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REVIEW : POTENTIAL UTILIZATION OF VARIOUS LIQUID WASTES AS A MEDIUM FOR THE GROWTH OF NATURAL FEED *Spirulina Platensis*

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ABSTRACT

Spirulina platensis is a type of blue-green algae that has many benefits, especially in the field of cultivation, namely as a source or mixture of natural feed for several fish commodities such as goldfish, tilapia and several other important aquaculture commodities. *Spirulina platensis* has a protein content of 70%, fat around (1.5-12)%, pigment chlorophyll (0.08%), beta carotene (0.23%) and xanthophile (0.15%) which are beneficial for the growth phases of aquaculture commodities. This review journal uses a literature review method, using several tools such as Google Scholar and Publish and Perish, with the aim of finding alternative mediums that can be used in the cultivation of *Spirulina platensis*. Palm Oil Mill Effluent (POME) waste, cultivation waste, tofu waste and domestic waste can be used as the main medium and mixed medium for the cultivation of *Spirulina platensis*. The growth of *Spirulina platensis* in POME waste was quite promising with a doubling time of 0.128/day, in cultivation waste the growth reached 717.4%, in tofu waste the growth *of Spirulina platensis* reached a cell density of 1,406 individuals/mL, and in domestic waste the doubling time occurred in culture 3.37%.

***Keywords*:** Spirulina platensis, Waste Water Treatment, Natural feed, Algae Cultivation, Cyanobacteria.

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1. INTRODUCTION

Spirulina sp, is a type of microalgae whose common is found in various types of environments both in marine, fresh and brackish waters (Buwono *et al* 2018). *Spirulina* sp is a type of Cyanophyta which is an autotrophic organism that is bluish-green in shape in a cylindrical shape that forms twisted colonies forming filaments resembling spirals (Haryati, 2008 in Buwono, 2018). The nutritional content of *Spirulina* sp is quite complete, namely 70% protein, around (1.5-12)% fat. Cahyaningsih and Subyakto (2009) in Buwono (2018) mentioned in their research that microalgae in general have an important role in aquaculture, because microalgae play a role as producers in the food chain in the waters. *Spirulina platensis* is commonly used as a natural feed. Usually *Spirulina platensis* is used as a mixture in fish pellets in the form of *Spirulin*a flour, a natural feed derived from *Spirulina platensis* is suitable for omnivore and herbivorous fish such as goldfish, tilapia and gourami fish. (Hidayah, 2017) *Spirulina platensis* culture media variously, one of which is POME waste, besides that formulation from various other wastes such as tofu waste, soy sauce waste, herbal medicine waste, and fish farming waste can also be carried out. *Spirulina* sp also plays a role as an additional feed for ornamental fish because the data adds coloring due to the pigments contained in it The pigments contained in *Spirulina* sp generally consist of chlorophyll (0.08%), beta carotene (0.23%) and xanthophils (0.15%). In addition to acting as a natural feed, *Spirulina* sp also acts as an immunostimulant (Utomo *et al.,* 2005 in Buwono, 2018). Cultures carried out on *Spirulina* sp are usually carried out with the aim of obtaining the highest cell abundance with optimal nutrient content. Kulltur *Spirulina* sp is usually done on a laboratory scale and can also be carried out en masse. The media commonly used in *Spirulina* sp culture is pro analyst media such as *Zarruk* media. However, the use of this medium is quite expensive to be mass-produced, therefore, it is necessary to have another medium at a lower price to support *Spirulina* sp, which is a type of microalgae that is commonly found in various types of environments both in marine, freshwater and brackish waters (Buwono *et al* 2018). *Spirulina* sp is a type of Cyanophyta which is an autotrophic organism that is bluish-green in shape in a cylindrical shape that forms twisted colonies forming filaments resembling spirals (Haryati, 2008 in Buwono, 2018). The nutritional content of *Spirulina* sp is quite complete, namely 70% protein, around (1.5-12)% fat. Cahyaningsih and Subyakto (2009) in Buwono (2018) mentioned in their research that microalgae in general have an important role in aquaculture, because microalgae play a role as producers in the food chain in the waters. *Spirulina platensis* is commonly used as a natural feed. Bias, *Spirulina platensis* is used as a mixture in fish pellets in the form of *Spirulina* flour, natural feed derived from *Spirulina platensis* is suitable for use in omnivorous and herbivorous fish such as goldfish, tilapia and gourami fish. (Hidayah, 2017) *Spirulina platensis* culture media variously, one of which is POME waste, besides that formulation from various other wastes such as tofu waste, soy sauce waste, herbal medicine waste, and fish farming waste can also be carried out. *Spirulina* sp also plays a role as an additional feed for ornamental fish because the data adds coloring due to the pigments contained in it The pigments contained in *Spirulina* sp generally consist of chlorophyll (0.08%), beta carotene (0.23%) and xanthophils (0.15%). In addition to acting as a natural feed, *Spirulina* sp also acts as an immunostimulant (Utomo *et al.,* 2005 in Buwono, 2018). Cultures carried out on *Spirulina* sp are usually carried out with the aim of obtaining the highest cell abundance with optimal nutrient content. Kulltur *Spirulina* sp is usually done on a laboratory scale and can also be carried out en masse. The media commonly used in *Spirulina* sp culture is pro analyst media such as *Zarruk* media.

However, the use of this medium is quite expensive to be mass-produced, therefore, it is necessary to have another medium at a lower price to support the culture of *Spirulina* sp in bulk. Some of the wastes that can be used as *Spirulina sp culture media* include POME waste, cultivation waste, guano orgqnik fertilizer, herbal medicine waste, tofu waste, domestic waste, soy sauce waste, and the mass addition of Spirulina sp *culture CO2ang* gas. Some of the wastes that can be used as *Spirulina sp culture media* include POME waste, cultivation waste, guano orgqnik fertilizer, herbal medicine waste, tofu waste, domestic waste, soy sauce waste, and the addition of $CO_{2\ gas}$

2. MATERIALS AND METHODS

2.1. Materials and Methods

The method used in writing this journal is a literature review method, using several tools such as Google Scholar and Publish and Perish by entering the keywords "Spirulina platensis natural feed" "Spirulina platensis natural feed cultivation techniques" "Spirulina platensis cultivation" "Spirulina platensis cultivation medium". 31 sources were obtained from research journals, theses, theses, and dissertations.

2.2. Results

The European Food Safety Agency (EFSA) and the Drug Administration Forum (FDA) released that some of the optimal conditions for the growth of Spirulina in culture tubs include a light period of 12/12 4000 Lux, temperature 30°C, inocnation volume, stirring speed, dissolved solids (10 – 60 g/L), pH (8.5 – 10.5), water quality which includes total micronutrients in it at least C, N, P, K, S, Mg, Na, Cl, Ca, Fe, Zn, Cu, Ni, Co dan Se). The growing medium that is usually used in the cultivation of *Spirulina platensis* is *a zarrouk* medium with the composition of most of the nutrients needed by *Spirulina platensis* for growth. However, the disadvantage of this medium is that it is quite expensive to reach 80 USD per liter, making it impossible to produce biomass on a large scale at the cost of a fairly expensive growing medium. Therefore, a formulation of Spirulina growing medium is needed in the hope of reducing maintenance costs or the cost of the growing medium. Several previous studies have shown positive results regarding several alternative mediums and growth medium formulations that are used to suppress high prices.

Ahsan *et al.*, (2008) in Nawal *et al.*, (2022) stated that Spirulina can be cultivated in several types of mediums, both industrial and agricultural waste, sugar factory waste, livestock waste, and other organic waste. However, the thing that needs to be considered when choosing waste as an alternative medium or formulation is the availability of inorganic minerals in it, besides that the factor that is considered is in terms of the price of the replacement medium or formulation to be used

2.3. Growth of *Spirullina platensis* in POME waste media

POME is a liquid waste from palm oil processing (CPO). POME has high characteristics of organic materials that are difficult to decompose, so if it is discharged directly into the water, it will affect the solubility of oxygen in the water which will endanger aquatic life. POME derived from palm oil waste used as a Spirulina culture medium is rich in organic and inorganic materials which are generally used as one of the supporting factors for Spirulina growth. Research on the use of POME waste as one of the growing medium *for Spirulina platensis* has been widely researched.

Research conducted by Sari *et al.*, 2022 proves that the use of POME waste as a growing medium *for Spirulina platensis* is able to increase the growth *of Spirulina platensis*. *Spirulina platensis* in this study was cultured in a batch of 1000 ml Erlenmeyer as a reactor using aeration and also a 24-hour TL lamp source as a lighting source. The growing medium in this study was POME+Urea (25 or 50 ppm), POME+TSP (25 or 50 ppm), POME+$NaHCO_3$ (200 or 400 ppm). The results of this study prove that the total density is 0.648, in addition to the use of *Spirulina platensis* can also reduce total carbon content by 93-98%, N content by 99-99.5%, and P by 92-97%.

Elvitriana *et al* (2017) researched the use of POME at different concentrations (10%, 30% and 50% Vinoculolum/Vmedia) as a growing medium *for Spirulina platensis*. Culture was carried out for 7 days after which several analyses were carried out to determine the growth rate *of Spirulina platensis* and several other components such as nutrient uptake and secondary metabolite content. The results obtained from this study are that the best POME waste concentration is 30% with 4x8 watt lighting for 8 hours, this treatment produces *Spirulina platensis biomass* as much as 1.2 grams/L, with the reduction rate of COD and BOD in POME waste reaching 40-80%.

POME waste that has not been treated has a fairly high organic waste content, so with such content it will not support the growth of *Spirulina platensis*. Several studies have made formulations between POME waste and several other nutrients. This is in line with the research of Mahdi *et al* (2022) who researched the formulation between POME waste with an additional 50 ppm NaHCO3 and 25 ppm $CO(NH2)_2$), microalgae require nutrients that are high enough to support growth, one of the important nutrients is C, N, and P, with a total ratio of 56:9:1, while the CNP ratio contained in POME waste is 47:7:1, The results of research conducted by Mahdi *et al* (2022) show that POME waste can replace C and N ratios of 83.9% and 77.7%, respectively.

Research on the formulation of POME waste with other additives has also been researched by Sari *et al* (2012) using POME waste with different concentrations (20%, 40% and 60%), with Urea (25 or 50 mg/L), $NaHCO_3$ (200 or 400 mg/L) and TSP (25 or 50 mg/L) which are given every 2 days. *Spirulina platensis* culture was carried out under different medium culture conditions, lighting using an 18-watt lamp, aerator in a 1000 ml Erlenmeyer batch. The results showed that the use of POME waste formulation as a growth medium *for Spirulina platensis* was effective in increasing the growth rate *of Spirulina platensis* by 0.128/day, with the best treatment of POME waste concentration of 20% and additional nutrients in the form of urea 25 ppm, TSP 50 ppm, and $NaHCO_3$ 200 ppm.

Several conditions show that POME waste used as a growing medium *Spirulina platensis* can be used as a growth medium directly without mixing with other media, but first POME waste goes through several dilutions to reduce the content of organic waste which is quite high. Research on this subject has been researched by Mutiah *et al* (2013) regarding the use of POME waste as a growing medium with different dilution and different cell densities of *Spirulina platensis* cultures. This research was conducted for 5 days in an open pond bioreactor and used lighting from 18 watt Philip fluorescent lamps for 8 hours. The dilution of POME waste tested was 3x, 4x, and 5x dilution, while the density of *Spirulina platensis* feed used was 0.443 g/L, 0.618 g/L, 0.952 g/L. The results showed that the best treatment to produce dry biomass of 0.9932 g/L was the treatment of *Spirulina platensis* feed of 0.443 g/L, while to produce a density of 0.7592 g/L was a 5x dilution treatment of POME waste.

2.4. Growth of *Spirulina platensis* in aquaculture waste media

Aquaculture waste contains several nutrients derived from the remaining metabolism of cultivated organisms and acts as nutrients in the growth of aquatic plants and microalgae. One of the nutrients that is widely found in aquaculture waste is N and P. Wahyuningsih *et al.,* (2015) stated that fish are only able to absorb 20-30% of nutrients derived from feed, and the rest decompose in the environment in the form of ammonia and organic proteins, the source of N in aquaculture waste while phosphorus (25-85)% comes from the rest of fish metabolism.

One of the uses of aquaculture waste as a growing medium *for Spirulina platensis* is the use of tilapia cultivation waste media for the growth of *Spirulina platensis* proven to increase growth by 717.4% on the 9th day of observation. In addition, *Spirulina platensis* can reduce ammonia levels by 5.27 mg/l, nitrate degradation by 2.2 mg/l, and phosphate degradation by 2.3 mg/l. This study was designed through several treatment schemes, namely positive control: 0 ml of waste + 3000 ml of sterile seawater; Negative control treatment (P0): 3000 mL of waste (without addition of spirulina), Treatment 1: 25% of waste (750 mL of waste + 2250 mL of sterile seawater); Treatment 2: 50% waste (1500 mL of waste + 1500 mL of sterile seawater); Treatment 3: 75% waste (2250 mL of waste + 750 mL of sterile seawater). Each treatment except the negative control treatment was added 500 mL of spirulina inoculant. (Mauretsa *et al.,* 2019)

Hartami *et al.,* (2022) researched the use of vannamei shrimp farming waste as a growing medium for *Spirulina platensis* cultivation with different concentrations. Some of the treatments used in this study include (A) Control; (B) 50% waste (1500 ml of waste + 1500 ml of water) + technical fertilizer + Spirulina inoculant; (C) 75% waste (2,250 ml of waste + 750 ml of water) technical fertilizer + Spirulina inoculant; (D) 100% waste + technical fertilizer + Spirulina inoculant., this study was carried out under the condition of culture salinity 18, pH 7-7.2, temperature 26-28oC, with 3 watt fluorine lamp lighting with a distance of 5 cm from the surface of the growing medium and periodization of light 12 light 12 dark, the study was carried out for 7 days according to the length of the life cycle *of Spirulina platensis* (Tinambunan *et al.,* 2017). The results of the study show that *Spirulina platensis* can reduce nitrate levels by up to 95%, phosphate by up to 85%, in addition, wastewater treatment for vannamei shrimp cultivation using *Spirulina platensis* is considered effective in increasing the yield of vannamei shrimp cultivation while maintaining the quality of cultivated water.

Another research conducted by Wuang *et al.,* (2015) regarding the use of fish farming waste that has a fairly high content of ammonia and nitrates as a growing medium *for Spirulina platensis* , not only used as a reducer of ammonia and nitrates contained in fish farming waste, further filtrate or growing medium *for Spirulina platensis* Used as a fertilizer for several commercial agricultural products such as red spinach, pak choy, Chinese cabbage kailan and white crown. The culture conditions in this study were set using a cultivation batch of 2 L, the average number of cells used per liter of fish farming waste was 2x104 algae cells used. The growth and absorption of nutrients were studied for 3 days with lighting using a fluorine lamp with a light intensity of 500-1000 lux placed at a distance of 8 cm above the surface of the cultivation medium, the culture temperature ranged from 28-30oC. The results of the study show that *Spirulina platensis* is effective in reducing ammonia and nitrate levels in fish farming waste and is effectively used as a biofertilizer in pak coy cultivation and arugula cultivation. Nogueira *et al.,* (2018) also researched the same thing, regarding the use of tilapia cultivation waste as a growing medium for *Spirulina platensis* cultivation.

The culture was carried out in a pool with a volume of 2,400 L using 200 Watt HQI Lamps artificial light with a light intensity of 440 mol.m-2.s-1, aeration of the pool of 800 L/hour and a pump with flows of 1000 L/hour. The formulation of the growing medium was carried out by adding 1,200 L of tilapia farming waste effluent combined with Jourdan media with the composition that can be seen in Table 1. The results showed that *Spirulina platensis* can reduce ammonia, nitrite, nitrate and total phosphate levels almost 0 on the 9th day of treatment, with a large culture scale can also be applied on an industrial scale in the future, and is a promising prospect for aquaculture wastewater retreatment at a fairly low price and maintenance.

Table 1. Jourdan Medium.

Reagent	Concentration (g.L-1)
Urea (CH4N2O)	0.07
Magnesium sulphate heptahydrate (MgSO4.7H2O)	0.2
Ferrous sulfate heptahydrate (FeSO4.7H2O)	0.005
Potassium sulfate (K2SO4)	1.0
Ammonium phosphate (NH4HPO4)	0.1
Potassium nitrate (KNO3)	2.0
Sodium chloride (NaCl)	10.0
Sodium bicarbonate (NaHCO3)	8.0

2.5. Growth of *Spirulina platensis* in tofu waste media

Tofu waste is usually in the form of organic liquid waste which generally has thick characteristics and an unpleasant odor, this if left without special waste treatment will result in water pollution and also serious air pollution. Most of the traditional tofu processing industry does not have a good waste treatment system, which then ends up being discharged into waterways and then empties into rivers and pollutes the river and the surrounding environment. Therefore, the treatment of tofu liquid waste should be a special and serious concern to be managed.

Tofu liquid waste has a very high content of organic materials and proteins, if managed properly and through the right pre-treatment, it has a promising opportunity to be used as one of the formulations of microalgae growth mediums including *Spirulina platensis*. Previous studies have discussed the use of tofu liquid waste as one of the mixtures of *Spirulina platensis growing medium*. Noriko *et al.*, (2011) investigated the use of tofu liquid waste in several concentration levels (10%, and 20%), with subcullutraries of 4:6, and 1:10, in a 500 mL Erlenmeyer batch, pH 9.5, irradiated using a 36-watt TL lamp with a continuous aerator. The results showed that the best treatment was the treatment of 10% tofu waste with a 4:6 subculture, the growth of *Spirulina platensis* in this study was observed with the color concentration in the culture batch, the treatment of 10% tofu liquid waste and 4:6 subculture produced a dark green color which indicated that there was an increase in number and indicated the occurrence of growth when compared to other treatments that tended to show yellow color Pias.

Maulana *et al.*, (2017) in their research on the use of tofu liquid waste as a new alternative to *Spirulina platensis growth medium*, tofu liquid waste in this study plays a role as a fertilizer that is fluoridated with EM4. Fermentation of liquid waste is carried out by formulating 32.4 ml of EM4 added with 615.6 ml aqueous and let sit for 5-7 days to breed microorganisms and activate microorganisms in EM4, after which as much as 648 ml of active EM4 and 12,312 ml of tofu liquid waste are mixed and fermented for 15 days, the water medium used has a salinity of 20 ppt (12.5 liters of seawater with a salinity of 32 ppt mixed with 7.5 liters of fresh water with a salinity of 0 ppt to the total volume 20 liters) as much as 20 liters per container. There were 5 treatments in this study with controls, treatment 1 15 mg/l of tofu waste fermentation, 20 mg/l of tofu liquid waste fermentation, 25 mg/l of tofu liquid waste fermentation, and 30 mg/l of tofu liquid waste fermentation. The results showed that the best treatment was the treatment of fermentation of tofu liquid waste of 30 mg/l with the average density *of Spirulina platensis* on the 8th day of observation was 1,406.75 individuals/ml.

Shaichurrozi and Jayanudin (2016) researched the use of 8%v/v tofu waste and 50%, 75%, and 100% synthetic materials as the growth medium *of Spirulina platensis*, the culture conditions in this study used an artificial light source that was used from an 18 watt TL lamp with a spacing distance of 15 cm from the surface of the growing medium, with a pH of 9. The results of the study prove that *Spirulina platensis* achieves maximum growth in 75% of synthetic materials reaching an optical density of 0.0759 with CNP nutrients that are successfully absorbed as much as 128:12:1, and even obtained the highest protein production up to 91.27%. The same thing was also researched by Simamora *et al.*, (2017) regarding the use of tofu waste as a growing medium for various kinds of microalgae, tofu liquid waste has a very high COD content, so it requires initial treatment first in the form of filtration to then be used as a growing medium. This study was designed with 5 treatments, treatment of 1 microalgae seed + *Spirulina platensis* + tofu waste, treatment 2 *Botryococcus braunii* + tofu waste, treatment 3 *Chlorella pyrenoidosa* + tofu waste, *Tetraselmis chuii* + tofu waste, *Botryococcus braunii* + tofu waste. The results showed that COD in treatment 1 using *Spirulina platensis* with an initial COD content of 4358.33 mg/L and after treatment using *Spirulina platensis* the COD content was 316.76 which means that the effectiveness of removal was 93.59%.

2.6. Growth of *Spirulina platensis* in Domestic waste media

Domestic waste in rivers generally comes from household and agricultural waste. Domestic waste generally has the character of solid waste and liquid waste. Domestic solid waste is usually treated using conventional methods or primary treatment and domestic organic waste is processed using secondary treatment. Secondary effluent contain inorganic phosphorus and nitrogen that usually caused eutrophication.

Secondary treatment usually uses the help of several organisms, one of which is *Spirulina platensis. Spirulina platensis* is a type of *Cyanobacteria* that is generally cultivated with the aim of obtaining high protein and high amounts of biomass. However, recently *Spirulina platensis* is used as water remediation. Some previous studies that have proven the treatment of domestic waste using *Spirulina platensis* include research conducted by Gardiyawasam *et al.,* (2022) domestic waste is used as a growing medium *for Spirulina platensis.* The culture conditions in outdoor with light intensity 19000 – 21730 lux, temperature 27.86–30.33 °C, the results showed that the biomass productivity reached 23.65 mg/L, doubling time 3.37/day, absorbed nitrate was 275 mg/L. The acidity degree was quite fluctuating from 8-11, a CO2 pump was used to optimize pH.

A similar study was conducted by Nguyen *et al.,* (2022) who examined the use of domestic waste as a growing medium *for Spirulina platensis* which is used as a waste water treatment agent. This study combined domestic waste and medium zarrouk as much as 250 mL, light intensity 5000 lux and temperature 25oC, light period: dark 8:16 hours, which was cultivated for 8 days with full aeration for 8 days. This study resulted in the conclusion that *Spirulina platensis* can absorb $N\text{-}NH4^{+}$, T-N, $P\text{-}PO4^{3-}$, T-P and COD well with the treatment efficiency of 96.37, 49.71, 67.05, 65.88 and 95.53%. The use of *Spirulina platensis* as one of the agents in domestic wastewater treatment has been widely researched, generally the use of *Spirulina platensis* in wastewater treatment is resistant to secondary to tertiary treatment. *Spirulina platensis* as a tertiatry treatment agent has been investigated by Chavan and Srikanth (2018)

3. CONCLUSIONS

Spirulina platensis is a type of algae that has many benefits, *Spirulina platensis* has a protein content of 70%, fat around (1.5-12)%, chlorophyll pigment (0.08%), beta carotene (0.23%) and xanthophils (0.15%). In addition, acting as a natural feed, *Spirulina* sp also acts as an immunostimulant. *Spirulina platensis* is usually cultivated using raw medium, *zarrouk* medium which is quite expensive, several studies have proven that *Spirulina platensis* can be cultivated using several mediums either purely using a substitute medium *for zarrouk* or a mixture between zarrouk and other mediums. Some of the mediums that have been proven to be used as a medium for *cultivating Spirulina platensis* are aquaculture waste, tofu waste, Palm Oil Mill Effluent (POME) waste, and domestic waste. The growth of *Spirulina platensis* in POME waste was quite promising with a doubling time of 0.128/day, in cultivation waste the growth reached 717.4%, in tofu waste the growth *of Spirulina platensis* reached a cell density of 1,406 individuals/mL, and in domestic waste the doubling time occurred in culture 3.37%. The concept of *Spirulina platensis* culture can be used as a waste phytoremediation concept. Some wastes used as a growth medium must go through the primary treatment stage which is usually in the form of physical treatment in the form of filtration, basically *Spirulina platensis* used as a phytoremediation agent can only be applied at the secondary and tertiary treatment stages. The effectiveness of *Spirulina platensis* as a phytoremediation agent is evidenced by the absorption of *Spirulina platensis* on the content of organic waste and inorganic waste in wastes used as a growing medium. *Spirulina platensis* can absorb levels of N, P, COD, BOD 99%, 97%, 44-77%, 80% respectively. *Spirulina platensis* in aquaculture waste can absorb ammonia, nitrate, and phosphate as much as 5.27 mg/L, 2.2 mg/L, and 2.3 mg/L, respectively. *Spirulina platensis* can absorb COD as much as 93.5%, while in aquaculture waste, *Spirulina platensis* absorbs TN and TP by 67.05% and 65.88%.

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