Indian Journal of Chemical Technology
Vol. 21, Sept-Nov 2014, pp. 386-391

Note

Effect of alkali treatment process parameters on semi refined carrageenan functional groups

S M Anisuzzaman[1]*, Awang Bono, Duduku Krishnaiah, Buhri Ariffin[2] & Ricky Lee[1]

[1]Chemical Engineering Programme, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

[2] Process Design Engineering – Physical Engineering, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

E-mail: anis_zaman@ums.edu.my

Received 3 August 2013; accepted 1 October 2014

Semi refined carrageenan (SRC) has been produced by alkali treatment from the seaweed Kappaphytus *alvarezii.* The effect of two alkali treatment process parameters; variation of potassium hydroxide (KOH) concentration and cooking temperature on SRC functional group has been investigated. The KOH concentration and temperature are in the range of 6-9%w/w and 60-80°C respectively at 60 min. The SRC powder is prepared using spray drying technique of SRC solution. The total absorption or compositions functional groups of SRC powder have been determined by Fourier transform infrared spectroscopy (FTIR). The analysis of experimental data shows that the investigated functional group composition of SRC produced is very much affected by KOH concentration. The predicted values of functional groups of sulphation, 3,6-anhydrous-G-galactose and D-galactose-4-sulphate are 1233.93, 928.41, 848.12 cm^{-1} respectively.

Keywords: Semi refined carrageenan, 3,6-Anhydrous-G-galactose, D-Galactose-4-sulphate, *Kappaphytus alvarezii*

Carrageenan is an important ingredient in the food manufacturing industries and widely used as a thickener, stabilizer or emulsifier[1,2]. It is produced from the seaweed through extraction process or grinding of dried seaweed. Semi refined carrageenan (SRC) is produced by alkali treatment of dried seaweed. It contains natural antioxidant compounds as well as nutritional materials, which can be utilized in a kaleidoscope of applications. For instance, the nutrients are vitamin, trace minerals, lipid, amino acid and antioxidant[3]. There are several types of carrageenans, such as kappa (κ), for iota (ι) and lambda (λ)[2,4]. The different types of carrageenan are predominantly developed by different seaweeds at different points in their respective life cycle[5]. Carrageenan is also the name initially given to a family of linear sulphated food grade polysaccharides obtained from red seaweeds (*Hypnea or Eucheuma* spp)[6]. The various family of polysaccharides however share a common galactan backbone of alternating 3-linked- β -D-galactopyranose (G-Unit) and 4-linked- β -D-galactopyranose (D-Unit)[7,8].

Carrageenan contains sulfate groups ($-OSO_3^-$) instead of hydroxyl groups as found in other seaweed products such agar. The fundamental structure of carrageenan consists of alternating (1-3)-linked β-D-galactopyranose and (1-4)-linked α-D-galactopyranose where galactose residues are partially sulphated at the positions of 2 and/or 6 and/or 3,6-anhydrided, depending or carrageenan type and which resulting on the relation of gelling properties. In the production of SRC, the dried seaweed or raw seaweed is subjected to certain process such as alkali treatment before it as dried and powderized. Alkali treatment is normally used for the gelling properties improvement[9,10]. During the treatment process, the hydroxide part of the reagent penetrates the seaweed and reduces the amount of sulphate in the carrageenan, which increases the 3,6-anhydrogalactose content[11]. The rising of 3,6-anhydrogalactose content in carrageenan improves the gel strength of carrageenan produced. The potassium part of the reagent combines with the carrageenan in the seaweed to produce a gel and this prevents the carrageenan from dissolving in the hot solution[12].

Literature shows that the extraction of carrageenan for 1 h heating at 50 to 70°C is more favourable to higher molecular weight and higher yield[9,12,13]. The variation and manipulation of process parameters in the alkaline treatment can also affect the functional groups on carrageenan such as the molecular weight and the chemical structure arrangement[14]. Spray drying is a method of producing a dry powder from a liquid or gel or slurry by rapidly drying with a hot gas. Spray drying is process involving the conversion of liquid feed into fine droplets exposing them to a hot drying systems[15]. A characteristic of liquid feed and dry air determines the properties of SRC powder produced. Hence, it is essential to optimize the process to obtain product with higher yield and quality[16,17].

Therefore, the present study is aimed to investigate the effect of alkaline treatment process parameters such as concentration of KOH and cooking temperature on the carrageenan functional group contents. In this investigation, three functional groups such as sulphation group, 3,6-anhydro-D-galactose and D-galactose-4-sulphate were selected. Besides that an optimization on selected process parameters was conducted to investigate the best conditions to get maximum content of certain functional group.

Experimental Section

Preparation of dry seaweed

Fresh seaweed was obtained from local farm and sun dried immediately after collection to protect from any degradation by micro-organism. Prior to the usage of sun dried seaweed, it was washed with distilled water in order to eliminate all epiphytes and encrusting material. Then the samples were chopped for about 10 to 20 mm in size for easy handling and to speed up drying rate. The chopping is also increased the surface area which helps to speed up the alkali penetration into the seaweed. Seaweed sample was then oven dried for 16 h at 60°C to avoid the decomposition of volatile components.

Alkali treatment of seaweed

Alkaline treatment was conducted by adding the oven dried seaweed sample into KOH solution and heated up in open cooker. The process parameters were set in the specific range. The KOH concentration and temperature were in the range of 6-9% w/w and 60-80°C respectively at 60 min. The solid loading to liquid solution was 25 g of seaweed sample and 400 mL of KOH solution. The alkali treated seaweed sample was then washed and dried for the SRC powder preparation.

Production of SRC powder

The carrageenan powder was prepared by spray drying of carrageenan solution. The solution was prepared by dissolving of dry SRC particle into distilled water and heated up to 90°C. The solid loading of dry SRC to distilled water was set at ratio of 1:18. The carrageenan solution was then fed into laboratory scale spray drier at flow rate of 8 mL/h. The hot air flow rate and the temperature were set at 40 m^3/h, and 130°C. The carrageenan powder produced was kept in air tight plastic container and stored in a desiccators containing silica gel before it was analyzed.

Determination of functional groups composition

A quantity of carrageenan powder was mixed with the KBr salt as suggested in the standard preparation of thin pellet of Fourier transform infrared spectroscopy (FTIR) analysis[18]. All IR spectra were recorded at room temperature (26°C ± 1°C) and the infrared spectra were recorded in the range of 450-4000 cm^{-1}. The resolution of the spectra was set at 4 cm^{-1} and the data spacing at 1.928 cm^{-1}. Each spectrum was displayed in terms of absorbance as calculated from the reflectance-absorbance spectrum using the OMNIC software.

Experimental design and Optimization

The experimental work was designed and arranged as central composite design (CCD) of response surface methodology (RSM). The process parameters chosen were set as the factors and the measured functional groups FTIR absorbance were set as responses. The experimental results were analyzed and optimized using Design-Expert® software version 7.0.0 (Refs 19, 20).

Results and Discussion

ANOVA analysis of the experimental data

The experimental arrangement was set to 20 runs with two factors and three responses. The KOH concentration and the cooking temperature were set as the factors, whereas the FTIR absorbencies of functional groups as the responses. The data of experimental arrangement and results are shown in Table 1.

The ANOVA analysis of the experimental data using Design Expert Package is presented as polynomial equations with actual factors of KOH concentration (A) and cooking temperature (B) for sulphation group (Eq. (1)), 3,6-anhydro-D-galactose group (Eq. (2)) and D-galactose-4-sulphate group (Eq. (3)). The equations obtained significant with respect to each factor towards the responses.

$$\text{Sulphation group}=1174.52–0.88A+1.70B+0.06AB-0.15A^2-0.01B^2 \quad \ldots (1)$$

$$\text{3,6-anhydro-D-galactose}=885.78+11.71A-0.02B-0.02AB– 0.71A^2 + 9.99e\text{-}04B^2 \quad \ldots (2)$$

$$\text{D-galactose-4-sulphate}=828.39+2.88A+0.28B–0.04AB–0.03A^2–2.69e\text{-}05B^2 \quad \ldots (3)$$

Effect of process parameters on SRC functional groups composition

The analysis of the effects of the changes of process parameters on composition of functional groups using Design Expert Package are presented in this section. Two parameters, factors interaction and 3D response surface plots were presented for each functional group.

The effect of process parameters on composition of sulphation group of SRC produced is presented in Fig. 1. It was observed that the sulphation group was increased with the increase of KOH concentration at elevated cooking temperature, whereas at lower cooking temperature, the sulphation group increased and formed a maximum before decreased as shown in Fig. 1 (a). The complete trend of the changes of sulphation group composition is due to the changes in process parameters as shown in Fig. 1 (b). The graphical presentation shows that the composition of sulphation group was significantly affected by the changes in process parameters such as cooking temperature and KOH concentration. However, at lower temperature the effect of KOH concentration on sulphation group composition is less significant

Table 1–Experimental run and the numerical results of the content of functional groups in SRC

Run	Factor A: Concentration of KOH (%w/w)	Factor B: Cooking temperature (°C)	Response 1: Sulphation Group (cm^{-1})	Response 2: 3,6-Anhydro-D-galactose (cm^{-1})	Response 3: D-Galactose-4-sulphate (cm^{-1})
1	6.00	60.00	1234.44	927.12	847.45
2	6.00	70.00	1233.04	926.34	848.16
3	6.00	70.00	1233.04	926.34	848.16
4	6.00	80.00	1229.20	927.58	848.85
5	6.00	80.00	1229.20	927.58	848.85
6	7.00	60.00	1228.92	928.77	848.08
7	7.00	60.00	1228.92	928.77	848.08
8	7.00	70.00	1234.94	928.49	847.97
9	7.00	70.00	1234.94	928.49	847.97
10	7.00	80.00	1232.30	928.20	848.14
11	8.00	60.00	1231.83	927.06	848.79
12	8.00	70.00	1233.46	928.22	848.10
13	8.00	70.00	1233.46	928.22	848.10
14	8.00	80.00	1234.92	927.97	847.26
15	8.00	80.00	1234.92	927.97	847.26
16	9.00	60.00	1233.41	927.00	848.12
17	9.00	60.00	1233.41	927.00	848.12
18	9.00	70.00	1232.75	926.80	847.72
19	9.00	70.00	1232.75	926.80	847.72
20	9.00	80.00	1232.38	926.07	847.57

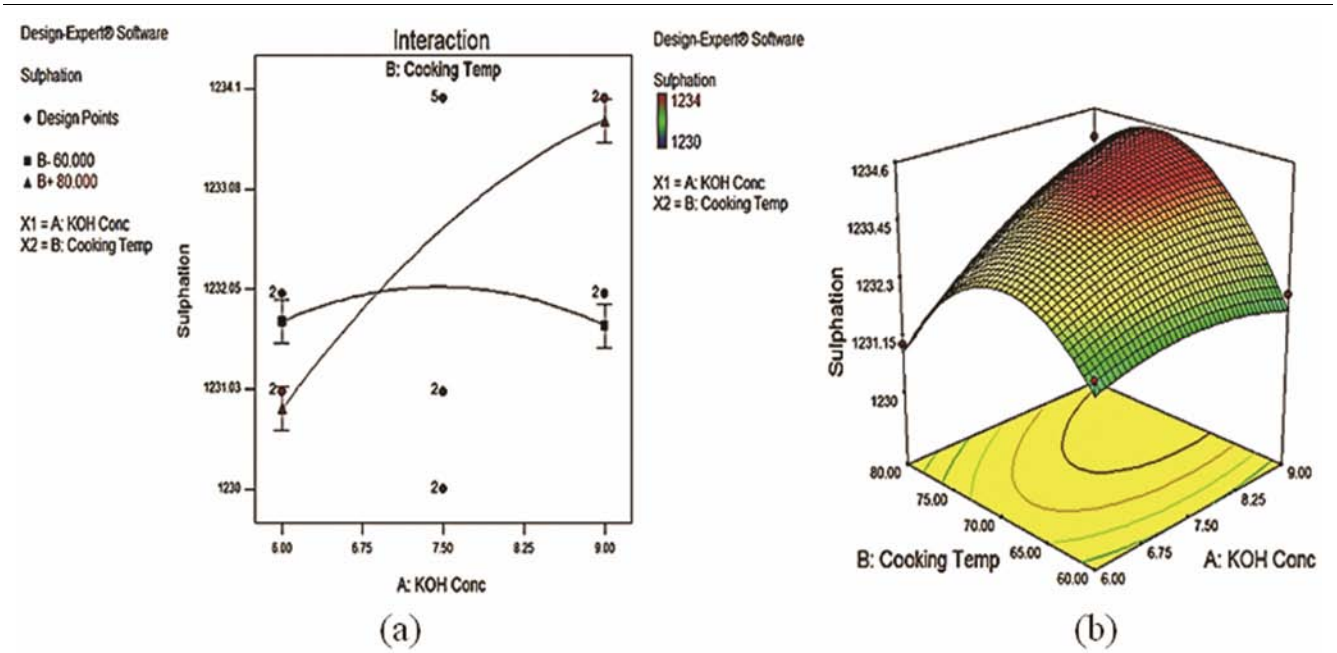

Fig. 1—Effect of concentration of KOH and cooking temperature (°C) on SRC sulphation group composition

compared to higher temperature. The variation of functional group is directly related to the gel properties of carrageenan. The observation on the variation of gel properties with the changes of processing parameters were reported previously[7].

Figure 2 represents the effect of process parameters on SRC composition of 3,6-anhydro-D-galactose functional group. It is observed that 3,6-anhydro-D-galactose composition increases and forms a maximum value before it decreased with the continuous increase in KOH concentration as shown in Fig. 2(a). The complete 3D graphical presentation of response surface for the process parameters of cooking temperature and KOH concentration is presented in Fig. 2(b). These figures show that the composition of 3,6-anhydro-D-galactose functional group in SRC produced was very much effected by the changes of KOH concentration but not the cooking temperature.

The effect of process parameters on the composition of SRC D-galactose-4-sulphate functional group composition is shown in Fig. 3. The

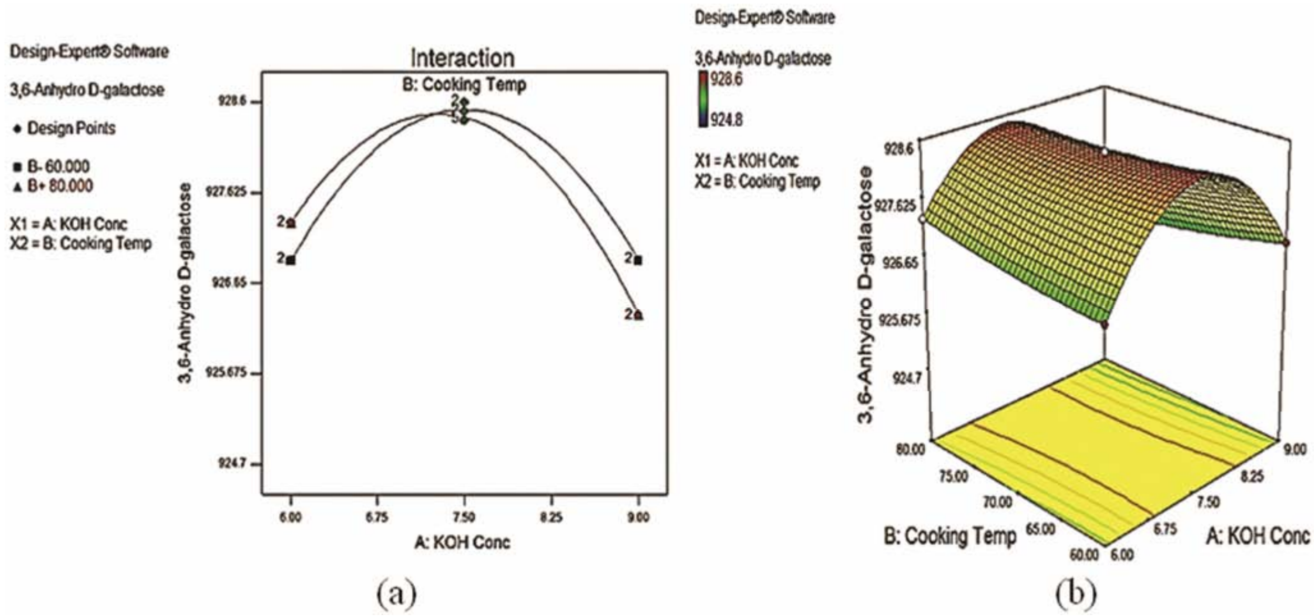

Fig. 2—Effect of concentration of KOH and cooking temperature (^{o}C) on the composition of SRC 3,6-anhydro-D-galactose functional group

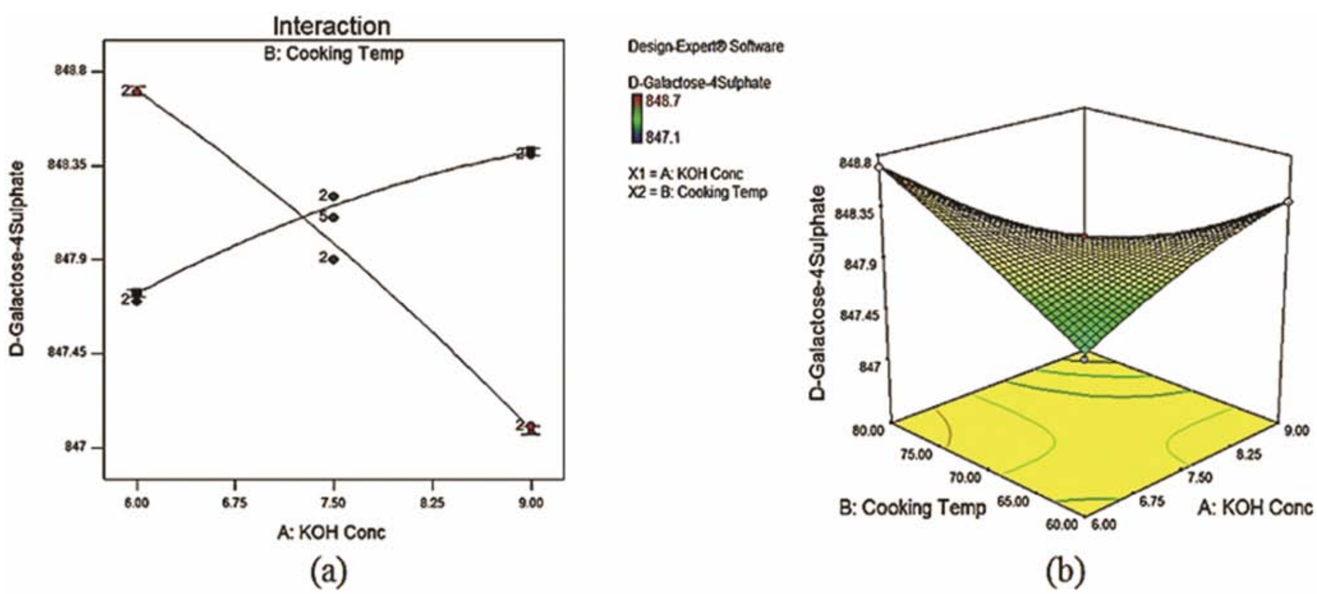

Fig. 3—Effect of concentration of KOH and cooking temperature (^{o}C) on the composition of SRC D-galactose-4-sulphate functional group

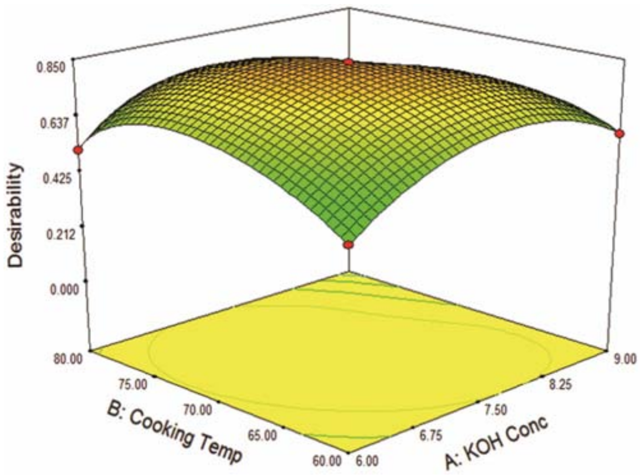

Fig. 4—3D response surface plot for the combined effect of KOH concentration and cooking temperature on desirability

composition of D-galactose-4-sulphate functional group is very much affected by the changes of cooking temperature and KOH concentration as shown in Fig. 3(a). The composition of D-galactose-4-sulphate functional group is increased with the increases of KOH concentration at lower cooking temperature whereas at higher temperature it behaved in opposite direction. The complete 3D response surface plot shows a clear twist on the composition of D-galactose-4-sulphate functional group as shown in Fig. 3(b).

Identification of process parameters for optimum or targeted composition of SRC functional group

With the setting up the experiment as CCD arrangement, the process parameters for the optimum or the targeted functional group composition can be identified. Setting up the investigated process parameters within the experimental range and the optimum condition of all SRC functional groups composition, the desirability of process parameters as a result of RSM analysis is shown in Fig. 4. The best optimum process condition for alkali treatment was found to be 7.35%w/w KOH concentration and 70.23°C cooking temperature. The numerical values of process parameters for the targeted SRC functional groups can also be obtained using similar analysis of RSM within Design Expert Software. The optimum value of functional group composition are 1233.93, 928.41, 848.12 cm^{-1} of sulphation, 3,6-anhydrous-G-galactose and D-galactose-4-sulphate respectively.

The best optimum process conditions for alkali treatment were found to be 7.35%w/w KOH concentration and 70.23°C cooking temperature. The numerical values of process parameters for the targeted SRC functional groups can also be obtained using similar analysis of RSM with Design Expert Software. The predicted values of functional groups of sulphation, 3,6-anhydrous-G-galactose and D-galactose-4-sulphate are 1233.93, 928.41, 848.12 cm^{-1} respectively.

Conclusion

The composition of functional groups in SRC plays an important role in the physical properties of the carrageenan gel. The control of the processing parameters such as cooking temperature and alkaline concentration are important in the determination of semi-refined carrageenan functional groups composition. The usage of statistical package, Design Expert software can simplify the experimental data in the determination of the targeted composition of SRC functional groups.

Acknowledgements

This research was fully supported by the Centre of Research & Innovation, Universiti Malaysia Sabah (Grant No. SLB0017-TK-1/2012) and is gratefully acknowledged.

References

1 McHugh D J, *A Guide to the Seaweed Industry. FAO Fisheries Technical Paper, T441 (*2003).
2 Campo V L, Kawano D F, da Silva Jr. D B & Carvolho I, *Carbohyd Polym,* 77 (2009) 167.
3 Gupta S, Cox S & Ghannam N A, *Food Sci Technol,* 44 (2010) 1266.
4 Webber V, de Carvalho S M & Barreto P L M, *Carbohyd Polym*, 90 (2012) 1744.
5 Rideout C S, Hill R, Bernabe M G & Markham, *U S Patent,* 813, 385, (1998).
6 Mehta A S, Mody K H, Iyer A & Ghosh P K, *Indian J Chem Technol,* 15 (2008) 45.
7 Bono A, Anisuzzaman S M & Ding O W, *J King Saud Uni - Eng Sci*, 26 (2014) 3.
8 Munoz J, Pelegrin Y F & Robledo D, *Aquaculture*, 239 (2004) 161.
9 Mishra D, Tripathy J & Behari K, *Carbohydr Polym*, *71* (2008) 524.
10 Anisuzzaman S M, Bono A, Susyana S, Ariffin B & Farm Y Y, *Development in Sustainable Chemical and Bioprocess Technology*, (Springer Book Series) (2013) 355
11 Mustapha S, Chandar H, Abidin Z Z, Saghravani R & Harun M Y, *J Sci Ind Res India,* 70 (2011) 865.
12 Kalinowski P, *A proposal to increase the value of farmed seaweed in Kaledupa,* (Sulawesi, Indonesia) (2007).
13 Montolalu R I, Tashiro Y, Matsukawa S & Ogawa H, *J Appl Phycol*, 20 (2007), 521.

14 Anisuzzaman S M, Bono A, Duduku K, Norazwienah A H & Hong Y W, *J Appl Sci,* 14 *(*2014) 1283.
15 Goula A M & Adamopoulos K G, *J Food Eng*, 78 (2005) 69.
16 Tonon R V, Brabet C & Hubinger M D, *J Food Eng*, 88 (2008) 411.
17 Bono A, Farm Y Y, Yasir S M., Arifin B & Jasni M N, *J Appl Sci,* 13 (2011) 2340.
18 Pereira L, Sousa A, Coelho H, Amado A M & Ribeiro-Claro P J A, *Biomol Eng*, *20* (2003) 223.
19 Bono A, Krishnaiah D & Rajin M, *Products and Process Optimization using Response Surface Methodology* (*Universiti Malaysia Sabah*, Malaysia) (2008).
20 Ahmad A L, Wong S S, Teng T T & Zuhairi A, *J Hazard Mater*, 145 (2006) 162.