Adaptive laboratory evolution of *Klebsiella pneumoniae* for improving 2,3-butanediol production

Hongbiao Li, Genlin Zhang, and Yanyan Dang

Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan, School of Chemistry and Chemical Engineering, Shihezi University, Shihezi, China

**ABSTRACT**

Microbial production of 2,3-butanediol is limited by the toxic components in the lignocellulose hydrolysate. To improve the 2,3-butanediol production via *Klebsiella pneumoniae* from cotton stalk hydrolysate, a method coupling a high tolerance of strain and detoxification of the hydrolysate was thus investigated in this study. The strain tolerance of *K. pneumoniae* to the cotton stalk hydrolysate was improved via an adaptive laboratory evolution, which involved a stepwise increase in the hydrolysate concentration in the medium. Compared with the initial strain, the resulting strain increased the biomass 3.2-fold in a medium of 20 g/L hydrolysate and produced 10.45 g/L of 2,3-butanediol at an optimal concentration of 60 g/L hydrolysate. After detoxification of cotton stalk hydrolysate, the cell metabolism of *K. pneumoniae* was further promoted, and the 2,3-butanediol production increased by 1.2 folds. Using fed-batch fermentation, the concentration of 2,3-butanediol reached 35.5 g/L with a yield of 0.43 g/g. The results demonstrated that the bioconversion of low-cost cotton stalk hydrolysates into 2,3-butanediol improves the economics of microbial 2,3-butanediol production.

**ARTICLE HISTORY**

Received 29 March 2016
Revised 2 June 2016
Accepted 3 June 2016

**KEYWORDS**

adaptive laboratory evolution; cotton stalk; fermentation; *Klebsiella pneumoniae*; 2,3-butanediol

**Introduction**

The increasing crude oil prices and the rising concept on national sustainability has result in the development of integrated biomass conversion processes to produce chemicals. As an important chemical feedstock, interest in microbial production of 2,3-butanediol (2,3-BDO) has increased significantly. This compound exhibits wide commercial applications as a liquid fuel, printing ink, synthetic rubber, plastic, flavoring agent and as a precursor for many synthetic polymers and resins. Additionally, 2,3-BDO has an extremely important economic value. In 2008, Chemical Week speculated that the worldwide demand of 2,3-BDO would grow at 6%–7% because of its expanding application. By 2011, the potential global market of 2, 3-BDO reached up to 32 million tones with a value of $43 billion.

At present, the biosynthesis of 2,3-BDO that is derived from renewable biomass offers an attractive environmentally-friendly and less energy-demanding alternative to conventional methods. Through a mixed acid fermentation pathway, microorganisms convert the precursor acetoine to 2,3-BDO by 2,3-butanediol dehydrogenase, which is accompanied by a number of soluble products including ethanol, acetate, lactate and formate. In nature, a number of microorganisms are capable of accumulating 2,3-BDO, of which a few such as *K. pneumoniae*, *K. oxytoca*, and *Serratia marcescens* are considered to have significant application potential. Taking advantage of a broad range of substrates such as glucose, xylose, fructose and arabinose, and the ability to achieve a more complete fermentation and a higher utilization of substrate, *Klebsiella* has been given extensively and considerably efforts to improve the production of 2, 3-BDO by glucose or sucrose. However, the large-scale production of 2,3-BDO still requires low-cost raw materials. In recent decades, considerable attention has been given to a low-cost lignocellulosic substrate to reduce the production costs of 2,3-BDO. Cellulose, hemicellulose and lignocellulose from agricultural biomass have been considered as the most
potential carbon sources for 2,3-BDO production.\textsuperscript{1,13} Nonetheless, the application of lignocellulosic substrates often results in a relatively low 2,3-BDO yield.\textsuperscript{15} A low tolerance of strains to inhibitors that are present in lignocellulosic hydrolysate is generally considered to be the key factor that limits fermentation.\textsuperscript{12} No reported strains can directly grow and produce 2,3-BDO in cellulose or hemicellulose hydrolysates without detoxification.

To generate robust strains that can use lignocellulose from residual biomass to improve the production of various alcohols, there is a growing interest in adaptive laboratory evolution.\textsuperscript{16,17} Here, \textit{K. pneumoniae} that was screened in our laboratory was used to produce 2,3-BDO from cotton stalk hydrolysate. To optimize the production of 2,3-BDO, adaptive laboratory evolution was employed to improve the tolerance of \textit{K. pneumoniae} to cotton stalk hydrolysate (Fig. 1). By coupling the detoxification process, the production of 2,3-BDO from \textit{K. pneumoniae} was significantly improved.

**Results and Discussion**

**Enhancement of the strain tolerance to cotton stalk hydrolysate**

The present study used the acid hydrolysate of cotton stalk contained 1.63 g/L phenol, 2.02 g/L acetic acid and 0.56 g/L furan derivative which included furfuraldehyde and 5-hydroxymethyl furfuraldehyde. To evaluate the effect of these compounds on the growth of \textit{K. pneumoniae}, cells were grown in a medium containing the cotton stalk hydrolysate at different concentrations. All concentrations of hydrolysates that were tested inhibited cell growth. The most severe inhibition in the medium was reached with 20 g/L cotton stalk hydrolysate, which caused a 12 h extension of the lag phase compared with that in LB medium culture (Fig. 2).

The adaptive laboratory evolution of yeast cells to lignocellulosic inhibitors is an effective method for improving inhibitor tolerance and fermentation performance, and it is effective for engineering yeast cells for biotechnological applications.\textsuperscript{18} In this study, a stepwise adaptation method was investigated by incubating \textit{K. pneumoniae} onto a liquid medium with a gradual increase in concentration of the cotton stalk hydrolysate.
hydrolysate. This resulted in a tolerant strain that could still live on media containing 80 g/L of cotton stalk hydrolysate. Compared with the wild type strain that grows in 20 g/L of cotton stalk hydrolysate, the biomass of the resulting strain exhibited a 3.2-fold increase (Fig. 2). After consuming 18.56 g/L of sugar, the 2,3-BDO concentration and yield were 5.16 g/L and 0.28 g/g, respectively (Table 1).

### Optimization of the cotton stalk hydrolysate concentration

The high substrate concentration likely inhibited the strain via low water activity or accumulated toxic substances. The data in Table 1 shows that both the growth of *K. pneumoniae* and accumulation of 2,3-BDO were obviously inhibited by the high concentration of hydrolysate. Particularly, for greater than 60 g/L of cotton stalk hydrolysate, the growth of the strain and the production of 2,3-BDO were both simultaneously inhibited. When the concentration of cotton stalk hydrolysate increased from 20 to 60 g/L, the ethanol and acetate simultaneously increased 1.1–1.3 folds. However, the acetate accumulation was more than the accumulation of ethanol. This variation in the accumulation of ethanol and acetate was similar under different cotton stalk hydrolysate concentrations (Table 1). Previous reports confirmed that a varied carbon source would yield different by-products; the yield of ethanol was the main by-product when the fermentation was performed with glucose as substrate, whereas the yield of acetate was significantly enhanced when xylose was the substrate. Acetate was verified not only as an inhibitor but also as a substrate in the 2,3-BDO fermentation process. Acetate may be an inducer in the 2,3-BDO metabolic process that induces the formation of acetalactate synthase. The acetalactate synthase converts some of the acetic acid to acetaldehyde that then condenses into acetoin and further produces 2,3-BDO.

### Increasing the 2, 3-BDO production via detoxification of cotton stalk hydrolysate

The adaptive laboratory evolution enhanced the tolerance of *K. pneumoniae* to cotton stalk hydrolysate, resulting in the normal growth of the strain; however, 2,3-BDO production was still affected by furan derivatives and phenolic compounds in the hydrolysate. Particularly, the furan derivatives affected the cell metabolism by inhibiting the glycolytic enzyme and alcohol dehydrogenase, and lowering the membrane permeability. To improve the 2,3-BDO production from hydrolysates, treatment processes to remove microbial inhibitors prior to fermentation are still required.

Therefore, a microwave-assisted heating-activated carbon adsorption method was employed to detoxify furfural and phenol from cotton stalk hydrolysate under the following conditions: activated carbon of 1%, microwave power of 330 W and detoxification time of 10 min. This processing removed 81.2% of furfural and 92.3% of phenol but only 10.6% of the total sugar loss. Table 2 shows the result of 48 h of

### Table 1. Influence of cotton stalk hydrolysate concentration on the 2,3-BDO fermentation.

| Hydrolysate (g/L) | Biomass (g/L) | Acetate (g/L) | Ethanol (g/L) | Sugar consumption (g/L) | 2,3-BDO | Concentration (g/L) | Yield (g/g) |
|------------------|---------------|---------------|---------------|-------------------------|---------|---------------------|------------|
| 20               | 0.84          | 2.88          | 1.03          | 18.56                   | 5.16    | 0.28                |
| 40               | 1.17          | 2.95          | 1.07          | 24.40                   | 9.32    | 0.38                |
| 60               | 1.20          | 3.13          | 1.42          | 36.66                   | 10.45   | 0.29                |
| 80               | 0.64          | 2.34          | 0.51          | 17.86                   | 4.22    | 0.24                |
| 100              | 0.45          | 2.25          | 0.47          | 13.25                   | 2.06    | 0.16                |

### Table 2. Influence of detoxification on the 2,3-BDO fermentation.

| Substrate (60 g/L) | Biomass | Acetate (g/L) | Ethanol (g/L) | Sugar consumption (g/L) | 2,3-BDO | Concentration (g/L) | Yield (g/g) |
|-------------------|---------|---------------|---------------|-------------------------|---------|---------------------|------------|
| Glucose           | 1.84    | 2.08          | 2.53          | 58.60                   | 25.10   | 0.43                |
| Detoxified hydrolysate | 1.77 | 3.62          | 2.44          | 54.33                   | 23.36   | 0.43                |
| Detoxified hydrolysate | 1.75 | 3.54          | 2.58          | 55.20                   | 23.50   | 0.43                |
| No detoxification hydrolysate | 1.22 | 3.00 | 1.26 | 35.86 | 10.20 | 0.28 |

*The fermentation results of the 30th generation tolerant *K. pneumonia*. 
Batch fermentation was inhibited by the high initial cotton stalk hydrolysate concentration. Thus, the fed-batch fermentation was performed in a 5 L bioreactor upon continuous stirring (200 rpm). In the fed-batch fermentation, the initial hydrolysate concentration was 60 g/L. After 30 h of fermentation, the concentrated detoxified hydrolysate containing 47.9 g/L glucose, 32.5 g/L xylose, 19.7 g/L arabinose, 0.6 g/L furfural and 1.2 g/L phenol was continuously added into the bioreactor to maintain a sugar concentration of 10–15 g/L. Figure 3 indicates that the growth of the strain was static after 50 h of fermentation in addition to the slowing of the 2,3-BDO production rate. Approximately 82.3 g/L of the total reducing sugar was consumed in the entire fed-batch culture. The final concentration of 2,3-BDO was 35.5 g/L with a yield of 0.43 g/g.

An industrial medium for economical 2,3-BDO production is what people expect. Ji et al. confirmed that glucose and xylose derived from lignocellulose as the carbon source in the developed medium can potentially improve the economics of microbial 2,3-BDO production. Thus far, various renewable-feedstock-based 2,3-BDO production methods have been reported (Table 3). A comparison of the results in the literature indicates that 2,3-BDO production from cotton stalk hydrolysate is effective (Table 3). K. pneumoniae has also been verified as a better 2,3-BDO producer when lignocellulose hydrolysate is used as the substrate. All analyses suggest that 2,3-BDO production from cotton stalk hydrolysate via fermentation is available for economic production from an industrial point of view.

### Table 3. Comparison of 2,3-BDO production from various renewable substrates by different microorganisms.

| Substrate                  | Strain                      | Mode        | BDO (g/L) | Yield (g/g) | Reference |
|----------------------------|-----------------------------|-------------|-----------|-------------|-----------|
| Bagasse                    | Klebsiella sp. Zmd30        | Batch       | 8.26      | 0.15         | [33]      |
| Rice straw                 | Klebsiella sp Zmd30         | Batch       | 24.6      | 0.62        | [33]      |
| Corn steep liquor          | K. oxytoca                  | Batch       | 23.4      | 0.428       | [28]      |
| Seaweed                    | Engineered E. coli         | Fed-batch   | 14.1      | 0.43         | [32]      |
| Corncob                    | K. oxytoca                  | Fed-batch   | 35.7      | 0.5          | [15]      |
| CelloDEXTRIN               | Engineered E. coli         | Fed-batch   | 5.5       | 0.54         | [34]      |
| Jerusalem Artichoke stalk  | K. pneumoniae               | Fed-batch   | 67.4      | 1.81         | [35]      |
| Cotton stalk               | K. pneumoniae               | Fed-batch   | 35.5      | 0.43         | This study |

*a* the molar ratio of 2,3-BDO to sugar  
*b* Mean g (2,3-BDO + acetoin) per (3 g stalks + 4 g tubers)
Conclusions

The objective of this study was to produce 2,3-BDO from cotton stalk hydrolysate using K. pneumoniae. The strain can resist 80 g/L of cotton stalk hydrolysate after direct cultivation with a stepwise increase in the hydrolysate solid medium. For the production of 2,3-BDO, the optimal concentration of hydrolysate is 60 g/L. The accumulation of ethanol and acetate was similar during the fermentation because of the co-metabolism of glucose and xylose from the cotton stalk hydrolysate. Detoxification efficiently improved the growing environment of the strain. Fed-batch fermentation indicated that the bioconversion of cotton stalk hydrolysate into 2,3-BDO is feasible.

Materials and methods

Preparation of the cotton stalk hydrolysate

Cotton stalk, containing 35% cellulose and 21% hemicellulose, was chosen as the raw material and was collected from a local farmer in Shihezi city in the Xinjiang Province of China. After it was milled in a muller and passed through a 40 mesh sieve, the cotton stalk was hydrolyzed to the hydrolysate according to the acid pretreatment method. The hydrolysate contained 22.8 g/L of reducing sugars, including 11.24 g/L of glucose and 7.62 g/L xylose.

Microorganism culture

K. pneumoniae was obtained from the Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan, Shihezi University, China. The strain was adapted via growth on the fermentation medium by gradually increasing the concentration of cotton stalk hydrolysate according to the acid pretreatment method. The hydrolysate contained 22.8 g/L of reducing sugars, including 11.24 g/L of glucose and 7.62 g/L xylose.

Analytical methods

The biomass was estimated by measuring the optical density at 600 nm. The concentration of the cotton stalk hydrolysate was characterized by the quality of the reducing sugars, which was determined using the DNS method. The concentrations of 2,3-BDO, glucose, xylose, ethanol and acetate were determined using a high-performance liquid chromatography system (SHIMADZU LC-10A) equipped with an Aminex HXP-87H column (Bio-Rad, Hercules, CA) and a differential refractive index detector. The working conditions included a temperature of 65°C, a mobile phase of 5 mM H2SO4 at a flow rate of 0.6 ml/min.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was financially supported by the National Natural Science Foundation of China (No. 21566032).

References

[1] Ji XJ, Huang H, Ouyang PK. Microbial 2,3-butanediol production: A state-of-the-art review. Biotechnology Advances 2011; 29:351–64; PMID:21272631; http://dx.doi.org/10.1016/j.biotechadv.2011.01.007
[2] Xiu ZL, Zeng AP. Present state and perspective of downstream processing of biologically produced 1,3-propanediol and 2,3-butanediol. Appl Microbiol Biot 2008; 78:917–26; http://dx.doi.org/10.1007/s00253-008-1387-4
[3] Petrov K, Petrova P. High production of 2,3-butanediol from glycerol by Klebsiella pneumoniae G31. Appl Microbiol Biot 2009; 84:659–65; http://dx.doi.org/10.1007/s00253-009-2004-x
[4] Kopke M, Mihalcea C, Liew FM, Tizard JH, Ali MS, Connolly JJ, Al-Sinawi B, Simpson SD. 2,3-Butanediol Production by Acetogenic Bacteria, an Alternative Route to Chemical Synthesis, Using Industrial Waste Gas. Appl Environ Microb 2011; 77:5467–75; http://dx.doi.org/10.1128/AEM.00253-10
[5] Hong E, Kim D, Kim J, Kim J, Yoon S, Rhi S, et al. Optimization of alkaline pretreatment on corn stover for enhanced production of 1,3-propanediol and 2,3-butanediol by Klebsiella pneumoniae AJ4. Biomass Bioenerg 2015; 77:177–85; http://dx.doi.org/10.1016/j.biombioe.2015.03.016
[6] Syu MJ. Biological production of 2,3-butanediol. Appl Microbiol Biot 2001; 55:10–8; http://dx.doi.org/10.1007/s00253-000-00486
[7] Frazer FR, McCaskey TA. Effect of components of acid-hydrolysed hardwood on conversion of d-xylose to 2,3-butanediol by Klebsiella pneumoniae. Enzyme Microb Technol 1991; 13:110–5; http://dx.doi.org/10.1016/0141-0229(91)90164-6
[8] Ma C, Wang A, Qin J, Li L, Ai X, Jiang T, Tang H, Xu P. Enhanced 2,3-butanediol production by Klebsiella pneumoniae SDM. Appl Microbiol Biot 2009; 82:49–57; http://dx.doi.org/10.1007/s00253-008-1732-7
[9] Wang AL, Wang Y, Jiang TY, Li LX, Ma CQ, Xu P. Production of 2,3-butanediol from corncob molasses, a waste...
by-product in xylitol production. Appl Microbiol Biot 2010; 87:965–70; http://dx.doi.org/10.1007/s00253-010-2557-8

[10] Zhang LY, Yang YL, Sun JA, Shen YL, Wei DZ, Zhu JW, Chu J. Microbial production of 2,3-butanediol by a mutagenized strain of Serratia marcescens H30. Bioresource Technol 2010; 101:1961–7; http://dx.doi.org/10.1016/j.biortech.2009.10.052

[11] De Mas C, Jansen NB, Tsao GT. Production of optically active 2,3-butanediol by Bacillus polymyxa. Biotechnol Bioeng 1988; 31:366–77; PMID:18584617; http://dx.doi.org/10.1002/bit.260310143

[12] Garg SK, Jain A. Fermentative Production of 2,3-Butanediol - a Review. Bioresource Technol 1995; 51:103–9; http://dx.doi.org/10.1016/0960-8524(94)00136-O

[13] Sun LH, Wang XD, Dai JY, Xiu ZL. Microbial production of 2,3-butanediol from Jerusalem artichoke tubers by Klebsiella pneumoniae. Appl Microbiol Biot 2009; 82:487–52; http://dx.doi.org/10.1002/bit.26025-008-1823-5

[14] Raguaskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL, et al. The path forward for biofuels and biomaterials. Science 2006; 314:484–9; PMID:16439654; http://dx.doi.org/10.1126/science.1114736

[15] Cheng K-K, Liu Q, Zhang J-A, Li J-P, Xu J-M, Wang XD, Dai JY, Xiu ZL. Effect of initial substrate concentration and aeration. J Chem Technol Biot 1990; 47:71–84; http://dx.doi.org/10.1002/jctb.280470109

[16] Alam S, Capit F, Weigand W, Hong J. Kinetics of 2,3-butanediol fermentation by Bacillus amyloliquefaciens: Effect of initial substrate concentration and aeration. J Chem Technol Biot 1990; 47:71–84; http://dx.doi.org/10.1002/jctb.280470109

[17] Wu J, Cheng KK, Wang GY, Li WY, Feng J, Zhang JA. Analysis of acetic acid, furfural and 5-hydroxymethylfurfural affecting 2,3-butanediol production using Klebsiella pneumoniae. J Chem Technol Biot 2013; 88:2239–43; http://dx.doi.org/10.1002/jctb.4094

[18] Chandel AK, Kapoor RK, Singh A, Kuhad RC. Detoxification of sugarcane bagasse hydrolysate improves ethanol production by Candida shehatae NCIM 3501. Bioresource Technol 2007; 98:1947–50; http://dx.doi.org/10.1016/j.biortech.2006.07.047

[19] Chandel AK, Silva SSD, Singh OV. Detoxification of lignocellulosic hydrolysates for improved bioethanol production, Biofuel production-Recent developments and prospects, 2011. Dr. Marco Aurelio Dos Santos Bernades (Ed.), InTech, DOI: 10.5772/16454.

[20] Deng H, Li C, Li F, Chen J. Optimized conditions for saccharification of cotton stalk hydrolysate after detoxification. Transactions of the Chinese Society of Agricultural Engineering 2011; 27:287–91

[21] Ge JP, Huang SF, Li XL, Pan C, Ping WX. Comparison of capacity for 2,3-butanediol production from corn cob hemicellulose hydrolysate fermentation by Klebsiella oxytoca HD79 and Klebsiella pneumoniae. Applied Mechanics and Materials: Trans Tech Publ, 2014; 672–674:147–53; http://dx.doi.org/10.1007/AMM.672-674.147

[22] Deng H, Li C, Li F, Chen J. Optimized conditions for saccharification of cotton stalk by alkali pretreatment. Transactions of the Chinese Society of Agricultural Engineering 2009; 25:208–12

[23] Yu EK, Saddler JN. Fed-batch approach to production of 2,3-butanediol by fermentation of cotton stalk hydrolysate after detoxification. Bioresource Technol 2009; 100:5214–8; http://dx.doi.org/10.1016/j.biortech.2009.05.036

[24] Li LX, Li K, Wang K, Chen C, Gao C, Ma CQ, Xu P. Efficient production of 2,3-butanediol from corn stover hydrolysate by using a thermophilic Bacillus licheniformis strain. Bioresource Technol 2014; 170:256–61; http://dx.doi.org/10.1016/j.biortech.2014.07.101

[25] Alam S, Capit F, Weigand W, Hong J. Kinetics of 2,3-butanediol fermentation by Bacillus amyloliquefaciens: Effect of initial substrate concentration and aeration. J Chem Technol Biot 1990; 47:71–84; http://dx.doi.org/10.1002/jctb.280470109

[26] Chandel AK, Kapoor RK, Singh A, Kuhad RC. Detoxification of sugarcane bagasse hydrolysate improves ethanol production by Candida shehatae NCIM 3501. Bioresource Technol 2007; 98:1947–50; http://dx.doi.org/10.1016/j.biortech.2006.07.047

[27] Chandel AK, Silva SSD, Singh OV. Detoxification of lignocellulosic hydrolysates for improved bioethanol production, Biofuel production-Recent developments and prospects, 2011. Dr. Marco Aurelio Dos Santos Bernades (Ed.), InTech, DOI: 10.5772/16454.

[28] Ge JP, Huang SF, Li XL, Pan C, Ping WX. Comparison of capacity for 2,3-butanediol production from corn cob hemicellulose hydrolysate fermentation by Klebsiella oxytoca HD79 and Klebsiella pneumoniae. Applied Mechanics and Materials: Trans Tech Publ, 2014; 672–674:147–53; http://dx.doi.org/10.1007/AMM.672-674.147

[29] Deng H, Li C, Li F, Chen J. Optimized conditions for saccharification of cotton stalk by alkali pretreatment. Transactions of the Chinese Society of Agricultural Engineering 2009; 25:208–12

[30] Yu EK, Saddler JN. Fed-batch approach to production of 2,3-butanediol by fermentation of cotton stalk hydrolysate after detoxification. Bioresource Technol 2009; 100:5214–8; http://dx.doi.org/10.1016/j.biortech.2009.05.036

[31] Silverstein RA, Chen Y, Sharma-Shivappa RR, Boyette MD, Osborne J. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresource Technol 2007; 98:3000–11; http://dx.doi.org/10.1016/j.biortech.2006.10.022

[32] Mazumdar S, Lee J, Oh MK. Microbial production of 2,3 butanediol from seaweed hydrolysate using metabolically engineered Escherichia coli. Bioresource Technol 2013;
[33] Wong CL, Huang CC, Lu WB, Chen WM, Chang JS. Producing 2,3-butanediol from agricultural waste using an indigenous Klebsiella sp Zmd30 strain. Biochem Eng J 2012; 69:32–40; http://dx.doi.org/10.1016/j.bej.2012.08.006

[34] Shin H-D, Yoon S-H, Wu J, Rutter C, Kim S-W, Chen RR. High-yield production of meso-2,3-butanediol from cellodextrin by engineered E. coli biocatalysts. Biotechnology Biofuels 2010; 3:11; http://dx.doi.org/10.1016/j.biortech.2010.06.041

[35] Li D, Dai JY, Xiu ZL. A novel strategy for integrated utilization of Jerusalem artichoke stalk and tuber for production of 2,3-butanediol by Klebsiella pneumoniae. Bioresource Technol 2010; 101:8342–7; http://dx.doi.org/10.1016/j.biortech.2010.06.041