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Use of an Isolated Facultative Thermophilic Alkaline Desulfuricant Strain for Decomposition of Thiosulfate

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The use of a desulfurization strain BYD-3, which isolates from soil and belongs to the thermophilic alkaline desulphuricant strain, for the decomposition of thiosulfate is reported. The strain can utilize thiosulphate as sulfur source and various organic compounds as carbon source. The colony of the strain is ivory-white and becomes primrose-yellow with protracting cultivated time. The cells of BYD-3 are motive and Gram negative. According to GenBank data, 16s rDNA results of BYD-3 are in good agreement with *Pseudomonas stutzeri* (100 %). For BYD-3, the optimum growth temperature is determined at 40 ºC, the optimum pH is found at 8.5 and the optimum rocking speed of sorting table is confirmed at 150 rpm. Under the optimum condition, the cells of BYD-3 can live for 36 h. Meanwhile, the concentration of thiosulphate in the media containing the cells of BYD-3 was decreased by 0.128 g/L from 3.2 g/L (percentage loss of content 96 %) in 28 h, meanwhile, sulfide was formed. These results suggest that the strain has potential application to the regeneration of the industrial solution containing thiosulphate.

Key Words: Thermophilic alkaline desulphurican, *Pseudomonas stutzeri*, Strain identification, DDS catalyst.

INTRODUCTION

Hydrogen sulfide is a malodorous gas and easily found in natural gas, coke-oven gas, shift gas and semi-water gas. Hydrogen sulfide is harmful to many industrial catalysts, so that H_2S had to be removed from various industrial gases[1]. Currently, the desulfurization is mainly achieved by adsorption[2,3], selective catalytic oxidation[4-6], wet scrubbing[7-9] and biological methods[10-12]. Compared with wet scrubbing, absorption and selective catalytic oxidation for H_2S removal, which have relatively high energy requirements or high chemical and disposal cost, the micro-organism desulfurization was paid more attention in various countries[13-19], because it presented lower cost, higher yield of second-produces and higher desulfurization efficiency.

Wei[20] developed the method of removal of sulfur compounds from gases by bio-chemical Fe-basic solution (DDS) to remove H_2S. In desulfurization processes, H_2S absorbed in DDS solution are oxidated into element sulfur using air. However, by-products, especially $Na_2S_2O_3$ produced in high amount, badly affect the desulfurization properties of DDS solution. Meanwhile, bacteria, which can decompose $Na_2S_2O_3$ into sulfur or sulfide, became an important part of DDS technology.

The reports on biological decomposition of $Na_2S_2O_3$ in the DDS technology are very less. Therefore, this work was mainly focused on providing some references for the biological decomposition of $Na_2S_2O_3$.

EXPERIMENTAL

The sample containing desulfurization strain was extracted from the neutral soil about 15 cm under the earth and isolated in sulfur synthetic medium.

The liquid medium was composed of $Na_2HPO_4{\cdot}7H_2O$ 7.9 g, metal salt solution 5 mL, KH_2PO_4 1.5 g, NH_4Cl 0.3 g, $Na_2S_2O_3{\cdot}5H_2O$ 10 g, $MgSO_4{\cdot}7H_2O$ 0.1 g per distilled water 1 L with pH 8.5-8.8 (controlled by 10 % Na_2CO_3). The strain was maintained on the sterilized solid medium added agar 16 g L^{-1} and conserved at 4 ºC. Before biological composition of $Na_2S_2O_3$ were carried out, the strain should be activated for about 2 h at room temperature.

Isolation and purification: The strain was inoculated on the solid medium for 2-7 d at 40 ºC. Meanwhile, the growth processes of the strain were observed and recorded. When the single colony was developed on the solid medium and transferred to polybasic culture medium for cultivation, the unanimous characteristics of the colony, figures, sizes and Gram's characteristics were presented. Then the strain was conserved as pure strain and named as BYD-3, which was cultivated on

the inclined solid medium, maintained at 4 ºC and inoculated after 1-2 months.

Re-screening method: To cultivate stains for the DDS technology, the experimental DDS solution sterilized at 121 ºC for 20 min was confected according to the industrial DDS solution. The strain was inoculated in the experimental DDS solution under the condition of 40 ºC and 150 rpm.

Identification of the isolate: The identifications of isolate were performed by conventional and chemotaxonomic analyses and 16s RNA gene sequencing. Sequencing of the 16s RNA genes and analyses of sequence were carried out at the Institute of Microbiology, Chinese Academy of Sciences, China.

Preferred experiments of cultivated conditions: 120 mL medium added with 10 mL cell solution was used to perform the preferred experiments by changing the conditions of temperature, rocking speed of sorting table and pH. The cell concentration was determined using visible spectra at 600 nm.

RESULTS AND DISCUSSION

Identification of the strain: The strain was named as BYD-3 and its characteristics have been ascertained through the physiological and biochemical experiments and motive experiment. The colony's diameter of the strain on the solid medium is 1-2 mm and the mid part of colony ivory-white and becomes primrose-yellow with protracting cultivated time. The cells of BYD-3 are motive and Gram negative. The strain can utilize thiosulphate as sulfur source and utilize various organic carbon as carbon source. The identification results of 16s rDNA were determined (Table-1).

The resulting sequences were compared to sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov). 16s rDNA results showed that the strain was in good agreement with *Pseudomonas stutzeri* (100%) (Table-2).

Blank experiment: The decomposition of $Na_2S_2O_3$ in the absence of bacteria was performed as blank experiment in the optimum conditions. The result shows that the concentration of $Na_2S_2O_3$ in the media keeps almost invariable in 28 h.

Growth temperature: The prepared cell solution was conserved under 4 ºC for the following experiments. 0.2 mL of solution was coated on the flat panel. The strain was inoculated and cultivated with the step of 5 ºC from 20 to 50 ºC; meanwhile, the strain's growth processes were recorded. So the step of 5 ºC was confirmed to optimize experimental temperature and 40 ºC was used as the basic temperature.

The effects of growth temperature on the strain are shown in Fig. 1. Fig.1 shows that $Y = n_{24}/n_0$ (n_{24} and n_0 represent the cell concentration of the strain at 24 h and 0 h, respectively) is 0.741 at 40 ºC and increases at other temperatures, but the increasing quantity is evidently lower than at 40 ºC. This phenomenon was due to the fact that the strain can play perfectly the role of enzyme at this temperature. At higher temperature, the enzyme of the strain loses the physiological action so that the strain cannot grow above 50 ºC. At lower temperature, on the contrary, the enzyme of the strain cannot have the physiological action also, so the strain grows slowly below 20 ºC.

Effect of pH: Based on the decomposition of medium, a series of media were prepared with pH step of 1 from 6 to 10 detected by a PHS-3 exact acidometer (Leici, Shanghai) and sterilized. 0.2 mL bacterial solutions was respectively coated on the above different solid culture media and then cultivated for 2-5 days at 40 ºC and the growth processes were recorded in detail.

TABLE-1

TGCTC CATGA TTCAG CGGCG GACGG GTGAG TAATG CCTAG GAATC TGCCT	50
GGTAG TGGGG GACAA CGTTT CGAAA GGAAC GCTAA TACCG CATAC GTCCT	100
ACGGG AGAAA GTGGG GGATC TTCGG ACCTC ACGCT ATCAG ATGAG CCTAG	150
GTCGG ATTAG CTAGT TGGTG AGGTA AAGGC TCACC AAGGC GACGA TCCGT	200
AACTG GTCTG AGAGG ATGAT CAGTC ACACT GGAAC TGAGA CACGG TCCAG	250
ACTCC TACGG GAGGC AGCAG TGGGG AATAT TGGAC AATGG GCGAA AGCCT	300
GATCC AGCCA TGCCG CGTGT GTGAA GAAGG TCTTC GGATT GTAAA GCACT	350
TTAAG TTGGG AGGAA GGGCA GTAAG TTAAT ACCTT GCTGT TTTGA CGTTA	400
CCAAC AGAAT AAGCA CCGGC TAACT TCGTG CCAGC AGCCG CGGTA ATACG	450
AAGGG TGCAA GCGTT AATCG GAATT ACTGG GCGTA AAGCG CGCGT AGGTG	500
GTTCG TTAAG TTGGA TGTGA AAGCC CCGGG CTCAA CCTGG GAACT GCATC	550
CAAAA CTGGC GAGCT AGAGT ATGGC AGAGG GTGGT GGAAT TTCCT GTGTA	600
GCGGT GAAAT GCGTA GATAT AGGAA GGAAC ACCAG TGGCG AAGGC GACCA	650
CCTGG GCTAA TACTG ACACT GAGGT GCGAA AGCGT GGGGA GCAAA CAGGA	700
TTAGA TACCC TGGTA GTCCA CGCCG TAAAC GATGT CGACT AGCCG TTGGG	750
ATCCT TGAGA TCTTA GTGGC GCAGC TAACG CATTA AGTCG ACCGC CTGGG	800
GAGTA CGGCC GCAAG GTTAA AACTC AAATG AATTG ACGGG GGCCC GCACA	850
AGCGG TGGAG CATGT GGTTT AATTC GAAGC AACGC GAAGA ACCTT ACCAG	900
GCCTT GACAT GCAGA GAACT TTCCA GAGAT GGATT GGTGC CTTCG GGAAC	950
TCTGA CACAG GTGCT GCATG GCTGT CGTCA GCTCG TGTCG TGAGA TGTTG	1000
GGTTA AGTCC CGTAA CGAGC GCAAC CCTTG TCCTT AGTTA CCAGC ACGTT	1050
AAGGT GGGCA CTCTA AGGAG ACTGC CGGTG ACAAA CCGGA GGAAG GTGGG	1100
GATGA CGTCA AGTCA TCATG GCCCT TACGG CCTGG GCTAC ACACG TGCTA	1150
CAATG GTCGG TACAA AGGGT TGCCA AGCCG CGAGG TGGAG CTAAT CCCAT	1200
AAAAC CGATC GTAGT CCGGA TCGCA GTCTG CAACT CGACT GCGTG AAGTC	1250
GGAAT CGCTA GTAAT CGTGA ATCAG AATGT CACGG TGAAT ACGTT CCCGG	1300
GCCTT GTACA CACCG CCCGT CACAC CATGG GAGTG GGTTG CTCCA GAAGT	1350
AGC	1353

The effects of growth pH on the strain are shown in Fig. 2. From Fig. 2, the optimum growth pH for the strain was confirmed at 8.5. The results were attributed to the strain, which can play commendably the role of enzyme at optimum acid condition, benefit the dissolution of CO_2 in culture medium and the strain's metabolism for the carbon source. At higher

TABLE-2
HOMOLOGOUS MICROORGANISMS WITH BYD3 IN 16S rDNA

Accession	Description	Max score	Total score	Query coverage (%)
FJ768951.1	*Pseudomonas stutzeri* 16S ribosomal RNA gene, partial sequence	2499	2499	100
FJ796076.1	*Pseudomonas* sp. HB09004 16S ribosomal RNA gene, partial sequence	2499	2499	100
FJ217184.1	*Pseudomonas* sp. BBAPs-01 16S ribosomal RNA gene, partial sequence	2499	2499	100
FJ217182.1	*Pseudomonas putida* strain BBAL5-01 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU883660.1	*Pseudomonas* sp. 42(2008) 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU849665.1	*Pseudomonas putida* 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU862300.1	Uncultured bacterium clone Feng5 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU628149.1	*Pseudomonas* sp. AG9 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU628147.1	*Pseudomonas* sp. AG7 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU628146.1	*Pseudomonas* sp. AG6 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU628145.1	*Pseudomonas* sp. AG5 16S ribosomal RNA gene, partial sequence	2499	2499	100
EU636773.1	*Pseudomonas stutzeri* strain BD-1 16S ribosomal RNA gene, partial sequence	2499	2499	100
AM945587.1	*Pseudomonas* sp. MOLA 357 partial 16S rRNA gene, culture collection, OLA:357	2499	2499	100
EU037276.1	*Pseudomonas* sp. G3DM-15 16S ribosomal RNA gene, partial sequence	2499	2499	100
EF542804.1	*Pseudomonas* sp. QZ1 16S ribosomal RNA gene, partial sequence	2499	2499	100
CP000304.1	*Pseudomonas stutzeri* A1501, complete genome	2499	2499	100
AY654828.1	*Mucus* bacterium 10 16S ribosomal RNA gene, partial sequence	2499	2499	100
AM084028.1	*Pseudomonas* sp. R-25343 16S rRNA gene, strain R-25343	2499	2499	100
AF094748.1	*Pseudomonas stutzeri* 16S ribosomal RNA gene, partial sequence	2499	2499	100
U26262.1	*Pseudomonas stutzeri* CCUG 11256 16S ribosomal RNA gene, complete sequence	2499	2499	100
U25432.1	*Pseudomonas stutzeri* 16S ribosomal RNA gene, complete sequence	2499	2499	100

pH condition, the physiological action of the amino acids in the cell was affected seriously because the basic complexes entered the cell under the effect of osmotic pressure when the concentration of basic complexes in the surrounding of cells was higher than in the cell. Under lower pH condition, on the contrary, the obvious decreasing dissolution capacity of CO_2 in culture medium affected the growth of the strain severely and the results explained commendably the phenomenon that the strain cannot grow below 6 of pH.

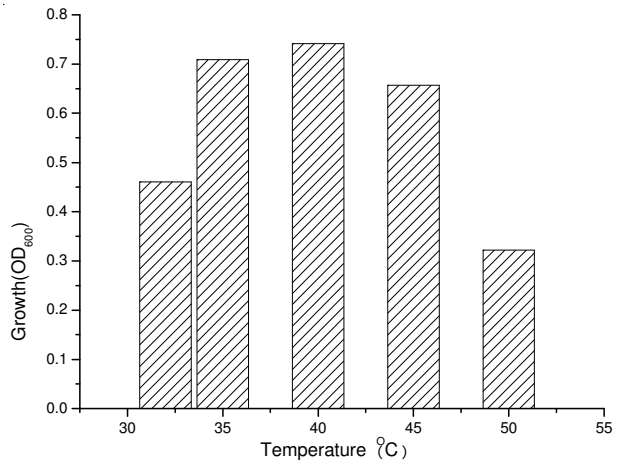

Fig. 1. Effect of growth temperature on the BYD-3 growth.

Effects of rotation speeds of sorting table on the growth of strain: The effects of rotation speeds of sorting table on the growth of the strain are shown in Fig. 3. From Fig. 3, the $Y = n_{24}/n_0$ was confirmed for 0.702 when the rocking speed of sorting table was kept at 150 rpm. This phenomenon was attributed to better dissolution capacity of O_2 and CO_2 at 150 rpm so that the strain can grow favourably under this condition. At higher rocking speed, the process benefits mass transfer of gases but does not benefit dissolution of O_2 and CO_2 in culture medium. At lower rocking speed, on the contrary, there is higher dissolution capacity for O_2 and CO_2 in culture medium, but lower dissolution capacity of O_2 and CO_2 in culture medium resulted from the lower rate of mass transfer. According to experimental result, the rocking speed of sorting table at 150 rpm was confirmed as the better condition for the dissolution capacity of O_2 and CO_2 and the process of mass transfer in culture medium.

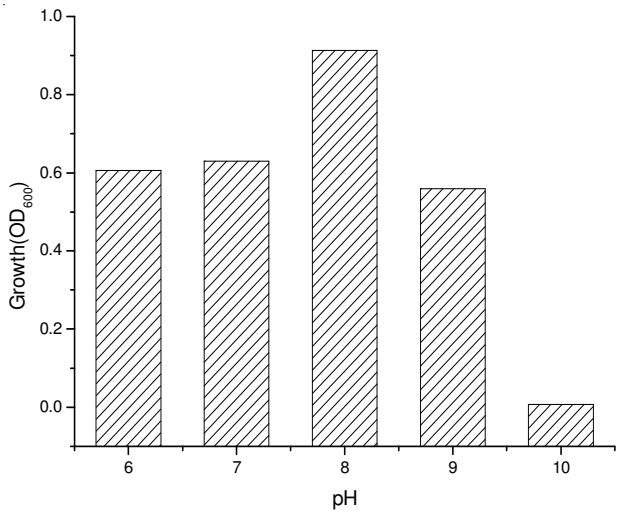

Fig. 2. Effect of growth pH on the BYD-3 growth

Then, the optimum cultivated conditions were determined as follows: the optimum temperature is at 40 ºC and the strain can grow between 20 and 50 ºC (there is slow growth below 20 ºC), the optimum cultivated pH condition is at 8.5 and the strain can grow at the pH between 6 and 10.

Determination of growth curve: The cell concentration was counted by visible spectra at 600 nm. Under the optimum cultivated condition, the strain was cultivated and the growth curve of the strain was plated and shown in Fig. 4.

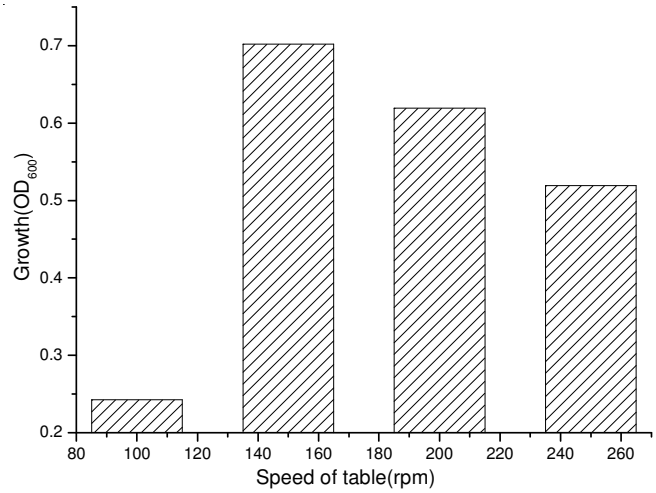

Fig. 3. Effects of rotation speeds of sorting table on the BYD-3 growth.

From Fig. 4, the lag phase of the strain appears at 0-5 h and the strain adapts themselves mainly to the cultivated condition in the lag phase. Exponential phase of the strain is presented at 5-25 h and the strain utilizes the nutriment in the medium for growth and to reproduce rapidly. In the following process, the growth of the strain enters the stationary phase at 25 h. Moreover, the medium cannot provide enough energy source for the growth of the strain, so the strain enters the death phase at 36 h and the cell concentration of the strain decreases obviously at this phase.

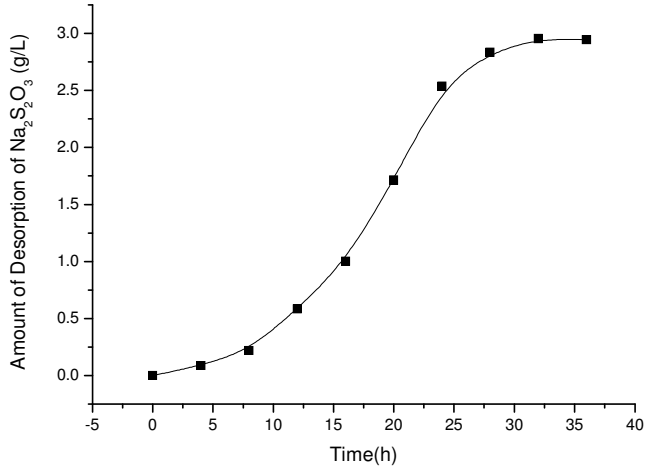

Fig. 4. Growth curve of BYD-3 under the optimum growth condition

Biological decomposition under optimum condition: Blank experimental and biological decomposition results are shown in Fig. 5. From Fig. 5, under the optimum decomposition condition, the concentration of $Na_2S_2O_3$ in the media was decreased by 0.128 g/L from 3.2 g/L (percentage loss of content 96 %) in 28 h. The and XRD spectrum showed the formation of sulfide.

Conclusion

The optimum cultivated condition for BYD-3 was confirmed as follows: temperature is at 40 ºC, pH is at 8.5 and rocking speed of sorting table is at 150 rpm. Under the optimum condition, the cells of BYD-3 can live for 36 h. According to GenBank data, 16s RNA results of BYD-3 are in good agreement with *Pseudomonas stutzeri* (100 %). Meanwhile, the concentration of $Na_2S_2O_3$ in media was decreased by 0.128 g/L from 3.2 g/L (percentage loss of content 96 %) in 28 h; meanwhile, sulfide was formed.

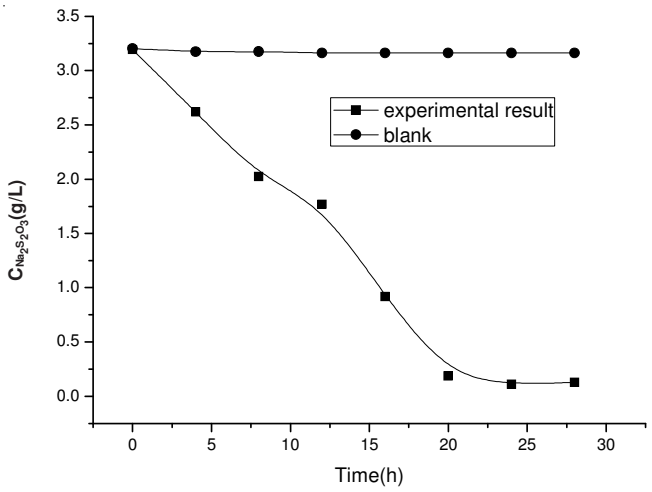

Fig. 5. Concentration changes of $Na_2S_2O_3$ in the growth process of strain BYD-3 under the optimum condition

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REFERENCES

1. M.C. Iliuta and F. Larachi, *J. Chem. Eng. Data*, **52**, 2 (2007).
2. I. Rosso, C. Galletti, M. Bizzi, G. Saracco and V. Specchia, *Ind. Eng. Chem. Res*., **42**, 1688 (2003).
3. S. Yasyerli, I. Ar, G. Dogu and T. Dogu, *Chem. Eng. Process*., **41**, 785 (2002).
4. A. Wingen, N. Anastasieviç, A. Hollnagel, D. Werner and F. Schüth, *J. Catal.*, **193**, 248 (2000).
5. Y.H. Xiao, S.D. Wang, D.Y. Wu and and Q. Yuan, *Sep. Purif. Technol.*, **59**, 326 (2008).
6. S. Bashkova, F.S. Baker, X.X. Wu, T.R. Armstrong and V. Schwartz, *Carbon*, **45**, 1354 (2007).
7. S. Ebrahimi, R. Kleerebezem, M.C.M. van Loosdrecht and J.J. Heijnen, *Chem. Eng. Sci.*, **58**, 417 (2003).
8. J.F. Demmink, A. Mehra and C.M. Beenackers, *Chem. Eng. Sci*., **57**, 1723 (2002).
9. T.C. Blessing, B.W. Wielinga, M.J. Morra and and S. Fendorf, *Environ. Technol. Sci*., **35**, 1599 (2001).
10. A.K. Michael, *FEMS Microbiol. Rev.*, **24**, 135 (1999).
11. A.B. Jensen and C. Webb, *Process Biochem*., **30**, 225 (1995).
12. A. Das, P. Roy and A.K. Mishra, *Lett. Appl. Microbiol*., **16**, 164 (1993).
13. P.T. Selvaraj, M.H. Little and E.N. Kaufman, *Biotech. Prog*., **13**, 583 (1997).
14. H.M. Lizama, P.A. Zielinski, L.D. Kerby and C.C. Abraham, *Biotech. Bioeng*., **77**, 111 (2002).
15. D.H. Park, J.M. Cha, H.W. Ryu, G.W. Lee, E.Y. Yu, J.I. Rhee, J.J. Park, S.W. Kim, I.W. Lee, Y.I. Joe, Y.W. Ryu, B.K. Hur, J.K. Park and K.M. Park, *Biochem. Eng. J*., **11**, 167 (2002).
16. J.M. Cha, W.S. Cha and J.H. Lee, *Process. Biochem*., **34**, 659 (1999).
17. N.P. Stamford, A.J.N. Silva, A.D.S. Freitas and J.T. A. Filho, *Bioresour. Tech*., **81**, 53 (2002).
18. K. Nakamura, M.A. Yudiarto, N. Kaneko, H. Kurosawa and Y. Amano *Appl. Microbial. Biotech*., **48**, 753 (1997).
19. A. Michael, *Adv. Env. Res*., **8**, 483 (2004).
20. X.H. Wei, Desulfurication & Decarburization Solution Activities. CN. 02130605. 2, (2002).