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5   **Effect of polysaccharide chain conformation on ultrasonic degradation of curdlan in**

6   **alkaline solution**

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Abstract: This study was to investigate the effects of polysaccharide chain conformation on ultrasonic degradation of curdlan, a high MW β-glucan with wide applications. The ultrasonic degradation was performed in alkaline solution at 0.1 M and 0.3 M NaOH, in which the curdlan chain was mainly in triple helical conformation and random coil form, respectively. The degradation rate was represented by the kinetic model, $1/M_t - 1/M_o = kt$, with the rate constant k increasing with the ultrasonic power. The degradation rate was much higher in 0.3 M NaOH than in 0.1 M NaOH, suggesting that curdlan in random coil conformation was more liable to degradation than in helical conformation. Curdlan in 0.1 M NaOH was changed from triple helices to single helices and eventually to random coils with a higher solubility. In summary, ultrasonic degradation of curdlan in alkaline solution had a close and complex relationship to the chain conformation changes.

1. Introduction

Curdlan is an exopolysaccharide produced by *Agrobacterium biobar*, *Alcaligenes faecalis* and some other bacteria (McIntosh & Stanisich, 2005; Zhang & Edgar, 2014; Liu et al., 2015). It is a water-insoluble linear β-1,3-glucan and soluble in alkaline condition. Curdlan has a high molecular weight with an average degree of polymerization about 450 and up to 12,000 monosaccharide units (Futatsuyama et al., 1999). It has a major application in the food industry because of the favorable rheological properties and various bioactivities (Zhang & Edgar, 2014). A useful and unique property of curdlan is its ability to form two distinct types of

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thermo-gels in aqueous media, high-set gel and low-set gel network (Cai & Zhang, 2017). In high-set gel, the polymer chain is predominately in triple helical conformation, while single helix is predominant in low-set gel network. In an alkaline solution, the triple helix of curdlan chain unwinds and conformational transition from helical structure to random coil occurs with increasing NaOH concentration from 0.19 M to 0.24 M (Nakata et al., 1998). Chemical modifications such as carboxymethylation, sulfation, phosphorylation, oxidation and esterification have been applied to prepare curdlan derivatives with good water solubility as well as bioactivity to widen its application (Cai & Zhang, 2017).

Power ultrasound (US) has been exploited as a simple and direct means for acceleration or activation of a wide range of chemical and physical processes (Mason & Lorimer, 2002; Tao & Sun, 2015). Depolymerization of high molecular weight polysaccharides is a useful approach for generation of lower molecular weight fractions with lower viscosity, higher water solubility and better functionality. Compared with other techniques for polysaccharide degradation, including thermal, mechanical, oxidative, and hydrolytic and high-energy radiation, US is a clean, non-hazardous, non-thermal mechanical means, which is most favorable for processing food grade products (Gogate & Prajapat, 2015). Because ultrasonic degradation of polysaccharide is mainly caused by the fluid shear forces from collapsing cavitation, it may reduce the molecular weight by splitting the most susceptible chemical bonds, but not cause significant change in chemical structure (Huang et al., 2015; Prajapat et al., 2016; Čížová et al., 2015; Zhi et al., 2017).

The US degradation process and rate of polysaccharides can be affected by the structure and properties of polysaccharides to be degraded. Although many previous studies have been

done on ultrasonic degradation of polysaccharides, few have addressed the effects of chain

conformation on the degradation process (Gogate & Prajapat, 2015; Tao & Sun, 2015). There

is still no reported study on the application of power US for modification or degradation of

natural curdlan. This study aims to investigate the effect of chain conformation on the US

degradation of curdlan in alkaline solution with different NaOH concentrations and to analyze

the degradation kinetics and mechanism.

2. Materials and methods

2.1 Materials

Curdlan powder from *Alcaligenes faecalis* was purchased from Sigma-Aldrich, USA. The

powder was stored at 4^oC before use. Curdlan solution was prepared by dissolving the sample

powder in 0.1 M and 0.3 M sodium hydroxide (NaOH) solution for 2 hours at room temperature

with constant stirring. Aqueous solutions were prepared with ultrapure water from a Milli-Q

water purification system (Millipore, Bedford, MA, USA). Congo red was purchased from

Shanghai Yuan Ye Biotechnology, Ltd. (Shanghai, China). All other reagents were of analytical

grade unless otherwise specified.

2.2 Ultrasonic treatment conditions of curdlan

Ultrasonic treatment (degradation) of curdlan was performed with a Model VCX-130

ultrasonic processor of 20 kHz frequency and 130 W maximum power and a probe horn of 6

mm tip diameter (Soncis & Materials Inc., Newton, USA). The actual power at a set amplitude

was determined by the calorimetric method as described previously (Cheung & Wu, 2013). The

89   curdlan sample solution (25 mL and 2 g/L) was contained in a 50 ml plastic centrifuge bottle

90   and the ultrasound probe was immersed in the solution at a fixed depth of 2 cm. The sample

91   bottle was placed in an ice bath to maintain a relatively low temperature of $40 \pm 5^{\circ}C$ throughout

92   the US treatment. The US processor was operated at three different amplitudes of 20%, 50%

93   and 70%, corresponding to power intensities of 10.6, 34.3 and 48.4 W/cm^2 tip surface,

94   respectively. The treatment period was varied from 2 to 60 minutes, after which the US-treated

95   curdlan solution was taken for measurement of various properties.

96

97   **2.3 Molecular weight determination by intrinsic viscosity measurement**

98   Intrinsic viscosity [η] of curdlan solution in NaOH was determined by the dilution method

99   (Yang & Zhang, 2009) and the viscosity was measured using an Ubbelohde viscometer at

100   $25.0\pm0.1^{\circ}C$ in a water bath. The viscosity-average molecular weight (M_v) of curdlan was

101   determined according to Mark-Houwink equation,

102       $[\eta] = KM_v{}^a$, (Eq. 1)

103   where $K = 0.0079$ and $a = 0.78$ for curdlan dissolved in 0.3 M NaOH at 25 $^{\circ}C$ (Nakata et al.,

104   1998), or $K = 0.0032$ and $a = 0.85$ for curdlan dissolved in 0.1 M NaOH at 25 $^{\circ}C$ (Futatsuyama

105   et al., 1999).

106

107   **2.4 Ultrasonic degradation kinetic model**

108   The reduction of curdlan molecular weight (M_v) with time during the US was fitted to the

109   following kinetic model for ultrasonic degradation process of polymer originally derived by

110   Malhotra (1986),

111 $$\frac{1}{M_t} - \frac{1}{M_0} = kt \quad \text{(Eq. 2)}$$

112   where t is the sonication time (min) and k is the rate constant (mol/g·min) of the ultrasound

113   degradation process, and M_0, M_t are the viscosity-average molecular weights (mol/g) at time 0

114   and time t, respectively. The value of k at a given US power intensity (W/cm^2) was determined

115   by linear regression fit of experimental data according to Eq.2.

116

117   **2.5 Gel formation by acidification**

118   A weak gel is usually formed when curdlan in alkaline solution is neutralized with the

119   addition of acid, due mainly to the single and triple-helical conformation of curdlan polymer

120   chain (Saito et al., 1977; Kanzawa et al., 1987). For evaluation of the US effect on the solubility

121   of curdlan molecules in a neutral solution, 36% HCl was added at an equal mole to NaOH to

122   the US-treated curdlan solution immediately after the US treatment for neutralization. The gel

123   formed after the neutralization was separated from the liquid solution by centrifugation at 6000

124   rpm for 15 min, and then dried at 70°C for 2 days for weight measurement.

125

126   **2.6 Congo red-curdlan complex test**

127   Congo red test was a simple and useful measurement of the high-ordered conformation of

128   polysaccharide chain in alkaline solution (Saito et al., 1977; Ogawa & Hatano 1978). The test

129   was performed of the curdlan solution before and after sonication at various NaOH

130   concentrations. Congo red was mixed with curdlan of 0.2% in 25 mL NaOH solution from 0.05

131   to 0.5 M NaOH before and after various sonication periods at a power intensity of 48.6 W/cm^2.

132   The solution was maintained at room temperature for 1 h and the absorbance spectrum from

133   400-700 nm was recorded on a UV-Vis spectrometer for detection of the maximum absorbance

134   wavelength (λ_{max}).

135

136   **3. Results and discussion**

137   **3.1 Effect of ultrasonic power on intrinsic viscosity of curdlan**

138   Fig.1 shows the decrease of intrinsic viscosity of curdlan with sonication time in 0.1 M and

139   0.3 M NaOH. The initial intrinsic viscosity of curdlan on 0.1 M NaOH (4.25 dL/g) was higher

140   than that in 0.3 M NaOH (2.50 dL/g), due probably to the change from helical conformation of

141   curdlan chain in 0.1 M NaOH to random coil in 0.3 M NaOH. With the relatively low alkaline

142   concentration, short degradation period and mild temperature applied in ultrasonic degradaiton,

143   significant changes in the curdlan structure should be minimum other than conformation

144   changes. According to the literature, extensive degradation of polysaccharides such as cellulose

145   in low alkaline concentrations can only take place at relatively high temperature (over 100°C)

146   or over prolonged periods of days, months or years (VanLoon and Glaus, 1997; Pavasars,

147   Hagberg, Boren, & Allard, 2003).

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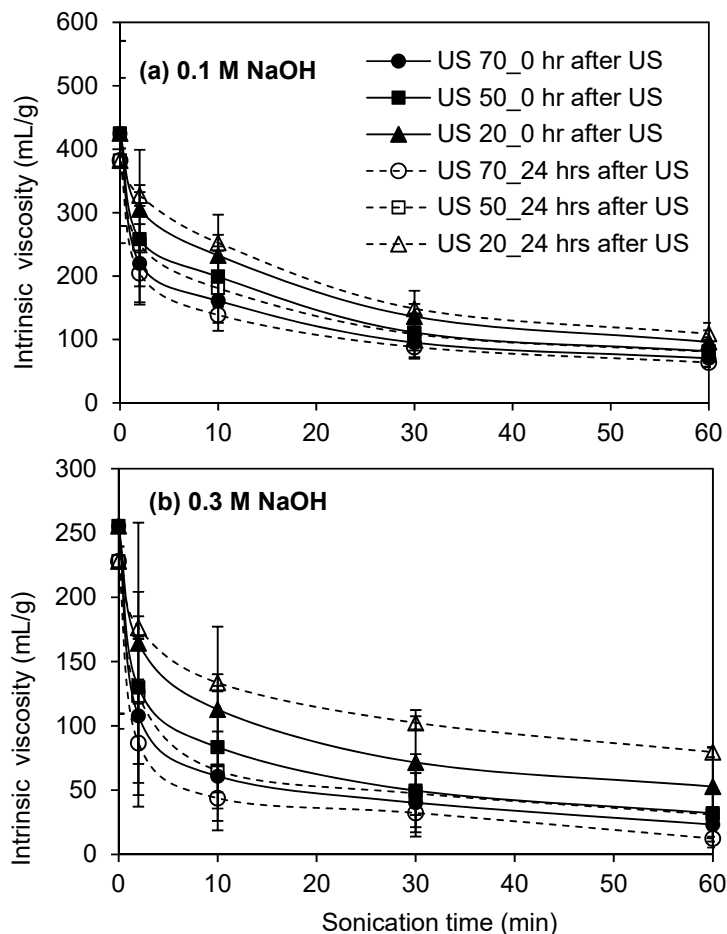
Fig. 1. Intrinsic viscosity change of curdlan solution with sonication time in (a) 0.1 M and (b) 0.3 M NaOH caused by ultrasound treatment at various power intensity (solid line: measured immediately after US treatment; dash line: measured 24 hours after US treatment).

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The intrinsic viscosity $[\eta]$ decreased rapidly within the initial 5-10 min and slowly in the later period of sonication. At 70% amplitude for instance, $[\eta]$ was reduced by about 70% in the fist 10 min and by about 90% in 60 min. The reduction of intrinsic viscosity was more rapid and significant at a higher US power intensity. The intrinsic viscosity value was not much different when the measurement was taken immedietely after sonication or 24 h later, suggesting an stable change by the US treatment. However, at a low US power intensity of 10.6

159   W/cm^2 correspoding to 20% amplitude the intrinsic viscosity measured 24 h later was slighty

160   higher than that measured immediately after US treatment.

161   Fig. 2 shows the variation of intrinsic viscosity with NaOH concentration in the curdlan

162   solution. The intrinsic viscosity of native curdlan decreased sharply with the increase in NaOH

163   concentration from 0.1 to 0.25 M and increased noticeably as the NaOH concentration

164   increased from 0.25 to 0.3 M. This intrinsic viscosity trend reflects the transition of

165   conformation from helical to random coil form (Nakata et al., 1998). In contrast, the curdlan

166   solution after 30 min US treatment had a much lower intrinsic viscosity and only changed

167   slightly with the increase in NaOH from 0.1 to 0.5 M, due mainly to the fact that the US treated

168   curdland was mainly present in random coil form. The conformational changes of curdlan with

169   US treatment and NaOH concentration were further examined in section 3.4.
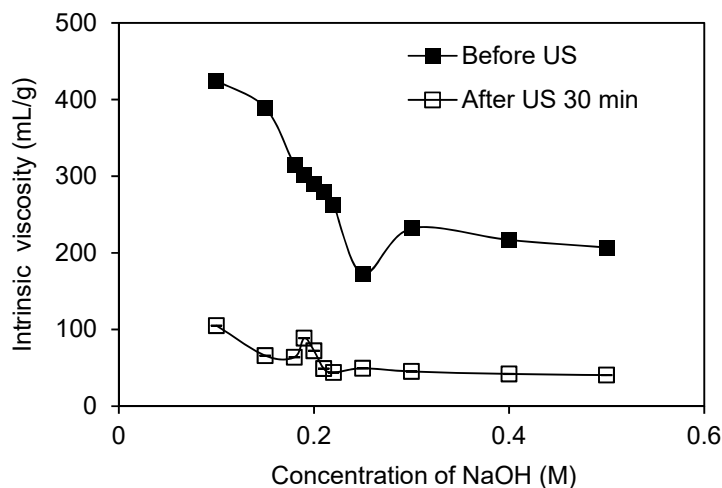
170

171   **Fig. 2.** Intrinsic viscosity change of native and US-treated curdlan with NaOH concentration

172   (curdlan 2 g/L; US intensity 48.4 W/cm^2 for 30 min).

173

174   **3.2 Ultrasonic degradation kinetics of curdlan**

175   Table 1 shows the viscosity-average molecular weight M_v of curdlan before and after

176   sonication in various conditions estimated from the intrinsic viscosity shown above. Table 2

177   shows the degradation rate constants and correlation coefficients derived by linear regression

178   fitting of the data of $1/M_v$ versus sonication time t to the kinetic model Eq.2. The rate constant

179   k increased with the US power intensity. In addition, k was much larger in 0.3 M NaOH than

180   in 0.1 M NaOH and it also increased with US power intensity more rapidly at 0.3 M NaOH.

181   This suggests that the random coil conformation of curdlan chain in 0.3 M NaOH is more

182   susceptible to degradation than the rigid triple helical conformation in 0.1 M NaOH.

Table 1. Viscosity-average molecular weight of curdlan in alkaline solution before and after ultrasonication (M_v estimated from measured [η] as described in section 2.3).

US intensity (W/cm^2)	US time (min)	M_v (0.1 M NaOH)	M_v (0.3 M NaOH)
(Original)	0	165,453	86,196
48.4 (US 70)	2	71,278	28,515
	10	47,697	13,692
	30	24,248	8,066
	60	16,500	3,984
34.3 (US 50)	2	87,309	36,220
	10	62,617	20,523
	30	29,590	10,547
	60	19,912	6,022
10.6 (US 20)	2	108,314	48,908
	10	76,345	30,186
	30	38,422	16,836
	60	24,596	11,415

Table 2. Degradation rate constant k (mol/g·min) and correlation coefficient (R^2) of curdlan under ultrasound treatment at different power intensity (W/cm^2).

US intensity (W/cm^2)	Rate constant, k (mol/g·min)		Correlation coefficient (R^2)	
	0.3 M NaOH	0.1 M NaOH	0.3 M NaOH	0.1 M NaOH
48.4	0.0040	0.0010	0.932	0.982
34.3	0.0026	0.0008	0.977	0.952
10.6	0.0014	0.0006	0.971	0.914

3.3 Effect of US on gel formation and solubility of curdlan in neutral condition

After sonication, the amount of gel formed by neutralization of the alkaline curdlan solution

191   was decreased (Fig.3). In other words, solubility of curdlan molecules in neutral solution was

192   increased by US treatment. The decrease in the gel formed in 0.3 M NaOH was more significant

193   than that in 0.1 M NaOH. In the alkaline solution of curdlan, gel forms by curdlan chains

194   through interaction with the sodium chloride salts (Saito *et al.*, 1990) so that more gels could

195   be formed at a higher NaOH concentration in which more sodium salts were formed during

196   acidification.
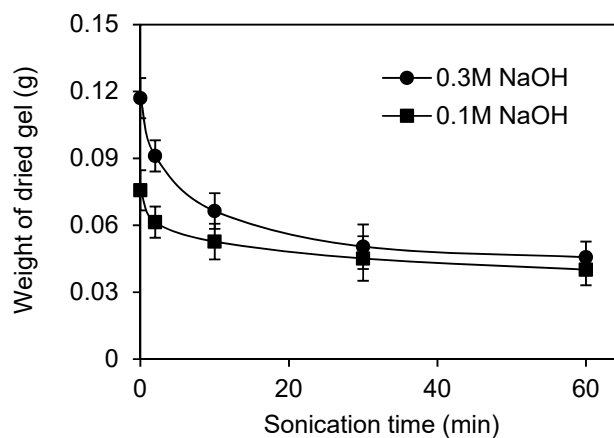
197
198   **Fig. 3.** Variation of the amount of gel formed by acidification for the curdlan solution (2 g/L)

199   after US treatment at 48.4 W/cm^2.

200

201   **3.4 Effect of US on curdlan chain conformation**

202   Fig.4 shows the results of Congo red test on curdlan before and after sonication. In the

203   NaOH concentration range 0.05-0.2 M, the λ_{max} of Congo red-curdlan solution was all higher

204   than Congo red solution. The increase in the absorption maximum or the red shift is attributed

205   to the formation of complex between Congo red molecules and the single helical structure of

206   the glucan chain (Ogawa & Hatano, 1978). Therefore, single helices existed in the curdlan

207   solution at NaOH concentrations below 0.2 M. The λ_{max} value of sonicated curdlan was lower

208   than that of the Congo red mixed with the native curdlan solution at a higher NaOH

209   concentration than 0.15 M, due probably to the disruption of the single helices, which hindered

210   the complex formation between the curdlan and Congo red molecules. In 0.1 M NaOH solution

211   when the curdlan polymer chain was mainly in triple-helix conformation, the corresponding

212   λ_{max} value of the US-treated curdlan attained a maximum at 30 min of sonication (Fig. 5). The

213   result suggests that the amount of single helices was increased in the curdlan solution after

214   sonication. However, a sharp decrease of the λ_{max} was detected after much longer period of

215   sonication for 60 min, due probably to the severe destruction and loss of the single helix
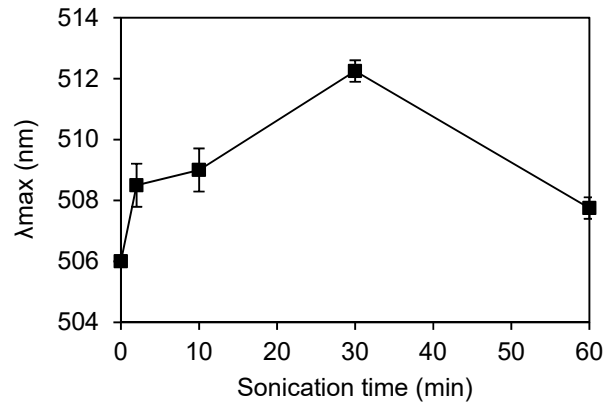
216   conformation.

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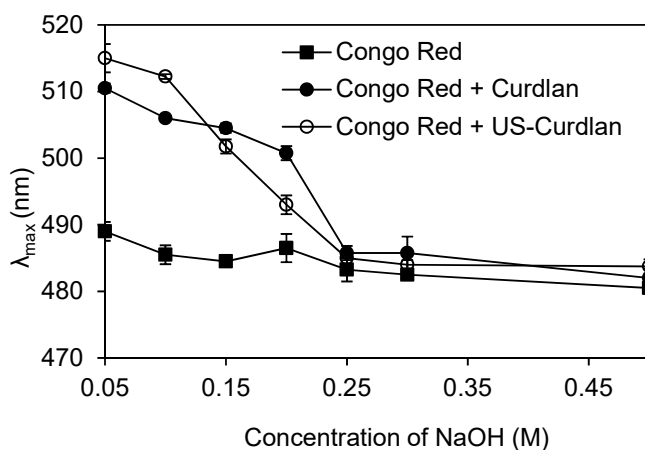
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220   **Fig. 4.** Variation of λ_{max} of Congo red solution alone (Congo red), Congo red with native
221   curdlan sample (Congo red + curdlan), and Congo red with sonicated curdlan at 48.4 W/cm^2
222   for 30 min (Congo red + US-Curdlan) at various NaOH concentrations.

223

Fig. 5. Variation of λ_{max} with sonication time of US-treated curdlan in the Congo red test (US intensity 48.4 W/cm^2 at 70% amplitude; curdlan in 0.1 M NaOH solution).

3.5 General discussion

Curdlan is a linear β-1,3-glucan and soluble in alkaline solution, and forms gels during acidification of the alkaline solution. The curdlan polymer chain is mainly present in single and triple helix conformation at low concentration of alkaline (0.01-0.19 M NaOH) and the helical structure can be disrupted, and unwounded into random coil structure at high alkaline concentrations (> 0.25 M NaOH) (Nakata et al., 1998; Zhang et al., 2003; Ogawa et al., 1972). High-intensity US can also cause disruption of the curdlan backbone in an alkaline solution, leading to a higher flexibility of the backbone (Gogate & Prajapat, 2015; Yan et al., 2015). Therefore, the triple helix structure is initially degraded into triplex or duplex loops and finally into random coils. The loose loops and random coils of curdlan chains have weaker intra- and inter-molecular interactions such as hydrogen bonds with the interstitial water molecules for the formation of micelles in the solution. Consequently, the amount of micelles decreases and the amount of random coil chains increases with prolonged US treatment. The intra/intermolecular hydrogen bonds between backbones are also weakened by increasing

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242 alkaline concentration. Curdlan in random coil conformation is more susceptible to the

243 ultrasonic degradation, as evidenced by the higher degradation rate constant k and more rapid

244 increase with the ultrasonic intensity in 0.3 M NaOH than in 0.1 M NaOH.

245   The notable decrease of viscosity with increasing US power and sonication time is

246 attributable to the conformation change of curdlan chain from relatively rigid triple helix to

247 more flexible single helix and random coil in 0.3 M NaOH solution. The Congo red test

248 provided direct evidence for the conformation changes. With the decrease of molecular weight

249 by ultrasonic degradation, more hydroxyl groups are exposed in solution, resulting in a higher

250 charge-to-mass ratio and stronger electrostatic interaction. The bound water per molecule also

251 increases, leading to higher water solubility and reduced gel formation in neutral condition.

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253

254 **4. Conclusions**

255   Power US was proven a simple and effective means for partial degradation of curdlan in

256 aqueous alkaline solution. The degradation rate was dependent on the alkaline (NaOH)

257 concentration, being higher at a higher NaOH concentration. Ultrasonic degradation of curdlan

258 caused conformational changes of the polysaccharide chain as well as reduction of intrinsic

259 viscosity and molecular weight. It is of interest to further investigate the sites of chain cleavage

260 occurring in the curdlan chain by ultrasonic degradation and to characterize the specific

261 structure of US-degraded curdlan.

262
263
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