Investigating the effect of nutrients on cocultivation of *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous*

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Abstract

Microalgae *Haematococcus pluvialis* and yeast *Xanthophyllomyces dendrorhous* are two microorganisms known for their ability to produce astaxanthin, a valuable carotenoid with huge applications in various industries. This study aimed to investigate the optimal culture medium for the coculture of these two species, focusing on providing appropriate nutrients for their growth, particularly before *H. pluvialis* enters the red phase. Growth curves of *H. pluvialis* and *X. dendrorhous* were obtained in their standard media, Bold's Basal Medium (BBM) and Yeast Malt (YM) medium, respectively. Four candidate media were prepared based on BBM and YM constituents: BBM with glucose (BG), BBM with glucose and malt (BGM), BBM with glucose and peptone (BGP), and BBM with glucose and yeast extract (BGY). Cell numbers of both species were compared after 6 days of coculture incubation. Results showed that *H. pluvialis* exhibited the highest cell densities in BGM and BGY media, reaching 1.22×10^5 and 1.488×10^5 cells/mL, respectively. In contrast, the highest growth of *X. dendrorhous* was observed in BG medium, with a maximum cell density of 3.8×10^5 cells/mL. BGM demonstrated the balanced growth for both species, while BGY resulted in the highest cell concentration for *H. pluvialis* and controlled the growth of *X. dendrorhous*. The study highlights the importance of selecting a culture medium that balances the growth of both species and ensures controlled nutrient competition for a productive co-culture system. These findings contribute to the development of efficient co-cultivation strategies to enhance the cell growth rate and productivity.

Keywords: *Haematococcus pluvialis*, *Xanthophyllomyces dendrorhous*, Co-culture, Microalgae, Yeast, Culture medium

Introduction

Cultivating multiple microbial species under controlled conditions, known as microbial co-culture, can boost productivity and efficiency in various biotechnology applications. This approach promotes symbiotic relationships among microorganisms, improving performance compared to monoculture. Microbial co-cultures offer numerous advantages in industrial biotechnology, including increased yields, improved product quality, and the ability to utilize cheaper

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substrates (Bader et al., 2010).

Microalgae and yeast co-cultures have gained significant attention because of their potential to enhance biomass production and valuable compound synthesis. These interactions are characterized by a symbiotic relationship in which microalgae act as oxygen generators for yeast, while yeast provides CO_2 and organic acids to microalgae (Arora et al., 2019). This mutually beneficial arrangement can increase productivity and reduce cultivation costs. Studies have shown that co-culturing microalgae and yeast can substantially improve their biomass and lipid production. For instance, a co-culture of *Rhodotorula glutinis* and *Scenedesmus obliquus* in a photobioreactor demonstrated a 40-50 % increase in biomass and a 60-70 % increase in total lipids compared to single culture batches (Yen et al., 2015). Similarly, a coculture of *Chlorella pyrenoidosa* and *Rhodotorula glutinis* at a 3:1 ratio achieved maximum biomass concentration and lipid productivity, with total fatty acid productivity reaching twice that of monoculture (Liu et al., 2018). Microalgae-yeast coculture interactions offer promising opportunities for improving biomass and enhancing the synthesis of valuable compounds. These synergistic effects can be attributed to gas exchange, nutrient sharing, and gene expression alterations (Arora et al., 2019; Karitani et al., 2024; Xu et al., 2024). The co-culture system also demonstrated synergistic effects on dissolved oxygen and pH levels, which were mutually adjusted by the two organisms (Liu et al., 2018). Despite the numerous advantages of co-culturing, it also presents disadvantages that need to be addressed. A key issue is maintaining an optimal inoculum ratio, as deviations can reduce efficiency or harm the culture (Karitani et al., 2024). Furthermore, in some cases organic acids produced by microalgae may inhibit yeast growth (Naseema Rasheed et al., 2023).

Medium optimization plays a crucial role in enhancing the performance of microalgae and yeast co-cultures. The composition of the cultivation medium significantly affects growth, metabolite production, and the symbiotic relationship between microalgae and yeast (Xu et al., 2024; Qin et al., 2019). Studies have shown that optimizing the macronutrient composition, particularly carbon and nitrogen sources, can substantially improve co-culture performance. For instance, the addition of glucose with peptone in the co-culture of *Chlorella vulgaris* or *Chlorella sorokiniana* with a strain of *Saccharomyces cerevisiae* significantly enhanced biomass production of the co-culture by approximately 2-fold compared to that in monocultures (Xu et al., 2024). Furthermore, the addition of *Rhodotorula glutinis* yeast residual cell-free medium to *Chlorella vulgaris* cultures enhanced the production of specific fatty acids, such as nervonic acid and behenic acid, by 9 and 6 times, respectively (Ashtiani et al., 2021). By optimizing the nutrient composition, researchers can enhance biomass production and synthesis of valuable metabolites. This approach improves the economic feasibility of microbial metabolite production and opens up new possibilities for sustainable biofuel and biochemical production (Arora et al., 2019; Kitcha and Cheirsilp, 2014).

Haematococcus pluvialis is a freshwater unicellular green microalga that is widely recognized as one of the best natural sources of astaxanthin (Lee et al., 2016). It has the most significant capacity to accumulate astaxanthin among non-genetically modified organisms, with the ability to compose up to 4-7 % of its total dry weight (Gherabli et al., 2023; Mota et al., 2022). *H. pluvialis* undergoes a unique lifecycle, transitioning from green vegetative motile cells (green phase) to red hematocysts under stress conditions (red phase), during which it develops a thick, rigid three-layered cell wall (Kim et al., 2022).

Xanthophyllomyces dendrorhous, formerly known as *Phaffia rhodozyma*, is also capable of synthesizing astaxanthin (Domínguez-Bocanegra et al., 2007; Rodríguez-Sáiz et al., 2010). Although both organisms are considered major sources of natural astaxanthin production, their cultivation methods and astaxanthin yields vary. *H. pluvialis* can produce up to 9.2 mg/g of astaxanthin under optimal conditions, wherease *X. dendrorhous* has achieved yields of up to 9 mg/g through genetic engineering and optimized fermenter conditions (Domínguez-Bocanegra et al., 2007; Gassel et al., 2014). Both *H. pluvialis* and *X. dendrorhous* are of significant interest to the biotechnology industry for the production of natural astaxanthin, a valuable carotenoid used in aquaculture, nutraceuticals, cosmetics, and pharmaceuticals (Rodríguez-Sáiz et al., 2010; Mota et al., 2022). Ongoing research focuses on improving cultivation techniques, enhancing astaxanthin yields, and developing cost-effective production methods to compete with synthetic astaxanthin.

Co-cultivation of *X. dendrorhous* and *H. pluvialis* has been explored as an innovative approach to enhance astaxanthin production while simultaneously addressing environmental concerns by in situ carbon dioxide fixation. This method exploits the complementary metabolic abilities of these two astaxanthin-producing microorganisms (Domínguez-Bocanegra et al., 2007). In a mixed culture system, CO_2 generated by *X. dendrorhous* during fermentation was fixed by *H. pluvialis* through photosynthesis. Concurrently, the oxygen produced by *H. pluvialis* stimulates growth and astaxanthin production in *X. dendrorhous*. This symbiotic relationship resulted in significantly increased biomass and astaxanthin concentrations compared to pure cultures of either species (Dong and Zhao, 2004). This co-culture approach presents a novel method for improving the yield of high-value bio-products while simultaneously achieving in situ CO_2 fixation. By combining the strengths of both microorganisms, this strategy addresses the limitations of individual cultures and offers a more sustainable and efficient means of improving production (Dong and Zhao, 2004).

This study aims to investigate the optimal culture medium for cocultivation of the two species, *H. pluvialis* and *X. dendrorhous*. The focus was on understanding how nutrients influence the cell growth of each species during coculture, particularly before the microalga transitioning into the red phase.

Material and methods

Inoculum preparation

The yeast *X. dendrorhous* (IBRC-M30167) was purchased as a lyophilized ampoule from the Iranian Biological Resource Center. The yeast was activated by adding Yeast Malt (YM) medium (10 g/L glucose (Merck, Germany), 5 g/L peptone, 3 g/L yeast extract, and 3 g/L malt extract (Quelab, Canada) to the ampoule and grown on YM Petri dishes (Villegas-Méndez et al., 2021). A sample of grown yeast was transferred into a 250 mL Erlenmeyer flask filled with 50 mL of YM medium. The mixture was then incubated for 48 h at 23 °C with continuous stirring at 110 rpm in an orbital shaker incubator (KTG, Iran). This culture served as the inoculum for subsequent experiments.

Liquid *H. pluvialis* was purchased from the algae bank of the Research Institute for Industrial Biotechnology, Academic Center for Education, Culture, and Research (ACECR), Mashhad, Khorasan Razavi Province, Iran. It was grown in a Bold's Basal Medium (BBM) (Samhat et al., 2024) and incubated for 14 days at 23 °C under 12 h daily white illumination (30 µml m^{-2} s^{-1}).

Growth curve of X. dendrorhous

To determine the growth curve of the yeast over 5 days, experiments were conducted in duplicate using 250 mL Erlenmeyer flasks containing 50 mL YM medium. The flasks were inoculated with 10 % (v/v) of the prepared inoculum suspension. The cultures were incubated at 23 ± 1 °C with continuous shaking at 110 rpm. Growth was monitored by measuring the optical density at 600 nm using a spectrophotometer and counting cell numbers with a hemocytometer at 24-hour intervals for five consecutive days. The samples were appropriately diluted when necessary to ensure that the readings fell within the linear range of the spectrophotometer. The obtained OD values and cell numbers were plotted against time to generate the yeast growth curve, allowing for the identification of different growth phases.

Growth curve of H. pluvialis

Growth curve experiments were conducted to determine the growth characteristics of microalgae. Microalgal cultures were inoculated with 10 % (v/v) inoculum into sterile 250 mL Erlenmeyer flasks containing 50 mL BBM medium. The flasks were incubated in a shaker incubator at 23 ± 1 °C under 12 h of daily illumination (30 µmol m^{-2} s^{-1}) and agitated manually three times per day. Cell density was measured daily using a hemocytometer, and the optical density (OD) was recorded at 680 nm using a spectrophotometer (UNICO S-2150, USA). Samples were collected every 24 h for 15 days. The experiment was performed in duplicate, and the average values were used to plot growth curves.

Preparation of media

Four candidate media were prepared based on BBM (standard medium of *H. pluvialis*) and the constituents of YM (standard medium of *X. dendrorhous*). Since yeast growth requires a carbon source, all media contain glucose. The media used were as follows.

1-BBM with the addition of 10 g/L of Glucose (BG)

2-BBM with 10 g/L glucose and 3 g/L Malt (BGM)

3- BBM with 10 g/L glucose and 5 g/L Peptone (BGP)

4-BBM with 10 g/L glucose and 3 g/L Yeast

extract (BGY).

The cell numbers of *H. pluvialis* and *X. dendrorhous* after 6 days of coculture incubation were compared with their standard medium, BBM, and YM, respectively.

Incubation

The experiments were performed in 250 mL Erlenmeyer flasks containing 50 mL of the prepared media. All flasks were started at an initial cell concentration of 2×10^4 cells/mL for each species. The flasks were kept in an illuminated shaker incubator for 6 days at 23 °C under 12 h of daily illumination (30 µmol m^{-2} s^{-1}) and shaken at 110 rpm. The growth of both microalgae and yeast was measured at the end of six days of incubation by counting cell numbers under a microscope using a hemocytometer, and all experiments were performed in duplicate.

Statistical analysis

All data are expressed as mean ± standard error of the mean. Statistical analysis was performed using the Minitab software. A one–way ANOVA analysis was performed to detect whether the data were significantly different by using a p-value of $p < 0.05$.

Results and Discussion

Growth curve of X. dendrorhous

The *X. dendrorhous* culture in YM medium demonstrated a typical growth curve, as indicated by both cell concentration and OD measurements (Figure 1). However, the initial lag phase was too short to be observed. The exponential growth phase occurred within the first 48 hours, during which the cell concentration increased from 1.94×10^6 cells/mL at inoculation to 1.5×10^8 cells/mL at the end of day 2. Following this period, the culture entered a stationary phase, with cell numbers stabilizing around 1.6×10^8 cells/mL after 24 h, and a slight decrease was observed to 1.4×10^8 cells/mL by the end of day 5. Parallel optical density measurements at 600 nm revealed similar growth patterns. The OD values increased from 0.185 at inoculation to 2.213 after 2 days, corresponding to the exponential growth phase, and remained constant until the end of the measurements, indicating the stationary phase.

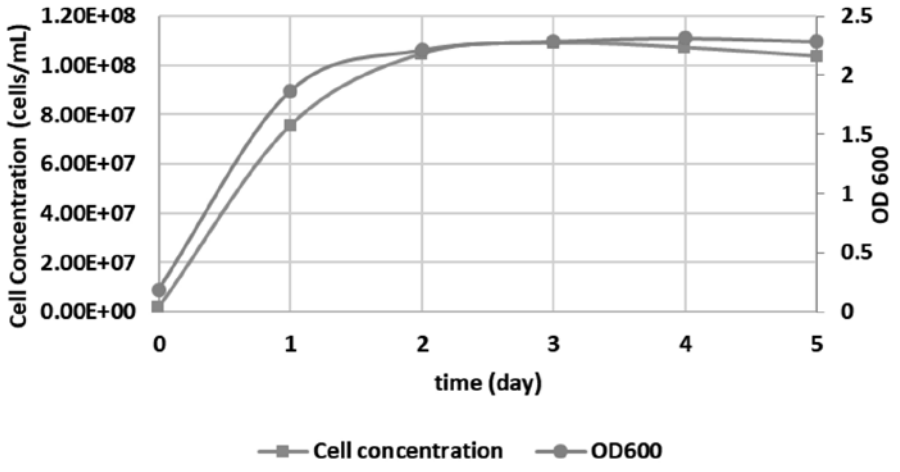

Fig. 1. Growth curve of *X. dendrorhous* in 5 days measured by cell concentration and optical density

X. dendrorhous typically exhibits a growth curve with distinct phases, including exponential and stationary phases (Castelblanco-Matiz et al., 2015). Recent studies have mainly focused on carotenoid biosynthesis, particularly astaxanthin, which is often induced during the late exponential growth phase (Lodato et al., 2007). Notably, growth curve and carotenoid production can be significantly influenced by various factors such as pH and nutrient sources, as well as availability. For example, the carbon source plays a crucial role in determining the timing of carotenoid biosynthesis. When grown on a non-fermentable carbon source, such as succinate, carotenoid production begins at the start of the growth cycle. It is approximately three times higher than when grown on glucose, a fermentable carbon source. In the presence of glucose, carotenoid production typically occurs at the end of the exponential phase (Wozniak et al., 2011).

The impact of nitrogen sources on carotenoid biosynthesis in *X. dendrorhous* is not thoroughly documented. However, the carbon to nitrogen (C/N) ratio in the growth medium significantly influences carotenoid production. As the C/N ratio increases, both cell growth and total astaxanthin accumulation increase, though the astaxanthin content per cell decreases. This indicates that the balance of carbon and nitrogen plays a crucial role in the process (Pan et al., 2017). Nitrogen sources such as peptone and yeast extract are known to enhance yeast metabolism and growth. For instance, in *Saccharomyces cerevisiae,* peptone increases biomass production (Da Cruz et al., 2002), while in *Penicillium canescens*, yeast extract is optimal for enzyme production, with the best results achieved by combining yeast extract and peptone (Bakri et al., 2003).

A comparison of the growth curves of the two species (Figures 1 and 2) shows that *X.*

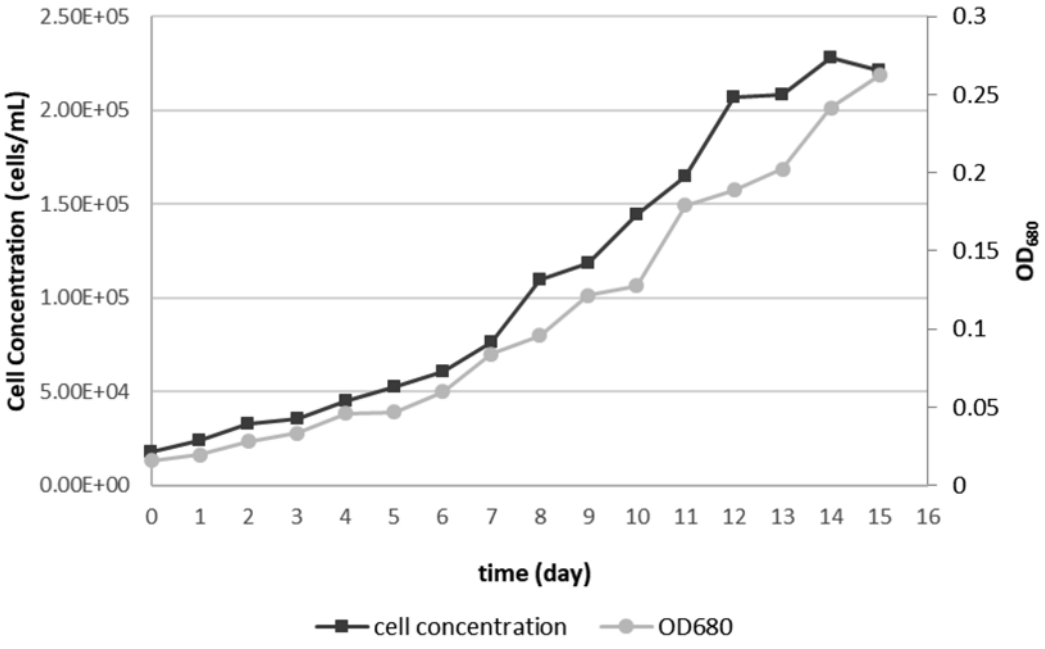

Fig. 2. Growth curve of *H. pluvialis* in 15 days measured by cell concentration and optical density

dendrorhous has a much faster growth rate than *H. pluvialis*, which necessitates the need to control the growth of yeast in the co-culture. Therefore, in this study, components of YM medium were added to BBM to propose different culture media for the controlled growth of *X. dendrorhous* in co-culture with *H. pluvialis*. This approach can be valuable for optimizing cultivation conditions to enhance biomass production.

Growth curve of H. pluvialis

The growth curve of *H. pluvialis* was monitored over 15 days using cell number and OD measurements (Figure 2).

The experiment began with an initial cell density of 1.75×10^4 cells/mL. A lag phase was observed during the first 3 days, with a minimal increase in cell number. Exponential growth started on day 4, with cell numbers increasing rapidly to reach 2.07×10^5 cells/mL by day 12. The growth rate decreased between days 12 and 15, indicating the start of the stationary growth phase. By day 15, the culture reached a final cell density of 2.21×10^5 cells/mL. The OD graph also showed the same growth pattern as the cell number, except for the stationary phase. The growth of *H. pluvialis* in BBM has been extensively studied, with several studies reporting favorable results. BBM has been found to provide optimal conditions for the vegetative growth of *H. pluvialis* compared to other common media, such as BG11 and 3NBBM (Nahidian et al., 2018). In autotrophic cultivation, BBM yielded the highest cell density of 1.5×10^5 cells/mL among the tested media (Tripathi et al., 1999). Interestingly, the growth curve of *H. pluvialis* in BBM can be further optimized by adjusting the nutrient concentrations. For instance, increasing phosphate levels in modified BBM led to up to an 86 % increase in growth rate and the highest cell density and the optimal concentrations of micronutrients such as boron (0.185 mM) and iron (0.046 mM) were found to enhance growth rates, although these optima depend on inoculum size (Nahidian et al., 2018). BBM consistently performed well for *H. pluvialis* growth, with a maximal biomass productivity of 86.54 mg/L/day (Zhao et al., 2019). However, the growth curve can be significantly improved by optimizing the nutrient concentrations, particularly phosphate, nitrogen and carbon. It's worth noting that while BBM supports excellent vegetative growth, other media may be more suitable for subsequent astaxanthin production in a two-stage cultivation strategy (Zhao et al., 2019; Fábregas et al., 2000). Wang et al. (2013) investigated the impact of varying concentrations of nitrate on biomass and astaxanthin production from *H. pluvialis*. The initial nitrate concentration significantly influenced the final biomass density; specifically, higher initial nitrogen concentrations in the culture led to greater final biomass density at the end of the 10-day culture period. The observed increases in final biomass density were attributed to both an increase in cell number and size (Wang et al., 2013).

Pang and Chen (2017) studied the effects of C5 organic carbon on the growth and cellular activity of *H. pluvialis*. They compared phototrophic, heterotrophic, and mixotrophic cultures using sodium acetate as the carbon source. The results showed

that the highest cell density was achieved under mixotrophic conditions, which was more than double that of the heterotrophic culture and higher than the phototrophic culture. This indicates that light is crucial for the growth and cell division of *H. pluvialis*. The researchers also examined nine different organic carbon sources, finding that ribose ,mannose ,fructose ,and sodium acetate resulted in the highest cellular densities (Pang and Chen, 2017).

BBM medium was selected as the base for potential co-culture media because of the necessity of excellent growth in the green phase of *H. pluvialis* for its co-culture with yeast.

Medium optimization for the co-culture

The results of the comparative study on cell concentration of *X. dendrorhous* and *H. pluvialis* in six different culture media (YM, BBM, BG, BGM, BGP, and BGY) are presented in Figure 3.

Growth of X. dendrorhous in different media in co-culture

Investigation of the growth of *X. dendrorhous* in the six different culture media in co-culture with *H. pluvialis* revealed that BG medium exhibited the highest cell numbers at the end of the co-cultivation, reaching a maximum of 3.8×10^5 cells/mL after 6 days (Figure 3). This was followed by BBM with glucose and peptone (BGP), which showed a peak cell density of 2.925×10^5 cells/mL. The BGM medium supported moderate growth at 1.725×10^5 cells/mL. In contrast, BGY and YM media showed controlled growth of yeast cells in the co-culture. The standard BBM medium without supplements showed no growth, with a final cell density of 5×10^3 cells/mL. This indicates that the additional nutrients, particularly the presence of glucose in the other media, significantly enhanced *X. dendrorhous* growth.

Growth of H. pluvialis in different media in co-culture

After six days of co-cultivation, *H. pluvialis* exhibited varying levels of cell growth across the six tested culture media (Figure

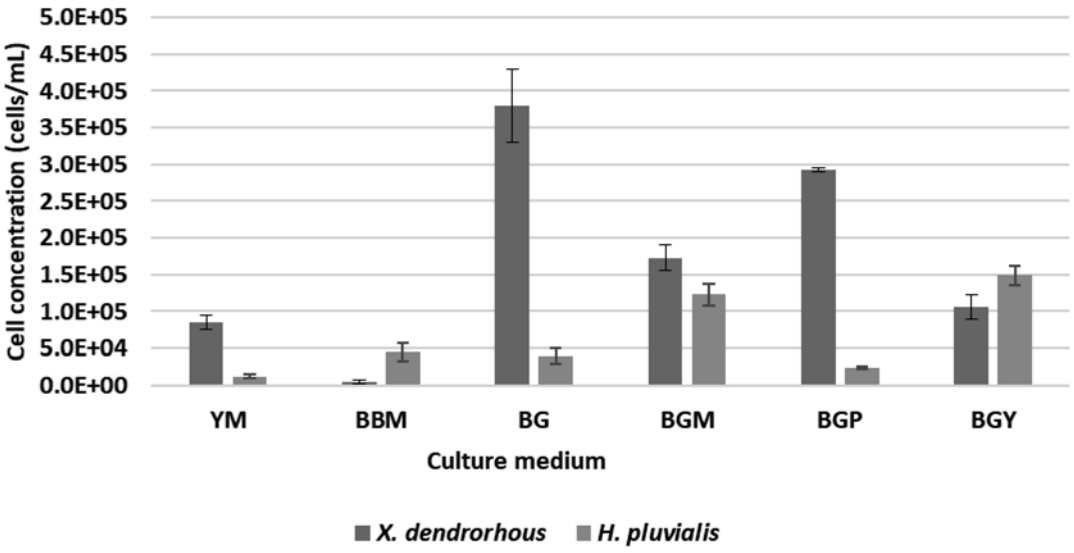

Fig. 3. Growth of *X. dendrorhous* and *H. pluvialis* in different culture media after 6 days of co-culture

3). The highest cell densities were observed in the BGM and BGY, with 1.22×10^5 and 1.488×10^5 cells/mL values, respectively. This was followed by BBM and BG, which had cell counts of 4.5×10^4 cells/mL and 4×10^4 cells/mL, respectively. The standard BBM medium demonstrated non-significant growth compared to the supplemented media. Among the variations in BBM, the addition of malt and yeast extract resulted significant increase in cell numbers, whereas peptone had the least impact on the growth enhancement of the microalga. As expected, the YM medium showed no growth compared to the BBM-based culture media, resulting in a final cell count of 1.25×10^4 cells/mL. These results indicate that supplementing BBM with certain compounds, specifically malt and yeast extract, significantly improves the growth of *H. pluvialis* in co-culture.

Comparison of the growth of H. pluvialis and X. dendrorhous in the co-culture

The growth of *X. dendrorhous* and *H. pluvialis* was compared across various coculture media (Figure 3), revealing distinct patterns and interactions between the two species. In the standard medium for *X. dendrorhous*, YM, the yeast's cell concentration was seven times higher than that of *H. pluvialis*. This indicates that *H. pluvialis* cannot utilize the complex organic nutrients present in YM. On the other hand, in BBM, the standard medium for *H. pluvialis*, *X. dendrorhous* showed no growth due to a lack of carbon sources, but the cell density of *H. pluvialis* increased to 2.25 times the initial cell concentration.

The BG medium allows both species to thrive effectively; however, the cell density of *X. dendrorhous* was 9.5 times higher than that of *H. pluvialis*. The yeast rapidly consumed all available glucose, which affected the growth of the microalga. *X. dendrorhous* is known to efficiently utilize glucose as a carbon source for growth. Marcoleta et al. (2011) noted that high glucose concentrations in the medium result in high cell growth but low carotenoid production, indicating that the yeast rapidly consumes glucose (Marcoleta et al., 2011). This suggests that while glucose is essential for yeast growth, the rapid consumption of glucose by *X. dendrorhous* may allow it to outcompete *H. pluvialis* in a co-culture situation, preventing the microalgae from utilizing the glucose present in the medium.

The rapid growth of yeast, driven by glucose consumption, leads to the expectation that the carbon dioxide produced during this process will promote the growth of microalgae. However, the results do not support this assumption. This indicates that the high levels of carbon dioxide generated in a short time from yeast growth are insufficient to meet the carbon needs of microalgae.

In the BGP medium, the high growth of *X. dendrorhous* also led to nutrient depletion, which hindered the growth of *H. pluvialis,* and similar to the BG medium, the CO_2 produced by the yeast in a short time was insufficient for the optimal growth of the microalga.

The BGM medium was superior for both species as it contained essential nutrients and promoted balanced growth. The similar cell concentrations observed in BGM for both species indicate its potential for creat-

ing a controlled co-culture environment. Interestingly, the BGY medium resulted in the highest cell concentration for *H. pluvialis* while effectively preventing the growth of *X. dendrorhous*. This suggests that the BGY medium facilitates efficient nutrient utilization and gas exchange between the two species.

It should be noted that, according to Figure 3, the presence of glucose in the medium is necessary for yeast growth, while malt and yeast extract are the most effective additives for enhancing microalgae growth.

A consistent pattern emerged across all media: when the growth of *X. dendrorhous* reached its maximum (as observed in BG and BGP media), the growth of *H. pluvialis* was inhibited due to competition for nutrients and inadequate CO_2 production. This highlights the importance of selecting a culture medium that balances the growth of both species, ensuring controlled nutrient competition and optimal gas exchange. In conclusion, a successful co-culture of *X. dendrorhous* and *H. pluvialis* necessitates a precisely formulated medium that controls the growth of the yeast while optimizing the growth of *H. pluvialis*. This balanced approach would facilitate controlled nutrient competition and the efficient utilization of synergistic gases (CO_2 and O_2) produced by each species, ultimately leading to a more productive co-culture system.

Dong and Zhao (2004) investigated the co-culture of *H. pluvialis* and *Phaffia rhodozyma* in BBM medium with varying glucose concentrations. They found that the biomass in the mixed cultures was higher than in pure cultures, particularly with glucose concentrations between 3-5 g/L, and it increased as glucose levels rose. *P. rhodozyma* also exhibited higher biomass at lower glucose concentrations, but saw a decline when concentrations exceeded 15 g/L, possibly due to the Crabtree effect. In contrast, *H. pluvialis* had low biomass concentrations, showing little variation across the tested glucose ranges (Dong and Zhao, 2004).

Recent studies have also shown that optimization of culture conditions, particularly the medium composition, plays a crucial role in the success of these co-culture systems. In a study conducted by Xu et al. (2024), it was shown that the addition of glucose and peptone significantly enhanced biomass production in *Chlorella-Saccharomyces* co-cultures, increasing it by approximately 2-fold compared to monocultures. Glucose supplementation alone led to a 3-fold increase in lipid content while restricting yeast growth. The combination of glucose and yeast extract benefited yeast monocultures but not the co-culture system (Xu et al., 2024). In another study, using food waste hydrolysate as a culture medium for *Rhodosporidium toruloides* and *Chlorella vulgaris* co-culture resulted in improved nitrogen utilization (23 % increase), reduced sugar utilization (17 % increase), and lipid production (12 % increase) compared with *R. toruloides* monoculture (Zeng et al., 2018). Additionally, utilizing different carbon and nitrogen sources can affect symbiotic relationships. Tian et al. (2020) demonstrated that a symbiotic yeast (*Cryptococcus sp*.) can hydrolyze sucrose, making it available for *Chlorella pyrenoidosa* under both heterotrophic and mixotrophic conditions.

Conclusion

This study investigated the optimal culture medium for the co-culture of *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous*, two microorganisms known for their ability to produce astaxanthin. Results showed that *H. pluvialis* exhibited the highest cell densities in BGM and BGY media, while *X. dendrorhous* growth was highest in BG medium. BGM demonstrated balanced growth for both species, while BGY resulted in the highest cell concentration for *H. pluvialis* and controlled growth of *X. dendrorhous*. The study highlights the importance of selecting a culture medium that balances the growth of both species, ensures controlled nutrient competition, and optimizes gas exchange for a productive co-culture system.

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