Effect of Pretreatment Process on Bioconversion of Kenaf (*Hibiscus cannabinus* L.) Core to Glucose

Sim Hong Ng,[a] Paridah Md. Tahir,[a,b]* Rosfarizan Mohamad,[a,c] Luqman C. Abdullah,[a,d] Adrian C.Y. Choo,[a]* and Yan Yee Liong [b]

Kenaf (*Hibiscus cannabinus* L.) is a renewable carbon-rich lignocellulosic resource for fermentable sugars. In this study, kenaf cores cultivar V36 from four-month-old stems were pretreated by i) physical, ii) physical and thermal, and iii) physical and chemical methods. The celluloses of pretreated kenaf core particles were then hydrolyzed into fermentable sugars by cellulase from *Trichoderma reesei* (C2730). The pretreated kenaf core particles were incubated for 48 h at 37 °C. The efficiency of bioconversion was mainly dependent on the pretreatments applied prior to the hydrolysis process. The effects of the pretreatments on kenaf core's lignin, holocellulose, and cellulose contents were also determined. Kenaf cores without pretreatment had 19.4% lignin, 86.2% holocellulose, and 47.4% alpha-cellulose. The combination of physical and chemical pretreatment on kenaf cores cultivar V36 resulted in a higher cellulose content (92.49%) and produced 50 times higher sugar concentration than the physical pretreatment.

Keywords: Lignocelluloses; Kenaf core material; Enzymatic hydrolysis; Fermentable sugar

Contact information: a: Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; b: Department of Forest Production, Faculty of Forestry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; c: Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; d: Department of Chemical Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
** Corresponding authors: parida@introp.upm.edu.my; ccy.adrian@gmail.com*

INTRODUCTION

Lignocellulosic material comprises lignin, hemicelluloses, and cellulose and is a sustainable and renewable resource that comes from the forest or agricultural and urban wastes. On average, seventy-five percent of lignocelluloses are made up of carbohydrates. This carbohydrate-rich material is now an essential source for fermentable sugar production. Hydrolysis of lignocellulosic materials into fermentable sugars to be further fermented into various products such as biochemicals and biofuels have gained focus among researchers because dependency on fossil fuels has a large impact on the environment. This dependency has forced governments across the world to seek alternative renewable, sustainable, and environmentally friendly sources to replace the present petro-chemical industries for energy, chemical, and material production. In this respect, biorefinery will become an important industrial sector in the near future.

Lignocelluloses can be converted into fermentable sugars through acid hydrolysis and enzymatic hydrolysis. The acid hydrolysis process is simple and rapid, and high sugar yield can be obtained, but this process suffers from high acid consumption and low recovery of the acid. Enzymatic hydrolysis results in high sugar yields because sugar

degradation can be avoided by use of enzymatic hydrolysis processes (Parisi 1989). The advantage of enzymatic hydrolysis is that through genetic engineering, new strains can be developed to help improve the enzymatic hydrolysis process. Thus, enzymatic hydrolysis, which is more environmentally friendly, is a better choice for the production of fermentable sugars.

Research on the hydrolysis process has shown that pretreatment is needed prior to hydrolysis in order to achieve higher reducing sugar yields (Kadam *et al.* 2008). Pretreatment can be categorized into physical pretreatment, thermal pretreatment, chemical pretreatment, biological pretreatment, or a combination of these. Converting lignocelluloses into reducing sugars is more difficult compared to sugars and starches because of its structure. Lignin and hemicellulose which surround the cellulose prevent the enzymes from attacking the cellulose. Pretreatment needs to be applied to disrupt or open up the lignin-carbohydrate complex in order to provide easier access for enzymes. This enhances the enzymatic hydrolysis process and increases the reducing sugar yield (Millett *et al.* 1976). A well pretreated substrate and an efficient cellulose system can contribute to the economical bioconversion of biomass feedstocks to products (Kadam 2008).

A major portion of fermentable sugars is derived from corn, grain, and sugar cane. In 2006, approximately 46 million m^3 of bioethanol was produced from these materials (Jørgensen *et al.* 2007). The conversion of agricultural crops to energy and chemicals affects food prices across the world. To solve this problem, there has been much research on the use of residues from agriculture and forest industries such as corn stalk, oil palm stem, sago palm stem, and waste paper.

In Malaysia, the main agricultural crops are oil palm and rubber trees. Recently, kenaf (*Hibiscus cannabinus* L.) was introduced to partially replace tobacco in Malaysia by 2010 (Anuar and Zuraida 2011 and Mohd Suhairil *et al.* 2012). Kenaf is a fast-growing agricultural crop planted for its fibre. Kenaf bast fibres are long and can be used in the pulp and paper or biocomposite industries. Kenaf core with shorter fibre length is usually produced for low end products such as animal bedding and absorbent materials for oil cleaning. According to Ohtani *et al.* (2001), kenaf is reported to contain high holocellulose (77.6%) and alpha cellulose (45.3%). Due to its high cellulose content, kenaf is a suitable raw material for fermentable sugar production.

In this study, the enzymes used to break-down cellulose into reducing sugars are cellulases. Cellulose hydrolysis is affected by the synergistic action of the cellulase components endoglucanase, exoglucanases, and β-glucosidases. The cellulases used were from *Trichoderma reesei* (C2730). Cellulase from *Trichoderma reesei* is one of the most studied cellulases, and it has been shown to be effective in converting cellulose into reducing sugars (Domingues *et al.* 2001; Suurnäkki *et al.* 2000). The aim of this study is to investigate the effect of pretreatments on the glucose yield from kenaf core cultivar V36 and the potential of kenaf core as a feedstock for the production of lactic acid.

EXPERIMENTAL

Substrates Preparation

Four-month-old kenaf cultivars V36 were obtained from Taman Pertanian Universiti, Universiti Putra Malaysia. The kenaf stems were debarked manually to obtain the core materials. After debarking, the kenaf cores were air-dried before being chipped and ground into a powder. The moisture content (MC) of the powder was 10 to 12%.

Pre-treatment

Physical pretreatment (HM)

Kenaf core chips were ground (Thomas Hammer-mill, USA) to powder form and sieved into four different sizes: 425 µm to 600 µm, 250µm to 425µm, 180 µm to 250 µm, and ≤180µm. The four fractions, taken together, accounted for essentially 100% of the original dry mass.

Physical and Thermal pretreatment (HMTH)

The physically pretreated kenaf (V36) core powders were soaked in distilled water and thermally treated by autoclaving the fibres at 121 °C, 15 psi for 15 min. After the fibres were filtered from the distilled water, the fibres were dried in an oven at 103 °C for 24 h.

Physical and Chemical pretreatment (HMCH)

The physically pretreated kenaf (V36) core powders were chemically pretreated by soaking them in alkaline solutions [NaOH 0.5% (HMCHN-0.5), NaOH 1.0 % (HMCHN-1.0), NaOH 10.0% (HMCHN10.0), and NaOH 17.5 % (HMCHN-17.5)] for 4 h at room temperature. The powders were then filtered and soaked in distilled water for 24 h. The pretreated material was soaked until it was free of NaOH (tested with litmus paper). The powders were then filtered again and dried in an oven at 103 °C for 24 h.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out by treating 1 g of dried pretreated kenaf powder in 100 mL distilled water with 0.5 mL of *Trichoderma reesei* (C2730) cellulase with FPU ≥ 700 units/g. The process was carried out in a shaker incubator at 200 rpm at 37 °C for 48 h.

Analysis Methods

Kenaf powders with sizes of 250 to 425 µm were selected for the analysis of their chemical constituents. The lignin, holocellulose, and alpha cellulose content of all treated kenaf core powders were determined according to the corresponding TAPPI Methods.

The samples or kenaf core powder were extracted with alcohol:acetone (1:2) solvent to obtain extractive-free fibres. The strong acid degraded the polysaccharides in the sample while the acid-insoluble lignin remained. The acid-insoluble lignin content in the samples was determined using 72% sulfuric acid. The holocellulose content in the samples was determined using sodium chlorite and 10% acetic acid. The holocellulose was air-dried, and then the alpha cellulose content was determined. The alpha cellulose content in the samples was determined using 17.5% NaOH. The alpha cellulose determination process was carried out in a 20 °C water bath.

The effect of the pretreatment on the morphology of the kenaf core powder surface was observed under Scanning Electron microscopy (SEM) at 1000X and 3000X magnification.

After enzymatic hydrolysis, the hydrolysate was centrifuged at 3,500 rpm for 30 min before being analyzed for glucose content using HPLC, Column Supelco LC NH-2, with a refractive index detector under conditions of 80% acetonitrile as mobile phase and 1.0 mL/min flow rate.

RESULTS AND DISCUSSION

Chemical Constituents of Kenaf Core Material

Table 1 shows the chemical constituents of kenaf cultivar V36 core materials. It was found that the alpha-cellulose content in kenaf core materials was higher than that of hardwoods and oil palm stem which are 40 to 45% (Rowell *et al.* 2005) and 29.2 % (Law and Wan Rosli 2002), respectively. The lignin content in kenaf core materials (19.4%) is higher than that in oil palm stem (18.8%) and is in the range of hardwoods (18 to 25%). The lignin content in kenaf core materials can decrease the effectiveness of the enzyme synergy action in the hydrolysis process because lignin can adsorb the enzymes, and therefore less enzymes will be available for the hydrolysis process. This decreases the reducing sugar yield (Jørgensen *et al.* 2007). Pretreatment is needed prior to the hydrolysis process to increase the alpha-cellulose content and reduce the lignin content in the kenaf core material for higher fermentable sugar yield.

Table 1. Chemical Constituents of Kenaf Core

Chemical composition	Kenaf core V 36 (%)	Hardwoods* (%)	Oil palm stem** (%)
Lignin	19.40	18-25	18.8
Holocellulose	86.19	65-70	45.7
Alpha-cellulose	47.40	40-50	29.2
Ash	4.28	0.2-0.8	2.3

Source: *Rowell *et al.* 2005; **Law and Wan Rosli 2002

Effect of Pretreatment on the Kenaf Core V36 Material's Chemical Constituents and Glucose Concentration

Figures 1 to 6 show SEM images at 1000X and 3000X magnifications. It can be seen (Fig 1 & 2) that HMCH pretreated Kenaf core V36 fibres have more open surface contact area and higher pore volume. It is proposed that this facilitates the enzymatic hydrolysis process. The smoother surface morphology of the particles might also increase the accessibility of the enzymes to the substrate, if one envisions the enzymes as mainly acting upon the broad external surface. NaOH successfully removed the other polysaccharides that may have protected the cellulose from enzymatic degradation.

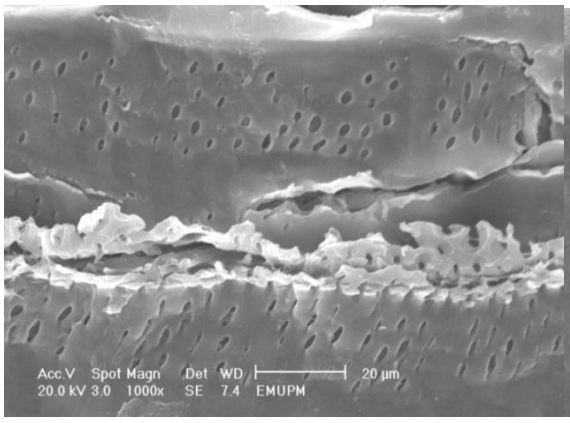

Fig. 1. HMCHN-17.5 pretreated kenaf core V36 under SEM at 1000X magnification

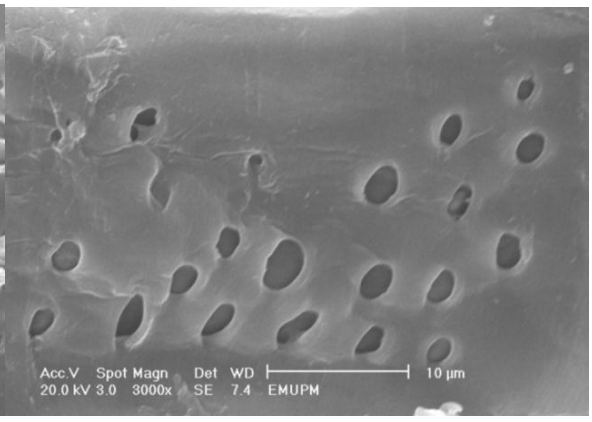

Fig. 2. HMCHN-17.5 pretreated kenaf core V36 under SEM at 3000X magnification

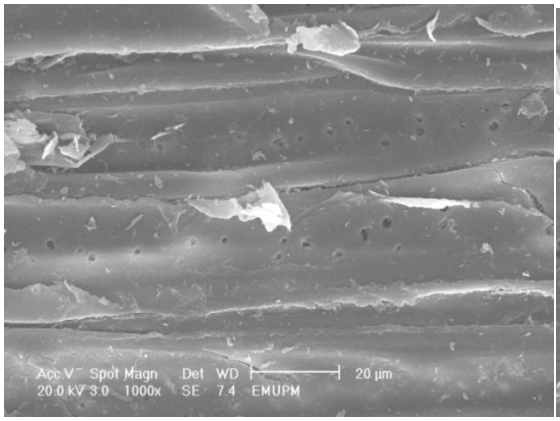

Fig. 3. HM pretreated kenaf core V36 under SEM at 1000X magnification

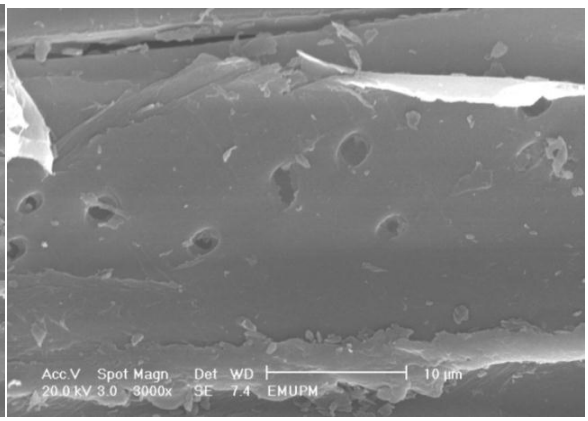

Fig. 4. HM pretreated kenaf core V36 under SEM at 3000X magnification

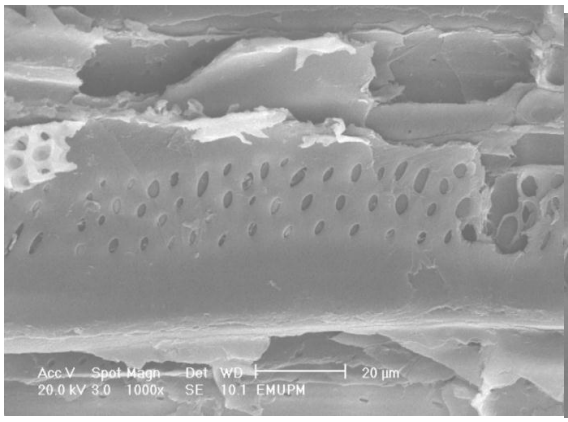

Fig. 5. HMTH pretreated kenaf core V36 under SEM at 1000X magnification

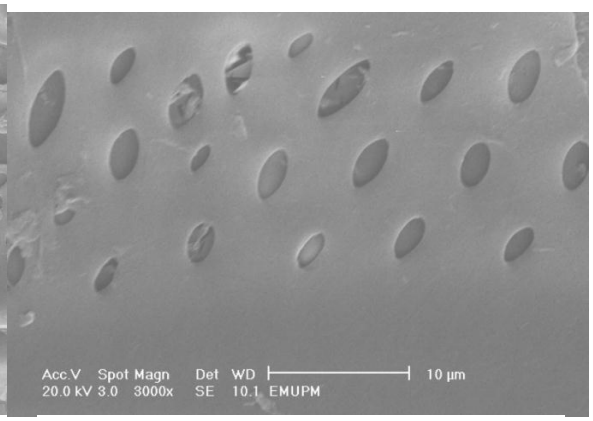

Fig. 6. HMTH pretreated kenaf core V36 under SEM at 3000X magnification

The chemical composition of the pretreated kenaf core material varied among different particle sizes and pretreatment applied. The HMCHN-17.5 treated kenaf core materials had the highest alpha-cellulose content (92%), and the HM treated kenaf core materials with 425 to 600 µm sized particles had the lowest alpha-cellulose content (47%) (Table 2). The use of sodium hydroxide (NaOH) in the pretreatment was effective in increasing the surface area availability of cellulose in the kenaf core material (Figs. 1 and 2). NaOH is a pre-swelling agent that can increase the accessibility of the kenaf core materials to hydrolysis reactions. In this study, the higher the concentration (17.5%) of NaOH used in the HMCH pretreatment, the higher the alpha-cellulose content obtained. The pretreatment increased the alpha cellulose composition in the pretreated kenaf core V36 fibres by about 43%. The effect of pretreatment on the lignin content for all the pretreated kenaf core V36 materials was relatively low (Table 3) because the temperature and chemicals used in the pretreatments does not contribute to lignin degradation.

Higher temperatures increase the solubility of lignin, but they also increase the solubility of hemicellulose. Lignin starts to soften and flow at 140 °C and solubilizes at 160 °C (Hendriks and Zeeman 2009). In this study, the temperature used in the HMTH pretreatment was 121 °C. Removal of lignin prior to the enzymatic hydrolysis process is important because lignin not only shields the cellulose microfibrils from enzyme syner-

gistic reactions but also attracts cellulase to its surface (Jørgensen *et al*. 2007). From Table 2, it can be observed that the finer the particle used in the same treatment, the higher the alpha-cellulose content obtained. NaOH reacts with the finer kenaf core V36 particles more effectively due to the higher surface area in the smaller sized particles.

Table 2. Effect of Pretreatment and Powder Size on the Alpha Cellulose Content (%) for the Pre-Treated Kenaf Core

Pretreatment	425-600 µm	250-425 µm	180-250 µm	<180 µm
HMCH-N17.5	81.8±0.3 ^{E}	83.2±1.0 ^{D}	88.4±0.8 ^{B}	91.5±0.9 ^{A}
HMCH-N10.0	79.4±0.1 ^{F}	80.0±0.8 ^{F}	86.7±0.8 ^{C}	88.8±0.2 ^{B}
HMCH- N1.0	70.4±0.2 ^{G}	69.3±0.1 ^{H}	70.4±0.5 ^{G}	70.4±0.1 ^{G}
HMCH-N0.5	63.1±0.3 ^{J}	63.3±0.3 ^{J}	68.5±0.1 ^{H}	67.9±0.1 ^{HI}
HMTH	46.5±0.5 ^{L}	46.6±0.5 ^{L}	49.8±0.3 ^{K}	50.1±0.3 ^{K}
HM	46.5±0.4 ^{L}	46.9±0.7 ^{L}	47.1±0.1 ^{L}	49.6±0.3 ^{K}

LSD = 0.804

Note: Means followed by the same letter in each row are not significantly different at p≤0.05 according to LSD.

Table 3. Effect of Pretreatment and Powder Size on the Lignin Content (%) for the Pretreated Kenaf Core

Pre-treatment	425-600 µm	250-425 µm	180-250 µm	<180 µm
HMCH-N17.5	27.8±0.2 ^{B}	26.5±0.4 ^{C}	26.1±0.2 ^{C}	29.5±0.6 ^{A}
HMCH-N10.0	26.6±0.5 ^{C}	26.8±0.3 ^{C}	27.1±0.6 ^{BC}	28.5±0.6 ^{B}
HMCH- N1.0	21.7±0.4 ^{D}	21.4±0.5 ^{D}	20.5±0.4 ^{DE}	21.1±0.2 ^{D}
HMCH-N0.5	21.6±0.6 ^{D}	20.4±0.2 ^{DE}	19.7±0.7 ^{E}	20.7±1.0 ^{DE}
HMTH	20.1±0.7 ^{E}	20.3±1.0 ^{E}	20.2±0.8 ^{E}	21.4±0.3 ^{D}
HM	20.0±0.3 ^{E}	19.4±0.6 ^{EF}	19.3±0.2 ^{EF}	20.6±0.2 ^{DE}

LSD = 0.89

Note: Means followed by the same letter in each row are not significantly different at p≤0.05 according to LSD.

Table 4. Effect of Pretreatment and Powder Size on the Holocellulose Content (%) for the Pretreated Kenaf Core

Pretreatment	425-600 µm	250-425 µm	180-250 µm	<180 µm
HMCH-N17.5	75.6±0.1 ^{G}	74.9±0.1 ^{GH}	76.0±0.8 ^{G}	74.6±0.6 ^{GH}
HMCH-N10.0	79.9±2.1 ^{E}	77.9±0.4 ^{F}	76.1±1.3 ^{G}	73.0±0.1 ^{H}
HMCH- N1.0	77.4±0.2 ^{FG}	73.8±0.2 ^{H}	72.7±0.1 ^{H}	73.6±0.7 ^{H}
HMCH-N0.5	76.3±0.4 ^{G}	74.5±0.3 ^{GH}	78.4±0.5 ^{F}	78.7±0.4 ^{F}
HMTH	86.4±0.6 ^{C}	86.0±0.8 ^{C}	87.6±0.4 ^{B}	88.3±0.7 ^{B}
HM	89.6±0.9 ^{A}	86.2±0.5 ^{C}	82.2±0.3 ^{D}	78.8±0.1 ^{F}

LSD = 1.13

Note: Means followed by the same letter in each row are not significantly different at p≤0.05 according to LSD.

Table 5 shows that pretreatment of the substrates with HMCH–N17.5 resulted in higher reducing sugar yield (4.4 g/L). Higher sugar yield was obtained due to the presence of a higher percentage of alpha-cellulose (92%) (Table 2) in the HMCH-N17.5 treated samples. The higher alpha-cellulose content in the substrates can facilitate the enzymatic hydrolysis process by providing higher available areas for reaction with the cellulose enzyme. HM pre-treatment resulted in the lowest reducing sugar yield due to the higher hemicelluloses content and lower alpha-cellulose content in the substrates

(Table 2). The presence of lignin and hemicelluloses in the microfibrils which surrounds the microfibril prevents the cellulose enzyme from accessing the cellulose surface (Jørgensen *et al.* 2007). Because of this reason, the amount of alpha-cellulose that can be converted into reducing sugar is relatively low.

Table 5. Effect of Pretreatment and Powder Size in the Glucose Yield (G/G) for the Pre-Treated Kenaf Core

Pretreatment	425-600 µm	250-425 µm	180-250 µm	<180 µm
HMCH-N17.5	0.335±0.006 ^{C}	0.412±0.002 ^{B}	0.423±0.001 ^{AB}	0.441±0.002 ^{A}
HMCH-N10.0	0.412±0.002 ^{B}	0.425±0.005 ^{AB}	0.432±0.002 ^{A}	0.439±0.003 ^{A}
HMCH- N1.0	0.224±0.005 ^{G}	0.233±0.003 ^{G}	0.270±0.002 ^{E}	0.260±0.002 ^{EF}
HMCH-N0.5	0.256±0.010 ^{F}	0.268±0.003 ^{E}	0.272±0.006 ^{E}	0.301±0.009 ^{D}
HMTH	0.200±0.008 ^{I}	0.213±0.100 ^{GH}	0.231±0.008 ^{G}	0.292±0.023 ^{D}
HM	0.008±0.001 ^{K}	0.004±0.001 ^{JK}	0.015±0.004 ^{J}	0.020±0.001 ^{J}

LSD = 0.113

Note: Means Followed by the same letter in each row are not significantly different at p≤0.05 according to LSD.

CONCLUSIONS

1. Pretreatments of the substrates contributed to higher fermentable sugar yield. The reduction of substrate size increases the surface contact area of the substrates to the enzymes, thus increasing the fermentable sugar yield. However, the yield decreases while decreasing the substrate size (from 60-80 to >80 mesh) for kenaf core material pretreated by HMCH-N1.0.
2. Kenaf core materials can be converted to fermentable sugars with the yield of 4.4 g/L. The pretreatment also helps to increase glucose yield and contribute to further applications in various processes in different sectors.
3. The use of kenaf core V36 as feedstock for enzymatic hydrolysis opens an avenue for lignocellulose materials in bioconversion technology.
4. The lignocelluloses which are renewable and sustainable are a promising feedstock for biochemicals and biofuels in the near future.

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