วารสารวิทยาศาสตร์บูรพา ปีที่ 27 (ฉบับที่ 1) มกราคม – เมษายน พ.ศ. 2565
BURAPHA SCIENCE JOURNAL Volume 27 (No.1) January – April 2022


# การหาสภาวะที่เหมาะสมในการเพาะเลี้ยง *Paramecium caudatum* โดยใช้วัสดุเหลือใช้ทางการเกษตร

# Optimization of *Paramecium caudatum* Cultivation Condition Using Agricultural Wastes

ทรงกลด ใบยา[1] บงกช บุญบูรพงศ์[2] และ สุรศักดิ์ ละลอกน้ำ[2*]
Songklod Baiya[1], Bongkoj Boonburapong[2] and Surasak Laloknam[2*]
[1] *ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ*
[2] *ภาควิชาวิทยาศาสตร์ทั่วไป คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ*
[1] *Department of Biology, Faculty of Sciences, Srinakharinwirot University*
[2] *Department of General Science, Faculty of Sciences, Srinakharinwirot University*

Received : 16 June 2021
Revised : 2 August 2021
Accepted : 26 August 2021

## บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อหาภาวะที่เหมาะสมในการเพาะเลี้ยง *Paramecium caudatum* โดยใช้วัสดุเหลือใช้ทางการเกษตรเป็นแหล่งคาร์บอนและไนโตรเจน โดยตรวจวัดปริมาณน้ำตาลรีดิวซ์และปริมาณโปรตีน พบว่า กากน้ำตาลมีปริมาณน้ำตาลรีดิวซ์สูงสุด รองลงมา คือ ฟางข้าว และกากถั่วเหลือง กากถั่วเหลืองมีปริมาณโปรตีนสูงสุด รองลงมา คือ กากน้ำตาลและฟางข้าว การศึกษาการเจริญเติบโตของ *P. caudatum* ในอาหารสูตรเดี่ยวที่มีความเข้มข้นของกากน้ำตาล กากถั่วเหลือง และ ฟางข้าว ความเข้มข้นร้อยละ 0.00 0.01 0.10 0.50 1.00 2.00 5.00 และ 10.00 โดยน้ำหนักต่อปริมาตร พบว่า กากน้ำตาลที่ ความเข้มข้นร้อยละ 0.10 โดยน้ำหนักต่อปริมาตร ทำให้ *P. caudatum* เจริญดีที่สุด กากถั่วเหลืองที่ความเข้มข้นร้อยละ 1.00 โดยน้ำหนักต่อปริมาตร ทำให้ *P. caudatum* เจริญดีที่สุด และ ฟางข้าวที่ความเข้มข้นร้อยละ 1.00 โดยน้ำหนักต่อปริมาตร ทำให้ *P. caudatum* เจริญดีที่สุด เมื่อทำการเตรียมอาหารสูตรผสมที่มีความเข้มข้นของกากน้ำตาล กากถั่วเหลือง และ ฟางข้าว ร้อยละ 0.10 1.00 และ 1.00 โดยน้ำหนักต่อปริมาตรและทำการศึกษาการเจริญของ *P. caudatum* เปรียบเทียบกับอาหารสูตรเดี่ยว พบว่า *P. caudatum* ที่เลี้ยงในอาหารสูตรผสมมีเจริญดีกว่าสูตรเดี่ยวอย่างมีนัยสำคัญ จากนั้นศึกษาผลของพีเอช 4-10 และอุณหภูมิ 10-40 °C ต่อการเจริญของ *P. caudatum* พบว่า ภาวะที่เหมาะสมในการเพาะเลี้ยง *P. caudatum* ในสูตรผสมที่ pH 6.0 และอุณหภูมิระหว่าง 25 และ 30 °C จากงานวิจัยนี้สรุปได้ว่าวัสดุเหลือใช้ทางการเกษตรสูตรเดี่ยวและสูตรผสมสามารถใช้เพาะเลี้ยง *P. caudatum* เพื่อนำไปใช้ประโยชน์ในด้านสิ่งแวดล้อมและอาหารสัตว์ต่อไป

**คำสำคัญ** : พารามีเซียม ; อาหารเพาะเชื้อ ; วัสดุเหลือใช้ทางการเกษตร

## Abstract

This research aimed to optimize the agricultural waste culture medium and conditions of *Paramecium caudatum*. Agricultural wastes, sugarcane molasses (SGCM), soybean meal (SB), and rice straw (RS) were determined total reducing sugar and total protein contents. The results showed that SGCM contains the highest total reducing sugar content and following SB and RS. SB had the highest total protein content and following SGCM and RS. The impact of the agricultural waste culture medium on *P. caudatum* growth was conducted by varying concentrations of each agricultural wastes, 0.00, 0.01 0.10, 0.50, 1.00, 2.00, 5.00, and 10.00% (w/v). The optimal concentrations of each agricultural wastes are 0.1% (w/v) of SGCM, 1.0% (w/v) of SB, and 1.0% (w/v) of RS, respectively. The impact of the combination of agricultural wastes medium (0.1% (w/v) of SGCM, 1.0% (w/v) of SB, and 1.0% (w/v) of RS) on *P. caudatum* growth was investigated compared with each agricultural waste medium. The *P. caudatum* growth in a combination medium was significantly increased compared with a single medium. The effect of pH (pH 4-10) and temperature (10-40 °C) on *P. caudatum* growth were determined. The optimal cultivation condition of *P. caudatum* was cultivated in the combination of agricultural wastes medium with pH of 6.0 and temperature ranged between 25 and 30 °C. This finding indicated that this new modification agricultural wastes culture medium is suitable for *P. caudatum* growth used in ecotoxicological studies and animal feed production.

**Keywords :** *Paramecium* ; culture medium ; agricultural wastes

*Corresponding author. E-mail : surasak2515@hotmail.com

## Introduction

*Paramecium*, single-cell free-living protozoans, are members of the phylum Ciliophora that are widespread in the aquatic environment. *Paramecium* usually feeds on microorganisms such as bacteria, yeast, and algae as carbon and nitrogen sources to promote growth. It has been widely used as a model organism in classrooms and laboratories to study biological processes and bioindicator organisms to follow changes in their habitats and ecotoxicological studies by monitoring physiological changes, cell motility, and cell organization (Edmiston Jr *et al.,* 1985; Madoni, 2000; Hemmersbach *et al.,* 2001; Miyoshi *et al.,* 2003; Momayezi *et al.,* 2004; Takahashi *et al.,* 2005; Rouabhi *et al*., 2006; Venkateswara *et al.,* 2007; Mortuza *et al*., 2009; Amanchi 2010; Duncan *et al.,* 2011; Mansano *et al.,* 2016). Because this unicellular protist is viewed with the naked eye and also with the microscope. According to research published, several basic *Paramecium* metabolic parameters show similarity with higher animals (Zharkova *et al.,* 2021). Moreover, the advantages of using *Paramecium* in the experiments are cheapness, high sensitivity to stimulants, and short-duration experiments. Recently, the *Paramecium* genome database was published and this genetic information is a valuable resource for future studies (Arnaiz *et al*., 2020).

In recent years, agricultural wastes have become the main source of pollution in the world (Wang *et al*., 2016). According to the research, these agricultural wastes have a very high nutrition potential and can be used for various applications (Ibrahim, 2015). Agricultural wastes management is considered to be an important step in environmental protection and agricultural development. The simplest agricultural wastes management is recycling utilization such as animal feed, organic fertilizers, and soil amendment. Moreover, these agricultural wastes are utilized as culture medium components for many organisms and substrate for biochemicals, enzyme, and biofuels production (Suo *et al*., 2011; Muhammad *et al*., 2012; Amin *et al*., 2014; Alavijeh & Yaghmaei, 2016; Gabhane *et al*., 2014; Pryshliak & Tokarchuk, 2020)

In Thailand, a common culture medium in protozoa experiments used for studies of *Paramecium* is the rice straw medium with unknown concentration. To date, no report has yet been performed on the growth of *Paramecium* using the combination of agricultural wastes medium. In this research, we determined the best *Paramecium* agricultural waste culture medium and conditions for *Paramecium caudatum* isolated from the Saen Saep canal near Srinakarinwirot University. Because these agricultural wastes can be a viable source of organic nutrients such as carbon, nitrogen, minerals, and various valuable chemicals. Thus, combining different agricultural wastes is an approach to enhance such biomass (Mondal *et al*., 2017). The present study aimed to 1) determine the total reducing sugars and protein contents in agricultural wastes including sugarcane molasses (SGCM), soybean meal (SB), and rice straw (RS) and used as exogenous carbon and nitrogen sources for

*Paramecium*'s culture medium 2) optimized the *Paramecium*'s agricultural waste culture medium and 3) optimize the *Paramecium* cultivation conditions. Therefore, we predicted the new modification *Paramecium* cultivation medium using agricultural wastes could be used for isolation and biomass production.

## Methods

*Paramecium Culture Preparation*

Samples of water were taken from the littoral zone of three sampling points in the Saen Saep canal, one of Bangkok's major drainage arteries, near Srinakharinwirot University, Bangkok from March to August 2020 as shown in Figure 1. Water samples were screened for *Paramecium* within 24 hr after sampling under the microscope. *Paramecium* was maintained on 1% (w/v) Rice straw medium (modified medium from SSVT) and classified based on morphology under a compound microscope according to Charubhun & Charubhun (2007). 1% (w/v) Rice straw medium was used for *Paramecium* inoculum preparation. The inoculation cultures were grown at room temperature (25 °C). Mid-log phase cells of *Paramecium* were subjected to use as starter cultures for experimental tests.


| Sampling points | Site Description | Location coordinates | |
|---|---|---|---|
| | | Latitude | Longitude |
| Point 1 | Asok drainage | 13°44’51” N | 100°33’47” E |
| Point 2 | Prasan Mit Pier | 13°44’50” N | 100°33’56” E |
| Point 3 | Prasan Mit drainage | 13°44’49” N | 100°34’02” E |

*Figure 1* Description of the *Paramecium* sampling points and location coordinates at the Saen Saep canal from March to August 2020.

*Paramecium* sp. was isolated from the Saen Saep canal near Srinakarinwirot University, Bangkok (13°44’51” N 100°33’47” E, 13°44’50” N 100°33’56” E, and 13°44’49” N 100°34’02” E) and classified as based on morphology under a compound microscope. The cell body of *P. caudatum* is spindle-shaped 200-300 micrometers long, the front portion is rounded and tapering at the posterior to a blunt point as shown in Figure 2. The pellicle is uniformly covered with cilia and has a long oral groove and two star-shaped contractile vacuoles.

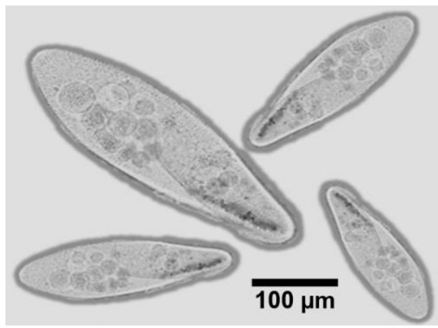


*Figure 2* Morphology of *Paramecium caudatum* isolated from Saen Saep canal. The scale bar is 100 micrometers.

*Preparation of agricultural waste stock solutions*

Agricultural wastes including SGCM, SB, and RS were obtained from Ayutthaya Province, Thailand. Sun-dried RS and SB were dried in a cabinet oven with air circulation at 60 °C for 48 h and ground to powder using a grinder. Ten grams of each agricultural wastes were weighed and extracted with 100 ml tap water. The mixtures were sterilized at 120 °C for 15 min and filtered using Whatman No. 1 filter paper. The supernatants were labeled as SGCM, SB, and RS stock solutions and subjected to determine total reducing sugars and protein contents according to the dinitrosalicialic acid (DNS) method (Miller, 1959) and the dye-binding method of Bradford (Bradford, 1976), respectively.

*Impact of the agricultural waste culture medium on Paramecium growth*

The stock solutions of each agricultural wastes were diluted to different concentrations (0.01, 0.05, 0.10, 0.50, 1.00, 2.00, 5.00 and 10.00% (w/v)) with tap water. Each agricultural waste culture medium was sterilized at 120 °C for 15 min. The initial density *Paramecium* level of 100 cells/mL was grown under various types of 200 mL of the agricultural waste medium at the different formational concentrations in 500 mL of conical flasks and left at room temperature. Cell growth was evaluated in triplicate each day for consecutive 30 days. 20 µL of each sample were taken with a micropipette and the numbers of live *P. caudatum* were counted under the compound microscope.

*Impact of the combination of agricultural waste culture medium on Paramecium growth*

The optimal concentrations of each agricultural waste culture medium were used to prepare the combination medium to test the impact of the combination of agricultural waste culture medium on *Paramecium* growth. The initial density *Paramecium* level of 100 cells/mL was grown in 200 mL of each treatment in 500 mL of conical flasks and left at room temperature. Cell growth was evaluated in triplicate each day for consecutive 30 days and investigated using the compound microscope.

*Effects of pH on Paramecium growth*

To study the effects of pH on *Paramecium* growth, the initial density *Paramecium* levels of 100 cells/mL cells was added to 200 ml of the combination of agricultural waste culture medium with different pH (pH 4-10) using 10 mM phosphate buffer in 500 mL of conical flasks and left at room temperature. Cell growth was evaluated in triplicate each day for consecutive 30 days. 20 μL of each sample were taken with a micropipette and the numbers of live *P. caudatum* were counted under the compound microscope.

*Effects of temperature on Paramecium growth*

To study the effects of temperature on *Paramecium* growth, cells were grown in the combination of agricultural waste culture medium at optimal pH value. The initial density *Paramecium* level of 100 cells/mL was grown in 200 mL of each treatment in 500 mL of conical flasks and incubated at 10, 15, 20, 25, 30, 35, and 40 °C. Cell growth was evaluated in triplicate each day for consecutive 30 days. 20 μL of each sample were taken with a micropipette and the numbers of live *P. caudatum* were counted under the compound microscope.

*Data analysis*

Data represent means ± SD for 3 independent experiments. Data analysis was conducted using one-way analysis of variance (ANOVA) with IBM SPSS Statistics for Windows V21.0 to test the effects of the culture media for each (Corp, 2012). Differences were considered significant at $p<0.05$. All experiments were carried out in triplicate.

## Results

*Determination of the total reducing sugars and protein contents in agricultural waste stock solutions*

The total reducing sugars and protein content of the agricultural wastes, including SGCM, SB, and RS, were determined as shown in Table 1. The results showed that the sugarcane molasses were enriched in reducing sugars, whereas soybean meal contained the highest amount of protein amount. The data showed that these agricultural wastes could be used as the carbon and nitrogen sources for the *Paramecium*'s growth.

***Table 1*** The total reducing sugars and protein contents of Agricultural waste stock solutions. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

| Agricultural waste stock solutions | Total reducing sugars content (mg/ml) | Total protein content (mg/ml) |
|---|---|---|
| 10%(w/v) Sugarcane molasses (SGCM) | 50.00±3.45 | 0.25±0.02 |
| 10%(w/v) Rice straws (RS) | 10.00±0.55 | 0.13±0.01 |
| 10%(w/v) Soybean meal (SB) | ND | 1.00±0.07 |

ND means that total reducing sugars content is undetectable through the DNS method

*Impact of the single agricultural waste culture medium on Paramecium growth*

The growth curves of the *Paramecium* cultured in each agricultural waste culture medium were shown in Figure 3. Cells grew exponentially and stopped 3-4 days after inoculation. The maximum cell density was obtained 4-5 days after inoculation. Cell density of *Paramecium* was increased in agricultural waste concentrations of 0.1% (w/v) sugarcane molasses (Figure 3A), 1.0% (w/v) soybean meal (Figure 3B) and 1.0% (w/v) rice straw (Figure 3C). High concentrations of three agricultural wastes inhibited *Paramecium* proliferation. Then, the decline phase seemed to start. The cell density of *Paramecium* in soybean meal medium was higher than in other culture media ($p$<0.05) (Figure 4).

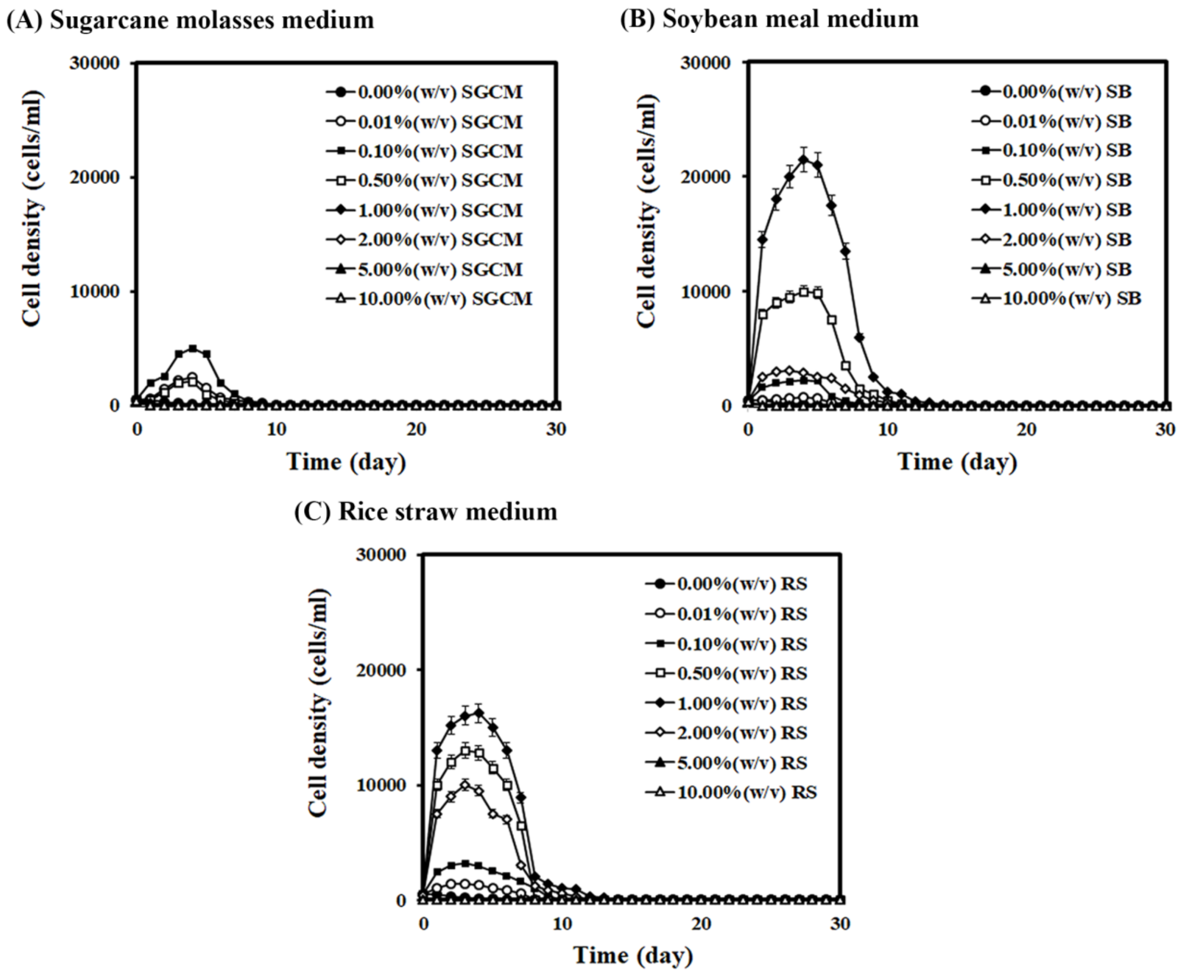


*Figure 3* The growth curves of *Paramecium* in each agricultural waste medium: (A) sugarcane molasses (SGCM), (B) soybean meal (SB), and (C) rice straw (RS) medium. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

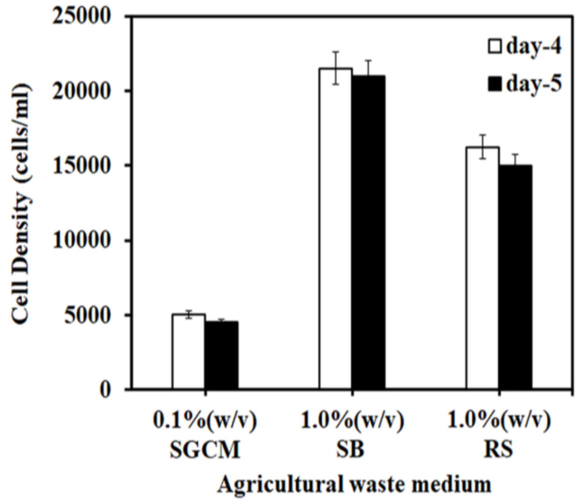


***Figure 4*** Comparison of *Paramecium* cell density under the optimal concentration of each medium at day4 and day5. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

*Impact of the combination of agricultural waste culture medium on Paramecium growth*

The optimal concentrations of SGCM (0.1% (w/v)), SB (1.0% (w/v)) and RS (1.0% (w/v)) was selected to prepare the combination medium. The growth curve of the *Paramecium* was shown in Figure 5. The results showed that the growth of the *Paramecium* in the combination medium was significantly increased ($p$<0.05) compared with other media. The maximum cell density was obtained 4 days after inoculation. The cell density of the *Paramecium* grown in the combination medium was significantly increased with 183, 1.69, 1.28, and 5.50 folds compared with tap water, 1.0% (w/v) rice straw medium, 1.0% (w/v) soybean meal medium, and 0.1% (w/v) sugarcane molasses medium, respectively. The combination medium supplies more nutrients than other media because the carbon and nitrogen sources directly affect biomass production.

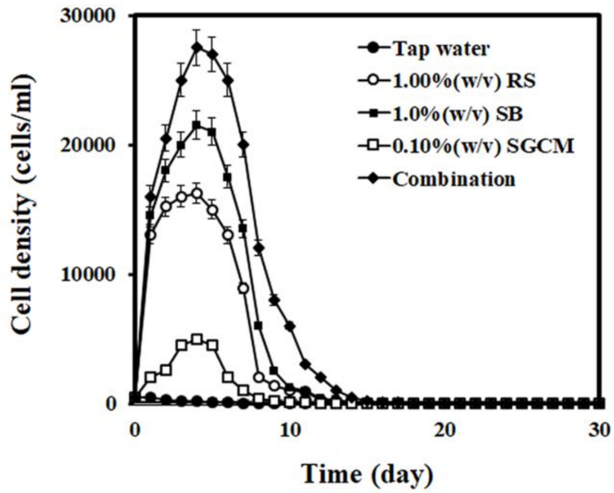


*Figure 5* Growth curve of *Paramecium* under combination medium to compare with other. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

*Effects of pH on Paramecium growth*

The effects of pH on *Paramecium* growth were studied using the combination medium with different pH values (pH 4-10) as shown in Figure 6. The results indicated that the cells thrived at a slightly acidic pH value of 6.0. The *Paramecium* growth rate was decreased to half of that under normal growth conditions at pH values of 4, 5, and 7. *Paramecium caudatum* mortality was 100% immediately when exposed to pH 9 and 10.

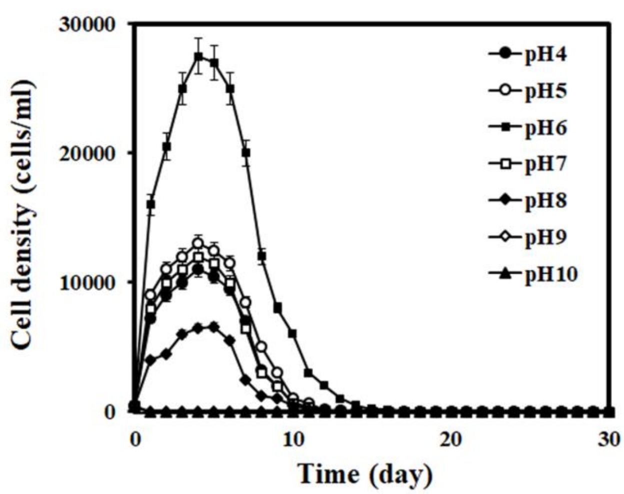


*Figure 6* Effects of pH on growth of *Paramecium* cells in combination medium. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

*Effects of temperature on Paramecium growth*

The effects of temperature on *Paramecium* growth were determined by culturing in the combination medium (pH 6.0) and placed in an incubator which was set to 10-40 °C as shown in Figure 7. The optimal temperature range for *Paramecium* growth was between 25-30 °C. Lower and higher temperature treatments decreased the growth rate of *Paramecium*.

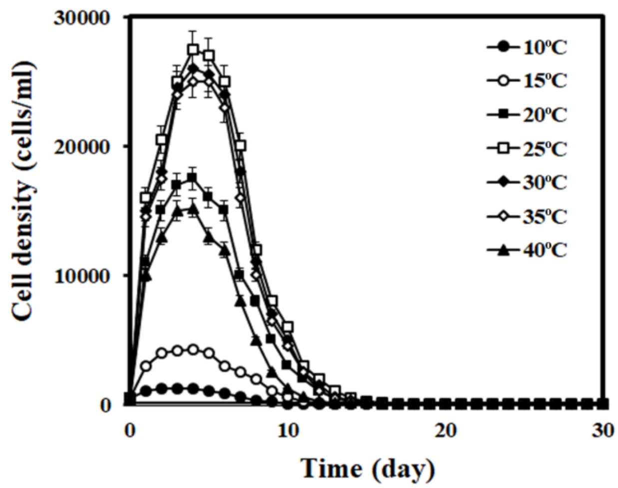


***Figure 7*** Effects of temperature on growth of *Paramecium* in combination medium, pH 6.0. Data represent means ± SD (n=3) for 3 independent experiments ($p$<0.05).

## Discussion

Agricultural wastes, sugarcane molasses, rice straw, and soybean meal, have been reported as the carbon sources for the growth of bacteria, green algae, and cyanobacteria (Piasecka *et al.,* 2017). The rice straw medium, the traditional *Paramecium* culture medium in Thailand, was used as control and the optimal concentration for *Paramecium* cultivation is at 1.0% (w/v) RS. Rice straw extracts are mainly composed of ash along with minor contents of protein, carbohydrate, and phenolic substances (Tangkhavanichi *et al.,* 2012; Satlewal *et al.,* 2018; Karimi *et al.,* 2014) that can be used as a carbon source for the growth of living organisms. Our results showed that the cell density of *Paramecium* in soybean meal medium was higher than other agricultural wastes medium. Because soybean meal, generally used as an organic fertilizer and common protein source for animal feed industry (approximately 44-48% crude protein), has a greater nutritional value in diets such as digestibility amino acids, minerals, and also phytonutrients (Ghorbani *et al.,* 2017; Baker *et al.,* 2011; Malhotra and Coupland, 2004). Moreover, several studies have reported that soybean meal supplementation has positive effects on animal growth (Ghorbani *et al.,* 2017; Baker *et al.,* 2011; Heikkinen *et al.,* 2006). Whereas, *Paramecium* grown in 0.1% (w/v) SGCM at Day4 showed a 3.25-fold reduction in cell density compared with control. Because sugarcane molasses mainly consists of fermentable sugars such as sucrose, glucose, fructose (approximately 50%), and non-sugar substances such as mineral and trace elements and bioactive compounds (Piasecka *et al.,* 2017; Cheah *et al.,* 2018). These sugar substances are the agents of both nutritional and osmotic effects. So, usage of high concentration sugarcane molasses is associated with numerous problems. Because it may induce osmotic stress and affects many metabolism pathways (Prajer, 2005).

Our results are in line with the previous studies such that the optimal pH and temperature range for the survival of *P. caudatum* is at pH 4.7-6.7 and 28-31 °C (Heydarnejad, 2008; Krenek, *et al.,* 2011; Krenek, *et al.,* 2012). Several studies showed that the optimum temperature of *P. caudatum* is ranging mostly between 15 °C and 25 °C. But *P. caudatum* inhabits the littoral zone of freshwater environments, which is affected by atmospheric temperature changes, especially during the summer season. Therefore, organisms of these habitats may exhibit higher optimum temperatures. *P. caudatum* mortality was 100% immediately when exposed to pH 4, 10.7, and 11.7 either in normoxic or hypoxic conditions. Moreover, pH and temperature are a major influence on solubility and bioavailability of heavy metals and others in sediments (Li *et al.,* 2013) and can affect the growth, survival, and morphological changes of *P. caudatum* (Weisse & Stadler, 2006; Weisse *et al.,* 2001; Lee, 1942; Duncan *et al.,* 2010; Krenek, *et al.,* 2012). Moreover, the pH and temperature tolerance of *P. caudatum* depend on the ranges of seasonal variation of pH and temperature at the *Paramecium* sampling points. Because the change of these parameters causes different variations including chemical, physicochemical, and biological processes.

## Conclusions

From our results, we can conclude that the application of agricultural wastes in culture medium provided positive results for the growth and biomass production of *Paramecium.* The combination medium (0.1% (w/v) SGCM, 1.0% (w/v) SB, and 1.0% (w/v) RS) is the new modification cultivation medium for isolation. These three can be used as alternative cheap carbon and nitrogen sources in laboratory conditions and animal feed production.

## Acknowledgments

This research was supported by grants from the Strategic Wisdom and Research Institute, Srinakharinwirot University for Routine to Research funding (128/2563). We are thankful to anonymous reviewers for their constructive feedback insightful suggestions and valuable comments on an earlier version of this paper in the International Research paper writing course organized by the Strategic Wisdom and Research Institute, Srinakharinwirot University. We are also thankful to the editor and reviewers, for their insightful suggestions and comments.

## References

Alavijeh, M.K., & Yaghmaei, S. (2016). Biochemical production of bioenergy from agricultural crops and residue in Iran. *Waste management*, *52,* 375-394.

Amanchi, N.R. (2010). A low cost microbiotest for screening behavioural and ecotoxicological responses of *Paramecium caudatum* and *Oxytricha fallax* to azadirachtin. *Advances in Applied Science Research*, *1*, 124-31.

Amin, M., Bhatti, H. N., Zuber, M., Bhatti, I. A., & Asgher, M. (2014). Potential use of agricultural wastes for the production of lipase by *Aspergillus melleus* under solid state fermentation. *J Anim Plant Sci*, *24,* 1430-7.

Arnaiz, O., Meyer, E., & Sperling, L. (2020). ParameciumDB 2019: integrating genomic data across the genus for functional and evolutionary biology. *Nucleic acids research*, *48*(D1), D599-D605.

Baker, K.M., Utterback, P.L., Parsons, C.M. & Stein H.H. (2011). Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. *Poultry Science*, *90*, 390-95.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*, 248-54.

Charubhun, B., & Charubhun, N. (2007). *Protozoa in Inland waters*. Bangkok: Kasetsart University press.

Cheah, W.Y., Show, P.L., Juan, J.C., Chang, J-S., & Ling, T.C. (2018). Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation. *Energy Conversion and Management*, *164*, 188-97.

Corp, IBM. (2012). IBM SPSS Statistics for Windows. New York.

Duncan, A.B., Fellous, S., Accot, R., Alart, M., Chantung Sobandi, K., Cosiaux, A., & Kaltz, O. (2010). Parasite - mediated protection against osmotic stress for *Paramecium caudatum* infected by *Holospora undulata* is host genotype specific. *FEMS microbiology ecology*, *74*, 353-60.

Duncan, A.B., Fellous, S., Quillery, E., & Kaltz, O. (2011). Adaptation of *Paramecium caudatum* to variable conditions of temperature stress. *Research in Microbiology*, *162*(9), 939-944.

Edmiston Jr, C.E., Goheen, M., Malaney, G.W., & Mills, W.L. (1985). Evaluation of carbamate toxicity: Acute toxicity in a culture of *Paramecium multimicronucleatum* upon exposure to aldicarb, carbaryl, and mexacarbate as measured by Warburg respirometry and acute plate assay. *Environmental research*, *36*, 338-50.

Gabhane, J., William, S. P., Gadhe, A., Rath, R., Vaidya, A. N., & Wate, S. (2014). Pretreatment of banana agricultural waste for bio-ethanol production: individual and interactive effects of acid and alkali pretreatments with autoclaving, microwave heating and ultrasonication. *Waste management*, *34*(2), 498-503.

Ghorbani Vaghei, R., Abolhasani, M.H., Ghorbani, R., & Matinfar, A. (2017). Production of soybean meal-based feed and its effect on growth performance of western white shrimp (*Litopenaeus vannamei*) in earthen pond. *Iranian Journal of Fisheries Sciences*, *17*, 578-86.

Heikkinen, J., Vielma, J., Kemiläinen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D., & Von Wright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *261*, 259-68.

Hemmersbach, R., Bromeis, B., Block, I., Bräucker, R., Krause, M., Freiberger, N., Stieber, C., & Wilczek, M. (2001). Paramecium—A model system for studying cellular graviperception. *Advances in Space Research*, *27*, 893-98.

Heydarnejad, M.S. (2008). Survival of *Paramecium caudatum* at various pH values and under normoxic and hypoxic conditions. *Pakistan journal of biological sciences*, *11*, 392.

Ibrahim, R.A. (2015). Tribological performance of polyester composites reinforced by agricultural wastes. *Tribology International*, *90*, 463-466.

Karimi, E., Mehrabanjoubani, P., Keshavarzian, M., Oskoueian, E., Jaafar, H.Z., & Abdolzadeh, A. (2014). Identification and quantification of phenolic and flavonoid components in straw and seed husk of some rice varieties (*Oryza sativa* L.) and their antioxidant properties. *Journal of the Science of Food and Agriculture*, *94*, 2324-30.

Krenek, S., Berendonk, T.U., & Petzoldt, T. (2011). Thermal performance curves of *Paramecium caudatum*: a model selection approach. *European Journal of Protistology*, *47*, 124-37.

Krenek, S., Petzoldt, T., & Berendonk, T.U. (2012). Coping with Temperature at the Warm Edge – Patterns of Thermal Adaptation in the Microbial Eukaryote *Paramecium caudatum*. *PLOS ONE*, *7*(3), e30598.

Lee, J.W. (1942). The effect of pH on food-vacuole formation in Paramecium. *Physiological Zoology*, *15*, 459-65.

Li, H., Shi, A., Li, M., & Zhang, X. (2013). Effect of pH, Temperature, Dissolved Oxygen, and Flow Rate of Overlying Water on Heavy Metals Release from Storm Sewer Sediments. *Journal of Chemistry*, 2013, 434012.

Madoni, P. (2000). The acute toxicity of nickel to freshwater ciliates. *Environmental Pollution*, *109*, 53-59.

Malhotra, A., & Coupland, J.N. (2004). The effect of surfactants on the solubility, zeta potential, and viscosity of soy protein isolates. *Food Hydrocolloids*, *18*, 101-08.

Mansano, A. S., Moreira, R. A., Pierozzi, M., Oliveira, T. M., Vieira, E. M., Rocha, O., & Regali-Seleghim, M. H. (2016). Effects of diuron and carbofuran pesticides in their pure and commercial forms on Paramecium caudatum: The use of protozoan in ecotoxicology. *Environmental pollution*, *213*, 160-172.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, *31*, 426-28.

Miyoshi, N., Kawano, T., Tanaka, M., Kadono, T., Kosaka, T., Kunimoto, M., Takahashi, T., & Hosoya, H. (2003). Use of *Paramecium* species in bioassays for environmental risk management: determination of IC50 values for water pollutants. *Journal of health science*, *49*, 429-35.

Momayezi, M., Albrecht, P., Plattner, H., & Schmidt, H.J. (2004). Temperature-induced change of variant surface antigen expression in Paramecium involves antigen release into the culture medium with considerable delay between transcription and surface expression. *Journal of membrane biology*, *200*, 15-23.

Mondal, M., Ghosh, A., Tiwari, O.N., Gayen, K., Das, P., Mandal, M.K., & Halder, G. (2017). Influence of carbon sources and light intensity on biomass and lipid production of *Chlorella sorokiniana* BTA 9031 isolated from coalfield under various nutritional modes. *Energy Conversion and Management*, *145*, 247-54.

Mortuza, M.G., Takahashi, T., Ueki, T., Kosaka, T. Michibata, H., & Hosoya, H. (2009). Comparison of hexavalent chromium bioaccumulation in five strains of paramecium, *Paramecium bursaria*. *Journal of Cell and Animal Biology*, *3,* 062-66.

Muhammad, I., Muhammad, N., Quratualain, S., & Shahjahan, B. (2012). Effect of medium composition on xylanase production by Bacillus subtilis using various agricultural wastes. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *12*(5), 561-565.

Piasecka, A., Krzemińska, I., & Tys, J. (2017). Enrichment of *Parachlorella kessleri* biomass with bioproducts: oil and protein by utilization of beet molasses. *Journal of Applied Phycology*, *29*, 1735-43.

Prajer, M. 2005. Glucose, sorbitol and insulin as exogenous agents that monitor the interautogamous interval (IAI) in *Paramecium primaurelia*. *Folia Biologica (Kraków)*, *53*(3-4), 215-21.

Pryshliak, N., & Tokarchuk, D. (2020). Socio-economic and environmental benefits of biofuel production development from agricultural waste in Ukraine. *Environmental & Socio-economic Studies*, *8*(1),18-27.

Rouabhi, R., Berrebbah, H., & Djebar, M.R. (2006). Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium* sp.. *African Journal of Biotechnology*, *5*, 45-48.

Satlewal, A., Agrawal, R., Bhagia, S., Das, P., & Ragauskas, A.J. (2018). Rice straw as a feedstock for biofuels: availability, recalcitrance, and chemical properties. *Biofuels, Bioproducts and Biorefining*, *12*, 83-107.

Suo, L.N., Sun, X.Y., & Li, S.Y. (2011). Use of organic agricultural wastes as growing media for the production of *Anthurium andraeanum* 'Pink Lady'. *The Journal of Horticultural Science and Biotechnology*, *86*(4), 366-370.

Takahashi, T., Yoshii, M., Kawano, T., Kosaka, T., & Hosoya, H. (2005). A new approach for the assessment of acrylamide toxicity using a green paramecium. *Toxicology in Vitro*, *19*, 99-105.

Tangkhavanich, B., Kobayashi, T., & Adachi, S. (2012). Properties of rice straw extract after subcritical water treatment. *Bioscience, biotechnology, and biochemistry*, 110983.

Venkateswara, R.J., Gunda, V.G., Srikanth, K., & Arepalli, S.K. (2007). Acute toxicity bioassay using *Paramecium caudatum*, a key member to study the effects of monocrotophos on swimming behaviour, morphology and reproduction. *Toxicological & Environmental Chemistry*, *89*, 307-17.

Wang, B., Dong, F., Chen, M., Zhu, J., Tan, J., Fu, X., Wang, Y., & Chen, S. (2016). Advances in recycling and utilization of agricultural wastes in China: Based on environmental risk, crucial pathways, influencing factors, policy mechanism. *Procedia environmental sciences*, *31*, 12-17.

Weisse, T., Karstens, N., Meyer, V.C.L., Janke, L., Lettner, S., & Teichgräber, K. (2001). Niche separation in common prostome freshwater ciliates: the effect of food and temperature. *Aquatic Microbial Ecology*, *26*, 167-79.

Weisse, T., & Stadler P. (2006). Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution. *Limnology and Oceanography*, *51*, 1708-15.

Zharkova, I.M., Safonova, Y.A., Grebenshchikov, A.V., Trufanova, Y.N., Kazimirova, Y.K., & Slepokurova, Y.I. (2021). Application of *Paramecium caudatum* for the assessment of energy costs for food raw materials and products digestibility. *In IOP Conference Series: Earth and Environmental Science*, *640*(6), 062006 1-8.