

**Training report of JICA's KCCP for The Project for  
Development of the Duckweed Holobiont Resource Values  
towards Thailand BCG Economy (Be-HoBiD)  
At Interdisciplinary Center for River Basin Environment,  
University of Yamanashi, Kōfu Campus**



**DR. CHAYANID WITTHAYAPHIROM**

**05-29 June 2023**

## **Acknowledgement**

First, I would like to thank scholarship from SATREPS and JICA for support and Professor Tadashi Toyama, Professor Kazuhiro Mori and his lab members for take care and help while I was there.

**Course Title:** Wastewater treatment using Duckweed Holobiont

Dr.Chayanid Witthayaphirom

### **Tentative course schedule:**

- 4 Jun: Depart from BKK
- 5 Jun: Arrive at Haneda and then Yamanashi
- 5-29 Jun: at University of Yamanashi
- 30 Jun: Depart from Yamanashi to Haneda
- 1 Jul: Depart from Haneda to BKK

## **Training in Japan**

**On June 06-14, 2023 Preparation medium and Duckweed culture**

On June 06, 2023. Toyama sensei introduced his laboratory.

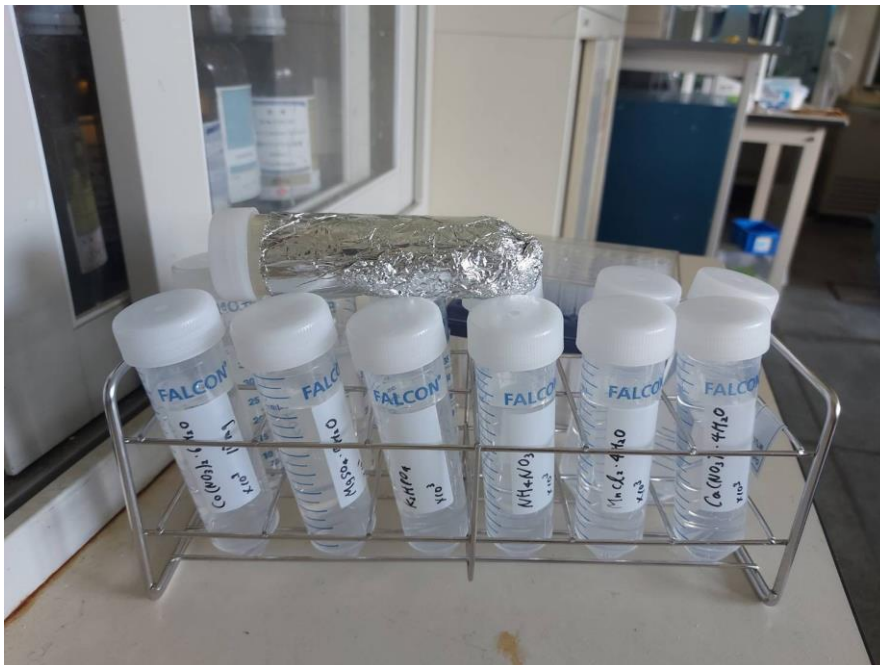




And I learned a recipe for duckweed. I started with Hutner medium preparation following the Toyama's sensei recipe.

-Weigh each chemical according to the recipe in each tube, then add 50 ml of DI, and keep it in room temperature.

- Pipette 1 ml each solution into 900 ml DI in 1000 ml beaker, adjust pH=7 (using 1N HCl or 1N NaOH) and add DI until it reaches 1000 ml. Then transfer 200 ml into a 500 ml flask. After that, sterilize the solution.



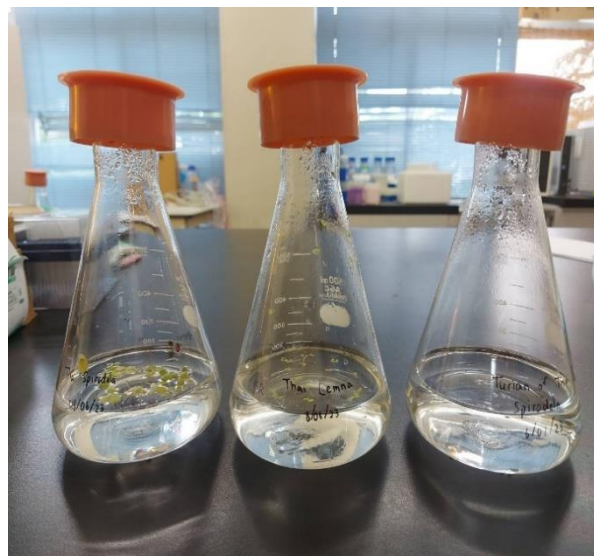
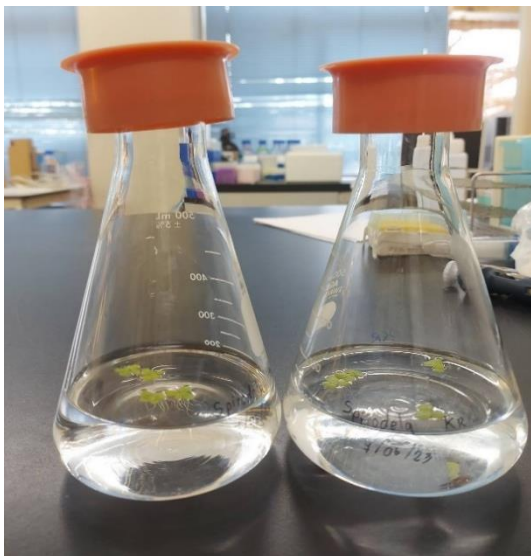




On June 07-08, 2023,

**Duckweed culture in Hutner's medium with duckweeds from the stock.**

- Burn the loop and wait for it to cool. Then transfer 4 duckweeds from the stock into a flask and incubate in the curing room.





On June 09, 2023

### Preparation Hoagland medium

-Weigh each chemical according to the recipe in each 50 ml tube, then add 50 ml of DI, except  $K_2SO_4$  which is mixed in a 500 ml beaker and kept in room temperature.

- Pipette 1 ml of each solution into 900 ml DI in 1000 ml beaker, adjust pH=7 and add DI until it reaches 1000 ml. Then transfer 200 ml into 500 ml flask. After that, sterilize the solution.





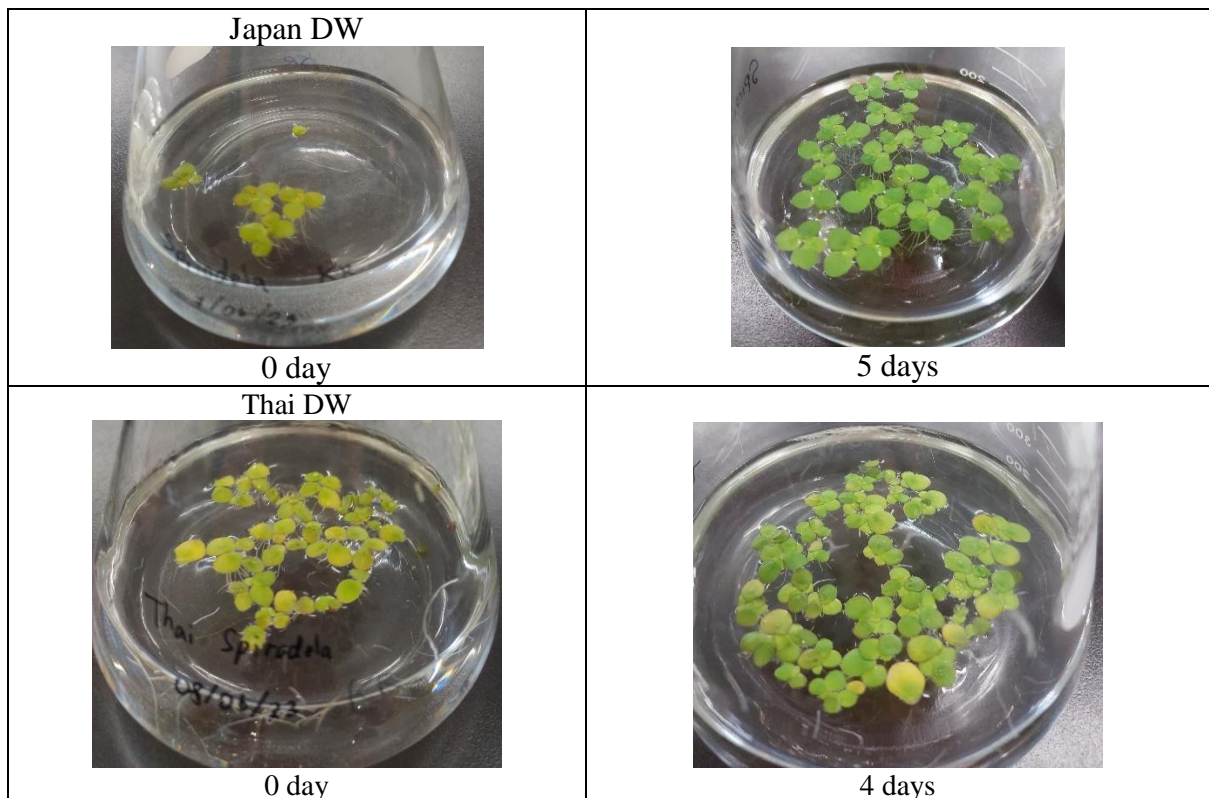
From June 12-16, 2023. Check the growth of duckweed

On June 12, 2023

Check growth of duckweed

On day 0, put 4 fronds in a 500 ml flask

After 5 days, the duckweed has increased leaf buds and there are 16 fronds in the 500 ml flask

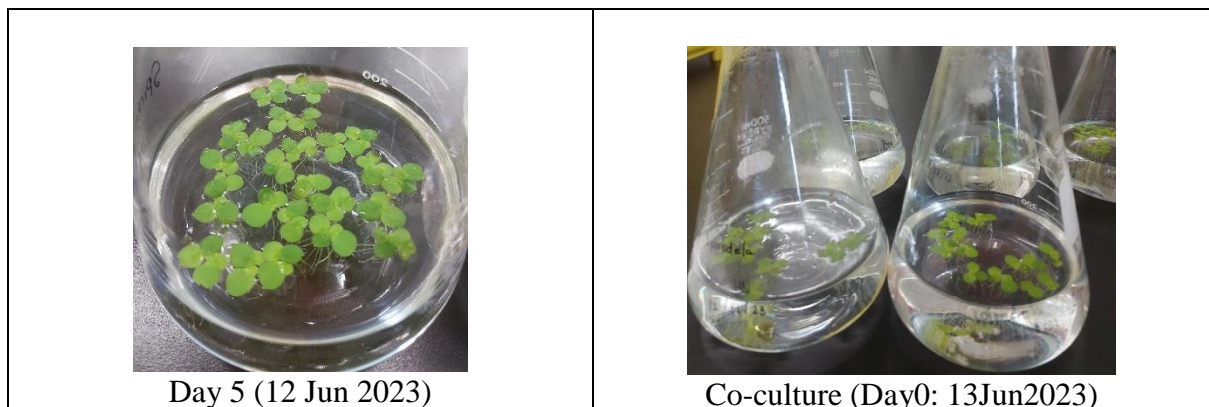


On June 13, 2023. Prepare Wastewater

-Prepare 2000 ml of Hutner medium and transfer it to 10 flasks for co-culture duckweed.

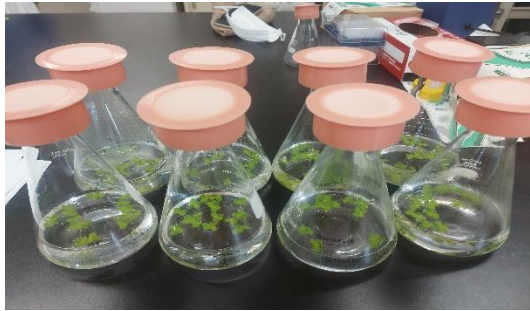
-Separate 1 flask of duckweed co-culture into 2 flasks

∴ resulting in a total 8 flasks



On June 14, 2023

Check the growth of co-culture duckweed.



Co-culture (Day1: 14 Jun 2023)

DWs were slightly growth

From June 15-29, 2023 Perform wastewater treatment using Duckweed Holobiont and environmental bacterial communities according to the wastewater treatment schedule.

### Schedule of wastewater treatment and environmental bacterial communities experiment plan in June 2023

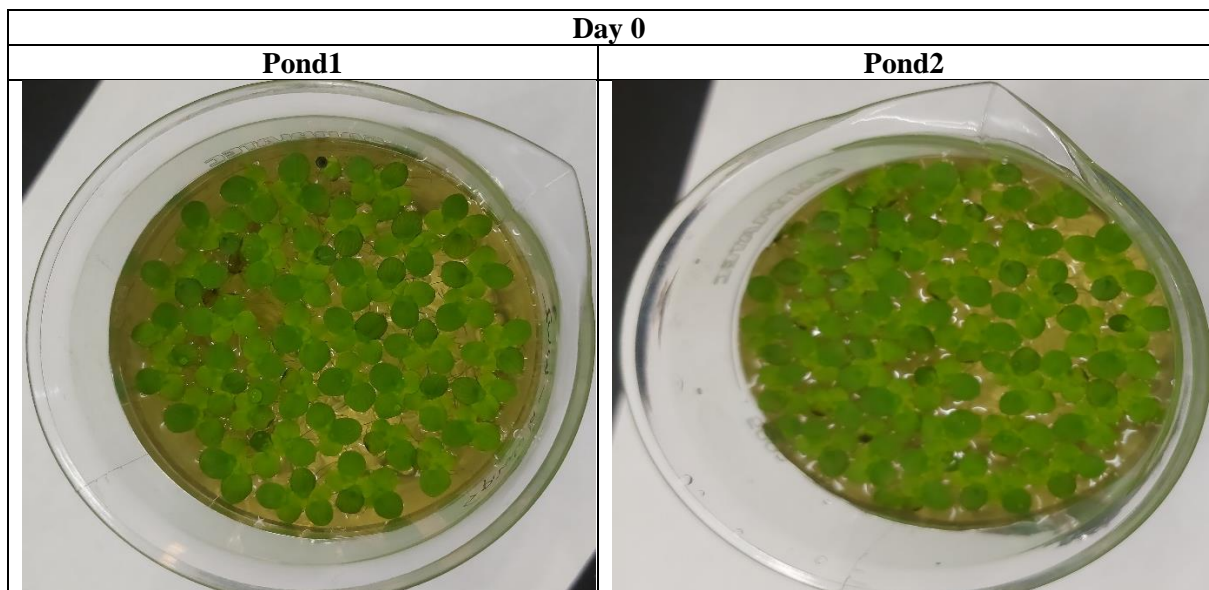
Mon	Tue	Wed	Thu	Fri	Sat	Sun
12	13	14	15 (day 0)	16 (day 1)	17 (day 2)	18 (day 3)
Preparation medium and Duckweed culture	Preparation medium and Duckweed culture	Preparation medium and Duckweed culture	wastewater treatment	wastewater treatment	wastewater treatment	wastewater treatment
Investigation the growth duckweed (number DW: 4 fronds to =16-18 fronds (day 5))	-prepare 1/10 Hutner medium 2000 ml for co-culture -co-culture DW from 1flask to 2 flasks	-prepare 1/10 Hutner medium -Monitor and compare the growth of DW (number of DW, dry wt., Chlorophyll content)	-DW increase the number of <i>Spirodela's</i> culture -Collect wastewater -Culture DW ( <i>Spirodela polyrhiza</i> , <i>Limna minor</i> ) -Monitor wastewater qualities (TOC, TN, microbes) -Take photo -The growth of DW (dry wt.)	-Monitor wastewater qualities (TOC, TN, microbes) --Take photo	-Monitor wastewater qualities (TOC, TN, microbes) --Take photo	-Monitor wastewater qualities (TOC, TN, microbes) -Take photo
19 (day 4)	20 (day 5)	21	22	23	24	25
wastewater treatment	wastewater treatment					
-Monitor wastewater qualities (TOC, TN, microbes) -Take photo	-Monitor wastewater qualities (TOC, TN, microbes) -Take photo -The growth of DW (dry wt.)					
26	27	28	29			
Environmental bacterial communities (day 11)	Incubating bacteria plate	Incubating bacteria plate	Incubating bacteria plate			



On June 15, 2023

### **Perform wastewater treatment**



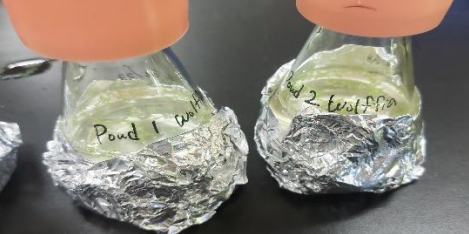
- Prepare enough co-culture DWs for wastewater experiment.
- Transfer 5 flasks co-culture DWs to a container box.
- Pick duckweed and place it in a 500 ml beaker with wastewater (2 repetitions)
- Inoculate in the room and collect 20 ml of wastewater in a 50 ml tube (day 0) and take a photo of duckweed growth.



### **Perform duckweed co-culture with environmental bacterial communities**


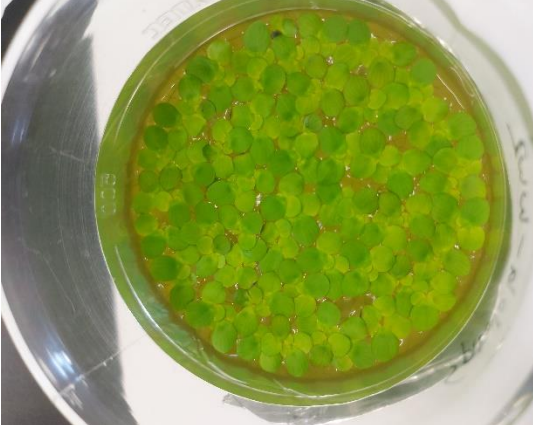
- Culture duckweed in water pond in flask (use sterilized Spirodela, Lemna, Wolffia) for 11 days.
- transfer 200 ml water pond from Spirodela to a 200ml flask and transfer 100 ml of water pond from both Lemna and Wolffia in 100 ml flask (2 repetitions).

On June 15, 2023 (Cont.)

Day 0	
Spirodela	
Lemna	
Wolffia	



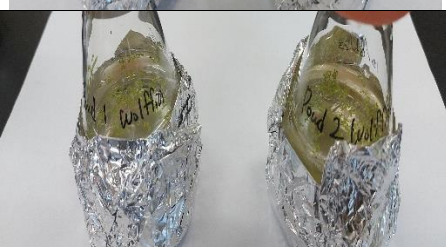
On June 16, 2023

-Collect 20 mL of wastewater in a 50 mL tube (day 1) and take a photo of duckweed growth.

Day 1	
Pond1	Pond2
	

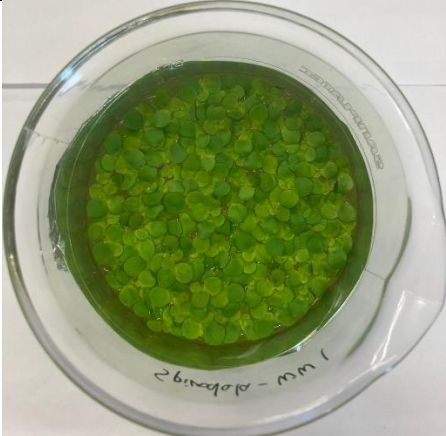

On June 16, 2023 (Cont.)

- Take a photo of duckweed growth for the environmental bacterial communities.

Day 1	
Spirodela	
Lemna	
Wolffia	

On June 17, 2023


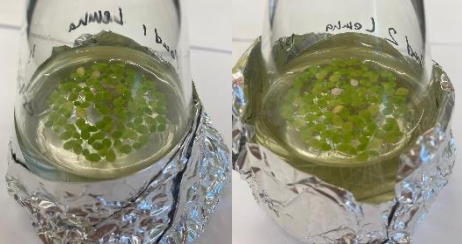
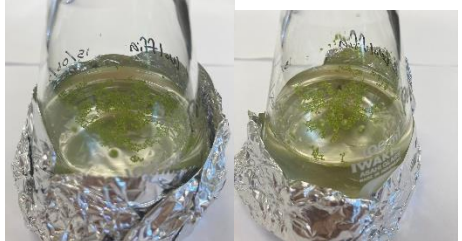
-Collect 20 mL of wastewater in a 50 mL tube (day 1) and take a photo of duckweed growth.

Day 2	
Pond1	Pond2
	





On June 17, 2023 (Cont.)

- Take a photo of duckweed growth for the environmental bacterial communities.

Day 2	
Spirodela	
Lemna	
Wolffia	

On June 18, 2023


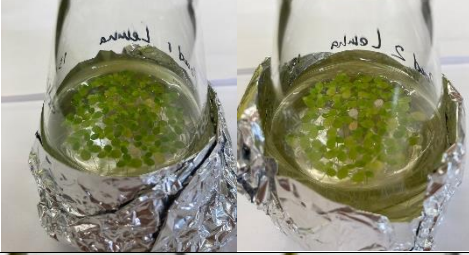

-Collect 20 mL of wastewater in a 50 mL tube (day 1) and take a photo of duckweed growth.

Day 3	
Pond1	Pond2
	




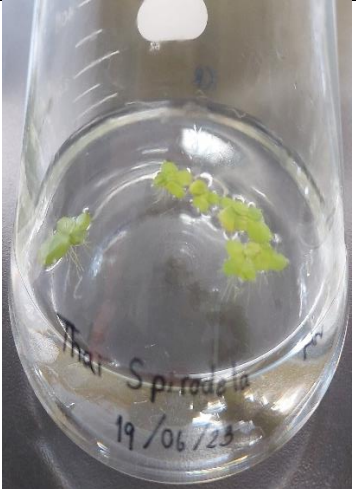

On June 18, 2023 (Cont.)

- Take a photo of duckweed growth for the environmental bacterial communities.

Day 3	
Spirodela	
Lemna	
Wolffia	

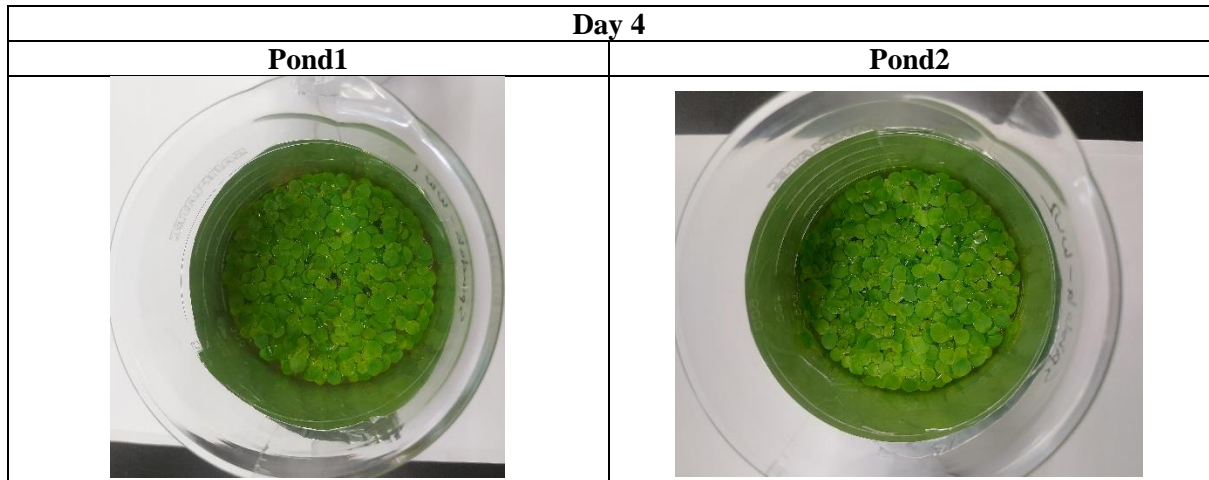
On June 19, 2023

-Co-culture duckweed (Japan from 13062023) and (Thai from 08062023)

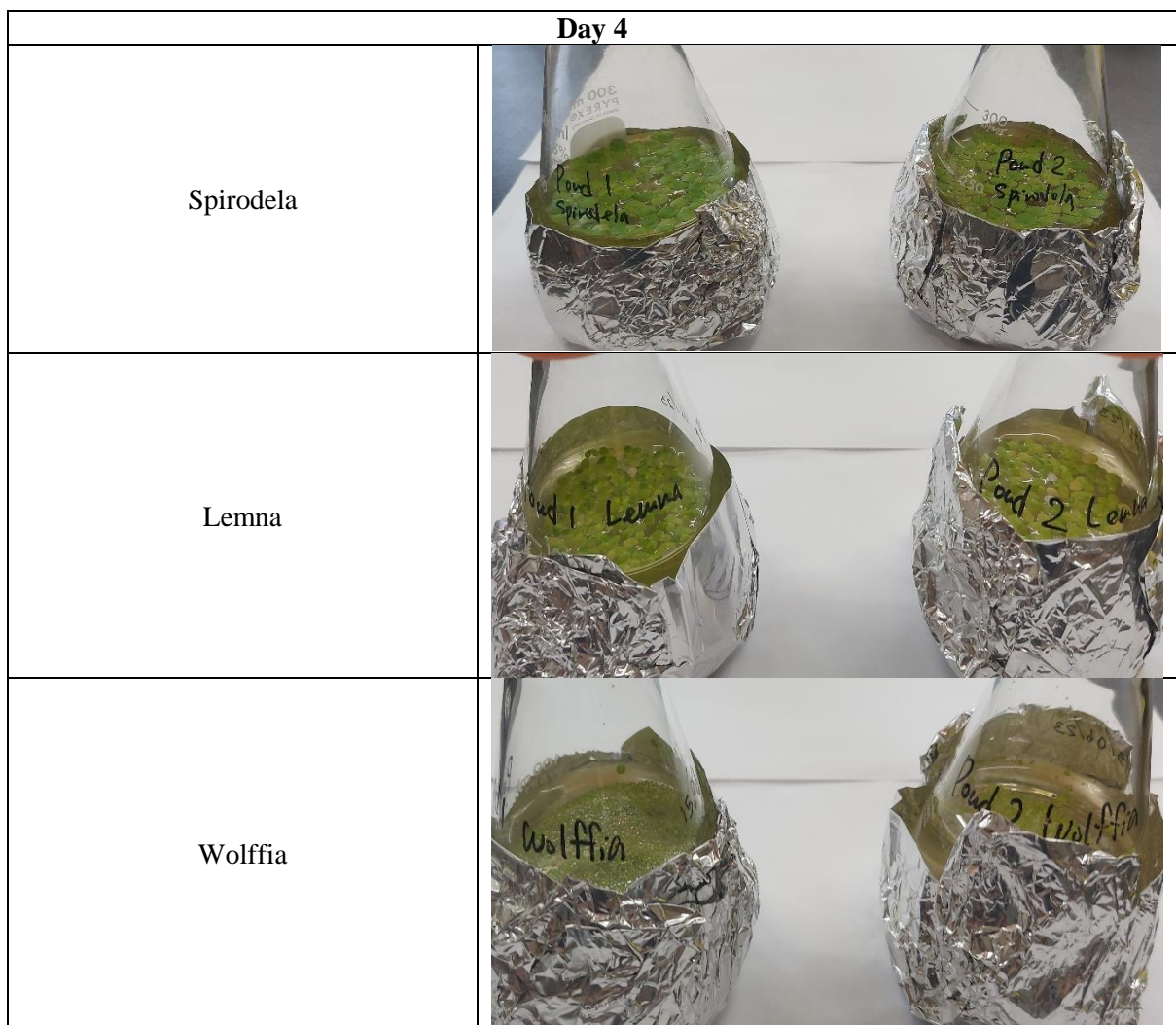
Japan, Spirodela	Thai, Spirodela	Thai, Lemna
		

On June 19, 2023(Cont.)

-Collect 20 mL of wastewater in a 50 mL tube (day 1) and take a photo of duckweed growth.

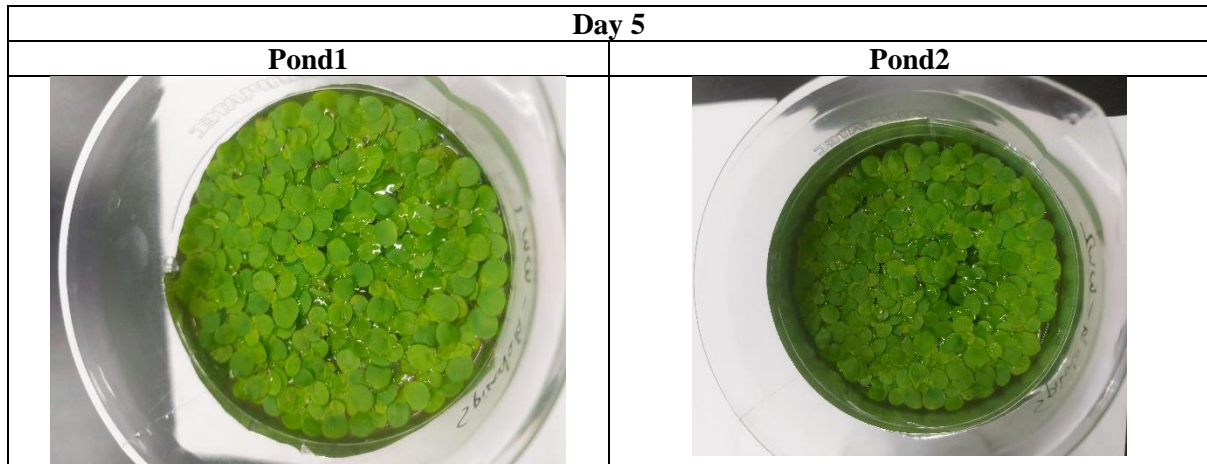


- Take a photo of duckweed growth for the environmental bacterial communities.

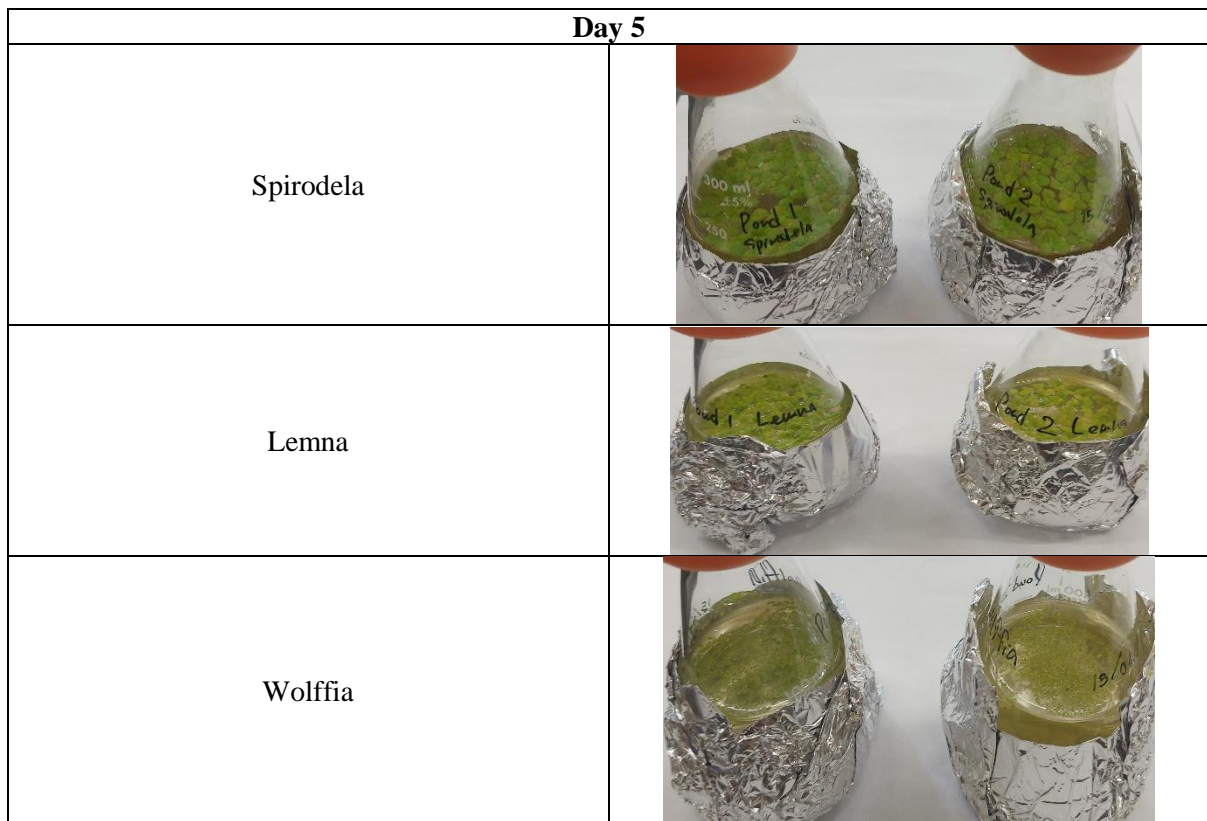


On June 20, 2023

-Collect 20 mL of wastewater in a 50 mL tube (day 1) and take a photo of duckweed growth.



- Take a photo of duckweed growth for the environmental bacterial communities.



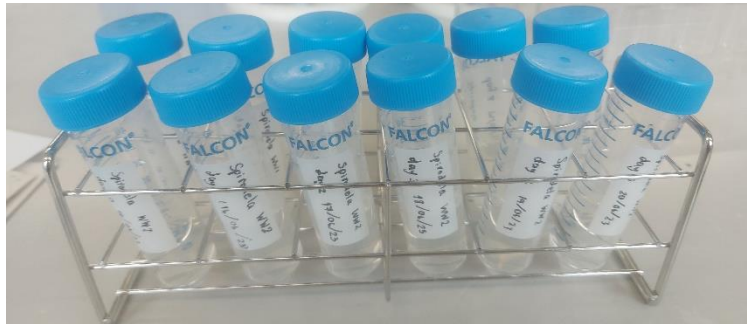
- Dry weight duckweed for wastewater treatment at 80°C 1 night.





On June 21, 2023

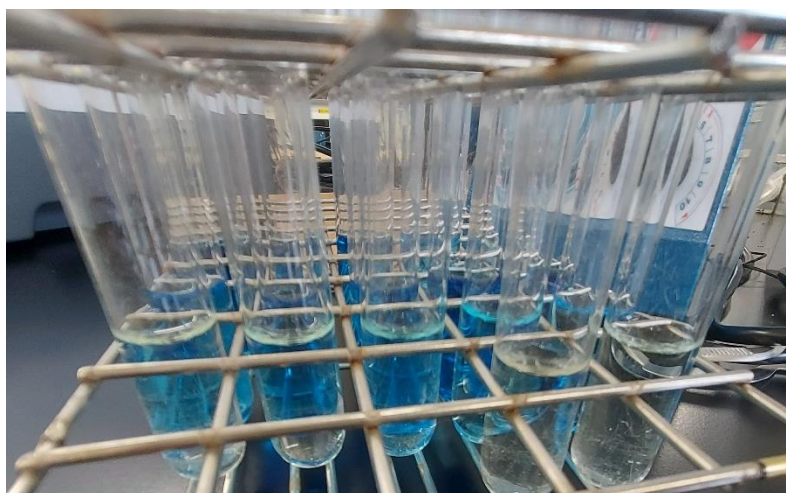
**Analysis TOC and  $\text{NH}_4^+$  for wastewater**



-TOC 15 ml in tube-TOC by TOC-L shimadzu



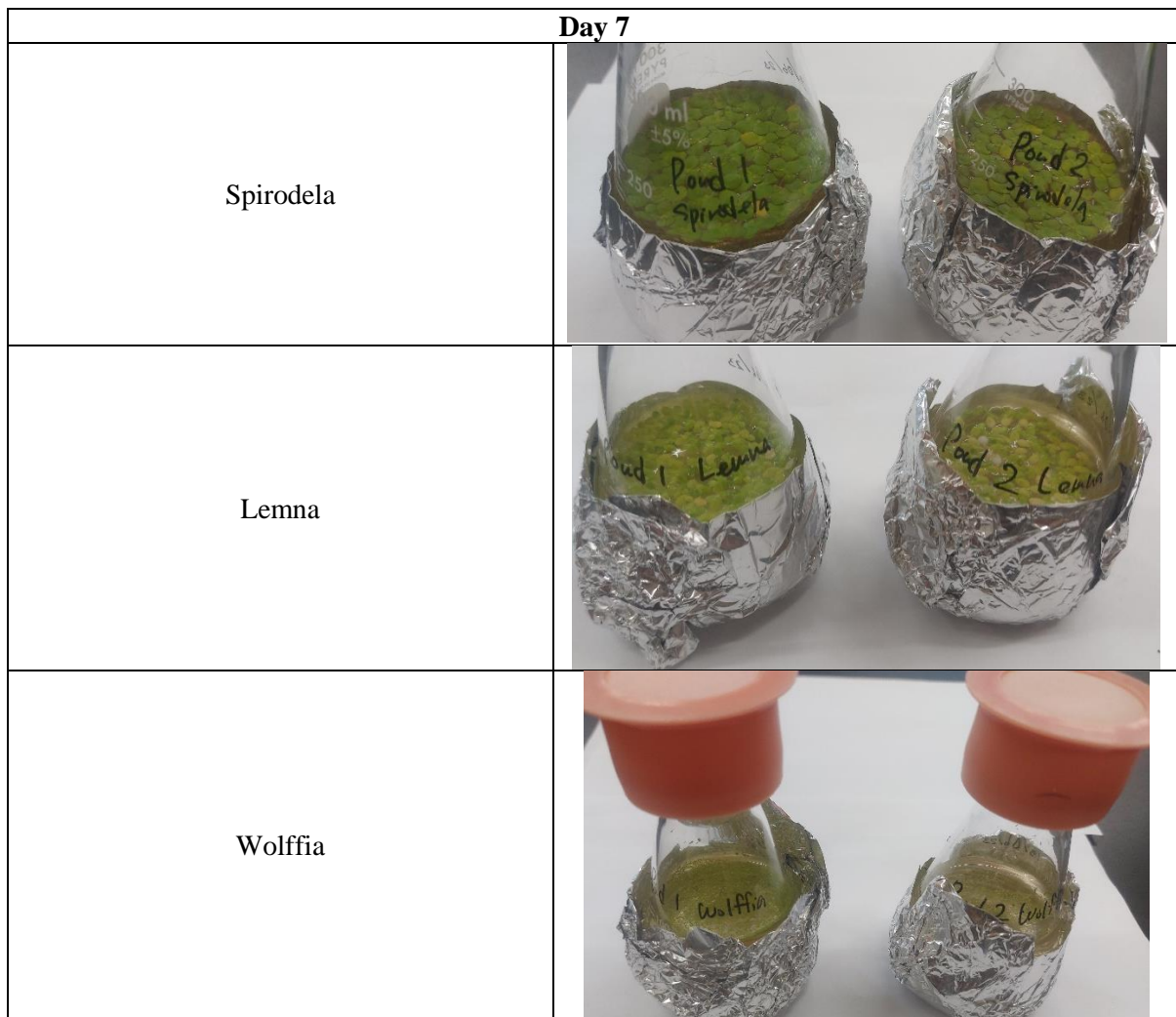
- $\text{NH}_4^+$  dilute 10 times





On June 22, 2023

- Take a photo of duckweed growth for the environmental bacterial communities.



On June 26, 2023

Analysis plate count for bacteria on 11 days in R2A agar @ 25°C



On June 27, 2023

Perform bacteria plate incubation

On June 28, 2023

Investigate bacteria plate incubation

On June 29, 2023

Investigate bacteria plate incubation

On June 30, 2023

Depart from Yamanashi to Haneda Airport

On July 01, 2023

Arrive in Thailand

## **Conclusion**

I found this training to be extremely valuable, as it provided me with a wealth of knowledge on topics such as co-culture, wastewater treatment, and environmental bacteria. The ideas and skills I have acquired will undoubtedly be applied to my duckweed research in Thailand (Group 5). I would like to express my heartfelt gratitude to SATREPS and JICA for their scholarship support, as well as Prof. Tadashi Toyama, Prof. Kazuhiro Mori, and their lab members for the exceptional training they provided.

Beyond the research aspect, I have been deeply impressed by the remarkable Japanese culture, cities, and the kindness of the Japanese people. Through this experience, I have not only gained research expertise and training but also developed a profound understanding of Japanese culture and values.