematic diagram of utilizing nitrate contaminated water for agriculture

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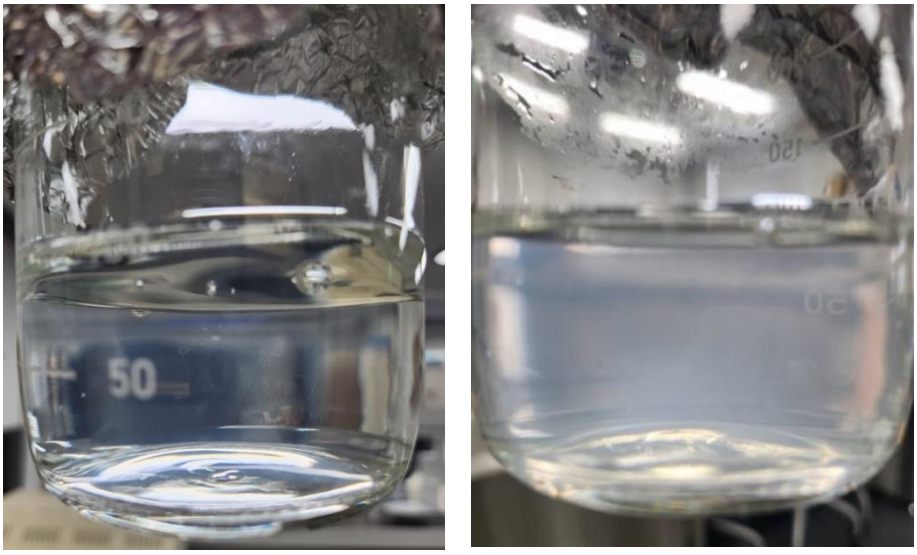
Appendix



The change of groundwater after the inoculating *B. subtilis* (left: before / right: after)

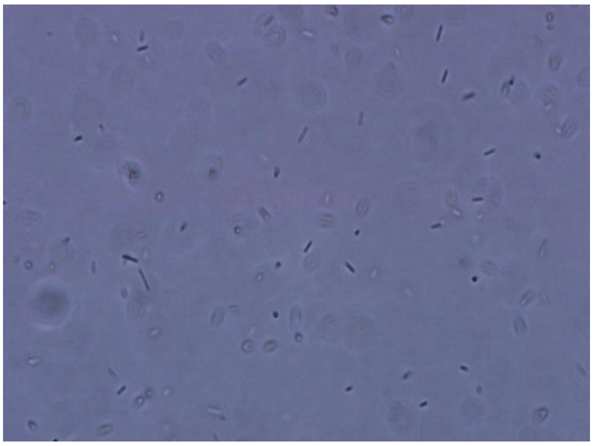


Image of *B. subtilis* incubated on groundwater medium (Light microscopy)

Preparation of drying of kidney beans after speciation