Comparison of cultivation of *Chlorella* sp. in effluent from rubber processing Blocks rubber and Concentrated latex factories

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Abstract:

The study "Comparison of cultivation of *Chlorella* sp. in effluent from rubber processing Blocks rubber and concentrated latex factories" aims to compare the cultivation of *Chlorella* sp. in effluent from rubber processing Blocks rubber and concentrated latex factories, examine the growth rate of *M. macrocopa* that acquired nutrients from *Chlorella* sp., and determine how effective *Chlorella* sp. is at improving the effluent's water quality. In the experiment, we separated the three experimental sets. Initially, the control set consists of MSG residue, field fertilizer, urea fertilizer, and drinking water. The wastewater from the manufacturing of concentrated latex is used in the second set. Blocks of rubber processing effluent are used in the third set. We spent a week in cultivation. On the first day, we fill 250 ml flasks with 100 ml of effluent and modify the water's pH to create the ideal environment for *Chlorella* sp. growth. We pour an additional 150 ml on the third day of the experiment. Every day, we monitored the water quality, counted the quantity of *Chlorella* sp. cells, and modified the temperature, light, and pH. After that, we fed *M. macrocopa Chlorella* sp. for three days. We gathered data on temperature, dissolved oxygen (DO), and surface temperature each day. Every experiment was conducted three times.

So, the results, the growth rates of *Chlorella* sp. from every set are (27.08 ± 1.73), (95.83 ± 0.35) and (29.17 ± 0.48) cells/ml respectively, the growth rates of *M. macrocopa* from every set are (27.08 ± 1.73), (95.83 ± 0.35) and (29.17 ± 0.48) cells/ ml respectively, the increasing rates of the weight of *M. macrocopa* from every set are (1.53 ± 1.24, 3.66 ± 1.15 and 3.13 ± 1.84 g) respectively. When data was analyzed statistically, it was found that the three sets of experiments were not statistically different from each other ($P<0.05$). The water quality before and after the experiment such as pH, from the control set, was 6 before and 7 after, the effluent from concentrated latex processing, was 6 before and 7 after, the effluent from Blocks rubber processing, was 6 before and 6.5 after which is according to the research questions. From the results, the effluent from concentrated latex processing can be an option to replace field fertilizer and urea fertilizer in cultivating *Chlorella* sp. .

Keywords: *Chlorella* sp./ *Moina macrocopa*/ effluent/ the rubber processing factory

Research Question:

1. Effluent from which rubber processing affects the growth rate of *Chlorella* sp. the best?
2. Effluent from which rubber processing affects the growth rate of *Moina macrocopa* the best?
3. After cultivating *Chlorella* sp., which sets have the best water quality?

Hypothesis

1. The best growth rate appears in *Chlorella* sp. grown in concentrated latex processing effluent.
2. The best growth rate appears in *Moina macrocopa* which was fed with *Chlorella* sp. grown in the effluent from Concentrated Latex processing.
3. After cultivating, the water quality is improved.

Introduction and Review of Literature

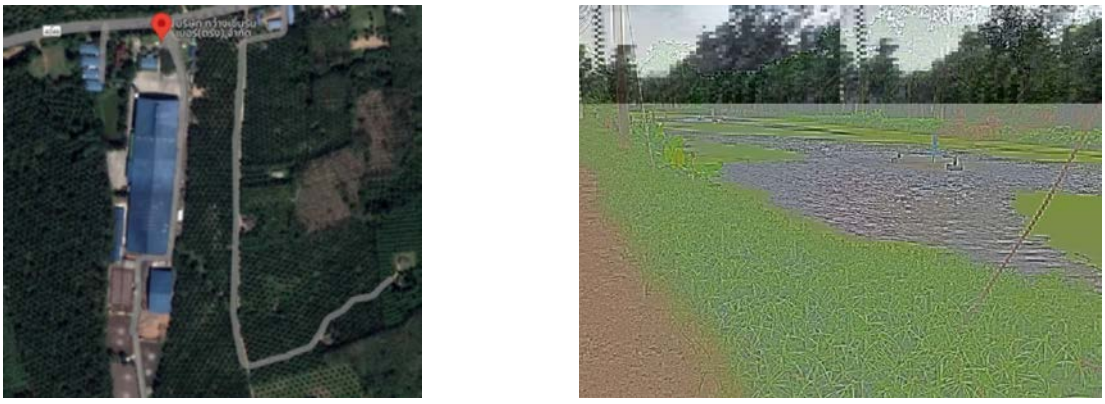
Among several industrial crops is the rubber tree, or Hevea *brasiliensis*. According to data from the Rubber Research Institute (2018), Thailand dominates the world in rubber exporting and owns the second-largest land of rubber farms. In particular, latex, which is made from the food transport tube that exists in rubber tree bark. Numerous rubber goods, including tires for heavy machinery, medical equipment, and household items like shoes, erasers, and balloons, can be made from latex. Tires are frequently made from blocks of rubber. Due to the several above factors, Thailand's rubber sector is greatly improving, and rubber planting is expanding throughout Thailand, particularly in the south, which experiences monsoons more frequently than other parts of the country, making the soil there more humid. As more farmers decide to plant rubber trees, more latex is being produced. Formic acid or sulfuric acid, which are added to the process to strengthen the rubber, make the water have an unpleasant odor and be more acidic. By research, it was discovered that *Chlorella* sp. could improve the quality of the water and grow in the effluent. Since *Chlorella* sp. is rich in protein, farmers frequently apply it as food for tiny aquatic animals like rotifers and *Moina macrocopa*. *Chlorella* sp. cultivation provides a variety of food recipes. A few of the recipes are expensive. Therefore, we want to find out *Chlorella* sp.'s capacity to spread in the wastewater from rubber manufacturing factories in order to address these issues.

Research Method and Materials *(including GLOBE Data!)*:

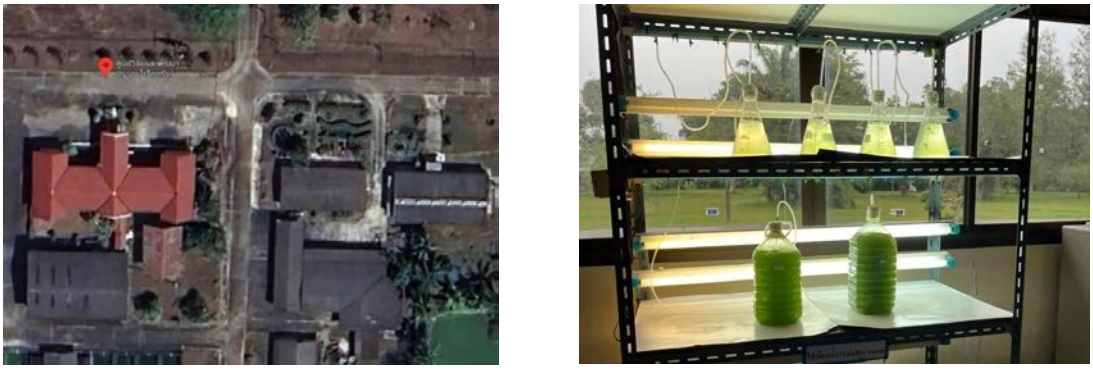
1) 250 ml flasks
2) Rubber stopper
3) Glass tube
4) Air pump
5) Basin
6) Filter paper
7) Fluorescent lamp
8) Calcium Hydroxide
9) Urea Fertilizer 46-0-0
10) Field Fertilizer 16-20-0
11) The effluent from Blocks rubber processing
12) The effluent from Concentrated latex processing

Study site

This study located 2 study sites, first is Guangken Rubber(Trang) Company Limited which is located in Na Mueang Phet Subdistrict, Sikao District, Trang Province on coordinates 7°33'28"N 99°25'31"E. Second is Trang Inland Fisheries Research and Development Center which is located in Natho Ming Subdistrict Mueang Trang District, Trang Province on coordinates 7°32'05"N 99°32'47"E.



Picture 1 : Guangken Rubber(Trang) Company Limited which is located in Na Mueang Phet Subdistrict, Sikao District, Trang Province

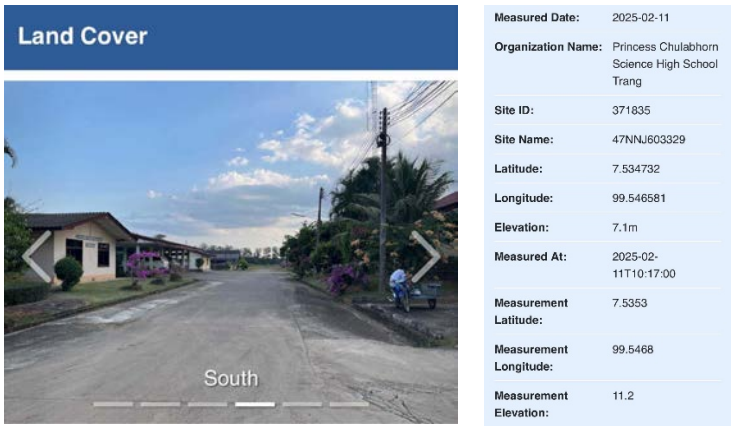


Picture 2 : Trang Inland Fisheries Research and Development Center which is located in Natho Ming Subdistrict Mueang Trang District, Trang Province

Data Collection of Water Quality

Measure water quality according to GLOBE protocols. So, we measured pH, DO, temperature, and surface temperature. As the following steps :

1) Located the point and collected the samples of the wastewater
2) Using Land Cover in GLOBE Observer Application
3) Collected the information about the water quality, such as pH by pH meter, dissolved oxygen by DO meter, temperature, and surface temperature by thermometer.
4) Repeated the previous step 3 times. Then, calculated to find the average.
5) Send the information to GLOBE Data Entry.



Picture 3 : Land Cover Observation

Research Method

1) The experimental sets

1.1 The control set, used drinking water, field fertilizer, urea fertilizer, and MSG residue as the nutrients for *Chlorella* sp.

1.2 The second set, used the effluent from concentrated latex processing.

1.3 The third set, used the effluent from blocks to rubber processing.

2) Preparing *Chlorella* sp. leavening

2.1 Clave 250 ml flasks, rubber stopper, glass tube at 180 degrees Celcius for 1 hour.

2.2 Pour 100 mL of drinking water into the flasks.

2.3 Add Calcium Hydroxide, urea fertilizer, field fertilizer, and MSG residue each 1 g in the water. Then, stirring the mixing.

2.4 Pour *Chlorella* sp. leavening from Trang Inland Fisheries Research and Development Center leavening 10 ml in the flasks.

2.5 Close flasks with rubber stopper. Then, pumping oxygen with air pumps and leaving the flasks with enough light for 3 days. Collect data about pH, DO, and monitor the concentration of the leavening.

2.6 The third day, add water 150 ml, but Calcium Hydroxide, field fertilizer, urea fertilizer, and MSG residue 2 g.

2.7 Give oxygen for another 3 days. Collect data about pH, DO, and monitor the concentration of the leavening.

3) Cultivating *Chlorella* sp.

3.1 Clave 250 ml flasks, rubber stopper, glass tube at 180 degrees Celsius for 1 hour.

3.2 The control set pour drinking water 100 ml in the flasks.

3.3 The second and third set pour the effluent from rubber processing 3 liters in the plastic pot.

3.4 Pour 10 mL of Chlorella sp. leavening in the flask .

3.5 Close the flask with rubber stopper. Collect data about pH, DO, and moniter the concentration.

3.6 In the third day, add water in the control set another 150 ml. But add field fertilizer, urea fertilizer, and Calcium Hydroxide each 2 g. Collect data about pH, DO, and moniter the concentration.

3.7 In the same day, add 150 mL of the effluent in the second and the third set. Collect data about pH, DO, and moniter the concentration for another 3 days.

3.8 Collecting the results such as pH, DO, the concentration, and the number of *Chlorella* sp. cells by using Sedge Wick Rafter Counting Chamber to look through the microscope every day.

4) Feeding *M. macrocopa*

4.1 Clean the 10.5 ml basins by using the cleaning product, HAITER. Then, leaving all basins with sunlight for 15 minutes.

4.2 Pour the drinking water 500 ml.

4.3 Add *M. macrocopa* 1.5 g.

4.4 Pour the *Chlorella* sp. which is cultivated in the effluent from rubber processing in the same basins.

4.5 Collect data such as pH, temperature, DO, and weigh the weight of *M. macrocopa* to study the growth rate.

5) Checking the growth of *Chlorella* sp.

5.1 Collect the 20 mL of samples from each set by random. Then, we take the samples to look through the microscope and use Sedge wick Rafter Counting Chamber to count the number of cells. We repeat the process for 7 days.

5.2 When finishing the experiment, comparing the density of *Chlorella* sp. cells with the control set and the day that started.

5.3 Measure water quality every day, according to GLOBE protocols. The parameters are

DO : Dip the probe into the samples.(Then, wait for 25-35 seconds to read by looking at the LCD of DO meter.)

pH : Dip the probe in the samples. Wait for a while. Then, read the correct pH from pH meter.

Surface temperature and water temperature : Dip the thermometer in the samples to measure water temperature. Use Infrared thermometer to measure surface water temperature.

5.4 The formulas used to calculate the growth of *Chlorella* sp. .

5.4.1 The density per water 1 ml (Cell Diversity/mL)

The density per water 1 ml = The counted cells × 25

5.4.2 Average Cell Diversity

The average density = The whole density / the repeated cells

5.4.3 Growth rate

Growth rate = The increasing density / the duration of the experiment

5.5 The formulas used to calculate the growth of *M. macrocopa*.

5.5.1 Weight Average

Weight Average = All weights / the repeated weight

5.5.2 Weight gain

Weight gain = The latest weight – The start weight

5.5.3 % Weight gain

WG(%) = (The latest weight – The start weight) × 100 / the start weight

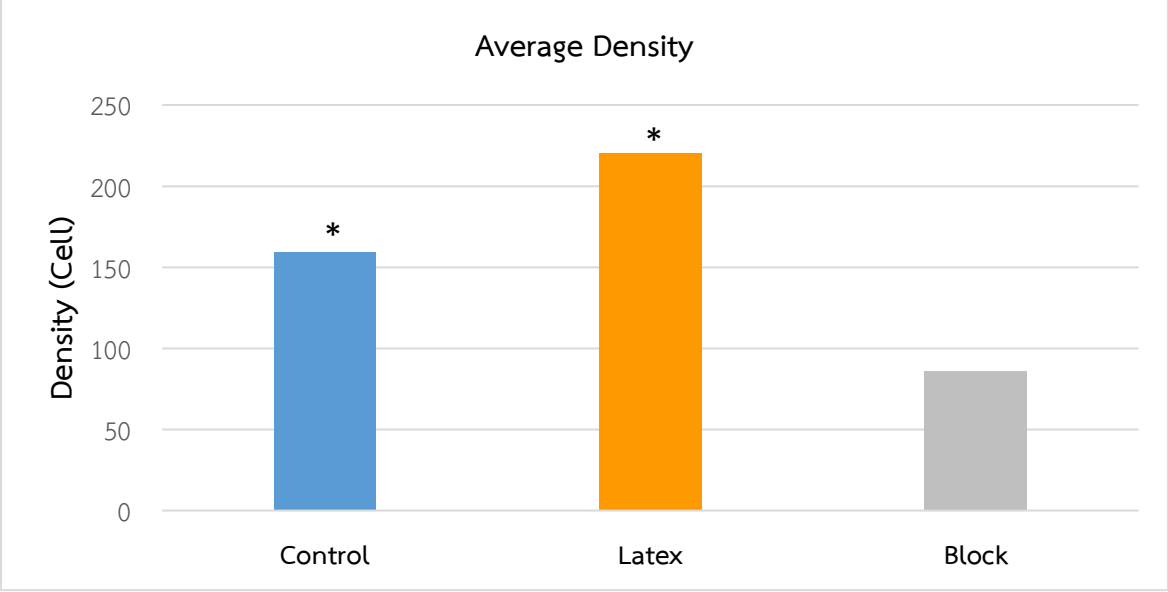
Data analysis

1) Compare the density of *Chlorella* sp. from each set.
2) Compare the weight of *M. macrocopa* with *Chlorella* sp., which cultivated from each set of the experiment, as its food.
3) Analyze the water quality in cultivating *Chlorella* sp. from each set.
4) Compare the density and the weight-average by using ANOVA one-way variance.

Results

The result from the study of the growth rate of *Chlorella* sp.

We found that there was a growth rate of *Chlorella* sp. after a week of cultivation. The second set has the best growth rate based on the **Picture 4**. The relative growth rates for each batch are 27.08 ± 1.73, 95.83 ± 0.35, and 29.17 ± 0.48 cells/ml. Furthermore, it keeps increasing over the course of the investigation. From, **Picture 4** indicates that the second set contains the highest density of *Chlorella* sp. The densities of each set are 141.67 ± 1.44 , 208.33 ± 5.20, and 375 ± 2.5 cells/ml, respectively.

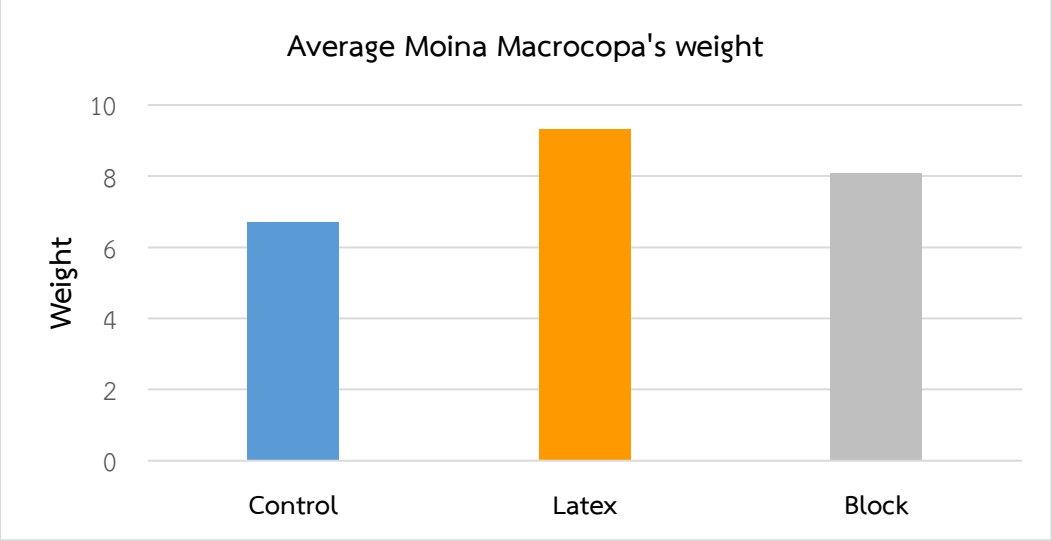


Note * There was statistical difference at 95% confidence ($p < 0.05$).

Picture 4 shows the density (cells) of *Chlorella* sp. that cultivated in the effluent from rubber processing for 7 days.

The result from the study of the growth rate of *M. macrocopa*.

After three days of raising *M. macrocopa*, we found that, as shown in **Picture 5**, the animal gained weight during the experiment, particularly in the second set. The corresponding grams of each set is 15.47, 13.88, and 9.10 g. The control set, the effluent from processing blocks of rubber, and *M. macrocopa* from concentrated latex processing also formed the most weight, respectively. The weight gain for each set is 1.53 ± 1.24, 3.66 ± 1.15, and 3.13 ± 1.84 g, respectively. *M. macrocopa* from concentrated latex processing has the highest percentage of weight gain, followed by the control set and the effluent from block rubber processing. The percentage of weight gained by each set was 107.06, 79.67, and 49.02 percent, in that order.

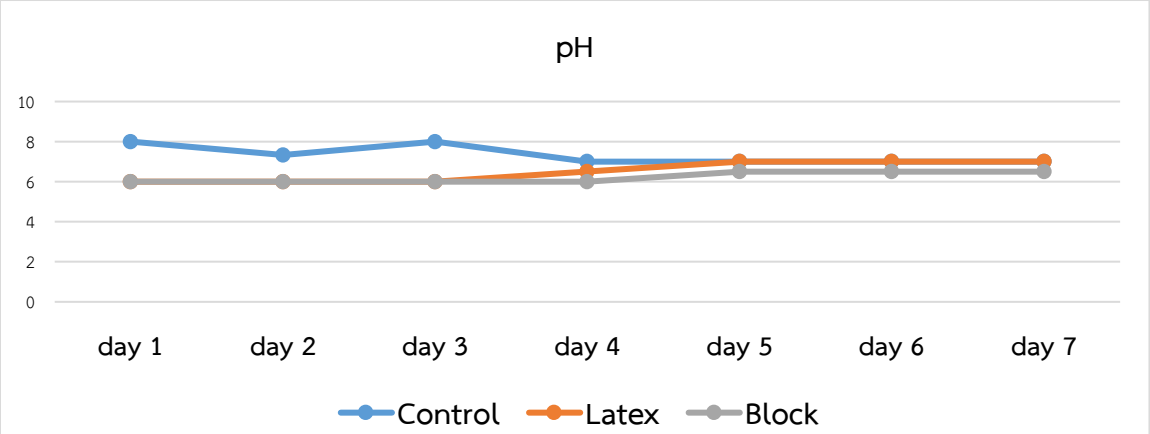


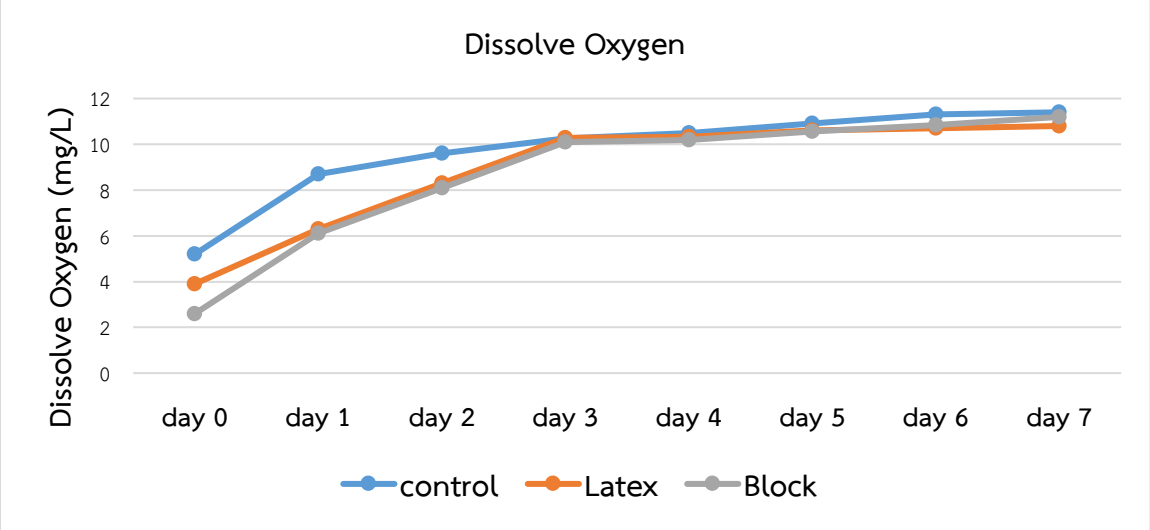
Note * There was no statistical difference at 95% confidence ($p < 0.05$).

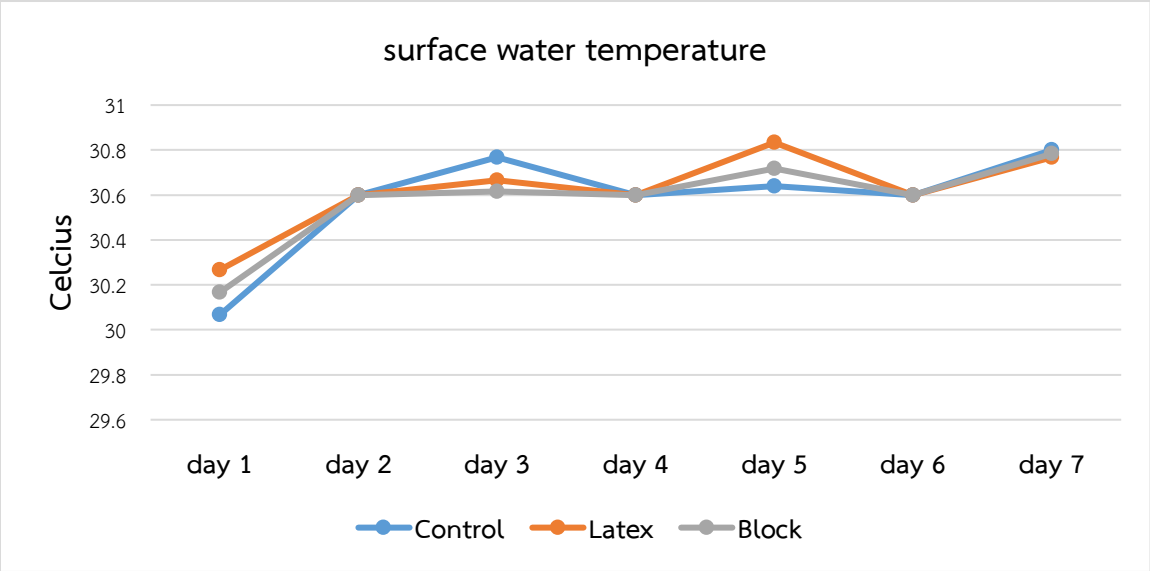
Picture 5 shows the average weight (gram) of *M. macrocopa* that feeds with Chlorella sp. as its food for 3 days.

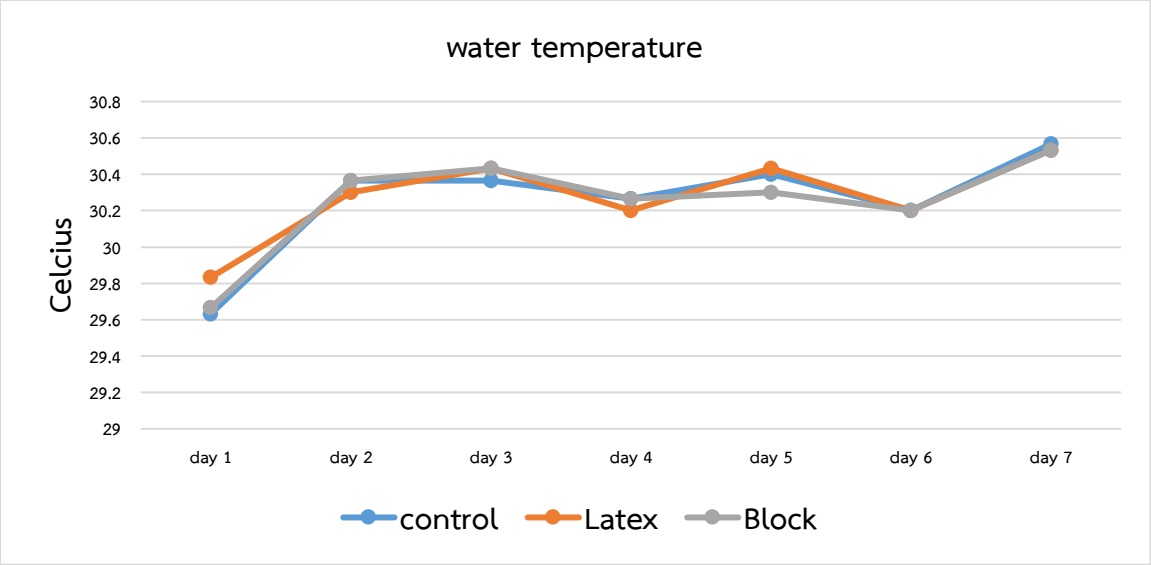
The result of the water quality after cultivating *Chlorella* sp. in the effluent from rubber processing.

The water's pH increases from 6.8 to 7 and 6.0 to 6.5, respectively, after the cultivation of *Chlorella* sp. in the effluent from concentrated latex processing and blocks rubber processing. This indicates that the third-set *Chlorella* sp. is more effective than the second-set at lowering acidity. In addition, there is an increase in dissolved oxygen (DO) from 3.9 to 10.8 mg/l and 2.6 to 11.2 mg/l, respectively. Thus, after blooming in both sets, the water quality becomes improved.


pH
10
8
6
4
2
0
day 1
day 2
day 3
day 4
day 5
day 6
day 7
Control
Latex
Block


Dissolve Oxygen
Dissolve Oxygen (mg/L)
12
10
8
6
4
2
0
day 0
day 1
day 2
day 3
day 4
day 5
day 6
day 7
control
Latex
Block


surface water temperature
Celcius
31
30.8
30.6
30.4
30.2
30
29.8
29.6
day 1
day 2
day 3
day 4
day 5
day 6
day 7
Control
Latex
Block



Picture 6,7,8,9 shows the water quality pH, DO, surface temperature, and water temperature

Discussion

According to the findings of the study "Comparison of cultivation of *Chlorella* sp. in effluent from rubber processing Blocks rubber and concentrated latex factories," the growth rate of *Chlorella* sp. is altered by the effluent from the rubber processing. The statistical analysis of the data reveals that the third set differs from the control set ($P<0.05$), whereas the second set does not show a statistically different from the control set ($P<0.05$). *M. macrocopa* can survive in the effluent when raised. According to statistical analysis, there is no significant difference between any of the sets ($P<0.05$). The growth rate varies in each set. It may rely on the nutrients, such as phosphorus and nitrogen, the cell's ability to reproduce, its enzymes, chlorophyll, or the process of photosynthesis in each.

Conclusion

The conclusion from the study, “Comparison of cultivation of *Chlorella* sp. in effluent from rubber processing Blocks rubber and concentrated latex factories,” indicates that the best set for *Chlorella* sp. to grow is the effluent from concentrated latex processing. Accordingly, each set contains 159.52±4.74, 220.24±2.88, and 85.71±1.78 cells/ml of *Chlorella* sp. The effluent from the concentrated latex processing set is not significantly different from the control set ($P<0.05$), according to statistical analysis of the data. However, there is a statistically significant difference ($P>0.05$) between the effluent from the blocks rubber set and the control set.

Also, the weight of *M. macrocopa* from the second set is the heaviest, the weight from the third set is the second heaviest, and the control set is the least heavy. The average weights from each

set are 6.71±1.4, 49.32±1.15, and 8.09±1.84 g, respectively. When analyzing the data statistically, all sets are not statistically different ($P<0.05$). The water quality, pH, DO, and temperature are better quality and suitable for cultivating *Chlorella* sp. .

According to the results Chlorella sp. can be grown most effectively by the effluent from concentrated latex processing. Furthermore, *Chlorella* sp. makes the effluent's water quality better.

Acknowledgements

We would like to thank our teacher, Mrs.Salamiyah Kittibunyatiwakorn, for helping and guiding us to complete the research process. Thank you to Guangken Rubber(Trang) Company Limited for providing the effluent and helping us to get all the information. Thank you to Trang Inland Fisheries Research and Development Center for providing *Chlorella* sp. leavening and place to cultivate *Chlorella* sp. . Thank you to Princess Chulabhorn's Science High School Trang for providing place to conducted an experiment. We would like to thank everybody that helps us get through the research process.

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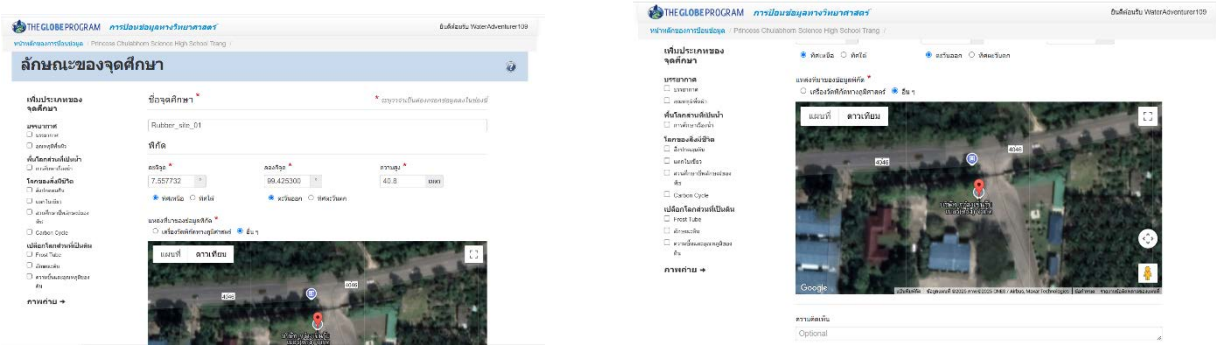
GLOBE's Database



Picture 10 : GLOBE DATA ENTRY

(Optional) Badge Descriptions/Justifications

I am a collaborator

This study has received assistance from the outside organization. Guangken Rubber(Trang) Company Limited, which located in Na Mueang Phet Subdistrict, Mueang Trang District, Trang Province, provided the effluent from rubber processing Blocks rubber and Concentrated latex. Trang Inland Fisheries Research and Development Center, which located in Na Toh Ming Subdistrict, Mueang Trang District, Trang Province, provided *Chlorella* sp. leavening and place to cultivated *Chlorella* sp. . We collected the information from our observations from both places in Globe Applications.

I make an impact

The farmers have to pay high costs for cultivating *Chlorella* sp. because they usually use field fertilizer and urea fertilizer. The effluent from rubber processing Blocks rubber and Concentrated latex factories has the proper nutrients for *Chlorella* sp. and it can grow in the effluent very well. Therefore, this study has the impact with the society, the farmers can choose to cultivate more *Chlorella* sp. because it increases their incomes and it doesn't have bad effects to environment.

I am a Data Scientist

In this study, we collected data to analyze and compare the statistical information to find differences and variations in data in each set of experiments, Including, conducted previous research to discuss and summarize experimental results.