Iranica Journal of Energy & Environment 2 (4): 320-325, 2011
ISSN 2079-2115
IJEE an Official Peer Reviewed Journal of Babol Noshirvani University of Technology
DOI:10.5829/idosi.ijee.2011.02.04.2391

BUT

Growth Kinetics and Demineralization of Shrimp Shell Using *Lactobacillus plantarum PTCC 1058* on Various Carbon Sources

[1]M. Khorrami, [1]G.D. Najafpour, [2]H. Younesi and [1]G.H. Amini

[1]Faculty of Chemical Engineering, Noushirvani University of Technology, Babol, Iran
[2]Department of Environmental Science, Faculty of Natural Resources and Marine Science, Tarbiat Modares University (TMU), Nour, Iran

(Received: August 18, 2011; Accepted: September 27, 2011)

Abstract: The present study has focused on the effect of various carbon sources such as glucose, sucrose and date syrup as natural carbon sources along with *Lactobacillus plantarum* microorganism on demineralization (DM) of shrimp shell. Logistic and Verhulst Equations were used for the determination of growth kinetic parameters. Maximum demineralization efficiency of 82% was obtained in the media contained date syrup. Data for fermentation with media contained date syrup were suitably fitted with both Verhulst and Logistic Equations. Kinetic data was obtained and Gompertz model for production of lactic acid was used. For the media contained date syrup as carbon source, maximum rate of acid production was obtained.

Key words: Date syrup % Demineralization % Growth kinetic % *Lactobacillus plantarum* % Chitosan

INTRODUCTION

Biowastes originated from marine food products such as carapace and head of the shrimp, crab and krill is considered to be a rich source of protein, calcium carbonate and chitin [1]. Chitin is a natural polysaccharide (poly $- (1P4)-N-acetyl-D glucosamine) and is the second most plentiful biopolymer after cellulose [2]. It is biodegradable, biocompatible and nontoxic; therefore, chitin and it's deacetylated derivative chitosan, has numerous applications in various fields, e.g. in food, agriculture, cosmetic, biomedicine, textile, water treatment and pharmacy [1, 3-5].

Conventionally, isolation of chitin from marine waste material involves acid treatment to dissolve calcium carbonate (demineralization) followed by alkaline extraction to solubilize proteins (deproteinization) [3]. These chemical processes are ecologically harmful because of the use strong acid and alkali solutions for the treatment of chitin properties such as weight and viscosity. Moreover, cost of the chemicals is another disadvantage of the chemical process [6,7].

In biological process, fermentation of crustacean shell biowaste using microorganisms resulted in production of lactic acid and protease. Lactic acid produces by breakdown of carbon source in fermentation medium [8,9]. One of the factors which affect on the process is using proper carbon source such as glucose, sucrose and etc. Use of cheap carbon source like date syrup in the fermentation process makes the process more economically feasible.

In several studies, use of microorganisms for demineralization of crustacean wastes have been reported; such as *Serratia marcescens FS-3* with 47% DM [10], *Bacillus subtilis* with 72% DM [11], *Pediococcus acidolactici* with 72.5% DM [12] and *Lactobacillus plantarum* used along with glacial acetic acid resulted in 81% demineralization [13].

The objective of present work is to investigate the effect of various carbon sources such as glucose, sucrose and date syrup on demineralization of shrimp shell. Moreover, kinetic parameters were determined by Verhulst and logistic kinetic models.

MATERIALS AND METHODS

Shrimp Shell Samples: The shrimp shell of fresh shrimp provided for the experiments were supplied from local market Tehran, Iran. The shells were separated from head and legs. They were washed for several times with tap water then dried at hot room (70°C). The dried shells were grinded to form fine uniform powder.

Corresponding Author: Dr. G.D. Najafpour, Faculty of Chemical Engineering, Noushirvani University of Technology, Babol, Iran. Fax: +98 111 321 0975.

Microorganism and Preparation of Inoculation: *Lactobacillus palantarum* PTCC 1058 was purchased from Persian type culture collection (PTCC), provided by Iranian Research Organization for Science and Technology (IROST). The MRS (Man Rogosa Sharpe) media was used for growing *L. plantarum*. The media was autoclaved at 121°C and pressure of 15 psig for 20 min. The inoculated culture was cultivated in an incubator shaker (Stuart, S1500 series, USA) at 30°C and agitation rate of 180 rpm for 24 hours.

Fermentation Process: The fermentation medium was composed of peptone, yeast extract, meat extract, K_2HPO_4, Na-acetate, $(NH_4)_2$-citrate, $MgSO_4$ and different sugar sources with concentration of 10, 5, 10, 2, 5, 2, 0.2 and 50 g/l, respectively. The prepared medium was autoclaved at 121°C and pressure level of 15 psig for 30 min. The sugar solution was separately autoclaved at the same condition. Shrimp shell powder (5% w/v) was autoclaved along with media in absence of sugar source [7, 14].

Batch fermentation was carried out in a 250 ml flask with media working volume of 100 ml in an incubator shaker at 30°C and 180 rpm. The prepared media was inoculated with 7% of seed culture and incubated for 182 hour. The samples were periodically withdrawn from the culture for determination of optical density, sugar consumption and variation of media pH.

After 182 hours of incubation, the fermented slurry was filtered through a paper filter (Whatman, England) paper-grade No. 41 to separate solid residues and washed with distilled water. Then filter cake was dried overnight in an oven (Binder, Germany) at 55°C.

Analytical Procedure: The cell population was determined by turbidity of the media using spectrophotometer. A calibration curve was developed based on cell dry weight with respect to optical density. Cell dry weight of the samples was determined based on calibration curve. The optical densities of the filtrates containing suspended cells were measured by spectrophotometer (Unico, USA) at wavelength of 620 nm (OD_{620nm}). For determination of carbohydrate concentration, 1.5 ml of samples were collected and the cells were settled by centrifugation at 7000 rpm for 7 min by a micro centrifuge (Hermle model: Z 233 M-2, Germany). The reduced sugar was determined by color-metric method using 3,5-dinitrosalicylic acid (DNS) method [15]. The pH values were measured using a pH meter (Hanna, Romania). Total titratable acidity (TTA) was determined by titration sample with 0.1 N NaOH to pH 8.4 [7]. The moisture content was measured by drying samples in an oven at 105°C till reach to constant weight. Ash content was determined by burning the samples in a crucible at 600°C in a furnace for 2 hours [15]. Demineralization efficiency was calculated using the following Equation, where AO and AR are the ash concentrations (g/g) before and after fermentation and O and R are the mass (g) of original sample and fermented residue, respectively [16]:

$$\text{Demineralization}(\%) = \frac{A_o o - A_R R}{A_o o} \times 100 \qquad (1)$$

RESULT AND DISCUSSION

Effect of pH and TTA: Fig. 1 shows variation of pH and TTA with respect to incubation time. In all cases, pH had decreasing trend which was too sharp in first 48 hours. The microorganisms utilized carbon source by breaking down their structure and produce acid via hydrolysis [8, 17]. This phenomenon is responsible for the removal of mineral content by precipitating them as carbon lactate. Among different carbon sources, date syrup has reached to the lowest pH level (3.9) at the end of fermentation process. As it is obvious from Fig. 1, TTA graphs have reverse trend in compare to pH variations. This fact expressed that acid production was increased while the pH value decreased. The maximum TTA was observed when date syrup was used as it was expected due to its lowest pH value. The media with date syrup as carbon source produced more organic acids than other carbon sources as shown in Fig. 2. Because of the date syrup sugars composition are fructose and glucose with small portion of sucrose then it can be easily utilize in fermentation process [18].

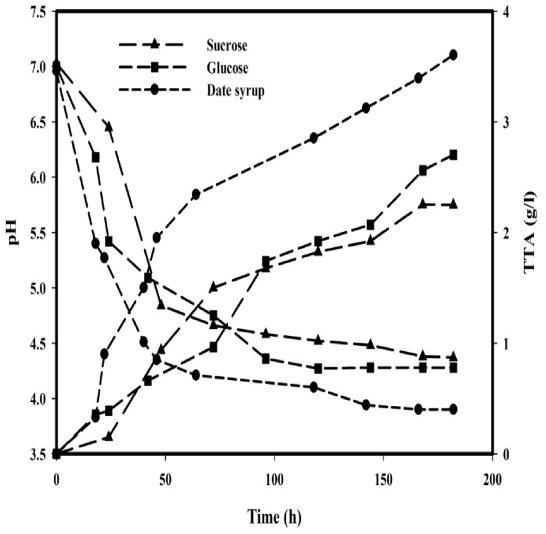

Fig. 1: Effect of incubation time on pH and TTA

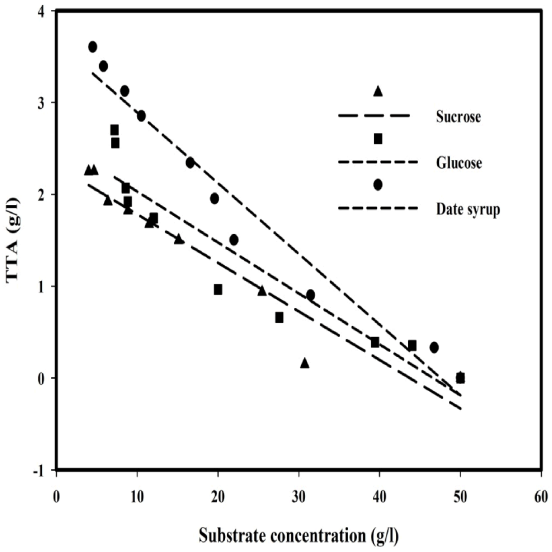

Fig. 2: Effect of substrate on TTA

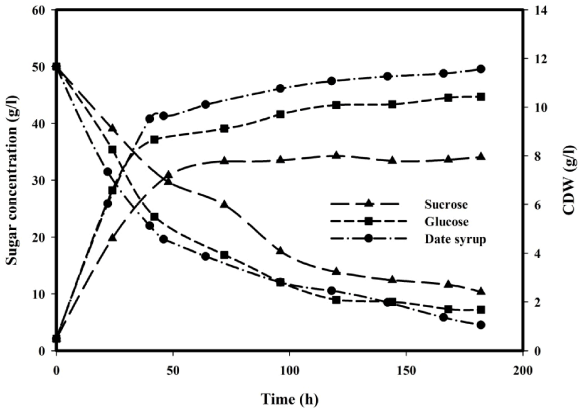

Fig. 3: Effect of incubation time on Substrate consumption and cell growth of *Lactobacillus plantarum*

Utilization of Substrate and Cell Growth: Substrates consumption and biomass production are shown in Fig. 3. As it can be observed, hydrolyzed date syrup was consumed much faster than sucrose and glucose. Moreover, the cell growth rate of medium containing date syrup media was more than two other mediums. The yields of biomass production (Equation 2) are 24, 23 and 18% for the media with date syrup, glucose and sucrose as carbon sources, respectively.

$$Y = \frac{X - X_o}{S_o - S} \quad (2)$$

Demineralization of Shrimp Shell: As it was described in the last section, reduction of pH and therefore production of acid in the media were responsible for the removal of minerals from shrimp shell powder. Figs. 1 and 2 showed that the amount of organic acid produced in a medium contained date syrup was more than the organic acids produced by glucose and sucrose. In order to determine the percentage of demineralization, ash content of chitins should be measured. Yields of demineralization process were 82, 75 and 71% for the media with date syrup, glucose and sucrose, respectively. The results showed that the highest yield was obtained when date syrup was used as carbon source.

Kinetic Studies: Substrates are consumed not only to supply the required energy but also for the cell growth and maintenance. The expression for substrate utilization with respect to time can be correlated by the first-order differential Equation described as follows:

$$\frac{dS}{dt} = K_s S \quad (3)$$

Where S is substrate concentration (gl^{-1}), *t* is incubation time (h) and K_s is the first order kinetic constant in (h^{-1}). Equation 4 was obtained by integrating Equation 3 where S_0 is the initial substrate concentration (gl^{-1})

$$S = S_0 \exp(-K_s t) \quad (4)$$

By taking natural logarithm from Equation 4, a linear model is obtained which is stated as follows:

$$Ln\left(\frac{S}{S_o}\right) = -K_s t \quad (5)$$

The data presented in Fig. 4 showed that the carbon source biodegradation has followed the first order kinetic model. The rate constants gradually decreased while

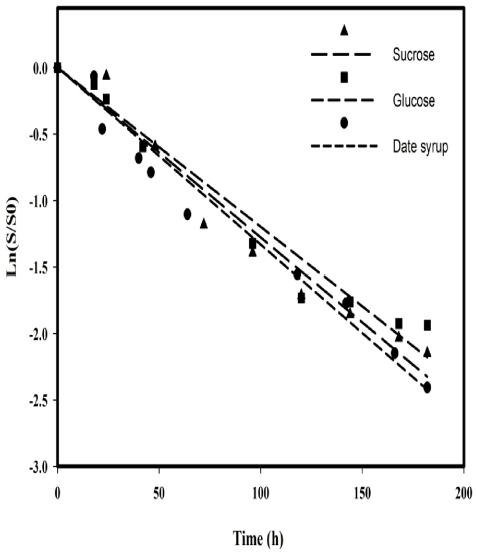

Fig. 4: Logarithmic model for substrate consumption

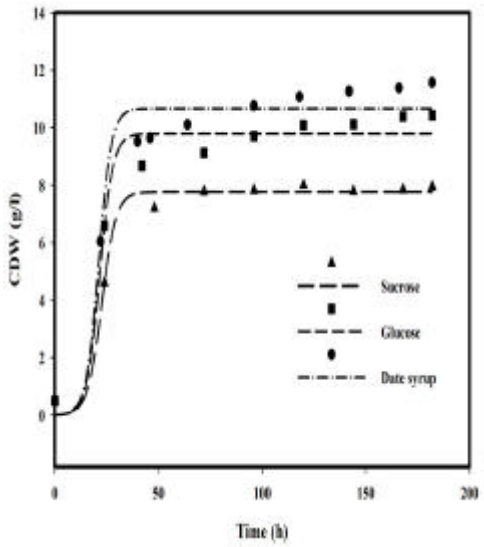

Fig. 5: Logistic kinetic model fitted with experimental data

the carbon source concentration was increased. The data were well fitted to a first order linear model with R^2 of greater than 0.95.

To evaluate quantitatively microbial growth kinetics, diverse mathematical models are used. In the present research work, in order to investigate growth kinetics, two different kinetic models, known as Logistic and Verhulst models were evaluated.

The growth rate of culture can be derived from Malthus law which relates cell growth to cell density as stated as follows:

$$\frac{dx}{dt} = mX \tag{6}$$

Where X is cell growth rate (glG^{1}), *t* is incubation time and μ is specific growth rate (hG^{1}) [19-20].

So far, various Equations have been proposed to support the microbial growth models. An example of these models is Logistic Equation which predicts lag phase, exponential growth rate and stationary cell population. The predicted specific growth rate by logistic model is described by the following Equation [19]:

$$m = m_{\max}\left(1\frac{X}{X_{\max}}\right) \tag{7}$$

Where, x_{max} presents the maximum cell dry weight concentration (glG^{1}). To obtain cell dry weight concentration; by substituting Equation 7 into Equation 6 and performing integration, the following Equation was obtained:

Table 1: Kinetic parameters

Carbon source	Sucrose	Glucose	Date syrup
Substrate consumption rate			
K_s (hG^{1})	0.013	0.012	0.013
R^2 (%)	0.957	0.962	0.97
Verhulst kinetic model			
μ_{max} ($g.lG^{1}.hG^{1}$)	0.080	0.097	0.085
x_{max} ($g.lG^{1}$)	8.824	10.87	12.330
R^2 (%)	0.911	0.978	0.972
Logistic kinetic model			
μ_{max} (hG^{1})	0.23	0.32	0.33
x_{max} ($g.lG^{1}$)	7.77	9.79	10.66
R^2 (%)	0.986	0.960	0.951

$$x = \frac{X_o \exp(m_{\max})}{1-\left(\frac{X_o}{X_{\max}}\right)(1-\exp(m_{\max}))} \tag{8}$$

Fig. 5 depicts a plot of logistic kinetic model with experimental data which were fitted well and followed the logistic model with R^2 value of higher than 0.95 in all carbon sources. The results showed that the regression analysis and kinetic parameters obtained were reasonably acceptable. Logistic model parameters such as maximum specific growth rate are summarized in Table1.

Verhulst kinetic model is another useful model which is a cell concentration dependent model. In this model maximum specific growth rate (μ_{max}) and maximum cell concentration (X_{max}) are kinetic parameters. Verhulst model is defined by the following Equation [21]:

$$m = m_{\max}\left(1-\frac{X}{X_{\max}}\right) \tag{9}$$

Kinetic parameters of the model are determined by plotting (μ) versus (X). The p lots of (μ) verse (X) for all carbon sources are depicted in Fig. 6. The kinetic parameters determined by the slope of (μ_{max}/X_m) and intercept of (μ_{max}) are summarized in Table 1. The obtained regression values for linear plot with different carbon sources were in the acceptable range (higher than 0.9).

Gompertz Model for Lactic Acid Kinetic Data: Gompertz model was used for the analysis of lactic acid production in demineralization process. This model is very similar to logistic model. The model is defined by the following Equation:

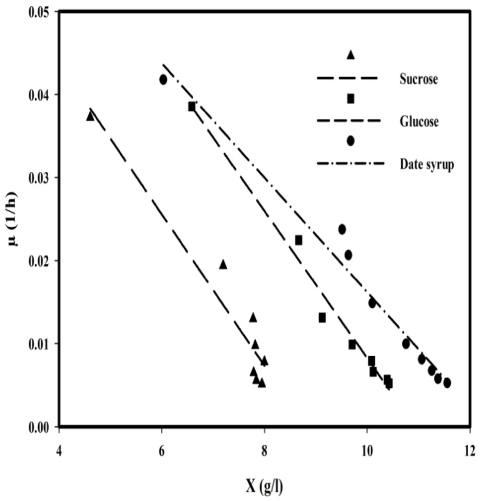

Fig. 6: Verhulst kinetic model fitted with experimental data

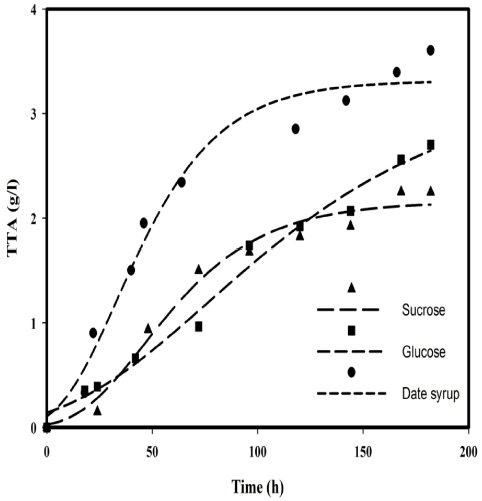

Fig. 7: Gompertz model fitted with experimental data of TTA

Table 2: Gompertz model parameters

	Sucrose	Glucose	Date syrup
P_{max}(g/l)	2.16	3.26	3.31
b (-)	4.41	3.13	3.43
k (1/h)	0.032	0.015	0.037
R^2 (%)	0.97	0.98	0.96
V_{max} (g/l/h)	0.025	0.018	0.045

$$P = p_{\max} \exp(-b\exp(-kt)) \quad (10)$$

Where P_{max} is the maximum product concentration, b is a constant for initial conditions and k is the acidification rate constant (Fig 7). The maximum rate of acid production was calculated by the following Equation [22]:

$$V_{\max} = 0.368\, kp_{\max} \quad (11)$$

The parameters for Gompertz model are shown in Table 2. In demineralization process, it was found that, acid production was maximized when date syrup as carbon source was used.

CONCLUSION

Demineralization of shrimp shell was successfully carried out by *lacatobacillus plantarum* with various carbon sources. Among three carbon sources (sucrose, glucose and date syrup) the highest demineralization yield (82%) and the lowest pH level were obtained in the media contained date syrup. Whereas extracted chitin with sucrose and glucose as carbon sources reached to 71 and 75% demineralization, respectively. Two cell concentration dependent kinetic models (Logistic and Verhulst) were well fitted with experimental data and the growth process followed the model. The regression value of both models for all carbon sources were reasonably acceptable (higher that 0.9). The maximum organic acid production was achieved using date syrup as carbon source.

ACKNOWLEDGMENT

Authors are thankful for the facilities provided in Biotechnology Research Center, Babol, Noushirvani University of Technology.

REFERENCES

1. Choorit, W., W. Patthanamanee and S. Manurakchinakorn, 2008. Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells. Bioresource Technol., 14(99): 6168-6173.
2. Ravi Kumar, M.N.V., 2000. A review of chitin and chitosan applications. Reactive and Functional Polymers, 1(46): 1-27.
3. Rinaudo, M., 2006. Chitin and chitosan: properties and applications. Prog. Polym. Sci., 7(31): 603-632.
4. Shirai, K., I. Guerrero, S. Huerta, G. Saucedo, A. Castillo, R. Obdulia Gonzalez and G.M. Hall, 2001. Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. Enzyme Microb. Technol., 4-5(28): 446-452.

5. Kim, W.J., W.G. Lee, K. Theodore and H.N. Chang, 2001. Optimization of culture conditions and continuous production of chitosan by the fungi, Absidia coerulea. Biotechnol. Bioprocess Eng., 1(6): 6-10.
6. Bautista, J.M., J. Jover, R. Gutierrez, O. Corpas, E. Cremades, F. Fontiveros, Iglesias, *et al*. 2001. Preparation of crayfish chitin by in situ lactic acid production. Process Biochem., 3(37): 229-234.
7. Oh, K.T., Y.J. Kim, V.N. Nguyen, W.J. Jung and R.D. Park, 2007. Demineralization of crab shell waste by Pseudomonas aeruginosa F722. Process Biochem., 7(42): 1069-1074.
8. Rao, M., J. Munoz and W. Stevens, 2000. Critical factors in chitin production by fermentation of shrimp biowaste. Appl. Microbiol. Biotechnol., 6(54): 808-813.
9. Zakaria, Z., G. Hall and G. Shama, 1998. Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. Process Biochem., 1(33): 1-6.
10. Jo, G., W. Jung, J. Kuk, K. Oh, Y. Kim and R. Park, 2008. Screening of protease-producing Serratia marcescens FS-3 and its application to deproteinization of crab shell waste for chitin extraction. Carbohydrate Polymers, 3(74): 504-508.
11. Sini, T., S. Santhosh and P. Mathew, 2007. Study on the production of chitin and chitosan from shrimp shell by using Bacillus subtilis fermentation. Carbohydrate Res.,16(342): 2423-2429.
12. Bhaskar, N., P. Suresh, P. Sakhare and N. Sachindra, 2007. Shrimp biowaste fermentation with Pediococcus acidolactici CFR2182: Optimization of fermentation conditions by response surface methodology and effect of optimized conditions on deproteination/demineralization and carotenoid recovery. Enzyme Microb. Technol., 5(40): 1427-1434.
13. Rao, M. and W. Stevens, 2006. Fermentation of shrimp biowaste under different salt concentrations with amylolytic and non-amylolytic Lactobacillus strains for chitin production. Food Technology and Biotechnol., 1(44): 83.
14. Oh, K.T., Y.J. Kim, N. Van Nguyen, W.J. Jung and R.D. Park, 2008. Effect of crab shell size on bio-demineralization with lactic acid-producing bacterium, Lactobacillus paracasei subsp. tolerans KCTC-3074. Biotechnol. Bioprocess Eng., 5(13): 566-570.
15. Thomas, L.C. and G.J. Chamberlin, 1980. Colorimetric chemical analytical methods. The Tintometer Ltd. Salisbury, England, pp: 85-87.
16. Jung, W., G. Jo, J. Kuk, Y. Kim, K. Oh and R. Park, 2007. Production of chitin from red crab shell waste by successive fermentation with Lactobacillus paracasei KCTC-3074 and Serratia marcescens FS-3. Carbohydr. Polym., 4(68): 746-750.
17. Sini, T.K., S. Santhosh and P.T. Mathew, 2007. Study on the production of chitin and chitosan from shrimp shell by using Bacillus subtilis fermentation. Carbohydr. Res., 16(342): 2423-2429.
18. Mostafazadeh, A.K., M. Sarshar, S. Javadian, M. Zarefard and Z.A. Haghighi, 2011. Separation of fructose and glucose from date syrup using resin chromatographic method: experimental data and mathematical modeling. Sep. Purif. Technol., pp: 72-78.
19. Najafpour, G.D., 2007. Biochemical engineering and biotechnology. Elsevier Science,
20. Bailey, J.E. and D.F. Ollis, 1986. Biochemical engineering fundamentals. McGraw-Hill Education,
21. Khavarpour, M., G.D. Najafpour and A.H.A. Ghoreyshi, 2011. Biodesulfurization of Natural Gas: Growth Kinetic Evaluation. Middle-East J. Scientific Res., 1(7): 22-29.
22. Cira, L.A., S. Huerta, G.M. Hall and K. Shirai, 2002. Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. Process Biochem., 12 (37): 1359-1366.