



Extracellular hydrolytic enzyme activities of indigenous actinomycetes on pretreated bagasse using choline acetate ionic liquid

Heri Satria^{*}, Yandri, Nurhasanah, Surtpto Dwi Yuwono, Dian Herasari

Department of Chemistry Faculty of Mathematics and Natural Sciences University of Lampung, Jl. Soemantri Brojonegoro No.1, Bandar Lampung, 35145, Indonesia

ARTICLE INFO

Keywords:

Hydrolysis
Extracellular enzyme
Bagasse
Ionic liquid
Choline acetate

ABSTRACT

Ionic liquids (ILs) pretreatment is currently becoming an attractive approach for improving the efficiency of enzymatic hydrolysis on lignocellulose. Cholinium acetate (ChOAc) ILs that contain choline cation combined with carboxylic acid-based anion is not only marked as biocompatible ILs but also known as a useful catalyst in the pretreatment of lignocellulosic biomass, which results in an increase of enzymatic saccharification rate of the biomass. In this study hydrolytic activities of cellulase and xylanase which produce extra-cellularly by isolated actinomycetes under the presence of ChOAc were investigated. ChOAc/biomass ratio (g/g) were set up between 0.0 and 3.0 for pretreatment followed by hydrolysis using cellulase and xylanase that excreted when actinomycetes were grown up in pretreated bagasse successively. Act-7 was used as inoculum in saccharification, and the optimum of ChOAc/biomass ratio was as identified to be 1, which achieved high saccharification with a yield of reducing sugar 74.58%. Since the cellulase activities reduced to around 30–70% while ChOAc/biomass ratio was increased to 2–3, in contrast, xylanase activities remain stable at the range of 58.97–75.19 U/mL. This experiment demonstrates the promising bio-hydrolysis of biomass in green technology to increase the yield of the biomass saccharification process.

1. Introduction

Due to its potential to renewable organic materials, lignocellulosic biomass was considered to provide as a low-cost feedstock for bioconversion into fermentable sugar which can be further utilized for bio-fuel and valuable chemicals production (Gomez et al., 2008; Uppugundla et al., 2014; Wu et al., 2016). This material primarily contains three bio-polymers particularly cellulose, hemicellulose and lignin with a low number part of trace components such as acetyl groups, phenolic substituents, and minerals unitedly. The quantities of these bio-polymers in biomass, depending on the source of biomass, commonly the biocomposite composed of cellulose, hemicellulose, and lignin with the various concentration of fractions biopolymers at 35–50%, 20–35%, 10–25% respectively. (Isikgor and Becer, 2015; Menon and Rao, 2012). The interaction of polymers in biomass produces the recalcitrance which comes from the crystallinity of cellulose, coating of cellulose by the lignin-hemicellulose matrix, and, hydrophobicity of lignin (Agbor et al., 2011; Isikgor and Becer, 2015; Mosier et al., 2005). Native lignocellulose structure forms strong recalcitrance to enzymatic hydrolysis and results in poor digestibility of

the material itself. A convenient pretreatment method is involved to overcome this limitation and makes the polymers easily accessible for enzyme digestion (Sun et al., 2016).

Ionic liquids (ILs) pretreatment, which is a kind of chemical pretreatment technique, has earned high interest after some types of ILs were invented as solvents that have the ability to soluble cellulose (Swatloski et al., 2002). ILs are organic salts generally consisted of organic cations and inorganic anions and the forms are in the liquid below 100 °C (Macfarlane and Kar, 2017; Welton, 2018). Recently, ILs have been pointed out to deconstruct the compact structure of lignocellulose. Some of ILs have been exhibited to dissolve lignin, hemicellulose, and cellulose so that the main polymers could be separated one and each other. Furthermore, the crystallinity of regenerated cellulose became diminish significantly after the cellulose was being soluble in the ILs. This fragile cellulose would be promoted more efficient enzymatic hydrolysis (Brandt et al., 2013). However, compared with the other addition of chemicals on pretreatment such as dilute acid or ammonia, the application of ILs on lignocellulosic pretreatment is not only have been shown more effective than conventional ones, but it

^{*} Corresponding author.

E-mail address: heri.satria@fmipa.unila.ac.id (H. Satria).

has also been proven to be applicable to various types of lignocellulosic biomass (Kilpeläinen et al., 2007; Brandt et al., 2013).

Recently among the type of ILS, imidazolium ILS type has been extensively studied in particular. In contrast, their abilities in the deconstruction of the lignocellulosic tight structure were faced by biocompatibility issues including their disadvantage points for example in cytotoxicity and biodegradability, and also in the high expense reason (Datta et al., 2010; Ouellet et al., 2011, 2015; Ninomiya et al., 2015a). On the other hand, compare with imidazolium ILS, some types of cholinium ILS have been apparent more biocompatible (Petkovic et al., 2009) and are also have a lower price generated by the low expense of the source cation (Plechova and Seddon, 2008). Cholinium ILS are composed of either choline cations associated with two commons of anions such as the amino acid-based (Hu et al., 2007), or carboxylic acid-based (Fukaya et al., 2007) ones. Many observations remarked the advantageous of cholinium ILS in the pretreatment of biomass that improves the hydrolysis of lignocellulosic biomass using cellulase (Ninomiya et al., 2015a, 2015b; 2015c, 2015d, 2014, 2013a; 2013b, 2013c). In addition, choline acetate (ChOAc), Fig. 1, has applied to pretreat bagasse in high-loading biomass (100 g/L) which has risen up the hydrolysis of cellulose and hemicellulose efficiency into 95% and 93% respectively (Ninomiya et al., 2018).

Since many previous studies conducted the enzymatic saccharification using pure cellulase and/or hemicellulase, there were fewer studies that investigated the application of native enzyme which secreted extracellularly by microorganisms when they cultivate on lignocellulosic biomass. However, this working was exploring the opportunity of impured extracellular hydrolytic enzyme which produces by some indigenous actinomycetes isolate in ChOAc-pretreated bagasse medium to enhance saccharification of cellulose and xylan contained therein. The aim of the study is to investigate the synergistic between a pretreatment of bagasse using a biocompatible ionic liquid (ChOAc) and enzymatic saccharification by applying the inoculum of actinomycetes that adequates to excrete extracellular cellulase and xylanase sequentially. The future prospect of this study is to develop a one-pot bioconversion of lignocellulosic biomass into fermentable sugar and its derivative product such as ethanol, organic acids, and other advanced chemicals.

2. Materials and methods

2.1. Materials

Row bagasse was obtained from useless sugar production residue of Sweet Indo Lampung (SIL) Company located in Lampung Province, Indonesia. The bagasse was dried and mashed up and screened using a sieve to obtain the less than 250 μm particle. The biomass was stored in an oven at 90 $^{\circ}\text{C}$ until used. Choline acetate was bought from Sigma-Aldrich and all chemicals classified in laboratory guaranteed reagent (GR), which purchased from a variety of commercial companies.

2.2. Isolation of actinomycetes and screening for cellulase and xylanase actinomycetes

Decomposed bagasse as a source of indigenous actinomycetes was collected directly from the waste area of SIL Company. One gram of

the bagasse then was transferred into 100 mL of saline water (NaCl 0.95% w/v) and was shaken using shaker incubator at 120 rpm for 60 min. The suspension which formed was subjected to serial dilutions up to 10^{-7} . Isolation of actinomycetes was conducted by pour plate methods in which each different dilution suspension was inoculated onto Yeast Maltose Agar (YMA) Medium (4 g/L of Yeast extract, 10 g/L of Malt extract powder, 4 g/L of Glucose, and 18 g/L Agar) in-appropriate in triplicate. The colony of actinomycetes was then identified base on morphology appearing after incubated for 7–14 days at 37 $^{\circ}\text{C}$.

Purification of actinomycetes was conducted by quadrant-streaking the single colony on a specific medium ISP-2 (Himedia) (Shirling and Gottlieb, 1966). The pure culture isolates are cultivated in the slant ISP-2 medium at 4 $^{\circ}\text{C}$ and glycerol stock was provided for the next examination.

Screening of cellulolytic and xylanolytic was examined by the Congo-Red method which the pure selected isolates were grown on YMA medium-enriched using 0.5% (w/v) carboxymethylcellulose/CMC (Sigma) and 0.5% (w/v) birchwood xylan (Sigma) respectively for 14 days. Ability to degrade CMC/birchwood xylan of the isolate was evaluated after the growth period by incubation with staining solution of Congo Red 0.1% (w/v) for 30 min and afterward was washed out using NaCl (5 M) (Amore et al., 2014, 2012; Teather and Wood, 1982). The clear halo zone around the colony of the isolate was signed as a positive isolate that has cellulase/xylanase activity.

The Yeast Maltose Broth (YMB) base medium used for enzyme production by the isolates contained the following components: 4 g/L of Yeast extract, 10 g/L of Malt extract powder, and 4 g/L of Glucose. The medium was enriched by 1% of CMC and 1% of Birchwood xylan to produce cellulase and xylanase respectively. All along with the production time, a repeatedly supernatant was collected for enzyme activity assay.

2.3. Pretreatment bagasse using choline acetate

Pretreatment of bagasse using ChOAc was applied according to the Ninomiya's group working (Ninomiya et al., 2018) with slight modifications. Definitely, ratios ChOAc/biomass (g/g) at 0, 0.5, 1, 1.5, 2, 2.5 and 3 for pretreatment of bagasse by ChOAc was conducted by immersing 5 g of bagasse powder with 0, 2.5, 5, 7.5, 10, 12.5 and 15 g of ChOAc in Erlenmeyer respectively. The mixture was then stirred gently using a magnetic stirrer for 60 min at room temperature. Furthermore, the pretreatment was conducted by using an autoclave at 110 $^{\circ}\text{C}$ for 1 h and the heating was extended in a thermal oven at 110 $^{\circ}\text{C}$ for 20 h. After this pretreatment step was complete, the mixture was cooled down at room temperature which placed on a clean bench.

2.4. Saccharification pretreated bagasse using actinomycetes

Actinomycetes medium was prepared by adding the previously pretreated bagasse as a biomass source ingredient. The pretreated bagasse was suspended well with 50 mL sterile YMB medium aseptically. Fermentation was then undertaken by inoculating 2 cork bore ($\text{O} = 5 \text{ mm}$) of cultivated actinomycetes into the previous suspension medium. Incubation was conducted using a horizontal shaker incubator at 37 $^{\circ}\text{C}$, 120 rpm. During the fermentation, a periodically liquid sample was taken up after 1–14 days. The collected samples were centrifuged at 6000 rpm for 5 min, obtained supernatants were transferred into sample labeled clean tube and was stored in a refrigerator for future analysis. The optimization of initial pH was conducted by setting up the initial pH at 6.0, 6.4, 7.0, 7.2, 7.6 and 8.0 using 0.2N $\text{Na}_2\text{HPO}_4 - \text{NaH}_2\text{PO}_4$ buffer solution.

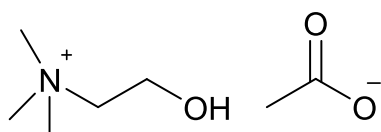


Fig. 1. Molecule structure of Choline acetate.

2.5. Cellulase and xylanase activity assay

The activity of cellulase and xylanase was examined according to the Carboxymethyl cellulose-ase/CMCase (Ghose, 1987) and Xylanase (Bailey et al., 1992) assay method respectively. The measurement of CMC-ase activity was conducted by adding 0.5 mL of the collected sample as a native enzyme into 0.5 mL of soluble 1% CMC in sodium citrate buffer (0.05M, pH 5.0) at 37 °C for 30 min. After finishing incubation, the reaction was cut off by pipetting 1.0 mL of 3,5-dinitrosalicylic acid (DNS) reagent and was then boiled at 95 °C for 15 min. The spectrophotometric method was applied to determine reducing sugars with DNS (Miller, 1959) using glucose as standard. The CMC-ase activity was calculated according to the amount of released glucose from CMC. Moreover, the examination of xylanase by mixing 0.5 mL of obtained crude xylanase enzyme filtrate sample with 0.5 mL of 1% birchwood xylan in 0.05M sodium citrate buffer (pH 5.0) at 37 °C for 30 min. The reaction was then terminated by adding 1.0 mL of DNS reagent and was then heated at 95 °C for 15 min. Xylose concentrations were estimated by using similar methods with reduction sugar analysis using xylose as standard. The xylanase activity was measured base on the amount of liberated xylose from xylan.

The unit of cellulase or xylanase activity (U/mL) was defined as amount of the enzyme that catalyze the CMC or birchwood xylan into 1 μ mol glucose or xylose as the product respectively per minute.

3. Results and discussion

3.1. Isolation and screening actinomycetes

Some indigenous actinomycetes from decomposed bagasse were successfully isolated on the YMA medium. Using a dilution series of initial samples to obtain a separated grown strain colony, the dilution factor at 10^{-5} was noted as the best suspension sample to be cultivated. The single different separate colonies grew on the YMA medium so that the single colony could be collected and characterized base on its shape and color appearance (Fig. 2).

Moreover, the purification step was conducted to collect a pure single colony that would be screened for their cellulolytic and xylanolytic ability. The actinomycetes strain which is able to hydrolyze cellulose and xylan was recognized by the formation of halo clear zone around the colony after applying Congo-Red staining on the se-



Fig. 2. Culturable actinomycetes on the YMA medium resulted from cultivated 10^{-5} suspension of decomposed bagasse.

lected cellulolytic or xylanolytic medium assay. In this working, seven actinomycetes isolate, namely Act-1 to Act-7, could grow consistently when they cultivated on the YMA medium and the isolate also exhibited hydrolytic activity on CMC-YMA and either on Xylan-YMA medium. In addition, cellulolytic and xylanolytic indexes were estimated base on a comparison between the diameter of a formed halo clear zone to the estimated diameter of the colony. The colony of strain has a diameter between 8 and 24 mm, and the diameter varies between the assay medium. The cellulolytic indexes were between 1.05 and 2.10 since the xylanolytic ones were 1.20–1.80 approximately. Table 1 describes a measurement both of cellulolytic and xylanolytic indexes of purified strains.

3.2. Cellulase and xylanase activities of actinomycetes

The plate assay using Congo-red staining has been widely applied in many studies to screen microorganisms were able to degrade cellulose and hemicellulose. Despite, Teather and Wood evaluated that the diameter of a formed halo zone was not always according to the enzyme-producing ability of microorganisms and also the activity degree (Teather and Wood, 1982). To confirm the ability to enzyme activities of isolate, in this study, the development of enzyme activities during cultivation actinomycetes was observed.

The observation of both cellulase and xylanase activities started to develop after seven days of the incubation period. It was detected that almost the highest activities of cellulase were seen on the thirteenth day and the activities saw decreasing after the fifteenth day. However, high activity of cellulase was detected over 20 U/mL to 50 U/mL from several isolates since the others have low activity under 15 U/mL. In addition, among seven isolates that have cultivated in batch culture, the cellulase activities from Act-1, Act-3, Act-7 were higher than the other isolate. The increasing of cellulase activity from these three isolates has a similar pattern which enlarged between eighth to the thirteenth day. The optimum cellulase activities of Act-1, Act-3, and Act-7 achieved to 35.34, 42.41, and 50.18 U/mL respectively and it tends to remain stable after the peak for the next three days production. It also appeared clearly, the cellulase activity from Act-7 was the highest one. The activity was over the others since the cellulase was released after tenth-day production with the range activity of 26.37–50.18 U/mL. All of the enzyme activities which are described in this work serve in Fig. 3.

Following the previous observations, the activity of xylanase along with the cultivation of selected actinomycetes strains was conducted. It was coming into sight that the xylanase activities were grown up initially after the eighth day of fermentation. The increasing of xylanase activity dramatically rose between the twelfth to the fourteenth day, which the highest one was seen on the fourteenth. Contrastly, it has been viewed that the xylanase activity of Act-4 and Act-5 isolate were higher than their cellulase activities, herein, both xylanase activities exceed the activity of Act-1. Comparing with its cellulase activity

Table 1
Indexes of cellulolytic and xylanolytic of strains.

Strains	Cellulolytic			Xylanolytic		
	D	d	Index	D	d	Index
	(mm)	(mm)		(mm)	(mm)	
Act-1	30	21	1.42	23	16	1.46
Act-2	19	18	1.05	10	8	1.20
Act-3	29	14	2.10	25	21	1.20
Act-4	38	32	1.20	26	24	1.10
Act-5	29	19	1.50	30	21	1.42
Act-6	39	28	1.38	18	12	1.46
Act-7	32	18	1.80	29	16	1.8

D; halo clear zone diameter; d; colony diameter.

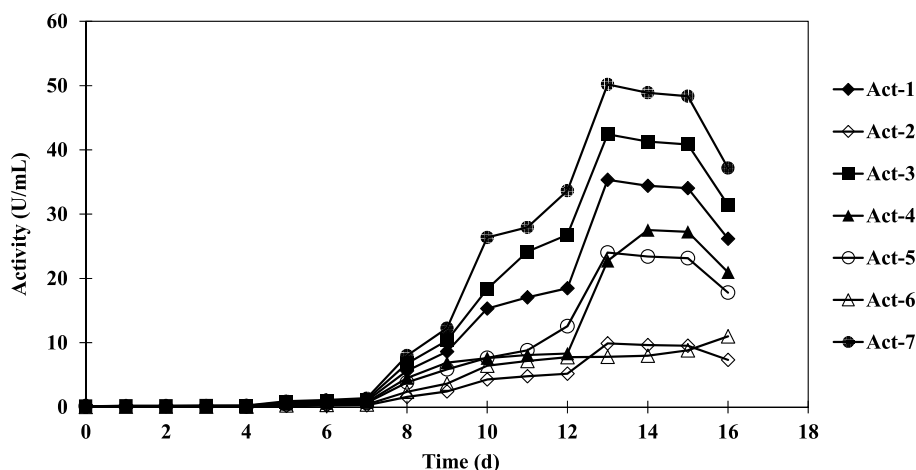


Fig. 3. Time course of cellulase production by selected actinomycetes strains in 1% CMC enriched YMB medium.

(35.33 U/mL), the xylanase activity of Act-1 was almost equal at 36.26 U/mL. Meanwhile, it was noted that xylanase activity from Act-3 and Act-7 rose above the other xylanase activity, and Act-7 has the highest xylanase activity. Fig. 4 summarizes the xylanase activities of actinomycetes strain Act-1 to Act-7 in the course of xylanase production.

Both of cellulase and xylanase activities declined after their maximum peak activities which it was coming after 14 days cultivation. The alighting could be associate to a condition after stationary growth phase of the actinomycetes isolate, whereas might be attributed to substrates depletion and/or the activity of contained proteases (Fatokun et al., 2016) or catabolic repression of byproduct such as cellobiose and glucose (El-Naggar, 2012). Lowering of carbon and nitrogen supply in the growth medium could affect the growth of the actinomycetes and production of excretion hydrolytic enzyme in the isolate were directly affected. On the other hands, the presence of hydrolytic product of bagasse such as cellobiose, glucose and xylose in sufficient concentration might be give a feedback inhibition which could inhibit enzyme activities (Andrić et al., 2010; Lammirato et al., 2010; Mithra and Padmaja, 2017).

Base on the highest enzyme activity of Act-7 isolate, it has been chosen as an enzyme source in the hydrolysis of the pretreated bagasse experiment. In previous procedure, the fermentation was conducted using YMB enriched medium that has initial pH at 6.4. To optimize the production of hydrolytic enzyme, in this study, the initial pH for both production of cellulase and xylanase were examined. Fig. 5 shows the activities of cellulase and xylanase that collected at 14

days in production period at different initial pH levels, and it exhibits the optimum initial pH was 7.0 that is the most suitable initial pH set up to achieve the peak of produced cellulase and xylanase at 79.97 and 95.95 U/mL respectively.

Screening of cellulolytic and xylanolytic and the character of its activity from microorganisms have already exposed by many researchers. Actinomycetes are famous as a source of many industrial enzymes including cellulase and xylanase and have the ability to release the hydrolytic enzyme (Lynd et al., 2002; Mukhtar et al., 2017). The hydrolytic activity can be quantified by a variety of methods and its value also diverse depending on the medium setting in the production procedure (Johnsen and Krause, 2014). Cellulase produced by isolated actinomycetes from a different region in India has examined which CMC-ase activity of actinomycetes strain NAA2 was the highest at 0.366 U/mL. It was resulted from the culture using modified Mandel's medium at 30 °C for seven days incubation (Saini et al., 2016). Furthermore, two *Streptomyces* strains isolated from Brazilian soil forest, M17a and M23, have cellulase activity at 235 and 95 U/mL respectively. In another study, by using modified Hosikoshi liquid medium, actinomycetes were secreted xylanase with range activities between 105 and 149 U/mL. It was also noted in many studies that the appropriate and optimum pH for cellulase and xylanase production using actinomycetes was nearly neutral (7.0) (Kulkarni et al., 1999; McCarthy et al., 1985; Lynd et al., 2002). The cellulase and xylanase activities in this working have been in the range of the activities and range of the initial pH production achieved by the previous researchers.

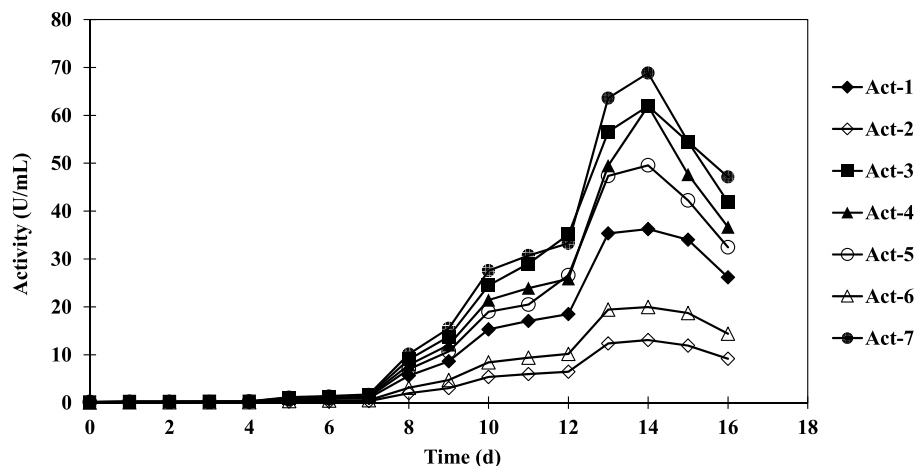


Fig. 4. Time course of xylanase production by selected actinomycetes strains in 1% Birchwood xylan enriched YMB medium.

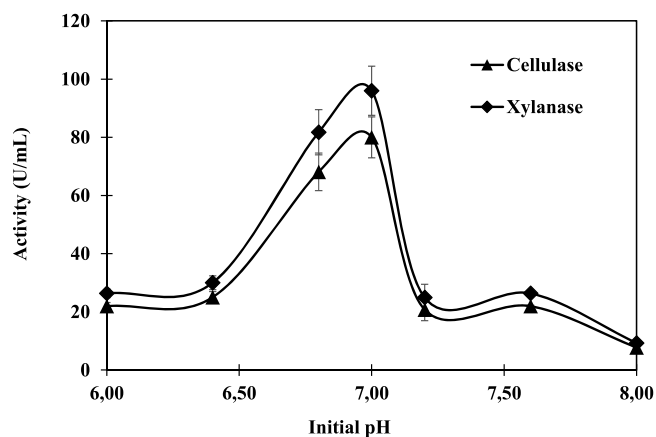


Fig. 5. The optimization of initial pH production in YMB medium that was enriched by 1% of CMC and 1% of Birchwood xylan to produce cellulase and xylanase by Act-7 respectively.

3.3. Cellulase and xylanase activities on saccharification in presence of ChOAc (Act-7)

The pretreatment of bagasse using ChOAc was then conducted to prepare a pretreated biomass substrate for hydrolysis enterprise. The procedure was applied to obtain similar substrate conditions to Ninomiya's group working (Ninomiya et al., 2018, 2015d). They mentioned that pretreatment using ChOAc up to ChOAc/biomass ratio at 1.5 (g/g) provided a sufficient specific surface area (SSA) herein it expected to accomplish enzyme accessibility for bagasse and also the decreasing of crystallinity index (CrI) of bagasse was 50–60%. Since that research used a pure hydrolytic enzyme, nevertheless this research applied a source of the hydrolytic enzyme from actinomycetes that secreted to the pretreated bagasse when they were grown up. Therefore, observation of the effect of the ChOAc amount used in pretreatment to hydrolytic enzyme activities was necessary to be explored. Adopting the previous optimum hydrolysis condition, the measurement of both cellulase and xylanase were examined. Fig. 6(a) and (b) describe the fluctuation of cellulase and xylanase activity in presence of ChOAc when they produced to hydrolyze pretreated bagasse in the growth medium respectively. The cellulase activities at the ChOAc/biomass ratios 0.0–1.5 (g/g) were observed stable since at the ratio 2 (g/g) the activity initiated to decline and the declining was clearly seen at the ratio 2.5–3.0 (g/g). The highest cellulase activity appears at the ratio was 1 (g/g). On the other hand, the xylanase activities seem stable at all of the ChOAc/biomass ratios, whereas the high activity was shown also at the ratio 1 (g/g). In order to explain the trend of the enzyme activities in Fig. 6 (a) and (b), the amount of ChOAc in the hydrolysis medium was calculated and it describes in Fig S-1 (in the electronic

supplementary information). It was pointed out that in the range presence of ChOAc between 0.0 and 0.2 g/mL, cellulase activities were settled at 46.23–66.40 U/mL, nevertheless, the activity dramatically reduced into 17.87 U/mL in the presence of ChOAc up to over 0.25 g/mL. On the contrary, the presence of ChOAc did not cause the xylanase activity to fluctuate, which the xylanase activity remain stable in the range of 58.97–75.19 U/mL. For all of the ChOAc/biomass ratios were given, from the standpoint of aspect the enzyme activity, this study acquired the optimum ratio was 1 (g/g) whereas the cellulase and the xylanase have activity at 66.40 U/mL and 75.19 U/mL respectively.

Moreover, in this step, the yield of reducing sugar was calculated base on the amount of released reducing sugar compare with carbohydrate (cellulose and xylan) content of bagasse (the composition of bagasse was depicted in Fig. S2). Fig. S3 presents the yield of reducing sugar from the saccharification of pretreated bagasse in this observation. It was confirmed that the highest obtained reducing sugar yield was achieved at the condition in which the highest both of cellulase and xylanase enzyme activity were produced. Turning into detail, the pretreatment by using the ChOAc/biomass ratio at 1 (g/g) resulted in the yield at 74.58%. Eventhough the yield of reducing sugar in this experiment was noted as a high result as a hydrolytic action of cellulase and xylanase, however it might also be involved by other hydrolytic enzymes.

ChOAc is categorized as biocompatible ILs (Petkovic et al., 2009) than imidazolium or the other traditional ILs. The effectiveness of ChOAc in using for pretreatment of lignocellulosic biomass which not only increases the accessibility of enzyme on saccharification of biomass already proved in many experiments but also has slight inhibitory impacts on microorganisms an enzyme while 1-ethyl-3-methylimidazolium (EmimOAc) acetate has negative effect significantly (Liu et al., 2012; Ninomiya et al., 2015b, 2015c, 2015d, 2013b, 2013c). In this study, we observed that up to 0.15 g/mL or equivalently with 1 M of ChOAc concentration did not affect the decrease in the enzyme activity significantly. Conversely, the other explorations have underlined that imidazolium ILs type even in millimolar was able to inhibit the activity saccharolytic enzymes (such as cellulase) and have significant toxicity to fermentative microorganisms (such as yeast) inoculated in bioethanol production from biomass (Datta et al., 2010; Ouellet et al., 2011, 2015). Although the future observation is involved to explain the effect of concentration ChOAc into the reducing of enzyme activity, however, at least this study was encouraging in the use of ChOAc in the successive process of bioconversion lignocellulose into bioethanol or the other valuable biochemicals using fermentative microorganisms.

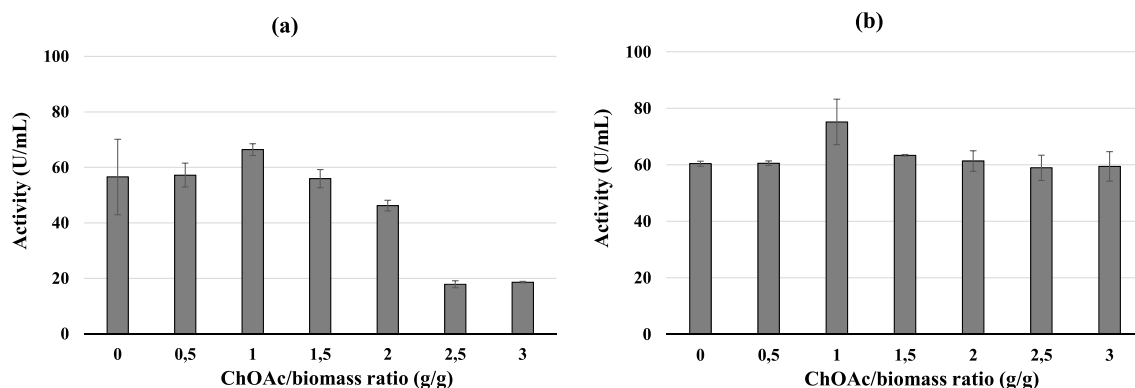


Fig. 6. The produced cellulase activity (a) and the xylanase activity (b) in the presence of remain ChOAc in pretreated bagasse.

4. Conclusions

This study found eight different indigenous actinomycetes that have shown both cellulolytic and xylanolytic activity. The halo clear zone on Congo-red plate assay suggested the isolates are capable to produce the hydrolytic enzyme that can employ cellulosic and hemi-cellulosic substrates. Among the strains tested, Act-7 exhibited the highest cellulase and xylanase activity at 66.40 and 75.19 U/mL respectively. In addition, the optimum initial pH for enzyme production using the YMB medium was 7.0, since the optimum time production was 14 days. Furthermore, in the presence of ChOAc up to 0.15 g/mL the cellulase activity showed stable, however, the cellulase activity starts to decline in the presence of 0.2 g/mL ChOAc and it appeared a dramatically decreasing of cellulase activity in occupancy of 0.25 g/mL ChOAc. On the other hand, the activity of xylanase was remaining in low fluctuation in various ChOAc concentration that was examined. The pretreatment of bagasse using ChOAc increased the yield of reducing sugar to the extent of 74.58%. This study has initiated the opportunity of a one-pot process in bioconversion of lignocellulose biomass using a biocompatible ionic liquid and potential indigenous actinomycetes.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgments

This study was supported by Directorate General of Research and Development Strengthening Ministry of Research Technology and Higher Education of Republic Indonesia that provided funding, under the contract number 065/SP2H/LT/DPRM/2019 for Heri Satria.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbac.2020.101503>.

References

- Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment : fundamentals toward application. *Biotechnol. Adv.* 29, 675–685. <https://doi.org/10.1016/j.biotechadv.2011.05.005>.
- Amore, A., Parameswaran, B., Kumar, R., Birolo, L., Vinciguerra, R., Marcolongo, L., Ionata, E., La, F., Faraco, V., 2014. Application of a new xylanase activity from *Bacillus amyloliquefaciens* XR44A in brewer's spent grain saccharification. *J. Chem. Technol. Biotechnol.* 90, 573–581. <https://doi.org/10.1002/jctb.4589>.
- Amore, A., Pepe, O., Ventorino, V., Birolo, L., Giangrande, C., Faraco, V., 2012. Industrial waste based compost as a source of novel cellulolytic strains and enzymes. *FEMS Microbiol. Lett.* 339, 93–101. <https://doi.org/10.1111/1574-6968.12057>.
- Andrić, P., Meyer, A.S., Jensen, P.A., Dam-Johansen, K., 2010. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes. *Biotechnol. Adv.* 28, 308–324. <https://doi.org/10.1016/j.biotechadv.2010.01.003>.
- Bailey, M.J., Biely, P., Kaisa, P., 1992. Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.* 23, 257–270. [https://doi.org/10.1016/0168-1656\(92\)90074-J](https://doi.org/10.1016/0168-1656(92)90074-J).
- Brandt, A., Gräsvik, J., Hallett, J.P., Welton, T., 2013. Deconstruction of lignocellulosic biomass with ionic liquids. *Green Chem.* 15, 550–583. <https://doi.org/10.1039/c2gc36364j>.
- Datta, S., Holmes, B., Park, J.I., Chen, Z., Dibble, D.C., Hadi, M., Blanch, H.W., Simmons, A., Saprà, R., 2010. Ionic liquid tolerant hyperthermophilic cellulases for biomass pretreatment and hydrolysis. *Green Chem.* 12, 338–345. <https://doi.org/10.1039/b916564a>.
- Fatokun, E.N., Nwodo, U.U., Okoh, A.I., 2016. Classical optimization of cellulase and xylanase production by a marine streptomyces species. *Appl. Sci.* 6, 1–14. <https://doi.org/10.3390/app6100286>.
- Fukaya, Y., Iizuka, Y., Sekikawa, K., Ohno, H., 2007. Bio ionic Liquids : room temperature ionic liquids composed wholly of biomaterials. *Green Chem.* 9, 1155–1157. <https://doi.org/10.1039/B706571J>.
- Ghose, T.K., 1987. Measurement of cellulase activities. *Pure Appl. Chem.* 59, 257–268. <https://doi.org/10.1351/pac198759020257>.
- Gomez, L.D., Steele-king, C.G., Mcqueen-mason, S.J., 2008. Sustainable liquid biofuels from biomass : the writing's on the walls. *New Phytol.* 178, 473–485. <https://doi.org/10.1111/j.1469-8137.2008.02422.x>.
- Hu, S., Jiang, T., Zhang, Z., Zhu, A., Han, B., 2007. Functional ionic liquid from biorenewable materials : synthesis and application as a catalyst in direct aldol reactions. *Tetrahedron Lett.* 48, 5613–5617. <https://doi.org/10.1016/j.tetlet.2007.06.051>.
- Isikgor, F.H., Becer, C.R., 2015. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polym. Chem.* 6, 4497–4559. <https://doi.org/10.1039/C5PY00263J>.
- Johnsen, H.R., Krause, K., 2014. Cellulase activity screening using pure carboxymethylcellulose: application to soluble cellulolytic samples and to plant tissue prints. *Int. J. Mol. Sci.* 15, 830–838. <https://doi.org/10.3390/ijms15010830>.
- Kilpeläinen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., Argyropoulos, D.S., 2007. Dissolution of wood in ionic liquids. *J. Agric. Food Chem.* 55, 9142–9148. <https://doi.org/10.1021/jf071692e>.
- Kulkarni, N., Shendye, A., Rao, M., 1999. Molecular and biotechnological aspects of xylanases. *FEMS Microbiol. Rev.* 23, 411–456. [https://doi.org/10.1016/S0168-6445\(99\)00066-6](https://doi.org/10.1016/S0168-6445(99)00066-6).
- Lammirato, C., Miltner, A., Wick, L.Y., Kästner, M., 2010. Hydrolysis of cellobiose by β -glucosidase in the presence of soil minerals - interactions at solid-liquid interfaces and effects on enzyme activity levels. *Soil Biol. Biochem.* 42, 2203–2210. <https://doi.org/10.1016/j.soilbio.2010.08.018>.
- Liu, Q., Hou, X., Zong, M., 2012. Ionic liquids from renewable biomaterials : synthesis , characterization and application in the pretreatment of biomass. *Green Chem.* 14, 304–307. <https://doi.org/10.1039/c2gc16128a>.
- Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.* 66, 506–577. <https://doi.org/10.1128/MMBR.66.3.506-577.2002>.
- Macfarlane, D., Kar, M., 2017. An introduction to ionic liquids. In: *Fundamentals of Ionic Liquids: from Chemistry to Applications*. Wiley Online Library, pp. 1–25. <https://doi.org/10.1002/9783527340033.ch1>.
- McCarthy, A.J., Peace, E., Broda, P., 1985. Studies on the extracellular xylanase activity of some thermophilic actinomycetes. *Appl. Microbiol. Biotechnol.* 21, 238–244. <https://doi.org/10.1007/BF00295129>.
- Menon, V., Rao, M., 2012. Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. *Prog. Energy Combust. Sci.* 38, 522–550. <https://doi.org/10.1016/j.pecs.2012.02.002>.
- Miller, G.L., 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426–428. <https://doi.org/10.1021/ac60147a030>.
- Mithra, M.G., Padmaja, G., 2017. Strategies for enzyme saving during saccharification of pretreated lignocellulose-starch biomass: effect of enzyme dosage and detoxification chemicals. *Heliyon* 3, e00384. <https://doi.org/10.1016/j.heliyon.2017.e00384>.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapfel, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96, 673–686. <https://doi.org/10.1016/j.biortech.2004.06.025>.
- Mukhtar, S., Zaheer, A., Aiysha, D., Abdulla Malik, K., Mehnaz, S., 2017. Actinomycetes: a source of industrially important enzymes. *J. Proteom. Bioinform.* 10, 316–319. <https://doi.org/10.4172/jpb.1000456>.
- Ninomiya, K., Inoue, K., Aomori, Y., Ohnishi, A., Ogino, C., Shimizu, N., Takahashi, K., 2015. Characterization of fractionated biomass component and recovered ionic liquid during repeated process of cholinium ionic liquid-assisted pretreatment and fractionation. *Chem. Eng. J.* 259, 323–329. <https://doi.org/10.1016/j.cej.2014.07.122>.
- Ninomiya, K., Kohori, A., Tatsumi, M., Osawa, K., Endo, T., Kakuchi, R., Ogino, C., Shimizu, N., Takahashi, K., 2015. Ionic liquid/ultrasound pretreatment and in situ enzymatic saccharification of bagasse using biocompatible cholinium ionic liquid. *Bioresour. Technol.* 176, 169–174. <https://doi.org/10.1016/j.biortech.2014.11.038>.
- Ninomiya, K., Ogino, C., Ishizaki, M., Yasuda, M., Shimizu, N., 2015. Effect of post-pretreatment washing on saccharification and co-fermentation from bagasse pretreated with biocompatible cholinium ionic liquid. *Biochem. Eng. J.* 103, 198–204. <https://doi.org/10.1016/j.bej.2015.08.002>.
- Ninomiya, K., Ohta, A., Omote, S., Ogino, C., Takahashi, K., Shimizu, N., 2013. Combined use of completely bio-derived cholinium ionic liquids and ultrasound irradiation for the pretreatment of lignocellulosic material to enhance enzymatic saccharification. *Chem. Eng. J.* 215–216, 811–818. <https://doi.org/10.1016/j.cej.2012.11.020>.
- Ninomiya, K., Omote, S., Ogino, C., Kuroda, K., Noguchi, M., Endo, T., 2015. Saccharification and ethanol fermentation from cholinium ionic liquid-pretreated bagasse with a different number of post-pretreatment washings. *Bioresour. Technol.* 189, 203–209. <https://doi.org/10.1016/j.biortech.2015.04.022>.
- Ninomiya, K., Soda, H., Ogino, C., Takahashi, K., Shimizu, N., 2013. Effect of ionic liquid weight ratio on pretreatment of bamboo powder prior to enzymatic saccharification. *Bioresour. Technol.* 128, 188–192. <https://doi.org/10.1016/j.biortech.2012.10.097>.
- Ninomiya, K., Utami, A.R.I., Tsuge, Y., Kuroda, K., Ogino, C., Taima, T., Saito, J., Kimizu, M., Takahashi, K., 2018. Pretreatment of bagasse with a minimum amount of cholinium ionic liquid for subsequent saccharification at high loading and co-fermentation for ethanol production. *Chem. Eng. J.* <https://doi.org/10.1016/j.cej.2017.10.113>.
- Ninomiya, K., Yamauchi, T., Kobayashi, M., Ogino, C., Shimizu, N., 2013. Cholinium carboxylate ionic liquids for pretreatment of lignocellulosic materials to enhance subsequent enzymatic saccharification. *Biochem. Eng. J.* 71, 25–29. <https://doi.org/10.1016/j.bej.2012.11.020>.

- 10.1016/j.bej.2012.11.012.
- Ninomiya, K., Yamauchi, T., Ogino, C., Shimizu, N., Takahashi, K., 2014. Microwave pretreatment of lignocellulosic material in cholinium ionic liquid for efficient enzymatic saccharification. *Biochem. Eng. J.* 90, 90–95. <https://doi.org/10.1016/j.bej.2014.05.013013>.
- El-Naggar, N.E.-A., 2012. Optimization of process parameters for the production of alkali-tolerant carboxymethyl cellulase by newly isolated *Streptomyces* sp. strain NEAE-D. *Afr. J. Biotechnol.* 11, 1185–1196. <https://doi.org/10.5897/ajb11.1819>.
- Ouellet, M., Datta, S., Dibble, C.D., Tamrakar, P.R., Benke, P.I., Li, C., Singh, S., Sale, K. L., Adams, P.D., Keasling, J.D., Simmons, B.A., Holmes, B.M., Mukhopadhyay, A., 2015. Impact of two ionic liquids, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium methylphosphonate, on *Saccharomyces cerevisiae*: metabolic, physiologic, and morphological investigations. *Impact of two ionic liquids, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium methylphosphonate, on Saccharomyces cerevisiae: metabolic, physiologic, and morphological investigations*. *Green Chem.* 13, 2743–2749. <https://doi.org/10.1186/s13068-015-0206-2>.
- Ouellet, M., Datta, S., Dibble, D.C., Tamrakar, P.R., Benke, P.I., Li, C., Singh, S., Sale, K. L., Adams, P.D., Keasling, J.D., Simmons, B.A., Holmes, B.M., Mukhopadhyay, A., 2011. Impact of ionic liquid pretreated plant biomass on *Saccharomyces cerevisiae* growth and biofuel production. *Green Chem.* 13, 2743–2749. <https://doi.org/10.1039/c1gc15327g>.
- Petkovic, M., Ferguson, J., Bohn, A., Trindade, J., Martins, I., Carvalho, M.B., Leit, M.C., Rodrigues, C., Garcia, H., Ferreira, R., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2009. Exploring fungal activity in the presence of ionic liquids. *Green Chem.* 11, 889–894. <https://doi.org/10.1039/b823225c>.
- Plechova, N.V., Seddon, K.R., 2008. Applications of ionic liquids in the chemical industry. *Chem. Soc.* 37, 123–150. <https://doi.org/10.1039/b006677j>.
- Saini, A., Aggarwal, N.K., Yadav, A., 2016. Cellulolytic potential of actinomycetes isolated from different habitats. *Bioeng. Biosci.* 4, 88–94. <https://doi.org/10.13189/bb.2016.040503>.
- Shirling, E.B., Gottlieb, D., 1966. Methods for characterization of streptomyces species. *Int. J. Syst. Bacteriol.* 16, 313–340. <https://doi.org/10.1099/00207713-16-3-313>.
- Sun, S., Sun, S., Cao, X., Sun, R., 2016. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour. Technol.* 199, 49–58. <https://doi.org/10.1016/j.biortech.2015.08.061>.
- Swatloski, R.P., Spear, S.K., Holbrey, J.D., Rogers, R.D., 2002. Dissolution of cellulose with ionic liquids. *J. Am. Chem. Soc.* 124, 4974–4975 [pii]. <https://doi.org/10.1021/ja025790m>.
- Teather, R.M., Wood, P.J., 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Environ. Microbiol.* 43, 777–780.
- Uppugundla, N., Da Costa Sousa, L., Chundawat, S.P.S., Yu, X., Simmons, B., Singh, S., Gao, X., Kumar, R., Wyman, C.E., Dale, B.E., Balan, V., 2014. A comparative study of ethanol production using dilute acid, ionic liquid and AFEX™ pretreated corn stover. *Biotechnol. Biofuels* 7, 1–14. <https://doi.org/10.1186/1754-6834-7-72>.
- Welton, T., 2018. Ionic liquids: a brief history. *Biophys. Rev.* 10, 691–706. <https://doi.org/10.1007/s12551-018-0419-2>.
- Wu, L., Moteki, T., Gokhale, A.A., Flaherty, D.W., Toste, F.D., 2016. Production of fuels and chemicals from biomass: condensation reactions and beyond. *Chem* 1, 32–58. <https://doi.org/10.1016/j.chempr.2016.05.002>.