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Cultivation of Natural Fish Feed *Scenedesmus dimorphus* (Turpin) Kützing 1834, in POME Waste with a Bioreactor System: A Review

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ABSTRACT

Microalgae are widely used as natural food for fish, one of which is *Scenedesmus dimorphus*. Aside from being a source of protein, microalgae are also a source of lipids, carbohydrates, vitamins, and pigments. The protein content in microalgae varies from species to species. Pome waste is waste that must be treated before disposal. Pome waste contains many nutrients that can pollute waters (nitrogen, phosphate, COD, BOD, TSS, and so on), but they are a food source for *Scenedesmus dimorphus*. Utilizing pome waste as a cultivation medium can reduce microalgae culture costs, reduce nutrient levels in waste, and grow microalgae optimally. Apart from waste, several factors affect the growth of *Scenedesmus* sp. namely synthetic medium, light intensity, and waste content. In this article, several researchers who have cultivated *Scenedesmus* sp. By utilizing pome waste and other factors, *Scenedesmus dimorphus* makes it possible to cultivate it in waste and can save costs. Apart from being used as natural fish feed, *Scenedesmus* sp. can also be used for aquatic phytoremediators.

Keywords**:** Natural fish feed, Nutrient, Phytoremediation, POME Waste, *Scenedesmus dimorphus*

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1. INTRODUCTION

Fishmeal is a common source used in fish feed to meet protein needs. However, sustainable consumption can reduce the commercial availability of fishmeal in large quantities [1]. Therefore, it is important to find a commercially sustainable substitute for fishmeal while maintaining feed quality. Algae are a source of protein, lipids, carbohydrates, vitamins, and pigments in the food chain [2]. Although the protein content of each species varies, in general microalgae contain essential amino acid compounds needed for better growth and survival of aquatic organisms [3]. Juvenile spot wolffish which were given fish meal as a control treatment, microalgae (*Scenedesmus obliquus*) 4% (AL4), 8% (AL8) and 12% (AL12) of fish feed during a 12-week trial, it yielded indications of optimal rapid cellular muscle growth of spotted wolffish in the administration of 4% *Scenedesmus obliquus* [4]. *Chlorella vulgaris* and *Scenedesmus obliquus* in the Autotrophic Biofloc Technology system can be an effective alternative source of amino acids for the growth of tilapia [5].

Indonesia is one of the largest palm oil-producing countries in the world. Along with the growth of the palm oil industry, the volume of POME (Palm Oil Mill Effluent) has also increased. POME waste requires further processing to avoid environmental pollution. H_2SO_4 or HNO_3 can hydrolyze POME and the results can be used for microalgae cultivation [6]. *Scenedesmus* sp. is a better microalgae in lipid production compared to *Spirulina* sp., or *Chlorella* sp. [7]. Microalgae cultivation can be carried out on synthetic media, with the addition of waste, or on the waste itself. *Scenedesmus dimorphus* is one of the many types of microalgae that can be cultured on additional pome media, by utilizing the nutrients in POME waste as a food source for microalgae to breed and survive. Indirectly there will be a symbiosis of mutualism between Scenedesmus microalgae and POME waste. Scenedesmus can take advantage of nutrient content to multiply, and POME waste will experience a decrease in water pollutant nutrients. So that Scenedesmus thrives and can be used as natural food as one of its benefits, and POME waste can be discharged into the waters because it meets the quality standards for wastewater quality. In this article, we will discuss the utilization of pome waste for *Scenedesmus dimorphus* microalgae culture with the aim of saving the cost of microalgae culture production, namely making the nutrient content of the waste as a food source in order to achieve optimal growth and the waste can meet the specified wastewater quality standards before being discharged into the waters. In other words, microalgae culture in waste media produces a win-win solution.

2. MATERIALS AND METHODS

The analysis was done in March 2023. The analytical method used was in the form of literature published internationally that were written in English and related to *Scenedesmus dimorphus* phytoremediation using pome waste. Apply relevant search keywords such as “bioreactor system”, “cultivation microalgae”, "natural feed fish", "phytoremediation", "POME waste", “Scenedesmus”, and "*Scenedesmus dimorphus*". And also collected literature from various sources such as Google Scholar, Elsevier, ScienceDirect, and Research Gate. With a systematic review method, which combines several previous primary research results to obtain accurate and proven facts. Use multiple search terms and term combinations; which resulted in the number of documents listed in Table 1. In total, we identified 69 potentially relevant academic articles, dissertations, thesis, and other international journal, all articles are written in English. All relevant journals then reviewed abstracts sections and results of academic articles, dissertations, thesis, and other international journals to identify those discussing *Scenedesmus dimorphus* culture in pome waste. This narrows the amount of relevant material to 34.

Table 1. Search Term and Number of Articles Found For Review

Search Terms	Initial Search Results	Relevant Literature
Scenedesmus dimorphus	10	6
Natural feed fish + Scenedesmus	6	4
Natural feed fish + *Scenedesmus dimorphus*	2	2
Phytoremediation	9	2
Phytoremediation + *Scenedesmus dimorphus*	8	4
Bioreactor system	7	1
POME waste + *Scenedesmus dimorphus*	12	7
Cultivation microalgae	5	2
Cultivation microalgae + *Scenedesmus dimorphus*	10	6

3. RESULT

3. 1. *Scenedesmus dimorphus*

The classification of *Scenedesmus dimorphus* is included in the phylum Chlorophyta, class Chlorophyceae, and genus Scenedesmus [8]. Scenedesmus is a microalga that generally lives in freshwater, measuring 8 - 12 micrometers, has no flagella, is unicellular, has a colony of 4 cells, is cylindrical in shape, and is green in color. There are spines or horns at the end of the colony. Colonies can consist of 2, 4, 8, or 18, where the cells are arranged in a zig-zag or linear manner. Cells can be crescent or ovoid in shape. Have different cell walls such as smooth, jagged, and thorny [9]. *Scenedesmus dimorphus* lives at pH 7 - 8, temperature 29 °C - 30 °C, and can reproduce asexually (split) and sexually (isogamy). *Scenedesmus dimorphus* contains 8 - 70% protein, 13 - 52% carbohydrates, and 6 - 40% lipids [10][11].

3. 2. *Scenedesmus* sp. culture

Growth of *Scenedesmus* sp. consists of several phases namely lag (adaptation state, no cell division), exponential/log phase (cell division begins to increase intensively, the end of this phase is best for harvesting), growth rate decrease phase (cell division decreases), stationary phase (reproduction rate and death in the same amount), and the death phase (death rate is greater than growth rate) where the curve of microalgae growth can be seen in the sigmoid curve Figure 1 [12]. In the growth and development of *Scenedesmus dimorphus* microalgae, there are internal and external factors that influence it. External factors such as pH, temperature, carbon dioxide, nutrients, and toxic compounds can affect the growth performance of *Scenedesmus dimorphus* if not considered during cultivation.

The media that can be used to culture Scenedesmus vary, you can use synthetic PHM media, Basal Bold Medium, wastewater media, or even a combination of synthetic media and wastewater. Laboratory-scale *Scenedesmus dimorphus* is generally cultivated in closed-pond bioreactors or photobioreactor systems. While on a large scale production usually uses an open pond system. A bioreactor is a membrane reactor technology that provides a biological environment used to treat wastewater containing micropollutants [13]. A photobioreactor is a closed-system bioreactor used to culture microalgae in water waste with the help of a light source to obtain biomass through photosynthesis [14]. In other words, the photobioreactor has two benefits, able to culture microalgae and being able to treat wastewater.

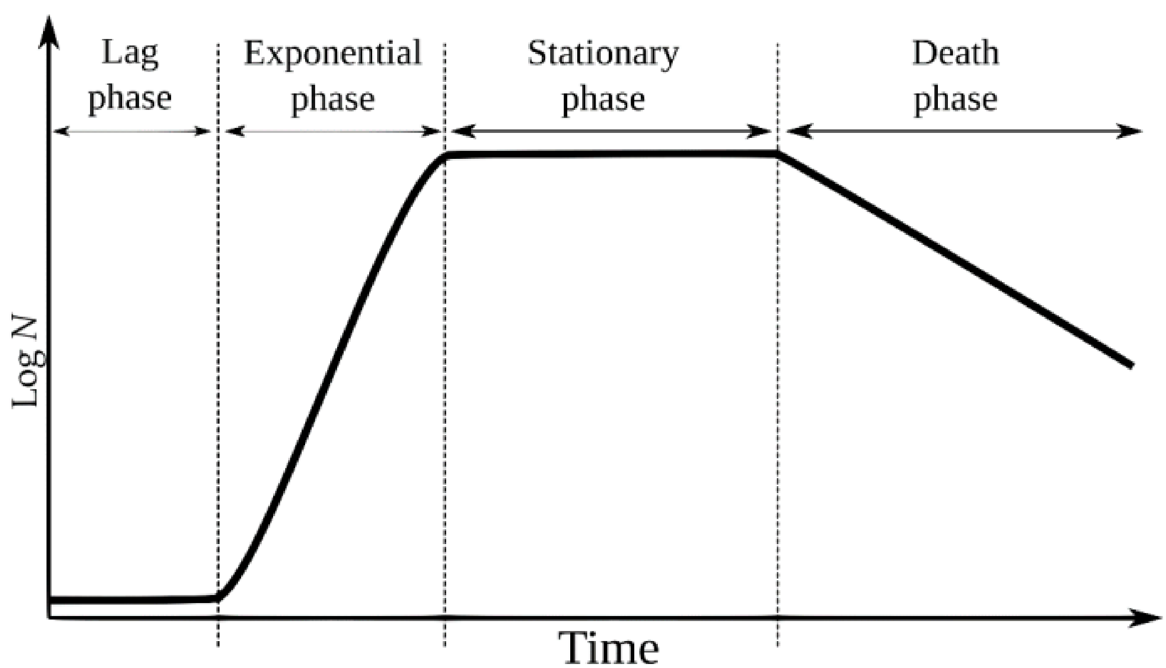

Figure 1. Microalge Growth Curve [12].

3. 3. POME waste

POME waste or Palm Liquid Waste, namely waste originating from palm oil processing that comes from condensate in the sterilization process, purification process water, hydro cyclone water, and factory washing water as a whole. Its characteristics are high viscosity, mud, high colloidal suspension, brownish color, and bad smell [15]. POME waste cannot be disposed of immediately because the nutrient content in it is still too high and can contaminate waters and if left without further processing can be dangerous and harm the environment. The characteristics of palm oil wastewater can be seen in Table 2.

Table 2. The Characteristics of Palm Oil Wastewater [16-18]

No.	Parameter	Unit	Range
1	*Biological Oxygen Demand* (BOD)	mg/L	10.250 - 65.714
2	*Chemical Oxygen Demand* (COD)	mg/L	15.000 - 102.696
3	*Total Suspended Solid* (TSS)	mg/L	5.000 - 54.000
4	*Total Solid* (TS)	mg/L	40.500 - 72.058
5	Oil dan Grease	mg/L	130 - 18.000
6	NH_3-N	mg/L	35 - 103
7	Total-N	mg/L	750 - 770
8	Temperature	℃	80 - 90
9	pH	-	3,4 - 5,2

With a lot of nutrient content in pome waste, it can be utilized by microalgae, especially *Scenedesmus dimorphus* as feed for breeding and growth so that cultivation does not require a lot of synthetic media and can reduce culture costs. This can also be a solution to reduce the level of pome waste so that it meets the nutritional content standards of pome waste and is not dangerous if disposed of into the waters. The standard discharge limits for pome wastewater can be used to determine the levels of pome wastewater that are safe to be disposed of and do not pollute the waters as shown in Table 3.

Table 3. Pome Wastewater Quality Standard [19]

Parameter	Unit	Maximum Range
BOD	mg/L	100
COD	mg/L	350
TS	mg/L	5.000
TSS	mg/L	400
Oil and Grease	mg/L	50
NH_3-N	mg/L	20
pH	-	5 - 9

Temperature	°C	45
Maximum Waste Discharge	m^3 / tons of production	2,5

3. 4. Previous research

Several researchers have conducted research on the culture of *Scenedesmus* sp. with the main media of pome waste and different treatments with different Scenedesmus growth results, as well as the effectiveness of different nutrient absorption. The following results from various studies can be seen in Table 4.

Table 4. The Productivity of *Scenedesmus* sp. With Various Composition and Waste Treatment

No	Type of *Scenedesmus*	Treatment	Result	Source
1.	*Scenedesmus dimorphus*	Landfill leachate was treated with NaClO, and diluted 5%, 10% and 15%.	The best results were with a dilution rate of 10%, nutrient removal reached 0,266 g/L in 10 days. The chemical oxygen requirement, ammonia nitrogen, total nitrate, and total phosphorus removal reached 81%, 80,1%, 72,1%, and 86%.	[20]
2.	*Scenedesmus dimorphus*	The use of anaerobic POME media.	Producing biomass ranges from 0,26 – 3,4 mg/L in DCW (Dry Cell Weight). *Scenedesmus dimorphus* showed that the reduction of ammonia nitrogen (NH_3–N) was 99,5%, ammonium (NH_4) 91,5%, Phosphorus 98,8%, PO_3–4 97,2%, COD 86% and 86,5 %BOD.	[21]
3.	*Scenedesmus obliquus*	Cultured at various concentrations of POME, it is 20%, 60%, 80%, 90%, 95%, and 100% while the inoculum concentration was 30%.	Growth rate decreased with increasing POME concentration (0,3545, 0,1792, 0,1566, 0,1268, 0,1158, and 0,1008/day), dry weight of biomass increased with increasing POME concentration (0,40, 1 ,20, 1,30, 1,77 , 2,28 and 2,45 g/L), and the lipid content also tended to increase with increasing POME concentration (13,10%, 36,59%, 36,65%, 50,37%, 60,40% and 64,98%).	[22]

4.	*Scenedesmus dimorphus*	POME was pretreated using H_2SO_4 or HNO_3 with concentrations of 5, 10 and 30%. Pre-treated POME is added to Bold Basal Medium (BBM). The treatment was 100% BBM (control), 5% pome+BBM, 10% pome+BBM, and 30% pome+BBM.	The optimum POME concentration is 10%, resulting in a high fat content, a lipid content value of 22%. The fatty acids obtained from *S. dimorphus* are palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1).	[23]
5.	*Scenedesmus dimorphus*	Using Bold Basal Medium (BBM) with the addition of various concentrations of POME 5%, 10% and 30% which has been pretreated with H_2SO_4 and HNO_3.	The addition of 10% POME in each pre-treatment on BBM media showed the best results from *Scendesmus dimorphus* microalgae. Total lipid content of microalgae cultured in POME pre-treated media 10% H_2SO_4 and HNO_3 were 21,5% and 22%, respectively.	[24]
6.	*Scenedesmus* sp.	Control of pome without microalgae, pome with *Scenedesmus* sp. UKM9, and pome with *Chlorella* sp. UKM2	Biomass productivity 0,0204 g/L/day. Reducing 52,42% phosphate, 37% TN, 74,4% Total Organic Carbon and 39,8% COD	[25]
7.	*Scenedesmus* sp.	Anaerobic POME with photoperiod of 24 hours:0 hours, 16 hours:8 hours, and 12 hours:12 hours (light:dark)	COD reduction 31% for 24 hours: 0 hours, NH_4^+ 97%, PO_4^{3-} 82%. 16h:8h reduction of COD 43%, NH_4^+ 96%, PO_4^{3-} 91%. 12h:12h reduced COD 57%, NH_4^+ 100%, and PO_4^{3-} 61%.	[26]
8.	*Scenedesmus dimorphus*	Cultivation of *S.dimorphus* on POME waste for 20 days. Experimental container The ratio	Cell density $5{,}2\times10^4$ cells/mL, ammonia reduced 37 mg/L (88,5%), total nitrogen decreased 87,5%, very high total phosphorus decreased by 96,8%, COD decreased 79%, BOD decreased 71,5%.	[27]

		of length to width is 2:1 and the volume is approx. 500 L		
9.	*Scenedesmus dimorphus*	Anaerobic POME uses a coagulation process (rice starch and tapioca flour) and an adsorption process (PKS activated carbon).	Growth rate and density of *Scenedesmus dimorphus* using POME waste with synthetic media (growth rate: 0,2862/day and optimal cell density on the 16th day: 160×10^4 cells/mL) and anaerobic (growth rate: 0,1721/day and cell density optimally on the 20th day: 90×10^4 cells/mL).	[28]

4. DISCUSSION

4. 1. *Scenedesmus* species

After the results of previous studies were obtained, there were two species of *Scenedesmus* sp. namely *Scenedesmus dimorphus* and *Scenedesmus obliquus*. The use of pome waste media with various additional treatments such as the addition of Basal Bold type synthetic media, photoperiod adjustment, and pome waste composition adjustment. Adding research sources regarding *Scenedesmus dimorphus* culture in leachate media or water from exposure to rainwater in landfills as a comparison to obtain optimal results for the growth of *Scenedesmus dimorphus*.

Scenedesmus dimorphus is often used as a potential microalga for bioremediation because *S. dimorphus* can remove contaminants from wastewater [29], is easily cultivable, can grow in fresh water and brackish water habitat [30], can naturally grow in a continental climate, it can grow in a wide range of temperatures, from 10 °C - 30 °C [31], regarded as one of the most important microalgae for biofuel feedstock due to its ability to grow in various types of wastewater along with its high biomass, lipid, and carbohydrate content [32].

4. 2. Reduction of nutrients

The highest growth of *Scenedesmus dimorphus* biomass with 10 - 20% pome waste media occurred as much as 0,2862 g/L/day [28] and *Scenedesmus obliquus* as much as 0,3545 g/L/day [22], whereas with 10% leachate media a biomass growth of 0,266 g/L for 10 days was obtained. The lipid content of *Scenedesmus dimorphus* was found to be 22% with 10% pome waste media [23]. The higher the pome waste composition included, the higher the lipid content in *Scenedesmus* sp. microalgae, but the less the amount of biomass growth from *Scenedesmus* sp. microalgae [22].

If averaged, *Scenedesmus dimorphus* can reduce various compounds such as 66,2% total nitrogen, 88,5% ammonia, 89,8% ammonia nitrogen, 94,4% ammonium, 82,1% total phosphate, Chemical Oxygen Demand (COD) 62,2%, Biological Oxygen Demand (BOD)) 79,7%, Total Organic Carbon (TOC) 74,4%, and Phosphorus 98,8% with a composition of pome waste 10 - 20%.

4. 3. External factors

Exponential growth of microalgae usually takes 3,5 hours to get multiple biomass from the beginning. Although the growth of microalgaes differ each the characteristics of the species, most of them get double biomass within 24 hours [33]. Requires optimal medium conditions to get optimal microalgae growth such as temperature, pH, nutrition, and so on. The majority of microalgae can live at a temperature of 16 – 35 °C with an optimal temperature of 24 - 30 °C. *Scenedesmus dimorphus* will grow optimally at 26 °C [34]. The average pH for the cultivation of most microalgae species is between 7 - 9, with an average optimum pH ranging from 8,2 – 8,7. *Scenedesmus* sp. was observed to be more productive at pH 7 to pH 8 [35]. Nutrition must also be able to meet the macronutrient and micronutrient needs of the *Scendesmus dimorphus* microalgae to obtain optimal growth results. Synthetic medium that are generally used for *Scenedesmus dimorphus* culture medium are Basal Bold Medium (BBM), and Provasoli for Haematococcus Medium (PHM), while the waste medium commonly used for *Scenedesmus dimorphus* are tofu liquid waste, and pome waste [22].

So it can be interpreted that besides the content of pome waste, several factors can also help the growth of *Scenedesmus* sp. such as the addition of synthetic medium used, optimal irradiation, the amount of waste concentration used, as well as other types of waste that have sufficient nutrient content for *Scenedesmus dimorphus*.

5. CONCLUSIONS

Based on the results obtained from various literature regarding the use of *Scenedesmus dimorphus* as natural fish feed, *Scenedesmus* sp. also capable of being a phytoremediator in waste, especially POME waste by utilizing its nutritional content as a food ingredient. *Scenedesmus dimorphus* cultured in pome waste can reduce total nitrogen by 66,2%, 88,5% ammonia, 89,8% ammonia nitrogen, 94,4% ammonium, 82,1% total phosphate, Chemical Oxygen Demand (COD) 62,2%, Biological Oxygen Demand (BOD) 79,7%, Total Organic Carbon (TOC) 74,4%, and Phosphorus 98,8% By saving production costs for *Scenedesmus* sp. because synthetic culture media are expensive. *Scenedesmus dimorphus* will grow at a pH of 7 – 8, a temperature of 29 °C - 30 °C, and absorb nutrients optimally in pome waste media compositions ranging from 10 - 20%.

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