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EDITORS

Dr. Emi Norzehan Mohamad Mahbob
Dr. Saiyidah Nafisah Hashim
Ms. Lili Widarti Zainuddin
Ms. Nurul Nazwa Mohammad
Ms. Rozaina Saleh

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PREFACE

Welcome to the 2nd International Science, Engineering and Technology Colloquium 2025 (ISETC 2025)!

It is with great pleasure that we present this year's colloquium, a distinguished gathering of scholars, researchers, and professionals dedicated to the advancement of applied sciences. Held virtually on 9th July 2025, ISETC 2025 serves as a platform to highlight cutting-edge research, foster meaningful networking, encourage collaborations, and explore innovative solutions to global challenges.

This year's theme, "The Power of Scientific Innovation: Intelligent Solutions for a Sustainable World" highlights our shared goal to solve global challenges through applied science. It celebrates innovation as a key to building a more sustainable and resilient future. We're proud to feature keynote speakers and presenters who bring fresh insights, bold ideas, and breakthroughs shaping tomorrow's world.

We would like to extend our heartfelt appreciation to the Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, for their outstanding support and assistance throughout the planning and execution of the event. Their dedication has been instrumental to the success of this colloquium. We would like to sincerely thank all participants and researchers for their valuable contributions. We also appreciate the reviewers for their careful evaluations and helpful feedback. Your hard work and support mean a lot to us.

As editors, we are proud to present this collection of proceedings. It is an honour to bring together the interesting and meaningful work of our speakers and authors. We hope the ideas shared will lead to new discoveries, encourage more research, and help solve global challenges through science and innovation.

Warm regards,

Dr. Emi Norzehan Mohamad Mahbob
Dr. Saiyidah Nafisah Hashim
Ms. Lili Widarti Zainuddin
Ms. Nurul Nazwa Mohammad
Ms. Rozaina Saleh

ISETC 2025 Proceedings Editors

CO-CULTIVATION STRATEGIES AND THEIR IMPACT ON MICROALGAL BIOMASS COMPOSITION

Nazlina Haiza Mohd Yasin*, Nur Azmiza Baharin

Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

**Corresponding author: nazlinayasin@ukm.edu.my*

Abstract: Microalgae are microorganisms capable of accumulating metabolites such as carbohydrates, lipids, proteins, pigments and others throughout their growth. These metabolites have various applications in the biotechnology field. Cultivating microalgae in a suitable nutrient-rich medium, such as Bold's Basal Medium (BBM), supports optimal growth. Most past research on co-cultivation has focused on interactions between microalgae and fungi or microalgae and bacteria. However, studies on microalgae–microalgae co-cultivation remain limited, despite its potential to significantly enhance metabolite production. Therefore, the main objective in this study is to compare microalgae growth potential and biochemical compositions (lipids, carbohydrates, proteins and pigments) in monoculture and co-cultivation of microalgae. Two microalgae namely *Chlorella* sp. (UKM2) and *Scenedesmus* sp. (UKM9) were used. The results show that the maximum specific growth rate (μmax) for co-cultivation was higher at 0.355 day^{-1}, compared to 0.314 day^{-1} and 0.230 day^{-1} for UKM2 and UKM9 in monoculture, respectively. The results on the metabolites showed that lipid compounds were highly produced by monoculture UKM2 (2.981 g/L), followed by co-culture (2.698 g/L) and monoculture UKM9 (1.3 g/L). Next, in terms of carbohydrate content, no significant difference was found in all experiments. Meanwhile, for pigment profiles including total chlorophyll and carotenoids, UKM2 in monoculture exhibited the highest concentrations. The interaction between microalgae in co-culture resulted in the highest protein production, reaching 4.39 g/L, compared to monocultures of UKM2 and UKM9. In conclusion, co-cultivation resulted in high growth rate corresponding to the enhanced protein production; certain metabolites were more abundantly produced from monoculture conditions. Moving forward, optimizing protein extraction methods is essential to maximize yield from microalgae co-cultures. Additionally, exploring strains compatibility and refining co-cultivation parameters will be key in advancing the production of targeted metabolites in microalgae biotechnology.

Keywords: *Microalgae, co-cultivation, metabolites, protein, monoculture*

INTRODUCTION

Microalgae are microscopic organisms commonly found in freshwater or saltwater environments, with the capability to produce biochemical compounds suitable for biotechnology applications (Yousuf, 2020). While the role of single microalgae species in biomass growth and metabolites production is well established, studies on co-cultivation between different microalgae species are limited. Co-cultivation strategies involving two or more microalgae species have the potential to promote synergistic interactions, leading to enhanced production of valuable compounds. Several co-cultivation approaches have been reported, often involving different organisms including eukaryotic multicellular organisms, fungi or bacteria (Mehes et al., 2012). To date, the co-cultivation studies that are often used involve the co-cultivation of microalgae-bacteria (Mohd Udaiyappan et al. 2020) and microalgae-fungi (Wrede et al., 2014), especially in wastewater treatment and biofuels production. The co-cultivation of two or more microalgae species frequently resulted in stable growth with higher product of interest. For example, Tajido-Nunez et al (2020) proved that the co-cultivation of *Chlorella vulgaris* and *Tetradesmus obliquus* resulted in higher nutrient removal in recirculating aquaculture systems. Meanwhile, Gupta and Marchetti (2024) showed the biomass productivity increased by 12-174% in co-cultivation of *Scenedesmus* sp. with filamentous microalgae, *Tribonema* sp. The combination of both microalgae also showed better performance in removing nutrients for dairy wastewater treatment. Their studies also proved that higher carbohydrate and lipid can be obtained with suitable harvesting time. Nur et al. (2022) also proved that the consortium of four different microalgae species (*Dunaliella sp, Spirulina* sp., *Nannochloropsis* sp, and *Chaetoceros calciltrans*) promote growth and lipid accumulation under outdoor cultivation in palm oil mill effluent. In this study, the microalgae *Chlorella* sp. (UKM2) and *Scenedesmus* sp. (UKM9) were used, and their performances were compared under monoculture and co-culture conditions. Monoculture experiments were conducted to determine the baseline levels of biochemical compounds produced by each species individually, while the co-culture setup was used to investigate changes in growth rate and biochemical composition resulting from the interaction between the two species. The interactions between UKM2 and UKM9 using general culture medium (BBM) without altering any culture conditions were evaluated in this study. The changes of microalgae metabolism in co-cultivation may result in the positive or negative impact towards growth, lipids, carbohydrates, proteins and pigments accumulation in microalgae biomass.

MATERIALS AND METHODS

Chlorella sp. (UKM2) and *Scenedesmus* sp. (UKM9) were cultured in 1 L Duran bottle with BBM, supplied with light source of 3000 lux and aeration rate of 1 L/min, and were kept at 25 °C as described by Jasni et al. (2020). All experiments were conducted under sterile conditions. For the monoculture experiments, 30% (v/v) of either UKM2 and UKM9 was independently cultured in 70% (v/v) BBM. In the co-culture set up, 15% (v/v) of each UKM2 and UKM9 was combined and cultured in 70% (v/v) BBM. Microalgae growth was assessed by monitoring optical density at 690 nm and fitted to the Modified Logistic Model using MATLAB R2020a as described by Ni Aznan et al. (2024). Lipid content in microalgae biomass was determined using the chloroform-methanol extraction method, as described by Folch et al. (1957). Carbohydrate content in the microalgal biomass was quantified using the phenol-sulphuric acid method (Laurens et al., 2012), while protein concentration was measured using Bradford assay (Bradford 1976). Pigment profiles, including chlorophyll a, chlorophyll b and carotenoids, were assessed following the method by Parsons and Strikland (1963). Statistical analysis was performed using SPSS Version 21.0 (SPSS Inc., Chicago, IL), with significance determined at $p<0.05$.

RESULTS AND DISCUSSION

Growth Analysis of Monoculture and Co-culture

Both monoculture and co-culture of UKM2 and UKM9 were cultivated for 18 days, with daily measurement of optical density. The growth data were fitted to a modified logistic equation to determine maximum specific growth rate (μmax) and lag phase (λ). As shown in Table 1, the co-cultivation of UKM2 and UKM9 exhibited a higher μmax (0.3548 day^{-1}) compared to the monoculture of UKM2 (0.3138 day^{-1}) and UKM9 (0.2299 day^{-1}). The higher growth observed in the co-cultivation experiments suggests that both microalgae strains are capable of thriving in the same growth medium. In general, co-culture systems promote mutualistic interactions, where microorganisms benefit from the exchange of nutrients and metabolic by-products (Rashid et al., 2019). Due to this reason, the co-culture system exhibited faster doubling time. Furthermore, no lag phase (λ) was detected from either monoculture or co-culture conditions, indicating the rapid adaptability of the microalgae to the nutrient-rich freshwater medium, such as BBM.

Table 1. Growth analysis of monoculture and co-culture experiments

Culture	**Maximum specific growth rate (μmax) (day^{-1})**	**Doubling time (day)**
Chlorella sp. (UKM2)	0.3138	0.96
Scenedesmus sp. (UKM9)	0.2299	1.31
Co-culture of *Chlorella* sp. (UKM2) and *Scenedesmus* sp. (UKM9)	0.3548	0.85

Biochemical Analysis

Carbohydrates and proteins are considered primary metabolites in microalgae which was produced along microalgae growth. Meanwhile, lipids are often classified as secondary metabolites. Figure 1 shows the carbohydrates, proteins and lipids concentration in microalgal biomass from monoculture and co-culture experiments after 18 days of cultivation. It was noted that no significant difference was observed from carbohydrate and protein content in all experiments. Microalgal carbohydrates were often utilized as a feedstock for biofuel production through anaerobic digestion process to produce bioethanol, biobutanol, biohydrogen and methane (Silvello et al., 2022). However, the carbohydrate content in this study was relatively low (0.27 - 0.28 g/L) in all conditions.

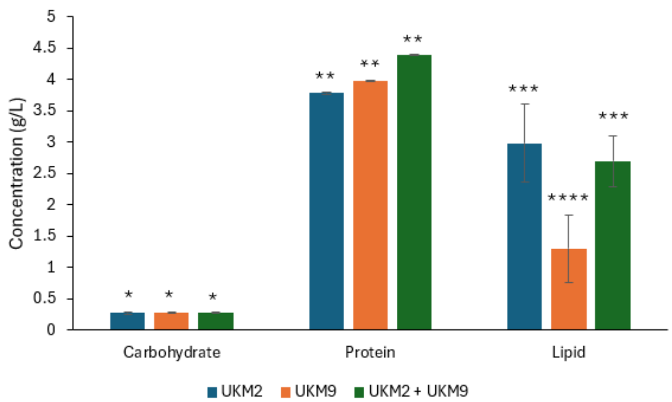

Figure 1. Carbohydrates, proteins and lipids concentration in microalgal biomass after 18 days of cultivation

Protein content was highest in the co-culture experiment corresponding to the higher growth under this condition. Proteins are essential components in human and animal nutritions. Microalgae are well known for its high protein content - up to 50-70% of their dry weight (Garcia-Encinas et al., 2025). This study highlights the opportunity of microalgae co-cultivation strategy for discovery of new distinct nutritional and health benefits from protein. Higher lipid content was observed for monoculture UKM2 (2.981 g/L), followed by co-culture (2.698 g/L) and monoculture UKM9 (1.3 g/L). Abiotic stress factors such as C/N ratios, light intensity and salinity might influence the accumulation of lipids in microalgae biomass (Liu et al., 2025). A two-stage strategy, initially producing biomass followed by nitrogen limitation in the second stage, is commonly used to increase lipid accumulation in microalgae biomass (Aziz et al., 2020). Moreover through this study, chlorophyll and carotenoid contents were higher in monoculture UKM2. The higher pigments in UKM2 exhibit higher photosynthetic metabolism through prolonged cultivation as reported by Nadzri et al. (2023).

CONCLUSIONS

The co-cultivation of UKM2 and UKM9 demonstrated their compatibility for growth in a shared medium, as evidenced by a higher maximum specific growth rate and shorter doubling time. Furthermore, co-culture conditions resulted in increased protein content in the microalgal biomass. However, other metabolites such as lipids and pigments were found in higher concentrations in the monoculture of UKM2. These findings suggest that further optimization strategies should be explored to enhance protein production in co-culture systems, potentially unlocking novel nutritional or functional benefits derived from microalgal proteins.

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SITI BASRIYAH SHAIK BAHARUDIN
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Setuju.

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