agriTECH, 45 (4) 2025, 397-406

Optimizing the pH, Temperature, and Nitrogen Source for Bacillus sp. 01-Mediated Oil Palm Empty Fruit Bunch Bioconversion

Hamka Nurkaya[1], A. Arbainsyah[1], Krishna Purnawan Candra[2,3]*, M. Marwati[2], Y. Yuliani[2]

[1]Samarinda State Polytechnic of Agriculture, Jl. Samratulangi, Kampus Sei Keledang, Samarinda 75131, Indonesia
[2]Department of Agricultural Product Technology, Agricultural Faculty of Mulawarman University,
Jl. Tanah Grogot, Kampus Gunung Kelua, Samarinda 75119, Indonesia
[3]Doctoral Program for Environmental Science of Mulawarman University, Jl. Sambaliung, Kampus Gunung Kelua,
Samarinda 75119, Indonesia
*Corresponding author: Krishna Purnawan Candra, Email: candra@faperta.unmul.ac.id

Submitted: January 7, 2025; Revised: June 13, 2025; Accepted: July 9, 2025

ABSTRACT

This study aims to optimize the fermentation conditions of *Bacillus sp*.01 for the simultaneous production of cellulase and reducing sugars from oil palm empty fruit bunch (OPEFB). Milled OPEFB was used as the sole carbohydrate source in a mineral salt medium to investigate the effects of temperature (30-45 °C), pH (5.0-8.0), and nitrogen sources on the fermentation performance of *Bacillus* sp.01. The results showed that the highest sugar and cellulase production were achieved at pH 7.0 and 35 °C, yielding 2.52 mg/mL and 0.93 U/mL, respectively, in a 100 mL batch system enriched with 1% milled OPEFB. Regression analysis showed that the optimum sugar and cellulase production conditions were pH 6.73 and 32.5°C. Organic nitrogen sources (beef extract and peptone) showed better performance in promoting sugar and cellulase production than inorganic nitrogen sources (NH_4NO_3 and $(NH_4)_2SO_4$). These results show the potential of using OPEFB as a substrate for bioconversion by Bacillus sp.01, which could contribute to developing sustainable waste management strategies in the palm oil industry.

Keywords: Cellulase; lignocellulolytic; nitrogen source; bioconversion

INTRODUCTION

The Oil Palm industry is growing rapidly in Southeast Asia, particularly Indonesia and Malaysia. In Indonesia, the total plantation area has increased by 7.04% in 4 years, from 14.33 million hectares in 2018 to 15.34 million hectares in 2022 (Directorate of Food Crops Horticulture and Estate Crops Statistics, 2023) and became 15.44 million hectares in 2023 (BPS-Statistics Indonesia, 2024). In 2022, Indonesia's Crude Palm Oil and Palm Kernel Oil production reached 46.82 and 9.36 million tons, respectively (Directorate of Food Crops Horticulture and Estate Crops Statistics, 2023). This increase is expected to lead to the production of more solid (Oil Palm Empty Fruit Bunch, OPEFB) and liquid waste (Palm Oil Mill Effluent, POME) of approximately 40.97 and 117.05 million tons, respectively. Wu et al. (2010) reported that POME generation was approximately 2.5 times the amount of CPO produced, while Susi et al. (2023) stated that the composition of alpha-cellulose of 0.89 times the amount of OPEFB generated.

OPEFB is a lignocellulose material that consists of 3 polymers, namely cellulose (52.7-83.7%), hemicellulose (5.6-27.17%), and lignin (1.7-16.8%) (Mohtar et al., 2017; Yimlamai et al., 2021). Lignin's function in cellulose structure is multifaceted. It provides structural support to plant cell walls (Welker

DOI: https://doi.org/10.22146/agritech.103499
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

et al., 2015), influences the efficiency of enzymatic hydrolysis of cellulose (dos Santos et al., 2019), and interacts with cellulose at the molecular level. Several studies have shown that innovation is needed to solve the solid-waste problem by converting lignocellulolytic materials into other valuable products. Appropriate bacteria and conditions are essential for the conversion of lignocellulolytic materials.

Previous studies have suggested the use of *Trichoderma harzianum* (Bagewaand di et al. 2018), *Bacillus licheniformis* (Helianti et al., 2014), and *Streptomyces* spp. Nurkaya et al. (2017) used OPEFB as a potential substrate for fermentation. However, indigenous microorganisms remain a critical factor in efficiently using OPEFB. The current study isolated a potential lignocellulolytic bacterium *Bacillus* sp.01. from the OPEFB in the palm oil industry of East Kalimantan Province, Indonesia. The bacterial fermentation system is preferred over the fungal fermentation system because of its simplicity. The optimal fermentation conditions (pH, temperature, and nitrogen source type of medium) of *Bacillus* sp.01 for simultaneous sugar and cellulase production using milled OPEFB as cellulose were reported. The optimization procedure was analyzed using a regression analysis. The results are expected to help overcome the waste problem among palm oil industries by increasing the efficiency of OPEFB utilization to support zero-waste production in the agroindustry.

METHODS

Materials

OPEFB was collected from a palm oil industry in Muara Badak Sub-District, Kutai Kartanegara District, East Kalimantan Province, Indonesia. The bunch was sterilized at 121 °C for 15 minutes, dried at 105 °C for 4 hours, and manually cut into small pieces with a knife. Subsequently, it was milled using a crusher machine (crusher machine multifunction PLC180, Maksindo, Indonesia) and sieved with a size of 100 mesh (fiber particle size of fiber–1-2 mm) (Nurkaya, 2013). Carboxymethyl cellulose (CMC), dinitro salicylic acid (DNS), KH_2PO_4, NaCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, and $(NH_4)_2SO_4$ were purchased from Sigma–Aldrich.

Isolation of Cellulolytic Bacteria

Cellulolytic bacteria were isolated using the CMC minimal agar method suggested by Ariffin et al. (2008). In addition, 1 gram of milled OPEFB was inoculated in CMC minimal medium (1.00 g KH_2PO_4, 0.50 g NaCl, 0.5 g $MgS0_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$, 0.03 g $(NH_4)_2SO_4$, and 5.0 g CMC diluted in 1 L distilled water) at pH 7.0. The culture was incubated for 10 days at 30°C with agitation at 200 rpm in a shaking water bath (VS-1205SWL-Vision, USA). After incubation, the culture was streaked onto CMC minimal medium agar and incubated at 30 °C. The clear zone (halo) surrounding the colonies was detected by staining with Congo red and de-staining the color 3 times with 1 M NaCl, each for 15 minutes. Cellulolytic bacteria were selected from colonies that showed a clear zone. The potential isolate named *Bacillus* sp.01 showed the best CMC-ase activity and was further reported in this study for cellulase production optimization by fermentation using OPEFB.

Optimization of the Cellulase Production Procedure

In this study, 1 mL CMC broth of overnight incubated *Bacillus* sp.01 was poured into 100 mL OPEFB medium, a mineral salt medium (1 g KH_2PO_4, 0.5 g NaCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_20$, 0.01 g $MnSO_4.H_2O$, 0.03 g $(NH_4)_2SO_4$ per L) enriched with 1.0 g OPEFB.

In the system with 100 mL and fermentation time up to 48 hours, the fermentation reached an optimum result for sugar and cellulase production at 24 hours (Figure 1a). Based on these results, the fermentation optimization procedure was carried out in a 100 mL system at 150 rpm and an incubation time of 24 hours.

The optimization conditions for sugar and cellulose production experiments were conducted separately in 3 steps, namely (i) investigation of the effect of pH (5, 6, 7, and 8), (ii) determination of fermentation temperature (30, 35, 40, and 45 °C), and (iii) application of different nitrogen sources (ammonium nitrate, ammonium sulfate, peptone, and beef extract) of 0.03 g/L medium.

Reducing Sugar and Cellulase Activity Assay

Sugar content reduction was determined using the DNS method (McCleary & McGeough, 2015). Cellulase activity against CMC was assayed according to Naseeb et al. (2015) with some modifications, such as one-half milliliter of supernatant was added to 0.5 ml of 1% CMC prepared in 0.05 M phosphate buffer (pH 7.0 in a 10 mL test tube and incubated at 50 °C for 60 minutes. The test tubes were closed during the assay. The supernatant was collected by centrifuging the fermenter at 10,000 rpm for 2 minutes (IEC Centre-M2 Centrifuge, USA). The reaction was stopped by adding 3.0 mL of DNS reagent and placing the tubes in a water bath at 100 °C. After 5 minutes, the tube was immediately transferred into a cold-water bath and placed at room temperature for 5 minutes each, then 10 mL of distilled water was added.

The reducing sugars in the mixture were read at 540 nm (UV-Vis Spectrophotometer Shimadzu U-1800, Japan) against a blank (using distilled water instead of sample/supernatant). In addition, 1 unit of cellulase activity was defined as the amount of enzyme that released 1 µmole per minutes of glucose from CMC.

Experimental Design and Data Analysis

Each experimental step was a single-factor experiment arranged in a completely randomized design, in 4 treatments (5.0, 6.0, 7.0, and 8.0 for pH; 30, 35, 40, and 45 °C for temperature; beef extract, peptone, ammonium sulfate, and ammonium nitrate for nitrogen sources) and repeated 3 times. Data were analyzed by non-parametric statistics using the Kruskal-Wallis test (the data did not normally distribute nor had equal variance) and plotted using regression analysis by SigmaPlot v.12.3. Dunn's test at alpha of 0.05 tested the significant differences between treatments. Polynomial regression analysis was used to optimize the conditions for simple sugar and cellulase production.

RESULTS AND DISCUSSION

Lignocellulose Composition of OPEFB

Chemical composition of the OPEFB was determined by proximate analysis according to AOAC (Alemayehu et al., 2021). The OPEFB from East Kalimantan consisted of 10.08% moisture content and 64.86% carbohydrate content, comprising cellulose, hemicellulose, and lignin of 34.89, 13.85, and 11.68%, respectively. Meanwhile, the protein, lipid, and ash content of the OPEFB were 9.56, 6.90, and 8.60%, respectively. Haqiqi et al. (2021) reported higher cellulose content of OPEFB from the same location (Muara Badak Sub-District, Kutai Kartanegara District, Indonesia). Medina et al. (2016) also reported that different methods could lead to varying results in the cellulose assay.

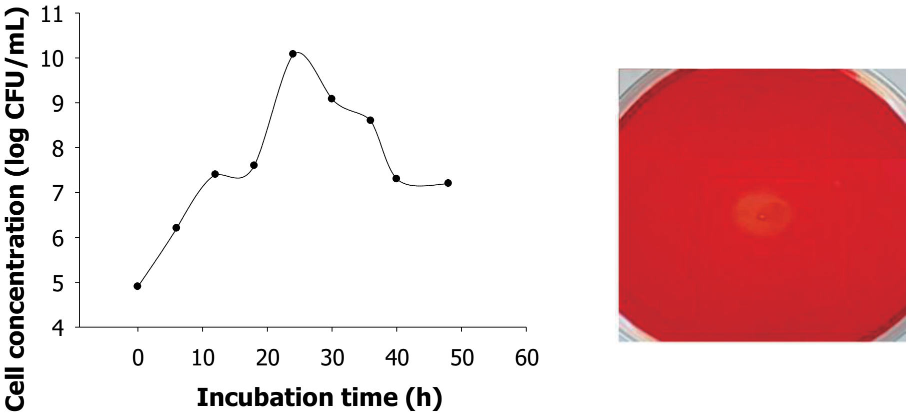
The chemical composition of OPEFB from Ecuador was 47, 25, and 25% for cellulose, hemicellulose, and lignin, respectively (Aguilar et al., 2022), while that from Malaysia was 52.7-59.7, 21.6-27.2, and 12.3-16.8%, respectively (Ibrahim et al., 2013; Mohtar et al., 2017), and from Thailand was 83.7, 5.6, and 1.7%, respectively (Yimlamai et al., 2021).

Lignocellulolytic Bacteria from Composting OPEFB

Among the cellulolytic bacteria, 9 colonies showed positive enzyme activity, and the potential 1 colony (*Bacillus* sp.01) that showed the largest clear zone on CMC minimal agar was selected to be explored by optimizing the cellulase activity in this study. *Bacillus* sp.01 was a gram-negative bacterium with a morphological colony of white color, irregular, translucent, with a low convex and undulated edge. The growth profile and the extracellular CMC-ase activity of the *Bacillus* sp.01 were presented in Figure 1.

In a 100 mL medium system, *Bacillus* sp. 01 showed an optimum growth at 24 hours (10.08 CFU/mL), while the *Bacillus* sp.01 received abundant food, specifically carbon sources, in the form of CMC. In addition, *Bacillus* sp.01 produced extracellular CMC-ase, as showed by a clear zone surrounding the colony.

Sikder et al. (2024) showed cellulose nanocrystals with a yield of 22. 53% (w/w) could be produced from OPEFB fibers using cellulases. The OPEFB acted as a



(a) (b)

Figure 1. Characteristics of *Bacillus* sp.01 isolates. Growth profile in 100 mL of OPEFB-medium (a) and CMC-ase activity in CMC-medium is shown by the yellowish circle area in the center of the medium (b).

potential inducer for cellulase production from *Bacillus* sp.01, as the species could grow well in the OPEFB medium (Figure 1a). The cellulase production from *E. coli, Bacillus, Pseudo*, and *Serratia* was induced by groundnut cake, coconut cake, rice bran, and soy cake (Sethi et al., 2013). However, lignocellulosic waste (straw) was a potential inducer of cellulase production by *Aspergillus terreus* (Narra et al., 2014). This study of *Bacillus* sp.01, an indigenous bacterium in composted OPEBF, led to the utilization and overcoming of the waste problem in the oil palm industry.

According to the results of this study, *Bacillus* spp. were commonly found in composts. During bacterial succession at 20 days of palm oil mill effluent (POME) composting, gram-negative *Bacillus* (*Lysinibacillus massiliensis*) dominated the bacterial population (Zainudin et al., 2014). Chandna et al. (2013) showed that the bacterial composition of waste from cereals processing (rice bran, wheat bran, rice husk, grass, and leaves enriched by cow dung, mustard oil cake, cow urine, and molasses) was dominated by *Bacillus* (78%), and 15% of the whole population was gram-negative. However, the complexity and diversity of the bacterial community in OPEFB compost remained uncertain. Kusumaningtyas et al. (2022) showed that uncultured gamma and beta proteobacteria, as well as many unclassified uncultured bacteria, were identified by molecular methods.

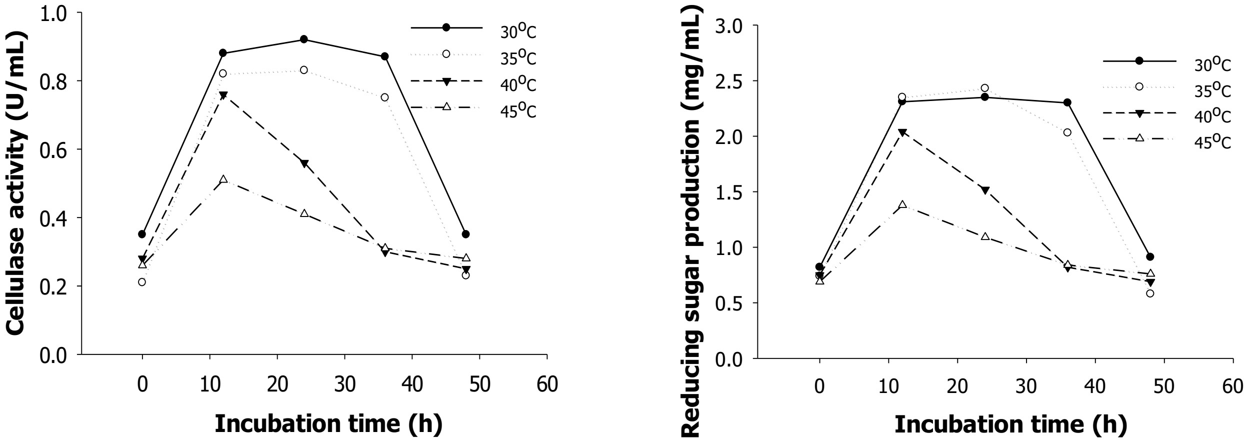
The *Bacillus* sp.01 produced cellulase, which was classified as a thermostable enzyme. In addition, the cellulase showed optimum activity at pH 7.0 and a temperature assay of 50 °C. Some reports showed that lignocellulolytic bacteria produced endoglucanase with a broad pH range. Chelab & Yang (2016) reported a *Bacillus* sp.RL1 had optimum assay conditions at pH 4.8 and 50.0 °C, while Mahmoud et al. (2014) reported a *Streptomyces* sp. with optimum enzyme assay at pH 6.0-6.5 and 50 °C.

Fungal cellulases were reported as potential sources for hydrolyzing the OPEFB (Onyia et al., 2023; Triwahyuni et al., 2024). However, some reports showed bacterial cellulases had significant potency in hydrolyzing the lignocellulose (Awan et al., 2023; Priya et al., 2016; Shyaula et al., 2023), including OPEFB (Amraini et al., 2023; Dini & Afriani, 2022; Gusmawartati & Sari, 2023).

Optimization of Fermentation Conditions for Using OPEFB as the Sole Carbon Source

The fermentation conditions for cellulase and reducing sugar production were optimized within 24 hours of incubation. Figure 2 showed that in a 100 mL system medium, *Bacillus* sp.01 produced the highest cellulase activity and reduced sugar at about 24 hours. Sreena et al. (2016) reported the same phenomenon for endoglucanase, exoglucanase, and FP-ase from *Bacillus subtilis* in a medium supplemented with 1% CMC, except for *glucosidase*, which showed a maximal result at 48 hours. However, Chelab & Yang (2016) reported a slightly longer incubation time for *Bacillus* sp.RL1 to produce endoglucanase during fermentation using 1% CMC as a substrate to reach the maximum result, which was in the 48-72 hours range.

The effect of fermentation conditions (pH of the medium, incubation temperature, and nitrogen source) after 24 hours of fermentation on the production of reducing sugar and cellulase using a medium



(a) (b)

Figure 2. Cellulase and reducing sugar production profile of *Bacillus* sp.01 isolate at different incubation temperatures. The experiment was conducted in 100 mL OPEFB medium with ammonium sulfate as the nitrogen source.

Table 1. Effect of fermentation conditions on reducing sugar and cellulase production using OPEFB

Treatment/Parameter	Level of treatments			
Incubation temp. (°C)*)	30.0	35.0	40.0	45.0
Reducing sugars (mg/mL)	2.351±0.037 b	2.426±0.074 b	1.523±0.108 ab	1.094±0.012 a
Cellulase activity (U/mL)	0.871±0.014 B	0.898±0.027 B	0.564±0.040 AB	0.405±0.004 A
pH medium**)	5.0	6.0	7.0	8.0
Reducing sugars (mg/mL)	1.819±0.007 a	2.195±0.071 ab	2.351±0.037 b	2.036±0.073 ab
Cellulase activity (U/mL)	0.674±0.003 A	0.813±0.026 AB	0.871±0.014 B	0.754±0.027 AB
Nitrogen source (0.03 g/L medium***)	Beef extract	Peptone	$(NH_4)_2SO_4$	$(NH_4)NO_3$
Reducing sugars (mg/mL)	2.494±.004 b	2.523±0.009 b	2.351±0.037 a	2.408±0.106 ab
Cellulase activity (U/mL)	0.924±0.001 B	0.935±0.003 B	0.871±0.014 A	0.892±0.039 AB

Note: Data (average ± SD) were calculated from two replicates and analyzed using the Kruskal-Wallis test. Data within the same row, followed by different letters, show significantly different (Dunn's test, $p<0.05$). Reducing sugars were determined by the DNS method. One unit of cellulase activity was expressed as one μmol reducing sugars released per minute. All the fermentation was conducted in a 100 mL system with 150 rpm for 24 h. *) The treatment was performed using OPEFB medium with $(NH_4)_2SO_4$ as nitrogen source at pH 7.0, **) The treatment was conducted using OPEFB medium with $(NH_4)_2SO_4$ as nitrogen source at 35 °C, ***) The treatment was performed using OPEFB medium with different nitrogen source of pH 7.0 at 35 °C.

supplemented with OPEFB as the sole carbon source was presented in Table 1.

Data in Table 1 showed the simultaneous production of reducing sugar and cellulase in a 100 mL OPEFB medium incubated for 24 hours. The best fermentation condition for reducing sugar and cellulase production was reached at the temperature of 35 °C, using the OPEBF medium set to pH 7.0, and finally enriching the medium with peptone as the nitrogen source. In addition, the optimization of the fermentation conditions (in 100 mL OPEFB medium for 24 h) for sugar and cellulase production was analyzed using the regression technique. This showed that the optimum result of fermentation using OPEFB medium was reached at an incubation temperature of 32.5 °C, a pH medium of 6.73, and enriched the medium with peptone as a nitrogen source. Based on these results, the OPEFB was a potential carbon source for *Bacillus* sp.01 in using the solid waste from the oil palm industry for various products, such as sugar and cellulose.

In this study, the limitation was that there were outlier data. The 2 replications' data were used instead of the 4 replicated data points available, and the use of 2 replications of data was based on considering removing the outlier data.

Effects of Fermentation Temperature

The optimum incubation temperature for cellulase production was studied by culturing cellulolytic bacteria isolates in 1% (w/v) OPEFB medium at several temperatures (30-45 °C). In addition, the *Bacillus* sp.01 was cultured in OPEFB at pH 7.0 and an agitation rate of 150 rpm for 2 days of incubation. Figure 3 showed the optimization of fermentation temperature for sugar and cellulose production by *Bacillus* sp.01 in a minimal medium supplemented by OPEFB powder as a cellulose source.

Production of reducing sugar and cellulase in the OPEFB medium at pH 7.0, 35 °C was slightly higher than that at 33 °C, but decreased intensively at 40 °C by approximately 37.22 and 37.19%, respectively. The same value of regression determination of both reducing sugar and cellulase production model ($R^2=0.9926$) showed that both parameters, namely production of reducing sugar and parallel with cellulase production, in the fermentation system of using OPEFB medium enriched with $(NH_4)_2SO_4$ as nitrogen source, were parallel regarding fermentation temperature. In addition, the fermentation system reached the optimum condition at 32.55 °C, which pointed about 2.60 mg/mL and 0.96 U/mL for sugar and cellulase, respectively. Some reports justified that lignocellulolytic bacteria expressed endoglucanase optimally at 30 to 40 °C. The optimization model was very significant, which was shown by the value of R^2 was very close to one.

Bacillus subtilis showed a cellulase activity of 0.204 U/mL against OPEFB at pH 7.0 and a temperature of 40 °C (Amraini et al., 2023). *Streptomyces. hirsutus* 12.3.A, *B. subtilis* and *B. subtilis* sp. RL1 optimally produced endoglucanase when cultured at 30, 35, and

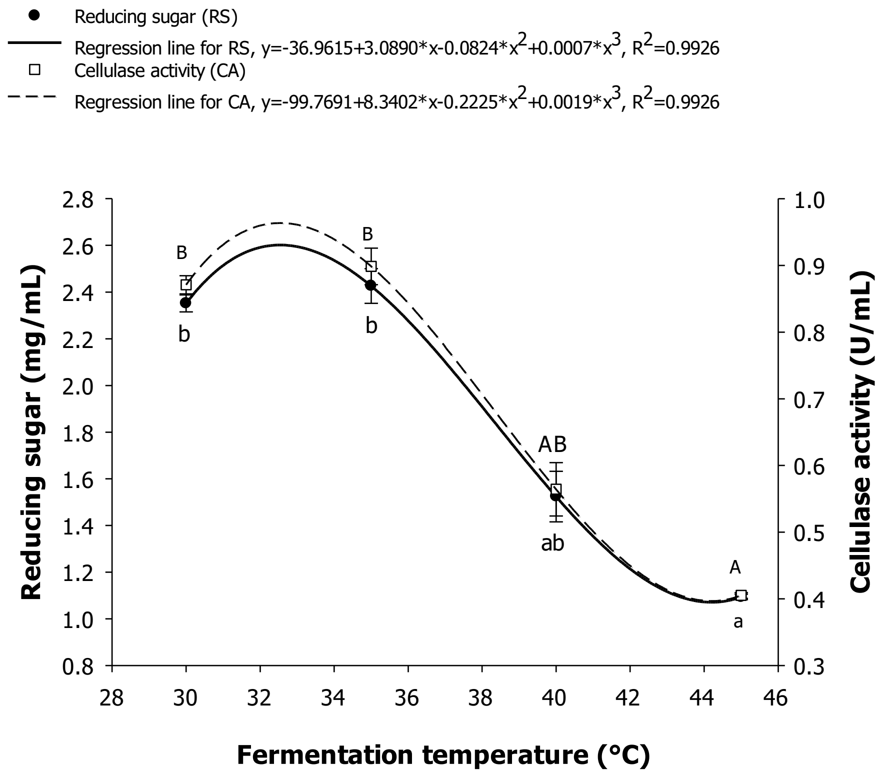


Figure 3. Optimization of fermentation temperature on production of reducing sugar and cellulase of *B. subtilis* sp.01. *See notes in Table 1 for detailed experiments.*

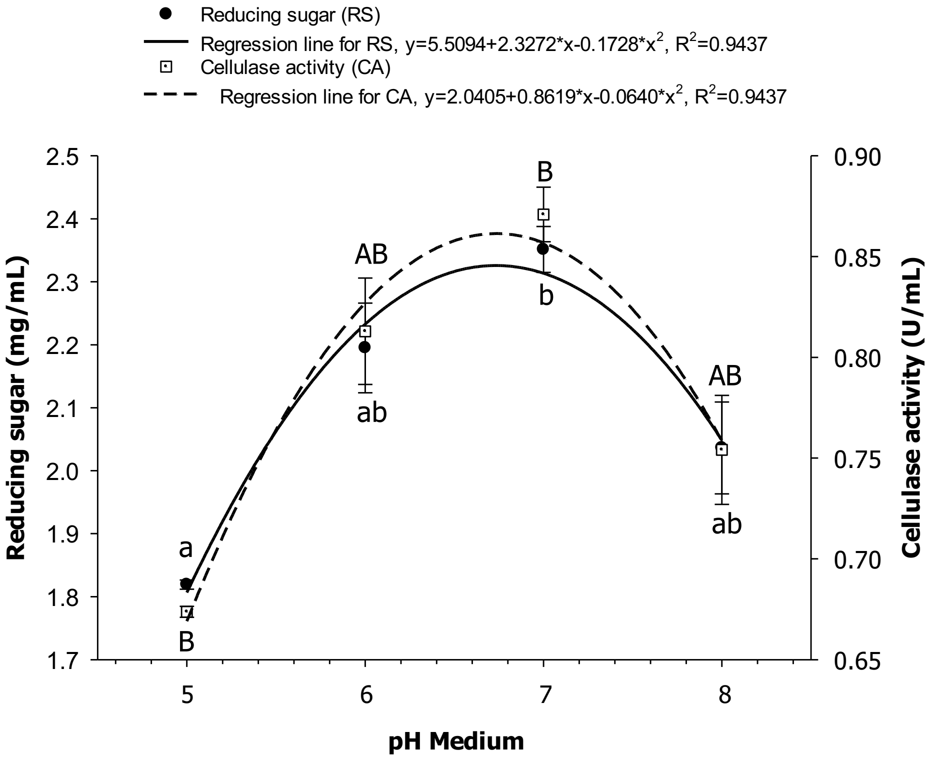


Figure 4. Optimization of pH Medium on production of reducing sugar and cellulase of *B. subtilis* sp.01. *See the note in Table 1 for a detailed experimental procedure.*

40 °C (Chelab & Yang, 2016; Nurkaya et al., 2017; Sethi et al., 2013).

Effect of pH Medium

Figure 4 showed the optimization of medium pH on sugar and cellulase production of *Bacillus* sp.01. The curves for reducing sugar and cellulase exhibited the same characteristics, giving the optimum pH medium for sugar and cellulase production of 6.73.

Using the OPEFB medium (1% OPEFB) at pH 7.0, the reducing sugar and cellulase production expressed as enzyme activity reached 2.35 mg/mL and 0.871 U/mL, respectively. This suggested that the yield of CMC-ase activity was 87.1 U/g dry OPEFB. The equal value of the regression determination of both the production of reducing sugar and the cellulase regression model (R^2=0.9437) showed that the pH medium showed the mimic effect. Incubation using OPEFB medium with $(NH_4)_2SO_4$ as nitrogen source at 35 °C maximized the production of sugar and cellulase to 2.33 mg/mL and 0.86 U/mL for reducing sugar and cellulase activity, respectively, at pH 6.73. A similar fermentation condition to *Bacillus* sp.01 in this experiment on cellulase production was reported. Soil isolated *Bacillus subtilis* grew in media containing 0.05% glucose, 0.075% peptone, 0.001% $FeSO_4$, 0.05% KH_2PO_4, and 0.05% $MgSO_4$ expressed the highest cellulase activity (0.55 U/mL) at pH 7.0 by adding coconut cake in the medium of pH range of 6.0 to 11.0 compare to by adding soy cake, groundnut cake, and wheat bran, which show cellulase activity of 0.44, 0.40, and 0.28 U/mL, respectively (Sethi et al., 2013). Chelab & Yang (2016) reported that the soil isolated *Bacillus* sp.RL1 expressed 18-fold (3.41 U/mL) and 14-fold (3.43 U/mL) for endoglucanase and glucosidase in pineapple shell containing beef extract medium at pH 7.0, compared to the activity expressed from the same medium with CMC and peptone instead. Another isolate from OPEFB, *Streptomycetes hirsutus* 12.3.A, produced the highest cellulase activity and reducing sugar content during fermentation at a pH medium of 7 (Nurkaya et al., 2017).

Effect of Nitrogen Source in The Medium

The effect of 4 types of nitrogen sources as growth medium on sugar and cellulase production of *Bacillus* sp.01 was shown in Figure 5.

Ammonium nitrate showed a slight increase in sugar and cellulase production of Bacillus sp.01 compared to ammonium sulfate. However, the organic nitrogen source (beef extract and peptone) was affected better. The peptone was the nitrogen source that showed the highest sugar and cellulase production of the *Bacillus* sp.01 in the OPEFB medium. A similar phenomenon was reported by Akintola et al. (2019) and Ariffin et al. (2008), that organic nitrogen increased cellulase production more than inorganic nitrogen of Enterobacter

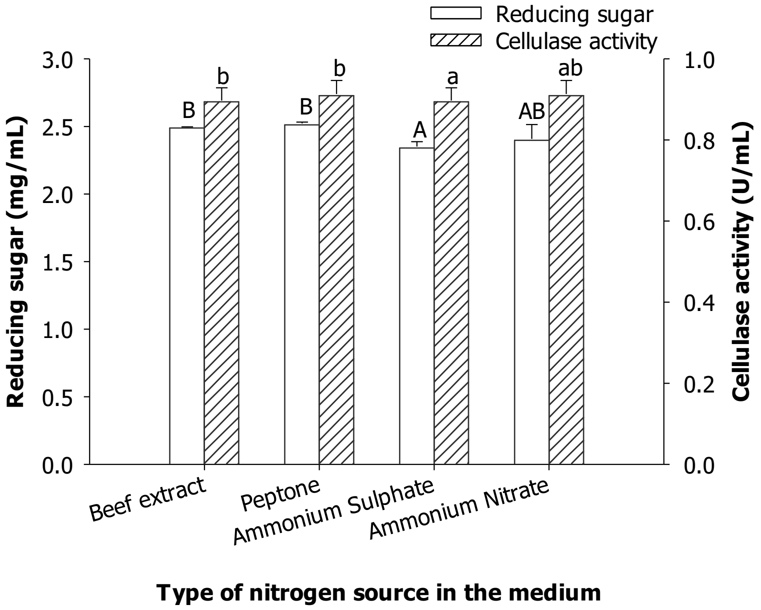


Figure 5. Effect of nitrogen source type on sugar and cellulase production of *B. subtilis* sp.01. *The Bar coded by different letters (uppercase for reducing sugar, and lowercase for cellulase activity) within the same bar type shows significantly different (Dunn's test, p<0.05). See the note in Table 1. detailed experimental procedure.*

cloacae and *Bacillus pumilus*, respectively. Different responses to some nitrogen sources were reported for bacterial cellulase. The organic nitrogen source increased the cellulase activity of *Bacillus* sp. in the row of yeast extract > beef extract > peptone (Thomas et al., 2018). Meanwhile, Mahmoud et al. (2014) and Sethi et al. (2013) showed that organic nitrogen repressed the production of cellulase of *Streptomyces* sp. and 4 other bacteria (*E. coli*, *Pseudomonas*, *Bacillus,* and *Serratia*), respectively. Among the inorganic nitrogen, the ammonium sulfate produced higher cellulase of *B. pumilus* than ammonium nitrate (Ariffin et al., 2008), which showed the opposite phenomenon experiment.

CONCLUSION

In conclusion, an indigenous bacterial species from composted OPEFB, *Bacillus* sp.01, has been isolated. In a system of 100 mL medium containing milled OPEFB, *Bacillus* sp.01 shows the optimum growth and production of cellulase after 24 h of fermentation. This species can simultaneously use milled OPEFB to produce simple sugar and cellulase. The optimal batch fermentation condition of *Bacillus* sp.01 to produce cellulase with higher activity and produce simple sugar in a 100 mL OPEFB medium system for 24 hours, 150 rpm is observed at pH 6.73 and 32.55 °C. Peptone is the best nitrogen source among the 2 other sources (beef extract, ammonium sulfate, and ammonium nitrate).

CONFLICT OF INTEREST

There is no conflict of interest between authors.

REFERENCES

Aguilar, A. D., Valle, V., Almeida-Naranjo, C. E., Naranjo, Á., Cadena, F., Kreiker, J., & Raggiotti, B. (2022). Characterization dataset of oil palm empty fruit bunch (OPEFB) fibers – Natural reinforcement/filler for materials development. *Data in Brief*, *45*(September). https://doi.org/10.1016/j.dib.2022.108618

Akintola, A. I., Oyedeji, O., Adewale, I. O., & Bakare, M. K. (2019). Production and physicochemical properties of thermostable, crude cellulase from enterobacter cloacae IP8 isolated from plant leaf litters of Lagerstroemia indica Linn. *Journal of Microbiology, Biotechnology and Food Sciences*, *8*(4), 989–994. https://doi.org/10.15414/jmbfs.2019.8.4.989-994

Alemayehu, G. F., Forsido, S. F., Tola, Y. B., Teshager, M. A., Assegie, A. A., & Amare, E. (2021). Proximate, mineral and anti-nutrient compositions of oat grains (Avena sativa) cultivated in Ethiopia: implications for nutrition and mineral bioavailability. *Heliyon*, *7*(8), e07722. https://doi.org/10.1016/j.heliyon.2021.e07722

Amraini, S. Z., Nazaris, N. N., Andrio, D., Mardhiansyah, M., & Helwani, Z. (2023). Utilization of empty palm fruit bunches as a carbon source for cellulase production to reduce solid waste from palm oil. *Leuser Journal of Environmental Studies*, *1*(1), 34–38. https://doi.org/10.60084/ljes.v1i1.41

Ariffin, H., Hassan, M. A., Shah, U. K. M., Abdullah, N., Ghazali, F. M., & Shirai, Y. (2008). Production of bacterial endoglucanase from pretreated oil palm empty fruit bunch by Bacillus pumilus EB3. *Journal of Bioscience and Bioengineering*, *106*(3), 231–236. https://doi.org/10.1263/jbb.106.231

Awan, M. O. U., Iqbal, A., Rashid, M. I., Irshad, U., Hafeez, F., Ullah, F., Irshad, M., Ondrasek, G., Mustac, I., & Nazir, R. (2023). Proficient lignocellulolytic novel bacterial isolates from diversified Galiyat forests of lower Himalaya. *Forests*, *14*(6), 1–16. https://doi.org/10.3390/f14061180

Bagewadi, Z. K., Mulla, S. I., & Ninnekar, H. Z. (2018). Optimization of endoglucanase production from Trichoderma harzianum strain HZN11 by central composite design under response surface methodology. *Biomass Conversion and Biorefinery*, *8*(2), 305–316. https://doi.org/10.1007/s13399-017-0285-3

BPS-Statistics Indonesia. (2024). *Plantation area by province 2023*. BPS-Statisstics Indonesia. https://www.bps.go.id/id/statistics-table/2/MTMxIzI=/luas-tanaman-perkebunan-menurut-provinsi--ribu-hektar-.html

Chandna, P., Nain, L., Singh, S., & Kuhad, R. C. (2013). Assessment of bacterial diversity during composting of agricultural byproducts. *BMC Microbiology*, *13*(99), pp.14. https://doi.org/10.1186/1471-2180-13-99

Chelab, R. L., & Yang, X. (2016). Optimization of the production of a b-1, 4-endoglucanase from a newly isolated Bacillus sp.RL1 by medium optimization and analysis different growth parameters. *Research Journal of Applied Sciences, Engineering and Technology*, *13*(8), 664–674. https://doi.org/10.19026/rjaset.13.3052

Dini, I. R., & Afriani, E. (2022). The effect of lignocellulolytic bacteria consortium on composting empty oil palm fruit bunches. *International Journal of Science and Research Archive*, *7*(1), 220–228. https://doi.org/10.30574/ijsra.2022.7.1.0130

Directorate of Food Crops Horticulture and Estate Crops Statistics. (2023). *Indonesia Oil Palm Statistics 2022* (D. of F. C. H. and E. C. Statitics (ed.); 16th ed.). BPS-Statistics Indonesia.

dos Santos, A. C., Ximenes, E., Kim, Y., & Ladisch, M. R. (2019). Lignin–Enzyme Interactions in the Hydrolysis of

Lignocellulosic Biomass. *Trends in Biotechnology*, *37*(5), 518–531. https://doi.org/10.1016/j.tibtech.2018.10.010

Gusmawartati, & Sari, P. R. (2023). Cellulolytic bacteria as decomposers in dry empty fruit bunches composting. *IOP Conference Series: Earth and Environmental Science*, *1188*(012002). https://doi.org/10.1088/1755-1315/1188/1/012002

Haqiqi, M. T., Bankeeree, W., Lotrakul, P., Pattananuwat, P., Punnapayak, H., Ramadhan, R., Kobayashi, T., Amirta, R., & Prasongsuk, S. (2021). Antioxidant and UV-Blocking Properties of a Carboxymethyl Cellulose-Lignin Composite Film Produced from Oil Palm Empty Fruit Bunch. *ACS Omega*, *6*(14), 9653–9666. https://doi.org/10.1021/acsomega.1c00249

Helianti, I., Ulfah, M., Nurhayati, N., & Mulyawati, L. (2014). Cloning, Sequencing, and Expression of the Gene Encoding a Family 9 Cellulase from Bacillus licheniformis F11 in Escherichia coli and Bacillus megaterium, and Characterization of the Recombinant Enzymes. *Microbiology Indonesia*, *8*(4), 147–160. https://doi.org/10.5454/mi.8.4.2

Ibrahim, M. F., Razak, M. N. A., Phang, L. Y., Hassan, M. A., & Abd-Aziz, S. (2013). Crude cellulase from oil palm empty fruit bunch by trichoderma asperellum UPM1 and aspergillus fumigatus UPM2 for fermentable sugars production. *Applied Biochemistry and Biotechnology*, *170*(6), 1320–1335. https://doi.org/10.1007/s12010-013-0275-2

Kusumaningtyas, P., Candra, K. P., & Sahid, A. (2022). Changes in microbial community structure and diversity during decomposition of oil palm empty fruit bunches at different decomposition sites in humid tropical oil palm plantation. *Agriculture and Natural Resources*, *56*, 511–524. https://doi.org/10.34044/j.anres.2022.56.3.08

Mahmoud, A.-M. Y., Asif, A. M. J.-F., & Hani, Z. A. (2014). Production, purification and characterization of cellulase from Streptomyces sp. *African Journal of Microbiology Research*, *8*(4), 348–354. https://doi.org/10.5897/ajmr2013.6500

McCleary, B. V., & McGeough, P. (2015). A Comparison of polysaccharide substrates and reducing sugar methods for the measurement of endo-1,4-β-xylanase. *Applied Biochemistry and Biotechnology*, *177*(5), 1152–1163. https://doi.org/10.1007/s12010-015-1803-z

Medina, J. D. C., Woiciechowski, A., Filho, A. Z., Nigam, P. S., Ramos, L. P., & Soccol, C. R. (2016). Steam explosion pretreatment of oil palm empty fruit bunches (EFB) using autocatalytic hydrolysis: A biorefinery approach. *Bioresource Technology*, *199*, 173–180. https://doi.org/10.1016/j.biortech.2015.08.126

Mohtar, S. S., Tengku Malim Busu, T. N. Z., Md Noor, A. M., Shaari, N., & Mat, H. (2017). An ionic liquid treatment and fractionation of cellulose, hemicellulose and lignin from oil palm empty fruit bunch. *Carbohydrate Polymers*, *166*, 291–299. https://doi.org/10.1016/j.carbpol.2017.02.102

Narra, M., Dixit, G., Divecha, J., Kumar, K., Madamwar, D., & Shah, A. R. (2014). Production, purification and characterization of a novel GH 12 family endoglucanase from aspergillus terreus and its application in enzymatic degradation of delignified rice straw. *International Biodeterioration and Biodegradation*, *88*, 150–161. https://doi.org/10.1016/j.ibiod.2013.12.016

Naseeb, S., Sohail, M., Ahmad, A., & Khan, S. A. (2015). Production of xylanases and cellulases by Aspergillus fumigatus MS16 using crude lignocellulosic substrates. *Pakistan Journal of Botany*, *47*(2), 779–784.

Nurkaya, H., Wathanachaiyingyong, O., Marwati, Chaiyanan, S., & Chaiyanan, S. (2017). Production of cellulase from palm oil industrial solid waste by Actinomycetes isolate 12.3.A. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*, *19*(3), 483–490.

Onyia, D. C., Onyeneke, E. C., Okunwaye, T., Okogbenin, E. A., Okogbenin, B. O., Idabie, E., Asiriuwa, N. U., & Anemene, H. (2023). Cellulolytic fungi from palm pressed fiber, empty fruit bunch, and palm trunk. *Asian Journal of Research in Biochemistry*, *12*(1), 8–15. https://doi.org/10.9734/ajrb/2023/v12i1224

Priya, I., Dhar, M. K., Bajaj, B. K., Koul, S., & Vakhlu, J. (2016). Cellulolytic activity of thermophilic Bacilli isolated from Tattapani hot spring sediment in North West Himalayas. *Indian Journal of Microbiology*, *56*(2), 228–231. https://doi.org/10.1007/s12088-016-0578-4

Sethi, S., Datta, A., Gupta, B. L., & Gupta, S. (2013). Optimization of cellulase production from bacteria isolated from soil. *ISRN Biotechnology*, *2013*(ID 985685), 1–7. https://doi.org/http://dx.doi.org/10.5402/2013/985685

Shyaula, M., Regmi, S., Khadka, D., Poudel, R. C., Dhakal, A., Koirala, D., Sijapati, J., Singh, A., & Maharjan, J. (2023). Characterization of thermostable cellulase from Bacillus licheniformis PANG L isolated from the Himalayan soil. *International Journal of Microbiology*, *2023*. https://doi.org/10.1155/2023/3615757

Sikder, M. B. H., Rashid, S. S., Rahim, M. H. A., Ramli, A. N. M., Roslan, R., Sasi, A. A., & Mustafa, A. H. (2024). Enzymatic cellulose nanocrystal production from pretreated palm oil empty fruit bunch fibers. In S. Z. Abidin, M. Y. Taib, Z. M. Zain, & K. Azman (Eds.), *materialstoday: PROCEEDINGS 6th National Conference for Postgraduate* (pp. 249–253). https://doi.org/10.1016/j.matpr.2023.10.115

Sreena, C. ., Vimal, K. ., & Denoj, S. (2016). Production of cellulases and xylanase from Bacillus subtilis Mu S1 isolated from protected areas of Munnar wildlife division.

Journal of Microbiology, Biotechnology and Food Sciences, *5*(6), 500–504. https://doi.org/10.15414/jmbfs.2016.5.6.500-504

Susi, S., Ainuri, M., Wagiman, W., Afan, M., & Falah, F. (2023). High-yield alpha-cellulose from oil palm empty fruit bunches by optimizing thermochemical delignification processes for use as microcrystalline cellulose. *International Journal of Biomaterials, 2023*(ID 9169431), p.15. https://doi.org/https://doi.org/10.1155/2023/9169431

Thomas, L., Ram, H., & Singh, V. P. (2018). Inducible cellulase production from an organic solvent tolerant Bacillus sp. SV1 and evolutionary divergence of endoglucanase in different species of the genus Bacillus. *Brazilian Journal of Microbiology*, *49*(2), 429–442. https://doi.org/10.1016/j.bjm.2017.05.010

Triwahyuni, E., Asri, I. P., Sugiwati, S., Wijaya, H. W., Anggraini, I. D., Marno, S., & Anindyawati, T. (2024). Cellulase production by Trichoderma reesei and its application in hydrolyzing oil palm rmpty fruit bunches. *Cellulose Chemistry and Technology*, *58*(5–6), 495–503. https://doi.org/10.35812/CelluloseChemTechnol.2024.58.46

Welker, C. M., Balasubramanian, V. K., Petti, C., Rai, K. M., De Bolt, S., & Mendu, V. (2015). Engineering plant biomass lignin content and composition for biofuels and bioproducts. *Energies*, *8*(8), 7654–7676. https://doi.org/10.3390/en8087654

Wu, T. Y., Mohammad, A. W., Jahim, J. M., & Anuar, N. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. *Journal of Environmental Management*, *91*(7), 1467–1490. https://doi.org/10.1016/j.jenvman.2010.02.008

Yimlamai, B., Choorit, W., Chisti, Y., & Prasertsan, P. (2021). Cellulose from oil palm empty fruit bunch fiber and its conversion to carboxymethylcellulose. *Journal of Chemical Technology and Biotechnology*, *96*(6), 1656–1666. https://doi.org/10.1002/jctb.6689

Zainudin, M. H. M., Hassan, M. A., Md Shah, U. K., Abdullah, N., Tokura, M., Yasueda, H., Shirai, Y., Sakai, K., & Baharuddin, A. S. (2014). Bacterial community structure and biochemical changes associated with composting of lignocellulosic oil palm empty fruit bunch. *BioResources*, *9*(1), 316–335.