

Cottonseed extract as a coagulant for water treatment

Mekayla DePaolis¹, Sophie De Respino¹, Laxmicharan Samineni², Scott Brighton¹, Manish Kumar^{1,*}

¹Department of Civil, Architectural and Environmental Engineering, University of Texas at Austin, Austin, Texas, 78712, United States

²Department of Chemical Engineering, University of Texas at Austin, Austin, Texas 78712, United States

*Corresponding author e-mail: manish.kumar@utexas.edu

Supporting Information

Table S1: Characteristics of untreated water used in jar tests

Characteristic	Concentration
Total Hardness (mg/L)	120
Total Chlorine (mg/L)	1
Free Chlorine (mg/L)	0.5
Bromine (mg/L)	1
Total Alkalinity (mg/L)	120
pH	8.125
TDS (mg/L)	259.5
DOC (mg/L)	3.0

Table S2: Characteristics of groundwater*

Characteristic	Concentration
Manganese (mg/L)	0.004
Iron (mg/L)	0.1
Magnesium (mg/L)	28
Calcium (mg/L)	66
pH	8.50
TOC (mg/L)	1.6

*Groundwater and characteristics provided by University of Illinois Urbana-Champaign.

Sludge generation. Jar tests were carried out at an initial turbidity of 94.4 ± 1.1 NTU to determine the amount of sludge generation for cottonseed serum and ferric. Following sedimentation, the supernatant was removed and the sludge was filtered through a $0.45 \mu\text{m}$

PVDF filter and dried at 60°C for 3 hours. The filter mass difference pre- and post-filtration was measured to determine the amount of sludge generated for each jar (200 mL), shown in Figure S1.

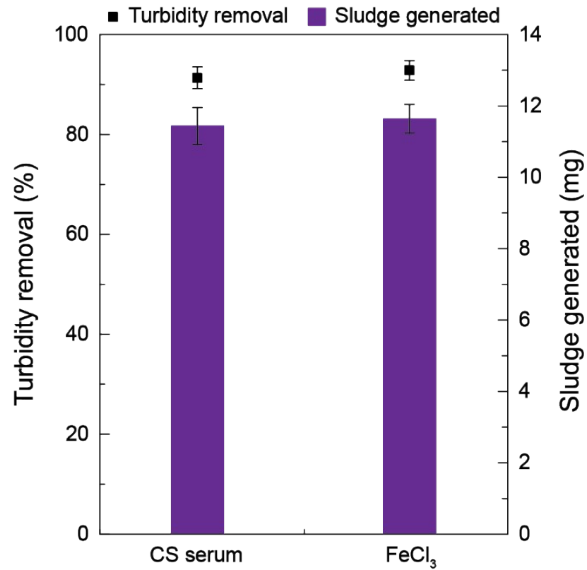


Figure S1: Cottonseed serum (CS serum) and FeCl₃ (5 mg/L dose) achieve 90% turbidity removal while generating the same amount of sludge

Table S3: Overview of initial jar test parameters

Experiment	Coagulant used	Cottonseed volume (mL)	Protein dose (mg/L)	Carbohydrate dose (mg/L)	Initial Turbidity (NTU)	Reference Figure
Increasing serum volume/protein dose	Cottonseed serum	0-10	0-3.83	Not measured	112±22	1A
Increasing initial turbidity		3	1.238		62.5-717	1B
Varying initial pH	Cottonseed serum and FeCl ₃	3	1.238		86.5±21.9	1C
Increasing serum age	Cottonseed serum	3	0.7-1.34	108±7.3	2A	
Evaluating DOC	Cottonseed serum and FeCl ₃	3	1.06±0.23	112.3±23.3	2B	
Carbohydrate isolate coagulation	Cottonseed serum carbohydrate	3	0.12±0.4	68.4±3.8	3A/B	

isolate

Protein isolate coagulation	Cottonseed serum protein isolate	3	1.49±.51	8.97±.29	69.8±4.2	3A/B
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Table S4: Final jar test water quality

Experiment	Coagulant used	Cottonseed volume (mL)	Protein dose (mg/L)	Initial Turbidity (NTU)	Final Turbidity (NTU)	Reference Figure
Increasing serum volume/protein dose	Cottonseed serum	0	0	112±22	94.9±11.2	1A
		2	0.77		17.0±7.66	
		4	1.53		12.1±4.55	
		6	2.30		10.4±5.47	
		8	3.07		8.4±2.59	
		10	3.83		8.0±4.55	
Increasing initial turbidity	Cottonseed serum	3	1.238	62.5±0.8	4.2±1.52	1B
				133±1.0	0.36±0.34	
				193±6.3	16.13±0.43	
				302±9.4	22.2±1.1	
				405±14.4	23.73±1.58	
				516±4.9	32.05±1.6	
591±6.9	38.2±2.0					
Varying initial pH	Cottonseed serum	3	1.238	72.9±4.8 (pH 6)	72.9±4.8 (pH 6)	1C
				21.1±4.6 (pH 7)	21.1±4.6 (pH 7)	
				13.1±2.1 (pH 8)	13.1±2.1 (pH 8)	
	FeCl ₃	0	0	111.6±7.1 (pH 8)	8.51±0.7 (pH 8.5)	1C
				98.6±1.4 (pH 8.5)	5.88±0.1 (pH 6)	
					4.78±0.3 (pH 7)	
Increasing serum age	Cottonseed serum	3	0.7-1.34	108±7.3	7.33 (2 d)	2A
					6.78 (35 d)	
					9.07 (118 d)	
					9.72 (218 d)	
Evaluating DOC	Cottonseed serum and	3	1.238	112.3±23.3	5.18±0.6 (CS) 3.59±0.06	2B

	FeCl ₃			(FeCl ₃)		
Carbohydrate isolate coagulation	Cottonseed serum carbohydrate isolate	3	0.12±0.4	69.8±4.2	64.23±3.9	3A/B
Protein isolate coagulation	Cottonseed serum protein isolate	3	1.49±.51	69.8±4.2	9.7±1.35	3A/B

Table S5: A review of bio-coagulants based on work done by *Kurniawan et al.*¹

Bio-coagulant	Destabilization mechanism	Performance	Reference
Crab shell	Cationic chitosan	16% turbidity removal at 1 mg/L dose (pH 8.1)	²
Crab shell	Cationic chitosan	94% turbidity removal at 2 mg/L dose (pH 7)	³
Shrimp shell	Cationic chitosan	96% oil removal (pH 4)	⁴
<i>Achatinoidea</i> shell	Unidentified protein(s)	99% turbidity removal with 4 g/L dose (pH 4)	⁵
Periwinkle shell	Chitin and unidentified protein(s)	84% at 100 mg/L dose (pH 4)	⁶
Snail shell	Cationic mineral surface	60% green dye removal at 100 mg/L (pH 4)	⁷
Alginate	Calcium alginate gel formation	98% turbidity removal with 80 mg/L Ca ²⁺ and 0.02 mg/L alginate	⁸
<i>Achromobacter xylooxidans</i> strain TERI L1	Exopolysaccharide production	75% turbidity removal with 10% (v/v) dose	⁹
<i>Bacillus agaradhaerens</i> C9		95% turbidity removal with 1.5 mg/L (pH 6.53)	¹⁰
<i>Bacillus mucilaginosus</i> ,		99% turbidity removal with 0.01% (v/v) dose	¹¹
<i>Chromobacterium violaceum</i> and <i>Citrobacter koseri</i>		42% turbidity removal with 1% (v/v) dose (LT-6, pH 6) and 40% turbidity removal with 1% (v/v) dose (LT-5, pH 3)	¹²
<i>Bacillus licheniformis</i> strain W7	Glycoprotein with high polysaccharide content	85% turbidity removal with 200 mg/L dose (pH 3)	¹³
<i>Bacillus salmalaya</i>	Protein-bound	83% turbidity with 2% dose	¹⁴

139SI-7	polysaccharide	(pH 7)	
<i>Bacillus velezensis</i>	Anionic polymer	35% turbidity removal with 100 mg/L (pH 4 and 8)	15
<i>Paenibacillus polymyxa</i>	Proteoglycan	94% turbidity removal with 0.1% (v/v) dose	16
<i>Aspergillus niger</i>	Polysaccharide	60% turbidity removal with 0.2% (v/v) dose	17
<i>Penicillium sp.</i> and <i>Trichoderma sp.</i>	Anionic fungi spores	51% (<i>Trichoderma</i>) and 55% (<i>Penicillium</i>) turbidity removal with 1% (v/v) dose	18
<i>Abelmoschus esculentus</i>	Anionic polysaccharide	97% turbidity removal with 3.2 mg/L (pH 6) and 88 mg/L Fe ³⁺	19
Dragon fruit foliage	Not reported	99.7% turbidity removal with 500 mg/L dose (pH 10)	20
<i>Moringa oleifera</i>	Cationic proteins (MO2.1 and MoCBP)	99.7% turbidity removal with 25 mg/L dose (pH 7.16)	21-23
<i>Ocimum basilicum</i> L.	Protonated groups on polysaccharide chains	58.8% turbidity removal with <i>O. basilicum</i> and alum (pH 7)	24
Rice starch	Polymers: amylase and amylopectin	84% TSS removal with 2 g/L dose (pH 3)	25

Table S6: Final pH of water after being treated with various doses of cottonseed serum

Volume serum added, mL	Average protein dose, mg/L	Final pH
0	0	8.03
2	0.766	7.92
4	1.533	8.14
6	2.299	8.19
8	3.065	8.32
10	3.831	8.6

Bio View:Identified Proteins	Accession Number	Alternate ID	Molecular Weight	8527
uncharacterized protein LOC107929052 OS=Gossypium hirsutum OX=3635 GN=LOC107929052 PE=3 SV=1	A0A1U8LMX7	LOC107929052	149 kDa	765
vicilin GC72-A OS=Gossypium hirsutum OX=3635 GN=LOC107928550 PE=3 SV=1	A0A1U8LQ34	LOC107928550	72 kDa	543
vicilin C72 OS=Gossypium hirsutum OX=3635 GN=LOC107928551 PE=3 SV=1	A0A1U8LLA0	LOC107928551	70 kDa	343
Lea4-A108 protein OS=Gossypium hirsutum OX=3635 GN=Lea4-D9 PE=2 SV=1	Q53WZ7	Lea4-D9	11 kDa	126
P04264 SWISS-PROT:P04264 Tax_Id=9606 Gene_Symbol=KRT1 Keratin, type II cytoskeletal 1	P04264		?	97
legumin B OS=Gossypium hirsutum OX=3635 GN=LOC107914926 PE=3 SV=1	A0A1U8KAE1	LOC107914926	59 kDa	85
sucrose-binding protein-like OS=Gossypium hirsutum OX=3635 GN=LOC107931543 PE=4 SV=1	A0A1U8LZX8	LOC107931543	62 kDa	82
sucrose-binding protein-like OS=Gossypium hirsutum OX=3635 GN=LOC107930549 PE=4 SV=1	A0A1U8LW A0	LOC107930549	62 kDa	78
P35908 SWISS-PROT:P35908 Tax_Id=9606 Gene_Symbol=KRT2 Keratin, type II cytoskeletal 2 epidermal	P35908		?	66
Embryogenesis abundant protein OS=Gossypium hirsutum OX=3635 GN=Lea2A-A PE=2 SV=1	Q03791	Lea2A-A	12 kDa	65
legumin A OS=Gossypium hirsutum OX=3635 GN=LOC107916455 PE=3 SV=1	A0A1U8KK K7	LOC107916455	58 kDa	63
P13645 SWISS-PROT:P13645 Tax_Id=9606 Gene_Symbol=KRT10 Keratin, type I cytoskeletal 10	P13645		?	58
protein SLE1-like OS=Gossypium hirsutum OX=3635 GN=LOC107890949 PE=4 SV=1	A0A1U8HY H9	LOC107890949	12 kDa	53
late embryogenesis abundant protein D-113-like OS=Gossypium hirsutum OX=3635 GN=LOC107891257 PE=3 SV=1	A0A1U8I5J0	LOC107891257	17 kDa	52
glucose and ribitol dehydrogenase homolog 1-like isoform X1 OS=Gossypium hirsutum OX=3635 GN=LOC107894345 PE=4 SV=1	A0A1U8IAI4	LOC107894345	38 kDa	50
Phosphoglycerate kinase OS=Gossypium hirsutum OX=3635 GN=LOC107922096 PE=3 SV=1	A0A1U8L251	LOC107922096	42 kDa	48
glucose and ribitol dehydrogenase homolog 1-like OS=Gossypium hirsutum OX=3635 GN=LOC107899107 PE=4 SV=1	A0A1U8IPZ7	LOC107899107	38 kDa	46

Table S7: Liquid Chromatography-Mass Spectroscopy results of cottonseed serum

late embryogenesis abundant protein D-29-like					
OS=Gossypium hirsutum OX=3635 GN=LOC107951546	A0A1U8NV	LOC1079			
PE=4 SV=1	X0	51546	35 kDa	41	
P35527 SWISS-PROT:P35527 Tax_Id=9606					
Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9	P35527		?	37	
OS=Gossypium hirsutum OX=3635 GN=LOC107927644					
PE=3 SV=1	A0A1U8LI10	27644	27 kDa	37	
late embryogenesis abundant protein D-34-like					
OS=Gossypium hirsutum OX=3635 GN=LOC107942670	A0A1U8MYI	LOC1079			
PE=3 SV=1	9	42670	27 kDa	37	
Phosphopyruvate hydratase OS=Gossypium hirsutum					
OX=3635 GN=LOC107905355 PE=3 SV=1	A0A1U8JAZ	LOC1079			
	9 (+1)	05355	48 kDa	34	
uncharacterized protein ECU03_1610-like					
OS=Gossypium hirsutum OX=3635 GN=LOC107924971	A0A1U8LCZ	LOC1079			
PE=4 SV=1	0 (+1)	24971	26 kDa	34	
Late embryogenesis abundant protein D-29					
OS=Gossypium hirsutum OX=3635 PE=3 SV=1	P13940		33 kDa	33	
alcohol dehydrogenase 1-like OS=Gossypium hirsutum					
OX=3635 GN=LOC107911678 PE=3 SV=1	A0A1U8JXP	LOC1079			
	4	11678	41 kDa	32	
Peroxiredoxin OS=Gossypium hirsutum OX=3635					
GN=LOC107916248 PE=3 SV=1	A0A1U8KEE	LOC1079			
	0	16248	24 kDa	31	
aldose reductase-like OS=Gossypium hirsutum OX=3635					
GN=LOC107959341 PE=3 SV=1	A0A1U8PI35	LOC1079			
		59341	36 kDa	31	
late embryogenesis abundant protein D-34-like					
OS=Gossypium hirsutum OX=3635 GN=LOC107923257	A0A1U8L2R	LOC1079			
PE=3 SV=1	8	23257	26 kDa	31	
leucine aminopeptidase 1-like OS=Gossypium hirsutum					
OX=3635 GN=LOC107888423 PE=3 SV=1	A0A1U8HPR	LOC1078			
	0	88423	54 kDa	28	
uncharacterized protein LOC107908186 OS=Gossypium					
hirsutum OX=3635 GN=LOC107908186 PE=3 SV=1	A0A1U8JKV	LOC1079			
	7	08186	59 kDa	28	
aldose reductase-like OS=Gossypium hirsutum OX=3635					
GN=LOC107940476 PE=4 SV=1	A0A1U8MU	LOC1079			
	Z9	40476	36 kDa	27	
serpin-ZX-like OS=Gossypium hirsutum OX=3635					
GN=LOC107940684 PE=3 SV=1	A0A1U8MV	LOC1079			
	L0	40684	43 kDa	27	
Malate dehydrogenase OS=Gossypium hirsutum					
OX=3635 GN=LOC107936241 PE=3 SV=1	A0A1U8MFT	LOC1079			
	2	36241	36 kDa	26	
P13647 SWISS-PROT:P13647 Tax_Id=9606					
Gene_Symbol=KRT5 Keratin, type II cytoskeletal 5	P13647		?	25	
late embryogenesis abundant protein D-7 isoform X2					
OS=Gossypium hirsutum OX=3635 GN=LOC107891681	A0A1U8I8C9	LOC1078			
PE=4 SV=1	(+1)	91681	13 kDa	24	

UTP--glucose-1-phosphate uridylyltransferase OS=Gossypium hirsutum OX=3635 GN=LOC107895238 PE=3 SV=1	A0A1U8II21 (+1)	LOC1078 95238	49 kDa	23
Peptidyl-prolyl cis-trans isomerase OS=Gossypium hirsutum OX=3635 GN=LOC107922329 PE=3 SV=1	A0A1U8KZH 3 (+1)	LOC1079 22329	18 kDa	23
em protein H5-like OS=Gossypium hirsutum OX=3635 GN=LOC107948294 PE=3 SV=1	A0A1U8NG V9	LOC1079 48294	10 kDa	22
P08779 SWISS-PROT:P08779 Tax_Id=9606 Gene_Symbol=KRT16 Keratin, type I cytoskeletal 16	P08779		?	22
uncharacterized protein LOC107962160 OS=Gossypium hirsutum OX=3635 GN=LOC107962160 PE=3 SV=1	A0A1U8PS0 7	LOC1079 62160	28 kDa	22
Annexin OS=Gossypium hirsutum OX=3635 GN=Anx1 PE=2 SV=1	Q69DC2	Anx1	36 kDa	22
probable aldo-keto reductase 1 OS=Gossypium hirsutum OX=3635 GN=LOC107894809 PE=4 SV=1	A0A1U8IBN 4	LOC1078 94809	38 kDa	22
heat shock 70 kDa protein OS=Gossypium hirsutum OX=3635 GN=LOC107936049 PE=3 SV=1	A0A1U8MB N0	LOC1079 36049	71 kDa	22
vicilin-like seed storage protein At2g28490 OS=Gossypium hirsutum OX=3635 GN=LOC107953208 PE=4 SV=1	A0A1U8NYI 4	LOC1079 53208	55 kDa	22
uncharacterized protein LOC107938538 OS=Gossypium hirsutum OX=3635 GN=LOC107938538 PE=3 SV=1	A0A1U8MN N0	LOC1079 38538	28 kDa	21
serpin-ZX-like OS=Gossypium hirsutum OX=3635 GN=LOC107887018 PE=3 SV=1	A0A1U8HK R0	LOC1078 87018	43 kDa	21
probable aldo-keto reductase 1 OS=Gossypium hirsutum OX=3635 GN=LOC107894822 PE=4 SV=1	A0A1U8IBN 7	LOC1078 94822	37 kDa	21
Fructose-bisphosphate aldolase OS=Gossypium hirsutum OX=3635 GN=LOC107899428 PE=3 SV=1	A0A1U8IW5 5 (+1)	LOC1078 99428	39 kDa	21
P02533 SWISS-PROT:P02533 Tax_Id=9606 Gene_Symbol=KRT14 Keratin, type I cytoskeletal 14	P02533		?	20
P48668 SWISS-PROT:P48668 Tax_Id=9606 Gene_Symbol=KRT6C Keratin, type II cytoskeletal 6C	P48668		?	20
embryonic protein DC-8-like OS=Gossypium hirsutum OX=3635 GN=LOC107908729 PE=3 SV=1	A0A1U8JT74	LOC1079 08729	63 kDa	20
seed biotin-containing protein SBP65-like isoform X1 OS=Gossypium hirsutum OX=3635 GN=LOC107924313 PE=4 SV=1	A0A1U8L9Y 4	LOC1079 24313	68 kDa	20
Glyceraldehyde-3-phosphate dehydrogenase OS=Gossypium hirsutum OX=3635 GN=LOC107924606 PE=2 SV=1	D2D308	LOC1079 24606	37 kDa	19

Carbonic anhydrase OS=Gossypium hirsutum OX=3635 GN=LOC107909667 PE=3 SV=1	A0A1U8JR79	LOC1079 09667	28 kDa	19
14-3-3 protein 6-like OS=Gossypium hirsutum OX=3635 GN=LOC107898790 PE=2 SV=1	B6RF01	LOC1078 98790	29 kDa	19
P00761 SWISS-PROT:P00761 TRYP_PIG Trypsin - Sus scrofa (Pig).	P00761	?		18
Glyceraldehyde-3-phosphate dehydrogenase OS=Gossypium hirsutum OX=3635 GN=LOC107957019 PE=2 SV=1	D2D332	LOC1079 57019	37 kDa	18
Peptidyl-prolyl cis-trans isomerase OS=Gossypium hirsutum OX=3635 GN=LOC107945048 PE=3 SV=1	A0A1U8N91 0	LOC1079 45048	18 kDa	18
basic 7S globulin-like OS=Gossypium hirsutum OX=3635 GN=LOC107894474 PE=4 SV=1	A0A1U8IAU 3	LOC1078 94474	47 kDa	18
embryonic protein DC-8-like OS=Gossypium hirsutum OX=3635 GN=LOC107889787 PE=3 SV=1	A0A1U8HV0 5	LOC1078 89787	63 kDa	18
vicilin-like seed storage protein At2g18540 isoform X1 OS=Gossypium hirsutum OX=3635 GN=LOC107896407 PE=4 SV=1	A0A1U8IG12 (+1)	LOC1078 96407	77 kDa	18
Malate dehydrogenase OS=Gossypium hirsutum OX=3635 GN=LOC107903665 PE=3 SV=1	A0A1U8J4P3	LOC1079 03665	35 kDa	18
uncharacterized protein LOC107905810 OS=Gossypium hirsutum OX=3635 GN=LOC107905810 PE=4 SV=1	A0A1U8JCM 6	LOC1079 05810	10 kDa	18
actin-7-like OS=Gossypium hirsutum OX=3635 GN=LOC107947058 PE=3 SV=1	A0A1U8ND N5	LOC1079 47058	42 kDa	18
ruBisCO large subunit-binding protein subunit alpha-like OS=Gossypium hirsutum OX=3635 GN=LOC107891664 PE=3 SV=1	A0A1U8I8B4 (+2)	LOC1078 91664	62 kDa	18

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flocculating protein from *Moringa oleifera* Lam. *Biochimica et Biophysica Acta (BBA) - General
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