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DOI:
10.2166/wh.2016.015

Document Version
Peer reviewed version

Citation for published version (Harvard):
Jones, A & Bridgeman, J 2016, 'An assessment of the use of native and denatured forms of okra seed proteins as coagulants in drinking water treatment', *Journal of Water and Health*. https://doi.org/10.2166/wh.2016.015

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

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Abstract

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

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

Introduction

Background

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

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

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

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

Extent of Okra plant applications

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

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

Protein denaturation

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

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

Materials and Methods

Collection and preparation of the Okra seed

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

Chemicals and reagents

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

Preparation and extraction of active compound in Okra seed

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

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

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

Denaturation of Okra salt extract

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

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

Water samples for coliform and E-coli tests

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

Preparation of the synthetic turbid water

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

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

Coagulation and flocculation test

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

pH meter (Mettler Toledo SevenGO, Switzerland). All experiments were conducted at room temperature (19±2ºC).

Results

Coagulation performance of native (non-denatured) Okra extracts

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

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

Effect of pH change on the performance of the OSEX

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

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

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

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

The effects of temperature variation to denature OSEX sample and its performance when treating synthetic water of initial turbidity of 200 NTU were considered by heating the extract to 60^{o}C for 6 hrs, 97^{o}C for 4 hrs and 140^{o}C for 2 hrs (Fig.6). Under this condition, all the 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^{o}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

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

Bacterial inactivation by okra crude extract

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

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

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

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

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

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

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

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

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

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

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

5.0 Conclusion

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

Funding

505 The first author appreciates and remains grateful to the Tertiary Education Trust Fund
506 (TETFund AST & D/2013/14) and Nigerian government for funding this research work.

507 Acknowledgements

508 The authors wish to acknowledge the assistance in the laboratory work given by Mr Mark
509 Carter of the School of Civil Engineering, University of Birmingham, UK.

510 Conflict of interest

511 The authors wish to declare that there are no conflicts of interest regarding the publication of
512 this paper. The present research work was not financially supported by any person or
513 organizations since the beginning to the end of this submitted work that may have any
514 influence whatsoever on this paper.

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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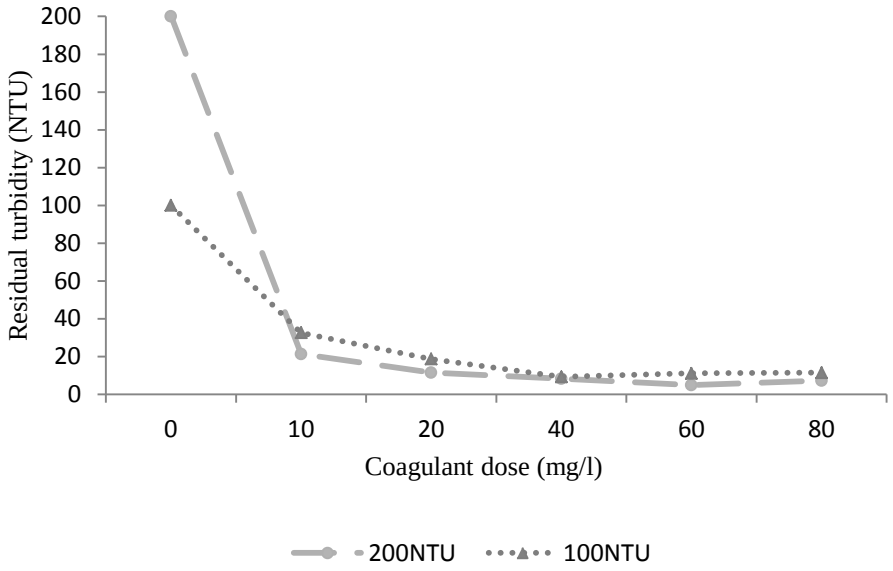
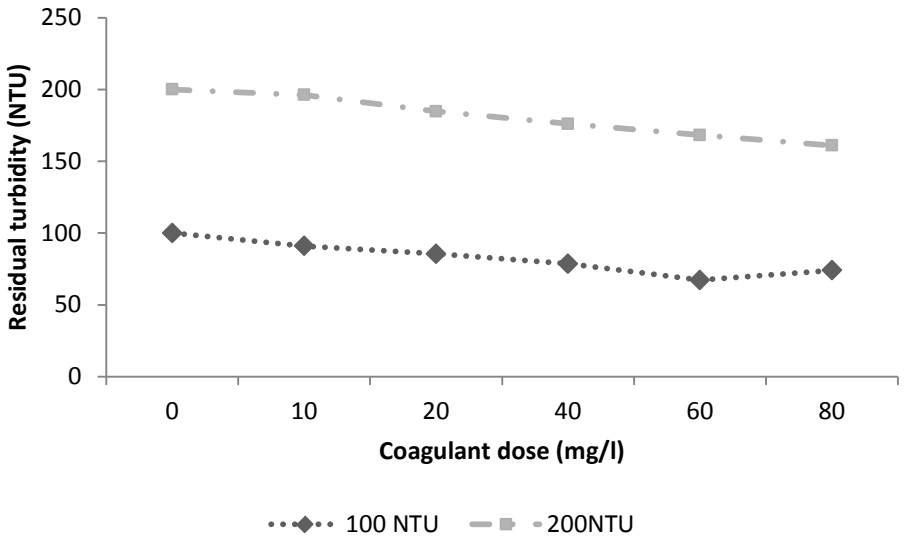
607 **List of figures and table**



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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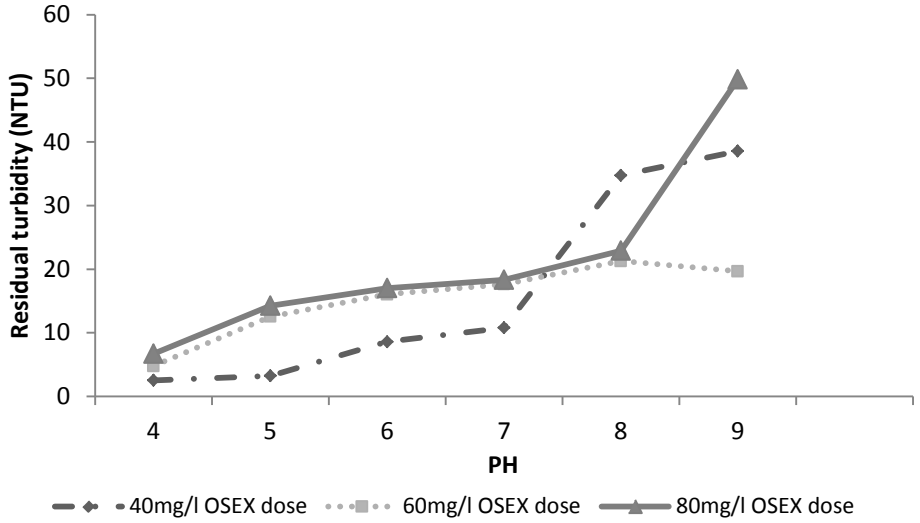

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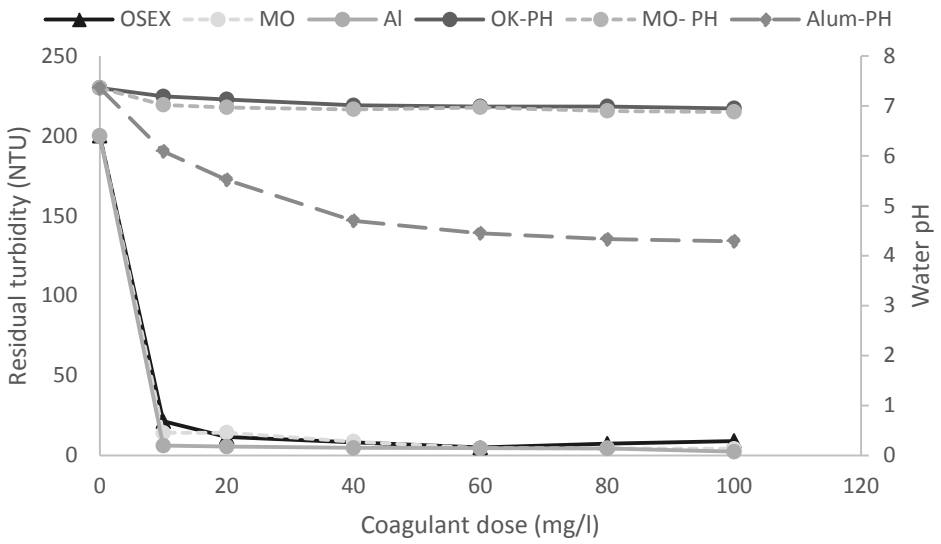
614 **Fig. 3** Influence of pH on turbidity removal using OSEX.



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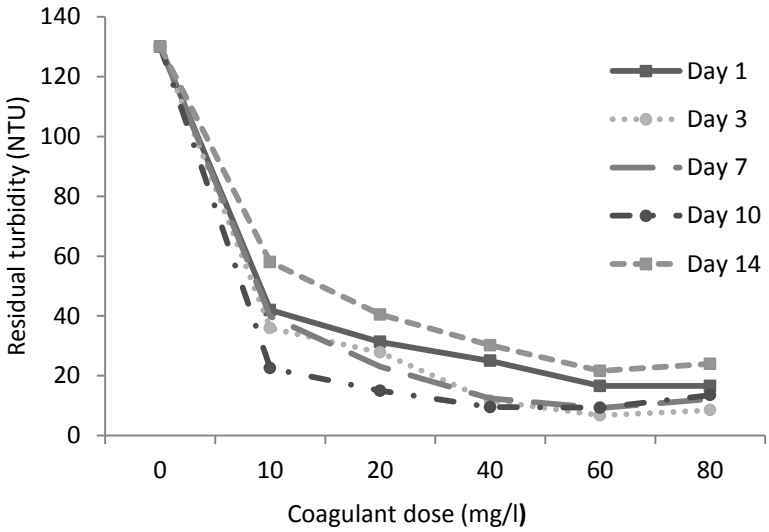
616 **Fig. 4** Effect of different coagulants doses on final water pH and turbidity.



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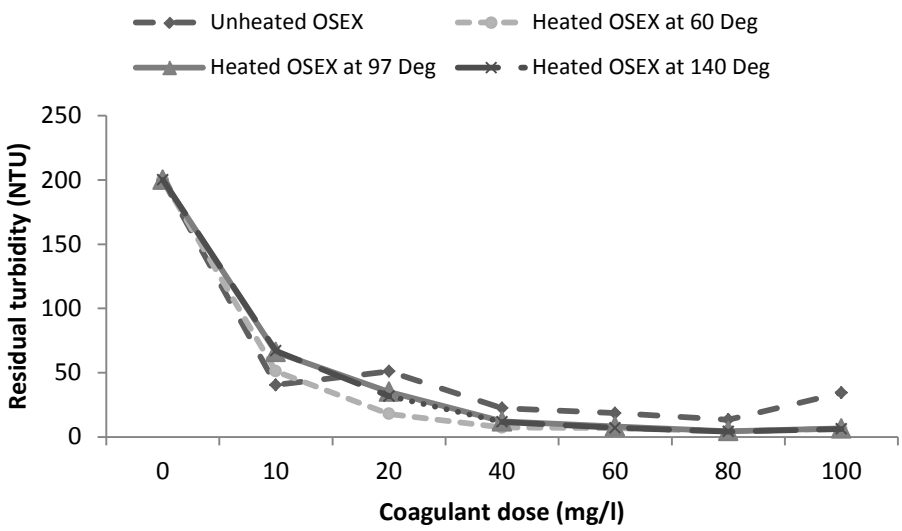
618 **Fig. 5** Removal of turbidity in synthetic water using stored OSEX.



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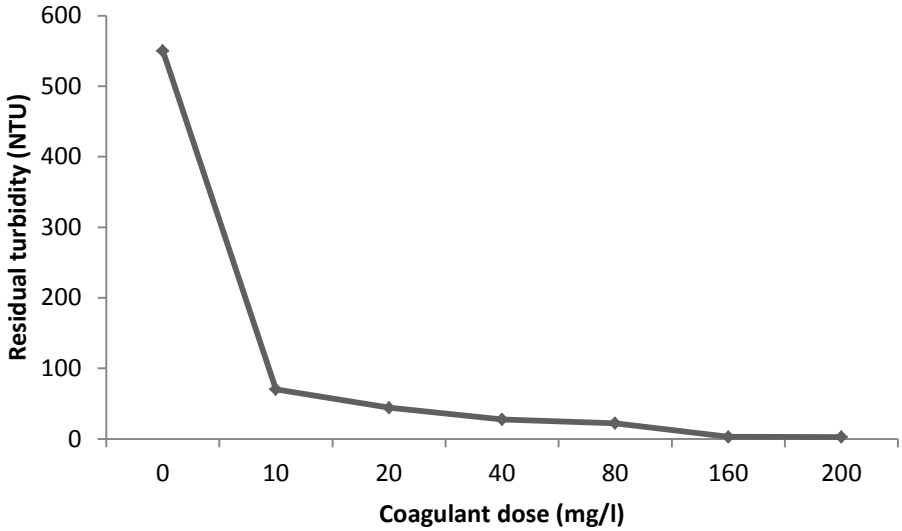
620 **Fig. 6** Removal of turbidity in synthetic water using thermal treated OSEX.



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

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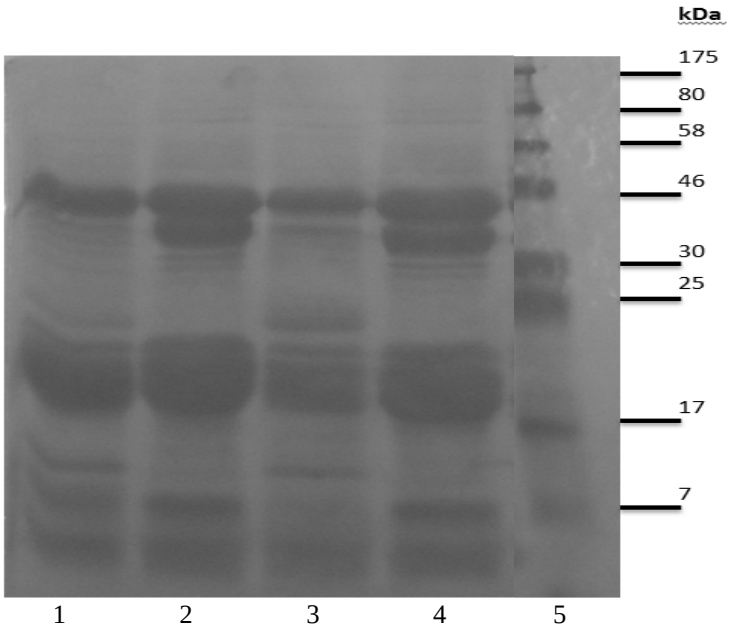

Fig. **8** Protein profiles of OSEX using 12% SDS PAGE analysis. Lane 1 untreated OSEX, Lane 2 heat treated OSEX at 140 °C for 2 hrs; Lane 3 stored OSEX sample for 3 days and Lane 4 heated OSEX at 60°C for 6 hrs and Lane 5 a Marker (New England, BioLab).

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
Number of positive wells for coliform:				
• 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**
Number of positive wells for E-coli:				
• 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**