Pretreated Sugarcane Bagasse Result in More Efficient Degradation by *Streptomyces* sp S2

Stanislaus Aditya Agung (✉ adityaagung14@gmail.com)

Bogor Agricultural University: Institut Pertanian Bogor <https://orcid.org/0000-0001-5753-5055>

Dede Heri Yuli Yanto

LIPi: Lembaga Ilmu Pengetahuan Indonesia

Anja Meryandini

Bogor Agricultural University: Institut Pertanian Bogor

Titi Candra Sunarti

Bogor Agricultural University: Institut Pertanian Bogor

Research Article

Keywords: Hydrogen peroxide treatment, alkaline-hydrothermal treatment, lignocellulose degradation, *Streptomyces* sp. S2, sugarcane bagasse

Posted Date: November 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1059961/v1>

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Abstract

Streptomyces genera plays important role in lignocellulose degradation. Many research founds *Streptomyces* has cellulolytic and ligninolytic enzymes that sufficient to degrade lignocellulosic materials. However, minimum lignocellulosic material condition that can efficiently degraded by *Streptomyces* sp. has not been fully understood. In this research, three pretreatment conditions (physical, alkaline-hydrothermal, and hydrogen-peroxide chemical treatments) of sugarcane bagasse used as lignocellulosic material, to further degraded by *Streptomyces* sp. S2. Lignocellulose component measurement conclude that raw (physical treated only) bagasse wasn't efficiently degraded by *Streptomyces* sp S2. Hydrogen-peroxide was effective on reducing both syringil and guaiacyl lignin, meanwhile alkaline-hydrothermal pretreatment was very effective on reducing syringil lignin. This study suggest that hydrogen-peroxide pretreatment can be used in many type of lignocellulosic material, which can be further degraded by *Streptomyces* sp. S2. Alkaline-hydrothermal preatreatment on the other hand is best suited to degrade lignocellulosic material that have high percentage of syringil lignin.

Introduction

Lignocellulose material consist of cellulose chained into hemicellulose, with lignin fill the gap between hemicellulose and cellulose fibers. In recent years lignocellulose materials gained attention to be used as feedstock for bioethanol, biogas, bioplastics or simple sugar (Azeredo et al.2015; Machado and Ferraz 2017; Fan et al. 2018; Sari et al.2021). Indonesia as agricultural country have big potential on using lignocellulose material, in 2017 alone Indonesia produce 150 million tonnes of dried rice grain, 30.000 thousands tonnes of corn and sugarcane about 2000 thousands tonnes (Kementarian Pertanian Republik Indonesia 2018). Sugarcane bagasse have biggest potential to be used for bioethanol based on high performance index and low environmental impact, also for bioplastics based on performance index (Sari et al. 2021). However, sugarcane bagasse consist of 17.52%–23.37% of lignin, which makes utilization of its cellulose to simple sugar hampered (Maryana et al.2014; Pin et al.2019). High degree of polymerization and high crystallinity of cellulose can also plays role in building sugarcane bagasse strength as lignocellulosic material, thus cellulose area that accesible become small (Lee et al. 2014).

Lignin can be reduced using bleaching and/ or pretreatment. Bleaching used in paper industry, which reduces lignin and change lignocellulose color to brighter color (white). Bleaching methods use chemical that contain chlorine or not, such as H_2O_2 , $NaClO_2$, or ClO_2 . Pretreatment on the other hand focus on reducing size of lignocellulose particle and reducing lignin contents. Pretreatment can be divided into physical (milling, extrusion, ultrasonic radiation), physicochemical (ammonia fiber explosion, supercritical CO_2). Chemical pretreatment can be done using acid or alkaline solution in high concentration or dilute, organosolv, ionic liquid or deep-eutectic-solvent (DES). Another type of pretreatment is biological pretreatment, that use whole organism (such as fungi or bacteria) or enzyme that can degrade lignin (Septevani et al.2018; Singh et al.2019; Baruah et al.2018).

Streptomyces sp. is Gram positive actinobacteria that abundant in soil and some other extreme environment such as deep sea, volcano and extreme cold environment like Artic. Most *Streptomyces* sp. are well known to produce secondary metabolites, such as antibacteria and anti-cancer, and also known to degrade substrate in dead-plants (Chater 2016; Sivalingam et al.2019). *Streptomyces* are substantial lignocellulose degrader alongside *Clostridium*, *Flavobacteria*, *Pseudomonas* and *Xanthomonas* for first three days of lignicellulose degradation (Ma et al., 2020). High number of binding sites that called CebR enable *Streptomyces* to have high cellulolytic activities. The CebR sites influence *Streptomyces* sp. to encode genes that associates for deconstruct plant biomass such as glycoside hydrolase, endoglucanase, cellobiohydrolase, β -glucosidase, mannanase, xylanase, chitinase and LPMO (lytic polysaccharide monooxygenase) (Book et al. 2016). Another finding conclude that ligninolytic *Streptomyces* sp. have high lignin degrading enzyme activities of lignin-peroxidase (LiP), aryl-alcohol oxidase (AAO), low laccase (Lac) and dye decoloring enzyme (DyP) activities, but don't have manganese-peroxidase (MnP) enzyme (Riyadi et al. 2020).

Many studies have found that *Streptomyces* sp. has sufficient amount of cellulolytic and ligninolytic enzyme, therefore *Streptomyces* sp. potentially used to degrade lignocellulose material. However efficiency of *Streptomyces* sp degradation on different lignocellulose condition hasn't been fully understood. This study use three sugarcane bagasse condition as lignocellulose (control, alkaline-hydrothermal pretreated, peroxide pretreated), then *Streptomyces* sp. degradation ability was tested on three bagasse conditon aforementioned above. In this study *Streptomyces* sp. S2 that was isolated from palm oil plantation, Jambi, Indonesia was used as biological pretreatment. Effect of *Streptomyces* sp S2 on sugarcane bagasse was evaluated its crude fiber content, lignocellulose component, crystallinity using X-ray diffraction, its chemical contents using Fourier Transform Infrared and lastly 3D structure using Scanning Electron Microscope (SEM).

Materials And Methods

Sugarcane Bagasse Preparation

Bagasse used in this research derived from the sugarcane juice seller in Bogor. Total of 40 kg (wet weight) sugarcane bagasse was cut, washed until clean then further sun-dried for 3 days and/or accelerated using oven until water content lower than 9%. All dried bagasse then milled using Wiley Mill, filtered through 40 mesh sieve. Bagasse that pass-through the sieve used for further treatment.

Alkaline-hydrothermal pretreatment

Every 100 g prepared sugarcane was treated using 1.5L sodium hydroxide (NaOH) 1M concentration (solid: liquid ratio 1:15) in 2L Erlenmeyer. Erlenmeyer was closed using plastic and rubber bands, before put into autoclave for 30 minutes at 121°C (Lemoes et al. 2018 modified temperature used). All of alkaline-autoclaved pretreated bagasse was neutralised using aquadest until the neutralised pH solution

is near or same with the aquadest used (pH 5.5). Neutralised sugarcane bagasse further filtered using filter paper before dried using oven between temperature 60–80°C.

Hydrogen peroxide pretreatment

Every 100 g sieved sugarcane bagasse was treated with total volume of 1L 6% (w/v) H₂O₂, 1% (w/v) trisodium citrate dihydrate, 1% NaOH (w/v) and 92% (w/v) aquadest in 2L Erlenmeyer. Erlenmeyer was closed using plastics that holed using skewers, for gas release that formed during pretreatment and tightened using rubber bands. Peroxide treatment was done in waterbath at 60°C for 90 minutes (Yan et al., 2019). Sugarcane bagasse then further neutralised using aquadest until the solution pH near or same with the aquadest used (pH 5.5). Neutralised bagasse was filtered through filter paper before dried using oven until water content lower than 9%.

Application using *Streptomyces* sp. S2

Culture preparation

Streptomyces sp. S2 that used confirmed to have some LPMO (Lytic Polysaccharide Mono-Oxygenase) enzyme activities (Utarti et al, 2020). *Streptomyces* sp.S2 was inoculated into ISP 4 agar medium, incubated for 5 days. Incubated *Streptomyces* sp. S2 then moved into 200 ml ISP 4 liquid medium (pH=6.0) using 10 mm diameter corkborer, incubated in 100 rpm shaker room temperature (27°C) for 14 days.

Degradation using *Streptomyces* sp. S2

On the 14th day of incubation, the liquid medium containing *Streptomyces* sp. S2 was inoculated into 500 ml Erlenmeyer that contains 15 g of previously sterilized (autoclaved 125°C, 40 minutes) sugarcane bagasse from previous pretreatment (control, alkaline-hydrothermal treated, peroxide treated). Erlenmeyer that contains solution of bagasse and *Streptomyces* then further incubated for another 4 days (total incubation 18 days in liquid ISP 4 solution). On 4th days of degradation, enzyme reaction was stopped by filtered the bagasse through filter paper then put the bagasse into freezer. Each pretreatment (control, alkaline-hydrothermal, hydrogen-peroxide) was treated duplo for *Streptomyces* sp. S2 pretreatment.

Fiber Characterization

Crude Fiber Measurement

Crude fiber was measured by Center Research of Biological Research and Biotechnology, Jl. Kamper, Bogor Agricultural University, Dramaga, Bogor, West Java, Indonesia. Amount of 1 g sample was dissolved in 1.25% H₂SO₄ 100ml, then boiled before destructed using mortar for 30 minutes. The destructed sample was filtered through a filter-paper and Buchner funnel. Residues that created washed

by 20–30 ml boiled water, then washed using 25 ml room temperature water. Residues that created after dissolving in H_2SO_4 was destructed again in 1.25% NaOH for 30 minutes and filtered as aforementioned method. Residues then washed by 1.25% boiled 25 ml H_2SO_4 , three times with 25 ml room temperature water, then with 25 ml alcohol consecutively. Sample that washed using alcohol then filtered and the residue with the filter paper was moved into porcelain cup (cawan). Porcelain cup containing sample and filter paper was dried in 130°C oven for 2 hours. Sample after oven was weighed as W1. Sample then heated in 600°C furnace for 30 minutes, dried sample after furnace was weighed as W2. Lignocellulose content calculated based on formula: Lignocellulose weight: W2-W1

$$\text{Lignocellulose content} = \frac{\text{lignocellulose weight}}{\text{sample weight}} \times 100\%$$

Neutral Detergent Fiber

Both Neutral Detergent Fiber and Acid Detergent Fiber was measured using AOAC (1998) method in Nutrition and Feed Technology Laboratory, Animal Husbandry Faculty, Dramaga, Bogor, West Java, Indonesia. Each 1 gram sample (a) and 100 ml neutral detergent solution added into 600 ml beaker. The solution and sample then heated until boils. Sample then extracted after 60 minutes boiling time, filtered through a fritted disc (b). Residues is washed using water and acetone, then further dried using 105°C until stable weight (c). Neutral detergent fiber calculated using formula:

$$\text{NDF}(\%) = \frac{c-b}{a} \times 100\%$$

Acid Detergent Fiber and Hemicellulose

Each 1 gram sample (a2) and 100 ml acid detergent solution added into 600 ml beaker, then heated until boils. Sample then extracted after 60 minutes boiling time, filtered through a fritted disc (b2). Residues is washed using water and acetone, then further dried using 105°C oven until stable weight (c2). Each acid detergent fiber and hemicellulose calculated using formula:

$$\text{ADF}(\%) = \frac{c2-b2}{a2} \times 100\%$$

$$\text{Hemicellulose}(\%) = \text{NDF}(\%) - \text{ADF}(\%)$$

Crystallinity Index Assay

Crystallinity index assay was done using XRD Shimadzu Maxima 7000 (Japan) in the Research Center of Biomaterials, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia. Sample was analyzed between 10° to 80° on continuous scanning at 2° speed per minute, with Cu radiation (1.506 Å). Background noise was separated from selected XRD pattern, then X-ray pattern was corrected and normalized using computer. Crystallinity index was calculated using formula: $(I_{cr} / (I_{cr} + I_a)) \times 100\%$, where I_{cr} is area of crystalline meanwhile I_a is area of amorph.

Functional Group Analysis

Fourier-transform analysis was done using Spectrum Two, Perkin Elmer (USA) on absorbance between 400–4000 cm^{-1} and spectrum area between 4 cm^{-1} and 16 scans. Wave intensity was based on percent of transmittance which calculated and observed later.

Morphological Characteristic

Each pretreatment sample was weighed around one gram (without repetition) and dehydrated using gradual ethanol (ethanol concentration: 50%, 70%, 80%, 90% and last 100%) before coated with gold. Each gold coated bagasse sample was observed under electron microscope SEM JEOL JSM-IT200 (Japan) on 200, 500 and 1000 times magnification at 3 kilo-Volt.

Result And Discussion

Lignocellulosic component of sugarcane bagasse

Crude fiber consist of insoluble and soluble fiber in which contain cellulose, hemicellulose, lignin and some amount of dextrin, β -glucan, mucilages, pectin, inulin, also oligosaccharides/ oligofructose (Slavin et al. 2009). Raw sugarcane bagasse used in this research have 31.90% crude fiber from total dry weight. *Streptomyces* sp. S2 alone ineffectively digest crude fiber in native sugarcane bagasse used, shown by not statistically different crude fiber amount compared to control. On the other side both alkaline-hydrothermal and hydrogen peroxide treatment does affect bagasse crude fiber, increased percentage of fiber by 19.96% and 10.95% respectively. *Streptomyces* sp. S2 increase amount of crude fiber by 5% after bagasse treated using alkaline-hydrothermal which is not statistically significant with alkaline-hydrothermal treated only. Decreased amount of crude fiber by 4.6% occur after peroxide bagasse further treated with *Streptomyces* sp S2, is statistically significant to bagasse that treated using peroxide only (Table 1).

Raw sugarcane bagasse that used consist of 31.83% hemicellulose, 46.65% cellulose and 9.90% lignin. *Streptomyces* sp. S2 degradation on raw bagasse wasn't effective, just slightly reduce cellulose to 44.14% and slightly increase hemicellulose and lignin to 32.28% and 10.78% respectively. Both alkaline-hydrothermal and hydrogen peroxide pretreatment reduce hemicellulose by 19.37% and 10.45% respectively. Cellulose content on the other hand increased by 31.56% and 4.99% after alkaline-hydrothermal and hydrogen peroxide pretreatment, respectively. Lignin concentration was reduced significantly by 4.08% after alkaline treatment, meanwhile peroxide treatment increase lignin significantly by 6.34%.

Increase in cellulose content and reduced lignin content after bagasse treated with alkaline was similar to Srivastava et al. (2017). On the other hand increased lignin after peroxide treatment was inconsistent compared to Yan et al. (2019) findings. Some possible explanation are sugarcane bagasse have high lignin enthalpy (around 130 kJ/mol), this makes sugarcane have high lignin thermal stability. This makes bagasse lignin harder to extracted using hydrogen-peroxide within same temperature, compared to biomass that have lower lignin thermal stability (Watkins et al. 2015; Chen et al. 2016). Another possible

explanation is due to acid nature of peroxide treatment, lignin was moved to the surface of bagasse (Xu et al 2017).

Contrary to the alkaline-hydrothermal pretreatment, further degradation of alkaline pretreated bagasse using *Streptomyces* sp.S2 was reduce cellulose content significantly by 4.86% from 78.21–73.35%. Its hemicellulose and lignin content on the other hand only slightly increased. *Streptomyces* sp. S2 degradation to peroxide pretreated bagasse furthermore increase celulose content by 1.52–53.16%, also increase hemicellulose significantly by 3.87–25.25%. Lignin after peroxide bagasse degraded using *Streptomyces* sp. S2 was reduced significantly compared to peroxide only treated bagasse by 6.48–9.76%, but not significant compared to control (raw) bagasse (Table 1). This is consistent and similar to Xu et al (2017) findings that use *Cupriavidus basilensis* to further degrade acid pretreated rice straw.

Table 1 Crude fiber and Lignocellulose Component of Sugarcane Bagasse

Treatment	Crude Fiber (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Control	31.90 ± 0.50	31.83 ± 0.74	46.65 ± 0.66	9.90 ± 0.55
Alkaline-hydrothermal (NaOH)	51.86 ± 1.09 ^b	12.46 ± 1.09 ^b	78.21 ± 0.42 ^b	5.82 ± 1.00 ^a
Hydrogen-Peroxide (H ₂ O ₂)	42.85 ± 0.93 ^b	21.38 ± 0.34 ^b	51.64 ± 0.59 ^a	16.24 ± 0.08 ^b
Control-Streptomyces	31.25 ± 1.03	32.28 ± 0.25	44.14 ± 0.91	10.78 ± 0.45
NaOH-Streptomyces	55.15 ± 0.67 ^b	13.20 ± 0.21 ^b	73.35 ± 0.21 ^{bb}	6.54 ± 0.31 ^a
H ₂ O ₂ -Streptomyces	38.25 ± 0.40 ^{ba}	25.25 ± 0.97 ^{ba}	53.16 ± 0.08 ^b	9.76 ± 0.18 ^{*b}

Hemicellulose value was based on neutral detergent fiber (NDF) substracted by acid detergent fiber (ADF), data not shown.

First value with upperscript letter ^(a,b) show statistically significant compared to control only, meanwhile second upperscript ^(a,b) show statistically significant from its previous treatment (control from control-*Streptomyces*, from NaOH- *Streptomyces*, H₂O₂ from H₂O₂ – *Streptomyces*).

(a) Statistically significant based on P (T< t) two-tail value on two sample t-test assuming equal variance, α=0,05

(b) Statistically significant based on P (T< t) two-tail value on two sample t-test assuming equal variance, α=0,01

Crystallinity index analysis

Raw sugarcane bagasse (control) used have crystallinity around 45%, means about 45% of cellulose content are distributed in well-ordered crystal lattice and the rest 54.66% are distributed unevenly

(amorph). Crystallinity after alkaline-hydrothermal pretreatment was reduced to 35.44% even the cellulose content increased indicates wider amorph area and loosened cellulose bonds. Peroxide pretreatment meanwhile increasing crystallinity of raw sugarcane bagasse by 5–50%, indicates increase in cellulose purity or more crystalline area.

Increase in crystalinity also mean wider specific surface area, that accesible to bacteria and can be further digested (Xu et al. 2017). Result after *Streptomyces* treatment indicates that well-ordered cellulose are harder to digest by enzymes, by looking at amount of reduced crystalinity of raw bagasse (9% after *Streptomyces* treatment only) was lower compared to 11% reduction after hydrogen peroxide bagasse (H₂O₂) treated by *Streptomyces*. Slight crystalinity increase after alkaline-hydrothermal (NaOH) bagasse treated with *Streptomyces* (from 35.44–36.95%) might indicate increase in cellulose purity after *Streptomyces* treatment (Figure 1 and Table 2).

Table 2. Crystalinity of each treatment based on X-ray diffraction (XRD) result

Treatment	Crystalline area (Icr)	Amorph area (Ia)	Crystalinity (%)
1.Control	34.488	41.58	45.338
2.alkaline (NaOH)	49.249	89.711	35.441
3.Peroxide (H ₂ O ₂)	62.257	60.048	50.903
4.Control-Streptomyces	24.657	44.113	35.854
5. NaOH-Streptomyces	45.285	77.258	36.954
6.H2O2-Streptomyces	27.899	42.496	39.632

Functional Group Analysis

The FTIR peak between 3500 and 3000 cm⁻¹ in control was higher than in alkaline and peroxide treated, indicates the hydrogen bonds in cellulose was cut after both pretreatment. Both control-*Streptomyces* and NaOH-*Streptomyces* have higher peak on 3300 cm⁻¹ after treated with *Streptomyces* sp S2 compared to its previous treatment, indicates that bonds between cellulose is stronger despite the cellulose content was lower compared to its previous treatment. On the other hand cellulose bond was weaker after hydrogen-peroxide bagasse was further treated using *Streptomyces* sp. S2, indicated from lower peak number 3300 cm⁻¹ on peroxide-*Streptomyces* bagasse compared to hydrogen-peroxide only. Simple carbonic compound just found in control confirmed with peak number 1726 cm⁻¹, which more likely to be ester (Nandiyanto *et al.*2019). This ester bonds was decreased after all pretreatment.

Present bands of raw bagasse around 1300-1200 cm⁻¹ with higher bands around 1300 compared to around 1200, indicates that sugarcane bagasse have higher S (syringyl) compared to (G) guaiacyl lignin (Watkins et al.2015). This confirms Miyamoto et al.(2018) results that sugarcane bagasse have higher syringyl lignin. Peak around 1300 was reduced after alkaline-hydrothermal pretreatment, meanwhile peak

around 1200 wasn't. This indicates that alkaline-hydrothermal pretreatment attack mainly on syringyl lignin, and not too effective on reduce guaiacyl lignin. Syringyl and guaiacyl bonds remains unchanged after alkaline pretreated bagasse further treated using *Streptomyces* sp. S2. Both peak around 1300-1200 was reduced after hydrogen-peroxide treatment, although the drop on 1300 peak not as much as the alkaline-hydrothermal treatment. This indicates weaker both syringyl and guaiacyl bonds despite high amount of lignin concentration remains after hydrogen-peroxide pretreatment. Both peak around 1300-1200 cm was even lower after peroxide bagasse treated with *Streptomyces* sp. S2, resulted in weaker syringyl and guaiacyl bonds (figure 2). This weaker bonds contribute to significant lignin content drop after peroxide bagasse treated with *Streptomyces* sp. S2, although not significant compared to control (table 1)

Bands around 1200- 800 shows especially, but not limited to hemicellulose, because this bands stacked with cellulose and lignin identifier bands (Gogna and Goacher 2018). Low bands around 1100- 900 in control and control-*Streptomyces* bagasse is similar to rice straw bands in Zulyadi et al (2016) research, which indicates hemicellulose still highly bonded to lignin. These 1100-900 peak was lower after alkaline and peroxide pretreatment, correspondingly to decrease in hemicellulose content. Peak on 1160 cm^{-1} was slightly increase after alkaline bagasse further degraded by *Streptomyces* sp S2, indicates bonds between hemicellulose remains unchanged. However, after peroxide bagasse further degraded by *Streptomyces*, peak on 1160 was weaker compared to hydrogen-peroxide only treated bagasse. This indicates some hemicellulose bonds was cut by *Streptomyces* sp S2, despite increase in hemicellulose content percentage. A peak on 897 cm^{-1} indicates β -glycosidic presence in sugarcane hemicellulose (Rashid et al. 2020). Peak number 832 on the other hand show aromatic ring on p-hydroxyphenyl propane in lignin (Portero- Barahona et al. 2019). Slight decrease in FTIR wavenumber of 832 cm^{-1} in all *Streptomyces* treatment sample except control-*Streptomyces*, indicates that *Streptomyces* sp. S2 can decrease p-hydroxyphenyl of lignin only after sugarcane treated with alkaline-hydrothermal and peroxide.

Morphological characteristic

Scanning electron photograph of physical-treated (control) sugarcane bagasse show regular and neatly bundled fiber (Figure 3A) with little to no difference of depth (Figure 3A2). Outer layer of alkaline-hydrothermal (NaOH) treated bagasse become unevenly folded, formed fabric-like folding and a big hole between the fiber (figure 3B). Higher magnification shows the folding in alkaline-hydrothermal treated bagasse are multi-layered (consist of several bundle of fiber, figure 3B2). This result was similar to wheat straw treated with 4% NaOH (Qi et al. 2018), despite different concentration and lignocellulose type of fiber used. Peroxide pretreated bagasse shown to have rough edges, shallow holes scattered around outer layer of fiber bundles and the bundles also have different depth in several area (figure 3C), compared to control that doesn't have different height of fiber bundles. Higher magnification show more detail of shallow holes formed around the bagasse fiber and rough edges formed around the bundles are also multi-layered (figure 3C2).

Streptomyces sp. degradation creates folding in control bagasse, some deep holes also formed around the bagasse. Bagasse after alkaline and *Streptomyces* treatment have smooth web-like fibers on its outer layer, some deep holes formed also can be seen (red arrows, figure 3E). Smooth layer of bagasse that exposed after alkaline-*Streptomyces* treatment might indicate lignin on outer surface of bagasse have completely removed (red arrows, figure 3D). Most outer area of bagasse after peroxide -*Streptomyces* have been completely removed, exposing another layer of hemicellulose and cellulose bundles. Deep holes that are formed by *Streptomyces* sp. S2 activities still can be seen (red arrows, figure 3F). Removal of lignin on outer layer of both alkaline and peroxide bagasse by *Streptomyces* sp. S2 is likely through bacterial digging mechanism, when *Streptomyces* sp S2 form holes and dig out the lignin. This mechanism similar to *Cupriavidus basilensis* that used to degrade rice straw (Xu et al. 2017)

Conclusion

Physical treated only sugarcane (control) cannot be further degraded *Streptomyces* sp. S2 degradation because have high lignin concentration with strong syringil and guaiacyl bonds, cellulose in high ordered crystal lattice, and neatly ordered fiber bundle as observed from lignocellulose content measurement, Fourier transform infrared, X-ray diffraction and Scanning electron microscope. *Streptomyces* sp. S2 can further degrade hydrogen-peroxide pretreated bagasse. Lower lignin percentage, weaker syringyl and guaiacyl lignin were found on peroxide-*Streptomyces* bagasse compared to hydrogen-peroxide only treated bagasse. Peroxide-*Streptomyces* treated bagasse also have higher cellulose and hemicellulose content compared to hydrogen- peroxide treated bagasse. Alkaline-hydrothermal pretreatment can significantly increase cellulose content, lowering both hemicellulose and lignin, specially syringil lignin. *Streptomyces* sp. S2 however, cannot further degrade alkaline treated bagasse, despite more smooth bagasse layer after its degradation. Alkaline-*Streptomyces* bagasse have lower amount of cellulose, similar syringil and guaiacyl lignin bonds, similar amount of lignin and hemicellulose compared to alkaline-hydrothermal only treated bagasse.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and Materials

All data generated or analysed during this study are included in this published article

Competing interest

The authors declare that they have no competing interest

Funding

This research was funded by the authors

Author contribution

SAA do the research, analyze and construct the article, DHYY help the crystalline until morphology analysis. AM and TCS conceive the study and help construct the article.

Acknowledgement

Not applicable

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Figures

Figure 1

XRD Graph used to measure crystalline area

Figure 2

Fourier Transform Infrared Graph

Figure 3

Scanning electron microscope (SEM) photograph (A and A2). Control (B and B2). Alkaline-autoclave (C and C2). Hydrogen peroxide (D). Control-Streptomyces (E). NaOH-Streptomyces (F) H2O2-Streptomyces

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