Production of Adipic Acid from Sugar Beet Residue by Combined Biological and Chemical Catalysis

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Adipic acid is one of the most important industrial dicarboxylic acids and is used mainly as a precursor to nylon-6,6. Currently, commercial adipic acid is produced primarily from benzene by a chemical route that is associated with environmental, health, and safety concerns. Herein, we report a new process to produce adipic acid from an inexpensive renewable feedstock, sugar beet residue by combining an engineered *Escherichia coli* strain and Re-based chemical catalysts. The engineered *E. coli* converted *p*-galacturonic acid to mucic acid, which was precipitated easily with acid, and the mucic acid was further converted to adipic acid by a deoxydehydration reaction catalyzed by an oxorhenium complex followed by a Pt/C-catalyzed hydrogenation reaction under mild conditions. A high selectivity to the free acid products was achieved by tuning the acidity of the Re-based catalysts. Finally, adipic acid was produced directly from sugar beet residue that was hydrolyzed enzymatically with engineered *E. coli* and two chemical catalysts in a yield of 8.4%, which signifies a new route for the production of adipic acid.

Adipic acid, the precursor of nylon-6,6, is one of the most important dicarboxylic acids in the chemical industry and has a market volume of 2.8 million ton per year\(^1\) with a growth rate of 3–5% per year. Commercial adipic acid is produced mainly from benzene-based petrochemical reactions, which generate huge amounts of the greenhouse gas nitrous oxide.\(^2\) To overcome this key drawback, many efforts have been made to produce adipic acid by more sustainable and environmentally friendly approaches.\(^3\) For example, researchers at Rennova developed a chemical route that comprised two heterogeneous catalytic steps for adipic acid production from glucose. Specifically, glucose was aerobically oxidized to gluconic acid that was in turn converted into adipic acid by hydrodeoxygenation with maximum yields of 66 and 89%, respectively.\(^4\) However, this chemical route was limited by the need for high temperature and pressure and the use of a supporting solvent. Scientists at Verdezyne genetically engineered yeast strains isolated from petroleum-contaminated soils to produce up to 50 g L\(^{-1}\) adipic acid from fatty acids derived from vegetable oil.\(^5\) In addition, Frost and co-workers designed and constructed a three-step biosynthetic pathway in which an *E. coli* mutant that lacked shikimate dehydrogenase converted 3-deoxyshikimic acid (DHS) to cis,cis-muconic acid.\(^6\) Up to 36.8 g L\(^{-1}\) cis,cis-muconic acid was produced from batch-fed fermentation in 22 mol% yield from glucose. Subsequently, cis,cis-muconic acid was hydrogenated chemically to adipic acid in a yield of 97%. Beckham and co-workers engineered *Pseudomonas putida* KT2440 to produce 13.5 g L\(^{-1}\) cis,cis-muconic acid from *p*-coumarate, which can be derived from lignin, in a yield of 67% that was then converted chemically to adipic acid with 97% conversion.\(^7\) Qian and co-workers designed a recombinant *E. coli* for a six-step pathway to reverse adipic acid degradation, which produced 639 \(\mu\)g L\(^{-1}\) of adipic acid directly from 10 g L\(^{-1}\) glucose.\(^8\) These biological routes used either glucose or fatty acids as substrates and in most cases showed low titers and yields.

Sugar beet residue is an abundant (~25 million ton yr\(^{-1}\)) and low-value byproduct of the beet-processing industry, which contains approximately 24% pectin, 24% cellulose, and 21% arabinan.\(^9\) *p*-Galacturonic acid, the principal monomer of pectin, has been converted into potential platform chemicals, which include meso-galactarate,\(^10\) keto-deoxy-L-galactonate,\(^11\) and L-galactonic acid\(^12\) by using engineered filamentous fungi. Recently, a consolidated bioprocess was developed to generate L-galactonic acid directly from orange peel.\(^13\) In this work, we developed a new route to produce adipic acid from sugar beet residue by combining biological catalysis and chemical catalysis (Scheme 1). Inspired by recent studies on the exploration of the synergy between biocatalysis and chemical catalysis (Scheme 1), we designed a new route to produce adipic acid from sugar beet residue by combining biological catalysis and chemical catalysis (Scheme 1).
Pseudomonas aeruginosa (Udh), which was identified in two 2016 lysates able to transform mucic acid (also known as meso-galactaric acid) to adipic acid,[15] we designed and constructed a whole-cell biocatalyst to convert d-galacturonic acid released from sugar beet residue into mucic acid. In addition, we developed and screened chemical catalysts that could catalyze the production of free adipic acid from a highly selective deoxydehydration (DODH) process using mucic acid as the substrate via muconic acid as the intermediate. The combination of these two types of catalysts yielded a green route to adipic acid from renewable biomass.

Development of recombinant E. coli-based biocatalyst

There are three distinct catabolic pathways for d-galacturonic acid in microorganisms.[16] In the oxidation pathway, d-galacturonic acid is oxidized to mucic acid by uronate dehydrogenase (Udh), which was identified in two Pseudomonas strains and Agrobacterium tumeifaciens.[17] In addition, Udh is able to convert d-glucuronic acid to d-glucaric acid.[18] Recently, three additional UdhS that could serve as alternative enzymes in our whole-cell biocatalyst were characterized.[19] We chose the Udh enzyme from A. tumefaciens because of its high activity towards d-galacturonic acid (the Michaelis–Menten constants for d-galacturonic acid are \( K_m = 0.5 \, \text{mM} \), \( V_{max} = 124 \, \text{U mg}^{-1} \)).[20] The overexpression of A. tumeifaciens udh directed by a T7-promoter-based pET system in E. coli BL21(DE3) was induced by 0.2 mM isopropyl \( \beta \)-D-thiogalactopyranoside (IPTG) and confirmed by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Figure S1). After induction, cells were collected by centrifugation and used as a whole-cell catalyst to convert 5, 10, and 20 g L\(^{-1}\) pure d-galacturonic acid into 4.3, 8.3, and 11.5 g L\(^{-1}\) mucic acid, respectively, in 48 h. These yields are much higher than that of the fungal strain Hypocrea jecorina QM6a \( \Delta \text{gar1} \, \text{udh} \) engineered previously, which produced 3.8 g L\(^{-1}\) mucic acid from 17.4 g L\(^{-1}\) d-galacturonic acid in 211 h at pH 5.0.[10] The titer of mucic acid in the engineered H. jecorina QM6a \( \Delta \text{gar1} \, \text{udh} \) was increased from 3.8 to 4.2 g L\(^{-1}\) after the addition of 2 g L\(^{-1}\) glucose to the reaction medium. An antagonistic effect was observed in our engineered E. coli strain. In theory, 1.08 g L\(^{-1}\) mucic acid should be produced from 1 g L\(^{-1}\) d-galacturonic acid if d-galacturonic acid was oxidized completely into mucic acid. We speculated that the yields we obtained were lower than the theoretical values were likely because of the native catabolism of d-galacturonic acid and mucic acid in E. coli in which d-galacturonic acid is isomerized to d-tagaturonate by uronate isomerase and mucic acid is catalyzed by d-galactarate dehydrogenase into 5-dehydro-4-deoxy-d-glucarate. Another reason might be that Udh is a NAD\(^{+}\)-dependent dehydrogenase, and the cellular NAD\(^{+}\) pool in the resting cells used might be insufficient to drive the reaction to completion.

To further improve mucic acid production from d-galacturonic acid, two endogenous genes, \( \text{uxaC} \) and \( \Delta \text{garD} \), which encode uronate isomerase, and \( \text{garD} \), which encodes d-galactarate dehydrogenase, were deleted from the E. coli BL21(DE3) genome by the lambda red-mediated gene disruption method[21] to yield E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \) strain. The primary monomers of sugar beet residue are glucose, L-arabinose, and d-galacturonic acid in almost equal dry weight ratio.[22] We mimicked this composition by using a mixture of 10 g L\(^{-1}\) glucose, 10 g L\(^{-1}\) L-arabinose, and 10 g L\(^{-1}\) d-galacturonic acid as the substrate to investigate the effect of \( \text{uxaC} \) and \( \text{garD} \) deletion on mucic acid production. E. coli BL21(DE3) \( \text{udh} \) (the E. coli BL21(DE3) strain that overexpresses A. tumeifaciens \( \text{udh} \)) and E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \, \text{udh} \) (the E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \) strain that overexpresses A. tumeifaciens \( \text{udh} \)) were cultured to prepare whole-cell biocatalysts. In 48 h, E. coli BL21(DE3) \( \text{udh} \) converted 68.5% of the d-galacturonic acid to mucic acid with a titer of 7.4 g L\(^{-1}\), whereas E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \, \text{udh} \) converted 95.4% of the d-galacturonic acid to mucic acid with a titer of 10.3 g L\(^{-1}\) (Figure 1). If we take the presence of glucose and L-arabinose into consideration, the yields of mucic acid were 22.8 and 31.8% for E. coli BL21(DE3) \( \text{udh} \) and E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \, \text{udh} \), respectively. This result demonstrated clearly that blocking the native catabolism of d-galacturonic acid and mucic acid in E. coli improved mucic acid pro-

![Scheme 1. Schematic representation of the green route for adipic acid synthesis from sugar beet residue by combining biological catalysis and chemical catalysis.](attachment:scheme_1.png)

![Figure 1. Effects of \( \text{uxaC} \) and \( \text{garD} \) deletions on mucic acid production. The substrates are 10 g L\(^{-1}\) d-galacturonic acid, 10 g L\(^{-1}\) glucose, and 10 g L\(^{-1}\) L-arabinose in 280 mM M9 medium. Resting cells with OD600 = 20 were used, and the pH was adjusted to neutral after each sampling point. WT is E. coli BL21(DE3) \( \text{udh} \) strain, and \( \Delta \text{uxaC} \, \Delta \text{garD} \) is E. coli BL21(DE3) \( \Delta \text{uxaC} \, \Delta \text{garD} \, \text{udh} \) strain.](attachment:figure_1.png)
duction and channeled almost all of the d-galacturonic acid to mucic acid. d-Galacturonic acid was consumed completely in both of the whole-cell reactions (Figure 1), which indicates that d-galacturonic acid entered its isomerization pathway in wild-type E. coli. In pH-controlled bioreactors, H. jecorina QM6a Δgar1 udh produced 5.9 g L⁻¹ of mucic acid at pH 6.5 and 2.6 g L⁻¹ of mucic acid at pH 4.0 from 9 g L⁻¹ d-galacturonic acid and 4 g L⁻¹ glucose.²⁰ We found a similar pH-dependent production in E. coli BL21(DE3) ΔuxaC ΔgarD udh, in which the mucic acid titer decreased if the reaction medium was acidified (Figure S2). This phenomenon could be explained by the characteristics of A. tumefaciens Udh, the optimal pH and temperature of which are 8.0 and 35 °C, respectively.²¹ Different from d-galacturonic acid, glucose and l-arabinose were both consumed during the biotransformation reactions (Figure S3).

Development of the Re-based chemical catalyst

In our DODH reaction of mucic acid (1) reported previously, only muconic acid esters (monoester 2 and diester 3) were produced with pure methyltrioxorhenium (MTO) catalyst (Scheme 2a),²¹a but from a chemical synthesis point-of-view, the free acid product is more desirable and cost efficient than the ester form. It was clear that the MTO-catalyzed DODH reaction and acidic Re-catalyzed esterification reaction are parallel and competitive, which indicates that the free acid selectivity of the DODH reaction could be improved by tuning the acidity of the Re-based catalysts. To identify cheaper and more convenient catalysts for the DODH reaction, several less acidic Re compounds were screened (Table S1).²⁰ If NH₄ReO₄ was employed for the DODH of 1 (Scheme 2), 82% yield of free muconic acid (4) and 16% yield of muconic acid monopentyl ester (2) were produced. The selectivity for the production of free muconic acid was much higher than 43% selectivity with MTO (5 mol%) at 150 °C and no selectivity with HReO₄ reported previously.²² Our result also demonstrated that the selectivity of the Re-catalyzed DODH of 1 to 4 or 2 was sensitive to the format of catalyst and reaction conditions. As a result of their different polarities, 4 and 2 were separated easily by extraction to give isolated yields of 79 and 16%, respectively. Kinetic studies of the DODH of 1 catalyzed by NH₄ReO₄ revealed that more than 65% of 1 was converted in the first 8 h and the selectivity to 4 was as high as 98% (Figure 2). After 8 h, the reaction proceeded at a slower rate and the total yields of 4 and 2 increased gradually, but with more 2 in the products. Thus, it is possible to obtain 4 in high selectivity by using NH₄ReO₄ as the catalyst and controlling the reaction time. So far, it has been demonstrated that by selection of the NH₄ReO₄ catalysts, free acid products could be achieved from sugar acids at high selectivity.

After we had achieved the high selectivity to 4 with NH₄ReO₄, further hydrogenation reactions towards adipic acid (5) was investigated. The NH₄ReO₄-catalyzed DODH reaction of 1 was terminated after 8 h. Compound 4 was isolated in 72% yield, and 20% of unreacted 1 was recovered by filtration (Scheme 2c). Thus, based on the converted 1, 4 was isolated in

Figure 2. Time course of the NH₄ReO₄-catalyzed DODH reactions. Reaction conditions: mucic acid (210.0 mg, 1.0 mmol), NH₄ReO₄ (13.4 mg, 0.05 mmol), 3-pentanol (20.0 mL), 120 °C, flowing N₂. Yield and conversion were determined by 1H NMR spectroscopy with an internal standard.

Scheme 2. DODH of mucic acid over a) MTO and b) NH₄ReO₄ and c) the highly selective procedure for the transformation of mucic acid to adipic acid.
90% yield. The hydrogenation of 4 was performed under mild conditions (room temperature, H2, 7 bar), and 92% isolated yield of 5 was obtained in 8 h. Notably, the hydrogenation reaction was conducted in water instead of organic solvent as reported previously.23 Despite the poor solubility of 4 in water, the hydrogenation reaction could still proceed as 5 dissolves well in water.

Production of adipic acid from mock substrate

To develop an integrated process that combines the engineered recombinant E. coli-based biocatalyst and the NH4ReO4 chemical catalyst, we sought to produce 5 from mock monosugar mixtures that mimic the composition of sugar beet residue. The composition of the sugar beet residue was first analyzed by diluted acid hydrolysis and found to comprise 22% glucose, 21% l-arabinose, and d-galacturonic acid mixed equally. The E. coli BL21(DE3) ΔuxaC ΔgarD udh strain was used to catalyze the conversion of d-galacturonic acid to 1 with 50 mL reaction volume in a 250 mL shake-flask. In 48 h, more than 99% of d-galacturonic acid in the mixture was converted to 1 (Figure S4), and unsurprisingly, glucose and l-arabinose were both consumed. Compared to test tubes, the relatively high yield of 1 here might be because of the better oxygen transfer in the shake-flask. Compound 1 was then recovered directly from the reaction supernatant by acid precipitation with a recovery yield of 91%. The resulting 1 was very pure (confirmed by NMR spectroscopy) and was then subjected to the Re-catalyzed DODH reaction to give the same high yield of 4 and 2 (99%). If we consider that we fed the cells with an equal mixture of glucose, l-arabinose, and d-galacturonic acid and the consecutive steps of the biological and chemical transformations, the overall yield of 4 and 2 here was 29.7%.

Production of adipic acid from sugar beet residue

Next we extended our studies to the real-world substrate, sugar beet residue. The composition of the sugar beet residue was first analyzed by diluted acid hydrolysis and found to comprise 22% glucose, 21% l-arabinose, and 21% d-galacturonic acid by dry weight, which was close to reported values. The composition of the sugar beet residue was produced from 7.8 g of sugar beet residue was 8.4%. The complex composition of sugar beet residue, the inefficient release of d-galacturonic acid, the reduced d-galacturonic acid conversion, and the lower purity of 1 accounted collectively for the decreased yield of 5 compared to that from the reaction of the mock substrate.

In conclusion, we developed a new route for the production of adipic acid from sugar beet residue. This route is a promising alternative for the valorization of beet residue and it could be extended to other pectin-rich biomass such as citrus peel. In this route, d-galacturonic acid was first released from beet residue through an enzymatic hydrolysis reaction and then converted to mucic acid by the engineered recombinant E. coli strain. The yield of mucic acid could be further improved by two approaches: the improvement of the enzymatic hydrolysis efficiency to release more d-galacturonic acid from sugar beet residue and the improvement of the specific activity of the uronate dehydrogenase by either protein engineering or bio-prospecting. Moreover, to better valorize the sugar beet residue, l-arabinose could also be converted to useful chemicals, such as rare sugar L-ribulose. The feasibility has been demonstrated by our preliminary results, but further optimizations are needed to find compatible reaction conditions for both d-galacturonic acid and L-arabinose conversion. The bioproduced mucic acid was precipitated easily and used for chemical conversion and its purity and reaction activity were comparable to that obtained commercially. Mucic acid was converted to adipic acid by a deoxydehydration reaction catalyzed by an ox-
orhenium complex reaction followed by a Pt/C-catalyzed hydride reduction reaction under mild conditions. The acidity of the orhenium catalysts determined the selectivity of the free acid products in the deoxydehydration reactions. We used modified Re catalysts to convert muconic acid to muconic acid and then to adipic acid with a high selectivity.

**Experimental Section**

**Materials, strains, and media**

All starting materials are commercially available and were used as received, unless otherwise indicated. *E. coli* BL21(DE3) and the pET-46 Ek/LIC Cloning Kit were purchased from Novagen (Germany). *E. coli* DH5α from Invitrogen (USA) was used for cloning and propagation of plasmids. All strains were cultured at 37°C and 250 rpm unless otherwise stated. All polymerase chain reactions (PCR) were conducted on Phusion High-Fidelity DNA Polymerase from Thermo Scientific (USA). QiAaprep Spin Miniprep Kit, QiAquick Gel Extraction Kit, and QiAquick PCR Purification Kit were from Qiagen (Germany). α-Galacturonic acid was obtained from Sigma–Aldrich (USA). Muccic acid (98%) and 3-pentanol (98%) were purchased from Aldrich. Other reagents involved were from Sigma or Merck. P-Bn 62 and PMF 63 were synthesized according to literature methods. M9 medium contained 10 g L⁻¹ glucose, was used for uronate dehydrogenase activity study, 1.0 mL of reaction mixture was taken at different time intervals and dried for NMR spectroscopy, and a known amount of methylene was added as an internal standard.

**Cloning of the uronate dehydrogenase gene**

The Agrobacterium tumefaciens Udh gene *udh* (GenBank accession number is BK006462) was cloned into a pET-46 vector by using the Ek/LIC Cloning Kit. The 798 bp *udh* ORF with overhang sequences at both the 5’ and 3’ ends was amplified from *A. tumefaciens* genomic DNA with the forward primer 5’-TCCGACATTAGAAGGTACGATTTTTGCGG-3’ and reverse primer 5’-AGGAA GAAAGCTCTCACAGGCATAGCACGAG-3’. The amplified DNA fragment was treated with a T4 DNA polymerase and assembled into the vector according to the manufacturer’s instructions to generate the recombinant plasmid pET46-udh. The correct sequence of *udh* was verified by the DNA sequencing service provided by Axil Scientific Pte Ltd, Singapore.

**Expression of Udh in E. coli BL21(DE3)**

*E. coli* BL21(DE3) was transformed with pET46-udh by heat shock to give the recombinant strain *E. coli* BL21(DE3) 98dh. This strain was cultured in M9-glucose medium for 24 h at 37°C, and the expression of Udh was induced by adding IPTG to a final concentration of 0.2 mM. This culture was continued for another 12 h before the cells were collected by centrifugation. Resting cells of OD600 = 2 were used in the conversion reaction from α-galacturonic acid to muconic acid, and reaction substrates of 5, 10, and 20 g L⁻¹ α-galacturonic acid was dissolved in M9 medium. Reactions were conducted at 37°C and 250 rpm for 2 days.

**Deletion of the uronate isomerase gene *uxaC* and ω-galactarate dehydrogenase gene *garD* in *E. coli* BL21(DE3)**

Gene knockout was conducted using the lambda red-mediated gene disruption method as described elsewhere. The cassette for the deletion of *uxaC* was amplified from the pKD4 template plasmid using a pair of primers: 5’-TGCCGACATTAGAAGGTACGATTTTTGCGG-3’ and 5’-TCCGACATTAGAAGGTACGATTTTTGCGG-3’. The correct sequence of ω-arabinose was purified on a gel. *E. coli* BL21(DE3) was transformed with helper plasmid pKD46. *E. coli* BL21(DE3)/pKD46 was grown in LB medium at 30°C to the early exponential phase, and lambda red recombinase was induced with the addition of 0.2% ω-arabinose. This strain was cultivated for another 3 h before it was made electrocompetent. The –1.6 kb deletion cassette was electropropagated into *E. coli* BL21(DE3)/pKD46 competent cells, and positive deletion colonies were selected on the kanamycin plate. The selected colonies were further verified by colony PCR. The kanamycin cassette was removed with the help of the pCP20 plasmid. Both pKD46 and pCP20 are temperature-sensitive plasmids and could be lost by heating to 42°C.

The deletion cassette for *garD* was amplified using a pair of primers: 5’-AACGACATTAGAAGGTACGATTTTTGCGG-3’ and 5’-ACGACATTAGAAGGTACGATTTTTGCGG-3’. All the other procedures were the same as for the deletion of the *uxaC* gene except that the targeting strain was *E. coli* BL21(DE3) ΔuxaC instead of wild type. The double deletion mutant was named as *E. coli* BL21(DE3) ΔuxaC ΔgarD.

**Production of muconic acid with *E. coli* BL21(DE3) ΔuxaC ΔgarD udh**

*E. coli* BL21(DE3) ΔuxaC ΔgarD was transformed with pET46-udh by heat shock to give the recombinant strain *E. coli* BL21(DE3) ΔuxaC ΔgarD udh. The strain was cultured in the same way as mentioned above to prepare a whole-cell biocatalyst. Resting cells of OD600 = 20 were used to catalyze the reactions from α-galacturonic acid to muconic acid in 280 mM modified M9 medium. Generally, the reaction substrates were a mixture of equal amounts of α-galacturonic acid, glucose, and ω-arabinose, such as 10, 20, and 40 g L⁻¹ of each sugar. Reactions were conducted at 37°C and 250 rpm for 2 days. Samples were aliquoted every few hours, and the reaction medium was adjusted to neutral pH with 3 M NaOH after each sampling point.

**Homogenous DODH reactions**

A mixture of muconic acid (1.0 mmol, 210.0 mg), Re catalyst (0.05 mmol), and 3-pretanol (20.0 mL) was heated to reflux (120°C) in a 50 mL flask under a flow of air or N₂. For the kinetic study, 1.0 mL of reaction mixture was taken at different time intervals and dried for NMR spectroscopy, and a known amount of methylene was added as an internal standard.
Conversion of mucic acid to adipic acid

In the first step of the DOH reaction, a mixture of mucic acid (2.0 mmol, 420.0 mg), NH₄ReO₄ (0.1 mmol, 26.8 mg), and 3-pentanol (40.0 mL) was heated to reflux (120 °C) in a 100 mL flask under N₂ atmosphere for 8 h. The reaction mixture was cooled to RT. The unconverted mucic acid was recovered by filtration and dried under vacuum at 50 °C overnight (42 mg, 20% of the initial amount). The filtrate was evaporated to dryness under reduced pressure. The solid was extracted into n-hexane (20 mL × 2) and then vacuum dried at 50 °C overnight to obtain muconic acid (204 mg, 72% yield). In the subsequent hydrogenation step, a mixture of muconic acid (100 mg, 0.7 mmol), 5.0% Pt/C (10.0 mg), and H₂O (10.0 mL) was charged into a Parr reactor. The reactor was sealed, purged with N₂ three times, and then pressurized with H₂ (7 bar). The reactor was stirred at RT (24 °C) for 8 h with overhead stirring before it was depressurized. The catalyst was separated by filtration, the water solvent was removed by evaporation, and adipic acid was obtained as a white solid (92 mg, 92% yield).

Production of mucic acid from sugar beet residue

Sugar beet residue was hydrolyzed by a combination of three enzymes, namely, cellulases from Trichoderma reesei ATCC 26921, pectinase from Aspergillus aculeatus, and Viscozyme L (a multi-enzyme complex that contains a wide range of carboxydrases, which includes arabanase, cellulase, β-glucanase, hemicellulase, and xylanase), all of which were obtained from Sigma–Aldrich, USA. The hydrolysis reaction was performed in 100 mM sodium citrate buffer (pH 5.0) at 50 °C and 200 rpm for 48 h in 250 mL baffled flask. The reaction suspension was then filtrated to recover the reaction medium and adjusted to pH 6.5 with stirring before it was depressurized. The catalyst was separated by filtration, the water solvent was removed by evaporation, and adipic acid was obtained as a white solid (92 mg, 92% yield).

HPLC analysis

The concentrations of α-galacturonic acid, mucic acid, glucose, and l-arabinose in the reaction solution were analyzed by using an HPLC system from Shimadzu, Japan. The HPLC was equipped with a Nucleogel® sugar 810H column (300 mm × 7.8 mm, Macherey-Nagel, Germany) linked to a Syngeri 4 μL Hydro-RP 80 A column (250 mm × 4.6 mm, Phenomenex, USA), and these two analytical columns were protected with a SecurityGuard guard cartridge from Phenomenex. Samples (10 μL) were injected and eluted with a mobile phase of 5 mM H₂SO₄ at an isocratic flow rate of 0.4 mL min⁻¹. The column temperature was maintained at 30 °C. α-Galacturonic acid and mucic acid were measured at 210 nm by using a SPD-M20A photodiode array UV/Vis detector (PDA), whereas glucose and l-arabinose were detected by using a RID-10A refractive index detector (RID).

Acknowledgements

We thank the Agency for Science, Technology and Research, Singapore for supporting various research projects in the Metabolic Engineering Research Laboratory (MERL) through the Visiting Investigator Programme to H.Z. This work was also supported by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research (A*STAR), Singapore) and Biomass-to-Chemicals Program (Science and Engineering Research Council, A*STAR, Singapore) (Y. Z.).

Keywords: biomass · biocatalysis · gene technology · homogeneous catalysis · rhenium


Received: January 20, 2016
Revised: February 13, 2016
Published online on 0000
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