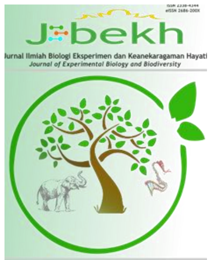
Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH)

Volume 10, Issue 2, November 2023



Analysis of Taurine Content in Microalgae *Tetraselmis* sp. Cultured at Different Salinities

[1]Eva Nur Afiah*, [1]Endang Linirin Widiastuti, [2]Henni Wijayanti Maharani

[1]Biology Department, Faculty of Mathematics and Natural Science, Universitas Lampung
[2]Fisheries Department, Faculty of Agriculture, Universitas Lampung
*Corresponding author: evae3671@gmail.com

Article History
Received: January 13, 2023
Accepted: October 3, 2023
Published: November 1, 2023

Abstract

Taurine is a non-essential sulfur-containing amino acid with the chemical formula C2H7NO3S. These compounds play an important role in maintaining the smooth running of various processes in the body. *Tetraselmis* sp. is one of the microalgae known to have a taurine biosynthetic pathway via the serine/sulfate pathway. The purpose of this study was to determine the effect of culture salinity on taurine content produced by microalgae *Tetraselmis* sp. which was dissolved using 96% ethanol. The samples obtained were then tested using UV-Vis spectrophotometry using standard synthetic taurine as a comparison. Based on the research results, the maximum absorption was obtained at a wavelength of 630 nm, and the regression equation $y = 0.001x + 0.033$ was obtained and had a correlation coefficient of $r = 1$. Based on the analysis results showed that all samples contained taurine. Samples at a salinity of 25 ppt contained an average of 2.82 ml/100g of taurine, while a culture salinity of 35 ppt contained an average of 4.03 ml/100g of taurine. From these data it is known that the highest taurine level was found at a culture salinity of 35 ppt, while the lowest taurine level was at a culture salinity of 25 ppt. This indicates that the higher the culture salinity, the higher the taurine content produced by the microalgae *Tetraselmis* sp. The phenomenon of increased taurine due to salinity stress is thought to be due to an increase in free amino acids in microalgae cells to produce metabolites that are used to defend themselves due to salinity stress. The function of taurine in this microalgae is thought to be an organic osmolyte in the cells of the *Tetraselmis* sp. microalgae.

Keywords: taurine, *Tetraselmis sp.*, osmotic stress.

How to Cite: E. N. Afiah, E. L. Widiastuti, and H. W. Maharani, "Analysis of Taurine Content in Microalgae *Tetraselmis* sp. Cultured at Different Salinities," *Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati (J-BEKH)*, vol. 10, no. 2. pp 53-60, 2023, doi:

INTRODUCTION

Osmotic stress is a physiological dysfunction caused by changes in the concentration of solutes surrounding a cell, leading to alterations in the movement of water across its cell membrane. This condition can result in cellular plasmolysis if the cell is unable to maintain its osmoregulation. Stress induced by high

ISSN 2338-4344
eISSN 2686-200X

NaCl concentrations has been studied in various marine algae. The presence of NaCl in the medium leads to an increase in cell size, loss of motility, and the production of a significant amount of extracellular mucus. The increase in cell size may reflect the accumulation of storage products such as starch or lipids, which are essential for membrane formation and provide components for cell wall synthesis. Meanwhile, the loss of motility and aggregation can result from the release of macromolecules. Additionally, taurine under salinity stress plays a series of roles in regulating calcium levels, as calcium plays a crucial role in salt tolerance, participating in the maintenance of membrane integrity and selective sodium transport [1].

According to the research by Tevatia et al. [2], *Tetraselmis* sp. demonstrates a taurine biosynthesis pathway, as evidenced by supplementation with serine and sulfate, which is capable of increasing the levels of cysteine, methionine (intermediates of the serine/sulfate pathway), and taurine. However, the levels of cysteine (precursor of the CDO/CSAD pathway), cysteine sulfinic acid, and hypotaurine remain unchanged.

Up to the present, there has been limited research on taurine from microalgae. Therefore, the author conducted this study to provide information on the taurine content in *Tetraselmis* sp. cultured under varying salinity conditions.

METHODS

This research was conducted from August to September 2019 in the Molecular Biology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung.

The equipment used in this research included a UV-Vis spectrophotometer, autoclave, thermometer, refractometer, pH meter, hemocytometer, centrifuge, aerator, microscope, oven, Erlenmeyer flask, beaker glass, measuring glass, test tubes, test tube rack, funnel, volumetric pipette, dropper pipette, filter paper, culture jars, heater, analytical balance, stirrer, and fluorescence lamp. The materials used in this research included *Tetraselmis* sp. microalgae culture, synthetic taurine, 96% ethanol, 70% alcohol, Conwy fertilizer, formalin, NaOH, calcium hypochlorite, NaCl, and distilled water.

Preparation of Microalgae Extract

This study utilized a culture derived from the *Tetraselmis* sp. microalgae parent obtained from the Lampung Aquaculture Development Center. *Tetraselmis* sp. was cultured for 7 days with varying salinity levels and three replications. Subsequently, microalgae were harvested by filtering the substrate from the settled microalgae with NaOH. The cultured solution was centrifuged, and the resulting pellet was collected to be placed in an oven for drying, yielding microalgae powder. Subsequently, 1 gram of the microalgae powder was weighed using an analytical balance and dissolved in 10 ml of 96% ethanol. The solution was then centrifuged once again.

Determination of Maximum Wavelength

The maximum wavelength was determined from the absorbance values of the taurine standard solution in the wavelength range of 400-800 nm using a UV-Vis spectrophotometer. A taurine standard solution with a concentration of 1 M was measured for absorbance using a UV-Vis spectrophotometer in the 400-800 nm wavelength range, with distilled water serving as the blank for absorption.

Preparation of Taurine Standard Curve

Taurine has a chemical formula of C2H7NO3S with a relative molecular mass (Mr) of 125. To prepare a 1 M taurine solution, 12.5 g of taurine powder is dissolved in 100 ml of distilled water and homogenized until completely dissolved. Subsequently, dilution is performed to create a 0.1 M taurine solution by taking 10 ml of the 1 M concentration solution and diluting it to 100 ml with distilled water. Each solution's absorbance values are measured at the maximum absorption wavelength, and a calibration curve is constructed to establish the relationship between taurine concentration (M) and its absorbance values.

Determination of Total Taurine Level

A volume of 1 ml of ethanol extract from *Tetraselmis* sp. microalgae is dissolved in 10 ml of distilled water until homogenized through three repetitions. The absorbance of each solution of ethanol extract from microalgae is measured using a UV-Vis spectrophotometer at the maximum absorption wavelength.

RESULTS AND DISCUSSION

Population density of microalgae *Tetraselmis* sp.

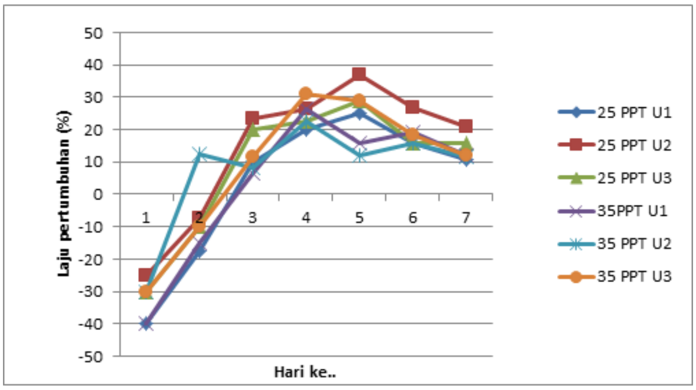
The highest population density of *Tetraselmis* sp. microalgae is found at a salinity of 25 ppt, with a population density reaching 225 x 104 cells/ml and an average cell count of 193.3 x 104 cells/ml. Meanwhile, at a salinity of 35 ppt, the population density is 185 x 104 cells/ml. However, among the three salinity levels, the culture does not exhibit a significant difference in population density.

Table 1. *Tetraselmis* sp. during 7 days of culture (10^4 cells/ml)

Treatment	Day						
Salinity	1	2	3	4	5	6	7
25 U1	$10x10^4$	$15x10^4$	$80x10^4$	$130x10^4$	$175x10^4$	$145x10^4$	$125x10^4$
25 U2	$15x10^4$	$25x10^4$	$110x10^4$	$145x10^4$	$225x10^4$	$200x10^4$	$185x10^4$
25 U3	$5x10^4$	$15x10^4$	$95x10^4$	$125x10^4$	$180x10^4$	$130x10^4$	$145x10^4$
Average	**$10x10^4$**	**$18.3x10^4$**	**$95x10^4$**	**$133.3x10^4$**	**$193.3x10^4$**	**$158.3x10^4$**	**$151.7x10^4$**
35 U1	$5x10^4$	$15x10^4$	$65x10^4$	$150x10^4$	$125x10^4$	$160x10^4$	$130x10^4$
35 U2	$5x10^4$	$10x10^4$	$60x10^4$	$125x10^4$	$95x10^4$	$130x10^4$	$115x10^4$
35 U3	$10x10^4$	$20x10^4$	$75x10^4$	16510^4	$185x10^4$	$150x10^4$	$125x10^4$
Average	**$6.7x10^4$**	**$15x10^4$**	**$66.7x10^4$**	**$146.7x10^4$**	**$135x10^4$**	**$147.7x10^4$**	**$123.3x10^4$**

Growth rate of microalgae *Tetraselmis* sp.

Figure 1 illustrates the growth rate of *Tetraselmis* sp. microalgae at culture salinities of 25 ppt and 35 ppt. From the graph, it is observed that the logarithmic growth phase at a salinity of 25 ppt begins from day 2 to day 5 of the culture, with growth rates reaching 25% in replication 1, 37% in replication 2, and 29% in replication 3. Subsequently, on days 6 and 7, *Tetraselmis* sp. microalgae undergo a stationary phase until the death phase the following day. At a salinity of 35 ppt, the logarithmic phase occurs from day 2 to day 4 of the culture, with growth rates reaching 26.2% in replication 1, 22.5% in replication 2, and 31.2% in replication 3



ISSN 2338-4344
eISSN 2686-200X

Figure 1. Growth rate of *Tetraselmis* sp. microalgae for 7 days.

Taurine standard curve

In the creation of this taurine standard curve, concentrations of 0.1 M and 1 M were utilized and analyzed using a spectrophotometer at a wavelength of 630 nm.

Linearity test

Table 2. Taurine standard linearity test

Concentration (M)	Replication	Absorbance	Average
0.1	1	0.034	0.034
	2	0.034	
	3	0.034	
1	1	0.034	0.035
	2	0.035	
	3	0.035	
Slop (b)		0.0339	
Axis intercept (a)		0.0011	
Coefficient of correlation (r)			

Then obtained the regression equation

y = a + bxy = 0.0011x+0.0339

r = 1

Taurine Assay Results with UV-Vis Spectrophotometer

Table 3. Taurine levels in *Tetraselmis* sp. at 25 ppt salinity (mg/100g).

Sample	Absorbance	Average Absorbance	Radar Total (mg/100g)	Average Radar (mg/100g)	Taurine Total (mg/100g)
25 ppt U1	0.035	0.036	1	2.52	2.82
	0.037		2.82		
	0.038		3.73		
25 ppt U2	0.036	0.037	1.91	3.12	
	0.037		2.82		
	0.039		4.64		
25 ppt U3	0.037	0.037	2.82	2.82	
	0.037		2.82		
	0.037		2.82		

The results of testing using a UV-Vis spectrophotometer on samples with a culture salinity of 25 ppt with 3 replicates and 3 replicates obtained average taurine levels in the first replicate of 2.52 mg/100g, 3.12 mg/100g in the second replicate and 2.82 mg/100g in the third replicate. With a total taurine content of 2.82 mg/100g.

Table 4. Taurine content in *Tetraselmis* sp. at 35 ppt salinity (mg/100g)

Sample	Absorbance	Average Absorbance	Radar Total (mg/100g)	Average Radar (mg/100g)	Taurine total (mg/100g)
	0.037		2.82		
35 ppt U1	0.036	0.036	1.91	2.21`	
	0.036		1.91		
	0.036		1.91		
35 ppt U2	0.040	0.038	5.55	3.43	4.03
	0.037		2.82		
	0.042		7.36		
35 ppt U3	0.040	0.041	5.55	6.45	
	0.041		6.42		

The table above shows the salinity of microalgae culture at 35 ppt salinity with 3 replicates and 3 replicates. From the data above, the average total taurine in replicate 1 was 2.21 mg/100g, replicate 2 was 3.43 mg/100g, and 6.45 mg/100g in replicate 3. With a total total taurine of 4.03mg/100g at 35 ppt salinity.

Discussions

Exposing *Tetraselmis* sp. microalgae to osmotic stress in the form of salinity for 7 days affects the population density and growth of the microalgae.

Based on the microalgae population density data, the highest population of microalgae is observed at a culture salinity of 25 ppt, while the lowest population is found at a salinity of 35 ppt. This aligns with the statement by [3], who explains that each microalga has specific self-protection mechanisms to maintain osmotic pressure within its cells. Direct changes in salinity can lead to alterations in osmotic pressure within the cell, disrupting cell activities. Additionally, salinity fluctuations can affect cytoplasmic pH and decrease enzyme activity within the cell.

According to [4], *Tetraselmis* sp. thrives optimally within the salinity range of 25-35 ppt. Most microalgae can tolerate environmental changes such as salinity variations within a wide range. Additionally, many microalgae can exhibit robust growth even at slightly lower salinity levels than those found in their natural habitats [5]. The variations in salinity levels also lead to changes in osmoregulation mechanisms (osmotic pressure), thereby inhibiting the development of vegetative cells and influencing the population density of microalgae.

The observations of *Tetraselmis* sp. microalgae growth under varied salinity levels over 7 days revealed diverse outcomes. This is likely due to the imposition of stress affecting the tolerance levels of *Tetraselmis* sp. microalgae to its growth environment. The highest average specific growth rates were recorded on the third and fifth days, attributed to the abundant nutrients in the culture medium, facilitating optimal microalgae growth.

The imposition of stress on *Tetraselmis* sp.

microalgae resulted in a lag growth phase lasting only one day, specifically on the first day. Pujiono [6] indicates that *Tetraselmis* sp. microalgae undergoes an adaptation phase ranging from the first day to the third day. Additionally, several factors influence the adaptation phase, including cell age, cell size, and the condition of the culture medium. If the cells are in a medium with suboptimal nutrient sources, microalgae will experience a more prolonged adaptation phase.

The determination of taurine levels in *Tetraselmis* sp. microalgae in this study was conducted using the UV-Vis spectrophotometer method.

From the data, the culture salinity with the highest taurine content is at 35 ppt, with a taurine concentration ranging around 4.03 mg/100 g of extract. This is due to an increase in the level of free proteins in microalgal cells as a defensive mechanism, affecting the production of taurine compounds. It is presumed that taurine plays a role as an organic osmolyte to protect the cells from osmotic stress. Meanwhile, the lowest taurine content is found in the 25 ppt culture salinity, with a concentration of approximately 2.82 mg/100 g of extract. This aligns with the initial hypothesis that stated taurine levels would increase with the addition of salt (salinity) in the culture medium.

Taurine is a non-essential sulfur-containing amino acid, but it does not belong to the protein group as it lacks the carboxyl group (-COOH) needed to form peptide bonds. A small portion of taurine forms di- or tripeptides with low molecular weights [7]. The taurine molecule consists of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) atoms, with the molecular formula C2H7NO3S. This compound plays a crucial role in maintaining the smooth functioning of various processes in the human and animal bodies. More than 50% of free amino acids in the heart are comprised of taurine. Taurine itself has a positive effect on the contraction of heart muscles, achieved through the regulation of calcium ion levels within the cells. The compound also assists in the movement of sodium, potassium, calcium, and magnesium ions in and out of cells, playing a role in the transmission of nerve impulses. Consequently, when there is stimulation from the Central Nervous System (CNS), it is then relayed to effector cells [8].

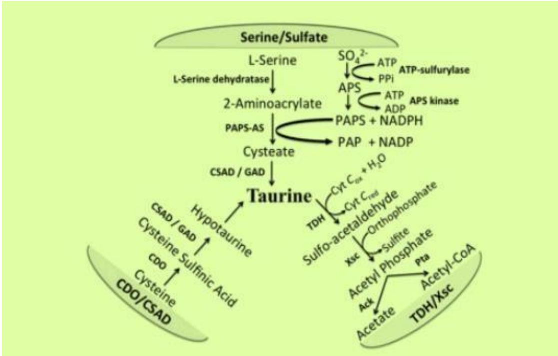


Figure 2. Taurine biosynthesis pathway[2].

In algae, taurine is synthesized through the serine/sulfate pathway. This pathway utilizes organic sulfate as the carbon backbone of L-serine. L-serine is converted into 2-aminoacrylate by the enzyme L-serine dehydratase. Subsequently, it is further converted into cysteate by PAPS-AS (3’-phosphoadenylyl sulfate: 2-aminocrylate C-sulfotransferase). Cysteate undergoes decarboxylation with the assistance of the CSAD/GAD enzyme, resulting in the formation of taurine [9].

There are two biosynthetic pathways for taurine from the precursor cysteine. In the first pathway, cysteine is converted into hypotaurine, which then undergoes dehydrogenation to form taurine. In the second pathway, cysteine is converted into L-cysteate, which is subsequently decarboxylated to taurine [10]. The first pathway is the primary biosynthetic route for taurine in many mammals, such as rabbits and rats. The second pathway is an alternative route that has been found in dogs [8].

One type of microalgae that possesses the taurine biosynthetic pathway is *Tetraselmis* sp. This is evident through the supplementation of serine or sulfate, which increases the levels of cysteine, methionine (intermediate of the serine/sulfate pathway), and taurine. However, the levels of cysteine (precursor of the CDO/CSAD pathway), cysteine sulfinic acid, and hypotaurine (intermediate of the CDO/CSAD pathway) remain unchanged. Additionally, an increase in the salinity of the culture can also elevate taurine levels, suggesting that the role of taurine in this microalgae is associated with being an organic osmolyte capable of protecting cells from osmotic stress [2].

The increase in taurine due to salinity stress is suspected to be a result of elevated levels of free amino acids when microalgae are under stress conditions. During such stress, microalgal cells actively produce various metabolites and defense systems to survive. Examples of these compounds include osmoprotectants such as proline, glycine betaine, and mannitol, which act as compounds tolerant to drought and salinity stress [11].

Stress induced by high concentrations of NaCl in the medium has been extensively studied in various marine algae. The presence of NaCl in the medium leads to an increase in cell size, loss of motility, and the production of a significant amount of extracellular mucus. The increase in cell size may reflect the accumulation of stored products such as starch or lipids, which are necessary for membrane formation and enhance components for cell wall formation. On the other hand, the loss of motility and aggregation can result from the release of macromolecules. Meanwhile, taurine under salinity stress plays a series of roles in regulating calcium levels. Calcium, being crucial for salt resistance, participates in maintaining membrane integrity and selective sodium transport [1].

CONCLUSION

Some of the conclusions obtained from this research are as follows, giving osmotic stress in the form of salinity affects the population density of *Tetraselmis* sp. microalgae cells. In this study, the salinity stress given was 25 ppt and 35 ppt. based on the results obtained, the highest population density was found in 25 ppt culture salinity with an average cell density of 193.3 x 10^4 cells/ml. While at 35 ppt culture salinity has the highest average number of cells of 147.7 x 10^4 cells/ml. Salinity stress affects the cell growth rateof microalgae *Tetraselmis* sp. At a culture salinity of 25 ppt, the growth rate in the highest logarithmic phase reached 25% in the 1st replicate, 37% in the 2nd replicate, and 29% in the 3rd replicate. Salinity stress also affects the taurine levels produced by microalgae *Tetraselmis* sp. Taurine levels in microalgae *Tetraselmis* sp.at 25 ppt culture salinity amounted to 2.82 mg/100g. While the taurine content of *Tetraselmis sp*. at 35 ppt culture salinity amounted to 4.03 mg/100g. This is thought to occur because taurine in microalgae *Tetraselmis* sp. functions as an organic osmolyte that can protect cells from osmotic stress..

ACKNOWLEDGMENTS

Thanks to LPPM University of Lampung for the grant given to carry out this research. Thanks to the supervisors who have guided the course of this research. Thanks to the laboratory assistants at the Microbiology Laboratory and Botany Laboratory of the Faculty of Mathematics and Natural Sciences, University of Lampung, who were willing to help provide tools and materials during the research. Thanks are also given to teammates for their help and support during the research work.

REFERENCES

[1] D. W. Rains, "Salt Transport by Plants in Relation to Salinity," *Annu Rev Plant Physiol*, vol. 23, no. 1, 2003, Accessed: Nov. 23, 2023. [Online]. Available:

https://www.researchgate.net/publication/234836622_Salt_Transport_by_Plants_in_Relation_to_Salinity

[2] R. Tevatia *et al.*, "The Taurine Biosynthetic Pathway of Microalgae," *Algal Res*, vol. 9, 2015.

[3] M. A. Borowitzka and L. J. Borowitzka, *Microalgal Biotechnology*. Cambridge University Press, 1988. Accessed: Nov. 23, 2023. [Online]. Available: https://books.google.co.id/books/about/Micro_algal_Biotechnology.html?id=FpprQgAACAAJ&redir_esc=y

[4] I. Rostini, "Kultur Fitoplankton (*Chlorella* sp. dan *Tetraselmis chuii*) Pada Skala Laboratorium," *Jurnal UNPAD*, vol. 9, no. 1, 2007.

[5] P. Leavens and P. Sorgeloos, *Manual on the production and use of live food for aquaculture*. Rome: Organization of The United Nation, 1996. Accessed: Nov. 23, 2023. [Online]. Available: http://aquacultura.org/upload/files/pdf/library/fao/Manual%20on%20the%20Production%20and%20Use%20of%20Live%20Food%20for%20Aquaculture.pdf

[6] S. Meisita, T. Tugiyono, E. Rusyani, and S. Murwani, "Perbedaan Pertumbuhan dan Kandungan Gizi Tetraselmis sp. Isolat dari Lampung Mangrove Center pada Kultur Skala Semi Massal dengan Konsentrasi TSP Berbeda," *Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH*, vol. 5, no. 2, 2019.

[7] J. A. Sturman, "Taurine in Development," *J Nutr*, vol. 118, no. 10, 1988, Accessed: Nov. 23, 2023. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S0022316622167635

[8] N. E. R. Ismail, S. Suheryanto, W. J. B. Kustomo, and Harsono, *Efektifitas Taurin dalam Memperbaiki Kinerja Ketahanan Kerja*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Republik Indonesia, 2005.

[9] A. N. Shikov, O. N. Pozharitskaya, A. S. Krishtopina, and V. G. Makarov, "Naphthoquinone pigments from sea urchins: chemistry and pharmacology," *Phytochemistry Reviews*, vol. 17, no. 3. Springer Netherlands, pp. 509–534, Jun. 01, 2018. doi: 10.1007/s11101-018-9547-3.

[10] Balai Besar Pengembangan Budidaya Laut (BBPBL), *Modul Petunjuk Teknis Kultur Pakan Alami di Balai Besar Pengembangan Budidaya Laut Lampung.* Balai Besar Pengembangan Budidaya Laut (BBPBL) Lampung, 2001.

[11] T. D. Kurnia and D. Suprihati, "Proline sebagai penanda ketahanan kekeringan dan salinitas pada gandum," Solo: Fakultas Pertanian Universitas Sebelas Maret, Solo, 2013. Accessed: Nov. 23, 2023. [Online]. Available: https://www.researchgate.net/publication/303971617_PROLINE_SEBAGAI_PENANDA_KETAHANAN_KEKERINGAN_DAN_SALINITAS_PADA_GANDUM