Settling of bentonite in gelatine solutions

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Abstract

New Zealand has a sizeable meat by-products processing industry, associated with significant aqueous effluent called stickwater. Stickwater has a biological oxygen demand of 50-150 g O_2/l and has to be treated prior to disposal. Currently, stickwater is dried and added to meat and bone meal in some inedible meat rendering plants. In edible rendering plants, the gelatin can be removed and the remaining broth is concentrated as a flavor enhancer. Where no further unit operations are carried out on stickwater, the stickwater must be treated to reduce the BOD. A medium size meat rendering plant in NZ can produce up to 30,000 L of stickwater at 2-5% solids per day [1]. In Hamilton, waste water treatment costs NZ$0.90 per kg solids or approximately NZ$1350 per day. In comparison, abattoir waste treatment costs NZ$ 0.23/kg in the US. [2].

Introduction

Extracting protein from stickwater can reduce the treatment charges by decreasing suspended solids and BOD. An example of a suitable treatment process is protein adsorption using nanoclays. Bentonite costs less than NZ$50 per ton [3] and has a gelatin adsorption capacity of up to 404 mg protein per gram bentonite (Table 3). For 30,000 kg stickwater, treatment using bentonite costs around NZ$74 per day (this excludes operating and other material costs).

Table 1. Properties of stickwater [2, 4, 5].

Fats (%)	Solid contents (%)	Protein (%)	BOD (g/l)	Organic-N_2 (g/m^3)
1-2	2-5	4-5	150	250

Bentonite is routinely used as a filler in plastics to improve or change the mechanical properties [6-11]. Bentonite has an overall negative charge which is pH independent [12] and is neutralized by a cation interlayer such as sodium or calcium ions [13-16]. Sodium and calcium bentonite typically consist of layered flat sheets with a spacing of about 1.2-1.5 nm between sheets, resulting in a filler with a very high aspect ratio when exfoliated and well-mixed with polymer [9, 17]. When completely exfoliated, bentonite can be used to form nano-composites that have improved properties compared to composites formed using micron-scale fillers [9].

The main protein in stickwater is gelatin, a denatured form of collagen. A collagen molecule consists of peptide chains of 1011 amino acids, with every third amino acid being glycine. 75% of the amino acids are hydrophobic and about 160 amino acids contain charged side groups that are distributed along its length [18]. Gelatin is unsuitable for forming plastics and films of any significant strength or durability without plasticization, crosslinking or reinforcement [19-24]. But due to the number of side groups gelatin has, it easily undergoes chemical crosslinking [21].

Experimental

In a laboratory experiment, gelatin prepared in phosphate buffer solution (PBS) was used as model stickwater. Gelatin solutions of 100 mL were made at concentrations of 4, 5, 6, 10, 15 and 20 mg/mL in 0.02M PBS at pH 3, 5.23, 7 and 9. Solution pH was adjusted using 1M HCl and 1M NaOH. 3 g bentonite was added to the gelatin solutions and the mixture was stirred on a magnetic stirrer. Three 1.5 mL samples were taken at 5, 10, 20, 40, 60 minutes and overnight and placed into 1.5 mL centrifuge tubes. The samples were centrifuged at 14 500 rpm for 2 minutes using an Eppendorf Minispin Plus. The supernatant was collected and analyzed for UV absorbance at a wavelength of 280 nm using a Pharmacia Biotech Ultrospec 2000. Bentonite adsorbed with gelatin 20 mg/mL were collected for XRD and TGA analysis.

Results and discussion

Table 2 summarizes the adsorption results. Protein adsorption ranged between 230 to 371 mg/g clay at 20 mg/ml starting gelatin concentration. Sodium bentonite/gelatin at pH 9 formed a gel and therefore solution protein concentration could not be measured. In all cases basal spacing (*d*-value) increased indicating some intercalation of gelatin between clay layers, and in most cases clay particle size increased suggesting some crosslinking or flocculation of clay particles by gelatin. Pellet density for calcium bentonite increased by 30% for pH 3 and 5.23 indicating that gelatin/clay can form a compact pellet, but in all other cases pellet density did not change significantly.

While bentonite is suitable for adsorbing protein from solution, recovering the bentonite-adsorbed-gelatin particles (CaGel and NaGel) from the solution could be challenging in large scale operations and using a large scale centrifuge may be cost prohibitive.

Table 2. Summary of adsorption findings.

Clay type	Gelatin adsorbed C_r (mg/g)	*d*-value (Å)	TGA (mg adsorbed organics / g clay)	Particle size (μm)	Pellet density (kg/m^3)
Calcium		15.5		14.16	1066.6
CaGel pH 3	298	17.9	399	35.06	1353.9
CaGel pH 5.23	235	18.8	375	35.63	1329.7
CaGel pH 7	342	19.8	468	42.18	1120.7
CaGel pH 9	371	19.9	520	13.53	1123.5
Sodium		12.4		4.01	1095.4
NaGel pH 3	359	19.9	502	32.1	1092.3
NaGel pH 5.23	404	21.9	538	15.19	1046.5
NaGel pH 7	273	23.1	499	5.89	1054.1
NaGel pH 9	gel	21.4	498	9.53	1070.7

A series of settling experiments were conducted to investigate the settling behavior of CaGel and NaGel in solution. The adsorption experiments were repeated at 1200 ml volume for all pHs at 0 and 20 mg/ml gelatin concentration (Ge20) in PBS solution. The equilibrium solution was transferred into a settling column and placed in a holding tank with circulating water heated to 37°C and 55°C (Fig. 1). 37°C was selected on basis that it is the lowest temperature for a 20 mg/ml gelatin solution to stay in aqueous form before gelling and 55°C is the temperature of fresh stickwater effluent at meat rendering plants. A 5 ml sample was withdrawn at 10, 30, 60, 90, 120 minute and overnight at five outlet points using separate syringes. The samples were analyzed for turbidity and protein concentration (UV absorbance at 280nm). The height of the pellet formation in the column was recorded at every sampling interval when visible.

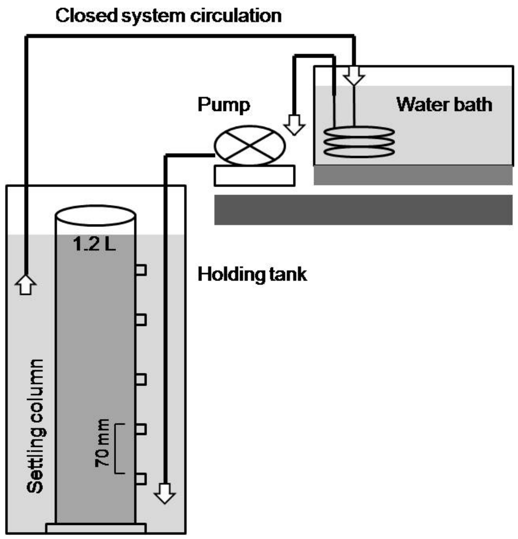


Fig.1. Process diagram for settling experiment.

Stoke's law (Eq. 1) was used to predict the theoretical settling velocity of the clay particles in gelatin solution (Table 3).

$$V_s = \frac{2}{9}\frac{(P_p - P_f) g R^2}{u} \quad (1)$$

Where V_s is the settling velocity of particle (m/s), P_p and P_f is the density of particle and fluid (kg/m^3), g is the gravitational acceleration (9.81 m/s^2), R is the particle radius (m) and u is the solution viscosity (kg/ms).

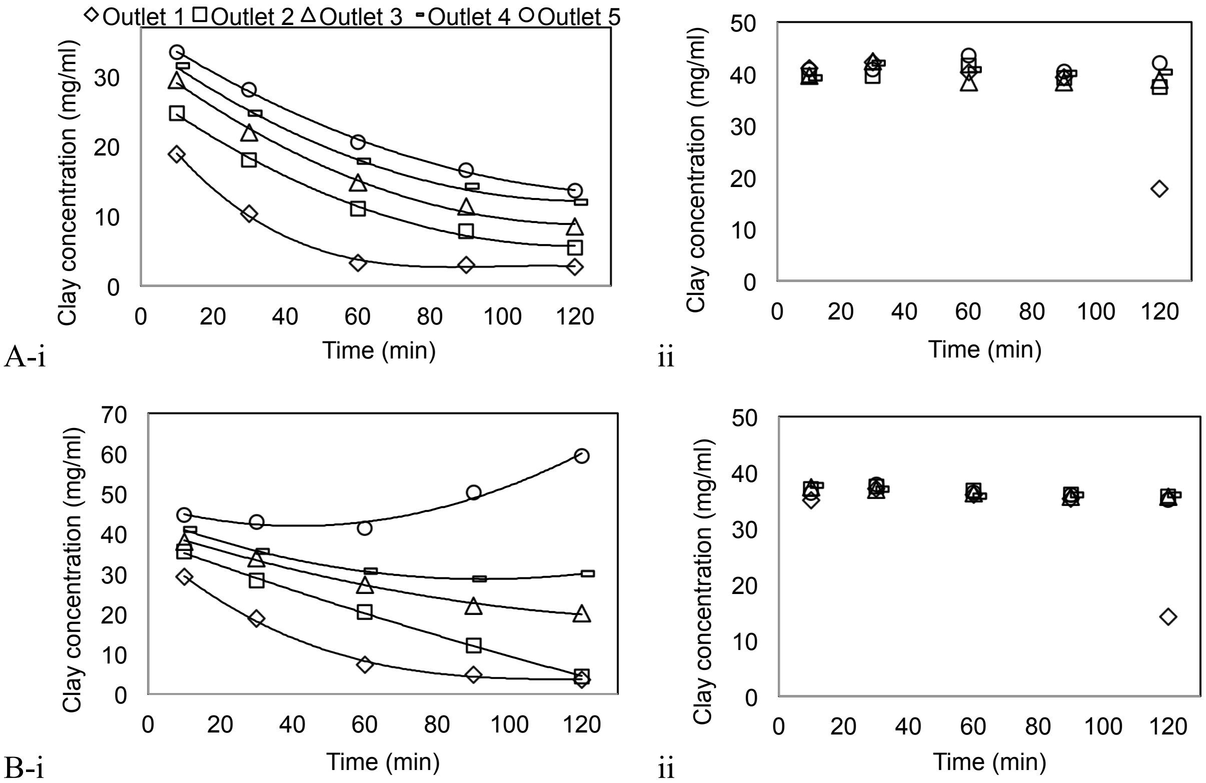
Table 3. Predicted settling velocity and time from Stoke's law at 37^{o}C in 40 cm settling column.

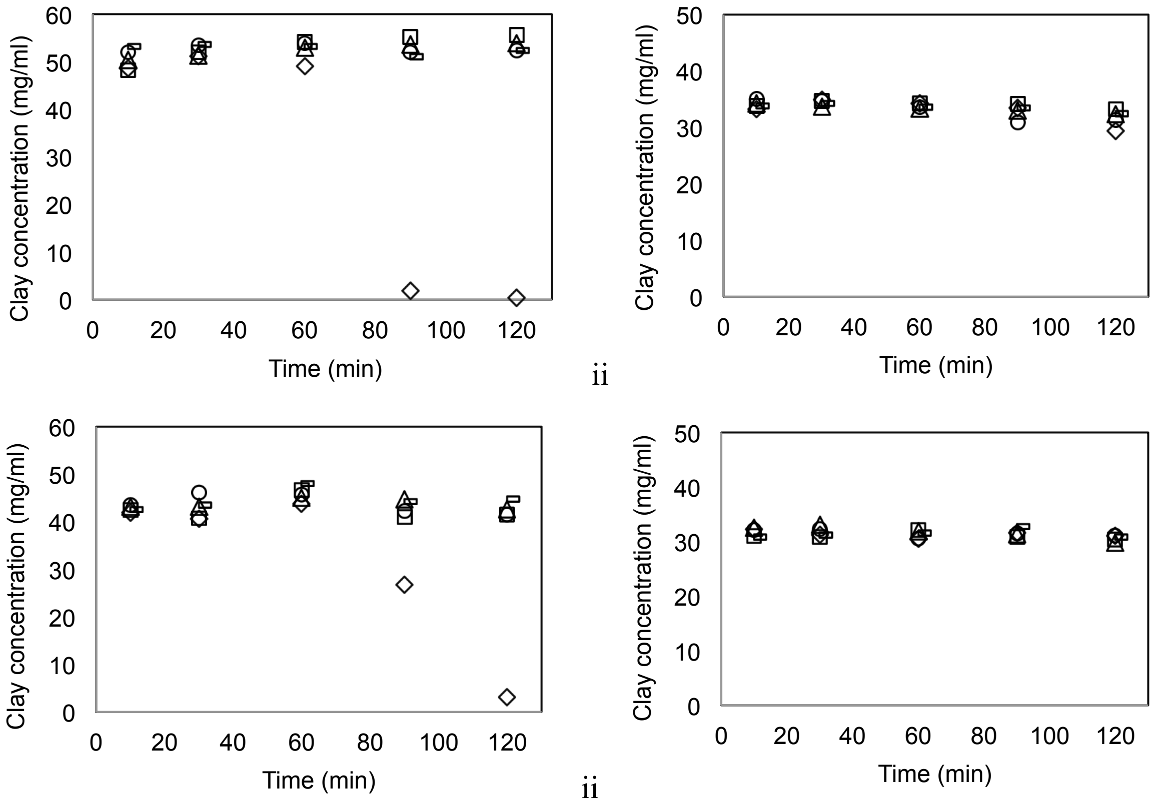
Solution (Ge20)			Calcium bentonite		Sodium bentonite	
pH	Density (kg/m^3)	Viscosity (kg/m.s)	V_s (m/s) x 10^{-5}	t (hr)	V_s (m/s) x 10^{-5}	t (hr)
3	988.7	0.001217	20.1	0.55	4.8	2.32
5.23	992.7	0.001552	15.0	0.74	0.4	25.49
7	991.9	0.001402	8.9	1.25	0.08	132.53
9	986.8	0.001142	1.2	9.31	0.36	30.57

Calcium bentonite/gelatin was predicted to have the best settling velocities and fastest settling times of 0.55 and 0.74 hours at pH 3 and 5.23 respectively. Longest settling time for calcium bentonite was at pH 9 at over 9 hours. Sodium bentonite/gelatine was predicted to have the longest settling times of up to 132 hours at pH 7.

Figure 2 summarized the settling behavior of the particles. Only CaGel at pH 3 and 5.23 settled. CaGel at pH 7, 9 and NaGel at all pHs stayed suspended. In PBS solution, sodium and calcium bentonite settled across all pHs (data not shown).

Looking at the density of the pellets (Table 2), CaGel at pH 3 and 5.23 are heavier indicating the settling is due to the density of the particles despite the lower amount of adsorbed gelatin. Predictions using Stoke’s law showed that all clay/gelatin suspensions would eventually settle, but when the suspensions were left overnight, there was no decrease in suspended solids. This suggests that the clay gelatin solutions at these pHs formed stable suspensions indicating repulsion between molecules.





C-i ii

D-i ii

Fig. 2. Settling experiment at 37°C and pH A) 3; B) 5.23; C) 7; D) 9; i) Calcium; ii) Sodium.

Conclusion

The settling behavior and adsorption were pH dependent, with calcium bentonite giving the best settling at pH 3 and 5.23 but the best adsorption at pH 7 and 9. Sodium bentonite gave the best adsorption at pH 5.23 and pH 3 without any settling for all pHs. Stickwater has a native pH of around 5.23, therefore sodium bentonite could be used at native pH to maximize the gelatin adsorption, while the pH should to be adjusted to pH 7 or 9 if calcium bentonite was used. However, calcium bentonite gives a much more compact pellet with a lower water content compared to sodium bentonite. Hence calcium bentonite is favoured in Europe for removing fines from wine [25]. In addition, calcium bentonite has a larger particle size than sodium bentonite which makes it more favorable for settling and decanting processes. At this stage, the simplicity of the model stickwater would probably be insufficient to predict the real gelatine adsorption onto bentonite. More variables such as salt competing effect and fat content can be introduced in the model solution for future work.

References

[1] Bioextracts, T. (2009), *Gel Bone Plant - Summary*, Taranaki Bioextracts, New Plymouth.
[2] Mittal, G. S. Treatment of wastewater from abattoirs before land application--a review. Bioresource Technology **2006**, *97*(9), 1119-1135.
[3] Commerce, N. Z. M. (1996), *New Zealand Annual Mining Review 1994*, Publicity Unit, Ministry of Commerce, Wellington.
[4] Bickers, P. O.; van Oostrom, A. J. Availability for denitrification of organic carbon in meat-processing wastestreams. Bioresource Technology **2000**, *73*(1), 53-58.
[5] Swan, J. E. Animal By-Product Processing. In *Encyclopedia of Food Science and Technology*; Francis, F. J., Ed.; John Wiley: New York, 2000; 35-42.
[6] Chen, B. Q.; Evans, J. R. G. Poly(epsilon-caprolactone)-clay nanocomposites: Structure and mechanical properties. Macromolecules **2006**, *39*(2), 747-754.
[7] Ma, X.-Y.; Liang, G.-Z.; Liu, H.-L.; Fei, J.-Y.; Huang, Y. Novel intercalated nanocomposites of polypropylene/organic-rectorite/polyethylene-octene elastomer: Rheology, crystallization kinetics, and thermal properties. Journal of Applied Polymer Science **2005**, *97*(5), 1915-1921.
[8] Manias, E.; Touny, A.; Wu, L.; Strawhecker, K.; Lu, B.; Chung, T. C. Polypropylene/Montmorillonite Nanocomposites. Review of the Synthetic Routes and Materials Properties. Chemistry of Materials **2001**, *13*(10), 3516-3523.
[9] Messersmith, P. B.; Giannelis, E. P. Synthesis and barrier properties of poly(ε-caprolactone)-layered silicate nanocomposites. Journal of Polymer Science Part A: Polymer Chemistry **1995**, *33*(7), 1047-1057.
[10] Yu, J.; Cui, G.; Wei, M.; Huang, J. Facile exfoliation of rectorite nanoplatelets in soy protein matrix and reinforced bionanocomposites thereof. Journal of Applied Polymer Science **2007**, *104*(5), 3367-3377.
[11] Zhu, J.; Morgan, A. B.; Lamelas, F. J.; Wilkie, C. A. Fire properties of polystyrene-clay nanocomposites. Chemistry of Materials **2001**, *13*(10), 3774-3780.
[12] Czímerová, A.; Bujdák, J.; Dohrmann, R. Traditional and novel methods for estimating the layer charge of smectites. Applied Clay Science **2006**, *34*(1-4), 2-13.
[13] Fusi, P.; Ristori, G. G.; Calamai, L.; Stotzky, G. Adsorption and binding of protein on "clean" (homoionic) and "dirty" (coated with Fe oxyhydroxides) montmorillonite, illite and kaolinite. Soil Biology and Biochemistry **1989**, *21*(7), 911-920.
[14] Güngör, N.; Karaoglan, S. Interactions of polyacrylamide polymer with bentonite in aqueous systems. Materials Letters **2001**, *48*(3-4), 168-175.
[15] Sun, X.; Li, C.; Wu, Z.; Xu, X.; Ren, L.; Zhao, H. Adsorption of Protein from Model Wine Solution by Different Bentonites. Chinese Journal of Chemical Engineering **2007**, *15*(5), 632-638.
[16] Tombácz, E.; Szekeres, M.; Baranyi, L.; Michéli, E. Surface modification of clay minerals by organic polyions. Colloids and Surfaces A: Physicochemical and Engineering Aspects **1998**, *141*(3), 379-384.
[17] Sinha Ray, S.; Okamoto, K.; Okamoto, M. Structure-Property Relationship in Biodegradable Poly(butylene succinate)/Layered Silicate Nanocomposites. Macromolecules **2003**, *36*(7), 2355-2367.
[18] Zamora, A. (2005), *Amino acid profiles of food proteins,* viewed January 2009 http://www.scientificpsychic.com/fitness/aminoacids1.html
[19] Apostolov, A. A.; Fakirov, S.; Evstatiev, M.; Hoffmann, J.; Friedrich, K. Biodegradable Laminates Based on Gelatin, 1. Macromolecular Materials and Engineering **2002**, *287*(10), 693-697.
[20] Apostolov, A. A.; Fakirov, S.; Hoffmann, J.; Friedrich, K. Biodegradable Laminates Based on Gelatin, 2. Macromolecular Materials and Engineering **2003**, *288*(3), 228-234.
[21] Bigi, A.; Cojazzi, G.; Panzavolta, S.; Roveri, N.; Rubini, K. Stabilization of gelatin films by crosslinking with genipin. Biomaterials **2002**, *23*(24), 4827-4832.
[22] de Carvalho, R. A.; Grosso, C. R. F. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocolloids **2004**, *18*(5), 717-726.
[23] Guerrero, P.; Stefani, P. M.; Ruseckaite, R. A.; de la Caba, K. Functional properties of films based on soy protein isolate and gelatin processed by compression molding. Journal of Food Engineering **2011**, *105*, 65-72.
[24] Natarajan, N.; Shashirekha, V.; Noorjahan, S. E.; Rameshkumar, M.; Rose, C.; Sastry, T. P. Fibrin-Chitosan-Gelatin Composite Film: Preparation and Characterization. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry **2005**, *42*(7), 945 - 953.
[25] Zoecklein, B. (1988), *Bentonite fining of juice and wine*, Virginia Polytechnic Institute and State University, Petersburg.