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Research Article

Comparison of the growth of Spirulina sp. in feed formulas containing rice straw fermentation liquid as an ingredient at different levels

Arom Tiaosakul[1]*, Sayam Raso[2], and Sukjai Rattanayurakorn[3]

Fisheries Department, Faculty of Agriculture and Technology, 145 Moo 15, Surin-Prasart Road, Nokmuang , Surin, Thailand

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Abstract

The objective of this research was to study the effects of different feed formulas on the growth of Spirulina and water quality in terms of temperature, pH and light intensity. The experiment was designed with completely randomized design (CRD), divided into 3 sets of treatments with different feed formulas with 3 replicates, using formula 1(Zarrouk's medium as control formula); formula 2(Zarrouk's medium mixed with 100 ml/1,000 ml of rice straw ferment liquid), and formula 3(Zarrouk's medium mixed with 200 ml/1,000 ml of rice straw ferment liquid) with 10 days cultured period provided with 24-hour light cycle. The growth efficiency was measured by measuring the average of Optical Density (OD), using a spectrophotometer at the wavelength of 560 nm. The results showed that the growth rate of Spirulina cultured with formula 1 was a statistically significantly different level of 95 % confidence ($p<0.05$) with, the growth rate of Spirulina cultured with formula 2 and 3. While the growth rate of Spirulina cultured with formulas 2 and 3 was not statistically different at a statistical significance level of 95 % confidence ($p>0.05$). In terms of water quality throughout the Spirulina culture, the temperature and pH values were in the range of 26.80±0.53 - 32.40±0.30 °C and 9.46±0.05 - 10.12±0.09, while the light intensity values were in the range of 1,125 - 1,950 lux, respectively

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Introduction

Rice straw is the part of the rice plant that remains after harvesting and removing the seeds. Rice straw contains the following primary and secondary nutrients: nitrogen (N) = 0.59 %, phosphorus (P) = 0.08 %, potassium (K) 1.56 %, calcium (Ca) 0.38 %, magnesium (Mg) 0.23 % and sulfur (S) 0.08 % (Terra-Gro Fertilizer Co., Ltd., 2023) It contains protein, dietary fiber and phosphorus (Spring Green Evolution Co., Ltd., 2023) total carbon content of 39.2 %, calcium 262 mg kg^{-1} and sodium 366 mg kg^{-1} (Abdelhamid, 2004). These elements are important for plant growth. Farmers generally use rice straw for agricultural purposes, such as as mulch to retain moisture, for animal feed, for compost or for plowing to increase organic matter which is a source of food and energy for beneficial microorganisms in the soil. In the fisheries sector, farmers use it as a food source for fish or as a fish food sandwich. By using rice straw as the main base

1 Corresponding Author: Fisheries Department, Faculty of Agriculture and Technology, 145 Moo 15, Surin-Prasart Road, Nokmuang , Surin, Thailand. E-mail: apermngam@gmail.com
2 Science in Creative biology, Faculty of Science and Technology, Surindra Rajabhat University, 186 Moo 1, Surin-Prasart Road, Nokmuang, Surin, 32000 , Thailand.
3Science and Mathematics Department, Faculty of Agriculture and Technology, 145 Moo 15, Surin-Prasart Road, Nokmuang, Surin, 32000, Thailand.

and placing it alternately with animal feces or other manures at the corners of the pond to provide a food source for the fish in the pond. Some farmers throw rice straw into the fish pond for about 3-5 days. The straw soaked in water will gradually decompose. Nile tilapia and other herbivorous fish such as carp, gourami, and striped catfish, etc., will eat the decompose rice straw until it is all gone. In addition to being food for herbivorous fish, rice straw soaked in water is also a hiding place and helps create algae and plankton, which are another type of fish food. In addition to helping solve the problem of smog caused by burning rice straw, it can also help save on fish farming costs and make fish meat firm and taste sweet like fish in natural water sources (Sridarat, 2012).

Spirulina is a blue-green algae, It is a single-celled algae that has a spiral shape. It is found in freshwater natural water sources, from cultured areas, and grows well in both clean and wastewater (Wongrat, 1996). This type of algae has a very high protein level, so it has received attention and is used as a supplement for health-conscious people and mixed in aquaculture formula feed (Bhattacharya and Shivaprakash, 2005) or used to replace protein sources from other sources (Boonsom, 1992). Spirulina contains a high protein content of 50-70 percent of dry cell weight and contains other important substances such as Phycocyanin, Allophycocyanin, Beta-carotene, Chlorophyll-a and unsaturated essential fatty acids (Vekataraman, 1983).

Most spirulina farming uses chemical fertilizers as a food source, which has high production costs, as an alternative to use as a supplement in ready-made aquatic animal feed with high production capacity, and to reduce the cost of cultivation, by finding natural food sources such as fermented animal waste or fermented plants (Chanchay and Chudarat, 2018).

Therefore, from the reasons mentioned above, to be a guideline to reduce the cost of spirulina cultivation with fermented water from rice straw, which is a plant residue left over from agriculture after harvesting, the data obtained from the study will be basic information to be applied to reduce the production cost of spirulina and can apply it to create benefits in the future.

Aim of the Study

This study aims to determine the effects of different feed formulas of the growth of *Spirulina* sp. in feed formulas containing rice straw fermentation liquid and water quality in terms of temperature, pH and light intensity.

Method

Preparation of water for culturing Spirulina algae Use tap water that has been left in a 50-liter plastic tank for 5 days and filter it with a thin cloth before using it in the experiment.

Preparation of Spirulina algae Spirulina algae, which was kindly provided by the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai Province, was cultivated in the laboratory using Zarrouk's medium to prepare for use in the experiment. Before using it, the algae were measured for cell density (Optical Density; OD) using a Spectrophotometer at a wavelength of 560 nanometers, to obtain an Optical Density (OD) value in the range of 0.80-0.90 for use in the study (adapted from Chuchet et al., 2010).

Preparation of rice straw fermentation solution Cut rice straw into small pieces and ferment with prepared tap water in a ratio of rice straw: water with ratio 1:10. Leave to ferment for 4 weeks.

Preparation of the control formula (Zarrouk's medium) adapted from Meng-Amphan and Nokham (2007) the solution consists of the following Sodium bicarbonate ($NaHCO_3$) 4.80 grams, Sodium hydroxide (NaOH) 1.12 grams, N-P-K fertilizer (16-16-16 formula), and Magnesium sulphate ($MgSO_4$) 1.62 grams respectively. These chemicals were dissolved in 600 milliliters of tap water. This process was repeated in 9 glass jars, each with a capacity of 3,000 milliliters, to prepare for the experiment.

The Completely Randomized Design (CRD) trial was planned, divided into 3 sets of 3 replicates each, as follows.

- Treatment 1 (T1) Feed formula 1 used Zarrouk's medium (control formula)
- Treatment 2 (T2) Feed formula 2 used Zarrouk's medium formula and 100 ml of rice straw fermentation liquid

➢ Treatment 3 (T3) Feed formula 3 used Zarrouk's medium formula and 200 ml of rice straw fermentation liquid

Add 200 ml of spirulina to every glass jar prepared above and adjust the water level in each glass jar to 1,000 ml with the prepared tap water. Place the glass jars in random positions, install an aeration set, and provide lighting with 120 cm long LED bulbs in every row of the glass jars throughout the 10 days.

Data collection and analysis

Water quality analysis analysed before the experiment and every day of algae cultivation, including pH and temperature (**°C**) using a multifunctional water analyzer model 9909 SP.

Light intensity by randomly measuring the light intensity at 3 points in the experimental area that received light from electric bulbs while growing spirulina algae by using the TECPEL 530 light intensity meter.

Optical Density (OD) measurement was modified from Promya and Saetan (2005) by randomly collecting algae samples from each experimental set using a Spectrophotometer (Jasco Model V-530, Japan) at a wavelength of 560 nm every day throughout the 10-day cultivation period to compare the algae growth rate (Growth rate, percentage; %) at a wavelength of 560 nanometers every day throughout the 10-day cultivation period to compare the algae growth rate (Growth rate, percentage; %) which is calculated by the following equation:

$$\text{Growth rate (\%)} = \frac{(OD_i - OD_o) \times 100}{OD_o}$$

When

OD_o = OD value of algae on day 0

OD_i = OD value of algae harvested on day i (i = 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10)

Data analysis of the growth rate and water quality data was analysed using the One-way analysis of variance (ANOVA) method according to the randomized experimental plan and the differences in the mean values were compared using the Honestly Significant Differences (Tukey's Test HSD) method at a 95 percent confidence level using the IBM SPSS Statistics V.26 ready-made program. The light intensity values will be reported as the mean values.

Results

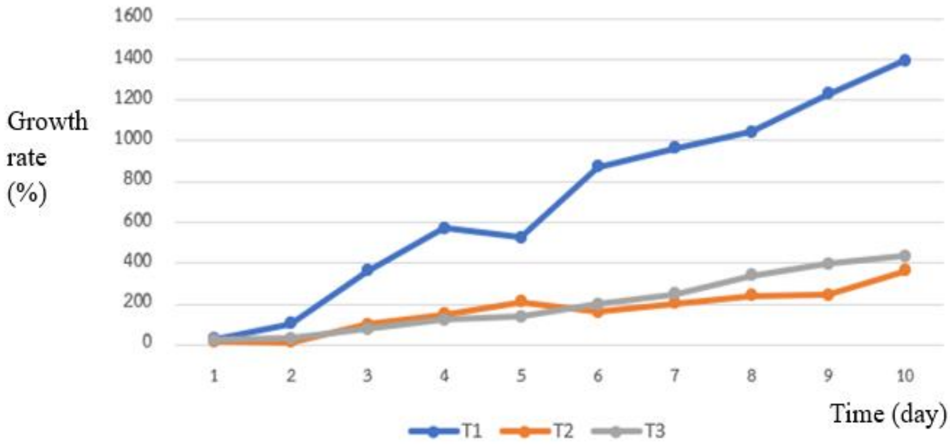
The results of the study comparison of *Spirulina* sp. cultivation yields in feed formulas containing rice straw fermentation as an ingredient at different levels showed that the growth rate of Spirulina algae with Feed formula 1 (T1) had an average growth rate of 9.46±0.05, 101.21±137.89, 363.53±125.26, and 573.05±198.01, 525.26±198.01, 871.27±176.37, 963.67±173.52, 1,044.51± 321.19, 1,228.52± 463.03 1,394.58±558.91 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, Feed formula 2 (T2) had an average growth rate of 15.04±28.66, 12.54±81.42, 100.07±68.76, 148.63±95.30,208.77± 137.50, 159.44±116.32, 201.19± 130.79, 240.64±193.23, 245.11± 135.44 and 361.42±201.54 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, Feed formula 3(T3) had average growth rates of 22.19± 33.41, 31.80±20.42, 77.94±89.52, 120.85± 107.84, 136.19±85.22, 197.60±124.39, 247.65±150.64, 338.00± 338.00±193.93, 395.53 ± 207.59 and 435.34± 238.54 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively (Table 4.1 and Figure 4.1). The growth rate of Spirulina algae after 10 days, Spirulina, algae cultured with Feed formula 1(T1) tended to have the highest growth rate (1,394.58±558.91 %) followed by Spirulina algae cultured with Feed formula 3 (T3) (435.34± 238.54 %) and 2 (T2) (361.42± 201.54 %), respectively.

When the growth rate data were analysed statistically, it was found that the growth rate of Spirulina cultured with Feed formula 1 was significantly different from the growth rate of Spirulina cultured with Feed formula 2 and 3 at a statistical significance of 95 % confidence level ($p < 0.05$). However, the growth rate of Spirulina cultured with Feed formula 2 and 3 was not statistically different at a statistical significance of 95 % confidence level ($p > 0.05$). (Table 4.1).

Table 1. Shows the average growth rate (%) of Spirulina algae over a period of 10 days

Days	Feed formula		
	1 (T1)	2 (T2)	3 (T3)
1	9.46±0.05[a]	15.04±28.66[b]	22.19±33.41[b]
2	101.21±137.89[a]	12.54±81.42[b]	31.80±20.42[b]
3	363.53±125.26[a]	100.07±68.76[b]	77.94±89.52[b]
4	573.05±198.01[a]	148.63±95.30[b]	120.85±107.84[b]
5	525.26±198.01[a]	208.77±137.50[b]	136.19±85.22[b]
6	871.27±176.37[a]	159.44±116.32[b]	197.60±124.39[b]
7	963.67±173.52[a]	201.19±130.79[b]	247.65±150.64[b]
8	1044.51±321.19[a]	240.64±193.23[b]	338.00±193.93[b]
9	1228.52±463.03[a]	245.11±135.44[b]	395.53±207.59[b]
10	1394.58±558.91[a]	361.42±201.54[b]	435.34±238.54[b]

Note: Identical letters on the same line are not statistically different at 95 % confidence.



Picture 1. The average growth rate (%) of Spirulina algae over a period of 10 days

Water quality in terms of acidity-alkalinity (pH) during from the beginning to the end of the experiment 10 days in the glass jars of all treatments, the results showed that the average pH values in Feed formula 1(T1) were 9.46±0.05, 9.67±0.03, 9.96±0.06, 9.70±0.02, 9.90±0.0, 9.79±0.03, 9.88±0.12 10.00±0.14, 10.01±0.23, 10.07±0.20 and 9.91±0.22; 9.51±0.05, 9.70±0.07, 9.65±0.06, 9.68±0.02, 9.81±0.05, 9.78±0.05, 9.91±0.05, 10.03±0.07, 10.00±0.15, 10.12±0.09 and 9.97±0.12 in Food formula 2(T2) and 9.58±0.01, 9.65±0.03, 9.70±0.02, 9.68±0.02, 9.81±0.02, 9.74±0.01, 9.86±0.04, 9.86±0.03, 9.81±0.08, 9.93±0.06 and 9.90±0.02 in Feed formula 3(T3), respectively (Table 2 and Figure 2 A).

The average pH value of all treatments was between 9.46±0.05 - 10.12±0.09, and when the data were analyzed statistically, it found that all treatments were not statistically different at 95 % confidence level ($p > 0.05$) (Table 2).

Table 2. Shows the average pH values during the cultivation of Sprulina algae with different formulas for 10 days

Days	**Feed formula**		
	1 (T1)	**2 (T2)**	**3 (T3)**
1	9.67±0.03^{a}	9.70±0.07^{a}	9.65±0.03^{a}
2	9.96±0.06^{a}	9.65±0.06^{a}	9.70±0.02^{a}
3	9.70±0.02^{a}	9.68±0.02^{a}	9.68±0.02^{a}
4	9.90±0.06^{a}	9.81±0.05^{a}	9.81±0.02^{a}
5	9.79±0.03^{a}	9.78±0.05^{a}	9.74±0.01^{a}
6	9.88±0.12^{a}	9.91±0.05^{a}	9.86±0.04^{a}
7	10.00±0.14^{a}	10.03±0.07^{a}	9.86±0.03^{a}
8	10.01±0.23^{a}	10.00±0.15^{a}	9.81±0.08^{a}
9	10.07±0.20^{a}	10.12±0.09^{a}	9.93±0.06^{a}
10	9.91±0.22^{a}	9.97±0.12^{a}	9.90±0.02^{a}

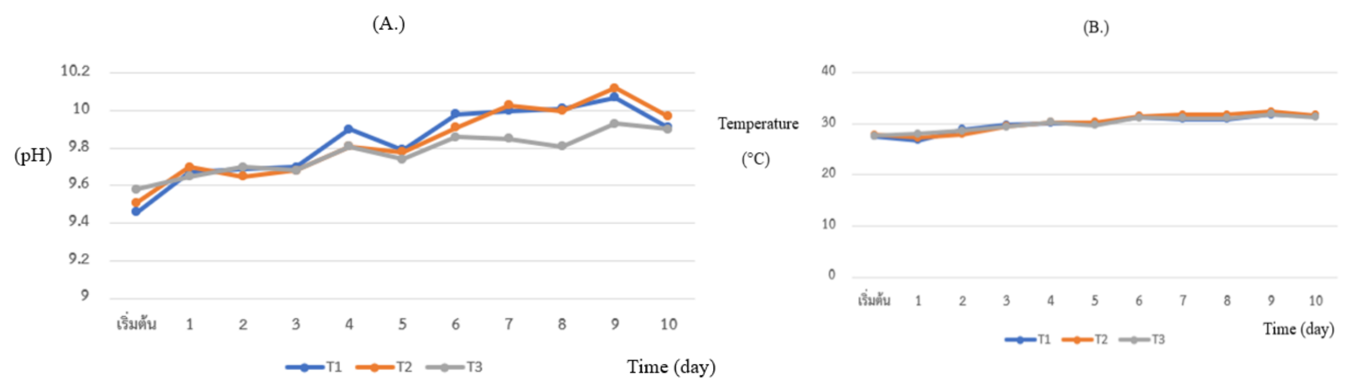
Note: Identical letters on the same line are not statistically different at 95 % confidence

For water quality in terms of temperature (°C) from the beginning to the end of the experiment 10 days in the glass jars of all treatments, the results showed that the average temperature values in Food formula 1(T1) were 27.57±0.47, 26.80±0.53, 28.93±0.31, 29.87±0.32, 30.10±0.17, 30.07±0.06, 31.30±0.56, 30.97±0.38, 30.97±0.38, 31.77±0.47 and 31.47±0.47 °C; 27.90±0.44, 27.27±0.31, 28.00±1.00, 39.57±0.21, 30.33±0.3,5 30.30±0.26, 31.50±0.87, 31.83±0.25, 31.83±0.25, 32.40±0.30 and 31.70±0.60 and 27.67±0.12, 28.03±0.38, 28.60±0.60, 29.43±0.65, 30.33±0.57, 29.77±0.75, 31.33±0.31, 31.10±0.53, 31.10±0.53, 31.90±0.72 and 31.30±0.92°C in Feed formula 3(T3), respectively (Table 3 and Figure 2 B). All treatments' average temperature (°C) values were between 26.80±0.53 and 32.40±0.30°C. When the data were analyzed statistically, it was found that all treatments were not statistically different at a 95 % confidence level ($p > 0.05$) (Table 3). While the average light intensity was in the range of 1,125 - 1,950 lux.

Table 3. Shows the average temperature (°C) values during the cultivation of Sprulina algae with different formulas for 10 days

Days	**Feed formula**		
	1 (T1)	**2 (T2)**	**3 (T3)**
1	26.80±0.53^{a}	27.27±0.31^{a}	28.03±0.38^{a}
2	28.93±0.31^{a}	28.00±1.00^{a}	28.60±0.60^{a}
3	29.87±0.32^{a}	39.57±0.21^{a}	29.43±0.65^{a}
4	30.10±0.17^{a}	30.33±0.35^{a}	30.33±0.57^{a}
5	30.07±0.06^{a}	30.30±0.26^{a}	29.77±0.75^{a}
6	31.30±0.56^{a}	31.50±0.87^{a}	31.33±0.31^{a}
7	30.97±0.38^{a}	31.83±0.25^{a}	31.10±0.53^{a}
8	30.97±0.38^{a}	31.83±0.25^{a}	31.10±0.53^{a}
9	31.77±0.47^{a}	32.40±0.30^{a}	31.90±0.72^{a}
10	31.47±0.47^{a}	31.70±0.60^{a}	31.30±0.92^{a}

Note: Identical letters on the same line are not statistically different at 95 percent confidence



Picture 2. Water quality in terms the average of acidity-alkalinity (pH): (A.) and the average temperature (°C): (B.) during the cultivation of Sprulina algae with different formulas for 10 days

Conclusion and Discussion

From the study of Comparison of the Growth of Spirulina sp. in feed Formulas Containing Rice Straw Fermentation Liquid as an Ingredient at Different Levels for 10 days, the results showed that the growth rate of Spirulina sp. cultured by feed formula 1 using Zarrouk's medium (control formula) was statistically different at 95 % confidence ($p<0.05$) with, Spirulina cultured by feed formula 2 (using Zarrouk's medium and 100 ml of rice straw fermentation liquid) and feed formula 3 (using Zarrouk's medium and 200 ml of rice straw fermentation liquid). While the growth rate of Spirulina cultured by feed formulas 2 and 3 was not statistically different at a statistical significance level of 95 % confidence ($p>0.05$).

A study of Tansakun and Chanthasin (1988) investigating the supplementation of certain primary nutrients, specifically $NaNO_3$ at various concentration levels, in wastewater from rubber factories yielded notable results. The growth rate of Spirulina algae reached its peak when $NaNO_3$ was added at a concentration of 2.5 grams per liter, surpassing the growth rate observed with standard nutrient media. Conversely, the addition of K_2HPO_4 and K_2SO_4 did not significantly impact the growth rate of Spirulina algae. In fact, increasing the concentration of these compounds to high levels resulted in a decrease in growth rate.

Furthermore, a comparative analysis was conducted between two nutrient combinations: $NaNO_3$ at 2.5 grams per liter combined with K_2HPO_4 at 0.1 grams per liter, and $NaNO_3$ at 2.5 grams per liter combined with K_2SO_4 at 0.1 grams per liter. The results indicated that both combinations yielded lower Spirulina growth rates compared to the standard Zarrouk's medium. A notable observation from this study was the correlation between nutrient solution pH and Spirulina growth rates. Nutrient formulations that promoted high growth rates in Spirulina were characterized by high pH values. Conversely, nutrient formulations associated with decreased Spirulina growth rates exhibited low pH values.

Laophongphich (1989) experimented with the cultivation of Spirulina (*S. platensis*) in distillery wastewater at concentrations of 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 percent. The medium was supplemented with chemical additives: 8.5 g/L $NaHCO_3$, 1.5 g/L NaNO3, 0.5 g/L K2HPO4, and 0.6 g/L N-P-K fertilizer (16-16-16 formula). The pH was adjusted to 10 ± 0.1, and the cultivation period lasted 24 days under both laboratory and outdoor conditions. The results indicated that in both environments, the algae exhibited optimal growth at a distillery wastewater concentration of 0.5 %.

Ciferri (1983); Venkataraman (1983) and Nakamura (1982) reported the pH levels observed during Spirulina cultivation of all nutrient formulations averaged between 9.46±0.05 and 10.12±0.09, which falls within the optimal range for Spirulina growth. As with the experiment of Chuchet et al. (2010) indicates that the ideal pH range for Spirulina growth is between 9.5 and 10.5, and Vincent and Silvester (1979) reported that Spirulina thrives in pH conditions ranging from 9 to 11, as this range facilitates optimum nutrient availability for the cyanobacterium growth and pH levels are closely related to the dissolution of carbon dioxide (Venkataraman, 1983) or the concentration of bicarbonate ions (Bunnak, 1986). These factors directly and indirectly influence the metabolic processes of algal cells

(Becker and Venkataraman, 1982). The pH levels affect photosynthetic processes and the activity of various enzymes involved in algal photosynthesis. But, in the present study, the pH levels ranged from 7.30 to 8.30, which is below the optimal range for Spirulina growth. While the temperature ranged from 26.80±0.53 to 32.40±0.30 °C. This temperature range is conducive to optimal Spirulina growth (Venkataraman, 1983).

The wavelengths that Spirulina can utilize photosynthesis are identical to those used by typical plants (Trissl, 1993). Increased light intensity enhances photosynthesis and accelerates cellular activity (Niangoran et al., 1974). When the light intensity is below 2000 lux, algae, and higher plants can only utilize approximately 20 % of the incident light energy (Bunnak, 1986). The algae's energy utilization efficiency decreases at light intensities exceeding 8000 lux. And, Nakamura (1982) suggests that the optimal light intensity for Spirulina growth is between 4,000-5,000 lux, while Tansakun and Chanthasin (1988) report a requirement of 3,500 lux for 16 hours per day. But, in the present study, throughout the experiment, the light intensity ranged from 1,125 lux to 1,950 lux., which is below the range for Spirulina growth as mentioned above.

Recommendations

Based on the above findings and conclusion, the following are the study's recommendations:

- In the cultivation of Spirulina using rice straw fermentation extract, it is suggested to supplement the culture medium with certain essential macronutrients, such as $NaNO_3$ and pH optimal range. This compound not only serves as a nutrient source for Spirulina, but also aids in elevating the pH level to a range between 9 and 11. This pH range is optimal for nutrient utilization, significantly impacting photosynthetic processes and the activity of various enzymes involved in algal photosynthesis.
- For optimal Spirulina cultivation, it is recommended to utilize areas with high light intensity or outdoor locations where illuminance exceeds 1,950 lux. The ideal light intensity range for cultivation is between 4,000 and 5,000 lux. To exceed this range can occur the photoinhibition.
- Rice straw, an agricultural by-product remaining after harvest, can be utilized in small quantities to produce fermentation extract. This application presents a potential avenue for reducing production costs in Spirulina cultivation in the future, offering an innovative approach to sustainable algal biotechnology in rural area application.

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