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1 **An assessment of the use of native and denatured forms of okra seed**
2 **proteins as coagulants in drinking water treatment**

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8

9 **Abstract**

10 The effects of temperature, storage time and water pH on the coagulation performance of
11 okra seed protein in water treatment were assessed. In a jar test experiment, okra salt extract
12 (OSEX) achieved a notable improvement in treatment efficiency with storage time and
13 showed good performance in quality after thermal treatment at 60, 97 and 140°C
14 temperatures for 6, 4 and 2 hours respectively. The performance improvement of more than
15 8% is considered to be due to the denaturation and subsequent removal of coagulation-
16 hindering proteins in okra seed. Furthermore, the results of a Sodium Dodecyl Sulphate
17 Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis show two distinctive bands of
18 protein responsible for the coagulation process after denaturation. It was further shown that at
19 optimal coagulant dose, the pH of the treated water remained unaffected as a result of the
20 protein's buffering capability during coagulation. Therefore, denatured okra seed exhibited
21 improved performance compared to the native crude extract and offers clear benefits as a
22 water treatment coagulant.

23 **Keywords:** coagulation, denaturation, okra, protein, seed, water treatment

24 **Introduction**

25 Background

26 Water treatment improves the quality of water supplied to the general public by eliminating
27 pathogens, turbidity and other contaminants in the raw water that may be harmful to human
28 health. The availability of good drinking water at all times enhances human development and
29 reduces the risk of contracting diseases that can emanate from polluted or contaminated
30 water. However, many communities in third world countries lack adequate access to potable
31 source of drinking water, with over 748 million poor people still lacking access to clean water
32 for domestic use (WHO and UNICEF, 2014). In Sub-Saharan Africa alone, the number of
33 communities that are not supplied with safe water today remains higher than in the 1990s.
34 Thus, water remains one of the greatest threats to mankind in the developing world. Water-
35 related disease has claimed over 1.8 million lives annually, mostly those of children under
36 five years of age, (WHO 2007). Efforts by government at all levels to tackle this problem
37 have failed in many third world countries due to lack of funds. Providing good water
38 infrastructure and skilled personnel to improve domestic water quality will save the lives of
39 many people in rural areas. To achieve this, coagulation and flocculation as a means of
40 domestic water purification should be enhanced. These processes are employed essentially to
41 improve the aggregation and settlement of particles that are later removed from sedimentation
42 and filtration units. The process is, however, dependent upon the ability of the coagulant and
43 flocculant aids to produce flocs with suitable characteristics. Coagulant and flocculant mixing
44 processes play a vital role in the transformation of particles into flocs (Duan and Gregory
45 2003) and the subsequent bridging of the flocs into bigger macro flocs. Aluminium sulphate
46 and ferric chloride are globally the most widely-used coagulants in drinking water treatment

47 (Duan and Gregory 2003). However, the presence of residual aluminium (Al) in the final
48 water has posed some concern to water and wastewater operators (Driscoll and Letterman
49 1988), as prolonged ingestion of aluminium in water has been linked to the development of
50 cognate decline in human brain and Alzheimer's disease (Gauthier et al. 2000). Martyn, et al.
51 (1989) reported that the rate of Alzheimer's disease in England and Wales was higher in areas
52 where the mean aluminium concentration exceeds 0.11mg/l than in locations with less than
53 0.01mg/l. Furthermore, some chemical polymers, e.g. acrylamide are also thought to be
54 carcinogenic (Malleleviale et al. 1984). In addition, the application of aluminium sulphate in
55 water treatment can consume the alkalinity and pH of the treated water that may result in
56 reduced coagulation efficiency.

57 However, in many developing countries, indigenous materials of natural plant and animal
58 origin have been used for decades in household water treatment. These materials, if properly
59 applied, have significant potential to increase the efficiency, and reduce the overall cost, of
60 water treatment in the developing world. Natural plant seeds are biodegradable, widely
61 available, environmentally friendly, and are non-toxic when consumed as food. Examples of
62 natural plant species previously studied include *Moringa oleifera* (MO) (Jahn 1988;
63 Ghebremichael et al. 2005), *Cactus* (Zhang et al. 2006) and *Mustard* seeds (Bodlund et al.
64 2014). Jahn (1988) used MO seed extract as a coagulant to alleviate domestic water supply
65 problems in the developing world. Madsen et al. (1987) also used MO extract as coagulant
66 and observed 99.5% reduction in turbidity accompanied by 80-99% reduction in coliform and
67 faecal coliform count. Additionally, it has been reported that the coagulation potential of
68 cactus plant is comparable to that of aluminium sulphate in removing turbidity in water with
69 low dose (Zhang et al 2006). Similarly, Bodlund et al. (2014) investigated the performance of
70 different mustard seeds in pond and synthetic water and reported a coagulation activity of
71 greater than 70% in mustard (large). To date, most research has focussed on the crude seed

72 extract and the purified seeds' proteins. Although MO has received more attention by
73 researchers than the other plant seeds, there are potentially many vegetable plants that could
74 perform as effectively and efficiently as MO in raw water treatment.

75 Extent of Okra plant applications

76 Okra is a plant widely grown in Nigeria and many other tropical regions of the world because
77 of its nutrients and ease of cultivation. It can grow under different environmental conditions
78 and reach maturity within three months of planting. Okra seed is a major source of protein,
79 vitamin, calcium and oil, and is capable of curing ulcers and providing relief from
80 haemorrhoids (Abidi et al., 2014). The okra pod contains carbohydrate and mucilaginous
81 substances capable of removing turbidity in water, treating tannery and industrial wastewater
82 (Agarwal et al. 2003). In their separate studies, De Jesus et al. (2013) and (Patale and Pandya,
83 2012) applied okra powder obtained from mature pod and achieved up to 99% reduction in
84 turbidity within 10 min sedimentation time, due to the presence of mucilage substances in the
85 pod. Conversely, the application of mallow and mucilage obtain from okra plant in water
86 treatment revealed a major drawback due to the addition of organic substances from the plant
87 in the final water (Anastasakis et al 2009). Furthermore, the flocculating performance of
88 various parts of okra plant including the seed was studied by (Fahmi et al. 2014) on 55 NTU
89 kaolin water and observed that, only 64.5% reduction in turbidity was achieved with okra
90 seed extract. Okra is one of the most consumed traditional vegetables, eaten fried or boiled,
91 steamed and may be added to salads, soups and stews. The extracted mucilage of Okra pod is
92 used as a suspending agent and as a pharmaceutical adjuvant in paracetamol and other drug
93 delivery (Sharma et al. 2013; Zaharuddin et al. 2014). It is also widely used in cosmetic,
94 pharmaceutical and food industries as preservative.

95 In natural plant seed extracts, the coagulating compounds reported in the literature concern
96 cationic protein (Ghebremichael et al. 2005). Studies have found the protein content in
97 defatted okra seed to be as high as 40-50% (Oyelade et al. 2003), whilst in crude form, okra
98 seed protein contents was found to be in the range of 23.8%-25.5%. Protein, in cationic state
99 can easily precipitate from solution with negatively charged substances. Okra seed protein
100 contains over 100 amino acids and more than eleven major amino acids including three
101 positively-charged (arginine, lysine and histidine), and the two anions of aspartic and
102 glutamic acids (Sami et al. 2013). The ϵ -amino group, the guanidine group and the imidazole
103 group of the corresponding lysine, arginine and histidine residues in okra can give proton
104 alkaline characteristics; they can bind the hydrogen ion and provide protein molecule a
105 positive charge after they are fully ionized.

106 Protein denaturation

107 A protein is said to become denatured when its folding structure is altered as a result of
108 exposure to certain elements of physical factors (e.g. heat), causing the protein to become
109 biologically inactive. Proteins also degrade and denature upon storage, with such
110 denaturation leading to visible aggregation and turbidity formation (Sharma and Luthra-
111 Guptasarma 2009). In some instances proteins can be renatured but in most cases the
112 denaturation is irreversible.

113 The research reported in this paper evaluates the performance of denatured Okra seed protein
114 compared to its native state as an alternative water treatment coagulant and disinfectant in
115 domestic water purification.

116

117 **Materials and Methods**

118 Collection and preparation of the Okra seed

119 A good quality seed of Okra was obtained at a local market in Hawul local government area
120 of Borno State-Nigeria. In this market, fresh and old seeds of high quality species of Okra are
121 readily available. The seeds were sorted, packaged and labelled appropriately for ease of
122 identification and transported to the UK for laboratory processing, preparation and analysis.
123 The seed was cleaned by washing with tap water in order to remove contaminants such as
124 dust, damaged seeds and plant debris which might affect the integrity of seeds during water
125 treatment. The seeds were then dried in an oven at 60°C for six hours before grinding.

126 Chemicals and reagents

127 Analytical grade chemicals and reagents (sodium chloride, sodium hydroxide, aluminium
128 sulphate and hydrogen chloride) were obtained from Fisher Scientific, UK and kaolinFluka-
129 60609, from Sigma Aldrich, Germany). Deionised (DI) water was used to prepare all the
130 suspensions and concentration solutions in this study.

131 Preparation and extraction of active compound in Okra seed

132 The seeds of okra were ground to fine powder using a laboratory miller (Tema mill,
133 Germany) for two minutes to obtain the desired powder. The resulting seed powders were
134 sieved through a set of stainless steel sieves (600 to 212µm). The powders retained on the
135 212 and 300µm were combined and used in the study.

136 The extract was prepared from the ground seed powders by adding 1.0 M NaCl solution to
137 the seed powder to make 2% (w/v) suspension, i.e. 2g of the seed powder in 100 ml NaCl.
138 The suspension was vigorously stirred using a magnetic stirrer for 15 minutes at room
139 temperature (19±2°C). In many tropical countries, room temperature ranges between 22 and
140 25°C. The suspension was then centrifuged at 4000 rpm for 10 minutes using a Heraeus

141 Megafuge16 (Thermo Scientific, Germany). The suspension was decanted and the residual
142 solids were dried in an oven at 50°C overnight. The weight of the dried solid material was
143 measured to ascertain the amount of seed powder used in making the suspension. The
144 decanted suspension was then filtered through a Whatman No. 42 filter paper and the filtrate
145 termed okra salt extract (OSEX). Similarly, the extract was prepared by dissolving 2g of the
146 seed powder in 100ml of deionised (DI) water to make 2% suspension, to extract the
147 coagulating compound in the seed. The suspension was stirred using a magnetic stirrer for 15
148 minutes and then centrifuged at 4000 rpm for 10 minutes. The suspension was decanted as in
149 OSEX and then filtered through a Whatman No. 42 filter paper and the filtrate termed okra
150 water extract (OWE). In addition, 2g of MO was dissolved in 100ml NaCl to make 2%
151 suspension of MO extract and used in the study. Protein concentration in the extracts was
152 estimated following (Bradford, 1976), where proteins absorbance was measured at 595nm
153 and its concentration determine on a standard Bovine Serum Albumin (BSA) curve.

154 Denaturation of Okra salt extract

155 The OSEX solutions were heated at different temperatures of 60, 97 and 140°C for 6, 4 and 2
156 hrs respectively, using a hot plate, because most people in developing countries uses
157 firewood as a source of cooking energy which is difficult to control, thus the need for a wider
158 temperature range. The heated samples were then centrifuged at 4500 rpm for 10 minutes and
159 were filtered through a Whatman no. 42 filter paper and used in the study. Similarly, the
160 extract was stored for 1, 3, 7, 10 and 14 days to denature the extract. Finally, the molecular
161 weight of the extracts and the denatured protein samples were determined on 12% Sodium
162 Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Protein sample was
163 resolved by SDS-PAGE (normal PAGE) and transferred to a nitrocellulose membrane
164 (Protran BA-85, Pierce Protein Biology). The membrane was blocked in 5% milk/1X TBST
165 (Tris-buffered Saline-TWEEN 20) and incubated on a rocker at room temperature for 30 min.

166 After the blocking; the membranes were incubated with primary antibody polyclonal goat
167 anti-GFP (AbD Serotec) 1:2000 of antibody in TBST overnight at 4°C. At the end of the
168 incubation, the membranes were washed three 3 with TBST at 5 min interval each and then
169 incubated with secondary antibody (polyclonal anti-Goat HRP) 1:1000 of antibody in TBST
170 for 1 hr at room temperature on a rocker. The membranes were washed again as done at the
171 end of primary antibody incubation. The blots were then incubated with West Pico
172 Chemiluminescent Substrate (Pierce) and then visualized with Gene Snap Software
173 (SynGene).

174 Water samples for coliform and E-coli tests

175 Natural water samples were collected from the Bourn Brook River adjacent to the University
176 of Birmingham in order to determine the bacterial inactivation capability of the heated OSEX
177 on coliform count and E-coli present in the water. The river water was spike with kaolin to
178 bring the turbidity level to 45 NTU. After the jar test, the experiment was conducted using
179 Colilert-18/Quanti-Tray (IDEX Inc. UK) for coliform/E-coli detection because of its ease of
180 operation, flexibility, accuracy and speed. Colilert 18 was added to the water sample in a
181 100ml sterilized vessel and the mixed reagent was then decanted into a tray, sealed and
182 incubated at 35°C for 18 hours. The presence of E-coli was determined with the help of a UV
183 probe. The number of positive wells was counted and then read off on the most probable
184 number (MPN) chat provided by IDEXX. The water sample was treated with two ranges of
185 OSEX doses (50 and 80 mg/l) and the results compared with aluminium sulphate as a
186 coagulant. The performance of OSEX in natural water environment in terms of turbidity
187 removal was also evaluated.

188 Preparation of the synthetic turbid water

189 Turbid water samples for jar test experiments were prepared by adding kaolin particles into
190 tap water. 40g of laboratory grade kaolin (Fluka and high grade, Sigma Aldrich) was added to
191 400ml of tap water and the suspension stirred for 30 minutes using a magnetic stirrer. The
192 suspension was made up to 1L by adding 600ml of tap water and then stirred for further 30
193 minutes. The suspension was allowed to stand for 24hr for the kaolin to hydrate. The
194 suspension was vigorously mixed for five minutes and the contents mixed with 30 litres of
195 tap water and allowed to stand overnight for particle settlement. The supernatant was
196 decanted and its turbidity measured. Depending on the level of turbidity required, the
197 supernatant was either diluted with tap water or concentrated with kaolin suspension.
198 Turbidity and pH were determined as initial values using standard methods before conducting
199 the jar test experiments. Buffer capacity (BC) was calculated following (Morr et al. 1973).

200 Where: $BC = \text{titrant (mg)}/\text{wt of protein} \times \Delta\text{pH}$

201

202 Coagulation and flocculation test

203 Jar tests were conducted using a standard apparatus comprising 6 1 litre beakers (Phipps and
204 Bird, 7790-900B USA) to evaluate the optimum coagulant dose for the coagulation tests. For
205 effective dispersion of the coagulant the water was rapidly mixed at 200rpm ($G = 240 \text{ s}^{-1}$) for
206 1 minute during which various doses of the coagulant were added to the beakers. The mixing
207 speed was then reduced to 30rpm ($G = 23 \text{ s}^{-1}$) for a further 30 minutes to simulate the
208 flocculation stage. The suspension was then allowed to stand undisturbed to facilitate
209 settlement for 1hour. A final treated water sample (10 ml) was drawn 2cm from the top
210 surface of the water in the beakers using a syringe. The turbidity of the water was then
211 measured using a turbidity meter (HI 93703, Hanna) and the water pH was measured with a

212 pH meter (Mettler Toledo SevenGO, Switzerland). All experiments were conducted at room
213 temperature ($19\pm 2^{\circ}\text{C}$).

214

215 **Results**

216 Coagulation performance of native (non-denatured) Okra extracts

217 The effect of varying native OSEX dose on turbidity removal was investigated, and results
218 are shown in Fig. 1. Different doses (0 – 80 mg/l) were applied to synthetic turbid water with
219 two levels of turbidity, 100 and 200 NTU using a standard jar test procedure. The advantage
220 of using the synthetic turbid water over river water was that it enables the study to simulate
221 the different levels of turbidity in water. The lowest residual turbidities were achieved with
222 40 mg/l (100 NTU) and 60 mg/l (200 NTU) doses of OSEX, with corresponding removal
223 efficiencies of 91 and 98% respectively. Furthermore, it was also observed that at coagulant
224 dose higher than 40mg/l, there was no reduction in residual turbidity in water with 100 NTU.
225 Similarly, when the coagulant dose in the high turbidity water (200 NTU) exceeded 60mg/l,
226 residual turbidity exhibited a modest increase. In addition, the study investigates the removal
227 of turbidity in water with 100 and 200 NTU using OWE as shown in Fig. 2. The result shows
228 that DI water extract did not yield any significant performance at the end of the treatment
229 process. Minimum residual turbidity was 67.25 NTU, representing only 33% turbidity
230 removal efficiency, using 40 mg/l dose of OWE sample while in the 200 NTU water, final
231 residual turbidity was 160 NTU, representing approximately 20% efficiency at maximum
232 dose of 80 mg/l used in the study. Further coagulant addition in the 100 NTU water also
233 resulted in re-stabilisation of colloids in water, indicating poor performance beyond 40 mg/l.
234 The typical protein concentration in OSEX was found to be 1.018 mg/ml while that of OWE
235 was 0.264 mg/ml. Therefore, the poor performance in OWE show that DI water is not a

236 strong solvent, aggressive enough to extract okra seed proteins while NaCl solution was
237 observed to be very effective in this regard due to salting in effect.

238 Effect of pH change on the performance of the OSEX

239 Different pH values were assessed in order to determine the optimum coagulation and
240 flocculation pH because of its importance in water treatment and also as it affects the stability
241 of protein. The OSEX dosage that achieved maximum turbidity removal was then
242 investigated. In addition, the effects of pH on the optimal dosage of the coagulant found in
243 the earlier experiments were also investigated. Specifically, different OSEX doses (40, 60
244 and 80 mg/l in the pH range 4 – 9) were assessed for turbidity removal on kaolin water with
245 original turbidity of 200 NTU and the results are shown in Fig. 3. Minimum residual turbidity
246 was found at pH of 4 and the maximum residual turbidity was found at pH 9 for the three
247 doses. At pH4, turbidity removals were 99, 98 and 97% for 40, 60 and 80mg/l doses
248 respectively. The effect of coagulant addition on final water pH during the jar tests was
249 considered for aluminium sulphate, MO and OSEX by incrementally dosing 10 –100 mg/l of
250 each coagulant to water with initial turbidity of 200 NTU. As the doses of each of the
251 coagulant increased the corresponding change in water pH was measured. Fig.4 shows that
252 the treated water pH was largely unaffected when the natural coagulants, MO and OSEX,
253 were used. This is because the pH of the water was buffered due to the presence of protein in
254 the seed during coagulation. The amount of proteins used for coagulation was 6.11mg in
255 OSEX and 7.9mg in MO with a BC of 0.016 and 0.017 respectively. However, alum dosing
256 to 40 mg/l produced an approximately linear reduction in pH from an initial value of pH 7.5
257 to pH 5, followed by a reduced rate of pH change (pH 5 to pH 4.5 when dosed from 40 to 100
258 mg/l). Similarly, as the doses of each of the coagulant increases the removal of turbidity also
259 increases in MO and alum treated water until maximum dosage 100 mg/l was reached. The
260 lowest residual turbidity in the final water of 4.29 and 4.08 NTU were achieved with (alum

261 and MO) and 4.89 NTU with OSEX using 60mg/l. The results show that all the coagulants
262 achieved approximately 98% turbidity removal. Fig. 4 shows that, all the coagulants
263 exhibited the same trend of behaviour in terms of turbidity removal. However, seed proteins
264 are amphoteric and contain both the basic and acidic amino groups which can buffer in
265 solution. Thus, in water treatment processes, the relationship between the initial and the final
266 water pH play an important role in determining the optimum pH required for coagulation.

267 Effects of denaturation by storage and temperature on the integrity of the OSEX

268 The effect of storage time on the performance of OSEX was considered in order to identify
269 the most appropriate time stock solution of OSEX will take before any deterioration in quality
270 as a coagulant. This is vital in developing countries, where electricity supply is a major
271 challenge, and the cost of obtaining modern, temperature-controlled storage facilities is
272 prohibitive. Fresh OSEX was prepared and stored in 200ml open beaker at room temperature
273 of 19 ± 2 °C between 1 and 14 days interval to observe its denaturation process. Performance
274 was assessed using OSEX which had been stored for 1, 3, 7, 10 and 14 days in terms of
275 turbidity removal. Fig. 5 shows that the performance of OSEX as coagulant increases with
276 storage time to day 10, after which its effectiveness in turbidity removal deteriorated.
277 Optimum performance was observed when OSEX was dosed at 40 mg/l. This yielded a
278 reduction of 92% from 130 NTU to less than 10 NTU. The coagulation performance at 60
279 mg/l dose was the same with that of 40 mg/l, and deterioration was observed when dose
280 exceeded 100 mg/l.

281 The effects of temperature variation to denature OSEX sample and its performance when
282 treating synthetic water of initial turbidity of 200 NTU were considered by heating the extract
283 to 60°C for 6 hrs, 97°C for 4 hrs and 140°C for 2 hrs (Fig.6). Under this condition, all the
284 extract samples were used immediately after preparation (fresh). The results show that at 80

285 mg/l, maximum turbidity removal efficiencies of >97% were recorded with all the denatured
286 extracts, whereas a maximum efficiency of 93% was recorded with non-denatured extract.
287 However, at lower coagulant dose, 10 mg/l, turbidity removal efficiency of the unheated
288 extract was approximately 80% while the heat treated samples recorded between (66 and 74)
289 performance. It is noteworthy that when the performance of the heat treated sample at 60°C
290 for 6 hrs was applied at a higher dose of 200 mg/l to a very high turbidity water (550 NTU),
291 the residual turbidity was observed to be 2.7 NTU representing a percentage removal
292 efficiency of >99% (Fig. 7). This is a typical water turbidity level in streams and rivers in the
293 tropics, especially in Sub Saharan Africa after rainfall event. Therefore, it is important to
294 investigate the performance of OSEX on very high turbidity water for people in developing
295 countries.

296 SDS-PAGE analysis of the three samples

297 SDS-PAGE analysis was conducted on the OSEX in order to obtain information on the
298 extract and the denatured samples as well as to determine the molecular weight (MW) of the
299 different fractions of OSEX. Here, OSEX denatured by heating and OSEX denatured by
300 storage together with non-denatured OSEX were analysed in order to provide information
301 regarding the stability of the different protein sizes after exposure to high temperature and
302 storage. The various bands and sizes of the proteins are depicted in Fig. 8. The SDS PAGE
303 results showed some similar distinctive protein bands with MW from 4 to 12 kDa in all the
304 samples. Faint bands were observed across the stored sample compared to the crude and
305 heated extracts. However, the densest bands were found in the heated samples at MW of 20
306 and 45 kDa. Interestingly however, the band with MW 43 kDa of protein in the heated
307 samples was not visible in either the crude extract or the stored samples. This is thought to be
308 a result of the removal of some overlapping proteins during heating which were absent in
309 both the crude and the stored samples. The concentration of proteins across all the bands is

310 higher in the heated sample than in crude extract and stored samples. However, despite the
311 faint band in the stored sample, the coagulation performance of the stored sample was found
312 to outperform that of non-denatured sample.

313 Bacterial inactivation by okra crude extract

314 The results of the bacterial reduction of coliforms and E-coli count using OSEX are
315 presented in Table 1. In this study, colillert-18 Quanti-Tray method was adopted to assess the
316 bacterial quality of the raw water before and after treatment with OSEX and the result
317 compared with that of aluminium sulphate. It is noteworthy that the number of large and
318 small positive well for coliform were the same in the raw water, before and after treatment
319 because it consist of multiple presence of microbes other than E-coli. In this case, the number
320 of coliform count was found to be 2419.6 MPN/100ml in both raw and treated water
321 respectively. However, there was substantial reduction in total E-coli count as observed in
322 Table 1, using 50 and 80 mg/l coagulant dosages. E-coli is a subset of total coliform, hence,
323 the result show that E-coli count/100ml in the raw water before treatment was 727.0/100ml
324 while after the treatment, the total E-coli count was reduced to 79.9/100ml and 54.5/100ml,
325 giving a percentage reduction in E-coli count of 89% and 93% respectively. In addition, at
326 optimum coagulant doses of 50 and 80mg/l, turbidity removals were 88% and 75%
327 respectively, in the final water. In both cases, there was no observed decline in total coliform
328 count after treatment, suggesting that some organic compounds in the seed were utilised as
329 substrate by other microbes in the water to aid growth. Further investigation using
330 aluminium sulphate as a coagulant achieved 86% reduction in E-coli count (from
331 727.0/100ml to 102.2/100ml) with a corresponding reduction of 28% in total coliform (from
332 2419.6 MPN/100ml to 1553.1 MPN/100ml). Similarly, there was substantial reduction in
333 turbidity by approximately 98% in final water treated with aluminium sulphate. The high

334 removal of turbidity in alum treated water may be responsible for the reduction in total
335 coliform count in this regard.

336 **Discussion**

337 In the work reported here, OSEX in its native form was used as a coagulant in treating water
338 with turbidity of 100 and 200 NTU. It was observed that an increase in OSEX dose resulted
339 in reduction of residual turbidity to a minimum value beyond which further addition of
340 coagulant caused an increased turbidity as a result of re-stabilisation of the colloids (Fig. 1).
341 At this point, there were more positively charged species than the available surface charges
342 on the colloids which encouraged stabilisation. At coagulant dose of 40 - 60 mg/l, maximum
343 turbidity reductions of 91 – 98% were observed, as shown in Fig.1. Maximum removal
344 efficiency was recorded in the high turbidity water (200 NTU) producing a residual turbidity
345 of 4.9 NTU, a value which is compliant with the WHO and Nigerian drinking water standards
346 of 5 NTU (SON 2007). This result is in agreement with finding reported by Katayon et al.
347 (2004) who used MO extract to evaluate the performance of MO in low and high turbidity
348 water. Turbidity removal was effective in high turbidity water because destabilization is
349 influenced by a high rate of colloidal interaction which encourages particle bridging (Gregory
350 and Duan 2001).

351 In the work reported here, it was observed that OWE indicated poor performance in terms of
352 turbidity removal. This means that, DI water is not aggressive enough to extract the
353 coagulating compounds while solvent such as NaCl solution was seen to be more aggressive
354 in extracting the coagulating protein. Fig. 2 shows that turbidity removal were 33% and 20%
355 when OWE was used to treat 100 and 200 NTU water compared with 91% and 98% achieved
356 with OSEX sample. Furthermore, protein concentration in OSEX was 3.8 times higher than
357 that in OWE. The effectiveness of OSEX may be due to the salting-in effect in the salt

358 extracts causing an increase in protein solubility and dissociation as reported by Okuda et al.
359 (1999), because NaCl solution has a substantial effect on the solubility of a protein.

360 To investigate further the character of okra protein, the relationship between coagulant doses
361 at different pH as it affect protein stability was investigated, as shown in Fig. 3, using the
362 optimal coagulant doses obtained in the previous tests. The results showed the effect of
363 turbidity removal at lower pH to be significant ($p < 0.05$), with a maximum of 99% efficiency
364 removal at pH of 4. This shows that low water pH had an important effect on the coagulation
365 of turbid water with OSEX. Whilst it is not practical to treat water at such a low pH, OSEX
366 has shown to perform effectively at pH of 6.5 - 7.5 as well. These were the ranges of water
367 pH tested in the previous experiments. Each protein has an optimal pH to attain its biological
368 function and its activity is normally affected by only a slight change in pH. Generally, an
369 acidic environment is considered to be conducive to the binding of a proton from the
370 dissociated carboxyl group and the transformation of protein into a cationic state, which is
371 responsible for the charge neutralization on particles in the water. It is known that inorganic
372 particles are negatively charged in aquatic environments and the net surface charge of
373 colloidal particles is reduced at low pH (Gregory 2005) which encourages the double layer
374 compression. While study has reported that natural coagulants are most effective in
375 coagulating water at pH in the region of pH 8 and above (Okuda, et al. 2001), the study
376 reported here observed the most effective pH to be lower, pH 4. This demonstrates that the
377 amino acid composition of proteins in different plants may have different coagulation
378 activities. The difference in coagulating property may be attributed to the type of protein in
379 okra seed although this requires further investigation.

380 The effect of coagulant addition on treated water pH and turbidity removal was investigated
381 as shown in Fig. 4. MO, the most studied natural plant; aluminium sulphate, a widely-used

382 synthetic coagulant in water treatment; and OSEX were each tested in water with a turbidity
383 of 200 NTU. The results show that all the coagulants achieved approximately 98% turbidity
384 removal efficiency with somewhat similar coagulation action. Turbidity reduction increases
385 as coagulant dose increases, indicating the level of charge neutralisation to be similar, though
386 the performance of OSEX was not as effective as that of MO and alum due to its high lipid
387 content. Furthermore, the results show that incremental dosing of both OSEX and MO
388 extracts yielded a plateau curve nature, meaning that the pH of the final water remained
389 unaffected from its initial pH of 7.36 whereas alum was found to depress the pH of the water
390 to pH 4.3. In aqueous solutions, amino acids contain weak α -amino groups (basic) and weak
391 α -carboxylic groups (acidic). Furthermore each of the basic and acidic amino groups contain
392 in its side chain an ionisable group and so the combined actions of free amino acids and other
393 amino acids in peptide linkages act as effective buffers during coagulation which resist a
394 change in pH of the water. Thus, natural coagulants offer an advantage over synthetic
395 coagulants since no chemical addition is needed to control the pH of the treated water.

396 The efficacy and integrity of OSEX after denaturation was assessed based on different
397 storage duration as shown in Fig. 5, and temperature as indicated in Fig 6. Interestingly, it
398 was observed that the coagulation efficiency of OSEX improved by approximately 8% with
399 storage time from the third to tenth days, even though the increase was not appreciable and
400 then degraded in quality thereafter. This suggests that, since there are many different sizes or
401 bands arising from heterogeneity of one or more active proteins in seed (Ghebremichael et al.
402 2005), some of the proteins which were eliminated during the storage due to denaturation are
403 protein compounds that hinder coagulation activity. However, the report presented here is not
404 in agreement with the results reported by Katayon et al. (2004), who noted a decrease in
405 turbidity removal efficiency of MO extracts stored longer than a day. This may be attributed
406 to the difference in protein compounds in okra and MO seeds, because many proteins can be

407 denatured within few hours of storage. Here, the degradation in performance after the tenth
408 day was caused by the aggregation, precipitation and repugnant odour emission from the
409 protein sample. It was observed during the course of the study that, there was also the issue of
410 physical protein agglomeration and adhesion on the container which could have added to the
411 degraded performance after the tenth day. A wide range of characteristics can be exhibited by
412 denatured proteins, from reduced solubility to communal aggregation.

413 OSEX was also heated (and so denatured) at different temperatures and its coagulation
414 efficiency evaluated after the heat treatment as shown in Fig. 6. It was observed that at the
415 lower coagulant dose of 10 mg/l, the efficiencies of the heat treated samples deteriorated
416 compared to the native sample. This could be due to the disruption of both the secondary and
417 tertiary structure of the proteins with only the primary structure available for activity which
418 might have low coagulation potential at a lower dose. However, there were improvements in
419 coagulation efficiencies at all doses above 10 mg/l. The highest performances were recorded
420 at 80mg/l for all the coagulants but the heated samples showed more than a 97% reduction in
421 turbidity compared to 93% for untreated sample (native). It was further observed that the
422 degree of improvement was rather varied across the denatured samples at doses of (20-
423 60mg/l) but still recorded approximately the same efficiency at 80mg/l. Thus, heating can
424 improve the coagulation potential of okra crude extract for people in developing countries as
425 home water treatment coagulant, where access to clean water is a big challenge. At a
426 coagulant dose of 100mg/l, all the samples deteriorated in performance compared to 80mg/l.
427 It is noteworthy that the deteriorated performance of the denatured samples still outperformed
428 the highest recorded efficiency of the non-denatured sample. This shows that the extract is
429 stable after heat treatment. Again, it was seen that heating could improve the effectiveness of
430 the filtration process. The time taken to filter 100ml of the heated sample was between 30 and
431 40 minutes compared to more than 6 hours for non-treated sample. This demonstrates that the

432 denaturation of proteins that are partially sensitive to storage time and temperature are
433 beneficial, since their removal during the process further improves the quality of the
434 coagulant protein. The two processes can therefore be considered as a simple protein
435 purification technology which can easily be adopted in developing countries.

436 An assessment of the performance of the denatured sample on a very high turbidity water of
437 550 NTU was undertaken. Fig. 7 shows the removal of turbidity was found to be more than
438 99% with a residual turbidity of 2.7 NTU at optimum coagulant dose of 200mg/l. This is
439 similar to river water turbidity found in most tropical countries of the world, especially after
440 heavy rainfall. Therefore, a higher coagulant dose may be required to achieve the WHO water
441 quality standard as shown in this experiment. Further work is required to assess the potential
442 of treating natural water which may contain natural organic matter as contaminants with Okra
443 extract.

444 The antibacterial activity of the denatured OSEX and alum was tested on contaminated river
445 water with low turbidity level (spike with kaolin), using collilert-18 Quanti-Tray method. The
446 Quanti-Tray test was conducted to assess the bacterial removal efficiency of the extract. The
447 result revealed that at coagulant doses of 50 and 80mg/l, the total E-coli count in the water
448 was reduced by 89% and 93% respectively, but the coliform counts remained unaffected after
449 the test, as shown in Table 1. On the other hand, when alum was used as coagulant, total E-
450 coli count in the water was reduced by 86% while there was also a 28% reduction in total
451 coliform count. It is clear here that the reduction in E-coli in OSEX treated water was due to
452 inactivation capability of the extract. Again the results revealed that some of the organic
453 compounds in the seed can served as substrates to many pathogens in the water which feed on
454 it and hence remain unaffected during the treatment. This result shows that E-coli are more
455 sensitive to the chemical compounds in the extract than the other microbes in the water.
456 Madsen et al. (1987) had shown a direct relationship between E-coli reduction and removal

457 of turbidity in treated water with MO extract at optimal coagulant dose. The Okra seed
458 contains phenol, alkaloids, flavonoids, saponins and ribosome-inactivation proteins (RIP)
459 (Kondo and Yoshikawa, 2007) which show clearly that the extract has pathogen inactivation
460 capability. Another possible reason for the reduction in E-coli count could be due to bacterial
461 attachment to the floc during sedimentation as reported by (Madsen et al. 1987). Furthermore,
462 turbidity removal was 88% at optimum coagulant dose of 50 mg/l while at a higher dose of
463 80mg/l, turbidity removal was only 75% with OSEX whereas alum achieved up to 98%
464 reduction in turbidity in the water after treatment. It can be deduced from the study that the
465 coagulation mechanism was the main cause of E-coli count reduction in alum treated water
466 while the reduction achieved with the extract was due to inactivation potential of OSEX as a
467 coagulant since the coliform count remain unaffected after treatment.

468 The different protein bands in crude extract (lane 1) and denatured samples of okra (by
469 heating – lanes 2 and 4, @ 140 and 60°C respectively) and storage (lane 3) were compared in
470 order to assess the effect of heat treatment and storage time on proteins denaturation (Fig.8).
471 The results show that the band in the CE with MW of 15kDa was removed following heat
472 treatment and was faint and less dense in the stored sample, indicating its susceptibility to
473 heating and storage. The concentration of the band between 17 and 21 kDa was more
474 discernible in the heat-treated samples than in the crude extract and stored samples. This
475 indicates many overlapping protein compounds that were removed during heating and
476 prompted the increase in coagulation efficiency. It is clear that the wider and denser band of
477 proteins around 43kDa in the heated samples was as a result of the effectiveness of the heat
478 treatment. This facilitated the removal of the overlapping proteins in the band that possessed
479 non-coagulating compounds. It is thought that this is the reason why there was increased
480 coagulation activity in the heat-treated sample compared with the crude extract. Bodlund et
481 al. (2014) also showed in a study where some Mustard seed species were heated at 95°C for 5

482 hours and observed the Mustard seeds to be thermos-stable, which resulted in increased
483 coagulation performances of the extracts. It was observed in this study that heating and
484 proper storage of the extract samples before employing in water treatment can effectively
485 remove the coagulation-hindering protein in the seed for effective performance.

486 5.0 Conclusion

- 487 ❖ Denaturing the protein of okra seed either by heating or storage destroyed both the
488 secondary and tertiary structure of the protein, yielded an increase in MW of 21 and
489 43 KDa and gave rise to improved coagulation performance. Denaturing is therefore,
490 considered advantageous to people in developing countries where access to clean
491 drinking water is still a major cause of death, though the increase was not much.
- 492 ❖ The crude extract sample of okra seed showed high coagulation activity in high
493 turbidity water than in low turbidity water. Although not as effective or efficient as
494 the denatured samples, it can still be considered as a good coagulant in terms of
495 turbidity removal in home water treatment.
- 496 ❖ The bacterial inactivation capability of the extract is notable, eliminating E-coli by
497 approximately 89% at optimum dose and 93% with a higher dosage, thought to be due
498 to the presence of saponins and ribosome-inactivating protein in the extract. Further
499 tests on its minimum inhibitory concentration on E-coli and other pathogenic bacteria
500 found in water are required to reveal its potential as disinfectant further.
- 501 ❖ Okra extract quality can be improved locally by simple heat treatment at household
502 level without any requirement for more sophisticated heating facilities to achieve the
503 desired improvement in treated water quality.

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510 Conflict of interest

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515 Author's contributions

516 The first author conducted the laboratory experiments and participated in the analysis of the
517 results and writing up of the paper.

518 The second author participated in reviewing the experimental procedures, results analysis and
519 writing the paper.

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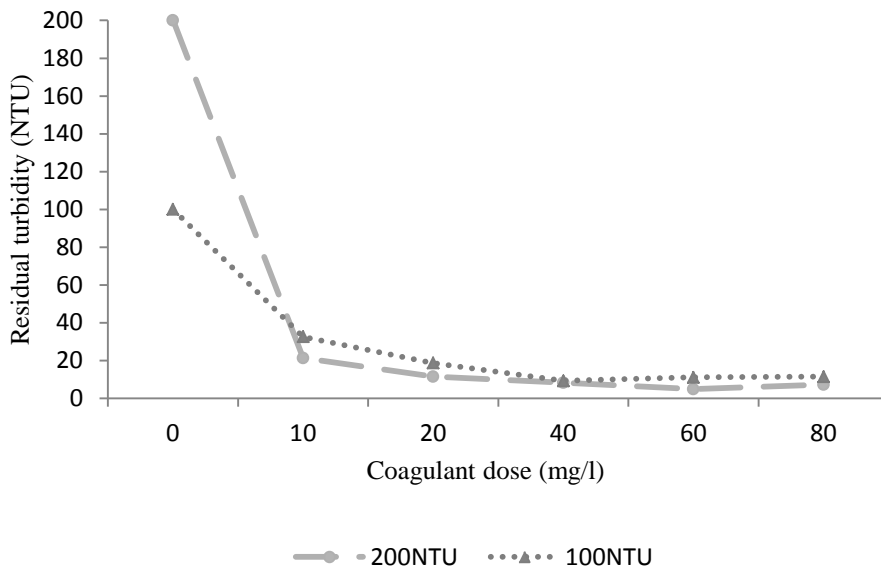
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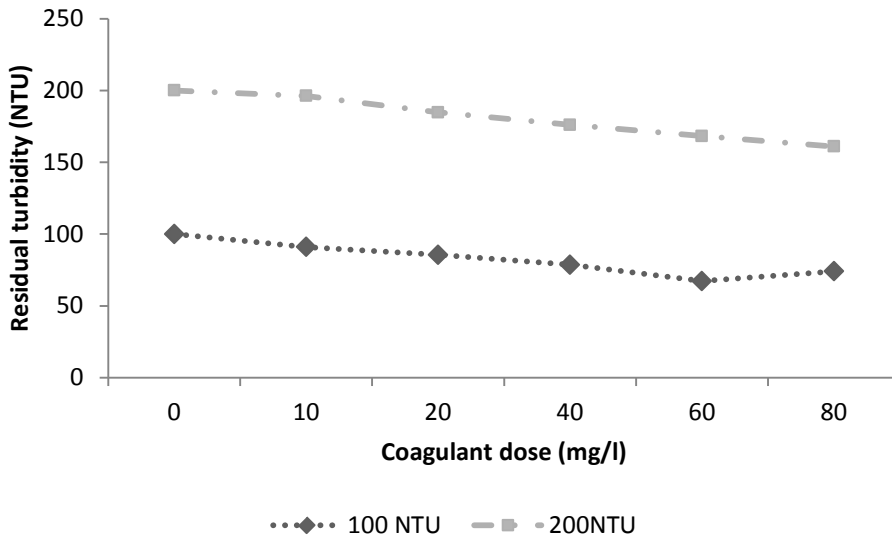
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607 **List of figures and table**



608

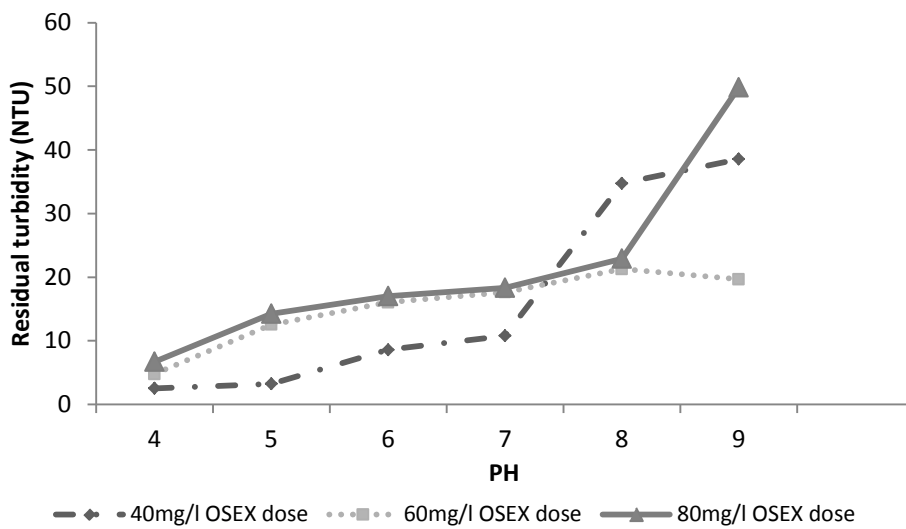
609 **Fig. 1** Performance of OSEX as a coagulant in treating turbid water.



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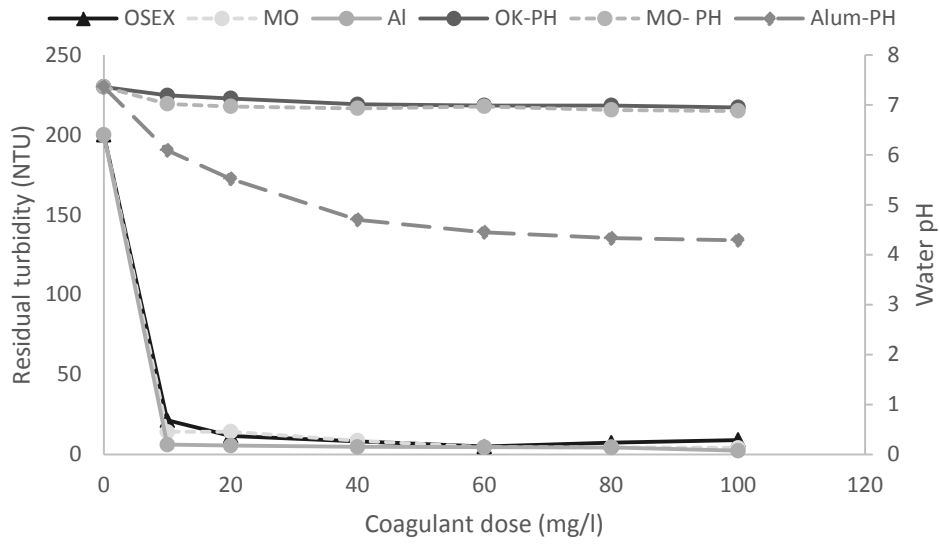
611 **Fig. 2** Performance of OWE as a coagulant in treating turbid water.

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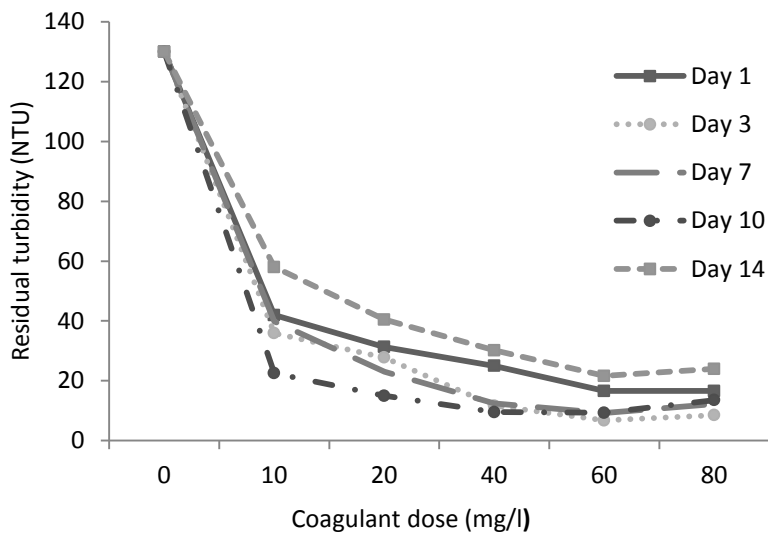
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614 **Fig. 3** Influence of pH on turbidity removal using OSEX.



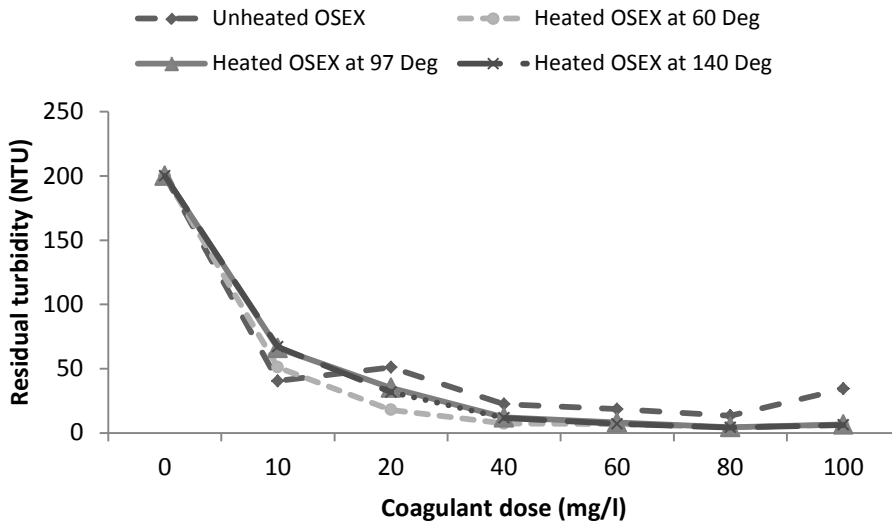
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616 **Fig. 4** Effect of different coagulants doses on final water pH and turbidity.



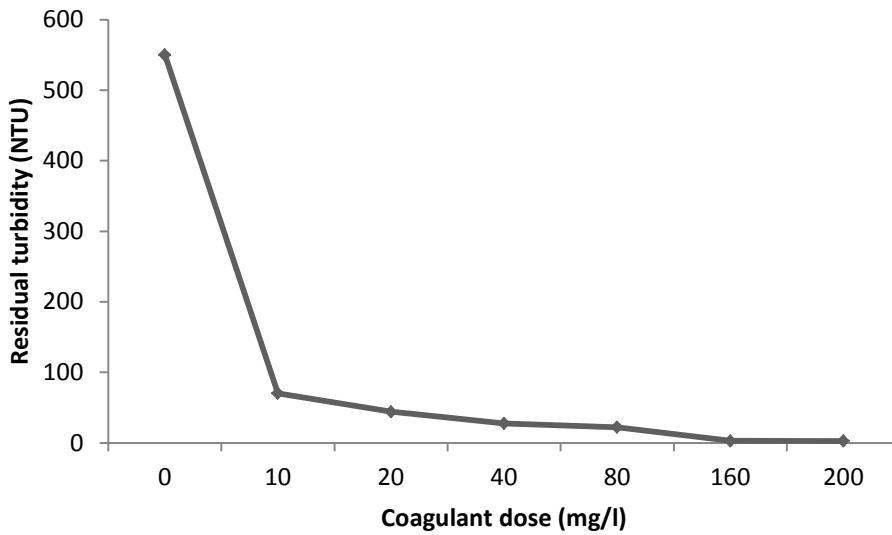
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618 **Fig. 5** Removal of turbidity in synthetic water using stored OSEX.



619

620 **Fig. 6** Removal of turbidity in synthetic water using thermal treated OSEX.



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622 **Fig. 7** Removal of turbidity in very high turbidity water using thermal treated OSEX.

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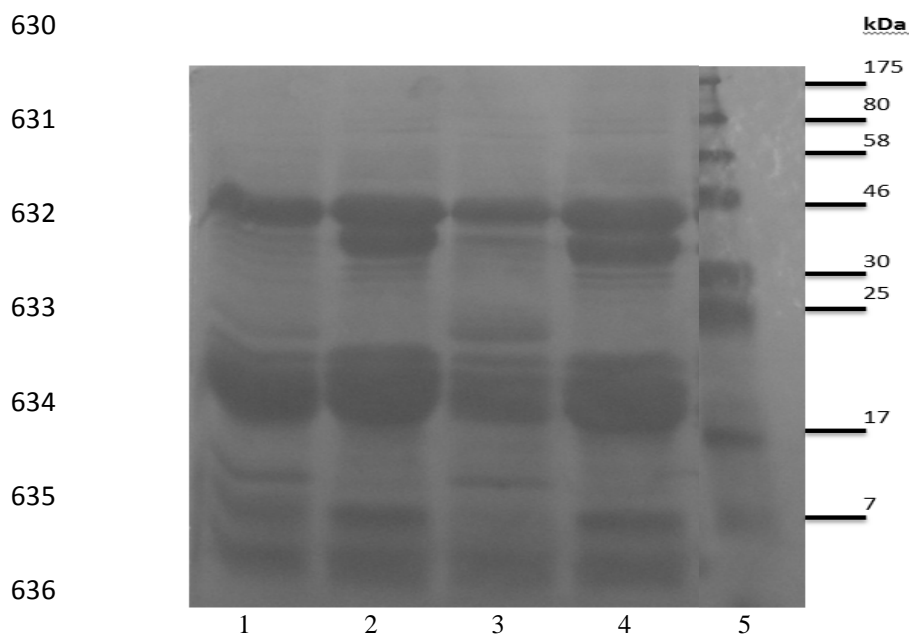
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638 **Fig. 8** Protein profiles of OSEX using 12% SDS PAGE analysis. Lane 1 untreated OSEX, Lane 2 heat treated
 639 OSEX at 140 °C for 2 hrs; Lane 3 stored OSEX sample for 3 days and Lane 4 heated OSEX at 60°C for 6 hrs
 640 and Lane 5 a Marker (New England, BioLab).

641

642 **Table 1 Removal of coliform and E-coli count in river water using OSEX**

Parameters	Raw water	OSEX-50mg/l	80mg/l	Alum-treated
Initial water turbidity (NTU)	45	5.6	11.4	0.92
<i>Number of positive wells for coliform:</i>				
• Large wells	49	49	49	49
• Small wells	48	48	48	45
Total coli form count (MPN/100ml)	2419.6	2419.6	2419.6	1732.9
Percentage reduction in coliform count (%)		0	0	28
<i>Number of positive wells for E-coli:</i>				
• Large wells	49	31	29	44
• Small wells	33	19	8	3
E-coli count (MPN/100ml)	727	79.9	54.5	102.2
Percentage reduction in E-coli count (%)		89	93	86

643