World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:6, No:12, 2012

The Composition of Rice bran Hydrolysate and Its possibility to use in the Ethanol Production by *Zymomonas Mobilis* Biofilm

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Abstract**—**Rice bran has been abandoned as agricultural waste for million tonnes per year in Thailand, therefore they have been proposed to be utilized as a rich carbon source in the production of bioethanol. Many toxic compounds are possibly released during the pretreatment of rice bran prior the fermentation process. This study aims to analyze on the availability of toxic compounds and the amount of glucose obtained from 2 different pretreatments using sulfuric acid and mixed cellulase enzymes (without and with delignification/ activated charcoal). The concentration of furfural, 5-hydroxymethyl furfural (5-HMF), levulinic acid, vanillin, syringaldehyde and4-hydroxybenzaldehyde (4-HB) and the percent acetic acid were found to be 0.0517 ± 0.049 mg/L, 0.032 ± 0.06 mg/L, 21074 ± 1685.62 mg/L, 126.265 ± 6.005 mg/L, 2.89 ± 0.30 mg/L, 0.37 ± 0.031mg/L and 0.72% under the pretreatment process without delignification/ activated charcoal treatment and 384.47 ± 99.02 g/L, 0.068 mg/L, 142107.62 ± 8664.6 mg/L, 0.19 mg/L, 5.43 ± 3.29 mg/L, 4.80 ± 0.76 mg/L and 0.254% under the pretreatment process with delignification/ activated charcoal treatment respectively. The presence of high concentration of acetic acid was found to impede the growth of *Zymomonas mobilis* strain TISTR 551 despite the present of high concentration of levulinic acid. *Z. mobilis* strain TISTR 551 was found to produce 8.96 ± 4.06 g/L of ethanol under 4 days fementation period in biofilm stage in which represented 40% theoretical yield.

Keywords**—**Rice bran, *Zymomonas mobilis*, biofilm, ethanol.

I. Introduction

THE use of bioethanol as an alternative fuel can reduce our demand on fossil fuel which has been kept risen up in price and also decrease the carbon dioxide emission which causes the greenhouse effect. Currently, the commercial bioethanol is commonly derived from edible part of food crops either sugarcane or corn in which leads to the escalation in food prices.

The alternative carbon source for bioethanol production is lignocellulosic materials which are remained as tremendous wastes in nature since lignocellulosic materials contain of up to 70% carbohydrates. Lignocellulosic complex composes of cellulose, hemicellulose and lignin. As Thailand produces million tonnes of rice per year so rice bran could be considerably used to produce value added products. However, the usage of lignocellulosic materials including rice bran requires the pretreatment process either physical or chemical prior the use of enzyme hydrolysis to degrade the materials to be fermentable sugars.

Diluted acid hydrolysis with sulfuric acid (H_2SO_4) and modified diluted acid methods with the physical pretreatment have been considered as a simple method as well as greatly used for pretreatment of lignocellulosic materials which provide the improvement in cellulose hydrolysis [1]. Rice hull and rice straw have been successfully pretreated with diluted acid and steam by given the high reaction rates and significant improves in the cellulose hydrolysis in the further step[2],[3],[4]. During the pretreatment process, cell growth inhibitor compounds such as 5- hydroxymethyl furfural (5-HMF) and furfurals are produced as degradation products of hexoses and pentoses, weak organic acids, phenolic compounds from lignin degradation are created in combination with the sugar degradation products [5],[6],[7]. The diluted acid hydrolysis has been found to be one the method that creates furans and phenol at high concentration [8]. These inhibitory compounds represent the inhibitory effect in both yeast and bacterial growth and enzyme that impedes the bioprocess of ethanol production which also leads to the decrease in the ethanol production rate and the total ethanol yield [9],[10]. Overliming with calcium hydroxide ($Ca(OH)_2$ and lignin removal have been found to be a cost effective processes for the detoxification of lignocellulosic hydrolysate to remove the volatile and non-volatile inhibitors such as furans and phenolic compounds [11].

Z. mobilis is a gram negative facultative anaerobic bacteria that has an ability to grow at high sugar concentration and represents a high ethanol productivity rate though the Entner-Doudoroff pathway using glucose as a substrate in which near the theoretical yield of ethanol. *Z. mobilis* also tolerates to ethanol concentration up to 13% (w/v) [12], [13]. Currently, *Z. mobilis* has been studied on its ability to produce ethanol from wheat bran extract that was added into the formulated medium to produce ethanol under the batch mode of planktonic cell or free cell[14]. However, the presence of toxic substances in the pretreated lignocellulosic materials substantially inhibited the ethanol producing efficiency by the microorganism. The toxic tolerance of *Z. mobilis* can be enhanced by the development of

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Z. mobilis biofilm and using biofilm application in the production of value added products in the presence of toxic compounds since biofilm differs in term of phenotypic characteristics which creates more tolerance to the stress than planktonic counterparts [15].

The aim of this research is to study on the compositions of rice bran hydrolysates that obtain from the pretreatment of with and without delignification/ activated charcoal. We also aim to study on the possibility of using rice bran hydrolysate in the production of ethanol by *Z. mobilis* TISTR 551 biofilm under the lab scale.

II. Procedure

A. Microorganism

Z. mobilis strain TISTR 551 obtained from Thailand Institute of scientific and Technological Research (TISTR). *Z. mobilis* was grown in yeast peptone glucose (YPG) medium (peptone 10 g, yeast extract 10 g and glucose 20 g per liter). The culture was grown at 30°C overnight to the OD_{600} of 1.0 for each study.

B. Preparation of Rice Bran Hydrolysate

Rice bran was treated in 10% w/v with 0.2 M potassium hydroxide (KOH) for 4 hours at the room temperature. Then the material was filtered through cheese cloth and repeatedly washed with the tap water until pH became neutral and dried at 85°C until the weight was constant. The total of 15% w/v of rice bran was then treated with 2% v/v diluted sulfuric acid (H_2SO_4) at 121°C for 30 minutes. After cooling down, the pH was adjusted to 6-6.5 with NaOH. The adjusted mixture was treated with cellulase enzyme (Novozyme, 29950 U/ml) for 72 hours at 55°C (2ml/ 100g of solid matter). The filtrated was collected by diatomaceous earth. The obtained rice bran hydrolysate was concentrated by boiling at 80°C to half of the volume. The overliming was processed using $Ca(OH)_2$ to adjust the pH to 10.5 at 90°C with frequently stirred. The hydrolysate was then filtered through diatomaceous earth and treated with 3.5% w/v activated charcoal for 1 hour at the room temperature. The total 7.5 g yeast extract and 10 g peptone were added per liter of hydrolysate obtained. The pH of the hydrolysate was brought back to 5.5 as optimum pH for *Z. mobilis* before the sterilization process prior running the fermentation. In another treatment, the initial lignin removal by KOH and later stage treatment with activated charcoal were ignored as indicated as without delignification/ activated charcoal process.

C. Analysis on the Compositions of Rice Bran Hydrolysate (HPLC)

The concentration of 5- hydroxymethylfurfural was analyzed using HPLC (Shimadzu) using Inertsil-OSD column (5 µm, 250 × 4.6 mm) maintained at 40°C. The mobile phase used was 5% acetonitrile in 0.2% phosphoric acid at the flow rate of 1ml/min. The detector used was UV detector at wavelength of 280 nm. The standard solutions used were 0.1, 0.5 and 1 mg/L HPLC grade 5- hydroxymethylfurfural. The concentration of levulinic acid, furfural, 4-hydroxybenzaldehyde, syringaldehyde and vanillin in the samples were analyzed by HPLC (Shimadzu) using inertsil C_{18} column (5 µm, 250 × 4.6 mm) maintained at 40°C. The mobile phase was acetonitrile- water (1:8 v/v) containing 1% acetic acid at the flow rate of 2 ml/min. The detector used was UV detector at wavelength of 280 nm. The standard solutions used were 10, 15 and 20 mg/L mixture of HPLC grade levulinic acid, furfural, 4-hydroxybenzaldehyde, syringaldehyde and vanillin.

The percentage of acetic acid was measured by the titration with 0.1N NaOH using phenolphthalein as an indicator.

D. Microorganism Quantitative Analysis on the Biofilm Formation

The quantitative analysis on the ability of *Z. mobilis* TISTR 551 on the biofilm formation or the cell adhesion was performed using crystal violet staining of the attached cells. The biofilm formation was done on polystyrene (PS) tube. The total of 10% (v/v) of the overnight bacterial culture (OD_{600nm}≈1.0) was inoculated into 2 ml of biofilm medium (10 fold diluted seed medium contains 20 g of glucose, 5 g yeast extract, 5g $(NH_4)_2SO_4$, 0.6 KH_2PO_4, 0.4 g NA_2HPO_4. $12H_2O$, 0.2 g $MgSO_4.7H_2O$ and 0.01g $CaCl_2$ per liter at pH 6.4) that was placed in the polystyrene (PS) tube. Biofilm was allowed to develop under the static condition at 30°C for the total of 4 consecutive days in which the medium was subsequently changed every day. The bacterial attachment was measured by using crystal violet staining by aspirated out the supernatant. The attached cells were stained with 1% crystal violet for 20 minutes and then destained the attached cells with 95% ethanol. The optical density OD_{595nm} was measured using a spectrophotometer [16]. The ability of *Z. mobilis* TISTR 551 in forming the biofilm was determined based on the intensity.

E. Ethanol Assay

Ethanol produced was analyzed by gas chromatography (GC) (HP Innowax Agilent 6890N) using Innowax column (29.8m × 0.25 mm x 0.25 µm) with a flame ionization detector (FID). The column temperature was 150°C, program run time 5.5 min, ethanol retention time about 1.9 min and the carrier gas was nitrogen (16 kPa), injector temperature 175°C, detector temperature 250°C, flow rate 40 ml/min, split ratio 1:50, velocity of H2 flow 60 ml/min, sample quantity 1 µl. One part of the supernatant was filtered by 0.22 µm cellulose acetate filters prior GC analysis.

F. Glucose Assay

The concentration of glucose was measured using glucose liquicolor kit manufactured by Human, germany.

G. Fermentation by Z. Mobilis Planktonic Cells

Ten percent overnight culture of *Z. mobilis* at OD_{600} approximately 1.0 was inoculated into rice bran hydrolysate as prepared from the above. The fermentation was performed for 4 days. The concentration of glucose and ethanol were analyzed before and after the fermentation. The experiment was performed triplicate.

H. Glucose Assay

Ten percent overnight culture of *Z. mobilis* at OD_{600} of 1.0 was inoculated into biofilm medium (10 fold diluted seed medium contains 20 g of glucose, 5 g yeast extract, 5g $(NH_4)_2SO_4$, 0.6 KH_2PO_4, 0.4 g NA_2HPO_4. $12H_2O$, 0.2 g $MgSO_4.7H_2O$ and 0.01g $CaCl_2$ per liter at pH 6.4) that was placed in the polystyrene (PS) tube. The culture was grown for 4 days in the biofilm medium in which the medium was subsequently changed every single day. At the end of day 4, the medium was replaced with rice bran hydrolysate as prepared from the above. The fermentation was set for 4 days. The concentration of glucose and ethanol were analyzed before and after the fermentation. The experiment was performed triplicate.

III. Result and Discussion

Rice bran is a byproduct of the rice processing in the worldwide. In order to use rice bran to produce bioethanol, rice bran needs to be pretreated. The pretreatment and enzymatic saccharification of rice bran cellulose and hemicelluloses to fermentable sugars and further ferment the rice bran hydrolyzate to ethanol is complicate. The physical, chemical and enzymatic processes need to be combined together to hydrolyze rice bran. Diluted acid hydrolysis and enzymatic process is the most common technique to depolymerize lignocellulosic material to fermentable sugars, however, the diluted acid produces many toxic compounds as by products. The purpose of this study was to analyze on the compositions of rice bran hydrolysates that were prepared from the diluted acid and enzymatic pretreatment processes in which delignification/ activated charcoal was applied and in the process without delignification/ activated charcoal. The potential of using rice bran hydrolysates from these pretreatments as substrates for ethanol production by *Z. mobilis* TISTR 551 planktonic cell and biofilm were compared in a lab scale.

The concentrations of glucose obtained from the two different pretreatment methods were approximately the same at 20 g/L. The rice bran hydrolysate that was obtained from the pretreatment without the delignification and activated charcoal was found to impede the growth and ethanol production by *Z. mobilis*. The delignification of rice bran was further applied prior the treatment with diluted acid and enzymatic process. Delignification by alkaline treatment was significantly found to break ester bond cross linking between hemicelluloses and lignin to remove lignin out from lignocellulosic material [17]. The activated charcoal was also applied after the overliming process to remove out toxic compounds. From the previous work suggested that overliming and activated charcoal treatments have shown the potential in the removal of furan and phenolic compounds from lignocellulosic hydrolysates[18]. Therefore, the delignification/ activated charcoal treatment was further applied to prepare rice bran hydrolysate in which it was hypothesized to remove more inhibitory compounds from the hydrolysate that impede the bacterial growth and ethanol production.

The compositions of the hydrolysate from the two different pretreatments were compared (TABLE I). Vanillin was found to be moderately decreased in the delignification/ activated charcoal treatment while furfural and levulinic acid concentration tended to be increased dramatically. Vanillin is normally generated by the partial breakdown of lignin[19]. Therefore, the delignification possibly reduced the concentration of vanillin since lignin was removed prior the diluted acid treatment. After the delignification, cellulose and hemicelluloses were more accessible to the diluted acid hydrolysis to produce more sugar compounds either hexose or pentose. Hence, this phenomenon probably created a potential in subsequently convert sugar compounds to furfural and levulinic acids in the diluted acid treatment because furfurals are normally produced as degraded products of hexoses and pentoses and levulinic acid is product from glucose, mannose and galactose[20]. There were no significant changed in the level of syringaldehyde, 5-HMF and 4-HB in delignification/ activated charcoal treatment. However, the delignification/ activated charcoal treatment efficiently reduced the concentration of acetic acid in the hydrolysate in which acetic acid probably be the most significant inhibitory compound in rice bran hydrolysate that inhibited the ethanol production by *Z. mobilis*. Acetic acid is one of the organic acid that is highly generated through the diluted acid pretreatment process from the degradation of lignin [21]. The presence of acetic acid results in acidification of the cytoplasms in which consequently increases in the energy demand to maintain the metabolic activity that causes the retarded in cell growth and development[22]. In some *Z. mobilis* strains, the final cell mass concentrations were found to be decreased linearly with the increase in the concentration of acetic acid over the range of 0-0.75% (w/v) in which the 50% reduction happened at about 0.5% (w/v) [23].

TABLE I
THE CONCENTRATION OF FURFURAL, 5-HYDROXYMETHYL FURFURAL, LEVULINIC ACID, VANILLIN, SYRINGALDEHYDE, 4- HYDROXYBENZALDEHYDE AND ACETIC ACID

Compounds (concentration)	With delignification/ activated charcoal	Without delignification/ activated charcoal
Furfural (mg/L or ppm)	384.47 ±99.015	0.0517 ± 0.049
5-Hydroxymethyl furfural (5-HMF) (mg/L or ppm)	0.068	0.332 ± 0.06
Levulinic acid (mg/L or ppm)	142107.62±8 664.6	21074 ± 1685.62
Vanillin (mg/L or ppm)	0.19 ± 0.0094	126.265 ± 6.005
Syringaldehyde (mg/L or ppm)	5.43 ± 3.29	2.8903 ± 0.303
4-Hydroxybenzaldehyde (4-HB) (mg/L or ppm)	4.806 ± 0.76	0.3729 ± 0.031
Acetic acid (%)	0.254 ± 0.075	0.72 ± 0.075

Therefore, cell growth and ethanol production were not found under the pretreatment process without delignification/ activated charcoal treatment in which acetic acid was found to be 0.72 ± 0.075.

The biofilm forming ability of *Z. mobilis* TISTR 551 on polystyrene surface was consistently increased from day 1 toward day 4 from the quantitative assay using crystal violet staining (Fig. 1). The higher the intensity of crystal violet toward day 4 represented the higher the biofilm formation or the higher the bacterial attachment. The biofilm formation could be visualized after the staining with crystal violet (Fig. 2). *Z. mobilis* is facultative anaerobic bacteria so biofilm was obviously developed on the air- liquid interface that could be the preference area for the bacterial attachment in which benefited the cells in terms of oxygen supply and nutrient accessibility from the top compare to the bottom of the tube. *Z. mobilis* TISTR 551 has shown its ability to form the biofilm on the plastic surface.

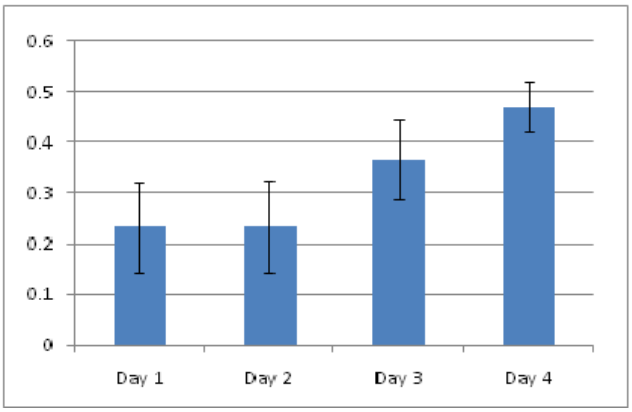

Fig. 1 *Z. mobilis* TISTR 551 Biofilm Formations on The Polystyrene Surface and The Quantitative Assay were Performed Using The Crystal Violet Staining (OD_{595}Measurement) Under The 4 Days Observation

Fig. 2 *Z. mobilis* TISTR 551 Biofilm Formations on The Polystyrene Surface After 4 Days Cultivation. The Bacterial Attachment was Stained with Crystal Violet

As in this work, *Z. mobilis* biofilm was hypothesized to be more tolerate to the inhibitory compounds that available in the rice bran hydrolysate than the planktonic cell or free cell, therefore, the fermentation efficiency under these two mode of growth were compared in same fermentation condition. The ethanol yields were found to be 0.066 ± 0.079 g/L in the planktonic cell culture and 8.95 ±4.06 g/L in the biofilm culture which represented 2% theoretical yield and 40% theoretical yield respectively (Table II). *Z. mobilis* biofilm produced high concentration of ethanol from rice bran hydrolysate while planktonic cells or free cell produced none. From the previous studies, ethanol productivity by microbes is normally inhibited in the presence of small concentration of by inhibitors from the pretreatment process [24], [25]. Extracellular polymeric substance (EPS) of the biofilm probably develops the resistant characteristic over the planktonic cell or free cell [26]. From this work the biofilm technique has shown its efficiency to be applied in the ethanol production from rice bran hydrolysate obtaining from diluted acid and enzymatic saccharification.

TABLE II
ETHANOL PRODUCTIONS FROM PLANKTONIC CELLS AND BIOFILM (4 DAYS OLD) OF *Z. MOBILIS* TISTR 551 USING RICE BRAN HYDROLYSATE FROM THE DELIGNIFICATION/ ACTIVATED CHARCOAL TREATMENT AS SUBSTRATE

Planktonic cell or Free cell		Biofilm	
Ethanol (g/L)	% Theoretical	Ethanol (g/L)	% Theoretical
0.066 ± 0.079	2%	8.95 ±4.06	40%

IV. CONCLUSION

Fermentation inhibitors that are generated during the pretreatment of rice bran with diluted sulfuric acid was found to effect on the cell growth and ethanol production especially in the pretreatment process that produced high concentration of acetic acid. The delignification/ activated charcoal treatment tended to significantly reduce the concentration of acetic acid in the rice bran hydrolysate. *Z. mobilis* TISTR 551 in biofilm form was found to be more tolerate to the presence inhibitors by producing higher ethanol yield than planktonic cell or free cell. Ethanol yield from biofilm mode of growth was 40% theoretical yield while the free cell produced only 2%. The future prospective on the process optimization can be done through the more effective pretreatment method to remove the remaining toxic compounds from the rice bran hydrolysate and apply the process in the biofilm reactor rather than the free cell reactor.

ACKNOWLEDGMENT

The financial support was accepted from The Thailand Research Fund (MRG5480014).

REFERENCES

[1] Sun, Y. and J. Cheng (2002). "Hydrolysis of lignocellulosic materials for ethanol production: a review." Bioresour Technol 83(1):
[2] Kim, J. H., D. E. Block, et al. (2010) "Conversion of rice straw to bio-based chemicals: an Integrated process using Lactobacillus brevis." Appl Microbiol Biotechnol 86(5): 1375-85.
[3] Saha, B. C., L. B. Iten, et al. (2005). "Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol." Biotechnol Prog 21(3): 816-22.
[4] Karimi K, E. G., Taherzadeh MJ.(2006). "Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with Mucorindicus, Rhizopus oryzae, and Saccharomyces cerevisiae." Enzyme and Microbial Technology 40: 138-144.

[5] Erdei, B., Barta Z., et al. (2010) "Ethanol production from mixtures of wheat straw and wheat meal."Biotechnol Biofuels 3: 16.
[6] Nilvebrant, N. O., A. Reimann, et al. (2001). "Detoxification of lignocellulose hydrolysates with ion-exchange resins." Appl Biochem Biotechnol 91-93: 35-49.
[7] Bjorklund, L., S. Larsson, et al. (2002). "Treatment with lignin residue: a novel method for detoxification of lignocellulose hydrolysates." Appl Biochem Biotechnol 98-100: 563-75.
[8] Klinke, H. B., A. B. Thomsen, et al. (2004). "Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass." Appl Microbiol Biotechnol 66(1): 10-26.
[9] Larsson S, P. E., Hahn-Hagerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant (1998). "The generation of fermentation inhibitors during dilute acid hydrolysis of softwood" 3-4: 151-159.
[10] Sakai, S., Y. Tsuchida, et al. (2007)."Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum R."Appl Environ Microbiol 73(7): 2349-53.
[11] Klinke H.B., T. A. B., Ahring B.K. (2004). "Inhibition of ethanol-producing yeast and bacteria by degradation product produced during pre-treatment of biomass." Appl Microbiol Biotechnol 66: 10-26.
[12] Rogers, P. L., K.L. Lee, D.E. Tribe (1979)."Kinetics of alcohol production by Zymomonas mobilis at high sugar concentration."Biotech.Lett.1: 165- 170.
[13] Dien, B. S., M. A. Cotta, et al. (2003). "Bacteria engineered for fuel ethanol production: current status." Appl Microbiol Biotechnol 63(3): 258-66.
[14] Shiva Soleimani, M. F. G., and SoheilShokri (2012)."Ethanol production by Zymomonas mobilis PTCC 1718 using low cost substrates." African Journal of Microbiology Research 6(4): 704-712.
[15] Li, X. Z., J. S. Webb, et al. (2006)."Enhanced benzaldehyde tolerance in Zymomonas mobilis biofilms and the potential of biofilm applications in fine-chemical production."Appl Environ Microbiol 72(2): 1639-44.
[16] Djordjevic D.,Wiedmann M. and McLandsborough L. A. (2002) "Microtiter Plate Assay for Assessment of Listeria monocytogenes Biofilm Formation." Appl. Environ. Microbiol. 68 (6) : 2950-2958
[17] Lee, J. (1997)."Biological conversion of lignocellulosic biomass to ethanol." Journal of Biotechnology 56: 1-24.
[18] Yadav, K. S., S. Naseeruddin, et al. (2011) "Bioethanol fermentation of concentrated rice Straw hydrolysate using co-culture of Saccharomyces cerevisiae and Pichia stipitis."Bioresour Technol 102(11): 6473-8.
[19] Sakai, S., Y. Tsuchida, et al. (2007)."Effect of lignocellulose-derived inhibitors on growth of and ethanol productionby growth-arrested Corynebacterium glutamicum R."Appl Environ Microbiol 73(7): 2349-53.
[20] Bjorklund, L., S. Larsson, et al. (2002). "Treatment with lignin residue: a novel method for detoxification of lignocellulose hydrolysates." Appl Biochem Biotechnol 98-100: 563-75.
[21] Bjorklund, L., S. Larsson, et al. (2002). "Treatment with lignin residue: a novel method for detoxification of lignocellulose hydrolysates." Appl Biochem Biotechnol 98-100: 563-75.
[22] Rousseau, H. G. L. J. D. (1993)."The Effect of Acetic Acid on Fuel Ethanol Production by Zymomonas." Applied Biochemistry and Biotechnology 39/40: 687-699
[23] Rousseau, H. G. L. A. J. D. (1998)."Improving Fermentation Performance of Recombinant Zymomonas in Acetic acid-Containing Media." Applied Biochemistry and Biotechnology 70-72: 161-172.
[24] J. P. Delgenes, R. Moletta, et al. (1996)."Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by Saccharomyces cerevisiae, Zymomonas mobilis, Pichia stipitis, and Candida shehatae." Enzyme and Microbial Technology 19: 220-225.
[25] Sakai, S., Y. Tsuchida, et al. (2007)."Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum R."Appl Environ Microbiol 73(7): 2349-53.
[26] Li, X. Z., J. S. Webb, et al. (2006)."Enhanced benzaldehyde tolerance in Zymomonas mobilis biofilms and the potential of biofilm applications in fine-chemical production."Appl Environ Microbiol 72(2): 1639-44.