

Communication

Bacterial Inactivation Kinetics of Dialdehyde Starch Aqueous Suspension

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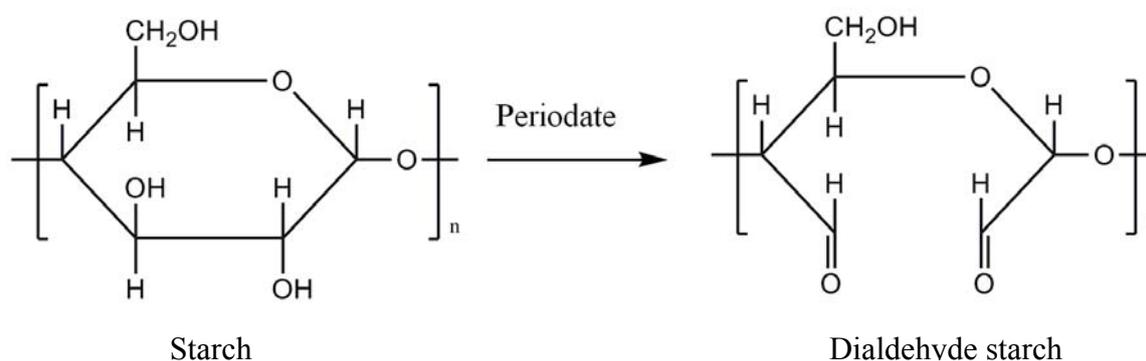
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Abstract: The bacterial inactivation kinetics of dialdehyde starch (DAS) aqueous suspension was studied by the relationship between the minimal lethal concentration (MLC) and the inactivation time at four different temperatures. The relationship between MLC and exposure time was found to follow the first-order Chick-Watson law. This first-order inactivation kinetics was modeled by pseudo-first order chemical reaction. This model was validated by the successful predication of the bacterial inactivation response at room temperature.

Keywords: antibacterial; bacteria; dialdehyde starch; inactivation kinetics; pseudo-first order chemical reaction

1. Introduction

Dialdehyde starch (DAS) is a starch derivative, prepared by the selective periodate oxidation of starch with the introduction of the dialdehyde functions, as shown in Figure 1 [1].

Figure 1. Periodate oxidation of starch to generate dialdehyde starch.

As a polymeric dialdehyde with the dialdehyde functionality similar to glutaraldehyde, DAS has been employed in the paper, textile and leather industrial applications as a crosslinking agent [2,3]. Unlike the high toxicity of glutaraldehyde [4], DAS has been reported to have very low toxicity [5-8]. The oral acute toxicity of 10 wt % DAS (100% oxidized) aqueous suspension was reported to be $LD_{50} \geq 6,800$ mg/kg in rats [8]. DAS has also been studied in biomedical applications such as a drug delivery carrier, a stent surface modifier to enhance protein absorption, and an absorbent to remove urea [9-11]. Compared to the well-studied antimicrobial activity of glutaraldehyde [12], the antimicrobial study of DAS has been limited. An early US patent described a method to prepare an antibacterial surface by incorporation of DAS granules to inhibit the growth of gram-positive bacteria [13]. In recent studies, DAS aqueous suspension has been found to show significant antimicrobial activities [14,15]. As a novel polymeric biocide, DAS is considered for its potential antibacterial application. It is important to understand the bacterial inactivation kinetics of DAS.

Bacterial inactivation kinetics depends on the type of the bacteria, type and concentration of the biocide as well as the associated inactivation mechanisms, and the environmental conditions such as the temperature and pH. Analysis and interpretation of bacterial inactivation kinetics, in general, are complicated. Survival curves, *i.e.*, the log reduction of bacteria *versus* exposure time are often plotted to interpret the bacterial inactivation kinetics [16]. Mathematical models of the inactivation kinetics have been reviewed, and some modified models recently have been proposed [16-19]. The fundamental concept of the inactivation kinetics is still based on the Chick-Watson Law, *i.e.*, $C^n \times t = K$, where C = concentration of biocide, n = coefficient of dilution, t = exposure time required to obtain a given level of inactivation, and K = empiric parameter that varies with biocide, bacteria and environmental condition [16].

In our previous study of the antibacterial activity of DAS aqueous suspension, the dominant inactivation activity was found from its dialdehyde functions, and the reactivity of the dialdehyde functionality was pH dependent [15]. The pH values of the as-prepared DAS aqueous suspensions in the studied concentration range were close to 3. The objective of this study was to investigate and model the bacterial inactivation kinetics of the as-prepared DAS aqueous suspension with a fixed pH at 3. Instead of recording the survival bacterial concentration *versus* exposure time, we chose to determine the relationship between the exposure time and the minimal lethal concentration (MLC) of DAS aqueous suspension in the antibacterial test. MLC in this study was defined as the lowest

concentration of DAS aqueous suspension to obtain no bacterial colony growth in the agar-plate method (for details see the experimental section).

2. Results and Discussion

2.1. DAS Concentration Change during Bacterial Inactivation

With its dialdehyde functions similar to glutaraldehyde, the DAS concentration in aqueous suspension can be determined using the same analytical method developed for the glutaraldehyde concentration in aqueous solution, *i.e.*, from the UV absorbance of its aldehyde functions at 235 nm [20]. The DAS aqueous suspension in our study exhibited absorbance maxima at 238 nm in its UV spectra. The relationship between the DAS concentration and its absorbance at 238 nm was found to be linear, and this linear relationship was employed to calculate the DAS concentration before and after the bacterial inactivation. The DAS concentration change during exposure time was negligible as illustrated in Table 1. This result indicated that the DAS was in excess during the inactivation. Similar results were also reported in the antibacterial study of glutaraldehyde [21], the uptake of glutaraldehyde by bacteria was found to be low compared to the bulk glutaraldehyde concentration.

Table 1. Dialdehyde starch (DAS) concentration during bacterial inactivation at room temperature.

Sample	Exposure time (h)	Absorbance at 238 nm	Calculated concentration (%)
0.35% DAS	-	0.2600	0.33
0.35% DAS w/ <i>S. aureus</i>	0	0.2589	0.33
	8	0.2578	0.33
0.7% DAS	-	0.5290	0.68
0.7% DAS w/ <i>E. coli</i>	0	0.5283	0.68
	10	0.5231	0.67

2.2. Bacterial Inactivation Kinetics

The storage temperature of the bacterial suspension and the inoculation temperature of bacteria in our study are 4 and 37 °C respectively. The test temperatures in our bacterial inactivation study were selected within the range of storage and inoculation temperatures, *i.e.*, 4, 14, 23 and 34 °C. The MLCs of the DAS aqueous suspensions against *E. coli* and *S. aureus* with various exposure times at these temperatures were illustrated in Figure 2(a,b). The obtained experimental data were fitted well by the equations in Table 2. The equations in Table 2 have general format as: $MLC \times t = \text{constant}$. The constant k was bacterial strain and temperature dependent. The DAS aqueous suspension was more effective against Gram-positive bacterium *S. aureus* than the Gram-negative bacterium *E. coli*. An increasing temperature accelerated the bacterial inactivation of the DAS aqueous suspension. The constants of the equations in Table 2 were used in the Arrhenius equation [$k = Ae^{(-E_a/RT)}$] to calculate the activation energy (E_a) of the DAS aqueous suspension against bacteria. The calculated activation energies were 16.6 and 17.4 kcal/mol for *E. coli* and *S. aureus* respectively, as listed in Table 2. These values were quite similar for the studied Gram-negative/positive bacteria and were very close to the

activation energy of glutaraldehyde against the *Bacillus subtilis* spore (20 kcal/mol) reported in the literature [22]. The similarity of the activation energy suggests that the inactivation mechanism of the DAS aqueous suspension is independent of the bacteria and may follow the similar inactivation mechanism as glutaraldehyde.

Figure 2. Minimal lethal concentration (MLC) of dialdehyde starch aqueous suspension against *E. coli* (a) and *S. aureus* (b) versus exposure time at four different temperatures, experimental data, × 4 °C, □ 14 °C, Δ 23 °C, ◇ 34 °C, and the fitted dot lines calculated from the equations in Table 2.

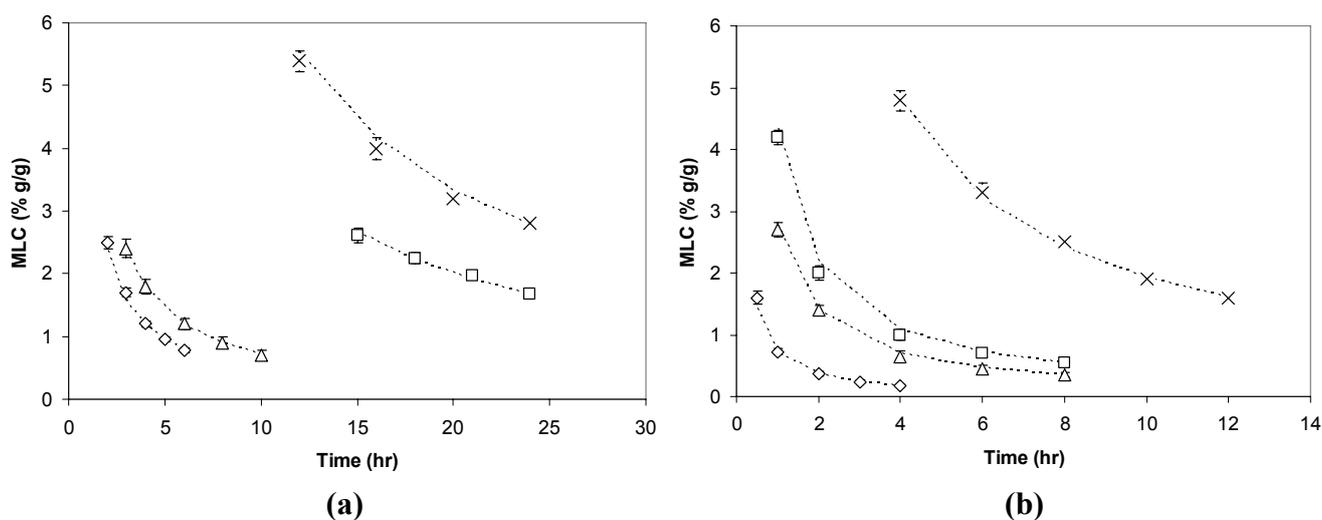


Table 2. Kinetic parameters of the dialdehyde starch aqueous suspension against bacteria.

Temperature (°C)	Kinetic equation	
	<i>E. coli</i>	<i>S. aureus</i>
4	MLC × t = 66.67	MLC × t = 19.23
14	MLC × t = 40.00	MLC × t = 4.35
23	MLC × t = 7.14	MLC × t = 2.78
34	MLC × t = 4.76	MLC × t = 0.72
Activation energy (kcal/mol)	16.6	17.4

The Chick-Watson model, *i.e.*, Equation (1), is generally accepted as the fundamental concept to describe the bacterial inactivation kinetics [16]. In many cases, the n value for Chick-Watson law is close to 1²³. Equation (1) can then be simplified to the first order kinetics Equation (2).

$$C^n \times t = K \tag{1}$$

$$C \times t = K \tag{2}$$

Ideally, under carefully controlled conditions, bacterial inactivation by chemical disinfectant may follow the first-order kinetics [23]. In practice, deviations from the first-order kinetics are caused by a number of factors, including bacterial growth conditions, bacterial aggregation and association with particulate materials in the suspension to affect the accessibility of a biocide to the targeted bacteria, biocide concentration change during inactivation and the effect of temperature and pH on the effective

biocide concentration [16,23]. Various models based on Chick-Watson law have been developed to fit the experimental results [16].

The aforementioned factors on the deviations from the first-order kinetics were limited in the current study. The concentration of the DAS was found to be in excess during the inactivation (Table 1). The physical states of bacteria (fresh prepared bacterial suspension) and the biocide (DAS aqueous suspension) remained the same in the selected test conditions. No other particulate materials were introduced in the test medium to affect the accessibility of DAS to bacteria. Though the antibacterial activities of glutaraldehyde and DAS have been reported to be pH-dependent [15,24], the pH was fixed at 3.0 in the test here. With these conditions, the bacterial inactivation kinetics of DAS aqueous suspension might be expected to follow the linear inactivation kinetics, which was confirmed in Table 2.

Inactivation of microorganisms by chemical disinfectants can be considered as a first-order chemical reaction [16,23]. During derivation of the Chick's law [Equation (2)], Chick observed that inactivation of bacteria by chemical disinfectant was analogous to an elementary bimolecular chemical reaction, in which the individual bacteria and chemical disinfectant represent the reactants [16,23]. The inactivation mechanism of the DAS aqueous suspension against bacteria has not investigated in the current kinetic study of bacterial inactivation. However, based on the similar dialdehyde functions between glutaraldehyde and DAS, it is reasonable to postulate that the bacterial inactivation of the DAS aqueous suspension follow the similar inactivation mechanism as glutaraldehyde, which involves strong association of dialdehyde functions with the reactive sites of bacteria [12,24]. This assumption is further supported by the inactivation kinetics study of glutaraldehyde in the literature. A linear inactivation kinetics of glutaraldehyde against *Bacillus subtilis* spore was observed and the obtained activation energy (20 kcal/mol) was very close to the calculated activation energy of the DAS aqueous suspension against bacteria here (Table 2) [22,25]. Based on the aforementioned inactivation assumption, the bacterial inactivation kinetics of the DAS aqueous suspension was modeled by Chick's chemical reaction approach.



By applying chemical reaction kinetics, the change of bacterial concentration during inactivation can be expressed as:

$$d[\text{Bacterium}]/dt = k[\text{DAS}][\text{Bacterium}] \quad (4)$$

The concentration of DAS was in excess during inactivation. This would give a pseudo-first order chemical reaction. After integration, one obtains:

$$\text{Log}[\text{Bacterium}]_t/[\text{Bacterium}]_0 = k[\text{DAS}] \times t \quad (5)$$

This can be rewritten as:

$$\text{Log reduction} = k[\text{DAS}] \times t \quad (6)$$

With initial bacterial concentration at 10^7 CFU/mL (colony forming unit), seven log reduction was considered to be the complete inactivation. Replacing the concentration of DAS by MLC, one obtains:

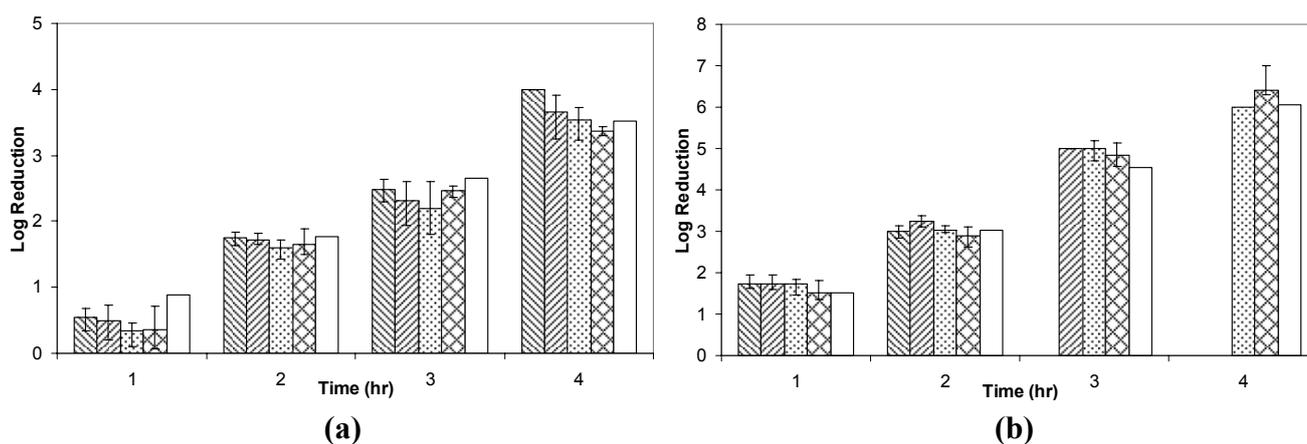
$$\text{MLC} \times t = 7/k \quad (7)$$

Equation (7) is the same format of the bacterial inactivation kinetic equations listed in Table 2. By comparison Equation (7) to the obtained experimental kinetic equations in Table 2, for the constant k in Equation (6) at 23 °C, we found $k = 0.98$ and $k = 2.52$ for *E. coli* and *S. aureus* respectively

The obtained mathematical model [Equation (6)] predicates that the log reduction of a test bacterium is the same for various initial bacterial concentrations when the exposure time, temperature and the concentration of the DAS aqueous suspension are the same. It also provides the time response of the bacterial inactivation by the DAS aqueous suspension with a known concentration.

A series of experiments were carried out to validate Equation (6). The initial bacterial concentrations were varied for a known concentration of the DAS aqueous suspension. The log reductions were recorded periodically with exposure time at 23 °C up to four hour. The obtained experimental results were compared to the calculated results from Equation (6) as shown in Figure 3. The selection of the DAS concentration in the validation was based on the statistically reliable count of the colony numbers with exposure time up to four hours at 23 °C. A lower DAS concentration was selected in the *S. aureus* test, as higher inactivation kinetic constant of DAS against *S. aureus* was found. Even with the lower concentration of the DAS aqueous suspension, when the initial *S. aureus* concentrations were at 10^4 CFU/mL and 10^5 CFU/mL, no bacterial colony was found in the agar-plate method at three and four hour exposure respectively. The results for these conditions were not presented in Figure 3(b). The calculated results predicated the experimental results of *S. aureus* and *E. coli* very well. This observation strongly supports the validity of the model for the bacterial inactivation kinetics of the DAS aqueous suspension.

Figure 3. Comparison of the experimental results of 0.9% dialdehyde starch aqueous suspension against *E. coli* (a) and 0.6% dialdehyde starch aqueous suspension against *S. aureus* (b) in various initial bacterial concentrations with the calculated results from Equation (6). ▨— 10^4 CFU/mL, ▩— 10^5 CFU/mL, ▤— 10^6 CFU/mL, ▥— 10^7 CFU/mL, □—Calculated.



3. Experimental Section

DAS was purchased from Sigma (P9265). The as-received DAS were amorphous granules with 90% oxidation extent [15]. In our study, the as-received DAS was used without further purification. Phosphate buffer saline (PBS) working solution (pH = 7.4) was prepared in house, and the

recipe for its preparation can be found in the previous publication [15]. All the PBS working solutions employed in the antibacterial study were autoclaved for sterilization.

DAS aqueous suspension was prepared by mixing the selected amount of DAS in the sterilized deionized water at 90–95 °C for two hours in an oil bath with reflux. The details of the preparation can be found in the earlier publication [15]. Different concentrations of the DAS aqueous suspensions were prepared from dilution of the as-prepared DAS aqueous suspensions in the sterilized deionized water. The pH of the DAS aqueous suspensions at difference concentration was close to 3. The final pH of the DAS test media were adjusted to 3.0 in the antibacterial kinetics study.

The bacterial strains employed were the Gram-negative bacterium, *Escherichia coli* (*E. coli*) C3000 ATCC 15597 and the Gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*), a laboratory strain received from the Department of Microbiology at the University of Florida. The bacterial suspensions were prepared according to the procedure by Kim *et al.* [26]. The bacteria were inoculated in Columbia broth overnight at 37 °C with constant agitation under the aerobic condition. The bacterial cells were collected by centrifugation at 500 g RCF (relative centrifugal force) for 10 min at 4 °C and washed three times with sterilized deionized water. The bacterial pellet was resuspended in sterilized deionized water after a final wash to obtain a $1\text{--}2 \times 10^9$ CFU/mL (colony forming unit) concentration.

The antibacterial tests were carried out by adding 0.1 mL bacterial aliquot into 9.9 g test medium with various DAS concentrations. PBS working solution (0.9 g) was included in the 9.9 g test medium. The pH of test medium was adjusted to 3 by drops of 0.1N HCl. PBS working solution was included to keep the pH constant at 3 during the antibacterial test. The mixtures were magnetically stirred for a specific time. The experiments were conducted in an incubator with the temperature kept constant at the set temperature. The sterilized deionized water (9 g) with PBS working solution (0.9 g), but without DAS was selected as the control test in our antibacterial study. At the selected temperatures, the specified exposure time had insignificant effect on the bacterial viability for the controlled test. Furthermore, at zero time incubation, the bacterial viability of the tested DAS medium was the same as that of the control test.

The log reduction, a terminology to describe the antibacterial activity, was defined as: $\text{Log reduction} = \text{Log } N_0 - \text{Log } N_t$. Where N_t was the number of the survival bacteria of the test sample at exposure time t and N_0 was the initial bacterial concentration of the test sample. The determined log reduction was the measurement of the overall bacterial inactivation by DAS aqueous suspension at pH of 3. The initial bacterial concentration in the antibacterial test was $1\text{--}2 \times 10^7$ CFU/mL. The bacterial viability test method was the agar plate-count method [26]. Samples were diluted in the PBS working solution on a serial 1/10th dilution. Aliquot (0.1 mL) from each of the dilution was placed on the nutrient tryptical soy agar. Each dilution was plated in triplicates. After 24 h incubation at 37 °C, the colonies that grew on the agar were counted to estimate the number of the viable bacteria. The bacterial viability of the tested DAS medium was the same as that of the control test at zero test time, DAS had no dose effect on the bacterial viability in the agar plate-count method. The lowest concentration of DAS aqueous suspension to obtain no bacterial colonies growth on the agar from the serial dilutions (0–2) was determined as the minimal lethal concentration (MLC). In the discussion of the inactivation kinetic model, for simplification, a 7-log reduction was considered to be the complete inactivation in the antibacterial test with $1\text{--}2 \times 10^7$ CFU/mL initial bacterial concentration. The MLCs

and log reductions were determined with at least triplicate replications and reported as a mean \pm standard error in the results.

The DAS test medium with a bacterial aliquot in the antibacterial test at room temperature were analyzed by a UV-Vis spectrometer (Perkin-Elmer Lambda 800) to determine the DAS concentration during the bacterial inactivation. A control sample set without adding bacterial aliquot was also included. All the analyzed samples were centrifuged to collect the DAS supernatant for the UV-vis analysis. The pH values of the analyzed DAS samples were kept at 3 during the sample preparation and UV-vis measurement. Quartz cuvettes were used for the UV-vis measurements.

4. Conclusions

The bacterial inactivation kinetics of DAS aqueous suspension, a novel polymeric biocide, was studied to further understand its antimicrobial activity. The survival response of bacteria to the exposure of DAS aqueous suspension was found to obey a first order bacterial inactivation kinetics. The response behavior of bacteria was modeled by the pseudo-first order chemical reaction kinetics. The established model was validated by well prediction of the bacterial survival response at room temperature.

References

1. Fiedorowicz, M.; Para, A. Structural and molecular properties of dialdehyde starch. *Carbohydr. Polym.* **2006**, *63*, 360-366.
2. Jane, J. Starch properties, modifications, and applications. *J. Macromol. Sci.-Pure Appl. Chem.* **1995**, *A32*, 751-757.
3. Ellis, R.P.; Cochrane, M.P.; Dale, M.F.B.; Duffus, C.M.; Lynn, A.; Morrison, I.M.; Prentice, R.D.M.; Swanston, J.S.; Tiller, S.A. Starch production and industrial use. *J. Sci. Food Agric.* **1998**, *77*, 289-311.
4. Beauchamp, R.O.; Stclair, M.B.G.; Fennell, T.R.; Clarke, D.O.; Morgan, K.T.; Kari, F.W. A critical-review of the toxicology of glutaraldehyde. *Crit. Rev. Toxicol.* **1992**, *22*, 143-174.
5. Gaddy, H.J.; Johannig, G.; Muhrer, M.E. Ruminant feed additive (DAS) toxicity studies using guinea-pigs. *J. Animal Sci.* **1975**, *41*, 400-400.
6. Gaddy, H.J.; Muhrer, M.E.; Johannin, G. Detoxification of NH_3 from Npn with oxidized starch. *J. Animal Sci.* **1974**, *39*, 238-238.
7. Wilson, R.H. Utilization and toxicity of dialdehyde-starches and dicarboxyl-starches. *Proc. Soc. Exp. Biol. Med.* **1959**, *102*, 735-737.
8. Radley, J.A. The Manufacture and Chemistry of Dialdehyde Starch. In *Starch Production Technology*; Radley, J.A., Ed.; Applied Science Publisher: London, UK, 1976.
9. Onyiriuka, E. Modification of polystyrene cell culture surfaces by grafting a thin film of starch. *J. Adhes. Sci. Technol.* **1996**, *10*, 617-633.
10. Shimizu, T.; Fujishige, S. A newly prepared surface-treated oxystarch for removal of urea. *J. Biomed. Mater. Res.* **1983**, *17*, 597-612.
11. Yu, D.M.; Xiao, S.Y.; Tong, C.Y.; Chen, L.; Liu, X.M. Dialdehyde starch nanoparticles: Preparation and application in drug carrier. *Chin. Sci. Bull.* **2007**, *52*, 2913-2918.

12. McDonnell, G.; Russell, A.D. Antiseptics and disinfectants: Activity, action, and resistance. *Clin. Microbiol. Rev.* **1999**, *12*, 147-179.
13. Siragusa, J.A. Method of Inhibiting Microbial Activity Using Insoluble Dialdehyde Polysaccharides. U.S. Patent 4,034,084, 5 July 1977.
14. Song, L.; Cruz, C.; Farrah, S.R.; Baney, R.H. Novel antiviral activity of dialdehyde starch. *Electron. J. Biotechnol.* **2009**, *12*, 1-5.
15. Song, L.; Sang, Y.J.; Cai, L.M.; Shi, Y.C.; Farrah, S.R.; Baney, R.H. The effect of cooking on the antibacterial activity of the dialdehyde starch suspensions. *Starch* **2010**, *62*, 458-466.
16. Weavers, L.K.; Wickramanayake, G.B. Kinetics of the Inactivation of Microorganisms. In *Disinfection, Sterilization, and Preservation*; Block, S.S., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2001; pp. 65-78.
17. Lambert, R.J.W. A model for the thermal inactivation of micro-organisms. *J. Appl. Microbiol.* **2003**, *95*, 500-507.
18. Peleg, M. Modeling and simulation of microbial survival during treatments with a dissipating lethal chemical agent. *Food Res. Int.* **2002**, *35*, 327-336.
19. Peleg, M.; Pechina, C.M. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 159-172.
20. Munton, T.J.; Russell, A.D. Aspects of action of glutaraldehyde on *Escherichia-coli*. *J. Appl. Bacteriol.* **1970**, *33*, 410-419.
21. Gorman, S.P.; Scott, E.M. Uptake and media reactivity of glutaraldehyde solutions related to structure and biocidal activity. *Microbios Lett.* **1977**, *5*, 163-169.
22. Sagripanti, J.L.; Bonifacino, A. Comparative sporicidal effect of liquid chemical germicides on three medical devices contaminated with spores of *Bacillus subtilis*. *Am. J. Infect. Control* **1996**, *24*, 364-371.
23. Hoff, J.C.; Akin, E.W. Microbial resistance to disinfectants: Mechanisms and significance. *Environ. Health Perspect.* **1986**, *69*, 7-13.
24. Gorman, S.P.; Scott, E.M.; Russell, A.D. Anti-microbial activity, uses and mechanism of action of glutaraldehyde. *J. Appl. Bacteriol.* **1980**, *48*, 161-190.
25. Retta, S.M.; Sagripanti, J.L. Modeling the inactivation kinetics of bacillus spores by glutaraldehyde. *Lett. Appl. Microbiol.* **2008**, *46*, 568-574.
26. Kim, Y.M.; Farrah, S.; Baney, R.H. Silanol—A novel class of antimicrobial agent. *Electron. J. Biotechnol.* **2006**, *9*, 176-180.