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Optimization of culture conditions for the production and activity of recombinant xylanase from microalgal platform

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ABSTRACT

Xylanases are enzymes responsible for the hydrolysis of the heteropolymer xylan. They have wide applications ranging from the bakery, animal husbandry, and textile to pulp and paper industry and biofuel productions. Recombinant xylanase production has been previously reported from different hosts such as bacteria and yeast. Microalgae offer a safe and cost-effective photosynthetic platform for producing recombinant proteins, including therapeutics and industrial enzymes. In this study, we optimized the production of recombinant xylanase expressed and secreted from the green alga, *Chlamydomonas reinhardtii*. The growth of the culture was optimized using response surface methodology (RSM) based on central composite design (CCD), with two numeric (culture incubation time and agitation rate) and one categoric (light intensity) factors. The optimum biomass concentration was obtained as 0.71 mg/mL from the CCD values. In addition, bubble column photobioreactors were set and compared for the culture growth, the protein concentration, and the enzyme activity under different light intensities and air flows. Increasing the aeration rate from 1 vvm to 2 vvm resulted in improved enzyme activity from 5330.5 U/g to 6277.7 U/g under 3500 lux illumination on the 3rd day of the culture. This study may lead to the further large-scale production of xylanase with high enzyme activity and reveal the advantage of the microalgae as a sustainable platform.

1. Introduction

Xylanases catalyse the hydrolysis of xylan, the second-most abundant polysaccharide and a major component in plant cell walls [1,2]. Due to its complex structure and heterogeneity, the complete degradation of xylan requires xylanase action. Endo-β-1,4-xylanase is the most significant xylanolytic enzyme, cleaving internal glycoside bonds in the xylan backbone, and thus reducing the degree of polymerization [3]. Xylanases are used in many different industries such as biofuel production, food and feed industry, bakery, textile, paper bleaching, and fruit juice clarification [4]. The market share of xylanase is estimated to reach 500 million dollars by 2023 [5,6], and the global xylanase market is expected to increase at 6.6% compound annual growth rate [7,8].

Xylanases are produced naturally by many organisms like bacteria, fungi, protozoa, gastropods, arthropods, and algae [9]. On the other hand, recombinant production of this enzyme is of interest to obtain higher yields and better enzymatic activities [9–12]. Homologous and heterologous expression of recombinant xylanase has been widely studied in various platforms such as bacteria, yeast, and filaments fungi [13–17]. As a recombinant platform, microalgae present certain advantages by being a fast-growing photosynthetic organism with no concern of pollen contamination, safe for human consumption, and easily scalable at low costs compared to other systems [18,19]. They offer a suitable host system for xylanase production with the advantage of eukaryotic machinery and photosynthetic nature. In the previous studies, the Xyn1 gene, responsible for endo-β-1–4-xylanase enzyme production in *Trichoderma reesei* (EC 3.2.1.8) [12,20] was successfully expressed in the green microalgae *Chlamydomonas reinhardtii* (cc1690) [21]. Its accumulation in the chloroplast was also studied in *C. reinhardtii*, and another green microalga, *Dunalilla tertiolecta* [22]. Since most xylanases require post-translational modifications such as disulfide-bond formation and glycosylation, the eukaryotic microalgal platforms stand out at this point, compared to the procaryotic systems.

Many genetic modification studies have been focused on the

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enhancement of xylanase expression, providing easy purification by adding protein tags, or improving stability against extreme conditions [9,23]. Apart from the genetic tools and techniques to improve the expression, optimizing the process was proven to enhance the production of the transgene [24]. Manipulation of the culture conditions can modulate algal metabolism by affecting the biosynthesis of compounds, increasing the accumulation of recombinant proteins as well as high-value metabolites such as carotenoids, lipids, and proteins [13,25,26].

In this study, we aimed to optimize the production and specific enzyme activity of recombinant xylanase expressed and secreted from the green alga, *Chlamydomonas reinhardtii*. The cell growth and the xylanase enzyme production of *C. reinhardtii* were investigated in bubble column photobioreactors to identify the optimum conditions for enhanced productivity of the secreted xylanase on large-scale. To the best of our knowledge, this is the first report regarding photobioreactor scale production of a recombinant xylanase from the green alga, *C. reinhardtii*.

2. Material and method

2.1. Expression of xylanase from C. reinhardtii

A cell-wall deficient strain of C. reinhardtii (cc3395) was previously transformed by the vector for the xylanase expression, including a gene cassette described by Rasala et al. [21]. Briefly, the coding region of the *Trichoderma reesei* Xyn1 gene (GenBank accession number: XM_006961749) was linked to the zeocin antibiotic resistance marker and FMDV cleavage peptide (ble-2A) and expressed from the nuclear genome under AR1 promoter with Ars1 secretion signal to accumulate xylanase in the culture media. A codon-optimized flag tag was inserted into the C-terminus of the Xyn1 protein sequence.

2.2. Immunoprecipitation of xylanase

The expression of xylanase enzyme was verified by the western blot analyses using Anti-Flag M2 affinity gel (Cat no: A2220 Sigma-Aldrich, USA) system. 50 mL of the culture media was separated from the cell pellet by centrifugation at 4100 rpm for 5 min at + 4 ℃ using a refrigerated centrifuge (NF 1200 R, Nüve, TR). 40 µL of resin was added to the supernatant and incubated for 1 h on a swing shaker (Duomax 1030, Heidolph, DE) at + 4 ℃. Cell pellet was sonicated in lysis buffer (cold TBS containing 0.05% Tween 20 and 1 mM phenylmthylsulfonyl fluoride) using a 3 mm sonicator probe tip (Bandelin Sonoplus 2070: 70 W HF power, 20 kHz). Lysed cells were centrifuged at 12,400 rpm for 30 min at 4 ℃ to separate the total soluble proteins. Equal amount of resin (40 µL) was added to the cell lysate for immunoprecipitation analysis of the cytoplasmic xylanase. Lysate was incubated with the resin on a roller shaker at 4 ℃ for 1 h. Flag resin was separated from the medium by centrifugation at 800 rpm for 1 min and transferred into an Eppendorf tube. 2x SDS-PAGE loading buffer was added on to the flag beads and incubated at 95 ℃ for 3 min to release and denature the protein. Protein was separated on 4–20% polyacrylamide gel (Miniprotean® TGXTM Precast Gels, BioRad, U.S.), and transferred to the nitrocellulose membrane at 10 volts overnight. The membrane was stained with Ponceau S solution to visualize the hole cell proteins and rinsed in Tris Buffered Saline-Tween 20 (TBS-T) solution before continuing to the antibody incubation. The membrane was incubated with Monoclonal Anti-flag M2-Alkalin Phosphatase (Cat. no: A9469, Sigma-Aldrich, USA) and developed with Alkaline Phosphatase Conjugate Substrate Kit (Cat. no:170–6432, Bio-Rad, USA).

2.3. Inoculum preparation

C. reinhardtii was maintained in TAP medium [27] with modifications. Briefly, 0.4 g NH_4Cl, 0.05 g $CaCl_2.2\ H_2O$, 0.1 g $MgSO_4.7\ H_2O$, 0, 0544 g KH_2PO_4, 0.1044 g K_2HPO_4, 2.42 g Tris-Base were dissolved in distilled water. 5 mL of trace mineral solution and 1 mL of glacial acetic acid were added to the culture, and the volume was adjusted to 1 L (pH 6.8). The media was autoclaved at 121 ℃ for 20 min. Preparation of trace mineral solution was as follows; 5 g disodium EDTA was dissolved in 400 mL distilled water. pH was neutralized to 6.5, and the following substances were dissolved with the following order: 0.5 g $FeSO_4.7\ H_2O$, 2.2 g $ZnSO_4.7\ H_2O$, 1.14 g H_3BO_3, 0.51 g $MnCl_2.4\ H_2O$, 0.016 g $CuSO_4.5\ H_2O$, 0.073 g $Na_2MoO_4.2\ H_2O$, 0.016 g $CoCl_2.6\ H_2O$. The final volume was adjusted to 500 mL and filtered through 0.22-µm vacuum cup filter.

The cultures were grown under a light intensity of 65 µE/m^2 s in an orbital shaker at 100 rpm at 24 ± 2 °C for 5 days and this culture was used as inoculum for all experiments. The specific growth rate (µ) of the cells was calculated from the initial logarithmic phase of growth as follows:

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (1)$$

where t_1 and t_2 (day) are the first and last days of the logarithmic growth phase and X_1 and X_2 (cells/mL) are the cell densities at the time of t_1 and t_2, respectively [28].

2.4. Optimization of microalgae production

Xylanase production was performed in 250 mL Erlenmeyer flasks containing 100 mL *C. reinhardtii* culture suspension at the cell concentration of 10^5 cells/mL. The flasks were incubated at 24 ± 2 °C in a rotary shaker incubator. Three different agitation rates (100, 120, 140 rpm) and two different light intensities (1500 and 3500 lux) were investigated as optimization parameters along with incubation time of 3, 5 and 7 days, to be optimized by response surface methodology (RSM). The central composite design was utilized to evaluate the effects of mentioned parameters on three responses including; biomass (mg/mL), xylanase specific enzyme activity (U/g) and protein content of culture supernatant (mg/L). An experimental plan consisted with 26 runs and the statistical analysis of the data was performed by using Design Expert 13 (Stat-Ease Inc., Minneapolis, USA). The following empirical second-order polynominal equation was used to define a predictive model to explain the effects of system variables on the response of biomass (mg/mL):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

where Y is the response of biomass (mg/mL), X_1, X_2 and X_3 are the factors of agitation rate (100, 120, 140 rpm), incubation time (3, 5 and 7 days) and light intensities (1500 and 3500 lux), respectively. β_0 is the intercept; β_1, β_2, β_3 are the coefficients of linear terms; β_{11}, β_{22} are the coefficients of squared terms while β_{12}, β_{13}, β_{23} are the coefficients of interactions among the independent variables.

The following empirical linear equation was used to define a predictive model to explain the effects of system variables on the response of protein content (mg/L):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \quad (3)$$

where Y is the response of protein content (mg/L), X_1, X_2 and X_3 are the factors of agitation rate (100, 120, 140 rpm), incubation time (3, 5 and 7 days) and light intensities (1500 and 3500 lux), respectively. β_0 is the intercept; β_1, β_2, β_3 are the coefficients of linear terms.

The following empirical linear equation was used to define a predictive model to explain the effects of system variables on the response of specific enzyme activity (U/g):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (4)$$

where Y is the response of specific enzyme activity (U/g), X_1, X_2 and

X_3 are the factors of agitation rate (100, 120, 140 rpm), incubation time (3, 5 and 7 days) and light intensities (1500 and 3500 lux), respectively. β_0 is the intercept; β_1, β_2, β_3 are the coefficients of linear terms; β_{12}, β_{13}, β_{23} are the coefficients of interactions among the independent variables, while β_{123} is the coefficient of interactions among the three independent variables.

2.5. Recombinant xylanase production in bubble column photobioreactor

The column photobioreactor consists of borosilicate glass tube (46 cm × 5 cm) with an inner thin aeration channel that goes to the bottom of photobioreactor. The total working volume was 500 mL for each photobioreactor, and the temperature was adjusted to 24 ± 2 °C. The agitation was performed through the air bubbles coming from aeration channel which was provided by an air pump. Different airflow rates (1.0 and 2.0 vvm) and light intensities (1500 and 3500 lux) were selected as experimental parameters for the photobioreactors.

2.6. Xylanase activity assay

The xylanase activity was determined by 3,5-Dinitrosalicylic acid (DNS) method with some modifications [29]. Breifly, 1.0% birchwood xylan was homogenized in 0.05 M Na-acetate buffer (pH 4.7) as substrate solution and 0.2 mL enzyme solution was mixed with 1.8 mL substrate in 50 °C for 5 min. Then, the reaction was stopped by the addition of 3 mL of DNS reagent and boiling for 15 min. The absorbance of the mixture was measured at 540 nm, after it cooled in ice-cold water for 1 min. For the calibration curve, xylose standard solutions were prepared in Na-acetate buffer at different concentrations and substituted with the enzyme. The slope of this calibration curve was used in the following enzyme activity calculation formula. One unit of xylanase activity (U) was defined as the amount of enzyme required to release 1 µmol of xylose equivalent per min. The xylanase activity was calculated using the following formula:

$$Activity \quad (U/L) = \frac{\left(\frac{ABS}{slope}\right) * V_{(enzyme+substrate)} * Dilution}{V_{enzyme} * t_{\min}} \tag{5}$$

2.7. Biomass and protein content determinations

50 mL culture from each sample was centrifuged, and cell pellets were dried in an incubator overnight at 60 °C. Dry cell pellets were weighed, and mg/mL values were calculated and recorded as biomass. Total protein content was determined by the Lowry protein assay, using *DC* Protein Assay Kit (Bio-Rad Laboratories, Inc). Bovine serum albumin (BSA) was utilized as the standard.

3. Results and discussion

3.1. Xyn1 expression from microalgae

In this study, a cell-wall deficient mutant strain of *C. reinhardtii* (cc3395) was used to optimize the xylanase production from the microalgal platform. Expression of xylanase was validated by the western blot analysis. Two protein bands at 25 kD and 36 kD were observed corresponding to the Xyn1 gene product. While the smaller protein band was the expected band of xylanase, the larger one may be the result of unreleased protein from the *ble-2A* peptide [21] (Fig. 1). He et al. (2019) demonstrated the effect of glycosylation on Xyn2 (gene from *T. reesei*) expressing *Pichia pastoris* for the xylanase production [30]. A significant decrease was observed in the enzymatic activity of the xylanase after deglycosylation of the enzyme [30]. Although yeast system is highly efficient to produce high quantities of recombinant enzymes, over-glycosylation of the proteins, and the methanol requirements of the process, make the microalgal platform safer and more economical compared to this platform.

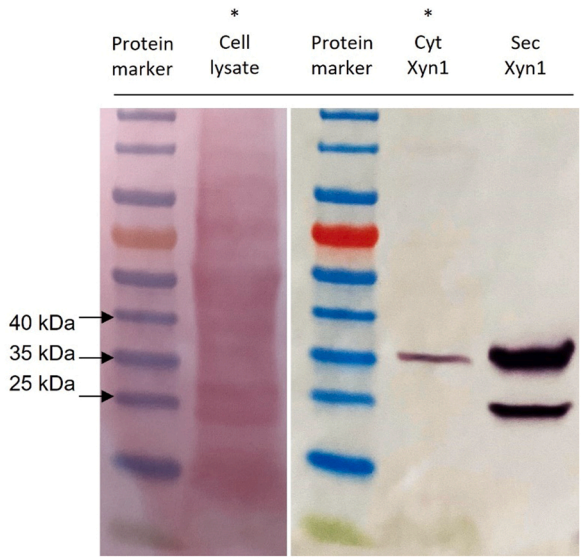


Fig. 1. Cell lysate: Ponceau S staining of the cell lysate on the transfer membrane. Cyt Xyn1: immunoprecipitation of the xylanase from the cell cytoplasm. Sec Xyn1: immunoprecipitation of the secreted xylanase (10x concentrated) from the culture media. * The same sample.

3.2. Optimisation of microalgae production

The central composite design for xylanase production by *C. reinhardtii* was shown in Table 1 together with the experimental biomass, protein content and specific enzyme activity results of the total 26 runs. The biomass of the culture, the protein concentration, and the enzyme activity were optimized using RSM based on central composite design, with two numeric (agitation rate:A and culture incubation time: B) and one categoric (light intensity:C) factors. Analysis of variance (ANOVA) for the response surface quadratic model was applied for biomass response and the accuracy of the model was manifested by R^2 (Table 2). It was calculated as 0.95 for biomass response indicating a very good correlation between the experimental and predicted values of the response. For protein contents and specific enzyme activity, linear fit models determined, and lack of fit values were not significant for both of the responses ($p < 0.05$). However, R^2 values of the linear fit models were 0.4928 and 0.5635 for protein content and specific enzyme activity, respectively, and lower than the acceptable range of 0.6. Therefore, CCD analyses were evaluated for the biomass only, and protein related responses were excluded from the design.

Incubation time of the culture appeared as the main factor for specific enzyme activity, presumably due to the increasing protease production from the cell over time. Specific enzyme activity was shown to be improved by both under the effect of light and agitation in RSM trials. Culture conditions at the low light intensity with slower agitation speed (1500 lux / 100 rpm) revealed similar results as high light and high-speed conditions (3500 lux / 140 rpm). The optimum conditions for the maximised biomass response were identified as 139.8 rpm and 6.997 days at 3500 lux by the RSM design (Fig. 2). The optimum biomass concentration was 0.71 mg/mL (desirability 1).

3.3. Recombinant xylanase production in bubble column photobioreactor

Bubble column photobioreactors were set and compared for the same responses under different airflow rates (1.0 and 2.0 vvm) and light intensities (1500 and 3500 lux). In photobioreactor experiments, as seen in Fig. 3, specific growth rates of *C. reinhardtii* were the same for both produced in photobioreactor aerated at 1.0 vvm, while increasing

Table 1
The central composite design for xylanase production by *C. reinthardii*.

Run	Factor 1 A: agitation rate (rpm)	Factor 2 B: incubation time (day)	Factor 3 C: light intensity (lux)	Response 1 Biomass (mg/mL)	Response 3 Protein content (mg/L)	Response 2 Specific enzyme activity (U/g)	Enzyme activity (U/L)
1	120	5	1500	0.36	72.84	2157.5	157.2
2	120	5	3500	0.40	83.71	1666.7	139.5
3	140	3	3500	0.18	61.97	4285.7	265.6
4	140	5	3500	0.60	72.84	3092.0	225.2
5	100	7	3500	0.64	147.84	1182.4	174.8
6	100	3	3500	0.48	63.05	3055.6	192.7
7	120	5	3500	0.34	34.79	4428.6	154.1
8	120	5	1500	0.36	140.23	535.7	75.1
9	100	5	3500	0.71	86.97	3218.4	279.9
10	120	5	1500	0.36	82.62	3283.1	271.2
11	120	3	1500	0.12	90.23	2861.1	258.2
12	140	3	1500	0.16	57.62	3275.9	188.8
13	120	3	3500	0.18	61.97	4700.0	291.3
14	120	5	3500	0.34	81.53	1524.4	124.3
15	120	7	1500	0.52	104.36	2802.2	292.4
16	120	5	1500	0.42	75.01	2100.0	157.5
17	120	7	3500	0.52	113.06	1293.2	146.2
18	100	5	1500	0.62	92.40	4700.5	434.3
19	120	5	3500	0.44	85.88	2558.1	219.7
20	140	5	1500	0.58	86.97	1580.5	137.5
21	140	7	1500	0.64	105.45	690.5	72.8
22	140	7	3500	0.68	132.62	1071.4	142.1
23	100	7	1500	0.56	109.79	772.7	84.8
24	100	3	1500	0.38	71.75	4548.6	326.4
25	120	5	1500	0.36	89.14	2949.4	262.9
26	120	5	3500	0.38	78.27	2692.3	210.7

Table 2
ANOVA for the experimental results of the central composite design of three responses biomass, protein content and specific enzyme activiy as a function of agitation rate (A), culture incubation time (B) and light intensity (C).

	Fit Model	Model	p-value	Lack of Fit	R^2
Biomass (mg/mL) = 0.39–0.045 A + 0.17B + 0.012 C + 0.08AB - 0.015AC - 005BC + 0.19A^2 - 0.098B^2	Quadratic	Significant	< 0.0001	Not significant	0.9481
Protein Concentration (mg/L) = 87.80–4.53 A + 25.54B - 2.84 C	Linear	Significant	0.0016	Not significant	0.4928
Specific enzyme activity (U/g) = 2577.94–290.18 A - 1242.88B + 96.58 C - 18.82AB + 455.63 AC - 172.84BC - 316.45 ABC	Linear	Significant	0.0189	Not significant	0.5635

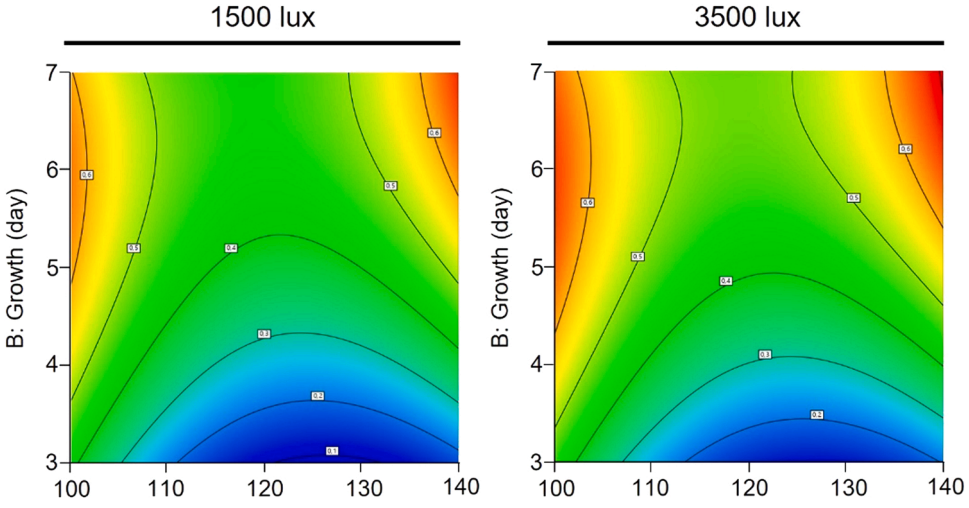
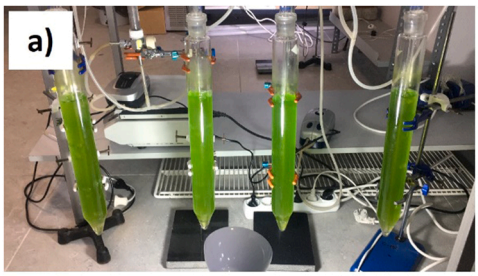
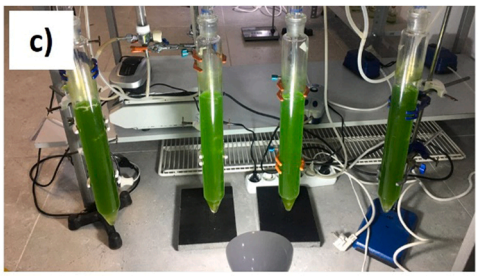


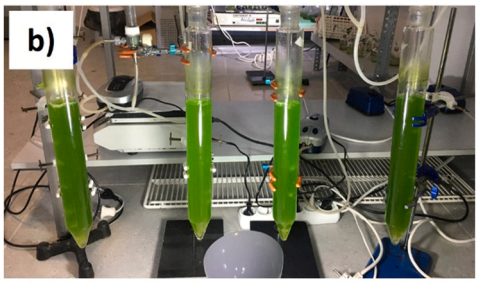
Fig. 2. Contour plots obtained from RSM trials for biomass in response to the changes in incubation time and agitation rate at low light (1500 lux) and high light (3500 lux) conditions.

aeration rate to 2.0 vvm caused different trends depending on the light intensity. Under the 2.0 vvm aeration rate, the specific growth rate improved to 0.125 day-1 for high light intensity (3500 lux) compared to the lower one (1500 lux).

Additionally, increasing the aeration rate from 1 vvm to 2 vvm resulted in improved enzyme activity (U/L) (Fig. S1) and specific







d)	Specific growth rate (μ)
1.0 vvm 1500 lux	0.1
1.0 vvm 3500 lux	0.1
2.0 vvm 1500 lux	0.075
2.0 vvm 3500 lux	0.125

Fig. 3. The images of aerated and illuminated bubble column photobioreactors from the 3rd (a), 5th (b), and 7th days cultures. Specific growth rates calculated for each photobioreactor (d).

enzyme activity (U/g) (Fig. 4). Since the specific enzyme activity is the ratio of enzymatic activity to protein content, the superior increase in protein content compared to the enzymatic activity rise resulted in the observation of declining specific enzyme activity of xylanase in the later days of the culture of *C. reinhardtii* produced in photobioreactors. The high light and high-aeration conditions (3500 lux / 2.0 vvm) on the 3rd day resulted in the maximum specific enzyme activity (6277.7 U/g) for the photobioreactor experiments, and it was followed by activity of 6048.2 U/g on the 5th day. This result has presented the achievement of xylanase enzyme activity produced in the photobioreactors compared to the bench-scale erlen-mayer cultures, where 4700.5 U/g was reached for the maximum specific enzyme activity. Even though, the high aeration rate beyond a certain critical limit can decrease the productivity of the bioprocess by providing less holding time to the air bubbles inside the culture media and creating oxygen limitation [31], the aeration rate of 2.0 vvm resulted in not exceeding to critical aeration limit for our bubble column photobioreactor under both illuminations. Similarly, increasing the light intensity from 1500 lux to 3500 lux provided a

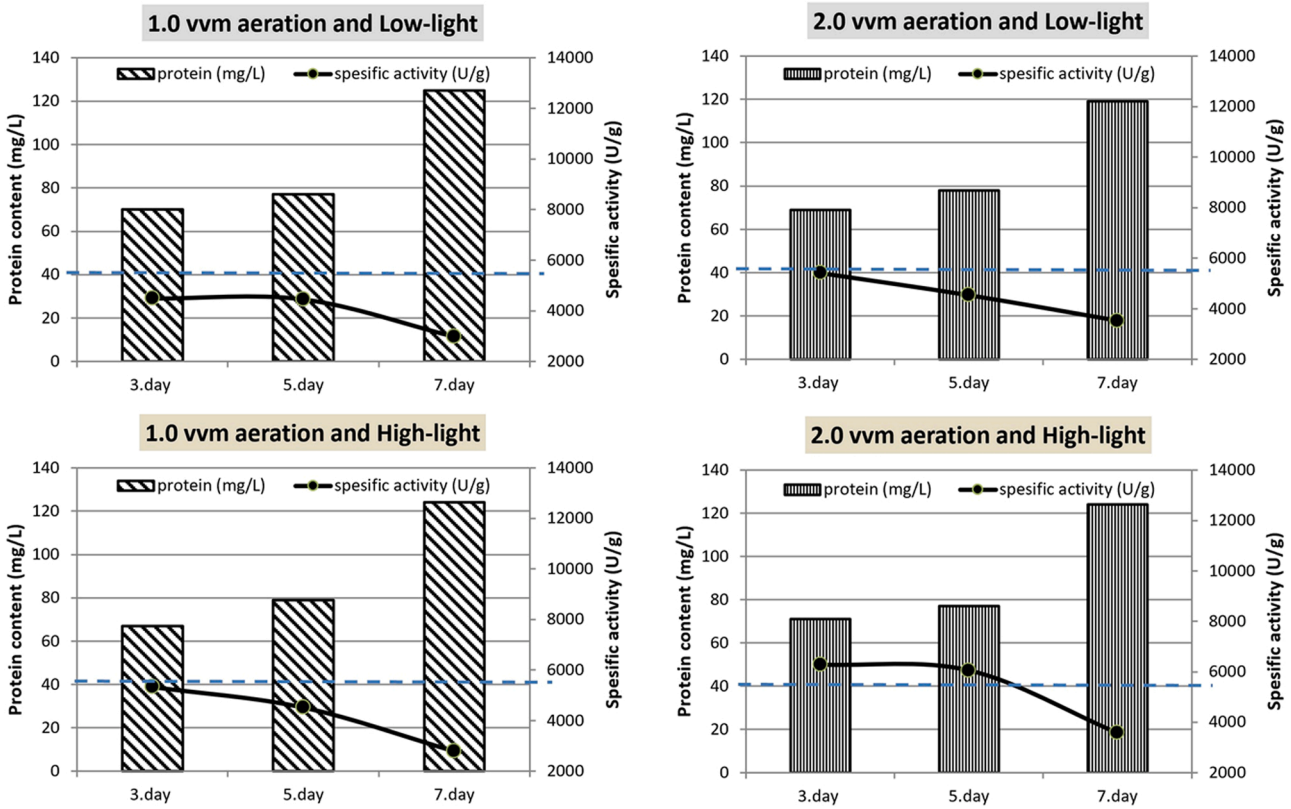


Fig. 4. Specific xylanase enzyme activity and protein concentration values of culture broth of *C. reinhardtii* produced in bubble column photobioreactors.

positive effect on xylanase activity produced in photobioreactors, especially in the earlier phases. The specific enzyme activity increased from 4489.8 U/g to 5330.5 U/g with the effect of high light on the 3rd day of the photobioreactor experiments with 1 vvm aeration rate. On the other hand, when the aeration rate was increased to 2 vvm, specific enzyme activity reached 5424.4 U/g under the low light conditions and hit the maximum value (6277.7 U/g) under higher illumination (3500 lux) (Fig. 4).

Yadav et al. [32] demonstrated the purification and characterization of extracellular xylanase obtained from optimized bacterial cultures, *Anoxybacillus kamchatkensis* NASTPD13 [32]. The crude bacterial xylanase showed a specific activity of 3.01 U/mg (3010 U/g). While the specific xylanase activity increased up to 3890 U/g after ammonium sulfate (80%) precipitation, following dialysis and column purification showed an 11-fold increase in specific activity up to 33,500.00 U/g [32]. The specific enzyme activity of crude xylanase extracellular extract synthesized by *Streptomyces* P12–137 strain also showed an increase from 10,884 U/g to 45,444 U/g after being subjected to ammonium sulphate precipitation (40–90%) followed by dialysis [33]. Georgianna et al. [22] produced xylanase in the chloroplast of *Dunaliella tertiolecta* and *C reinhartdii* with a specific activity of 57.79 ± 10.04 U/mg (approximately 1% of the total algal protein), and 276.58 U/mg, respectively [22]. Although the enzyme production levels of algae are lower than the bacterial and yeast expression systems, the ease of scale-up and low production costs of photosynthetic microalgae will also make it a competitive platform for xylanase and other recombinant enzymes. Microorganisms such as bacteria, yeasts and filamentous fungi naturally secrete xylanase with higher specific enzyme activity. However, these organisms also secrete other cellulose-degrading enzymes, such as cellulases, whose activities could have adverse effects on industrial applications [21]. Therefore, heterologous expression of this enzyme would be a better choice where only specific activity of the xylanase gene product is desired.

4. Conclusion

Recombinant DNA technology is a powerful tool to enhance the production of xylanase enzymes with specific activity from various host organisms. There have been several studies focused on the improvement of gene expression from microalgal platform from both molecular and process engineering perspectives. In this research, we identified the effect of three important factors of algal culture, namely, light intensity, agitation speed, and incubation time of the culture on biomass. Optimum biomass value was 0.71 mg/mL. The xylanase production of *C. reinhardtii* in bubble column PBR reached 6277.7 U/g under 3500 lux illumination and 2.0 vvm aeration rate. Further optimizations in large scale production of xylanase may reveal the advantage of the microalgae platform as an edible, safe and cost effective-system.

CRediT authorship contribution statement

AY, EIA, AD, and SS planned the experimental design, SPM supervised the molecular analysis, AY conducted the molecular analyses and RSM trials. AY and EIA performed the PBR setup and analyses, EIA and AD performed the enzyme activity tests. AY, EIA and AD wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2023.108967.

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