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Screening and identification of cellulolytic halotolerant strain and the degradation of wastewater

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ABSTRACT

This study was the first to evaluate the treatment of cotton pulp wastewater by isolated cellulolytic halotolerant bacteria. A cellulolytic halotolerant bacterium was screened from the marine mud domesticated by cotton pulp wastewater using Congo red staining. The strain was obtained as a *Bacillus paralicheniformis* by physiological and biochemical identification and 16S rRNA sequence analysis. The strain has an optimum growth salinity of 2% and can be grown using carboxymethylcellulose (CMC) or corncob as a carbon source. Maximum cellulase activities of FPase 0.143 U/mL, CMCase 0.189 U/mL, β-glucosidase 0.158 U/mL, respectively were achieved in a pH of 6–7 and a temperature of 35°C with 60 h. The chemical oxygen demand removal rate of the bacterium on the diluted cotton pulp wastewater for 6 consecutive days was 71.8%. These results contribute to the development of sustainable bioprocesses approaching a biorefinery concept. These results help to develop sustainable biological processes in the chemical fiber industry.

Keywords: Cellulolytic halotolerant; Congo red staining; Cellulase activities; Cotton pulp wastewater

1. Introduction

Cotton pulp is one of the important raw materials for the chemical fiber industry. The chemical oxygen demand (COD) concentration of the cotton pulp wastewater generated in the production process can reach 200,000 mg/L, and the concentration of the integrated wastewater is 1,500–3,000 mg/L [1]. Compared with other types of black liquor, cotton pulp wastewater has a deep color, high alkalinity, and high salt content. Its organic pollutants are complex, contain a lot of cellulose, oligosaccharides, fatty alcohols, and few lignin [2]. The main degradation of cellulose is through dilute acid hydrolysis and enzymatic treatment. Enzymatic saccharification is considered a promising technology compared to other methods [3]. The use of cellulolytic bacteria to consume cellulose itself as a carbon source is considered to be the most valuable energy substance in the future [4,5].

Insoluble cellulose macromolecular substrates are unable to be transported intracellularly, and most aerobic bacteria and fungi achieve efficient hydrolysis of cellulose by secreting large amounts of free extracellular cellulose [6]. Cellulases are the classification of three enzymes, which composed of endoglucanase, exoglucanase, and β-glucosidase [7]. Different enzymes act on different structural units and they act synergistically to cleavage β-1,4-glycosidic bonds in cellulose chains under mild conditions and with high specificity [8–10].

A certain concentration of inorganic salt is essential for maintaining microbial growth and plays a key role in adjusting osmotic pressure, maintaining cell stability and

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promoting enzyme activity [11]. However, excessive salinity has a negative impact on the internal structure of cellular molecules such as metabolites, extracellular polymers and biological enzymes [12,13]. The high-salt marine environment has proven to be a rich source of microbes containing industrially important enzymes. Hong et al. [14] evaluated 50 fungi species from the marine environment and found that *Arthrinium saccharicola* exhibited FPase, CMCase, and β-glucosidase of 0.39, 0.38, and 1.04 U/mL. Gunny et al. [15] found FPase activity of a cellulolytic halophilic fungus *Aspergillus terreus* was 0.029 U/mL. Other marine-derived cellulolytic bacteria included *Cladosporium sphaerospermum* [16], *Bacillus subtilis* [17], *Vibrio alginolyticus*, *Pseudomonas stutzeri* and *Klebsiella oxytoca* [18]. The use of cotton pulp wastewater for cellulolytic halotolerant bacteria screening was not yet described in the scientific literature. Other wastewaters such as domestic wastewater have been described as producers of cellulases [19,20]. In the future, the use of halotolerant microorganisms to treat high-salt wastewater is still a hot topic.

In this work, a cellulolytic halotolerant bacterium was screened from the marine mud acclimated to cotton pulp wastewater. The production capacity of three kinds of cellulase under different submerged fermentation (SmF) conditions was studied and the optimum conditions for cellulase production were determined. Then the continuous degradation of cotton pulp wastewater inoculated this strain was evaluated and expressed by the COD removal rate. Screening of cellulolytic halotolerant bacteria from cotton pulp wastewater has rarely evaluated so far.

2. Methods

2.1. Screening of cellulolytic halotolerant bacteria

Cellulolytic halotolerant bacteria isolated from Qingdao marine mud and diluted cotton pulp wastewater were screened for cellulase enzyme by Congo red staining. The carboxymethylcellulose (CMC) solid medium was composed of CMC (10 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), KH_2PO_4C, Na_2HPO_4 (1 g/L), peptone (10 g/L), yeast extract (1 g/L), NaCl (20 g/L), agar (20 g/L) and pH was 7.2–7.4. The solid medium was autoclaved (20 min, at 121°C) and the enriched culture solution was inoculated on CMC solid medium. After cultivating for 2 d at 30°C, strains capable of growing on CMC solid medium were selected and purification culture was continued. The hydrolysis zones were observed by immersing the plates with a 0.1% Congo red aqueous solution for 15 min followed by decolorizing with 1 M NaCl for 15 min [21]. The diameter of the hydrolysis zones (*D*) and the diameter of the strains (*d*) was recorded, then *D*/*d* was used as the standard for the primary screening.

2.2. Submerged fermentation

The SmF medium was composed of CMC (10 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), KH_2PO_4C, Na_2HPO_4 (1 g/L), peptone (5 g/L), yeast extract (5 g/L), NaCl (20 g/L) and pH was set at 7.2–7.4 before autoclaved (20 min, at 121°C). The flasks were inoculated strains and cultured in an orbital shaker for 2 d at 35°C and 150 rpm.

2.3. Enzyme extraction

The liquid culture was stirred for 1 h and then filtered and centrifuged at 5,000 rpm for 10 min. The supernatant was enzyme extraction.

2.4. Measurements of cellulase activities

Cellulase activities were measured by 3,5-dinitrosalicylic acid (DNS) method, through the concentration of the released reducing sugars [22,23]. Three types of enzyme activity were measured (i) FPase, (ii) CMCase, and (iii) β-glucosidase. The FPase activity unit was defined as the amount of enzyme required to release 1 μmol of glucose per minute after 1 h of enzymatic hydrolysis. Measurement of FPase activity which contains Whatman No. 1 filter paper strip, 1.0 cm × 6.0 cm (= 50 mg) was carried by 1 mL of crude extract and 1 mL of 10 mM phosphate buffer (pH 7.0). The CMCase and β-glucosidase activity unit was defined as the amount of enzyme required to release one μmol of glucose per minute after 0.5 h of enzymatic hydrolysis. For the measurement of CMCase activity, the assay mixture contained 1 mL of crude extract and 1 mL of 1% CMC in 10 mM phosphate buffer (pH 7.0) was used. For the measurement of β-glucosidase activity, the assay mixture contained 1 mL of crude extract and 1 mL of 0.5% salicin in 10 mM phosphate buffer (pH 7.0) was used. The enzyme–substrate mixture was incubated at 50°C for 60, 30, and 30 min, respectively for FPase, CMCase, and β-glucosidase assay. The reaction was stopped by adding 1.5 mL of DNS, boiled vigorously for 5 min and cooled to make up of 25 mL. The optical absorbance was read at 540 nm against reagent blank using a spectrophotometer.

2.5. Sequencing of 16S rRNA gene

The strain was sent to Qingdao PersonalGene Technology Co., Ltd., (Qingdao, China) to extract DNA. The 16S rRNA gene fragment of this strain was amplified with the upstream primer of 27F and the downstream primer of 1492R. The similarity search was carried out in silico using BLAST of NCBI. Identification at the species level was defined by a 16S rRNA gene sequence similarity of ≥99% with the sequence of the type strain in GenBank.

2.6. Determination of optimal salt concentration for cellulase activities

In order to determine the effect of salt concentration on cellulase activities in the SmF medium, the dominant strain was cultured in 100 mL of the SmF medium. Determination of cellulase activities in the range of 0%–4% (w/v) NaCl.

2.7. Quantification of enzyme activities on different carbon sources and nitrogen sources

The dominant strain was grown in SmF medium containing CMC, avicel (MCC), wheat straw, corncob and mixture (mixed wheat straw and corncob with 1:1) as a carbon source (1% w/v). Wheat straw and corncob were used without pre-treatment with acid or base. The strain

inoculated in 100 mL minimal medium containing one of the carbon sources and incubated at 35°C on a shaker at 130 rpm for 5 d Sampling was performed every 24 h to estimate the enzyme activity. The maximum enzyme activity was selected as the final result.

Nitrogen source group included $(NH_4)_2SO_4$, NH_4Cl, yeast extract, peptone and mixture (mixed yeast extract and peptone with 1:1) (1% w/v). Determination of cellulase activities by the above method.

2.8. Improved cellulose production in SmF

The influence of pH, temperature, and cultivation time in cellulase production during the growth of the dominant strain was evaluated. The cultivation time studied was 12–96 h (intervals of 12 h), the pH range was 5–9 and the temperature range was 25°C–50°C (intervals of 5°C).

2.9. Wastewater degradation performance

The dominant strain was inoculated with a 10% inoculum into a flask containing 100 mL of diluted cotton pulp wastewater. The flask was incubated at optimum conditions of enzyme production for 6 d and COD concentration was measured every day.

3. Results and discussion

3.1. Screening and identification of cellulolytic halotolerant bacterium

Bacterial communities play an important role in the carbon cycle in the ocean [24]. A total of 8 halotolerant strains were subjected for the cellulose activity and 5 strains showed hydrolysis zones. The hydrolysis zone diameter (*D*) and strain diameter (*d*) were measured and the value of *D*/*d* was calculated (Table 1). The larger the *D*/*d* ratio, the higher the enzyme production capacity of the strain and the stronger the ability to degrade cellulose. Although the No.1 strain had a faster growth rate (maximum *d* value), its ability to degrade cellulose was so poor. The larger *D*/*d* values were showed in the No.2, No.4, and No.5 strain. Further rescreening was required due to large errors in visual observation and measurement.

Table 2 shows all five strains have enzymes of different components to degrade cellulose. Further, No.5 strain was screened for the study as it showed high production of extracellular enzymes. Through characterized morphologically and biochemically, dominated bacterial as a gram-positive. The surface of the colony was smooth, the center was milky white and the edges were pale yellow. Comparative 16S rRNA gene sequences analyses showed that 100% similarity with *Bacillus paralicheniformis* (Table 3) and therefore classified as *Bacillus* sp., belongs to phylum *Firmicutes*, class *Bacilli*, Order *Bacillales*, and family *Bacillaceae*.

Table 1
CMC-Na hydrolytic ability of strains

Strain	*D* value (cm)	*d* value (cm)	*D*/*d*
No.1	0.90	0.80	1.13
No.2	0.40	0.20	2.00
No.3	0.70	0.50	1.40
No.4	0.60	0.25	2.40
No.5	0.30	0.25	2.00

3.2. Effect of NaCl concentration on cellulase production

Different NaCl concentration of SmF from 0.5% to 4% (w/v) were tested to determine the level of NaCl concentration for the maximum production of cellulolytic halotolerant bacteria.

The optimum NaCl concentration of medium for FPase and β-glucosidase production was found to be 2% (w/v) and the highest CMCase activity was observed at 3% (w/v) (Fig. 1). On the whole, cellulases produced by *Bacillus paralicheniformis* was increased up to 2% (w/v), and it started to decrease gradually when the concentration was increased from 2.0% to 4.0% (w/v) thus indicating that the optimum NaCl concentration was at 2.0% (w/v). The synthesis of this enzyme may be related to optimal growth at the optimum salt concentration [25]. In a high-salt environment, the separation of bacteria from the plasmolysis led to death and the enzyme content decreased. Similar results appeared in previous reports that salt may be indispensable for bacterial growth and enzyme production of halophilic bacteria [26]. The results showed that NaCl is one of the important components of the marine bacterial growth medium, which affects the activity of marine bacterial cellulases.

3.3. Effect of different carbon sources on cellulases production

The dominant bacterium was inoculated to some inexpensive cellulosic materials like wheat straw and corncob as the only carbon source. *Bacillus paralicheniformis* showed the greatest activity of FPase, CMCase, and β-glucosidase with a corncob, CMC, and corncob followed by CMC, corncob, and CMC (Fig. 2). The adsorption trend of cellulase was different during the hydrolysis of different substrates [27]. Corncob could be effectively degraded by various marine bacteria [28]. It has been reported that CMC as the substrate induces the production of cellulase via activating cellulase activator molecule to regulate the protein and eventually degrade cellulose [29]. Wheat straw pretreated with acid or alkali is used for cellulase production and also for simultaneous saccharification and fermentation to produce bioethanol [30,31]. In addition, acid/base treatment has

Table 2
Cellulase activities of strains

Strain	FPase (U/mL)	CMCase (U/mL)	β-glucosidase (U/mL)
No.1	0.036 ± 0.003	0.029 ± 0.008	0.031 ± 0.007
No.2	0.039 ± 0.002	0.023 ± 0.003	0.024 ± 0.003
No.3	0.037 ± 0.001	0.021 ± 0.002	0.032 ± 0.002
No.4	0.035 ± 0.001	0.019 ± 0.002	0.021 ± 0.003
No.5	0.136 ± 0.007	0.123 ± 0.006	0.103 ± 0.006

Table 3
16S rRNA partial sequence of *Bacillus paralicheniformis*

Bacillus paralicheniformis strain BRM043907 16S ribosomal RNA gene, partial sequence
Sequence ID: MH305356.1; *Length*: 1514; *Number of matches*: 1
Related information
Range 1: 16 to 1471

Score	Expect	Identities	Gaps	Strand
2689 bits (1456)	0.0	1456/1456 (100%)	0/1456 (0%)	Plus/Minus

CTTCGGCGGCTGGCTCCAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGG
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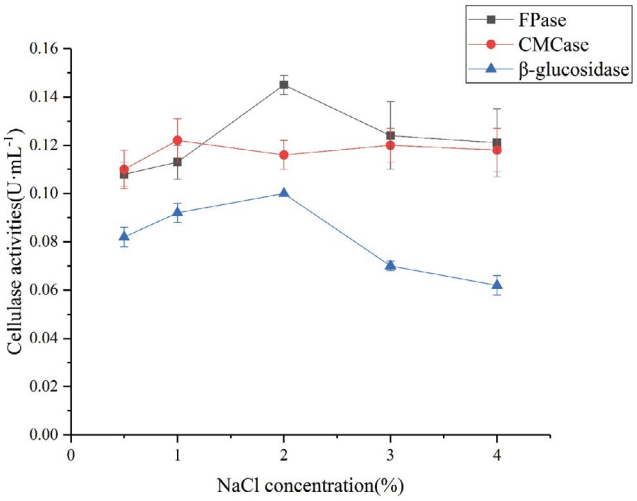

Fig. 1. Cellulase activities of cellulase produced by *Bacillus paralicheniformis* in the SmF containing different NaCl concentrations.

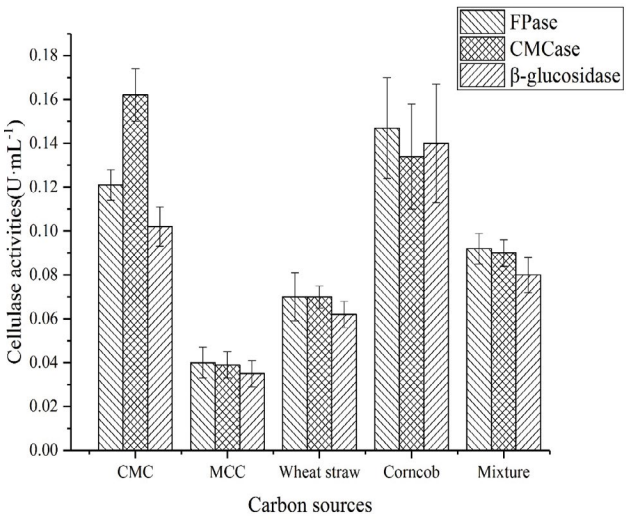

Fig. 2. Cellulase activities produced by *Bacillus paralicheniformis* in the SmF containing different carbon sources.

adverse effects on the environment. The screened bacterial isolates have the ability to bind cellulose biomass without acid/base treatment, and the ability to produce cellulase efficiently would be a competing approach in business.

3.4. Effect of different nitrogen source on cellulases production

According to Fig. 3, yeast extract provided the highest cellulase activities (FPase 0.144 ± 0.012 U/mL: CMCase 0.145 ± 0.005 U/mL; β-glucosidase 0.137 ± 0.004 U/mL), which were 1.4, 1.2, and 1.4 times obtained with cellulase activities provided by mixture (FPase 0.103 ± 0.002 U/mL: CMCase 0.123 ± 0.014 U/mL; β-glucosidase 0.101 ± 0.006 U/mL), which were the formulation of SmF medium. Other studies showed that peptone can provide the highest cellulase activities, and urea will inhibit the production of the enzyme [32]. So, yeast extract was used in future research.

3.5. Effect of cultivation time on cellulases production

Once the ability of *Bacillus paralicheniformis* to produce cellulases was determined, the optimal culture conditions for bacterium as a cellulase producer were studied. The optimum cultivation period for *Bacillus paralicheniformis* was 48–60 h. CMCase and β-glucosidase reached a maximum value of 0.183 and 0.157 U/mL respectively at 60 h, and the activity of FPase reached a maximum value of 0.141 U/mL at 48 h (Fig. 4). The incubation time was generally shorter than the 2–21 d found in the literature [33,34]. After 72 h, the cellulase activities began to decrease significantly. The relative activities of CMCase and β-glucosidase were 78.5% and 88.6% at 84 h, and FPase relative activity was 88.7% at 96 h. After 96 h, cellulase activities were maintained at a stable stage, which may be the decomposition of cellulose to produce glucose and fibrinose. Because of catabolite repression, enzymes are no longer consumed and cellulose is no longer broken down. Studies were shown that glucose and fibrinose are considered to have negative effects on cellulase production [35]. In order to maximize the use of cellulose and produce cellulase with stronger enzymatic activity, it is necessary to remove the cellobiose and glucose produced to maintain the growth level of *Bacillus* to provide uninterrupted stress conditions to stimulate higher cellulase production [36,37].

3.6. Effect of initial pH on cellulases production

Adjusting the initial pH of the SmF medium between 5 and 9 resulted in different types of cellulases tested exhibiting different regular patterns. The CMCase activity showed almost the same activity value over the entire pH range, even with a relative activity of 75.8% at a pH of 9. The optimum pH for FPase and β-glucosidase activities were 6 and 7 respectively, and the relative activities under acidic conditions were only 20.1% and 19.5% (Fig. 5). *Bacillus paralicheniformis* was more suitable for growth under weak alkaline conditions, and the three types of cellulases can maintain a relative activity of about 61.9% to 88.0% in the pH range of 7–9. *Salinivibrio* sp. strain NTU-05 [38], *Gracilibacillus* sp.

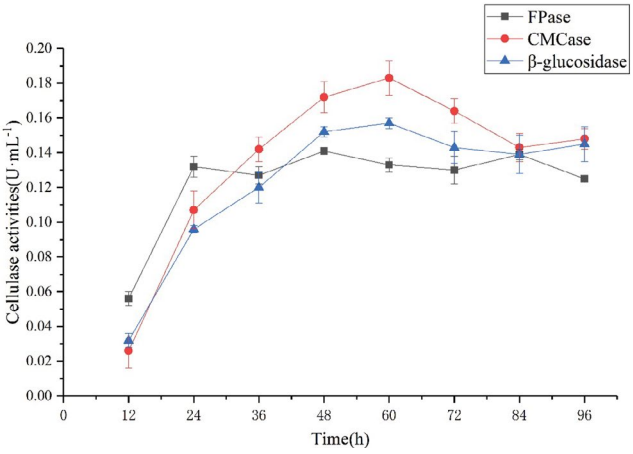

Fig. 4. Cellulase activities produced by *Bacillus paralicheniformis* along with the cultivation period in SmF.

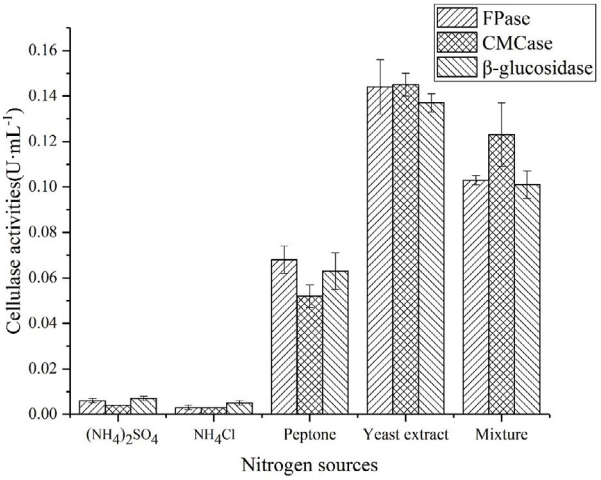

Fig. 3. Cellulase activities produced by *Bacillus paralicheniformis* in the SmF containing different nitrogen sources.

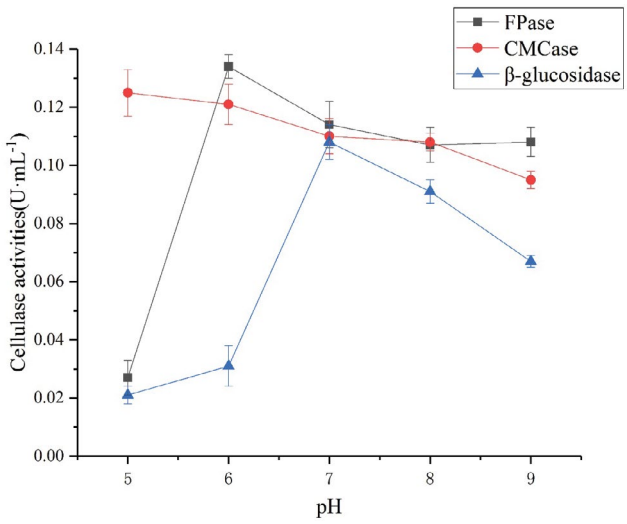

Fig. 5. Cellulase activities produced by *Bacillus paralicheniformis* with different initial pH in SmF.

SK1 [26] and *Bacillus* sp. L1 [39] also showed similar extreme halotolerance. Another study found that cellulases from *M. racemosus* CBMAI 847 exhibited two optimal pH values (5 and 8) [40]. Marine-derived fungus *Cladosporium sphaerospermum* achieved good cellulase production when the pH of the medium was adjusted to 4 [16]. Therefore, these reports showed that marine strains can biosynthesis cellulases under both acidic and alkaline conditions. Extreme pH condition also affects the binding of enzymes and substrates. Strong acids and bases inhibit the metabolic capacity of the strain and weaken the ability of the strain to degrade cellulose.

3.7. Effect of temperature on cellulases production

The temperature was one of the important factors affecting the enzymatic reaction. Increasing the temperature can accelerate the rate of movement between molecules, and the probability of collision between molecules becomes larger, thereby accelerating the rate of reaction. However, higher temperatures caused the enzyme protein to be gradually denatured and inactivated, thereby reducing the rate of enzymatic reaction [41]. The FPase activity showed good relative activity between 25°C and 45°C, which optimum activity reached 0.14 U/mL at 45°C and the relative activity between 35°C and 45°C was above 90%. CMCase activity has the widest temperature adaptability, maintaining 80% relative activity between 30°C and 50°C and reaching a maximum at 35°C. The β-glucosidase was sensitive to temperature and the optimum temperature was 35°C (Fig. 6). The relative activity of three types of cellulases during the test was maintained at more than 50%. In general, the optimal temperature for *Bacillus* is found in the range of 30°C–45°C, which can be explained by the fact that the strain is a mesophilic microorganism with better growth between 20°C and 40°C. In general, the optimal temperature for *Bacillus paralicheniformis* was found in the range of 30°C–45°C, which can be explained by the fact that the strain was a mesophilic microorganism with better growth between 20°C and 40°C

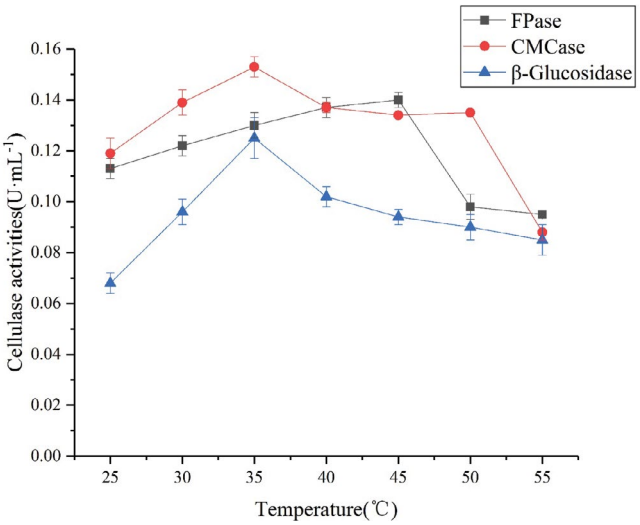

Fig. 6. Cellulase activities produced by *Bacillus paralicheniformis* with different cultivation temperature in SmF.

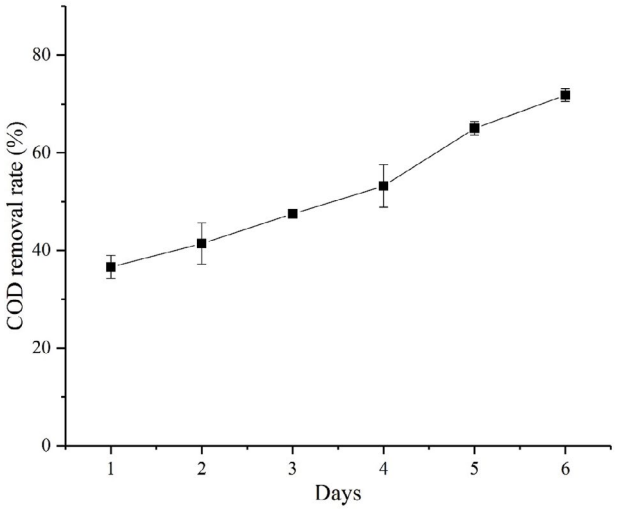

Fig. 7. Removal rates of COD.

[42]. CMCase with thermal stability had great potential for reducing production costs and increasing volumetric productivity. The speed and efficiency of the first step of CMCase catalyzing cellulose hydrolysis was critical to the success of the entire process [43].

3.8. Degradation performance of COD

According to the above experiment, the optimal production conditions of the cellulases were obtained. Inoculation of *Bacillus paralicheniformis* in the optimal SmF medium, the three cellulase activities can be obtained as follows: FPase 0.143 U/mL, CMCase 0.189 U/mL, β-glucosidase 0.158 U/mL. The wastewater was treated with diluted cotton pulp wastewater (2% salinity, 1,900 mg/L COD) as experimental water, and the treated water was continuously measured for 6 d. *Bacillus paralicheniformis* has a good treatment efficiency for diluted cotton pulp wastewater, and the highest treatment efficiency reached 71.8% on the 6th day (Fig. 7). It is reported that internal circulation anaerobic reactor better treated diluted cotton pulp wastewater with solute chemical oxygen demand concentration of 3,500 mg/L, the removal rate reached 68% [5].

4. Conclusion

Bacillus paralicheniformis was found to efficiently utilized marine mud and cotton pulp wastewater produced cellulases. To explore the production of cellulases by bacteria in SmF under different conditions. The isolated cellulolytic halotolerant bacterium showed thermo-stability and alkali resistance. The optimal cellulose (FPase 0.143 U/mL, CMCase 0.189 U/mL, β-glucosidase 0.158 U/mL) production can be obtained under optimal conditions using CMC and corncob as the carbon source. The COD removal rate of the bacterium on the diluted cotton pulp wastewater for 6 consecutive days was 71.8%.

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References

[1] G. Wang, T. Xiang, Y. Zheng, Analysis on water pollution control technology in the viscose fibre business, Arid Environ. Monit., 21 (2007) 176–180.
[2] H. Liu, B. Pang, J. Zhou, Y. Han, J. Lu, H. Li, H. Wang, Comparative study of pretreated corn stover for sugar production using cotton pulping black liquor (CPBL) instead of sodium hydroxide, Ind. Crops Prod., 84 (2016) 97–103.
[3] M.N. De Almeida, D.L. Falkoski, V.M. Guimarães, S.T. De Rezende, Study of gamba grass as carbon source for cellulase production by *Fusarium verticillioides* and its application on sugarcane bagasse saccharification, Ind. Crops Prod., 133 (2019) 33–43.
[4] S. Zu, W.Z. Li, M. Zhang, Z. Li, Z. Wang, H. Jameel, H.M. Chang, Pretreatment of corn stover for sugar production using dilute hydrochloric acid followed by lime, Bioresour. Technol., 152 (2014) 364–370.
[5] P. Cui, X. Zhou, Y. Zhang, The feasibility study of cotton pulp wastewater treatment with IC anaerobic reactor, Procedia Environ. Sci., 11 (2011) 686–692.
[6] L. Liu, Y. Hu, P. Wen, N. Li, M. Zong, B. Ou-Yang, H. Wu, Evaluating the effects of biocompatible cholinium ionic liquids on microbial lipid production by *Trichosporon fermentans*, Biotechnol. Biofuels, 8 (2015) 119.
[7] N.S. Naghavi, G. Emtiazi, N. Karimian, Partial purification and immobilization of cellulase enzymes from the fungus *Aspergillus terreus* isolated from rotten wood, J. Life Sci. Technol., 1 (2013) 7–9.
[8] R. Saini, J.K. Saini, M. Adsul, A.K. Patel, A. Mathur, D. Tuli, R.R. Singhania, Enhanced cellulase production by *Penicillium oxalicum* for bio-ethanol application, Bioresour. Technol., 188 (2015) 240–246.
[9] F.M. Medie, G.J. Davies, M. Drancourt, B. Henrissat, Genome analyses highlight the different biological roles of cellulases, Nat. Rev. Microbiol., 10 (2012) 227.
[10] H. Watanabe, G. Tokuda, Cellulolytic systems in insects, Annu. Rev. Entomol., 55 (2010) 609–632.
[11] M. Gatti, F. García-Usach, A. Seco, J. Ferrer, Wastewater COD characterization: analysis of respirometric and physical-chemical methods for determining biodegradable organic matter fractions, J. Chem. Technol. Biotechnol., 85 (2010) 536–544.
[12] H. He, Y. Chen, X. Li, Y. Cheng, C. Yang, G. Zeng, Influence of salinity on microorganisms in activated sludge processes: a review, Int. Biodeterior. Biodegrad., 119 (2017) 520–527.
[13] E.H. Seck, A. Diop, N. Armstrong, J. Delerce, P.E. Fournier, D. Raoult, S. Khelaifia, Microbial culturomics to isolate halophilic bacteria from table salt: genome sequence and description of the moderately halophilic bacterium *Bacillus salis* sp. nov, New Microbes New Infect., 23 (2018) 28–38.
[14] J.H. Hong, S. Jang, Y.M. Heo, M. Min, H. Lee, Y.M. Lee, H. Lee, J.J. Kim, Investigation of marine-derived fungal diversity and their exploitable biological activities, Mar. Drugs, 13 (2015) 4137–4155.
[15] A.A. Gunny, D. Arbain, P. Jamal, R.E. Gumba, Improvement of halophilic cellulase production from locally isolated fungal strain, Saudi J. Biol. Sci., 22 (2015) 476–483.
[16] N. Trivedi, C.R.K. Reddy, R. Radulovich, B. Jha, Solid state fermentation (SSF)-derived cellulase for saccharification of the green seaweed Ulva for bioethanol production, Algal Res., 9 (2015) 48–54.
[17] W.K. Hegazy, M.S. Abdel-Salam, A.A. Hussain, H.H. Abo-Ghalia, S.S. Hafez, Improvement of cellulose degradation by cloning of endo-beta-1,3–1,4 glucanase (bgls) gene from *Bacillus subtilis* BTN7A strain, J. Genet. Eng. Biotechnol., 16 (2018) 281–285.
[18] A. Augustine, I. Joseph, Four novel strains of cellulolytic symbiotic bacteria isolated and characterized from GI tract of marine fishes of various feeding habits, Biocatal. Agric. Biotechnol., 16 (2018) 706–714.
[19] N. Libardi, C.R. Soccol, J.C. De Carvalho, L.P. De Souza Vandenberghe, Simultaneous cellulase production using domestic wastewater and bioprocess effluent treatment - a biorefinery approach, Bioresour. Technol., 276 (2019) 42–50.
[20] S. Sarsaiya, S.K. Awasthi, M.K. Awasthi, A.K. Awasthi, S. Mishra, J. Chen, The dynamic of cellulase activity of fungi inhabiting organic municipal solid waste, Bioresour. Technol., 251 (2018) 411–415.
[21] K. Apun, B.C. Jong, M.A. Salleh, Screening and isolation of a cellulolytic and amylolytic *Bacillus* from sago pith waste, J. Gen. Appl. Microbiol., 46 (2000) 263–267.
[22] D.E. Eveleigh, M. Mandels, R. Andreotti, C. Roche, Measurement of saccharifying cellulase, Biotechnol. Biofuels, 2 (2009) 1–8.
[23] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Biochem., 31 (1959) 426–428.
[24] L.R. Lynd, P.J. Weimer, W.H. Van Zyl, I.S. Pretorius, Microbial cellulose utilization: fundamentals and biotechnology, Microbiol. Mol. Biol. Rev., 66 (2002) 506–77.
[25] K. Harshvardhan, A. Mishra, B. Jha, Purification and characterization of cellulase from a marine *Bacillus* sp. H1666: a potential agent for single step saccharification of seaweed biomass, J. Mol. Catal. B: Enzym., 93 (2013) 51–56.
[26] H.-Y. Yu, X. Li, Alkali-stable cellulase from a halophilic isolate, *Gracilibacillus* sp. SK1 and its application in lignocellulosic saccharification for ethanol production, Biomass Bioenergy, 81 (2015) 19–25.
[27] C. Cai, Y. Jin, Y. Pang, Q. Ke, W. Qiu, X. Qiu, Y. Qin, H. Lou, Tracing cellulase components in hydrolyzate during the enzymatic hydrolysis of corncob residue and its analysis, Bioresour. Technol. Rep., 4 (2018) 137–144.
[28] X. Liu, Y. Liu, Z. Jiang, H. Liu, S. Yang, Q. Yan, Biochemical characterization of a novel xylanase from *Paenibacillus barengoltzii* and its application in xylooligosaccharides production from corncobs, Food Chem., 264 (2018) 310–318.
[29] S.A. Sheweita, A. Ichi-Ishi, J.S. Park, C. Liu, L.M. Malburg, R.H. Doi, Characterization of engF, a gene for a non-cellulosomal *Clostridium cellulovorans* endoglucanase, Gene (Amsterdam), 182 (1991) 0–167.
[30] X. Shao, M. Jin, A. Guseva, C. Liu, V. Balan, D. Hogsett, B.E. Dale, L. Lynd, Conversion for Avicel and AFEX pretreated corn stover by *Clostridium thermocellum* and simultaneous saccharification and fermentation: insights into microbial conversion of pretreated cellulosic biomass, Bioresour. Technol., 102 (2011) 8040–8045.
[31] J.Q. Zhu, L. Qin, W.C. Li, J. Zhang, J. Bao, Y.D. Huang, B.Z. Li, Y.J. Yuan, Simultaneous saccharification and co-fermentation of dry diluted acid pretreated corn stover at high dry matter loading: overcoming the inhibitors by non-tolerant yeast, Bioresour. Technol., 198 (2015) 39–46.
[32] N. Libardi, C.R. Soccol, A. Góes-Neto, J.D. Oliveira, L.P.D.S. Vandenberghe, Domestic wastewater as substrate for cellulase production by *Trichoderma harzianum*, Process Biochem., 57 (2017) 190–199.
[33] G.S. Dhillon, S. Kaur, S.K. Brar, M. Verma, Potential of apple pomace as a solid substrate for fungal cellulase and hemicellulase bioproduction through solid-state fermentation, Ind. Crops Prod., 38 (2012) 6–13.
[34] F. Mostafa, A. Abd El Aty, Enzyme activities of the marine-derived fungus *Alternaria alternata* cultivated on selected agricultural wastes, J. Appl. Biol. Sci., 7 (2013) 48–55.
[35] N.K. Ramamoorthy, T.R. Sambavi, S. Renganathan, A study on cellulase production from a mixture of lignocellulosic wastes, Process Biochem., 83 (2019) 148–158.
[36] K.M.V. Nogueira, M. do Nascimento Costa, R.G. de Paula, F.C. Mendonça-Natividade, R. Ricci-Azevedo, R.N. Silva, Evidence of cAMP involvement in cellobiohydrolase expression and secretion by *Trichoderma reesei* in presence of the inducer sophorose, BMC Microbiol., 15 (2015) 195.

[37] A. Schuster, D. Tisch, V. Seidl-Seiboth, C.P. Kubicek, M. Schmoll, Roles of protein kinase A and adenylate cyclase in light-modulated cellulase regulation in *Trichoderma reesei*, Appl. Environ. Microbiol., 78 (2012) 2168–2178.
[38] C.-Y. Wang, Y.-R. Hsieh, C.-C. Ng, H. Chan, H.-T. Lin, W.-S. Tzeng, Y.-T. Shyu, Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU-05, Enzyme Microb. Technol., 44 (2009) 373–379.
[39] M. Irfan, A. Tayyab, F. Hasan, S. Khan, M. Badshah, A.A. Shah, Production and characterization of organic solvent-tolerant cellulase from *Bacillus amyloliquefaciens* AK9 isolated from hot spring, Appl. Biochem. Biotechnol., 182 (2017) 1390–1402.
[40] D.A. Santos, M.M. Oliveira, A.A.S. Curvelo, L.P. Fonseca, A.L.M. Porto, Hydrolysis of cellulose from sugarcane bagasse by cellulases from marine-derived fungi strains, Int. Biodeterior. Biodegrad., 121 (2017) 66–78.
[41] N.P. Marques, J. de Cassia Pereira, E. Gomes, R. da Silva, A.R. Araújo, H. Ferreira, A. Rodrigues, K.J. Dussán, D.A. Bocchini, Cellulases and xylanases production by endophytic fungi by solid state fermentation using lignocellulosic substrates and enzymatic saccharification of pretreated sugarcane bagasse, Ind. Crops Prod., 122 (2018) 66–75.
[42] H.K. Dillon, P.A. Heinsohn, J. Miller, Field Guide for the Determination of Biological Contaminants in Environmental Samples, American Industrial Hygiene Association, Biosafety Committee, AIHA-Rocky Mountain Section, P.O. Box: 746461, Arvada, CO 80006, 1996.
[43] F. Akram, I.U. Haq, W. Imran, H. Mukhtar, Insight perspectives of thermostable endoglucanases for bioethanol production: a review, Renewable Energy, 122 (2018) 225–238.