SYMBIOTIC PHOTOBIOREACTOR USING IMMOBILIZED MICROALGAE-YEAST CONSORTIUM FOR *SACCHAROMYCES CEREVISIAE* AND *CHLORELLA VULGARIS* BIOMASS PRODUCTION

Nguyen Tiet Minh Nhat, Nguyen Thuy Kim Anh, Vu Tran Khanh Linh
Ho Chi Minh City University of Technology and Education, Vietnam

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ABSTRACT

A photobioreactor for symbiotic growths of Saccharomyces cerevisiae and Chlorella vulgaris was developed. In this photobioreactor, yeast cells were freely suspended in medium while microalgal cells were entrapped in alginate beads in the same medium for separate biomass production. The symbiotic relationship was demonstrated as reflected by the photoautotrophic growth of C. vulgaris using CO_2 provided by S. cerevisiae and the biodegradation of 2000 mg/L glucose by S. cerevisiae utilizing photosynthetic O_2 produced by C. vulgaris. Yeast biomass productivities were 67.75 mg/L-h, 74.70 mg/L-h and 68.53 mg/L-h in three successive 8-hour photo-aeration cycles, significantly higher than that obtained in non-aerated yeast culture. Microalgal cell concentration also increased 84% after 24 hours of cultivation in the symbiotic photobioreactor, significantly higher than those grown in the medium without external carbon source. Enhanced yeast biomass productivity was obtained in symbiotic medium supplemented with 4000 mg/L glucose. The highest yeast biomass productivity of 99.82 mg/L-h was obtained at an initial microalgal concentration of 110.52 mg/L.

Keywords: *Saccharomyces cerevisiae; Chlorella vulgaris; biomass production; symbiotic culture; photobioreactor; photosynthetic aeration.*

1. INTRODUCTION

Saccharomyces cerevisiae has been widely used in various sectors of food industry such as fermentation starter in bread and alcoholic beverage productions, or as an alternative protein a source in animal feed or human nutrition [1,2]. The production of *S. cerevisiae* biomass, mainly in the form of baker's yeast involves a series of bioreactors with the increase in volume and is highly aerated in later stages to obtain higher biomass productivity and eliminate ethanol formation [3,4]. Because of the low solubility of oxygen in fermentation broth [5], aeration and agitation are maintained continuously during the growth phase, which causes considerable costs for production [6,7]. In addition, the high concentration of CO_2 accumulated during cellular respiration may also cause detrimental effect on yeast growth [8]. Therefore, increasing dissolved oxygen, decreasing CO_2 concentration and reducing production costs are major problems that need to be solved.

Microalgae are phototrophic micro-organisms that can sequester CO2 to form biomass and produce O2 during photosynthesis [9,10]. Owing to their chemical composition, microalgae biomass has become potential resources for numerous fields including food, feed, biopigment, drug and biofuel productions during the past few decades [11,12]. For instance, Taiwan Chlorella Manufacturing CO., LTD has cultivated Chlorella vulgaris in open ponds to harvest biomass that is used for food and feed supplement [13,14]. Although open ponds are cheaper to build and easier to operate, the major limitations in these systems include unstable growth conditions, evaporation losses, greater contamination risk and diffusion of CO2 to the atmosphere, resulting in low biomass productivity [15]. These problems with open ponds can be overcome by using closed-photobioreactors for microalgal biomass production [15,16]. However, the accumulation of dissolved oxygen produced during photosynthesis

could inhibit photosynthesis [17,18]. The high capital investment and production costs are also other drawbacks of closed-photobioreactors. One of the reasons for high microalgae production cost is attributed to the cost for CO2 supply including CO2 gas and energy consumption of aeration [19].

A consortium of yeast and microalgae in one closed-photobioreactor can tackle the aforementioned drawbacks of respective monocultures [20,21]. This association exploits microalgal photosynthesis to provide sufficient O2 for heterotrophic yeast; CO2 released from yeast respiration is then assimilated by the microalgae during their photosynthesis [22]. The symbiotic microalgae – yeast process hence allows cost – effective aeration, limits the detrimental effects of CO2 and O2 build-ups on yeast and microalgae, respectively, and effectively supports cell growths [21,22]. Moreover, the greenhouse effect can be mitigated due to the use of CO2 by microalgae. The symbiotic approach between yeast and microalgae have been documented in the literature. For examples, a few studies reported that the mixed cultivation of the yeast Rhodotorula glutinis and microalgae Spirulina platensis significantly enhanced total biomass and lipid production [23,24]. Shu et al. (2013) also reported that the symbiosis between Saccharomyces cerevisiae and Chlorella sp. improved cellular biomass and oil accumulation. However, when growing the two species together, the physical segregation of the microalgae and yeast is a primary concern for downstream processing, which could lead to huge cost of final bio – products.

To overcome the mentioned drawback in mixed culture of microalgae and yeast, Chlorella vulgaris cells can be immobilized in alginate beads to physically separate the two cell types while still allowing the symbiosis between them due to the permeability, biocompatibility and transparency of the alginate beads [26-28]. The bacteria – immobilized microalgae symbiosis was successfully applied for photosynthetic aeration in biological wastewater treatment [29]. As far as we concerned, there are few studies involving the independent – harvest of the two species for various purposes in food industry. Hence, the aim of this study is to evaluate the feasibility of culturing immobilized Chlorella vulgaris and freely suspended Saccharomyces cerevisiae in a closed-photobioreactor.

2. MATERIALS AND METHODS

2.1 Microorganisms and culture conditions

Chlorella vulgaris was purchased from Research Institue for Aquaculture No 2 (District 1, HCMC), while Saccharomyces cerevisiae was purchased from AB Mauri Viet Nam in the form of Instant Dry Yeast. S.cerevisiae was maintained on Yeast extract peptone dextrose (YPD) agar plate at 4 oC. The compostion of YPD medium contained (g/L) glucose, 20; yeast extract, 10; peptone, 20. The preculture was prepared by tranferring stock culture from agar plate to 500 mL Erlenmeyer flask containing 300 mL synthetic medium. The compostion of synthetic medium included (g/L) glucose, 20; yeast extract, 1; KH2PO4, 5; (NH4)2HPO4, 2; MgSO4.7H2O, 2 [30]. The seed culture was agitated at 140 rpm at room temperature in a magnetic stirrer for 24 hours.

C.vulgaris was cultivated in Bold's Basal Medium (BBM) [31]. C.vulgaris stock culture was preserved on BBM agar plate at 4 oC. Prior to inoculation, stock culture was tranferred from agar plate to 500 mL Erlenmeyer flask containing 300 mL BBM with 1000 mg/L NaHCO3. The flask was fitted by non – absortbent cotton wool bung. The seed culture was agitated with a magnetic stirrer at 140 rpm at room temperature. Continuous light intensity using Light-Emitting diodes (LEDs) at 300 µmolphoton/m2-s for 4 – 5 days.

Symbiotic medium made from BBM with 1 g/L yeast extract, 2 g/L (NH4)2SO4, 2 g/L glucose and adjusted to pH = 6.6 was used throughout the study. All the chemicals used in this research were of analytical grade.

Encapsulation of C.vulgaris in alginate beads

Chlorella vulgaris cells, incubated under autotrophic mode, were harvested by centrifuging at 4500 rpm for 20 minutes. Sodium alginate was dissolved in distilled water at concentration 7% (w/v) and then was autoclaved before vigorously mixing with microalgal cells in order to create a uniform suspension. Thirty milliliters of finished suspension were dripped into 3% (w/v) CaCl2 solution using peristaltic pump to form spherical beads. The beads were then left to harden in CaCl2 for 1 hour, washed with distilled water twice before conducting experiments. In order that the microalgal cells population could be determined, the beads were dissolved in 5% (w/v) EDTA solution.

Effects of air rate on yeast biomass productivity

The yeast cells were inoculated at a concentration of 58.42 mg/L in a 500-mL Erlenmeyer flask containing 300 mL symbiotic medium. The cell culture was agitated with a magnetic stirrer at 140 rpm at room temperature and was aerated at a rate of 1.125 L/min. A control experiment was also conducted under identical condition but without aeration to compare yeast biomass productivity. Yeast samples were periodically collected to determine biomass concentration, and glucose concentration. Ethanol concentration was measured at the end of each experiment.

Growth of microalgae in the presence of sodium bicarbonate and glucose

C.vulgaris cells are capable to grow under autotrophic and heterotrophic modes. It is unexpected that microalgae would compete sugar with yeast, which may lower yeast biomass productivity. Hence, this experiment was carried out to compare the glucose consumption rate of suspended and immobilized microalgae. Besides, another experiment was also conducted to investigate the growth performance of C. vulgaris in the presence of inorganic carbon (NaHCO3) under autotrophic condition.

Alginate encapsulated cells and suspended cells were inoculated at a concentration of 147.36 mg/L into 500-mL flasks containing 300 mL symbiotic medium supplemented with 2000 mg/L glucose. In the other experiment, the microalgal cells were inoculated at a concentration of 147.36 mg/L in a 500 mL Erlenmeyer flask containing 300 mL symbiotic medium and 1000 mg/L NaHCO3. A control experiment was conducted without carbon source under identical conditions to compare biomass productivity. All the flasks were continuously stirred and illuminated at the intensity of 300 μmolphoton/m2-s. Microalgal samples were periodically collected to determine biomass concentration, and glucose concentration.

Proof – of – concept experiment

In the proof – of – concept study, the photobioreactor operation was conducted in a 500 mL Erlenmeyer flask containing 300 mL symbiotic medium supplemented with 2000 mg/L glucose. The flask was fitted by silicone bung to block the gas exchange between the flask and the surroundings. The bioreactor was agitated with a magnetic stirrer at 140 rpm at room temperature. Continuous light intensity using LEDs was provided at 300 μmolphoton/m2-s. The symbiotic medium was changed every 8 hours to supply fresh nutrients to the yeast, but the same alginate beads were reused in two more subsequent cycles of the experiment. In the first batch, suspended yeast and immobilized microalgae were cultured at concentrations of 58.42 mg/L and 147.36 mg/L, respectively. Every 8 hours, the spent yeast culture was withdrawn and replaced with fresh symbiotic medium supplemented with 2000 mg/L glucose. Yeast cells attached on the alginate beads were served as the inoculum for subsequent cycles. A control experiment was also conducted under the same condition with the blank beads (beads without microalgae).

Yeast samples were periodically collected to measure yeast biomass concentration, glucose concentration, while six beads were picked from the flask at the end of each cycle to determine the number of cells per bead. Ethanol concentration was also measured at the end of each cycle.

Effects of glucose concentration and initial microalgal biomass on symbiotic photobioreactor performance

The effects of glucose concentration on the symbiotic photobioreactor performance was investigated by increasing initial glucose concentration up to 4000 mg/L. The experimental design was similar to that of proof – of – concept experiment, except only two consecutive 12-hour cycles were conducted. A control experiment was also conducted in which blank beads were used.

The effects of initial microalgal biomass on the consortium was investigated at 110.52 mg/L, 147.36 mg/L, 184.20 mg/L. The experimental design was similar to that of proof – of – concept experiment.

Analytical methods

Microalgae cell counts were conducted using Improved Neubauer haemocytometer. S.cerevisiae and C.vulgaris growth was monitored by OD measurement at 600 nm and 540, respectively, using spectrophotometer (Halo Vis 20, China). The OD600 was used to compute the yeast biomass concentration was as follow: dry cell weight (mg/L) = 292.1 x OD600 (R2 = 0.9906), while the OD540 was used to compute the microalgal biomass concentration was as follow: dry cell weight (mg/L) = 368.4 x OD540 (R2 = 0.9925). Biomass productivity (mg/L-h) was caculated from P = (Xt – Xo)/(t – to), where where Xo and Xt are the biomass concentrations (mg/L) at the beginning (to) and at the end (t) of each batch [32].

Glucose concentration was measured using 3,5 dinitrosalicylic method [33]. Ethanol concentration was measured using dichromate oxidation method [34]. pH was measured using pH meter (Mettler Toledo, US).

Results and discussion

Effects of aeration rate on yeast biomass productivity

Effects of aeration rate on S.cerevisiae growth was first conducted to understand its roles on yeast biomass production.

It can be seen in Figure 1 that growth was much higher without aeration as compared to the one under aeration rate of 1.125 L/minute. Results from Figure 1A dictate that high aeration rate not only decreased biomass concentration, but also caused negative effect on yeast growth. According to Pinheiro et al. (2000), overfeeding of oxygen may inhibit the growth of aerobic microorganism, this phenomenon was known as oxidative stress [35]. Because of its highly reactive characteristic, oxygen is easily partially reduced in mitochondrial respiration to form free radical molecules such as superoxide anion radical (O2-), hydrogen peroxide (H2O2), and hydroxyl radical (HO-) [36]. The increase of dissolved oxygen in the broth under vigorous aeration would lead to the formation of numerous active oxygen species. These molecules may cause impressive damage to cellular constituents including enzymes, nuleic acids and lipids. Hence, yeast cells must have spent the abundance of energy released from glucose to neutralize the free radicals, and this means that glucose used for synthesizing biomass decreased magnificently [37]. Although dissolved oxygen is one of the important factors that have the decisive effect on biomass productivity [1], the control of air flux must be carefully taken into account.

Figure 1B shows that the glucose bio–degradation rate without aeration was much faster than that with the presence of aeration (288 mg/L-h vs 251 mg/L-h). When glucose was exhausted at the 7^{th} hour, the growth rate of yeast with the absence of aeration started to decline. However, according to Pérez

Torrado et al. (2009), yeast could oxidize produced ethanol for biomass formation in the presence of oxygen, when sucrose was depleted [38]. To switch from fermentation to respiration, and to limit ethanol formation for biomass production, an oxygenic photosynthetic microalgae species could be integrated into the yeast culture.

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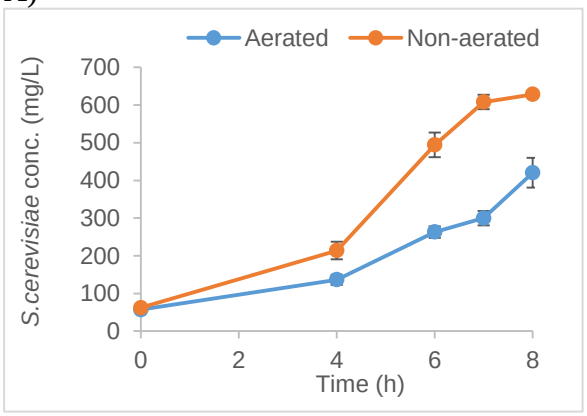

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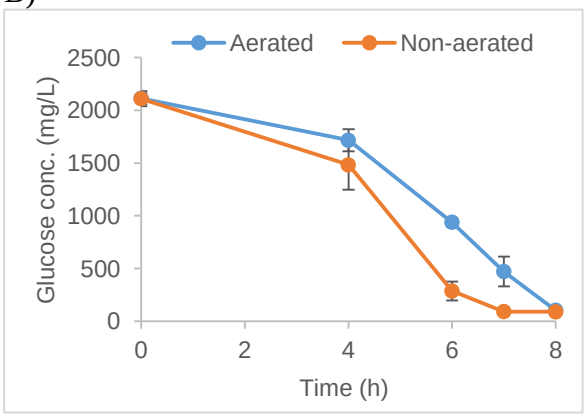

Fig. 1. *Effects of air flux on (A) growth rate and (B) glucose consumption rate of S.cerevisiae.*

2.2 Growth of microalgae in the presence of sodium bicarbonate and glucose

Inorganic carbon is one of the most essential factors attributed to photosynthesis. The experiment was conducted to understand the role of inorganic carbon in microalgal growth under autotrophic condition (Figure 2). Results show that there was a significant difference in biomass concentrations between B1 (without external inorganic carbon source) and B2 (with external inorganic carbon source). In particular, the biomass concentration only reached 175.7 mg/L after 60 hours of incubation in B1. This means that there was inadequate CO_2 in the headspace (approximately 0.0378%) to support the rapid growth of *C.vulgaris*. On the contrary, the growth rate of microalgae utilizing $NaHCO_3$ as a carbon source was significantly improved, which increased from 147 mg/L to 380.7 mg/L within 60 hours in B2. To conclude, it is obvious that inorganic carbon plays vital role in the biomass production under autotrophic mode.

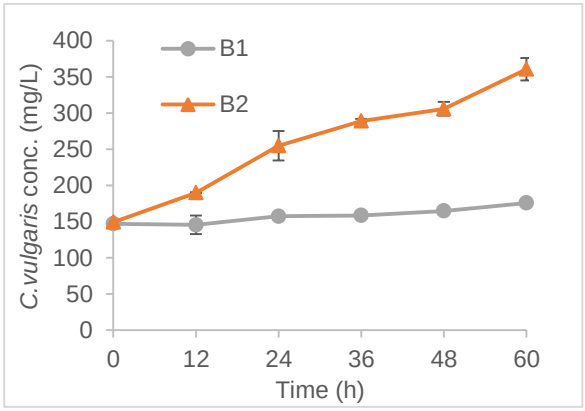

Fig. 2. *Effects of inorganic carbon on the growth of suspended C.vulgaris under headspace condition (B1) and with $NaHCO_3$ (B2).*

Because C.vulgaris can grow under heterotrophic and autotrophic modes, B3 and B4 experiments were done to investigate the glucose utilization rate of suspended and immoblized *C.vulgaris*. Figure 3 shows the trend of remaining glucose within 60 hours. The results show that suspended microalgae consumed glucose faster than immobilized ones. After 36 hours, the remaining glucose concentration in suspended microalgal cell culture was 136.75 mg/L. By contrast, the consumption of glucose in B4 experiment remained unchanged within the first 12 hours due to the hindrance of alginate barriers; and after that the glucose consumption rate started to increase. However, both suspended and immobilized *C.vulgaris* did not completely metabolize glucose over the 60 hours of cultivation.

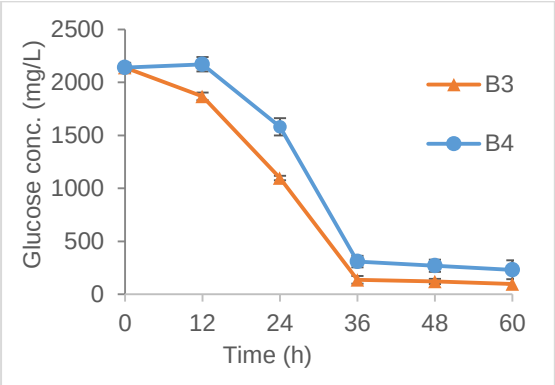

Fig. 3. *Comparison of glucose consumption by suspended microalgae (B3) and immobilized microalgae (B4).*

It is unexpected that there is a competition for organic carbon source between S. cerevisiae and C. vulgaris in a binary culture, because of their capabilities of assimilating organic compounds. However, results in this study indicated that with the same initial glucose concentration, glucose comsumption rate of S. cerevisiae was much faster than that of immobilized C. vulgaris. Compared with the complete glucose degradation of S. cerevisiae within 8 hours of cultivation (Figure 1), the initial glucose concentration remained unchanged in first 12 hours in immobilized C. vulgaris culture. After 60 hours of incubation, the remaining glucose concentration was still as high as 232 mg/L (B4, Figure 3). The alginate gel had played a vital role in preventing the diffusion of glucose into the beads, contributing to the decrease in glucose uptake rate of microalgae. Despite there was no entrapment of C.vulgaris into alginate beads in B3, the glucose consumption rate was not much faster than that of immobilized C. vulgaris (Figure 3).

In a symbiotic consortium, it is proposed that yeast would quickly consume glucose and simultaneously produce CO2 that can easily diffuse into alginate matrix to become the inorganic carbon source for autotrophic microalgae. With the advantages of obviating the glucose consumption of microalgae and still ensuring the gas exchange between O2 and CO2, encapsulation of microalgae in alginate beads may offer a promising method for separate harvesting of microalgal biomass from the yeast – microalgae consortium.

2.3 Proof – of – concept

To justify the feasibility of symbiotic relationship between immobilized C. vulgaris and suspended S. cerevisiae, the closed-photobioreactor was operated according to procedure presented in Section 2.5.

Figure 4A shows a comparison of yeast biomass concentration with and without photosynthetic aeration. It can be seen that there was no difference in yeast biomass productivity in cycle 1 (0h – 8h). However, the yeast biomass productivity of cycle 2 (74.70 mg/L-h) and cycle 3 (68.53 mg/L-h) improved significantly in the presence of C. vulgaris, which was much higher than that of cycle 2 (49.82 mg/L-h) and cycle 3 (50.29 mg/L-h) where yeast cells were cultivated alone. From Figure 1 and cycle 1 – Figure 4A, it is clear that yeast could use dissolved oxygen (DO) in medium for its growth, but the yeast growth rate was significantly improved in the 2nd and 3rd cycle in the presence of microalgal beads (Figure 4A). Hence, this clearly indicated that, without external aeration, yeast growth rate in cycle 2-3 of Control sample could have been hindered by the deficiency in oxygen supply, however autotrophic microalgae, or photosynthetic aeration, had tackled this problem in the symbiotic photobioreactor.

Figure 4C shows the ethanol production at the end of each cycle. It was found that the increase in biomass productivity coincided with the decrease in ethanol formation (Figure 4A, Figure 4C). This could be attributed to the conversion of glucose into biomass by S. cerevisiae in the presence of oxygen. According to Pérez-Torrado (2009), low oxygen level (below 2%) leads to the higher ethanol formation than that under aeration condition (0.5 – 1.5 kg/cm3). Without mechanical aeration, the yeast grew markedly in mixed culture than in monoculture (Figure 4A), probably because of advantageous photosynthetic aeration by immobilized microalgae. It was also reported

that in symbiotic culture, ethanol still exists to a certain extent when glucose concentration exceeds 0.08 mM [39]. This phenomenon occurs when the overflow of pyruvate produced from glycolytic pathway inhibits enzyme pyruvate dehydrogenase (PDH), inducing the conversion of pyruvate into ethanol and CO2 by pyruvate decarboxylase (PDC) [40].

The results reconfirmed the vital role of oxygen in enhancing yeast biomass productivity. However, the requirement of air flow rate in appropriate intensity is one of the most important issues for not causing negative effect on yeast (Figure 1A). Moreover, the growth of cells also depends on the agitation speed which influences the diffusion of oxygen from gas phase into microogranism. The consortium in this study may propose the solutions to this problem, because algal oxygen from alginate beads can penetrate easily into yeast culture, which contributes to both elevating the yeast growth rate and reducing the cost of mechanical aeration.

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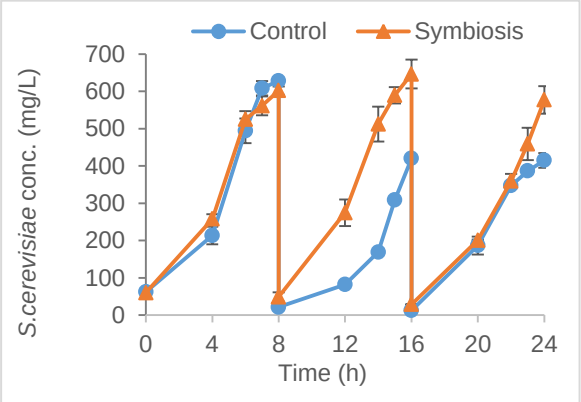

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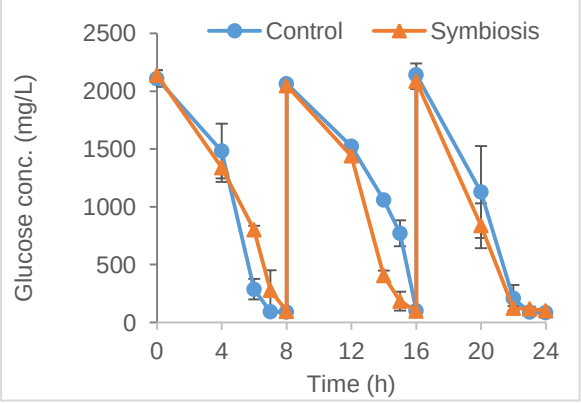

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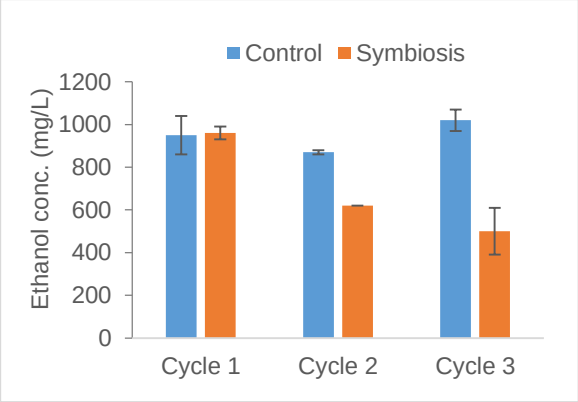

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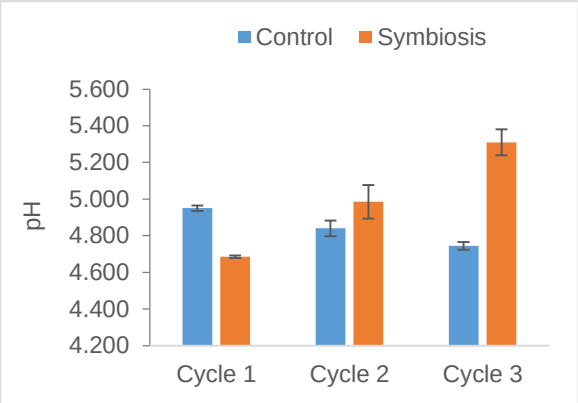

Fig. 4. *Effects of photosynthetic aeration on A) yeast growth, B) glucose uptake rate, C) ethanol formation, D) pH. Control indicates control samples where blank (without C.vulgaris) beads were used.*

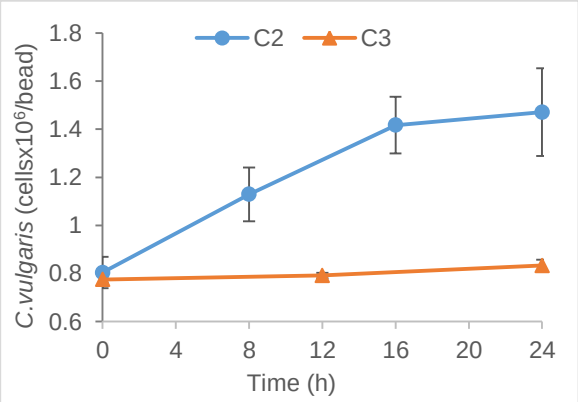

Fig. 5. *C. vulgaris growth with no carbon source (C3) and 2000 mg/L glucose (C2).*

Results in Figure 5 also show that in the symbiotic photobioreactor, microalgal concentration significantly increased. After 24 hours of cultivation, the final microalgal

concentration in the symbiotic photobioreactor was 1.47 x 106 cells/bead, while there was low microalgal growth observed in the beads in the non-symbiotic photobioreactor with no external carbon source. The result demonstrated that CO2 from yeast respiration was used as an inorganic carbon source by microalgae for their photosynthesis, because CO2 in the headspace was insufficient to support the microalgal growth (Figure 2), and glucose hardly diffuse into beads owing to the hindrance of alginate gel (Figure 3). In addition, immobilized microalgae was precultured under phototrophic condition before culturing into symbiotic photobioreactor, it is unseemly for C.vulgaris to switch the mode from phototrophy to mixotrophy or heterotrophy under oxygen starvation condition [29].

In terms of culture pH, there was a significant difference between yeast monoculture and coculture experiments (Figure 4D). The pH of coculture was higher than that of monoculture because of CO2 stripping by microalgal photosynthesis. The formation of acetic acid and lactic acid by yeast cells gave rise to the decrease in pH and then limited the optimum growth of microorganisms including yeast [4]; but the fixation of CO2 from HCO3- by microalgae makes the medium more alkaline (H2O + HCO3- → C (biomass) + OH-), which reduced this harmful effect on yeast growth [22, 41].

Results of the operation of the symbiotic photobioreactor indicated that the gas exchange between freely suspended S. cerevisiae culture and immobilized C. vulgaris effectively supported both yeast and microalgae biomass production and the reduction of ethanol concentration. Moreover, this interaction can tackle the problems of axenic monoculture which could adversely affect the biomass productivity. For example, the high levels of CO2 (above 0.016 M) could cause harmful effect on yeast due to the formation of HCO3- which inhibits many enzymes including succinate dehydrogenase – an enzyme in Krebs cycle, and could affect the permeability of the cell membrane [37]. Hence, the critical issue is to reduce the CO2 concentration in the yeast culture. Microalgae, meanwhile, can successfully convert HCO3- into biomass [41]. Likewise, high O2 levels (> 20%) also inhibits microalgal growth [22, 42]. It is reported that yeast can produce 0.042 – 0.130 M dissolved CO2 and the photosynthetic efficiency of microalgae increases when atmospheric oxygen drops from 21% to 1 – 3% [22]. Due to the gas exchange in this binary system of yeast-immobilized microalgae, the inhibition of CO2 on yeast and O2 on microalgae were significantly reduced.

2.4 Effects of glucose concentration on symbiotic bioreactor performance

The increase in glucose concentration would lead to higher demand of dissolved oxygen. Figure 6 shows the growth performance of yeast in monoculture (without microalgal beads) and in symbiotic photobioreactor supplement with 4000 mg/L glucose. The biomass productivities in cycle 1 (0h – 12h) of monoculture and coculture were 96.46 mg/L-h and 80.50 mg/L-h, respectively. In contrast to that trend, the yeast biomass productivity in cycle 2 of the coculture was much higher than that in the monoculture (93.84 mg/L-h vs 73.09 mg/L-h). It was found that the oxygen produced by microaglae was sufficient for yeast respiration to completely degrade 4000 mg/L glucose within 12 hours. Ethanol formation decreased in the 2nd cycle of binary culture, while there was no significant difference in ethanol concentration between each batch of yeast monoculture. Again, this clearly indicated the presence of symbiosis relationship between yeast and microalgae in the co-culture in which most of glucose was used for yeast biomass production rather than ethanol formation. Similar trend was observed in the study of Yen et al. (2015), the increase in glucose concentration also led to the increase in yeast growth rate.

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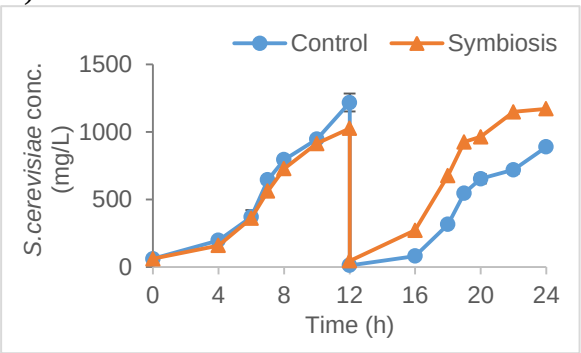

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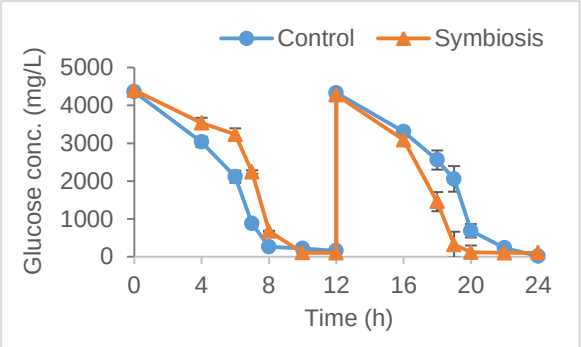

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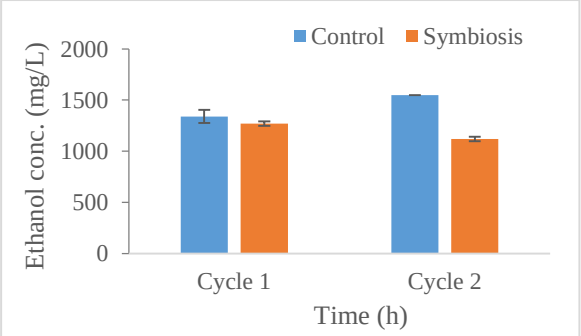

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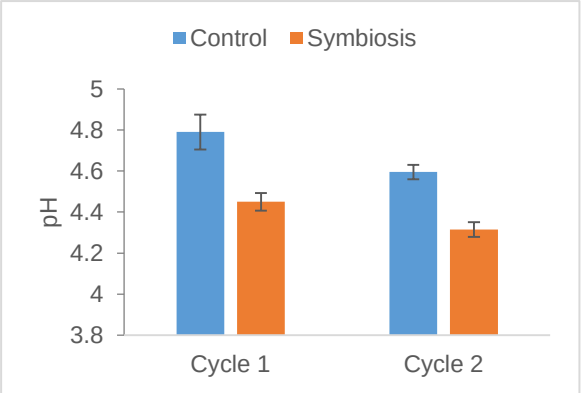

Fig. 6. *Effects of initial 4000 mg/L glucose on A) yeast growth, B) glucose uptake rate, C) ethanol formation, D) pH. Control indicates control sample where blank beads (without C.vulgaris) were used.*

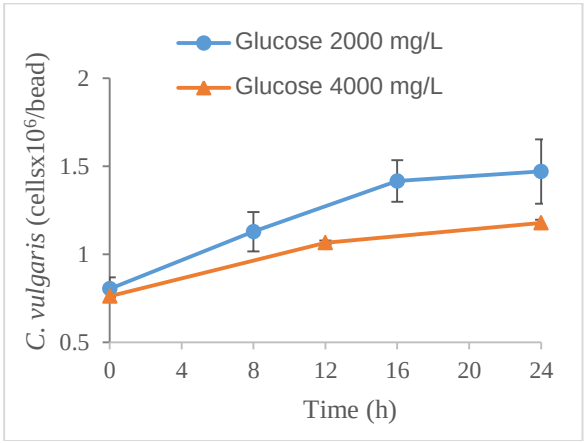

Fig. 7. *Cells density of C.vulgaris at different glucose concentration*

Figure 6D shows a pH comparison between yeast monoculture and co-culture. It is found that pH was fairly low in the mixed culture, implying the outgrowth of yeast over microalgae. However, the photosynthetic aeration by microalgae could sufficiently support yeast growth and development. Although enhanced yeast biomass production could produce more CO_2, this did not stimulate algal growth (Figure 7). The results depicted that the glucose concentration plays vital role in balancing the symbiotic relationship between yeast and microalgae.

2.5 Effects of initial microalgal biomass on symbiotic bioreactor performance

The initial microalgal biomass at different concentrations of 110.52 mg/L (D1), 147.36 mg/L (D2) and 184.20 mg/L (D3) was investigated to evaluate the performance of the symbiotic photobioreactor. As can be seen in Figure 8A, there was no discrepancy in yeast biomass productivities in cycle 1 of D1, D2 and D3. However, the yeast biomass productivity reached maximum (99.82 mg/L-h) in cycle 2 of D1. Likewise, yeast growth in cycle 3 shows similar trend to that of cycle 1. It is noticeable that the glucose uptake rate in cycle 2 of D1 was relatively higher than those of D2 and D3 (Figure 8B, C), whereas the pH and ethanol concentration in cycle 2 of D1 were rather low compared to those of D2 and D3. These results prove that yeast cells were growing rapidly and dissolved

oxygen produced by microalgae photosynthesis was sufficient to support yeast growth. The enhanced initial microalgal cells (187.20 mg/L), by contrast, did not enhance yeast biomass productivity as compared to that of Proof-of-concept experiment (Figure 8A).

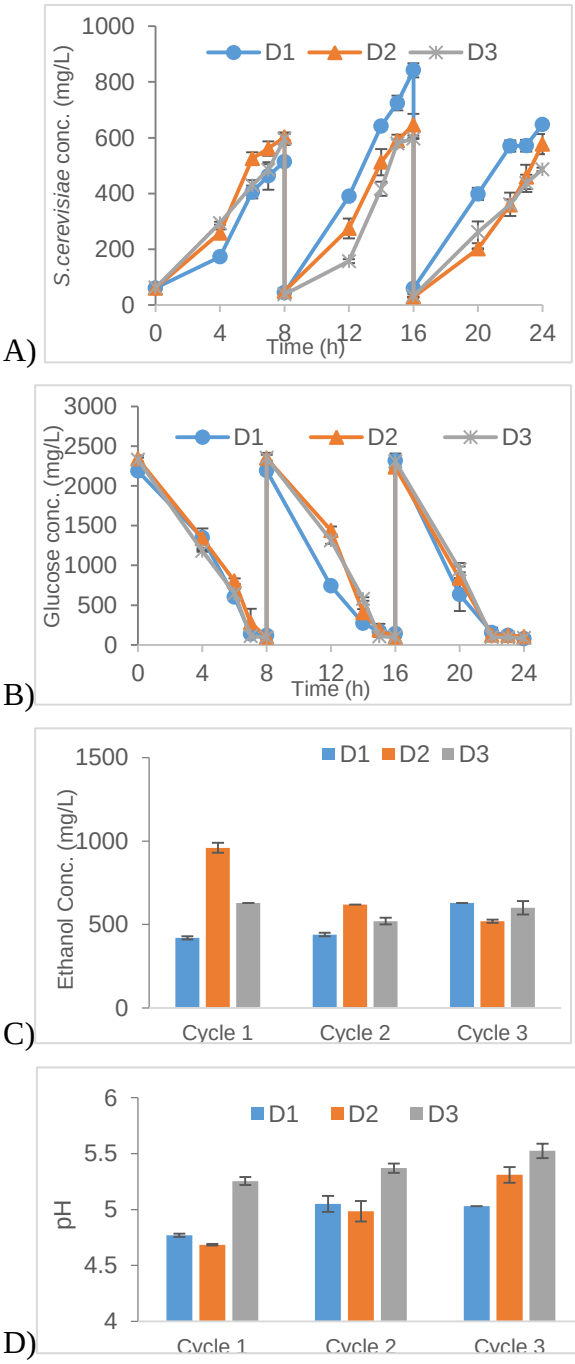

Fig. 8. *Effects of photosynthetic aeration at different concentration on A) the yeast growth, B) glucose uptake rate, C) ethanol formation, D) pH. (D1-110.52 mg/L, D2-147.36 mg/L, D3-184.20 mg/L).*

The reason of this phenomenon could be attributed to the insufficient nutrients in symbiotic medium and ineffective photosynthetic aeration due to self-shading [44].

Figure 9 shows cells density of Chlorella vulgaris over 24 hours at different initial cell concentrations. It can be observed in D1 that the fast growth of microalgae was obtained from hour 8 - 16, which coincided with the maximum yeast biomass productivity obtained in cycle 2. The enhanced microalgae cell concentration in D3 caused an increase in pH up to 5.5 (Figure 8D), which was not suitable for yeast growth [45]. Results of this experiment further confirm the symbiotic relationship between alginate-encapsulated C. vulgaris and freely suspended S. cerevisiae cells, however the photosynthetic aeration still sufficiently supported yeast aerobic respiration at lower microalgal concentrations (110.52 mg/L and 147.36 mg/L).

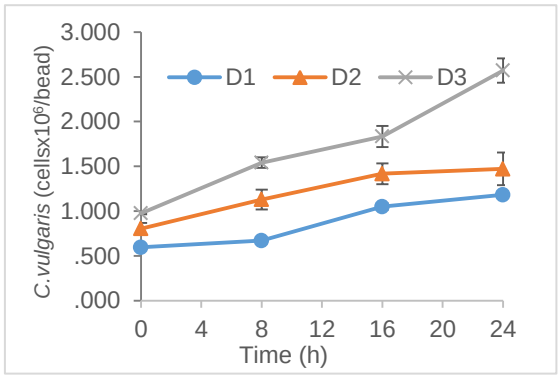

Fig. 9. *Cells density of Chlorella vulgaris over incubation times. (D1-110.52 mg/L, D2-147.36 mg/L, D3-184.20 mg/L)*

3. CONCLUSION

The symbiosis between alginate-encapsulated *C. vulgaris* and suspended *S. cerevisiae* has been successfully demonstrated. Because the two cultures were separated in the same closed-photobioreactor, clean yeast biomass and microalgal biomass could be easily harvested for various applications. In addition, this symbiotic relationship also allows cost-effective aeration and limits the risk of greenhouse gas emission, indicating the applicability of the photobioreactor model.

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Corresponding author:
Vu Tran Khanh Linh
Ho Chi Minh City University of Technology and Education
E-mail: linhvtk@hcmute.edu.vn