Intensification of Recombinant Laccase Production from Sago Bioethanol Liquid Waste and Evaluation of The Enzyme for Synthetic Dye Decolourisation

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Abstract

Purpose: Laccases are polyphenol oxidases that have diverse industrial applications. High industrial demand of laccases has necessitated the development of cost-effective production of recombinant laccases. One of the ways is by utilizing waste stream that has valuable composition for supporting microbial growth and metabolite expression. This work aims to intensify the production of recombinant laccases in *Pichia pastoris* GS115 using sago bioethanol liquid waste (SBLW) as a feedstock.

Method: Production of laccases from SBLW was intensified by the addition of glycerol ranging from 0.5% - 2.5% (w/v). The fermentation performance was assessed based on the biomass concentration and laccase activity. The resulting laccases produced were further evaluated in terms of the capability of the enzyme to decolourise Remazol Brilliant Blue R dye.

Results: The results showed that supplementation of SBLW with 2% (w/v) glycerol yielded improvements of 1.9-fold and 2.1-fold of biomass concentration and laccase activity, respectively in comparison to that achieved by fermentations using the standard Buffered Methanol-Complex Medium. Furthermore, the laccases produced using the optimal SBLW medium yielded a decolourisation percentage of 68.6% under non-optimised conditions and in the absence of mediators. The value represents 91% of the decolourising capability of laccases produced using the standard BMMH.

Conclusion: In general, this works represents a further step towards low-cost production of recombinant laccases using renewable feedstocks.

Keywords: Bioethanol waste; Dye decolourisation; Glycerol; Laccases; Sago fibre; Vinasse.

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Introduction

Laccases are ligninolytic enzymes that catalyse oxidative reactions of many phenol compounds and amines. The enzyme serves as one of the important biocatalysts for many industrial applications, particularly in the environmental and bioenergy fields (Singh and Gupta, 2020). Laccases have been extensively used for wastewater treatment, degradation of lignocellulosic residues (Mehrabian, et al., 2018) and xenobiotics (Darvishi, et al., 2018). In spite of the extensive uses and high demand of laccases, the industrial production of

laccases is often hampered by several factors. One of them is the low production of natural laccases. Hence, improved production of laccases using recombinant organisms is seen as a solution to enhance the industrial production of the enzyme. The use of engineered laccase producing strains also enables the consumption of wider types of carbon sources such as sucrose (Madzak, et al., 2005) and glycerol (Mamat, et al., 2021). This in turn will allow the adoption of wider range of fermentation feedstock for laccase production.

Another bottleneck that hampers the production of industrial laccases is the high cost of production (Singh and Arya, 2019), which is mainly due to the use of expensive synthetic fermentation media for cultivating laccase producing organisms. Amongst the popular strategies to reduce the production cost is the adoption of alternative fermentation feedstocks of which agricultural and industrial wastes have increasingly become amongst the preferred options (Sharma, et al., 2017; Wang, et al., 2019). One of the potential wastes yet still understudied is liquid waste from bioethanol fermentation, which comprises several useful nutrients for fermentations (Suhaili, et al., 2019). Currently, the liquid waste from bioethanol fermentation is mainly disposed to water streams causing severe ecological impacts.

In our previous work, we reported the feasibility of using sago bioethanol liquid waste (SBLW) for recombinant laccase production by *Pichia pastoris* GS115 (Mamat, et al., 2021). Our results showed that the highest activity of recombinant laccases produced from SBLW represented 73% of that obtained using the standard synthetic medium. In the same work, we have also addressed the influence of yeast extract on *P. pastoris* GS115 fermentation whereby 1% (w/v) was concluded as the best concentration of yeast extract for laccase production. Nonetheless, the SBLW medium formulation has yet to be fully optimised. Thus, continuous optimisation of the fermentation performance is therefore crucial. Furthermore, the efficacy of recombinant laccases produced from novel feedstocks should also be evaluated to ensure the applicability of the enzyme.

In this study, we investigate the feasibility of enhancing recombinant laccase production in *P. pastoris* GS115 by adding glycerol at different concentrations to SBLW medium. The previous optimal medium formulation as reported by Mamat, et al. (2021) namely 40% (v/v) SBLW enriched with 1% (w/v) yeast extract was used as the basic medium. SBLW was produced artificially by mimicking the actual production of bioethanol using sago fibre whereby the resulting spent broth was recovered upon the removal of ethanol by distillation. The best formulation of glycerol for *P. pastoris* GS115 fermentation was determined based on the maximum biomass concentration and laccase activity. Following that, laccases produced from fermentations using the optimal medium formulation was further evaluated in terms of the efficacy of the enzyme to decolourise Remazol Brilliant Blue R (RBBR), a widely used dye in the textile industries. To the best of our knowledge, this report is amongst the earliest to report the production of recombinant laccases from sago bioethanol waste stream and the evaluation of the enzyme for bioremediation application.

Materials and Methods

Microorganisms

Saccharomyces cerevisiae was used for the bioethanol fermentation. Recombinant *Pichia pastoris* GS115 (His-, Mut+) carrying pPICZB/eLcc1 expression vector harbouring laccase

gene derived from *Marasmius cladophyllus* (Sing, 2017) was used for laccase fermentation. The two microorganisms were obtained from Biochemistry Laboratory and Molecular Genetics Laboratory of Faculty of Resource Science Technology, UNIMAS, respectively.

Media

Sago fibre was supplied by Herdson Sago Mill, Pusa, Sarawak. Initially, sago fibre was pre-treated and hydrolysed according to a procedure by Awang-Adeni and co-workers (2013), producing sago fibre hydrolysate (SFH), which was then used as a feedstock for bioethanol fermentation. Buffered Methanol Glycerol Histidine (BMGH) consisting of 0.1 M potassium phosphate, 0.02% (w/v) biotin (ACROS, China), 13.4% (w/v) yeast nitrogen base (YNB) (BD Difco, France) and 0.4% (w/v) histidine (Sigma-Aldrich, USA) was used as a seed culture medium for *P. pastoris* GS115. BMMH consisting of 0.1 M potassium phosphate, 1.34% (w/v) YNB, 0.02% (w/v) biotin, 0.004% (w/v) histidine and 0.5 % (v/v) methanol (Merck, Germany) was used as the standard medium for producing laccases by the recombinant *P. pastoris* GS115. Before sterilisation, the initial pH of BMGH and BMMH was adjusted to 6.

Preparation of *Saccharomyces cerevisiae* seed culture

One loop of the single yeast colony from the culture grown on PDA agar was transferred into 100 mL sterile inoculum medium consisting of 20 gL^{-1} glucose and 5 gL^{-1} yeast extract. The culture was then incubated on an orbital incubator shaker (Hotech Instruments Corp, Taiwan) at 30 °C at 100 rpm for 9 to 12 h. Once the optical density (OD) of the culture reached 0.5 to 0.6, the culture was harvested and centrifuged at 10,000 rpm for 10 min to obtain the yeast pellet. Lastly, the recovered yeast pellet was used to inoculate fresh fermentation media.

Bioethanol fermentation and recovery of SBLW

Bioethanol fermentation by *S. cerevisiae* was carried out in 1-L shake flasks with a working volume of 500 mL. SHH with the initial concentration of glucose standardised to 55 gL^{-1} supplemented with 5 gL^{-1} yeast extract was used the feedstock. The initial pH of the culture was adjusted to 5.5. The cultures were shaken on an orbital shaker (Hotech Instruments Corp, Taiwan) at 150 rpm and at 30 ^{o}C for 48 h. The cell growth and bioethanol production were monitored by withdrawing aliquots of the culture broth every 12 h. Following the bioethanol fermentation, the culture broth was centrifuged at 10 000 rpm for 10 min. The liquid portion was recovered and heated at 80 ^{o}C using a water evaporator to distill the ethanol. The resulting liquid (SBLW) was initially characterised for its glycerol concentration using High Performance Liquid Chromatography (HPLC). After that, it was autoclaved and diluted to 40% (v/v) where BMMH was used as the solvent (Mamat et al., 2021). The diluted SBLW was then used as a feedstock for *P. pastoris* GS115 fermentation.

Preparation of *Pichia pastoris* GS115 seed culture

A single colony of *P. pastoris* GS115 was transferred into 40 mL of sterile BMGH medium in 100-mL baffled shake flask. The seed culture was then shaken overnight on an orbital shaker at 170 rpm at 30 C. When the OD of the culture reached 5 to 6, the culture was then withdrawn and centrifuged at 4000 rpm for 10 min before the resulting pellet was used as an inoculum for *P. pastoris* GS115 fermentation. In all fermentations, the seed culture was standardised to 10% (v/v).

Pichia pastoris GS115 fermentation

The fermentations were conducted using BMMH as the control medium and 40% (v/v) SBLW with 1% (w/v) yeast extract as proposed by Mamat, et al. (2021). In the latter formulation, the effect of different concentrations of glycerol (0.5% - 2.5% (w/v) on the fermentation performance was evaluated. All cultures were shaken at 170 rpm on an orbital shaker at 30 °C. Absolute methanol to a final concentration of 0.5% (v/v) was added to the cultures every 24 hour. All fermentations were conducted in triplicate. Throughout the fermentation, aliquots of culture broth were removed every 48 hours for cell growth and laccase assay.

In vitro decolourisation of Remazol Brilliant Blue R

The crude laccases produced using the optimised SBLW medium and BMMH were tested for in vitro decolourisation of Remazol Brilliant Blue R (RBBR) (Sing, 2017). The reaction was performed in a test tube, containing 1.98 mL of the crude laccases and 0.02 mL of 200 mgL^{-1} RBBR dye making a final volume of 2 mL. The reaction mixture was incubated for 15 days at room temperature. The decolourisation was monitored every three days by measuring the absorbance of the reaction mixture at 595 nm using a UV mini-1240v spectrophotometer. The reaction was conducted in triplicate. The decolourisation percentage of RBBR was calculated according to Equation 1:

$$\textit{Decolourisation percentage}\ \ (\%) = \frac{Ac - As}{Ac}\ x\ 100 \qquad \text{Equation 1}$$

Where A_c is the absorbance of the initial absorption of RBBR on Day 0 and A_s is the absorbance of the final absorption of RBBR on Day 15.

Analytical methods

Optical density measurement

The measurement of the OD of the culture broth was determined at 600 nm using a spectrophotometer (UVmini 1240, Shimadzu). Whenever required, the culture broth was diluted with distilled water to ensure the OD readings remained in the range of 0.1 to 0.9. The OD value was then translated to an equivalent dry cell weight (DCW) according to an established standard curve.

Laccase assay

The laccase activity was determined based on 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (Sing, 2017). About 0.4 mL of culture supernatant consisting of crude laccase was mixed with 0.1 mL 5 mM ABTS and 0.5 mL of 0.1 M sodium acetate buffer (pH 5). The reaction mixture was incubated for 2 min at room temperature before its absorbance value was measured at 420 nm using a spectrophotometer. Boiled culture supernatant was used to replace the crude laccase in the blank sample. The laccase activity was determined using Equation 2.

$$\textit{Enzyme activity}\ \left(\frac{U}{mL}\right) = \frac{\Delta A\ \times V\ \times 10^3}{v\ \times\ \varepsilon\ \times d} \qquad \text{Equation 2}$$

Where ΔA denotes absorbance change (min^{-1}), V is total reaction volume (mL), v is enzymatic extract volume (mL), ε is molar absorptivity (36000 $M^{-1}cm^{-1}$) and d is cuvette diameter (cm). One unit of activity (U) is equivalent to the amount of laccases that oxidises 1 µmol of ABTS per min.

Statistical analysis

The data were statistically analysed using SPSS Statistics 23 Software (IBM, United States). The means were compared using Tukey's test via one-way analysis of variance (ANOVA). The results were considered significantly different when the p-value was below 0.05.

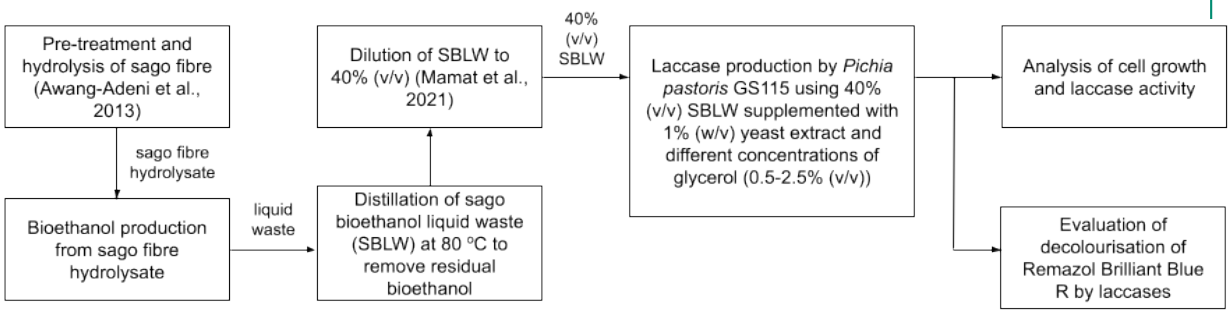

Figure 1. Overview of the workflow of the research

Results and Discussion

Effect of supplementation of optimised SBLW medium with different concentrations of glycerol on *Pichia pastoris* GS115 growth

Having identified the optimal concentration of SBLW medium for laccase production by *P. pastoris* GS115 in our preceding report (Mamat, et al., 2021), our subsequent work aims to determine the influence of supplementing the SBLW medium with glycerol on the performance of *P. pastoris* GS115 fermentation. The best formulation as concluded by Mamat, et al. (2021), which comprises 40% (v/v) SBLW and 1% (w/v) yeast extract, was used as the basic medium. The range of glycerol concentration added to the optimised SBLW medium was from 0.5% to 2.5% (v/v). Figure 2 shows the comparison of *P. pastoris* GS115 growth when cultivated using BMMH and SBLW-based media.

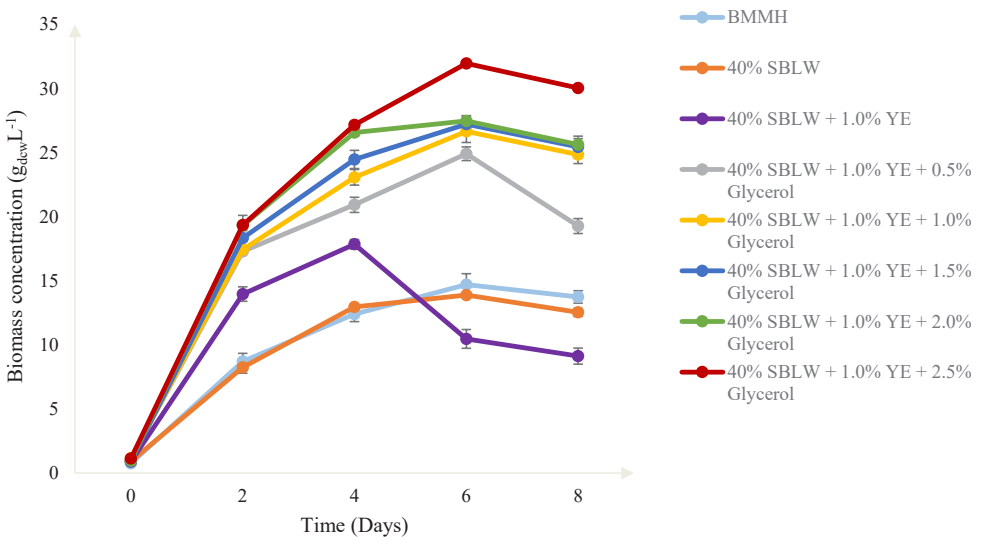

Figure 2. Comparison of batch fermentation kinetics of *P. pastoris* GS115 when cultured in BMMH, non-supplemented 40% (v/v) SBLW, 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and glycerol at different concentrations. Error bars indicate one standard deviation about the mean (n=3). Key: YE-yeast extract

The results showed that in general, all cultures supplemented with glycerol with the concentration between 0.5% and 2.5% showed an enhancement of maximum biomass concentration in comparison to that achieved in cultures using BMMH and SBLW medium without glycerol. The highest increment was shown by the cultures that were added with 2.5% (v/v) glycerol, which were 2.2 and 1.8-fold over that achieved in fermentations using BMMH and SBLW medium without glycerol, respectively.

Table 1 outlines the specific growth rate along with maximum biomass concentration obtained from fermentations using BMMH and SBLW-based media. Generally, the specific growth rate increased about 0.9 to 1.2-fold when the fermentation medium containing 40% (v/v) SBLW and 1.0% (w/v) yeast extract was added with 0.5% to 2.5% (v/v) glycerol. Adding 2.5% (v/v) glycerol to the SBLW medium during *P. pastoris* GS115 fermentation has resulted in the maximum specific growth rate (0.22 h^{-1}) representing 2.2 and 1.2-fold enhancements to that achieved by cultures using BMMH and SBLW medium without glycerol.

Table 1. Kinetic parameters for *P. pastoris* GS115 growth when cultured on BMMH, 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and glycerol at different concentrations. Key: YE-yeast extract

Medium	Specific growth rate, μ (h^{-1})	Maximum biomass concentration, X_{max} (g_{dcw} L^{-1})
Standard BMMH	0.10	14.70
40% SBLW + 1.0% YE	0.19	17.85
40% SBLW + 1.0% YE + %0.5 glycerol	0.17	24.91
40% SBLW + 1.0% YE + %1.0 glycerol	0.19	26.65
40% SBLW + 1.0% YE + %1.5 glycerol	0.19	27.22
40% SBLW + 1.0% YE + %2.0 glycerol	0.19	27.47
40% SBLW + 1.0% YE + %2.5 glycerol	0.22	31.96

BMMH is widely known as a standard synthetic medium for growing *P. pastoris*. The idea of transitioning to using alternative media for fermentation is due to the high cost of fossil-based media. Hence, the use of renewable feedstocks such as liquid stream from bioethanol production, which comprises useful fermentable components, is seen as an alternative strategy to reduce the fermentation cost. Although SBLW contains certain amount of residual glycerol (~3.25 gL^{-1}) (Mamat, et al., 2021), the amount may not be sufficient to maximise the production of biomass and recombinant laccases by *P. pastoris* GS115. As shown in this work, the residual glycerol in SBLW when used for growing *P. pastoris* GS115 only supported the maximum biomass concentration of 17.85 $g_{dcw}L^{-1}$. The increment of maximum biomass concentration as well as specific growth rate when additional glycerol was added to the SBLW containing cultures clearly suggest the significant benefit of supplementing SBLW medium with glycerol. Our results are in agreement with some previous works as reported in the literature (Jungo, et al., 2007; Tang, et al., 2009).

Glycerol has gained an increasing interest in its adoption as an alternative carbon source for many microorganisms due to several advantages such as its low cost due to its high availability. *Pichia pastoris* used in this study is a methylotrophic yeast, which depends on methanol as a carbon and energy source. However, the yeast can also consume glycerol as an alternative carbon source as the strain is glycerol-dependent (Arias, et al. 2017). The use of glycerol for *P. pastoris* fermentation can repress the protein expression whilst allowing maximum cell growth during the log phase (Zepeda, et al., 2008).

Below the growth inhibitory concentration, increasing the glycerol concentration in the fermentation media may result in an increasing biomass concentration. However, selection of the optimal glycerol concentration for any bioproductions should also consider the effect of glycerol on the production of the target protein. Given the low cost of SBLW despite the addition of glycerol that was proven beneficial for increasing the maximum biomass concentration during *P. pastoris* GS115 fermentation, the cost of the whole aforementioned fermentation medium is still considered cheaper than the synthetic BMMH medium.

Effect of supplementation of SBLW medium with glycerol on recombinant laccase activity

The corresponding laccase activity from fermentations using 40% (v/v) SBLW with 1.0% (w/v) yeast extract supplemented with different concentrations of glycerol was determined. Figure 3 shows the comparison of the activity of laccases produced by *P. pastoris* GS115 when cultured in BMMH, non-supplemented 40% (v/v) SBLW and 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and different concentrations of glycerol.

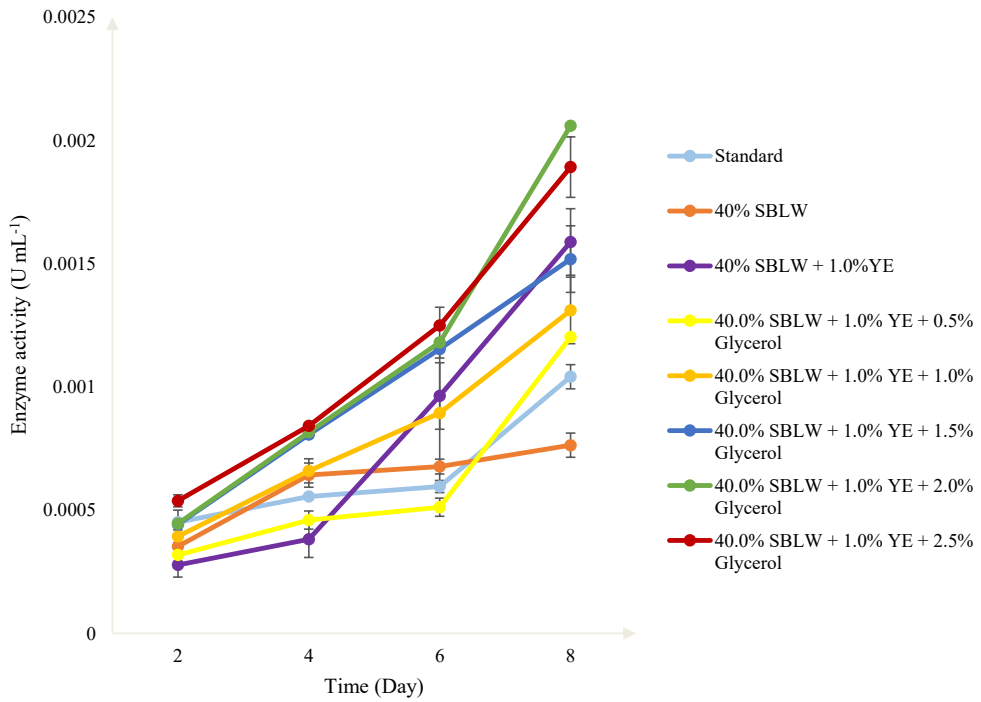

Figure 3. Comparison of laccase activity of *P. pastoris* GS115 when cultured in BMMH, unsupplemented 40% (v/v) SBLW, 40% (v/v) SBLW supplemented with 1.0% (w/v) YE and 40% (v/v) SBLW supplemented with 1.0% (w/v) YE and glycerol at different concentrations. Error bars indicate one standard deviation about the mean (n=3). Key: YE-yeast extract

The results showed that adding 0.5% to 1.5% (v/v) glycerol to 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract had no significant benefit on laccase activity in comparison to that attained in cultures using BMMH and 40% (v/v) SBLW in the absence of glycerol. However, addition of glycerol in the range of 2.0% to 2.5% (v/v) to *P. pastoris* GS115 fermentations containing SBLW-based media was found to have a positive effect on laccase activity. The highest laccase activity was attained in fermentations using 40% (v/v) SBLW supplemented with 1% (w/v) yeast extract and 2.0% (v/v) glycerol. The maximum laccase activity attained from the aforementioned medium represented 2.1-fold and 1.3-fold over that achieved in fermentations using BMMH and 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract only.

The enhancement of laccase activity as a result of supplementation of SBLW-based medium with glycerol as shown in this work, can be associated with the high reduction degree of glycerol (4.27) compared to other sugars where this in turn facilitates the yield of metabolites in higher amount (da Silva, et al., 2009). Additionally, the increased enyzme activity with supplementation of glycerol could also be attributed to co-assimilation of different carbon source by *P. pastoris* as evident in some earlier studies when carbon such as methanol and glycerol (Sola, et al., 2007) or methanol and glucose (Paulová, et al., 2012) are both added into the culture medium. The results in this work are in parallel with some literature reports (Rodríguez Couto, et al., 2006; Teerapatsakul, et al, 2007; Li et al., 2011; Kenkebashvili, et al., 2012). Considering both maximum biomass concentration and laccase activity, it can be concluded that the best formulation of fermentation medium for recombinant laccase production by *P. pastoris* GS115 in this study is 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and 2.0% (v/v) glycerol.

Determination of the efficacy of laccases produced from the optimised SBLW medium

Upon the successful production of laccases from SBLW, the subsequent aim was to evaluate the efficacy of laccases produced to decolourise a synthetic dye. RBBR was chosen since the dye is amongst the most widely used industrial dyes. RBBR is an anthracene derivative and it belongs to a group of organopollutants that are frequently poisonous and recalcitrant (Xin, et al., 2013). Crude laccases produced from *P. pastoris* GS115 fermentations using the optimal SBLW medium (40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and 2.0% (v/v) glycerol) was evaluated for in vitro RBBR decolourisation. Figure 4 depicts the UV-spectrophotometer analysis of RBBR dye before and after the decolourisation by laccases produced from the fermentations using BMMH and 40% (v/v) SBLW supplemented with 1.0% (w/v) yeast extract and 2.0% (v/v) glycerol.

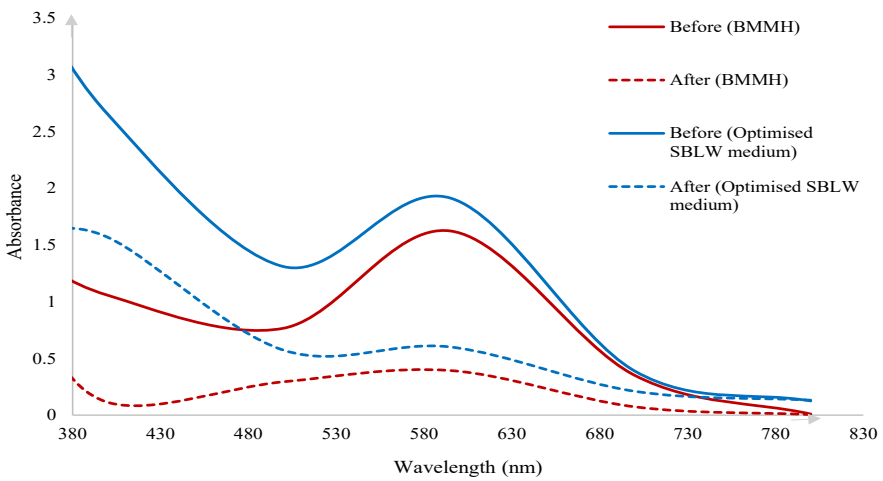

Figure 4. UV-spectrophotometer analysis of RBBR dye before and after the decolourisation by crude laccases obtained from fermentations using BMMH and the optimised SBLW medium

The profile shows that the absorbance spectrum of the reaction mixtures consisting of crude laccases produced from BMMH and the optimised SBLW medium reduced after the incubation period. The decrease of the absorbance values and change of the absorbance spectrum clearly indicates degradative colour reduction of RBBR dye by the crude laccases. The RBBR decolourisation rates by laccases produced from BMMH and the optimised SBLW medium were 75.8% and 68.6%, respectively. The values are within the range of what has been reported by previous works (Forootanfar, et al., 2012; Yadav, et al., 2021). It is worth mentioning that the decolourisation rates achieved by laccases in this study were obtained under non-optimised conditions and in the absence of mediators. It is expected that the rate can be enhanced upon the optimisation of the enzymatic reaction. The structure of the dye and enzyme, as well as the redox potential of the enzyme play important roles in determining the rate of decolourisation (Xin, et al., 2013). The dye can be efficiently degraded, and the decolourisation rate can be enhanced by the addition of natural redox mediators such as acetosyringone and syringaldehyde to the reaction mixture (Mani, et al., 2018). Moreover, the types of laccases producing organism also influence the physiological functions of laccases produced (Janusz, et al., 2020).

In general, the ability of the laccases produced from SBLW to decolourise RBBR as shown in this work despite its relatively longer incubation period suggests that the recombinant laccases were successfully expressed as a functional protein with the dye decolourising ability that is comparable to recombinant laccases produced using the standard media. Besides that, this preliminary result also shows that the recombinant laccases produced from bioethanol liquid waste have a promising potential for bioremediation application.

Conclusion

In summary, this work has shown the feasibility of intensifying the recombinant laccases produced using 40% (v/v) SBLW medium with 1.0% (w/v) yeast extract and 2.0% (v/v) glycerol. The addition of these two synthetic medium components to SBLW is still less expensive than using the entire standard synthetic medium for producing laccases. In addition, we have also demonstrated the promising utility of recombinant laccases produced from the optimised SBLW medium with the RBBR decolourisation percentage of 68.6% under non-optimised conditions and in the absence of mediators. Future work should consider the scale-up of recombinant laccase production using the optimised SBLW medium and the optimisation of dye degradation reaction by the resulting laccases.

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تكثيف إنتاج اللاكيز المؤتلف من نفايات ساجو بيوإيثانول السائلة وتقييم الإنزيم لإزالة اللون من الصبغة الاصطناعية

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المُستَخلَص

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الهدف: اللاكسات هي أوكسيديز بوليفينول لها تطبيقات صناعية متنوعة. ارتفاع الطلب الصناعي على اللاتكس يحتاج إلى تطوير وإنتاج فعّال من حيث التكلفة اللاكزات المؤتلف. تتمثل إحدى الطرق في استخدام تيار النفايات الذي يحتوي على تركيبة قيمة لدعم نمو الميكروبات والتعبير عن المستقلبات. يهدف هذا العمل إلى تكثيف إنتاج اللاكزات المؤتلف في بيشيا بسطورس باستخدام نفايات ساجو بيوإيثانول السائلة كمادة وسيطة.

الطريقة: تم تكثيف إنتاج اللاكزات من ساجو بيوإيثانول النفايات السائلة بإضافة الجلسرين تتراوح من 0.5 ٪ - 2.5 ٪ (وزن / حجم). تم تقييم أداء التخمير على أساس الكتلة الحيوية التركيز ونشاط اللاكيز. تم تقييم اللاكسات الناتجة من حيث قدرة الإنزيم على إزالة لون صبغة ريمازول بريليانت بلو آر.

النتائج: أظهرت النتائج أن مكملات ساجو بيوإيثانول النفايات السائلة مع 2 ٪ (وزن / حجم) من الجلسرين أسفرت عن تحسينات بمقدار 1.9 ضعف و 2.1 ضعف من تركيز الكتلة الحيوية ونشاط اللاكاز ، على التوالي بالمقارنة مع تلك التي تم تحقيقها عن طريق التخمير باستخدام معيار مجمع الميثانول المعياري. علاوة على ذلك ، يتم إنتاج اللاكسات باستخدام الأمثل ساجو بيوإيثانول النفايات السائلة أنتج الوسط نسبة إزالة اللون 68.6 ٪ في ظل ظروف غير محسّنة وفي غياب وسطاء. تمثل القيمة 91 ٪ من قدرة إزالة اللون من اللاكزات المنتجة باستخدام المعيار (مخزنة الحد الأدنى من هيستيدين الميثانول).

االستنتاج: بشكل عام، يمثل هذا العمل خطوة أخرى نحو إنتاج منخفض التكلفة من اللاكسيتات المؤتلفة باستخدام المواد الأولية المتجددة.

الكلمات المفتاحية: نفايات الإيثانول الحيوي، إزالة لون الصبغة، الجلسرين، اللاكسيس، اليياف الساجو، الفيناس.