Green Chemistry

An effective hybrid strategy for converting rice straw to furoic acid by tandem catalysis via Sn-sepiolite combined with recombinant E. coli whole cells harboring horse liver alcohol dehydrogenase

Journal:	*Green Chemistry*
Manuscript ID	GC-ART-07-2019-002499.R1
Article Type:	Paper
Date Submitted by the Author:	05-Sep-2019

Complete List of Authors:	Peng, Bo; Hubei University Ma, Cuiluan; Hubei University Zhang, Pengqi; Hubei University Wu, Changqing; Hubei University Wang, Ziwei; Hubei University Li, Aitao; Hubei University He, Yucai; Hubei University; Changzhou University Yang, Bin; Washington State University

SCHOLARONE™
Manuscripts

1 # An effective hybrid strategy for converting rice straw to furoic acid
2 # by tandem catalysis via Sn-sepiolite combined with recombinant *E.*
3 # *coli* whole cells harboring horse liver alcohol dehydrogenase

4 Bo Peng[a,c#], Cui-Luan Ma[a,#], Peng-Qi Zhang[a,#], Chang-Qing Wu[a], Zi-Wei Wang[a],
5 Ai-Tao Li[a], Yu-Cai He*[a,b,c], Bin Yang[c]

6 [a] *State Key Laboratory of Biocatalysis and Enzyme Engineering, Hubei Collaborative*
7 *Innovation Center for Green Transformation of Bio-resources, Hubei Key Laboratory*
8 *of Industrial Biotechnology, College of Life Sciences, Hubei University, Wuhan, P.R.*
9 *China.*

10 [b] *Advanced Catalysis and Green Manufacturing Collaborative Innovation Center,*
11 *National & Local Joint Engineering Research Center on High Efficient Biorefinery*
12 *and High Quality Utilization of Biomass, Changzhou University, Changzhou, P.R.*
13 *China*

14 [c] *Bioproducts, Sciences and Engineering Laboratory, Department of Biological*
15 *Systems Engineering, Washington State University, Richland, USA*

16

17 # Bo Peng, Cui-Luan Ma and Peng-Qi Zhang contributed equally to this work.

18

19 **Corresponding authors emails:** *Email:* ***heyucai2001@163.com*** (Y. He)

1

2 **Abstract:** The upgrading of biomass-derived furfural into high-value bio-based
3 chemicals has attracted interest. In this case, the conversion of rice straw into furfural
4 was firstly performed using tin-loaded sepiolite (Sn-sepiolite) as a catalyst. Acidified
5 solid acid Sn-sepiolite (3.0 wt%) converted alkali-pretreated dewaxed rice straw into
6 furfural at 42.2% yield at 170 °C for 20 min. Moreover, biomass-derived furfural
7 could be completely converted into furoic acid with recombinant immobilized *E. coli*
8 TS whole-cells within 96 h at pH 7.0 and 30 °C, respectively. Finally, efficient
9 recycling and reuse of the Sn-sepiolite catalyst and immobilized TS whole-cell
10 biocatalyst were developed for the synthesis of furoic acid from rice straw in the
11 one-pot reaction system. In summary, an effective one-pot chemo-enzymatic
12 synthesis of furoic acid from renewable biomass was successfully developed using
13 ambient conditions.

14

15 **Keywords:** Sn-sepiolite; Rice straw; Hemicellulose; Furfural; Furoic acid; Horse
16 liver alcohol dehydrogenase.

1 **Introduction**

2 Owing to increasing environmental issues and demand for alternatives to fossil
3 fuels,[1-3] lignocellulosic materials have become attractive renewable sources, attracting
4 worldwide attention to the production of biofuels, functional materials and bio-based
5 chemicals.[4-10] Cellulose (40-50 wt%), hemicellulose (25-35 wt%) and lignin (15-35
6 wt%) are the main building blocks of biomass.[11-15] Hemicellulose in biomass is one of
7 these complicated heterogeneous polymers and is composed of C5 and C6 sugars
8 (such as xylose, arabinose, mannose, glucose, etc.).[16,17] Arabinoxylan is a major
9 henmicellulose in the mature tissues of grass plants. Arabinose substitution degree of
10 xylans may affect lignocellulose digestibility. The global production of rice residue is
11 estimated around 9.0×10^9 tons per year.[18] Rice straw, like other biomasses, consists
12 of cellulose, hemicellulose and lignin. Additionally, it contains about 20% of silica
13 [19,20] Silica has been reported as the negative factor on biomass digestion.[6,7] It is of
14 great interest to effectively convert rice straw to bio-based products.

15 The conversion of abundantly available and inexpensive biomass into bio-based
16 chemicals has attracted much attention.[8-10] Furan products were known as one kind of
17 important bio-based chemicals. The bio-based production of furfural (FAL), which
18 can be derived from hemicellulose in biomass, has been known for decades. Its
19 downstream furan products are not only plentiful but also have high economic
20 value.[21,22] Conventionally, FAL is prepared via the acid-hydrolysis of biomass with
21 homogeneous mineral acids (e.g., H_2SO_4, H_3PO_4, and HCl). However, high loadings

1 of mineral acids can cause serious equipment corrosion, and FAL, byproducts and
2 mineral acids are hard to recover. Recently, heterogeneous solid acids (modified
3 SO_4^{2-}/SnO_2-argil, Amberlyst, SO_4^{2-}/SnO_2-kaoline, niobates, resin, silica, zeolites,
4 hydroxylated MgF_2, TiO_2, ZrO_2, SnO_2, Al_2O_3, etc.) have attracted great attention for
5 synthesizing FAL due to their high catalytic activity, low corrosivity, renewability,
6 and reusability compared with traditional mineral acid catalysts.[16,22-25] A FAL yield of
7 68% was reached from xylose using acidic ionic liquid (IL) supported on magnetic
8 γ-Al_2O_3 (Fe_3O_4@Al_2O_3-(BAIL-Al)) in DMSO-water (5:1, v:v) at 140 °C for 3 h.[26]
9 Using H-Beta zeolite as catalyst, the FAL yield from corn fiber, composed of 45
10 wt% C_5 sugars, was 62% at 160 ^{o}C in γ-valerolactone-water (9:1, v:v).[27]

11 Furoic acid (FA), a heterocyclic carboxylic acid, is known as one of the most
12 important upgrading products of FAL, which is a versatile raw material used for
13 synthesizing various agricultural, pharmaceutical and industrial chemicals.[21] The salt
14 or ester of FA is known as furoate. FA is an organic compound that is found mostly in
15 food products as a preservative and a flavoring agent. Other uses for FA include nylon
16 preparation and optic technologies. FA can be synthesized by the oxidation of either
17 furfuryl alcohol (FOL) or FAL via the chemical or biochemical approaches.[28,29]
18 Industrially, the Cannizaro reaction of FAL is used to produce FA accompanied by
19 the byproduct FOL in an aqueous alkaline media. Oxidating FAL using O_2 over a
20 catalyst is not an ideal approach because not only does FAL undergo oxidation into
21 FA, but also a competitive nucleus oxidation is undergone of the cleavage furan ring.
22 Nowadays, biocatalysis has been emerging as a promising alternative to the traditional

1 chemical approach for the oxidation of these inherently unstable bio-based furans due
2 to its high catalytic activity and selectivity under mild performance conditions.
3 *Acetobacter rancens* IFO3297 could convert 12 mmol FAL into FA at 95% yield
4 within 30 h.[30] *Gluconobacter oxydans* (ATCC 621H) could oxidize both FAL and
5 FOL into FA under mild conditions. Fed-batch bioprocess gave FA over 40 g/L, and
6 38 g/L FA was reached within 24 h in the COS-SSTR system.[31] Furans are known as
7 potential inhibitors towards enzymes and microorganisms,[32] thus, high loadings of
8 furans usually encounter low biocatalytic efficiency. In addition, there is a limited
9 amount of information for the effective chemo-enzymatic conversion of biomass into
10 FA via tandem catalysis under mild conditions.

11 Horse liver alcohol dehydrogenase (HLADH), an NAD-dependent enzyme, has a
12 broad substrate specificity[33] which is active on a variety of alcohols and
13 acetaldehydes.[34] In this study, one-pot chemo-enzymatic conversion of rice straw was
14 attempted in order to synthesize FA via the tandem catalysis with solid acid
15 Sn-sepiolite and whole-cells harboring HLADH under the mild condition. Firstly,
16 acidified Sn-sepiolite was used for the conversion of rice straw to FAL in a one-pot
17 manner. Furthermore, various reaction parameters (e.g., reaction temperature, reaction
18 time, heterogeneous solid acid loading, etc.) were investigated on the FAL production
19 in the aqueous media. Furthermore, recombinant *E. coli* cells harboring Horse Liver
20 Alcohol Dehydrogenase (HLDAH) were constructed (Fig. 1), and several bioreaction
21 parameters (e.g., bioreaction temperature, bioreaction time, metal ion additives, etc.)
22 were optimized to enhance the biocatalytic activity of recombinant *E. coli* whole-cells

1 harboring HLADH. Moreover, the one-pot conversion of rice straw to FA via
2 sequential catalysis with a solid acid Sn-sepiolite catalyst and HLADH whole-cell
3 biocatalyst was developed (Fig. 2). Finally, the recyclability of solid acid Sn-sepiolite
4 catalyst and immobilized whole-cell biocatalyst was tested in the developed one-pot
5 reaction system.

6 Results and discussion

7 Characterization of solid acid catalyst

8 Sepiolite ($Mg_8Si_{12}O_{30}(OH)_2$), a complex magnesium silicate, is a non-swelling,
9 lightweight, porous clay. [35] In this study, solid acid catalyst Sn-sepiolite was prepared
10 for the conversion of rice straw into FAL. The BET for assessing the surface areas of
11 microporous materials was presented in Table 1, which indicated that Sn-sepiolite
12 showed a large surface area (45.9 m^2/g) and pore volume (0.04 cm^3/g) compared to
13 fresh sepiolite. Soluble components in sepiolite might be dissolved by the solvent
14 during the preparation of solid Sn-sepiolite catalyst, resulting in the increase of pore
15 volume and surface area of Sn-sepiolite. Compared to sepiolite, Sn-sepiolite had a
16 smaller pore size (3.5 nm). SEM illustrated that both the sepiolite and Sn-sepiolite had
17 a relatively uniform dispersion of particles (Fig. 3a). The results of FT-IR spectra
18 revealed the difference between fresh sepiolite and Sn-sepiolite (Fig. 3b). The peak at
19 near 2,500 cm^{-1} is associated with the -OH stretching vibration of –COOH, and the
20 peak at near 1,750 cm^{-1} is ascribed with the C-O stretching vibration. They were
21 disappeared after the preparation of Sn-sepiolite. The range near 1,420 cm^{-1} and 1,620

1 cm^{-1} is associated with the -OH bending mode of adsorbed water (H_2O) molecules on
2 the sepiolite surface and the stretching vibration of zeolite water in sepiolite,
3 respectively. The peak at about 900-1200 cm^{-1}, which is ascribed to the stretching
4 vibration of S=O double bond, increased after the preparation of Sn-sepiolite. XRD
5 revealed that tin ions slightly changed the structure of sepiolite (Fig. 3c). The intensity
6 of Sn-sepiolite decreased compared with fresh sepiolite. The formation of SnO_2 might
7 disperse in sepiolite. Raman spectra indicated that the peaks of Sn-sepiolite are
8 significantly weaker than those of fresh sepiolite. The difference in the 1250-1500
9 cm^{-1} range is ascribed to the C-H bending in sepiolite, which decreased after the
10 preparation of Sn-sepiolite.

11 To effectively synthesize FAL from biomass, it was necessary to obtain the
12 optimum pretreatment conditions via solid acid catalysis.[22,36] Several pretreatment
13 parameters, including solid acid dosage, pretreatment temperature, and
14 pretreatment time for converting alkali pretreatment of dewaxed rice straw were
15 investigated on the FAL production. Different dosages of Sn-sepiolite (2.0 to 4.0
16 wt%, pH 1.0) were used in the alkali pretreatment of dewaxed rice straw into FAL at
17 160-180 ^{o}C for 5-40 min. The highest FAL concentration of 97.8 mM was obtained at
18 42.2% yield within 20 min at 170 ^{o}C with the Sn-sepiolite catalyst (3.0 wt%, pH 1.0)
19 (Fig. 4a & 4b). To ensure if the silica in the pretreated rice straw could play a role
20 with the Sn-sepiolite catalyst, different loadings of SiO_2 (0-3.0 wt%) were mixed with
21 solid acid Sn-sepiolite (3.0 wt%). It was found that SiO_2 had no effects on the furfural
22 production (data not shown).

1

2 **Optimization of converting FAL into FA with recombinant *E. coli* whole cells**
3 **harboring HLADH**

4 Biocatalysis represents an attractive route to produce value-added intermediates with
5 high selectivity under ambient reaction conditions.[3,22,31,34] In this study, recombinant *E.*
6 *coli* HL and its mutants (*E. coli* IS, *E. coli* KG, and *E. coli* TS) were constructed for
7 the bioconversion of FAL at pH 7.0 and 30 °C. Biotransformation of 25 mM FAL for
8 96 h, 89-96% yields of FA were obtained using whole-cells of *E. coli* HL, *E. coli* IS,
9 and *E. coli* KG as biocatalysts (Fig. S1†). It was found that recombinant *E. coli* TS
10 had highest FAL-oxidizing activity. 25 mM FAL was completely converted into FA
11 within 72 h. To further enhance the FAL-oxidizing activity of recombinant *E. coli* TS,
12 the whole-cell biocatalytic parameters (e.g., bioreaction temperature, bioreaction pH,
13 metal ion additive, substrate FAL dosage, etc.) were optimized. Effects of biological
14 reaction temperature (20-45 °C) and reaction pH (5.0-9.0) were investigated on the
15 FAL-oxidizing activity using whole-cells (0.1 g/mL, wet weight) as biocatalysts.
16 Reaction temperature (20-45 °C) influenced FAL-oxidizing activity of whole-cells
17 significantly (Fig. 5a). The whole-cells displayed the best catalytic activity at 30 °C.
18 Reaction pH (5.0-9.0) exerted a significant effect on the FAL-oxidizing activity (Fig.
19 5b). Recombinant *E. coli* TS cells displayed good FAL-oxidizing activities only
20 within a narrow pH range (pH 6.5-7.0). Furthermore, thermostability and pH stability
21 of TS cells in whole-cells were investigated at different reaction temperatures (4, 30
22 and 45 °C) and reaction pH values (6.5, 7.0 and 7.5). Different biological reaction pH

1 and temperature had significant effects on the stability of TS cells (Fig. 5c & 5d). The
2 half-life values of HLADH in TS cells were calculated based on the thermostability
3 and pH stability. At 30 °C, $t_{1/2(pH\ 6.5)}$, $t_{1/2(pH\ 7.0)}$, and $t_{1/2(pH\ 7.5)}$ were 22.1, 38.7 and 11.6
4 h; respectively. At pH 7.0, $t_{1/2(4\ °C)}$, $t_{1/2(30\ °C)}$, and $t_{1/2(45\ °C)}$ were 142, 38.7 and 3.45 h,
5 respectively.

6 Several metal salts (2.5 mM) including $MgCl_2$, $FeCl_3$, $MnCl_2$, $CoCl_2$, $CaCl_2$, $SnCl_4$,
7 $ZnCl_2$, $CuCl_2$ were chosen as additives for the investigation on the effects of
8 converting FAL at pH 7.0 and 30 °C. $MgCl_2$, $FeCl_3$, $MnCl_2$, $CoCl_2$, $CaCl_2$, $SnCl_4$,
9 $CuCl_2$ (2.5 mM) had clear inhibition on the FAL-oxidizing activity (Fig. 6a). Cu^{2+}
10 caused the strong inhibition on the biocatalytic activity and 18% of enzyme activity
11 was remained, which indicated that HLADH had low tolerance on Cu^{2+}. Notably,
12 $ZnCl_2$ (2.5 mM) had positively effect the biocatalytic activity. Different dosages of
13 $ZnCl_2$ (0-20 mM) were investigated on the FAL-oxidizing activity (Fig. 6b). By
14 increasing the concentration of $ZnCl_2$ from 0 to 5.0 mM, the biocatalytic activity
15 increased gradually. At over 5.0 mM, the biocatalytic activity decreased. In case of
16 20.0 mM Zn^{2+}, 20% of enzyme activity was inhibited. Clearly, $ZnCl_2$ (5.0 mM) could
17 promote the highest biocatalytic activity by 1.4-folds.

18 In the bioreaction media, solid acid Sn-sepiolite might have significantly effects on
19 the FAL-oxidizing activity. It was found that Sn-sepiolite loadings (0-4.0 wt%) had
20 no inhibition on the FAL-oxidizing activity of recombinant *E. coli* TS whole-cells
21 harboring HLADH (Fig. 6c), which faciliated the bioconversion of biomass-derived
22 FAL without the removal of Sn-sepiolite. Thus, one-pot chemo-enzymatic conversion

1 of biomass to to FA could be developed by tandem catalysis via Sn-sepiolite
2 combined with recombinant *E. coli* whole cells harboring HLADH.

3 Based on above experiment results, the optimum biological reaction pH, reaction
4 temperature, and metal ion additve were pH 7.0, 30 °C, and $ZnCl_2$ (5 mM),
5 respectively. Compared to the synthesis of FA via Cannizaro reaction of FAL in an
6 aqueous alkaline media, [27,28] biocatalysis approach is of great interest due to its
7 performance under mild conditions.

8 **Effects of FAL, FOL and FA loadings on the FAL-oxidizing activity**

9 The success of a biocatalytic and biotransformation process strongly depends on the
10 tolerance of the biocatalyst towards high substrate and product concentrations.[37,38]
11 Unfortunately, the substrate FAL and its derivatives are well-known inhibitors to
12 microorganisms.[32] Firstly, the substrate FAL tolerance of whole-cells of recombinant
13 *E. coli* TS strain was evaluated at 30 °C and pH 7.0 (Fig. 7a). When FAL loadings
14 were ≤ 75 mM. FA was obtained in excellent analytical yields (> 99.9%) within 120 h.
15 However, FA yields were obtained at 80.1% and 40.2% when the FAL concentrations
16 were 100 and 150 mM, respectively. During the biotransformation of 10-150 mM
17 FAL, FOL was detected (Fig. S2†). At ≤ 75 mM FAL, FOL fomed faster than FA
18 (Fig. S3†). The former was obtained at the high concentration at 6 h. After 6 h, FOL
19 concentration decreased and FA concentration increased gradually. FOL was further
20 oxidized into FA. Using 100 and 150 mM FAL as substrates, FOL formed at the high
21 concentration of 70.5 mM (24 h) and 112 mM (72 h), respectively. After 144 h, FA

1 was obtained at 50.9-80.0 mM. These results suggested that the optimal substrate
2 FAL loading for synthesis of FA was 75 mM. This substrate concentration was higher
3 than the existing biocatalytic processes for the synthesis of FA from FAL with *G.*
4 *oxydans* strain (ATCC 621H).[31] *G. oxydans* could convert 6.5 g/L (68.4 mM) of FAL
5 into 7.5 g/L FA with a yield of 98.9%.

6 Furthermore, FOL (10-200 mM) was attempted as substrate for synthesizing FA
7 (Fig. S4†). It was found that 10 and 75 mM FOL could completely oxidized into FA
8 at 24 and 120 h (Fig. 7b), respectively. Low FA yields were obtained at over 100 mM
9 FAL loadings. In this study, biosynthesis of FA derived from FOL and FAL (75 mM)
10 via the biological oxidation was successfully demonstrated. Moreover, the tolerance
11 of whole-cells towards FA was evaluated when 10-200 mM FA was initially added
12 into aqueous media containing 75 mM FAL (Fig. 7c). No obvious inhibition was
13 detected in the presence of $\leq$ 75 mM FA. By adding FA at over 100 mM, product
14 inhibition was found during the biotransformation of 75 mM FAL. FA yields
15 decreased from 84.9% to 6.3% with further increasing FOL concentrations from 100
16 to 200 mM, and FA yields decreased from 42.2% to 2.5% with further increasing FA
17 loadings from 100 to 200 mM.

18 **Chemo-enzymatic synthesis FA from rice straw**

19 Catalytic upgrading of biomass-derived FAL and its derivatives for the production of
20 high-value products is currently of great interest.[39-44] In this study, catalysis of rice
21 straw with acidified Sn-sepiolite in the aqueous media via hydrolysis and dehydration

1 in one-pot reaction system will provide cheap FAL for the catalytic upgrading of FAL
2 into FA.
3 Under the established conditions above, the solid acid Sn-sepiolite catalyst
4 mediated rice straw pretreatment (hydrolysis/dehydration) into FAL and recombinant
5 *E. coli* TS whole-cells catalyzed the biotransformation of FAL into FA were then
6 combined to test the conversion of rice straw to FA in sequential one-pot manner. As
7 indicated in Fig. 8, acidified Sn-sepiolite converted rice straw to give 97.8 mM FAL
8 within 20 min. After simple pH adjustment with NaOH (2 M) and dilution with KPB
9 (pH 7.0), *E. coli* whole-cells harboring HLADH was added to initiate the bioreaction
10 of prepared FAL liquor without removal of Sn-sepiolite. Bioconversion for another 96
11 h, FAL (75.0 mM) was completely oxidized into FA. Furthermore, the aqueous FA
12 liquor was separated by filtration, followed by extraction with the same volume
13 of ethyl acetate three times. FA was isolated in the yield of 93.5% based on
14 FAL, and its structure was confirmed with HPLC and ^{1}H NMR (300 MHz) (Fig.
15 S5†): δ10.43 (s, 1H), 7.66 (q, 1H, *J*=0.8 Hz), 7.35 (dd, 1H, *J*=3.5 Hz, *J*=0.8 Hz), 6.57
16 (q, 1H, *J*=1.8 Hz).
17 Clearly, one-pot conversion of rice straw into FA via sequential catalysis with
18 Sn-sepiolite at 170 ^{o}C for 20 min and whole-cells harboring HLADH at 30 ^{o}C and pH
19 7.0 was successfully demonstrated. Zhou et al reported that 10 g/L of FAL could be
20 oxidized into FA with 90% yield.[31] To our knowlegement, it was the first report that
21 chemo-enzymatic catalytic synthesis of FA with high yield was derived from
22 renewable biomass in one-pot manner under mild condition.

1 **Recycling of Sn-sepiolite catalyst and immobilized biocatalyst**

2 Development of reusable and sustainable catalyst is of great importance for potential
3 industrial application.[22,45-47] Recycle use of Sn-sepiolite and immobilized *E. coli* TS
4 whole-cells was conducted for converting rice straw in one-pot manner reaction
5 system. In a 100-mL sealed stainless steel reactor, dry rice straw powder (40-60 mesh,
6 3.0 g) was mixed with 40 mL aqueous media containing Sn-sepiolite (3.0 wt%, pH
7 1.0) at 170 ^{o}C for 20 min under the agitation of 500 rpm, and then immobilized
8 whole-cells (wet weight of 13.3 g, which corresponds to 0.68 g dry cell weight) were
9 added into the prepared FAL liquor (pH 7.0) for further bioconversion without
10 removal of Sn-sepiolite. Biotransformation for 96 h at 30 ^{o}C, the FA liquor was
11 collected from Sn-sepiolite and immobilized TS whole-cells by simple filtration. The
12 recoveried immobilized beeds were washed three times with saline (0.8 wt%, NaCl)
13 and reused in the next batch bioconversion of FAL liquor. The solid Sn-sepiolite
14 catalysts and rice straw residues were treated in Muffle furnace to remove biomass
15 and other residues. Recovered Sn-sepiolite catalysts were reused for next batch of
16 FAL synthesis. Recycled Sn-sepiolite catalysts and immobilized whole-cell
17 biocatalysts were attempted to conduct for the next batch of chemo-enzymatic
18 conversion in one-pot reaction system.

19 To test the stability of Sn-sepiolite catalyst, tin-based solid acid Sn-sepiolite was
20 recycled and reused for six times to pretreat rice straw into FAL (Fig. 9a). The FAL
21 yields gradually decreased after each recycle of Sn-sepiolite. After six runs, FAL
22 yield decreased from 42.2% to 34.9%, indicating a comparable stable recycle

capacity. As indicated in Fig. 9b, the immobilized whole-cells showed good recyclability. The prepared FAL liquor obtained from the conversion of biomass via the catalysis with recoveried Sn-sepiolite was chosen as substrate, > 99.9% FA yields for the first 2 cycles and remained at 90.5% of original productivity after 6th bioconversion of FAL liquor. Both solid Sn-sepiolite catalysts and immobilized whole-cell biocatalysts had stable catalytic ability, and an efficient recycling and reuse was developed for the chemo-enzymatic synthesis of FA from rice straw in the one-pot reaction media.

Conclusions

The present study concluded that high FAL yield of 42.2% was achieved from rice straw with acidified Sn-sepiolite (3.0 wt% dosage) in the aqueous media at 170 ^{o}C for 20 min. Moreover, one-pot conversion of alkali pretreatment of dewaxed rice straw to FA at 42.2% yield via tandem catalysis with acidified solid acid Sn-sepiolite and recombinant *E. coli* TS whole cells (or immobilized whole-cells) harboring horse liver alcohol dehydrogenase was developed within 96 h at pH 7.0 and 30 ^{o}C. Clearly, this one-pot strategy provides an effective approach for converting biomass to FA, which has high potential application.

Experimental section

Materials and strains

1 Isopropyl β-*D*-1-thiogalactopyranoside (IPTG, >99%) and kanamycin disulfate salt
2 (>99%) were obtained from Sangon (Shanghai, P.R. China). Tryptone and yeast
3 extract were purchased from OXOID (Shanghai, P.R. China). Primer STAR Max
4 DNA Polymerase and restriction enzyme Dpn I were bought from Takara (Shanghai,
5 P.R. China). T5 Exonuclease was obtained from New England Biolabs (Beverley,
6 MA). Plasmid Miniprep Purification Kit and DNA Clean/Extraction Kit were
7 obtained from Genemark (USA). Oligonucleotide primers synthesizing and DNA
8 sequencing were conducted by Genecreate (Wuhan, P.R. China). Sepiolite, NaCl,
9 K_2HPO_4, KH_2PO_4, $SnCl_4$•$5H_2O$ (≥ 99%), sepiolite, furfural (FAL) and other
10 chemicals were purchased from Sinopharm Group Chemical Reagent Co., Ltd.
11 (Shanghai, P.R. China).

12 *E. coli* DH5a and *E. coli* BL21 (DE3) were used as hosts for gene cloning and
13 protein expression, respectively, and were grown in LB broth or LB agar plate at 37
14 ^{o}C with kanamycin (50 μg/mL). The plasmid pRSFDuet-1 (Novagen, Germany) was
15 used as vector for gene cloning and protein expression.

16 **Construction of recombinant *E. coli* cells harboring Horse Liver Alcohol**
17 **Dehydrogenase**

18 Enzyme gene of Horse Liver Alcohol Dehydrogenase (HLADH) from *Equus caballus*
19 (horse) was synthesized by Genecreate (Wuhan, P.R. China) with codon optimization
20 (Table S1†).

1 The fragment of HLADH and the linear plasmid backbone were amplified by using
2 the synthesized gene HLADH and empty plasmid pRSFDuet-1 as templates,
3 respectively. The primers used with 15 bp homologous ends were shown in Table S2†.
4 The two PCR fragments with homologous ends were ligated to give the plasmid
5 pRSFDuet-HLADH by using T5 exonuclease to promote the efficiency, the resulted
6 pRSFDuet-HLADH was transformed into *E. coli* DH5α (Fig. 1a). Recombinant *E.*
7 *coli* HL was then plated on LB agar plate containing 50 μg/mL kanamycin.

8 HLADH in recombinant *E. coli* HL was further mutated (Fig. 1b). The double
9 mutants I224S and I269S (mutation of isoleucine 224 and isoleucine 269 to serine),
10 mutant K228G (mutation of lysine 228 to glycine), and mutant T178S (mutation of
11 threonine 178 to serine) were constructed according to a two-step PCR strategy. [48]
12 Using pRSFDuet-HLADH as a template, primers with mutation sites were designed,
13 and reverse primers were designed at 150 bp intervals. The polymerase synthesized
14 two long fragments between the two primers, and then two long fragments.
15 Amplification of the entire plasmid template as a primer. The expression proteins of
16 HL, IS, KG, and TS were all 40.0 kDa on SDS-PAGE (Fig. 1c, line 2-5). The target
17 HLADH proteins were verified with HPLC/MS (Fig. S6†). MS Raw data were
18 assayed with Proteome Discoverer software. Data were searched against the
19 UniProtEcoli and horseprotein database.

20 **Preparation of whole-cell catalysts**

1 Recombinant *E. coli* cells were inoculated to 3.0 mL LB (Luria-Bertani) broth (10 g/L
2 tryptone, 5 g/L yeat extract, 5 g/L NaCl) containing 50 μg/mL kanamycin and grown
3 at 37 °C for 6-8 h. The pre-culture (500 μL) was transferred into 50 mL TB (Terrific
4 Broth) medium (4 mL/L glycerol, 12 g/L tryptone, 24 g/L yeast extract, 17 mM
5 KH_2PO_4, and 72 mM K_2HPO_4) containing kanamycin (50 μg/mL). Cells were grown
6 at 37 °C and 220 rpm to OD_{600} about of 0.6~0.8, and then induced by addition of
7 isopropyl β-*D*-thiogalactoside (IPTG) to a concentration of 0.5 mM. The cells
8 continued to grow for another 14-16 h at 25 °C. After cultivation, the cells were
9 harvested by centrifugation (5000 *g*, 4 °C, 10 min), washed twice with potassium
10 phosphate buffer (KPB, 100 mM, pH 7.0) and then used as catalysts in the subsequent
11 biotranformations.

12 **Conversion of biomass to FAL with solid acid Sn-sepiolite**

13 Solid Sn-sepiolite catalyst was prepared with $SnCl_4 \cdot 5H_2O$ (47.0 g) and sepiolite
14 (105.0 g) as previous procedure.[49] Acidified Sn-sepiolite could convert rice straw into
15 FAL.

16 Rice straw (40-60 mesh; 37.8% glucan, 29.2% xylan, 13.6% lignin) was soaked
17 with acetone-ethanol (2:1, v:v) in a Soxhlet apparatus for 6 h and dried in an oven at
18 60 °C for 18 h to obtain dewaxed rice straw. This dewaxed sample (40 g) was soaked
19 with 800 mL NaOH (1.0%) at 60 °C for 4 h. After the spent liquor was cooled to the
20 room temperature, the sediment was separated by filtration and further washed with
21 ethanol-water (7:3, v:v) for three times, and the solid was dried in an oven at 60 °C for

1 18 h to obtain alkali pretreatment of dewaxed rice straw. Silica in biomass was
2 removed after this pretreatment.

3 3.0 g dry pretreated rice straw powder (40–60 mesh) with 2-4 wt% solid acid
4 Sn-sepiolite catalyst and 40 mL water (pH 1.0) was incubated in a 100-mL sealed
5 stainless steel reactor at 160-180 °C for 5-40 min.

6 **Conversion of commercial and biomass-derived FAL to FA**

7 To biotransform FAL liquor derived from rice straw powder (3.0 g, dry weight; 40-60
8 mesh) via catalysis with Sn-sepiolite (3.0 wt%) in a 100-mL sealed stainless steel
9 reactor (500 rpm) containing 40 mL aqueous media at 170 °C and pH 1.0 for 20 min,
10 recombinant *E. coli* whole-cells harboring HLADH (0.1 g/mL, wet weight) were
11 added into this reactor (500 rpm) in the absence and presence of Sn-sepiolite at 25-45
12 °C and pH 6.0-9.0.

13 The immobilized recombinant *E. coli* whole-cells were prepared using the
14 previously reported carrageenan immobilization procedure.[37] 20.0 g dry cells were
15 mixed well with 200 mL phosphate buffer (100 mM, pH 7.0) at 50 °C, and then this
16 mixture was mixed with carrageenan (5.0 wt.%) at 50 °C. The obtained
17 cell/carrageenan solution was then dripped into phosphate buffer (100 mM, pH 7.0) to
18 form beads (～ 2.5 mm diameter). The immobilized beads were further cross-linked
19 with polyethylenimine and glutaraldehyde, producing immobilized whole-cell
20 catalysts. Immobilized whole-cells (wet weight of 13.3 g, which corresponds to 0.68 g

1 dry cell weight) were added to FAL liquor for biotransformation without removal of
2 Sn-sepiolite at 30 ^{o}C and pH 7.0.

3 **Analytical methods**

4 Sn-sepiolite samples were assayed with Fourier transform infrared spectrums (FT-IR),
5 X-ray diffraction (XRD), scanning electron microscope (SEM), and Raman and
6 Brunauer-Emmett-Teller (BET) as previously reported procedures.[48] FOL and FA
7 were assayed with HPLC (Model 2695, Waters Corporation, Milford, MA) equipped
8 with an Waters Nova-Pak column (Parl No.WAT044245), which was eluted with
9 CH_3OH : 0.4 wt% $(NH_4)_2SO_4$ (5 : 95, v:v) and detected at 254 nm. The FAL was
10 determined with HPLC as previously reported methods.[31] All experiments were
11 repeated three times. Error bars indicate the standard error of the mean.

12

13 **Acknowledgements**

14 This work was financially supported by the Natural Science Foundation of China
15 (No. 21978072) and the Advanced Catalysis and Green Manufacturing Collaborative
16 Innovation Center (2018). This work was also supported by the U.S. Department of
17 Energy (DOE), the Office of Energy Efficiency & Renewable Energy (EERE)
18 Awards (DE-EE0007104 and DE-EE0006112),with the Bioproducts, Science &
19 Engineering Laboratory and Department of Biological Systems Engineering at
20 Washington State University.

1 **Conflicts of interest**

2 There are no conflicts of interest to declare.

3

4 **References**

5 [1] Z. Li, X. Guo, X. Feng, C. Li, *Chem. Eng. J.,* 2015, **263**, 249-256.

6 [2] M. Jin, Y. Liu, L. da Costa Sousa, B.E. Dale, V. Balan, *Biotechnol. Bioeng.,*
7 2017, **114**, 1713-1720.

8 [3] J. Xu, B. Wu, L. Hu, Z. Wu, N. Xu, B. Dai, J. He, *Chem. Eng. J.,* 2015,
9 **267**,163-169.

10 [4] L. Zhang, Y. Pu, J. R. Cort,, A. J., Ragauskas, B. Yang, *ACS Sustain. Chem.*
11 *Eng*., 2016, **4**, 6618-6628.

12 [5] L. Liu, H. M. Chang, H. Jameel, S. Park, *Bioresour. Technol*., 2018, **252**,
13 165-171.

14 [6] F. Li, M. Zhang, K. Guo, Z. Hu, R. Zhang, Y. Feng, X. Yi, W. Zou, L. Wang, C.
15 Wu, J. Tian, T. Lu, G. Xie, L. Peng, *Plant Biotechnol. J.,* 2015, **13**, 514-525.

16 [7] Z. Dan Sun, Y. Li, J. Wang, Y. Tu, Y. Wang, Z. Hu, S. Zhou, L. Wang, G. Xie, J.
17 Huang, A. Alam, L. Peng, *Bioresour. Technol.,* 2017, **243**, 957-965.

18 [8] S. M. Bruce, Z. W. Zong, A. Chatzidimitriou, L. E. Avci, J. Q. Bond, M. A.
19 Carreon, S. G. Wettstein, *J. Mol. Catal. A-Chem*., 2016, **422**, 18-22.

20 [9] R. Karinen, K. Vilonen, M. Niemelä, *ChemSusChem*, 2011, **4**, 1002-1016.

21 [10] J. N. Chheda, Y. Román-Leshkov, J. A. Dumesic, *Green Chem.*, 2007, **9**,
22 342-350.

23 [11] H. Yuan, J. Li, H. Shin, G. Du, J. Chen, Z. Shi, L. Liu, *Bioresour. Technol.,*
24 2018, **247**, 1184-1188.

1 [12] H. Chen, H. Ruan, X. Lu, J. Fu, T. Langrish, X, Lu, *Chem. Eng. J.*, 2018, **333**,
2 434-442.

3 [13] L. Xian, Z. Li, A. X. Tang, Y. M. Qin, Q. Y. Li, H. B. Liu, Y. Y. Liu, *Bioresour.*
4 *Technol.,* 2019, **285**, 121293.
5 [14] X. Li, K. Peng, Q. Xia, X. Liu, Y. Wang, *Chem. Eng. J.*, 2018, **332**, 528-536.
6 [15] M. Jin, C. Gunawan, V. Balan, M. Lau, B. E. Dale, *Bioresour. Technol.,* 2012,
7 **20**, 587-594.
8 [16] S. J. You, E. D. Park, M. J. Park, *Korean J. Chem. Eng.,* 2016, **33**, 806-811.
9 [17] W. Xing, G. Xu, J. Dong, R. Han, Y. Ni, *Chem. Eng. J.,* 2018, 333, 712-720.

10 [18] R. Chandra, H. Takeuchi, T. Hasegawa, *Appl. Energy,* 2012, 94, 129-140.

11 [19] H. Zhang, X. Zhao, X. Ding, H. Lei, X. Chen, D. An, Y. Li, Z. Wang, Bioresour.
12 *Technol*., 2010, 101, 1263-1267.

13 [20] J. Umeda, K. Kondoh, *Ind. Crop Prod.,* 2010, 32, 539-544.

14 [21] N. S. Kumar, V. C. Srivastava, S. Basu, *Indian Chem. Eng.,* 2013, **55**, 153-164.

15 [22] X. X. Xue, J. H. Di, Y. C. He, B. Q. Wang, C. L. Ma, *Appl. Biochem.*
16 *Biotechnol.,* 2018, **185**, 42-54.

17 [23] Z. Ma, X. Wu, Z. Si, D. Weng, *Appl. Catal. B: Enviorn.,* 2015, **197**, 380-394.

18 [24] T. Zhang, W. Li, S. An, F. Huang, Q. Liu, *Bioresour. Technol.,* 2018, **264**,
19 261-267.
20 [25] H. Zhou, Y. Sha, Z. Xiao, L. Li, J. Hao, K. Yang, N. Li, R. He, Q. Liu, *Int. J.*
21 *Coal Sci. Technol.,* 2018, **5**, 464-472.
22 [26] C. Wu, W. Yuan, Y. Huang, Y. Xia, H. Yang, H. Wang, X. Liu, *Catal. Lett.,*
23 2017, **147**, 953-963.
24 [27] J. M. R. Gallo, D. M.Alonso, M. A. Mellmer, J. H. Yeap, H. C. Wong, J. A.
25 Dumesic, *Top. Catal.,* 2013, **56**, 1775-1781.

1 [28] N. K. Gupta, A. Fukuoka, K. Nakajima, *ACS Sustainable Chem. Eng.*, 2018, **6**,
2 3434-3442.

3 [29] P. Verdeguer, N. Merat, A. Gaset, *Appl. Catal. A: Gen.,* 1994, **112**, 1-11.

4 [30] K. Mitsukura, Y. Sato, T. Yoshida, T. Nagasawa, *Biotechnol. Lett.* 2004, **26**,
5 1643-1648.

6 [31] X. Zhou, X. Zhou, Y. Xu, R. R. Chen, *J. Chem. Technol. Biotechnol.,* 2017, **92**,
7 1285-1289

8 [32] J. J. Dong, R. Z. Han, G. C. Xu, L. Gong, W. R. Xing, Y. Ni, *Bioresour.*
9 *Technol.,* 2018, **259**, 40-45.

10 [33] D. H. Park, B. V. J. Plapp, *J. Biol. Chem.,* 1991, **266**, 13296-13302.

11 [34] H. Jia, M. Zong, H. Yu, N. Li, *ChemSusChem,* 2017, **10**, 3524-3528.

12 [35] C. Su, W. Li, M. Chen, Z. Huang, L. Wu, *Bioresour. Technol.,* 2016, **200**,
13 1065-1072.

14 [36] H. Zhou, Y. Sha, Z. Xiao, L. Li, J. Hao, K. Yang, N. Li, R. He, Q. Liu, *Int. J.*
15 *Coal Sci. Technol.,* 2018, **5**, 464-472.

16 [37] Y. He, J. Xu, J. Su, L. Zhou, *Appl. Biochem. Biotechnol.,* 2010, **160**, 1428-1440.

17 [38] X. J. Zhang, H. Z. Deng, N. Liu, Y. C. Gong, Z. Q. Liu, Y. G. Zheng, *Bioresour.*
18 *Technol.,* 2019, **276**, 154-160.

19 [39] Z. H. Xu, A. D. Cheng, X. P. Xing, M. H. Zong, N. Li, *Bioresour. Technol.,*
20 2018, **262**, 177-183.

21 [40] L. Yan, R. Ma, H. Wei, L. Li, B. Zou, Y. Xu, *Bioresour. Technol.,* 2019, **279**,
22 84-91.

23 [41] L. Hu, T. Li, J. Xu, A, He, X, Tang, X. Chu, J. Xu, *Chem. Eng. J.,* 2018, **352**,
24 110-119.

25 [42] Y. M. Li, X. Y. Zhang, N. Li, P. Xu, W. Y. Lou, M. H. Zong, *ChemSusChem*,
26 2017, **10**, 372-378.

1 [43] K. Fulajtárova, T. Soták, M. Hronec, I. Vávra, E. Dobrocka, M. Omastová,
2 *Appl. Catal. A-Gen*, 2015, **502**, 78-85.

3 [44] C. Perego, D. Bianchi, Biomass upgrading through acid–base catalysis, *Chem.*
4 *Eng. J.*, 2010, **161**, 314-322.

5 [45] N. Miletić, A. Nastasović, K. Loos, *Bioresour. Technol.,* 2012, **115**, 126-135.

6 [46] G. W. Zheng, J. H. Xu, *Curr. Opin. Biotechnol.,*2011, **22**, 784–-92.

7 [47] M. Zhou, X. Ju, X., L.Z. Li, L. Yan, X. Xu, J. Chen, *Appl. Microb. Biotechnol.,*
8 2019, **103**, 2483-2492.

9 [48] A. Li, C. Acevedo-Rocha, M. Reetz, *Appl. Microb. Biotechnol.*, 2018, **201**,
10 6095-6103.

11 [49] Y. He, C. Jiang, J. Jiang, J. Di, F. Liu, Y. Ding, Q. Qing, C. Ma, *Bioresour.*
12 *Technol.,* 2017, **238,** 698-705.

1 # Figure Captions

2 **Fig. 1.** Construction procedure of the pRSFDUET-HLADH [The
3 pRSFDUET-HLADH was obtained by T5 exonuclease, the T5 exonuclease cleaves
4 the DNA base from the 5' end to the 3' end, revealing the single-stranded DNA
5 homology arm, and complementing the HLADH by the intracellular ligase of
6 *Escherichia coli* DH5α. Homologous arm connection, resulting in
7 DH5α-pRSFDUET-HLADH] (a); The mutation of pRSFDUET-HLADH [The
8 process of mutation Primers with mutation sites were designed. Using
9 pRSFDUET-HLADH as a template, a pair of long primers were formed by DNA
10 polymerase (Primer star Mix), and a long primer was used as a new primer to amplify
11 the mutant dsDNA, and then digested with *Dpn* I to get the mutant plasmid] (b);
12 Selected SDS-PAGE analysis for *E. coli* (HL, TS, IS, KG) . Lane M: protein standard
13 markers; lane 1: *E. coli* HL expressing HLADH only; lane 2: *E. coli* TS expressing
14 T178S only; Lane 3: *E. coli* IS expressing I224S and I269S only. Lane 4: *E. coli* KG
15 expressing K228G only (c).

16

17 **Fig. 2.** Scheme for one-pot conversion of rice straw to FA via sequential catalysis
18 with solid acid Sn-sepiolite catalyst and HLADH whole-cell biocatalyst.

19

20 **Fig. 3.** SEM (A), FT-IR (B), XRD (C) and Raman (D) images of sepiolite (a) and
21 Sn-sepiolite (b).

22

23 **Fig. 4.** Effects of catalyst Sn-sepiolite loading (a), reaction temperature and reaction
24 time (b) on the FAL yield. [Conditions: 3 g alkali pretreatment of dewaxed rice straw
25 was mixed with a certain amount of Sn-sepiolite (2.0-4.0 wt%) in a sealed stainless
26 steel reactor at 170 ^{o}C for 20 min (a); 3 g alkali pretreatment of dewaxed rice straw
27 was mixed with a certain amount of Sn-sepiolite (3.6 wt%) in a sealed stainless steel
28 reactor at 160-180 ^{o}C for 5-40 min (b)].

29

30 **Fig. 5.** Effects of various reaction pH on biocatalytic activity of *E. coli* TS whole-cells
31 (a); Effects of various reaction temperature on biocatalytic activity of *E. coli* TS
32 whole-cells (b); Effects of pH stability on biocatalytic activity of *E. coli* TS
33 whole-cells (c); Effects of thermostability on biocatalytic activity of *E. coli* TS
34 whole-cells (d).

35

1 **Fig. 6.** Effects of various metal ion additives on biocatalytic activity of *E. coli* TS
2 whole-cells (a); Effects of Zn^{2+} loadings on biocatalytic activity of *E. coli* TS
3 whole-cells (b); Effects of Sn-sepiolite loadings on the biocatalytic activity of *E. coli*
4 TS whole-cells (c).

5

6 **Fig. 7.** Effects of various FAL loadings (a), FOL loadings (b), and FA loadings (c) on
7 biocatalytic activity of *E. coli* TS whole-cells.

8

9 **Fig. 8.** Time courses for the biological conversion of rice straw-derived FAL with *E.*
10 *coli* TS whole-cell biocatalysts.

11

12 **Fig. 9.** Recycling and reusing of solid Sn-sepiolite catalyst (a) and immobilized *E.*
13 *coli* TS whole-cell biocatalysts (b).

14

15
Table Captions

16

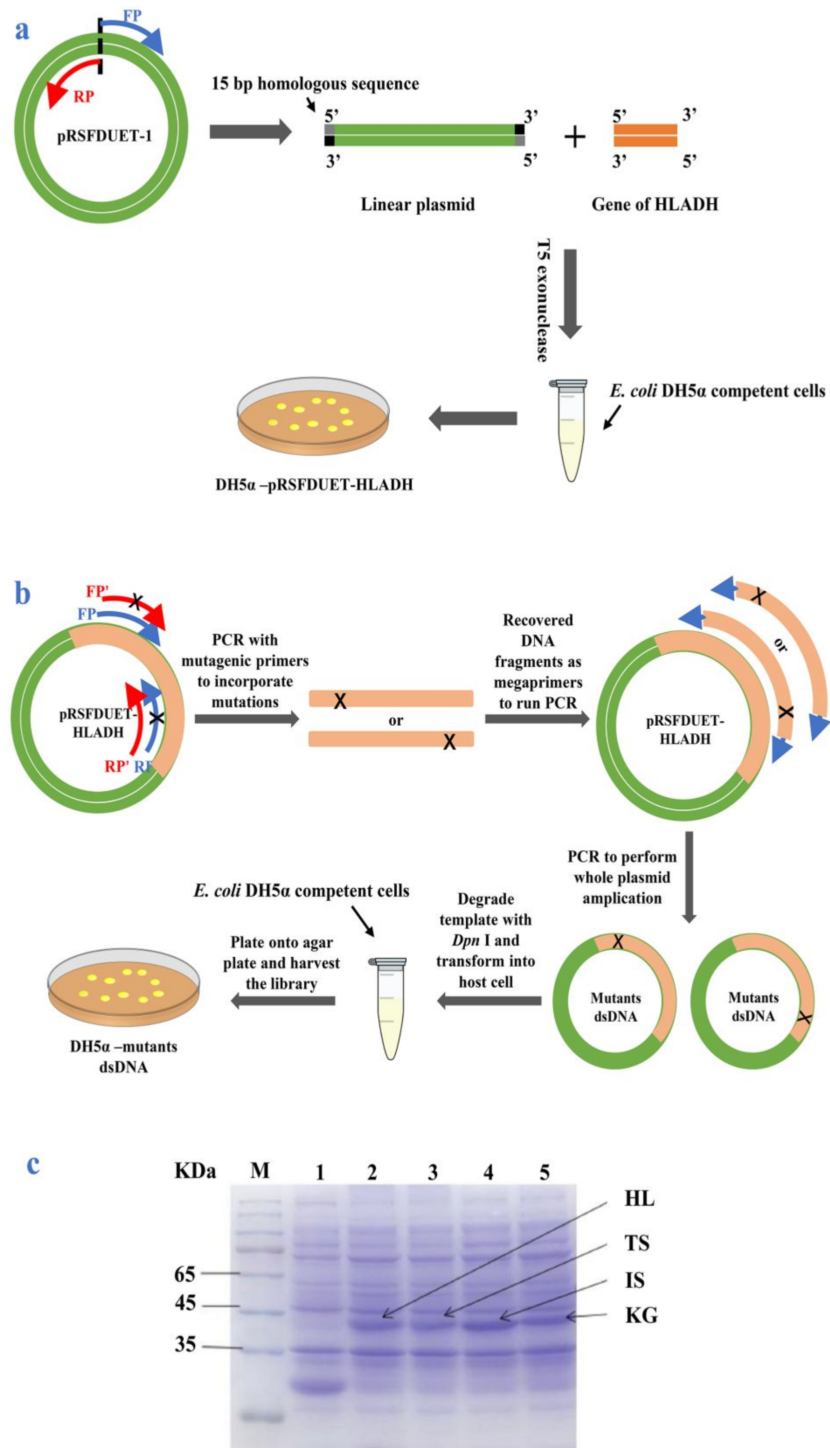
17 **Table 1.** Characterizations of Sn-sepiolite and fresh sepiolite.

18

19

1

2



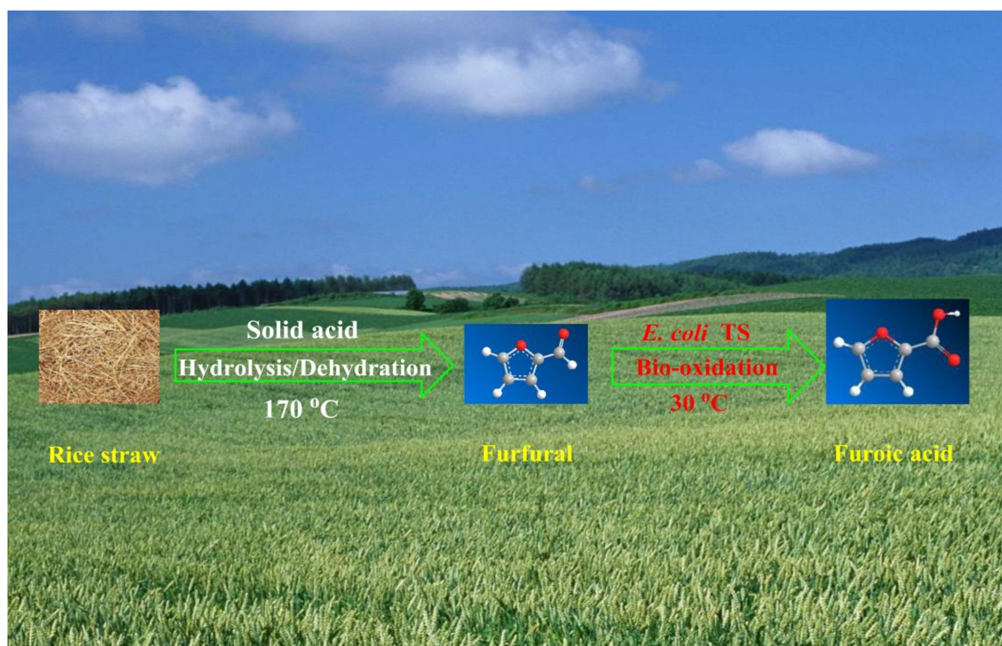
3

4

Fig. 1.

1

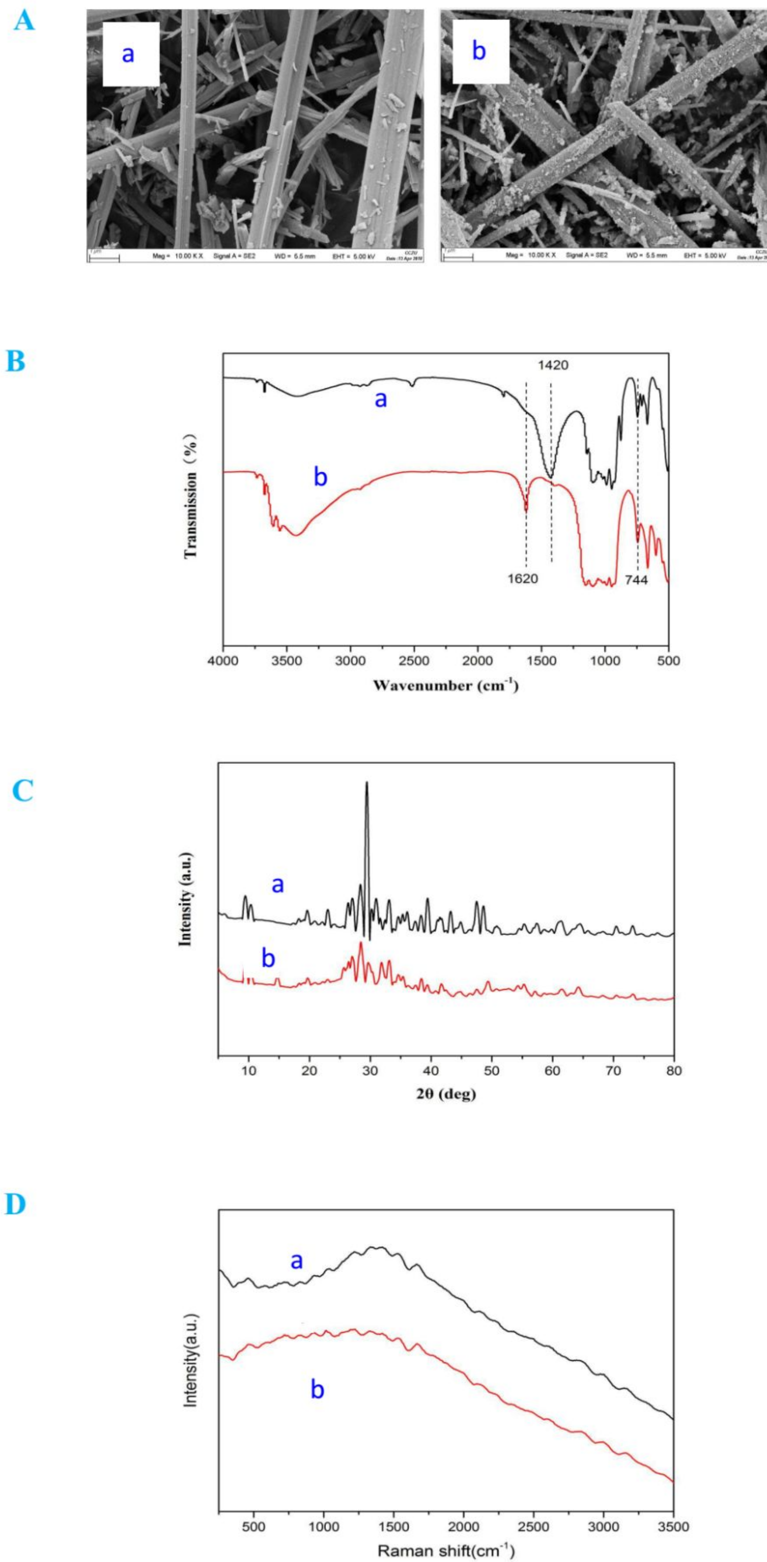
2



3

4 **Fig. 2.**

5



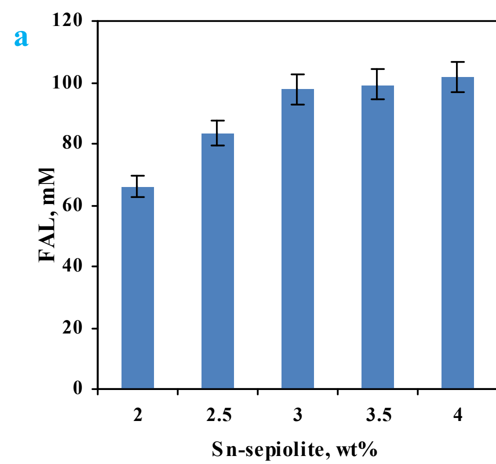
1

2 **Fig. 3.**

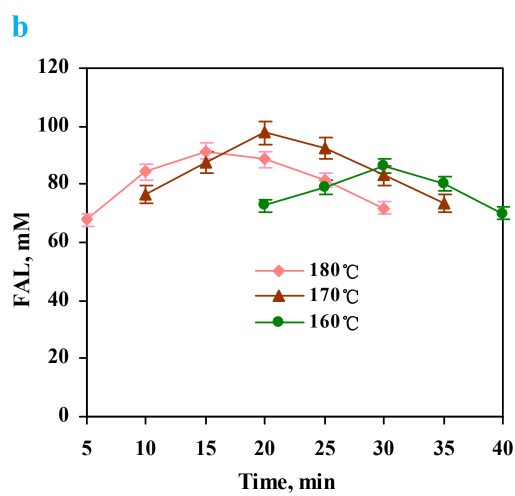
3

1

2



3

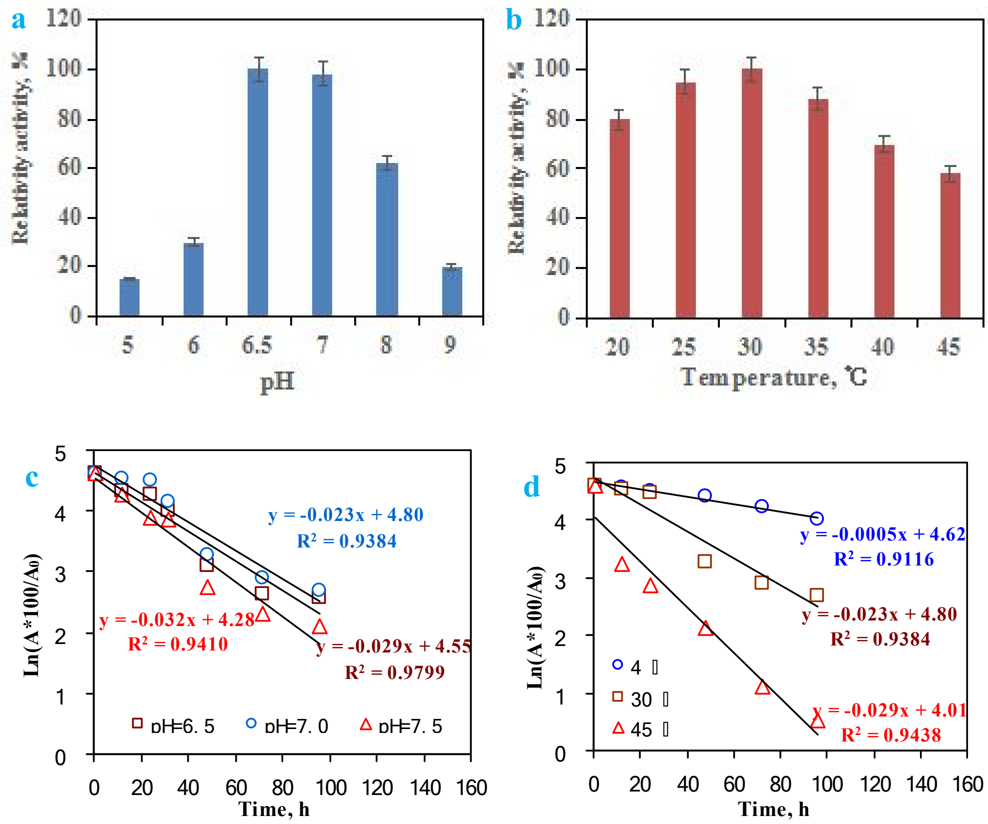


4

5 **Fig. 4.**

1

2

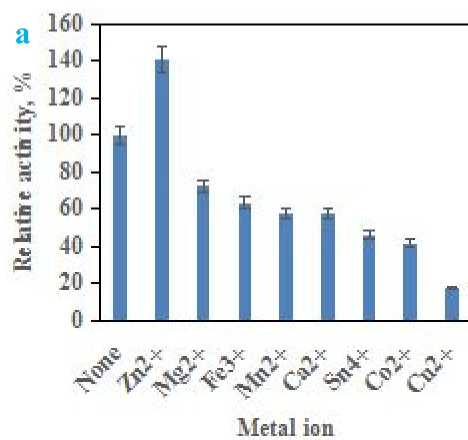


3

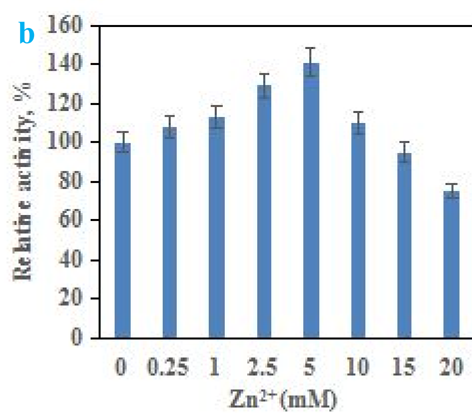
4

5 **Fig. 5.**

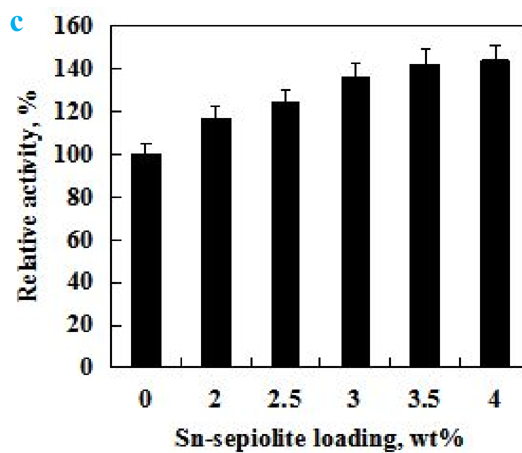
1



2

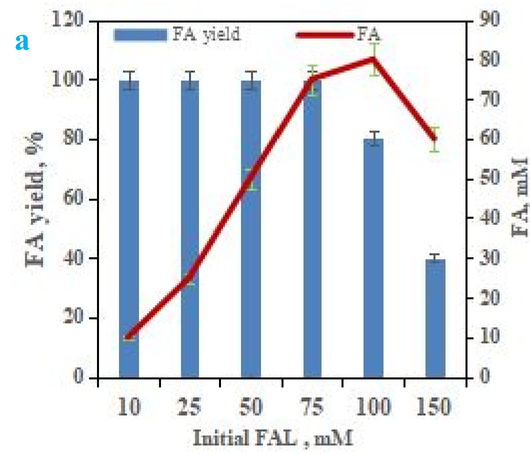


3

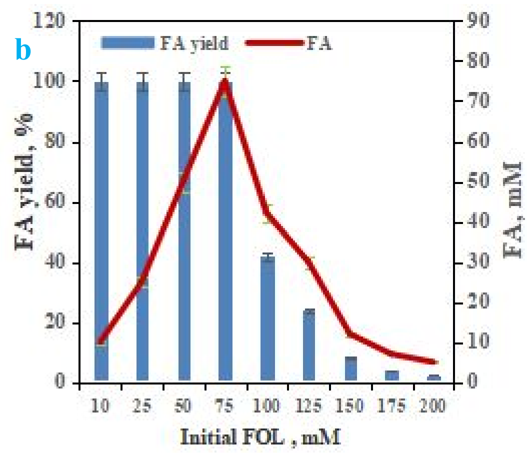


4

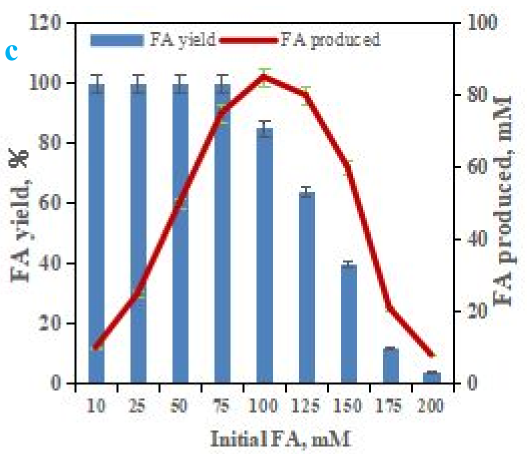
5 **Fig. 6.**



1



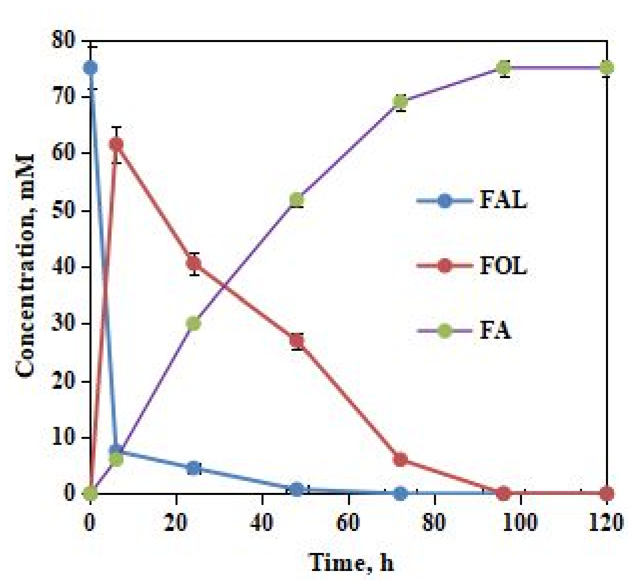
2



3

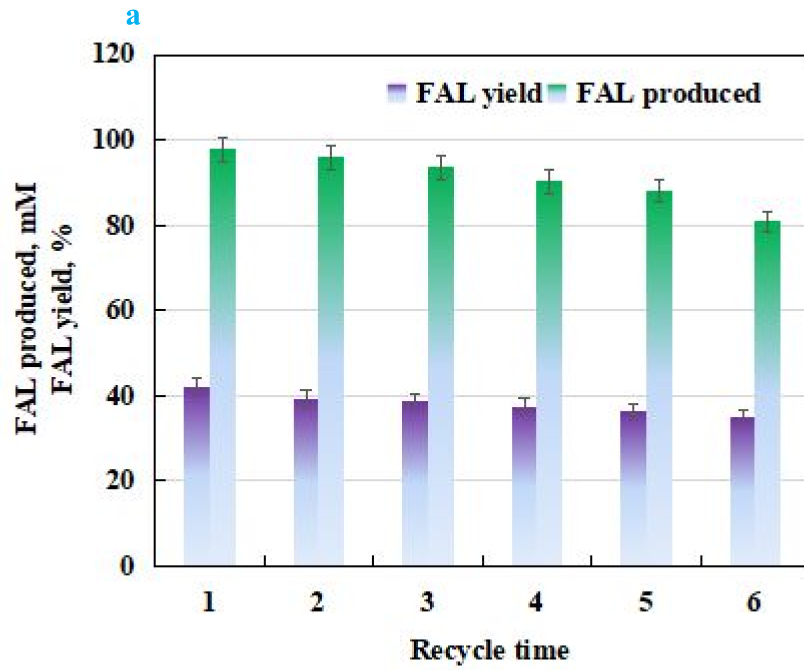
4 **Fig. 7.**

1

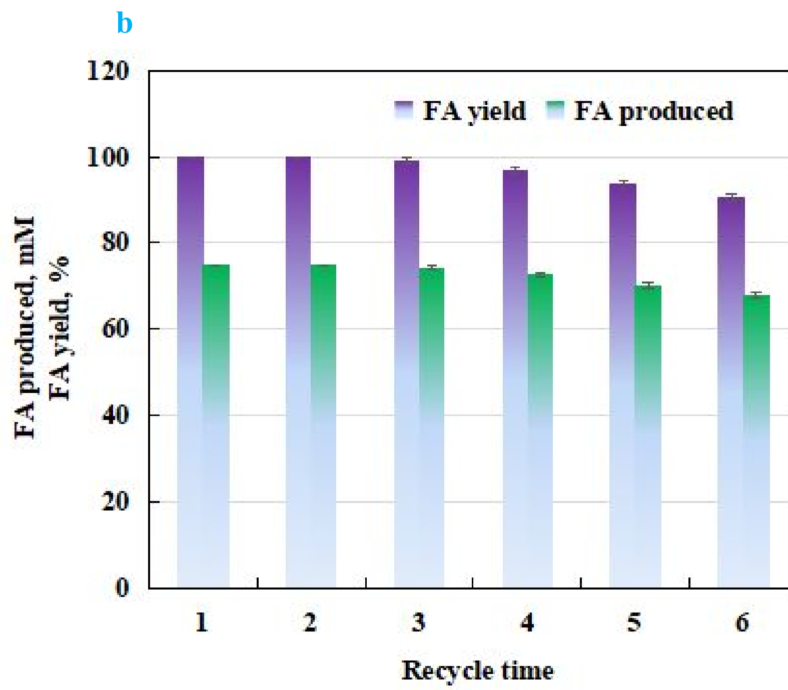


2

3 **Fig. 8.**



1



2

3 **Fig. 9.**

1

2

3 **Table 1.** Characterizations of Sn-sepiolite and fresh sepiolite.

Sample	BET surface area, m^2/g	Pore volume, cm^3/g	Pore size, nm
Fresh sepiolite	4.2 ± 0.9	0.01 ± 0.003	12.6 ± 0.6
Sn-sepiolite	45.9 ± 4.7	0.04 ± 0.007	3.5 ± 0.3

4