KTH Land and Water
Resources Engineering

MORINGA SEED AND PUMICE AS ALTERNATIVE NATURAL MATERIALS FOR DRINKING WATER TREATMENT

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November 2004

TRITA-LWR PHD 1013

ISSN 1650-8602
ISRN KTH/LWR/PHD 1013-SE
ISBN 91-7283-906-6

To Azeb, Elion and Esey

ኣብ ክንዲ እቲ ሰናይ ዝገበረለይ ኩሉስ ንእግዝኣብሄር እንታይ ውሬታ ክመልሰሉ እየ?
(መዝ. 116፥12)

How can I repay the LORD for all his acts of kindness to me? (Psalms 116:12)

ABSTRACT

Pumice and the *Moringa oleifera* (MO) seed were investigated as alternative natural materials for drinking water treatment based on problems identified at the Stretta Vaudetto water treatment plant in Eritrea.
Lab and pilot scale studies showed that pumice was a suitable alternative material for dual media filtration. Conversion of the sand filters at Stretta Vaudetto to pumice-sand media would significantly improve performance of the filtration units.
The coagulant protein from the MO seed was purified in a single-step ion exchange purification method. The parameters for batch purification were optimized that can be readily scaled up. This will promote its use in water treatment.
A small volume coagulation assay method was developed that simplified and expedited the coagulation activity experiments. MO coagulant protein (MOCP) possessed considerable coagulation and sludge conditioning properties as alum. It also showed antimicrobial effects against bacteria, some of which are antibiotic resistant. The coagulation and antimicrobial properties of MOCP render it important in water treatment.

Key words: antimicrobial effects; coagulant protein; dual media filtration; ion exchange; *Moringa oleifera;* pumice; sludge conditioning; Stretta Vaudetto; water treatment.

LIST OF PAPERS APPENDED

I. Ghebremichael, A. K. and Hultman, B. (2003). Low cost water treatment using natural materials: a case study for Asmara. *Vatten,* **59** (2) 81-87.

II. Ghebremichael, A. K. and Hultman, B. (2002). An investigation of the suitability of pumice for improving filter performance at Stretta Vaudetto water treatment plant. International Symposium on Research for Nation Development: appropriate technology, Asmara, Eritrea, June 2002, conference record.

III. Ghebremichael, A. K. and Hultman, B. (2004). Alum sludge dewatering using *Moringa oleifera* as a conditioner. *Water Air and Soil Pollution*, **158**: 153-167

IV. Ghebremichael, A. K., Gunaratna, K. R., Henriksson, H., Brumer, H. and Dalhammar, G. (2004). A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. (Submitted to Water Research.)

V. Ghebremichael, A. K., Gunaratna, K. R., and Dalhammar, G. (2004). *Moringa oleifera* for simultaneous coagulation and disinfection in water purification. In: Proceedings of the Canadian Water Resources Association 57th annual conference, Montreal, Canada, June 16-18, 2004, (CD-ROM).

VI. Ghebremichael, A. K., Gunaratna, K. R., and Dalhammar, G. (2004). Single-step ion exchange purification of the coagulant protein from *Moringa oleifera* seed.
(Submitted to Protein Journal)

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ABBREVIATIONS

CAE	Crude Ammonium Acetate Extract
CFU	Colony Forming Units
CM	CarboxyMethyle
COD	Chemical Oxygen Demand
CSE	Crude Salt Extract
CST	Capillary Suction Time
CWE	Crude Water Extract
DBPs	Disinfection By Products
DEAE	DiEthylAminoEthyl
ES	Effective Size
FRL	Filter Run Length
IEF	IsoElectric Focusing
IEX	Ion Exchange
LBB	Buffered Lauryl Broth
MIC	Minimum Inhibitory Concentration
MO	*Moringa oleifera*
MOCP	*Moringa oleifera* Coagulant Protein
MRCST	Multi Radii Capillary Suction Time
MS	Mass Spectrometry
NOM	Natural Organic Matter
OD	Optical Density
pI	Isoelectric Point
SDS-PAGE	Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis
SRF	Specific Resistance to Filtration
UC	Uniformity Coefficient
UFW	Unaccounted For Water
WHO	World Health Organization
WPC	Water Production per Cycle

1 INTRODUCTION

1.1 Background

About one billion people lack safe drinking water and more than six million people (of which 2 million are children) die from diarrhoea every year (Postnote, 2002). The situation persists and it will continue to cause substantial loss of human lives unless it is seriously dealt with at all levels. In the developing countries treatment plants are expensive, the ability to pay for services is minimal and skills as well as technology are scarce. In order to alleviate the prevailing difficulties, approaches should focus on sustainable water treatment systems that are low cost, robust and require minimal maintenance and operator skills. Locally available materials can be exploited towards achieving sustainable safe potable water supply.

Drinking water treatment involves a number of unit processes depending on the quality of the water source, affordability and existing guidelines or standards. The cost involved in achieving the desired level of treatment depends, among other things, on the cost and availability of chemicals. Commonly used chemicals for the various treatment units are synthetic organic and inorganic substances. In many places these are expensive and they have to be imported in hard currency. Many of the chemicals are also associated with human health and environmental problems (Crapper et al., 1973; Christopher et al., 1995; Kaggwa, 2001) and a number of them have been regulated for use in water treatment systems. Natural materials can minimize or avoid the concerns and significantly reduce treatment cost if available locally. This thesis presents a study on the use of natural materials for coagulation, sludge conditioning and filtration.

1.2 Motivation

The water supply system in Asmara, Eritrea, has a number of problems in the treatment and distribution of potable water to the community. The studies in this thesis stem from problems identified at Stretta Vaudetto, one of the three water treatment plants in Asmara. The treatment plant was commissioned in 1941 and at the existing condition it is capable of treating at approximately half the design capacity (SAUR International, 1997). In order to satisfy the increasing water demand of the city, however, it is often overloaded. Consequently, the treatment efficiency is compromised and the treated water quality does not always meet the guideline values. During the rainy season, treated water turbidity is often very high and occurrences of waterborne disease are common. The problems are mainly attributed to failures in the design and operation of the coagulation and filtration units. Sludge is directly disposed off to a recipient (reservoir), which is one of the water sources for the treatment plant. The need for upgrading the treatment plant is vital in order to produce good quality water, to increase the treatment capacity and to properly manage the sludge. However, the cost of chemicals and alternative filter media impede achieving the desired upgrading needs. The focus is, therefore, directed toward the use of locally available materials. The situation at Stretta Vaudetto is typical of water treatment systems in developing countries and the results of this study can be applied to a number of similar situations.

1.3 Objectives

This thesis presents an investigation on the suitability of pumice for dual media filtration and the *Moringa oleifera* (MO) seed for coagulation and sludge conditioning. Material characteristics of pumice were investigated. Moreover lab and pilot scale column studies were carried out to assess its filtration performance in comparison with anthracite coal and sand.

Studies were carried out to assess effectiveness of the MO seed for coagulation and sludge conditioning. Moreover its antimicrobial effect on a number of gram-positive and gram-negative bacteria was investigated. The study aimed to isolate, characterize and purify the coagulant protein from the seed. Large-scale purification of the coagulant protein remains a challenge and one of the main objectives of this study was to develop a simple and inexpensive purification method that can be easily scaled up. Effectiveness of the ion exchange (IEX) purification in removing organic and nutrient loads from the crude extract was also assessed.

Specific objectives of this thesis are summarized as follows:

1. To assess the existing situation of Stretta Vaudetto and identify core problems.
2. To study the use of pumice, from the local area, for dual media filtration and to compare it with commercial anthracite coal and sand from the filters at Stretta Vaudetto.
3. To purify and characterize the coagulant protein from the MO seed and to optimise parameters for batch IEX purification required for large volume protein production. Moreover, to assess effectiveness of the purification in removing organic and nutrient contents from the crude extract.
4. To assess the coagulation and antimicrobial effects of the purified protein from the MO seed.
5. To study the use of MO seed extract for drinking water treatment sludge conditioning and compare it with alum and synthetic polyelectrolytes.

1.4 Thesis outline

Theory and practice of coagulation, filtration, disinfection and sludge conditioning are presented in section 2. Uses of pumice, MO and other natural materials for various treatment processes are described in section 3. Section 4 details the materials and methods used in the study. Overview of the water supply situation in Asmara, specific problems at Stretta Vaudetto and suggested alternatives for improving the performance of the treatment plant are discussed in section 5. Material characterization of pumice, lab and pilot scale column filtration studies are presented in section 6. Discussions on the characterization and purification of the coagulant protein from the MO seed as well its coagulation, sludge conditioning and antimicrobial effects are presented in sections 7 and 8. Conclusions of the studies and recommendation for further studies are presented in sections 9 and 10, respectively.

2 CONVENTIONAL SURFACE WATER TREATMENT

Conventional drinking water treatment includes, but is not limited to: coagulation, flocculation, sedimentation, filtration and disinfection. Coagulation and filtration are the most critical unit processes (other than disinfection) determining success or failure of the whole system and they are the bottlenecks for upgrading treatment plants. The two units are so closely linked that the design of one affects the other. When they are well designed and operated, other units, such as flocculation and sedimentation, may not be required (Conley, 1961) and the burden on disinfection is significantly reduced. Therefore much emphasis is put on the proper design and operation of these units.

2.1 Coagulation: principles and practice

Coagulation is often the first unit process in water treatment and it is very crucial for the removal of suspended and dissolved particles. It is the act of destabilizing stable colloidal particles in suspension. Destabilized particles are then flocculated for expedient removal in the sedimentation and/or filtration units. In most cases coagulation is optimised for the removal of inorganic colloidal particles. It is also used for the removal of dissolved natural organic matter by the process of enhanced coagulation (Gregor et al., 1997). Proper coagulation followed by sedimentation and filtration can achieve more than 99% reduction in bacteria and viruses (Faust and Aly, 1998).

Colloids in natural waters are predominantly negatively charged and they are stable by virtue of the hydration or electrostatic charge on their surfaces. Destabilization of colloidal particles can be effected by one of the following mechanisms: double layer compression, adsorption and charge neutralization, entrapment in precipitates (sweep flocculation), and interparticle bridging (Faust and Aly, 1998). Hydrolysing metal salts, based on aluminium or iron, are very widely used as coagulants in water treatment. The high cationic charge of the two metal salts makes them effective for destabilizing colloids. These salts bring about destabilization by adsorption and charge neutralization as well as by particle entrapment (Duan and Gregory, 2003). Performance of the metal salts is significantly influenced by the pH of the solution and they have a good coagulation effect in certain pH ranges only. Although the chemicals are very effective and widely used, they have certain drawbacks: they influence the pH value of the water, increase the soluble residues and increase volume and metal content of the sludge. With aluminium salts, there is a concern of associated Alzheimer's disease and similar health related problems (Crapper et al., 1973; Miller et al., 1984). Pre-hydrolyzed forms of the metals such as polyaluminum chloride and polyaluminosilicate sulphate are also effective coagulants. Compared to aluminium and iron salts, these are more effective, produce strong flocs and result in less sludge volume, albeit expensive (Duan and Gregory, 2003).

Organic polyelectrolytes are also commonly used as primary coagulants or coagulant aids. They destabilize particles by charge neutralization and interparticle bridging and produce large flocs (due to the bridging effect) compared to metal salts. Based on material cost, polyelectrolytes are more expensive than aluminium and iron salts, but overall operating costs can be lower because of a reduced need for pH adjustment, lower sludge volumes and reduced disposal costs. Polyelectrolytes can be either synthetic or natural. In practice, most polyelectrolytes in water treatment are synthetic. Even though they are effective coagulants, public health concerns of the monomers (as acrylamides) may hinder their use (Christopher et al., 1995; DeJongh et al., 1999). In this thesis a natural coagulant from MO seed is investigated as an alternative to the above mentioned chemicals.

Optimum coagulant dosage is commonly estimated from jar test analysis using 1 or 2 L volume beakers equipped with mechanical stirrers. Since jar test analysis requires large volumes of sample and coagulant dosage it may not be convenient for studying and comparing large number of samples. In this respect small volume coagulation experiments would be convenient to facilitate the data acquisition process and save coagulant samples. This can be performed by measuring optical density (OD) of clay suspension at 500 nm before and after coagulant addition (OD_{500}, Paper IV & VI). This method not only reduces the volume of clay suspension sample and coagulant dosage requirements, but also makes simultaneous analysis of large number of samples possible. It is suitable to easily screen out active and non-active coagulants and to study settling characteristics of the flocs by continuously recording OD_{500}.

2.2 Granular media filtration

2.2.1 *Theory and practice*

Granular media filtration is a physical, chemical and in some cases biological process for removing suspended solids, including bacteria, precipitated hardness and precipitated iron and manganese, from water by passage through porous media. Filtration processes are so complex that a quantitative understanding of solids removal is far from complete and the existing mathematical models do not describe the filtration process fully. Design and operational parameters are almost always determined from data collected from lab and pilot scale studies.

Granular media filtration involves a number of mechanisms, which include transport, attachment and detachment steps. The transport and detachment mechanisms are basically physical processes while the attachment mechanism is influenced by physical and chemical variables (O'Melia and Stumm, 1967; Schulz and Okun, 1984; HDR Engineering Inc., 2001). Solids in the liquid phase are brought to the surfaces of the filter media by the transport mechanisms, which include screening, interception, inertial forces, gravitational settling, diffusion and hydrodynamic conditions. The transport mechanisms and the efficiency of solids removal depend on physical characteristics such as size distribution of the filter medium, filtration rate, temperature, and properties of the suspended solids. Previously removed (or attached) solids are detached mainly as a result of increased interstitial velocity during filtration and by the shearing action during backwashing. High interstitial velocity may occur either when the pore spaces are clogged (due to solids removal) or when the loading rate is suddenly increased (hydraulic shock) and this may lead to turbidity breakthrough.

Chemistry of the water and surface of the filter media play a significant role in the attachment mechanism hence chemical pretreatment (coagulation) influences filtration performance (Amirtharajah, 1988). Attachment can be considered similar to the coagulation process and the mechanisms can be explained by the theory of double layer interaction and chemical bridging theory (Faust and Aly, 1998). Consequently, particles much smaller than the voids are readily removed during granular media filtration. Optimum coagulation is crucial for efficient filtration (Conley, 1961; Robeck et al., 1964; Amirtharajah, 1988; Logsdon et al., 1993; Logsdon, 2000; Emelko 2003) and it is more significant than the filtration parameters, such as, filter media and filtration rate (Conley, 1961; Robeck et al., 1964; Ghosh et al., 1994). Failure in the coagulation process leads to poor filter performance, especially in dual or multi media filters where solids penetrate deep into the filter media. It is therefore important that the coagulation and filtration units are designed simultaneously (Kawamura, 1975).

Granular media filtration is usually the final solids removal step in the treatment train and plays a critical role in safeguarding the microbial quality of the treated water. The multiple-barrier approach, comprising of water shed management, pre-treatment (coagulation, flocculation and sedimentation) and filtration has been very effective in reducing the concentration of microorganisms and hence the burden over disinfection. This is particularly important for the removal of protozoans, which are resistant to disinfectants (Emelko, 2003). Filters can remove more than 99% bacteria (Culp, 1986; Koivunen et al., 2003), *Cryptosporadium* and *Giardia* (Nieminiski & Ongerth, 1995). When the pre-treatment and filtration processes are properly carried out disinfectant requirement and the risk of disinfection-by-products (DBPs) formation are reduced.

2.2.2 Mono-medium and multimedia rapid sand filtration

Ideal media gradation for efficient filtration is a coarse-to-fine arrangement of grains in the direction of water flow. This type of media gradation can be approximately achieved either by reversing the direction of flow (in a mono-medium filters) or by using multimedia filter system composed of varying grain sizes having different specific gravities. Such type of filter media gradation substantially improves the distribution of solids removal in large part of the bed depth, consequently maximizing utilization of the available solids holding capacity. In conventional rapid sand filters the gradation is fine-to-course and most of the solids are removed at the top few centimeters (AWWA and ASCE, 1998). This results in early headloss build-up and short filter run length. In such cases frequent filter washing is necessitated even though the filtrate quality satisfies standard limits. The filters at Stretta Vaudetto are typical examples of rapid sand filter with filter run lengths of less than 6 h. Comparison of the gradation of a mono-medium and dual media filtration systems is shown in Fig. 1. In the dual media filter there is more storage volume to be exhausted before the headloss reaches terminal value. Moreover, the large interstice volumes at the top layer allow deep penetration of particles resulting in effective usage of significant depth of the filter media. In the mono-medium column, on the other hand, finer grains are packed at the top and provide smaller void volumes, hence smaller storage capacity for solids removal.

There are two approaches that can be adopted to overcome the problems in conventional rapid sand filtration (Qasim et al., 2000): 1) to replace the mono-medium by a dual or multi media filter system and 2) to use a coarse medium deep bed filter with uniformity coefficient (UC) close to one. When deep bed mono-medium filters are used, the rate of backwash should be such that the medium is not stratified. Such types of filters are generally washed using air or air/water backwash systems (AWWA and ASCE, 1998). Upgrading a rapid sand filter to deep bed mono-medium cannot be simply achieved with replacing the filter medium alone. Additional modifications may be required to the filter basin and backwash system. On the other hand, existing rapid sand filters can be expanded at least up to double the capacity with only the nominal expense of replacing sand with dual or mixed media (HDR Engineering Inc., 2001). Therefore it is technically and economically more convenient to upgrade existing rapid sand filters to dual media.

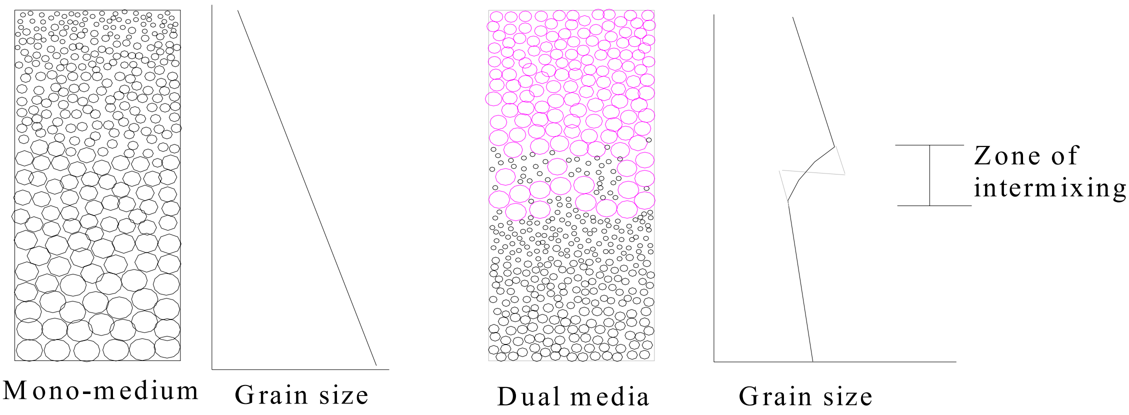


Fig. 1 Comparison of media gradation in mono-medium and dual media filters (After Metcalf and Eddy Inc., 2003).

In the case of Stretta Vaudetto, lack of air wash system and limited filter basin depth (Paper I, II) do not warrant simple conversion into deep bed filter system.

Dual media filters reduce the rate of headloss development, thus increase the filter run length (Conley and Pitman, 1960; Conley, 1961). By converting a rapid sand filter to dual or multi media filter, loading rates can be increased by 50% to more than 100% without compromising performance (Robeck et al., 1964; Laughlin & Duvall, 1968; Paramasivam et al., 1973; Kawamura, 1975; Ranade et al., 1976; Ranade & Gagdil, 1981). Anthracite coal is the most commonly used upper layer material in dual or multi media filtration. In many places in developing countries, it is expensive and has to be imported with hard currency. Taking the limited financial resources into account, the search for locally available low cost alternatives becomes essential.

2.3 Disinfection practice

Commonly used chemical disinfectants in water treatment are chlorine, chlorine dioxide, chloramines and ozone. Although chlorine is very effective and relatively inexpensive, it forms a range of DBPs that are of public health concern. To date more than 250 different types of DBPs have been identified, which are formed by the reaction of disinfectants with natural organic matter (NOM) or inorganic substances such as bromide ion (Sadiq and Rodriguez, 2004). Effective ways to avoid the risks associated with DBPs are either to remove the precursors before disinfection or to use alternative disinfectants. Ozone, chloramines and chlorine dioxide are also believed to produce hazardous DBPs (Sadiq and Rodriguez, 2004). Recently UV irradiation is becoming an important disinfection alternative albeit expensive. It is often used in conjunction with a second disinfectant to maintain sufficient residual in the distribution system. However, studies have indicated that in the presence of nitrate, UV disinfection may give rise to nitrite formation in excess of the standard limits (Mole et al., 1999).

Risks of DBPs have become so significant that zero or minimal use of chemical disinfectants (especially chlorine) is being practiced (van der Hoek et al., 1999). Even though there exist significant concern of DBPs, microbiological quality of drinking water cannot be compromised and existing practices seek a balance between microbial and chemical risks. The search for low cost disinfectants that maintain acceptable microbiological quality, and that avoid chemical risks is one of the biggest challenges facing the water treatment industry.

2.4 Sludge conditioning and dewatering

In many places it is common practice to dispose sludge from drinking water treatment plants with little or no treatment at all. Direct disposal of untreated sludge to the environment affects the recipient as a result of solids deposition and chemical composition of the sludge (Kaggwa et al., 2001). Consequently stringent effluent discharge standards are coming into effect and thus proper management of the sludge becomes inevitable. Drinking water treatment sludge consists of 90 to 99.9% water (Committee report, 1978; Metcalf and Eddy Inc., 2003) and it is difficult to dewater (Knocke and Wakeland, 1983; HDR Engineering Inc., 2001). The water content in sludge can be grouped into four classes (Vesilind and Hsu, 1997): 1) free water, 2) interstitial water, 3) vicinal water, and 4) water of hydration. The first two can be removed by gravity and mechanical drainage systems. The third type of water can be removed by compaction and deformation depending on solids concentration. Water of hydration can only be released by thermochemical destruction of the particles. This indicates that there is a limit to the amount of water that can be released from sludge by mechanical means. Thickening, conditioning and dewatering can achieve 70-90% water content reduction (Dharmappa et al., 1997).

Conditioning significantly improves sludge dewatering characteristics (Novak and Langford, 1977). The two most commonly used conditioning systems are chemical addition and heat treatment, the former being more economical. Effects of sludge conditioning are assessed by the dewatering rates and floc strength. These parameters are estimated from studies of capillary suction time (CST), specific resistance to filtration (SRF), shear strength, gravity drainage rate and cake solids concentration. SRF can be estimated from the standard Buchner funnel test or the multi radii CST (MRCST) apparatus (Fig. 2). The former is cumbersome and requires qualified operators. If a suitable correlation is established between the two methods, the MRCST can be conveniently used (Paper III). Weak flocs break down easily during subsequent transport and processing and this results in sludge that is difficult to dewater. Conditioning significantly improves floc strength and subsequent sludge handling.

Dewatering is carried out by mechanical systems or by natural processes. Sand drying beds have been widely used in areas where cheaper land and favorable climatic conditions exist. They are economical for drying large sludge volumes and do not require mechanical equipment, skilled operators and frequent attention. Significant reduction in the land requirement can be achieved by first conditioning the sludge to enhance the drainage capacity.

Dewatering characteristics and floc strength depend, among other things, on the type of chemical conditioners used. Commonly used chemicals include synthetic organic polyelectrolytes and inorganic chemicals such as aluminium sulphate (alum), ferric chloride and lime. These chemicals may be expensive and have negative impacts on sludge handling and the environment. Compared to the synthetic organic polymers, inorganic salts are less expensive but they increase the dry sludge solids by 20 to 30% (Metcalf and Eddy Inc., 2003). The high cost and environmental impact of the commonly used chemical conditioners may be alleviated if locally available alternative natural materials can be used.

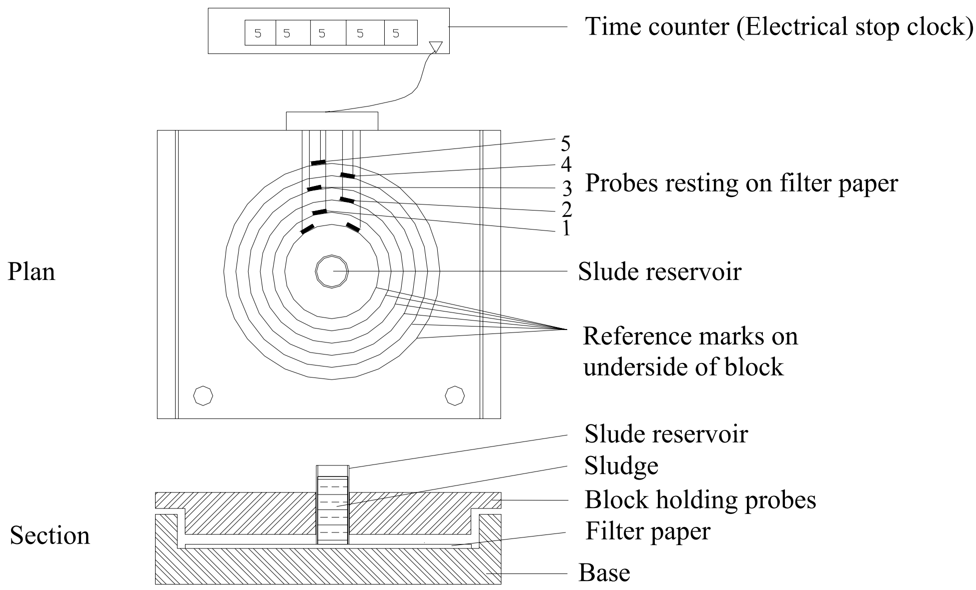


Fig. 2 MRCST apparatus. The timer starts to count when the advancing water interface in the filter paper reaches the first set of probes. The CST value is the time for the water-front to reach probe No 2. The radial position of each probe is such that equal amount of filtrate volume is dewatered from the sludge in order for the water-front to travel between adjacent probes.

Combination of low cost conditioning chemicals and sand drying beds would significantly reduce the sludge handling cost. This may encourage the proper management of sludge from water treatment plants.

3 ALTERNATIVE NATURAL MATERIALS IN WATER TREATMENT

3.1 Natural materials in water treatment applications

Natural materials have been used in water treatment since ancient times. But lack of knowledge on the exact nature and mechanism by which they work has impeded their wide spread application and they have been unable to compete with the commonly used chemicals. In recent years there has been a resurgence of interest to use natural materials due to cost and associated health and environmental concerns of synthetic organic polymers and inorganic chemicals. A number of effective coagulants have been identified from plant origin. Some of the common ones include MO (Olsen, 1987; Jahn, 1988), nirmali (Tripathi et al., 1976), okra (Al-Samawi and Shokrala, 1996), *Cactus latifaira* and *Prosopis juliflora* (Diaz et al., 1999), tannin from valonia (Özacar and Sengil, 2000) apricot, peach kernel and beans (Jahn, 2001), and maize (Raghuwanshi et al., 2002). Bhole (1995) compared 10 natural coagulants from plant seeds. The study indicated that maize and rice had good coagulation effects when used as primary coagulants or coagulant aid. One of the natural coagulants from animal origin is chitosan. It is a high molecular weight polyelectrolyte derived from deacetylated chitin. Chitin is cellulose like biopolymer widely distributed in nature, especially in insects, fungi, yeasts and shells of crabs and shellfish. Chitosan possesses effective coagulation properties and it can be used in water and wastewater treatment (Pan et al., 1999; Divakaran & Pillai, 2001). It has also been reported that chitosan possesses antimicrobial properties (Liu et al., 2000; Chung et al., 2003).

By using natural coagulants, considerable savings in chemicals and sludge handling cost may be achieved. Al-Samawi and Shokrala (1996) reported that 50-90% of alum requirement could be saved when okra was used as a primary coagulant or coagulant aid. Apart from being less expensive, natural coagulants produce readily biodegradable and less voluminous sludge. For example, sludge produced from MO coagulated turbid water is only 20-30% that of alum treated counterpart (Ndabigengesere et al., 1995; Narasiah et al., 2002).

There are very few reports on the use of natural coagulants for sludge conditioning. Ademiluyi (1988) studied the use of MO for sewage sludge conditioning. The study reported that MO had comparable conditioning effect as ferric chloride and aluminium sulphate. The biodegradable nature of MO makes it a preferred option over the others.

In dual or multi media granular filtration anthracite coal and garnet are often used. In many places these materials are not commonly available and they are expensive. Some of the locally available filter media, which have been used in a single or multi media filtration include: crushed coconut shells, burned rice husk (Frankel, 1974) and crushed apricot shell (Aksogan et al., 2003). Paramasivam et al. (1973) reported that high-grade bituminous coal, used in dual media filtration, could significantly improve the rate of filtration and that it could be a good substitute for anthracite coal. Pumice is also one of the natural materials that can be used for such purposes.

3.2 Pumice

Pyrochlastic materials are accumulations of fragments of rock thrown out by volcanic explosions that occur as a result of escaping gas, which has been confined under pressure. Pumice is a porous pyrochlastic igneous rock with extremely abundant cavities that render it lightweight material. The cavities vary in size and form depending on the composition and extent of entrapped gas in the magma. Some types of pumice are characterized by elongated, tubular parallel vesicles, whereas others have more or less spherical cavities (Ross and Smith, 1961). When the vesicles are open and interconnected the pumice becomes easily water logged and sinks in water.

Pumice is commonly used as a lightweight construction material and as an abrasive in cleaning, smoothening, polishing and in medical (dental) application. The low specific gravity and high porosity of pumice make it important for a number of applications in water and wastewater treatment processes. Pumice is used as a filter medium in water treatment (Gimbel, 1982; Andrievskaya et al., 1989; Farizoglu et al., 2003), as a support material for microbial growth in wastewater treatment (Şen and Demirer 2003; Kocadagistan et al., 2004) and for adsorbing phosphorus (Njau et al., 2003; Renman, 2004). The rough surface and porous structure are believed to provide a large number of attachment sites for microbial growth. Thus pumice can be used suitably as a media for biofiltration in water treatment systems.

In multi-media filtration the upper layer should be of low specific gravity so that the approximate coarse-to-fine media gradation is maintained throughout the filtration process. Because of its lower specific gravity, pumice would be a convenient material for dual or multi media filtration with sand. The use of pumice in dual media filtration is reported by Pumex UK Ltd. (2000).

There are two major pumice deposit locations in Eritrea. One is in the Durko area (Central highlands) and the second is in Alid area (Eastern lowlands of Dankalia). Planimetric analysis indicated that the pumice deposit site in Alid ranges between 9 and 10 square kilometers. In this study samples were collected from Alid (Paper II).

3.3 Moringa oleifera

3.3.1 Description and uses

MO is among the 14 species of trees that belong to the genus *Moringaceae*. It is native to North India and is the best known of all the species. It is drought resistant and grows in hot semi arid regions with annual rainfall of 250-1500 mm as well as in humid area with annual rainfall in excess of 3000 mm (Folkard, 2000). It grows fast and develops to a full tree within one year of its plantation.

MO is a multi purpose tree with most of its parts being useful for a number of applications and it is being referred to as the ‘miracle tree’ (Fuglie, 1999). The pods, leaves and flowers are important sources of food in some areas of India and Africa. The leaves are specially, rich in vitamins, minerals and proteins and they are considered to be important to fight malnutrition. The roots and other parts of the tree are used in traditional medicine. Oil can be extracted from the seed and used in food preparation, fine lubrication of delicate machines and in the cosmetics industry. The dried pods and husks (Fig. 3 and 4) can be pyrolysed for activated carbon production. Pollard et al. (1995) and Warhurst et al. (1997) were able to produce a good quality activated carbon (from MO seed husk) using single-step steam pyrolysis.

The coagulant is obtained from the by-product of oil extraction (Fig. 4). After coagulant extraction, the residue can be used as a fertilizer or processed for animal fodder. The multiple uses of the plant indicate significant potential of MO for commercial applications and it is becoming an important income generator. Technically speaking the part that is used for water treatment is a waste product and it can be acquired at a very low cost. Studies on the coagulation, sludge conditioning and antimicrobial activity of this material are presented in Paper III-V.

Fig. 3 Moringa oleifera pods and seed kernel. The coagulant is extracted from the seed kernel. The pods and husks can be pyrolysed for activated carbon production.

The crude MO seed extract is commonly used for water purification at household level in some areas. For instance villagers in Sudan have been traditionally using the MO seed to purify water from the Nile River (Jahn, 1988). Recently efforts are being made to use it for water purification at treatment plants for community water supply. Several studies have reported the use of crude and purified extracts from the seed for coagulation (Olsen, 1987; Jahn, 1988; Sutherland et al., 1990; Ndabigengesere et al., 1995; Muyibi and Evison, 1995a; Nkhata, 2001) and for hardness removal (Muyibi and Evison, 1995b). Muyibi and Evison (1995a) reported that MO could achieve turbidity removal between 92 and 99%. Coagulation effectiveness of MO varies depending on the initial turbidity. It is very effective for high turbidity waters and shows similar coagulation effects as alum, however, the effectiveness reduces for low turbidity waters (Sutherland et al., 1990). The use of MO as a primary coagulant is more appropriate for surface waters with excessive turbidity particularly during the rainy seasons. For low turbidity water it may be effectively used as a coagulant aid. Comparative coagulation study between alum and MO are presented in paper V.

Compared to the commonly used coagulant chemicals, MO has a number of advantages:

- it is of low cost
- it produces biodegradable sludge
- it produces lower sludge volume
- it does not affect the pH of the water.

The above listed advantages make MO a consumer and environmentally friendly low cost alternative with significant potential both in developing and developed countries.

Apart from turbidity removal MO also possesses antimicrobial properties (Olsen, 1987; Madsen et al., 1987). The mechanism by which MO acts upon-microorganisms is not yet fully understood. Broin et al. (2002) reported that a recombinant MO protein was able to flocculate gram-positive and gram-negative bacterial cells. In this case -microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water (Casey, 1997). On the other hand, MO may also directly act upon microorganisms and result in growth inhibition.

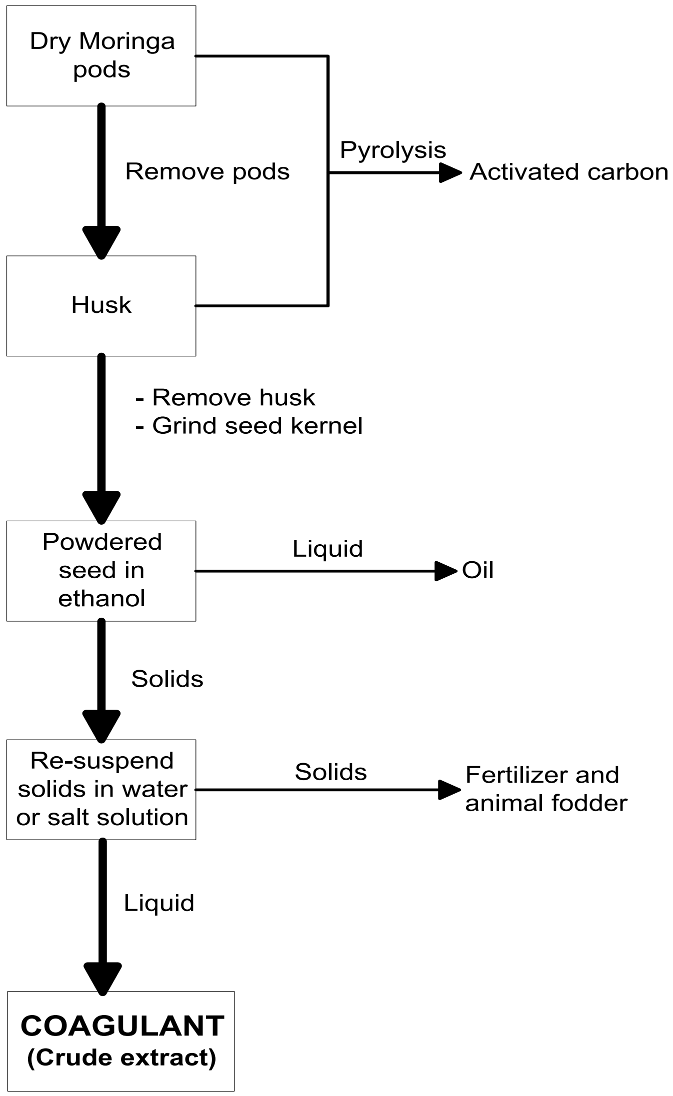


Fig. 4 Extraction of the coagulant component from the seed. The coagulant is a by-product of oil production. Note that oil can also be removed by other means such as pressing.

For example, Sutherland et al. (1990) reported that MO could inhibit replication of bacteriophage. Caceres et al. (1991) also observed growth inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Most of the reports on the antimicrobial effect of MO are based on crude extract (Olsen, 1987; Madsen et al., 1987; Eilert et al., 1981). From the crude extract it is difficult to identify the exact nature of the component that carries out the effect. Eilert et al. (1981) attributed the antimicrobial effects to the compound 4(α-L-Rhamnosyloxy) benzyl isothiocyanate synthesized by the plant. Others have also reported antimicrobial effects of recombinant (heterologous) form of MO protein expressed in *E. coli* (Broin et al., 2002; Suarez et al., 2003). Reports on the antimicrobial effects of the protein purified from MO seed are very rare. As the effects of a recombinant and natural proteins may differ, antimicrobial studies of the purified protein (from the seed) are deemed necessary. Such studies are presented in Paper IV and V.

The flocculation effect on colloidal particles and cells as well as the growth inhibition on microorganisms imply that the protein from MO may be very effective for both coagulation and disinfection in water treatment.

3.3.2 Characteristics of the coagulant component from the MO seed

In recent years the use of the MO seed for water treatment applications is gaining popularity and ongoing research is attempting to characterize and purify the coagulant component (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995, Okuda et al., 2001a; Paper IV-VI). The nature and characteristics of the component, which has coagulation and antimicrobial effects has been reported differently by a number of researchers. It was described as a water-soluble cationic peptide with isoelectric point (pI) above 10 and molecular mass of 6.5 kDa (Gassenschmidt et al., 1995), and 13 kDa (Ndabigengesere et al., 1995). The theoretical pI of the protein as estimated from the amino acid sequence is 12.6 (Broin et al., 2002). On the other hand, a non-protein and non-polysaccharide coagulant compound, with molecular mass of 3 kDa, has been identified from a salt extract solution (Okuda et al., 2001a). In the subsequent research, the coagulant was purified using anion exchange resin.

More than one coagulant peptide has been isolated from the seed and the sequence of one of them (identified as $MO_{2.1}$) has been established (Gassenschmidt et al., 1995). This suggests that a number of coagulant proteins are present, which may differ in one or more amino acid residues. A recombinant form of $MO_{2.1}$ protein was expressed in *E. coli* and it was found to have good flocculation and antimicrobial properties (Broin et al., 2002; Suarez et al., 2003). Seeds from different sources (geographic locations) exhibit varying coagulation performance (Narasiah et al., 2002), which may have to do with differences in the protein content and development of the seed. The complete array of proteins from the seed that posses the coagulation and antimicrobial property has not been fully identified. This entails the need for extensive research to identify and characterize the whole range of proteins with their amino acid sequences and structure.

The coagulation mechanism of the MO coagulant protein (MOCP) has been explained in different ways. It has been described as adsorption and charge neutralization (Ndabigengesere et al., 1995; Gassenschmidt et al., 1995) and inter-particle bridging (Muyibi and Evison, 1995a). The coagulation mechanism of the non-protein organic compound was attributed to enmeshment by net-like structure (Okuda et al., 2001b). Flocculation by inter-particle bridging is mainly characteristics of high molecular weight polyelectrolytes. Due to the small size of MOCP (6.5-13 kDa) bridging effect may not be considered as the likely coagulation mechanism. The high positive charge (pI above 10) and small size may suggest that the main destabilization mechanism could be adsorption and charge neutralization.

MOCP can be extracted by water or salt solutions (commonly NaCl). The amount and effectiveness of the coagulant from salt and water extraction methods vary significantly. In crude form, salt extract shows better coagulation performance than the corresponding water extract (Okuda et al., 1999). This may be explained by the presence of higher amount of soluble protein due to salting-in phenomenon. However, purification of MOCP from the crude salt extract may not be technically and economically feasible. In the case of IEX purification, for example, ionic strength of the crude extract has to be lowered to that of the equilibration buffer in order to maximize binding efficiency. This entails either handling of large sample volume (as a result of dilution) or the need for desalting prior to purification.

3.3.3 *Challenges for large-scale water treatment application*

The main drawback in using crude MO extract for large-scale water treatment application is the release of organic matter and nutrients (nitrate and phosphate) to the water (Ndabigengesere and Narasiah, 1998; Okuda et al., 2001a; Bawa et al., 2001). Most of the coagulation and antimicrobial studies of MO are based on lab scale experiments and household level applications. There are very few reports where the crude extract has been used in pilot and full-scale water treatment studies (McConnachie et al., 1999; Noor et al., 2002; Folkard et al., 2001). The studies, however, did not address the impact of the crude extract on the organic and nutrient loads as well as DBPs formation.

The presence of organic matter in water significantly influences performance of unit processes (oxidation, coagulation and adsorption), consumes disinfectant chemicals, forms DBPs and becomes a substrate for biological re-growth (affecting biological stability). It plays a role in the transport and concentration of inorganic and organic pollutants and imparts color, taste and odour. Removal of NOM from drinking water sources can be realized by enhanced coagulation, carbon adsorption, IEX and membrane filtration, which are often expensive (Randtke, 1988; Cheng et al., 1995; Crozes et al., 1995; Gregor et al., 1997; Jacangelo et al., 1995; Ødegaard et al., 1999; Bolto et al., 2002). For instance, Cheng et al. (1995) and Crozes et al. (1995) stated that the removal of NOM by enhanced coagulation increase operational cost as a result of increased sludge volume, and increased requirement of chemicals for coagulation and alkalinity adjustment. High level of nitrate concentration can cause methemoglobinemia in infants and the maximum concentration level in drinking water is limited to 10 mg/L (Faust and Aly, 1998). The presence of phosphate is more important in wastewater than in drinking water. In wastewater treatment plants, phosphorus removal is necessary to comply with stringent effluent standards.

The organic and nutrient release from the seed can be avoided either by purifying the coagulant component or by removing the released substances from the water. In the prior option the substances of concern are removed before any complications are inflicted to the water treatment system (prevention is better than cure). The latter option is not preferred since the removal of organic matter and nutrients from the water complicates the treatment process and increases costs. The methods employed to purify MOCP so far are cumbersome and involve a number of steps (Ndabigengesere et al., 1995; Okuda et al., 2001a). Scale-up of the methods would be capital intensive and require complex facilities. Despite the multiple purposes of MO and its availability, expensive purification of the coagulant hinders its use in large-scale water treatment plants. Large volume production of the coagulant protein remains a big challenge. Development of simple and inexpensive IEX purification that can be easily scaled up is discussed in Paper IV and VI.

3.3.4 *Protein purification by IEX method*

Proteins vary from each other in size, shape, charge, hydrophobicity, solubility, and biological activity. Purification processes make use of these properties to get good quality products using efficient procedures. Some of the commonly used purification methods include: precipitation, ion exchange adsorption, hydrophobic interaction, affinity chromatography, gel filtration, electrophoresis and ultrafiltration. IEX is the most commonly used chromatographic technique in protein purification (Karlsson et al., 1989; Roe, 1989).

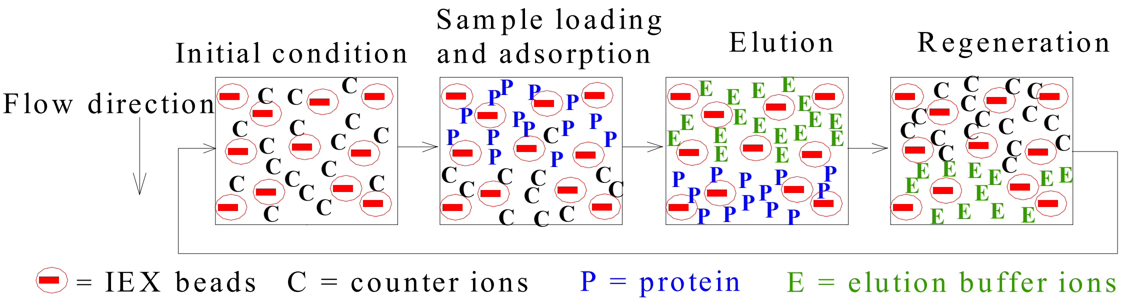


Fig. 5 Protein purification by IEX process. The different stages in the process, that is, from equilibration to regeneration are shown.

This stems, in part from its ease of use and scale-up, wide applicability, high resolving power, high capacity and low cost.

Protein purification in IEX chromatography is based on the reversible adsorption of the proteins to immobilized functional groups attached to the matrix. The functional groups are either positively charged (anion exchanger) or negatively charged (cation exchanger) and they are associated with mobile counter-ions, which can be reversibly exchanged with other ions of the same charge. The interaction between proteins and the functional groups depends on such factors as the net and surface charge distribution of the protein, ionic strength, pH and presence of other additives such as organic solvents (Karlsson et al., 1989). The most commonly used functional groups are diethylaminoethyl (DEAE) and carboxymethyl (CM) in anion and cation exchangers, respectively. Proteins are amphoteric, that is, they may have net positive or negative charge depending on whether the buffer pH is below or above the pI, respectively. Knowledge of pI is important to decide on the type of ion exchanger and buffer for optimum purification. Proteins with high pI value are effectively adsorbed to cation exchanger and those with low pI are adsorbed to anion exchanger.

Adsorbed proteins are normally eluted from the matrix by modifying either the pH or ionic strength of the aqueous buffer. This can be carried out in step or gradient elution procedure. Salt gradient is the most commonly used procedure for elution (Scopes, 1994). As the ionic strength increases, proteins start to elute in the order of their binding strength, the least strongly binding proteins being eluted first. In practice elution by pH gradient is not commonly used due to titration of both protein and IEX functional groups as pH is altered (Roe, 1989; Scopes, 1994). When pH gradient is used for elution, in the absence of very high buffering capacity, large pH changes occur as proteins are eluted and these result in poor resolution (Scopes, 1994).

IEX experiments are mainly performed in five steps (Fig. 5): equilibration, sample loading, adsorption, elution and regeneration. The IEX matrix is first equilibrated to have the starting pH and ionic strength that allow binding. The sample is then loaded and the proteins start to adsorb to the matrix. Once the proteins are adsorbed the matrix is washed with the equilibration buffer to remove non-adsorbed proteins. This is followed by elution to collect the proteins of interest. The final step is to regenerate the IEX matrix by removing substances not eluted in the previous experimental conditions and to re-equilibrate the matrix for the next purification.

IEX separation may be carried out either in a column, a batch procedure or expanded bed adsorption. The principles and procedures are similar in all cases. For isolation and characterization studies small column IEX chromatography are widely used. They are very efficient and versatile where step or gradient elution can be easily adopted. Batch separation method is employed for large volume purification. It offers a number of advantages compared to column chromatography. The merits include (Pharmacia Biotech, 1994): large volumes of dilute samples can be loaded at one time, binding of the sample is quicker, there is no need to remove particulate matter, it is suitable for highly viscous samples, there is no clogging and swelling-shrinkage problem as in columns and it can be carried out using readily available facilities.

4 MATERIALS AND METHODS

4.1 Filtration

4.1.1 Material characterization

Properties of the pumice obtained from Alid, Eritrea, were first investigated to check if it satisfies the requirements of filtering materials. Specific gravity was measured according to Mandal and Divishikar (1995). Acid solubility measurement was performed according to AWWA (1998). Durability test was conducted by packing pumice in a column and backwashing it for 100 hours (Paramasivam et al., 1973). Material strength was determined by scratching it with the different minerals in the Moh's scale.

4.1.2 Lab and pilot scale studies

Laboratory and pilot scale column filtration studies were carried out to compare performances of pumice, anthracite coal and sand. Pumice rock samples were crushed and sieved to the desired size gradation. Grade 2 anthracite coal was obtained from BETWS Anthracite Limited (United Kingdom). The effective size (ES), UC and media depth of filter materials used in the lab and pilot scale studies are given in Table 1.

Lab scale filtration studies were carried out in two Perspex plastic columns each 12 cm internal diameter and 50 cm high. In the case of dual media filtration, pumice and anthracite coal were used in the upper layer. Filtration performances were compared at varying loading rates. Comparisons were made in terms of headloss, turbidity and media expansion.

Pilot scale studies for a dual media (pumice-sand) and a mono-medium (sand from the filter units at the treatment plant) were carried out at Stretta Vaudetto treatment plant using 20-25 cm diameter and 200 cm high PVC columns at loading rates of 3.0, 5.5 and 7.5 m/h. The columns were fitted with piezometers at the inlet and effluent points. To ensure uniform backwashing and effluent collection, nozzles were provided over which 10 cm deep graded gravel was placed. Influent water was abstracted from the outlet point of the sedimentation tanks (just before the filters). Backwash water was taken from the main supply line to the city. Data collected include influent and effluent turbidity, headloss, filter run length (FRL) and water production per cycle (WPC).

The ES of pumice was estimated from Equation (1) so that the pumice and sand media grains would have the same settling velocity during backwashing so as to maintain the required gradation and avoid material loss (Kawamura 1975; Qasim et al., 2000).

$$d_2 = d_1 \left(\frac{S_{g1} - \rho}{S_{g2} - \rho} \right)^{2/3} \qquad (1)$$

Where

d_2 = effective size of the media with a specific gravity of S_{g2}, mm

d_1 = effective size of the media with a specific gravity of S_{g1}, mm

ρ = specific gravity of water

Assuming sand of effective size 0.50, as recommended for small-scale plants (Qasim et al., 2000) and specific gravities of sand and pumice being 2.65 and 1.6, respectively, the effective size of pumice was estimated to be approximately 1.0 mm. Sand and pumice depths in the columns were set to 30 cm and 60 cm, respectively.

Table 1 Size and gradation parameters of the sand, pumice and anthracite coal used in the lab and pilot scale studies.

	Lab scale			Pilot scale	
Parameters	Sand	Pumice	Anthracite	Sand	Pumice
Dual media					
ES (mm)	0.55	1.1	1.0	0.5	1.0
UC	1.6	1.6	1.42	1.5	1.4
Depth (cm)	4	8	8	30	60
Mono medium					
ES (mm)	0.55	-	-	0.67	-
UC	1.6	-	-	2.34	-
Depth (cm)	12	-	-	90	-

4.2 Sludge conditioning and dewatering

Sludge samples were obtained from the sedimentation tanks at Lovö drinking water treatment plant in Stockholm. The conditioning chemicals used in the study were crude salt extract from MO seed (10%, w/v), analytical grade alum (5% w/v solution), and synthetic polyelectrolytes (1%, w/v). The polyelectrolytes were Praestol 650 TR (cation) and 2540 TR (anion) (Degussa, Stockhuasen Nordic). Dried MO seeds were obtained from Kenya and Senegal. Conditioning studies were performed using 500 mL sludge volume in a miniflocculator jar test apparatus (Kemira, Kemwater) having three 1-liter size beakers coupled with stirrers and speed control. Rapid mixing and slow mixing were done at 250 rpm for 20 s and 40 rpm for 2 minutes, respectively. Dewatering characteristics of the conditioned sludge were studied in terms of drainage in sand bed, floc strength, CST and SRF.

Sand drainage experiments were carried out in three Perspex glass columns each 3.0 cm diameter and 60 cm high. The columns were packed with 0.3-1.0 mm size and 12 cm deep sand. Rate of filtration, cake solids concentration and total volume and turbidity of the filtrate were estimated. The effect of sludge application depth on dewatering performance was studied. Turbidity was measured using Hach turbidity meter (Model 2001A). Solids concentration was estimated according to the standard methods (APHA, AWWA and WEF, 1995).

The parameters SRF and CST were determined from an MRCST unit (model TW 166 Triton Electronics Ltd.). Sludge was applied in stainless steel tubes (with an inner diameter of 10 mm and 18 mm) and the time for water moisture to travel between concentric circles (probes) in the CST filter paper was recorded. Filtration studies were also carried out using a 9 cm diameter Buchner funnel at a suction pressure of 49 kPa. SRF values obtained from the MRCST unit (18 mm diameter reservoir) correlated well with the data from the Buchner funnel experiments. Hence SRF data reported in this study are from MRCST unit. SRF was calculated using Equation 2 (Novak and Langford, 1977; Özacar and Sengil, 2000).

$$SRF = \frac{2A^2Pb}{C\mu} \qquad (2)$$

Where

A is the area of the filter cake, m^2

P is the filtration pressure, N/m^2

μ is the viscosity of filtrate, Ns/m^2

C is sludge solids concentration, kg/m^3, and

b is the slope of the plot of time over filtrate volume against filtrate volume, s/m^6.

Shear strength experiments were carried out using a standard shear-test apparatus (Triton-WRC Stirrer timer type 131) where the conditioned sludge was stirred at about 1000 rpm for stirring times of 10, 40 and 100 sec. Shear strength of the flocs was analysed from the CST and SRF values of each stirring time.

4.3 Purification and characterization of MOCP

4.3.1 Coagulant extraction from the seed

Dry MO seeds were obtained from Senegal and stored at room temperature. The seeds were shelled just before the extraction and the kernel was powdered using a kitchen blender. Oil was removed by mixing the powder in 95% ethanol (5-10%, w/v) for 30 min and the solids were separated by centrifugation and dried at room temperature. From the dried samples, 5% (w/v) solutions were prepared using distilled water, NaCl solution or ammonium acetate buffer, stirred for 30 min and filtered through Whatman paper No 3 and 0.45 µm fiberglass. The filtrates are termed crude extracts.

4.3.2 IEX purification

MOCP was purified from the crude extracts by IEX column chromatography and batch adsorption method. IEX chromatography was carried out in a 1 mL High-trap CM sepharose fast flow cation exchange column (Bio-Rad, Sweden) on an Äkta explorer (Pharmacia Biotech). The column was equilibrated with 50 mM ammonium acetate buffer, pH 7 and step elution was carried out using 1 M of the same buffer. Batch IEX purification was conducted using CM sepharose fast flow weak cation exchanger with bead size 45-165 µm (Bio-Rad, Sweden). Adsorption and elution parameters such as pH, ionic strength and volume were estimated in small volume experiments to optimise the process for scale-up. Adsorption kinetics of the protein on the IEX matrix was studied and equilibrium constants were calculated from Langmuir equilibrium model given below.

$$\frac{C_e}{q_e} = \frac{1}{bX_m} + \frac{C_e}{X_m} \qquad (3)$$

Where

C_e is amount of protein in solution at equilibrium, mg/L

q_e is amount of protein adsorbed per weight of adsorbent, mg/g

b is a constant related to the heat of adsorption, mL/g

X_m is the maximum adsorption capacity, mg/g

4.3.3 Characterization

MOCP was isolated by native polyacrylamide gel electrophoresis (native-PAGE). The experiment was carried out according to Hultmark et al (1983) using a Mini-PROTEAN 2 apparatus (Bio-Rad). The gel was cut at 0.5 cm horizontally and the protein was eluted into milli-Q water or 50 mM phosphate buffer.

Molecular mass was estimated using 10% sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, UK, 1970). This was also used to study purity of the protein. pI was determined from isoelectric focusing (IEF) (Model 111 mini IEF cell, Bio-Rad) run with ampholytes in the pH range 8 to 10.

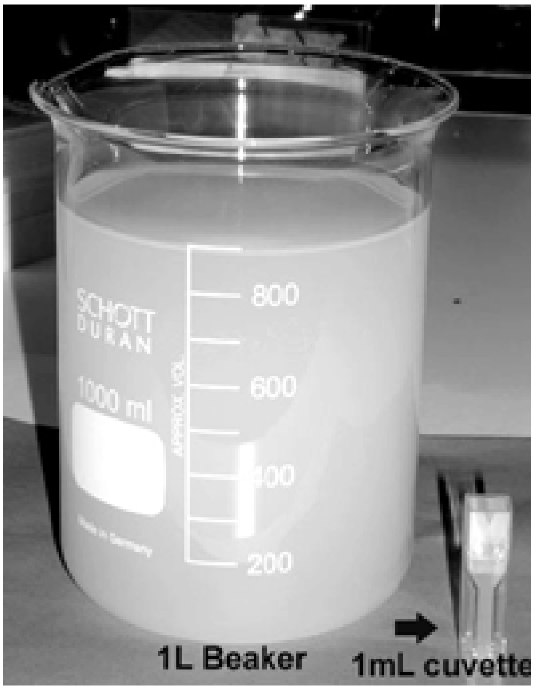


Fig. 6 Comparing sample sizes of jar test and cuvette method. In this study the 1 mL cuvette assay method was used. Coagulation activity was assessed in terms of absorbance reduction (OD_{500}). It was possible to compare large number of samples simultaneously.

Amino acid sequence of MOCP was determined from mass spectrometry (MS) analysis (Waters Corporation, Micromass MS Technologies, Manchester, U.K.). Protein content was estimated by the dye-binding method [Bradford, 1976] using bovine serum albumin as a standard solution. Thermal resistance of the coagulant protein was studied by boiling crude extracts at temperatures ranging from 60 to 100°C for 0.5 to 5 h. Samples were filtered through 0.45 μm fiber glass and tested for coagulation activity.

4.3.4 Coagulation activity

Small volume (1mL) coagulation activity assay method was developed using kaoline clay suspension to identify and compare the coagulation effect of different chemicals, such as crude MO extract, MOCP, alum and cecropin A (Fig. 6). Coagulant solutions (10 μL) were added to 1 mL volume clay suspension in a semi-micro plastic cuvette (10x4x45 mm, Sarsted Aktiengesellschaft & Co, Germany) and homogenized instantly. This was allowed to settle for 1 h and OD at 500 nm (OD_{500}) was measured using a UV-Visible spectrophotometer (Cary 50 Bio). For precise coagulation efficiency estimation, sample volume of 200 μL, from the top of the 1 mL cuvette, was transferred to a quartz glass cuvette (type 105.200-QS, 10 mm light path, HELLMA) for OD_{500} measurement. The stock clay solution was prepared as 1% (w/v) using tap water, stirred for 1 h and decanted after 24 h settling. Dilutions were prepared to get the desired initial turbidity suspension.

4.3.5 Antimicrobial property

Antimicrobial studies were carried out in terms of colony forming units (CFU), cell flocculation and growth kinetics on a number of gram-positive and gram-negative bacterial species (paper IV and V). Bacterial cultures were grown in 0.2 M phosphate buffered Lauryl broth (LBB) medium at 37°C overnight with continuous shaking. The cultures were diluted with 10 times diluted LBB to get the desired microbial density (OD_{620}) in each experiment.

In the studies of flocculation effect and CFU, MOCP and Cecropin A were added to the suspension cultures and incubated at 37°C for 2 h. Cell aggregations were analyzed under the microscope (Olympus BX51 with AnalySIS) and images were captured using a Sony PC 120 camera attached to the microscope. Samples from the 2 h incubated suspension were diluted 10 to 10^6-fold and aliquots were uniformly spread on nutrient agar plates and incubated at 37°C overnight for colony counting. In growth curve study, overnight grown bacterial cultures diluted in LBB, were inoculated in 96-well microtiter plate (Greiner bio-one) with different amounts of MOCP in a total volume of 150 μL. Plates were incubated at 37°C in FLUOstar* OPTIMA (LabVision, Sweden) spectrophotometer set to shake the culture before every measurement and record OD_{620} at 10 min interval.

4.3.6 Organic and nutrient contents

The amount of organic load released from MO extract into water was determined in terms of chemical oxygen demand (COD). Samples for COD measurement were obtained from coagulation experiments (jar test). Different fractions, from the IEX purification, were used for coagulating clay suspension. COD measurement was performed in Hach DR/2010 spectrophotometer using the low range (0-150 mg/L) COD digestion vials (Hach company). Procedure was followed according to the manufacturer's manual (Hach company, 1990).

Phosphate and nitrate contents (in the crude, non-adsorbed and purified samples) were determined by the flow injection analysis method in Aquatec 5400 analyzer (FOSS, Sweden) with automatic sampler (5027 sampler). Data were analysed using the Aquatec software.

5 OVERVIEW OF WATER SUPPLY AND TREATMENT SITUATIONS IN ASMARA (PAPER I)

The water supply system in Asmara, as a whole, and the treatment plant at Stretta Vaudetto, in particular, are experiencing a number of design and operational problems. Consequently the quality and quantity of distributed water do not meet the requirements of consumers and the targets set by the authorities.

Unaccounted for water (UFW) of the system amounts to 40% of the total water production (SAUR International, 1997). This is mainly due to leakage in the distribution system. The aged distribution pipes are also causes for inferior water quality at consumers' taps due to intrusion of pollutants from the surrounding soil and fall-off from corroded metal pipes. For example iron content and turbidity values are sometimes significantly higher at consumers' taps compared to the corresponding values at the treatment plant (Fig. 7). The recent renovation work (replacing the existing pipes with PVC material) is believed to have significantly reduced the amount of UFW and improved the water quality.

Stretta Vaudetto has a design capacity of 8000 m^3/d and comprises of cascade aeration, coagulation, flocculation, sedimentation, filtration and chlorination (Fig 8). Water from the different sources (Adi-Sciacca, Mai-Serwa, Adi-Nefas, Vagli Gnecchi, and Stretta Vaudetto) is first discharged into an inlet box and then it flows over cascade aerators at the bottom of which turbulent mixing occurs. Aluminum sulphate (alum) is added at this point for rapid mixing. Horizontal flow baffled channels are used for flocculation followed by two sets of sedimentation tanks. The second sets of sedimentation tanks are redundant with a very low turbidity removal efficiencies (approximately 1.5%). Settled water finally passes through a rapid sand filter before it is chlorinated in the clean water reservoir. Backwashing is carried out by water alone from an elevated reservoir. The treatment plant runs exclusively by gravity flow (hydraulic system) and this minimizes power consumption and overall operating cost.

Among the treatment plants in Asmara, Stretta Vaudetto has the most serious problems. Performance of the treatment plant is not satisfactory mainly due to lack of proper design and operation of the coagulation and filtration units. At the existing situation it can only handle flows much less than the design capacity and in many cases, the treated water quality is not compliant with the World Health Organization (WHO) guidelines. Moreover, direct disposal of sludge from the sedimentation tanks and filter wash to the recipient water body is of concern.

The amount of coagulant dosage applied is often below the optimum required. This is partly due to the lack of laboratory facilities (jar test apparatus) and problems in the preparation and dosing of the coagulant solution. The amount of alum dosage is estimated simply by visual observation of the raw water quality. The main reason, however, is lack of financial resources to import alum. From jar test analysis, carried out in this study, the average dry season optimum coagulant dosage was 35 mg/L. However, the coagulant dosage applied at the treatment plant was only 20 mg/L. The situation was worse during the rainy season when the coagulant added was only 40-60 mg/L whereas the optimum dose was found to be 80-100 mg/L. This significantly affects the performance of the sedimentation tanks and filters.

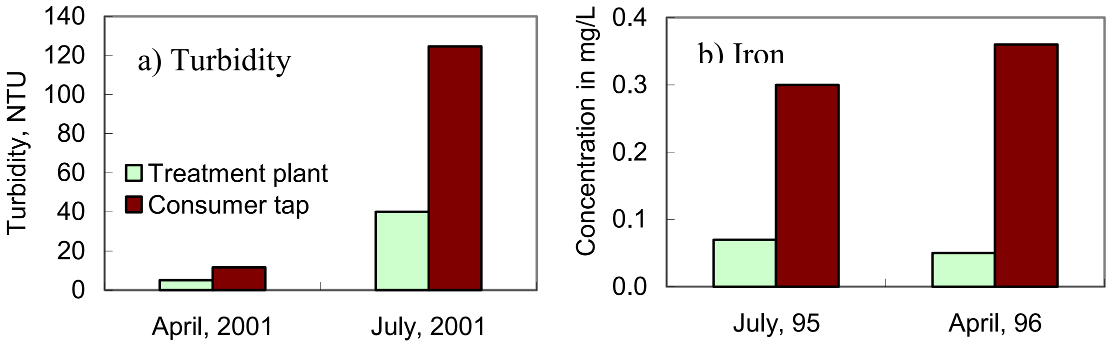


Fig. 7 Turbidity and concentration of iron in the treated water and at consumers' taps (Part of the data was obtained from records of Water Resources Department (2001)).

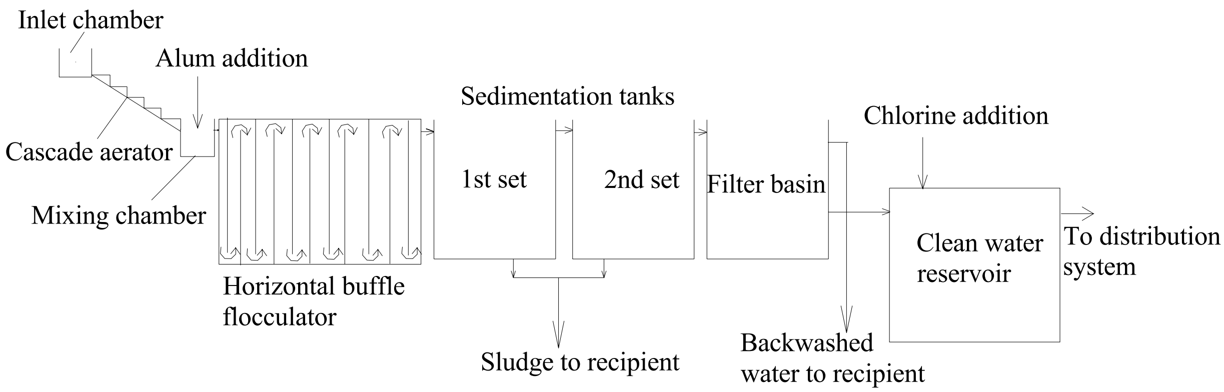


Fig. 8 Layout of Stretta Vaudetto water treatment plant.

Unsatisfactory performance of the filtration system is attributed to inappropriately selected sand medium, inadequate backwashing and poor pre-treatment. The filters have 1 m deep sand medium with ES (effective size) of 0.67 mm and UC (uniformity coefficient) of 2.36. The large UC value of the medium leads to a fine-to-course gradation and hence early headloss development. The rate of headloss development is so high that filter run lengths do not exceed 6 h. The filter backwash is not augmented by surface wash; hence effective cleaning of the sand medium is not achieved. Core samples from the filter basins showed considerable amount of mudballs, which are thought to have vital contribution to the short run lengths.

The average turbidity removal efficiency of the whole system, during the rainy period (in 2002) was 41% and in a number of cases the treated water turbidity exceeded 20 NTU. Turbidity value as high as 247 NTU has been reported (Water Resources Department, 2001). During the rainy season the water quality often does not meet the WHO guidelines and water borne disease outbreaks are common.

In order to improve performance of the treatment plant, suggestions were made to conduct in depth studies on locally available alternative materials that could be used in the coagulation and filtration units. The materials investigated were pumice for filtration and MO seed for coagulation and sludge conditioning. These can be incorporated into the system without major changes in the design and operation of the treatment plant.

6 PUMICE FOR DUAL MEDIA FILTRATION (PAPER II)

6.1 Material characteristics

The pumice from Alid, Eritrea, was first investigated for its suitability as a filter media and its characteristics were compared against recommended values. The specific gravity of pumice was estimated to be 1.6, which is similar to the values recommended for bituminous and anthracite coals (Paramasivam et al., 1973; AWWA, 1998). Hence it can be used as an upper layer on top of sand (specific gravity 2.65) and maintain the desired media gradation (coarse-to-fine in the direction of flow). Acid solubility of the pumice is about 4.8%, which is within the recommended limit for anthracite coal (5%, AWWA, 1998). Filter media with high acid solubility may result in material loss in low pH waters. Strength of pumice was estimated by the amount of attrition loss during extended backwashing and by scratching it against the minerals in the Moh's scale. The hardness value on the Moh's scale is 2.5. Although this is less than the recommended value for anthracite coal (2.7, AWWA, 1998), results of backwashing studies indicated that the material is sufficiently strong with attrition loss of only 1.8% during backwashing. Therefore it can be said that the material is durable enough not to require frequent replacement. In general, the study of material characteristics indicated that the pumice is suitable for multi media filtration. Pumice from Italy (Pumex UK Ltd., 2000) and Turkey (Farizoglu et al., 2003) have been reported to be suitable for filtration applications.

6.2 Column filtration: lab-scale

Comparative study between pumice and anthracite coal indicated that both materials had similar performances in terms of headloss development and filtrate quality. Backwashing studies also indicated that the rate of media expansion of the two materials was not significantly different (Fig. 9). Comparisons were also made between mono-medium (sand) and dual media (pumice-sand). In the mono-medium column most of the removal occurred in the top part of the sand. In the dual media, however, turbidity removal was distributed in the pumice and sand layers and the headloss in the pumice layer was only a small portion of the total. These indicate that the course-to-fine gradation of the dual media system allow deep penetration of suspended solids such that large percentage of void volume is used.

6.3 Column filtration: pilot-scale

During the pilot scale study data were collected for dry season (phase 1) and wet season (phase 2) periods. Summaries of the study for the two phases are presented in Table 2 and 3. The average FRL (filter run length) of the dual media column was more than double that of the mono-medium column and the WPC (water production per cycle) increased by 61%, while the effluent quality from both columns was similar. Studies have reported that dual media filters increase the loading rates of mono-medium by 50% to over 100% (Laughlin and Duvall, 1968; Paramasivam et al., 1973). The increase in FRL of the dual media column may be attributed to the higher particle storage capacity provided by the coarser pumice layer. Pumice has also the added advantage of high porosity and surface characteristics. It shows better filtration performance, compared to other materials such as sand, due to the rough surface properties (Gimbel, 1982; Andrievskaya et al., 1989).

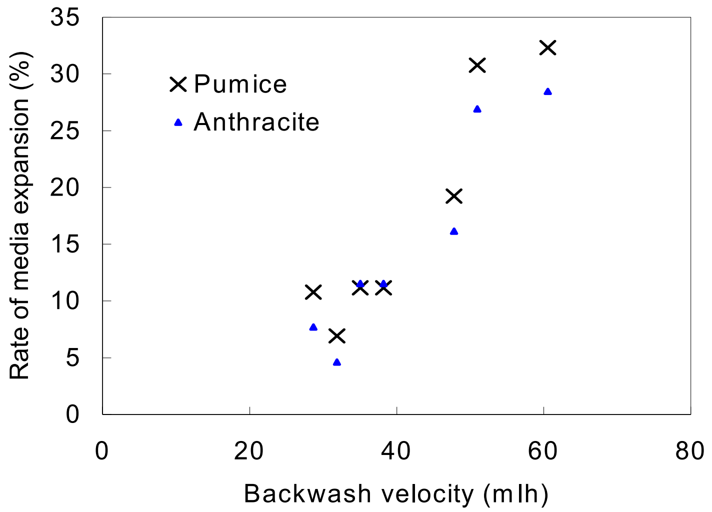


Fig. 9 Comparison of media expansion of pumice and anthracite coal during backwashing under varying velocities.

Table 2 Summary of results from the pilot plant column filtration study (phase 1).

Flow rate m/h	No of runs	Influent turbidity (NTU)	Effluent turbidity (NTU)	Headloss (cm)		WPC (m^3)	FRL (h)
				Clean	Terminal		
Dual media (Pumice-sand)							
3.0	8	3.52 ± 0.4	0.68 ± 0.1	7.4 ± 0.9	53.5 ± 3.0	5.27 ± 0.6	73.3 ± 7.9
5.5	9	4.61 ± 1.6	1.32 ± 0.5	12.0 ± 3.2	55.2 ± 6.8	5.73 ± 1.5	43.4 ± 11.4
7.5	8	2.85 ± 0.7	0.77 ± 0.2	15.7 ± 2.0	58.9± 5.2	3.92 ±0.5	21.7 ± 2.6
Mono medium (sand from existing filters)							
3.0	8	3.52 ± 0.4	0.74± 0.2	10.6± 0.7	80.5± 2.9	3.43 ± 0.9	31.7 ± 8.3
5.5	9	4.61 ± 1.6	1.37± 0.9	13.7 ± 4.2	77.3 ± 9.6	3.28 ± 0.7	16.6 ± 3.5
7.5	8	2.85 ± 0.7	0.59± 0.1	20.8 ± 2.1	81.0 ± 2.4	2.56 ±0.5	9.47 ± 2.2

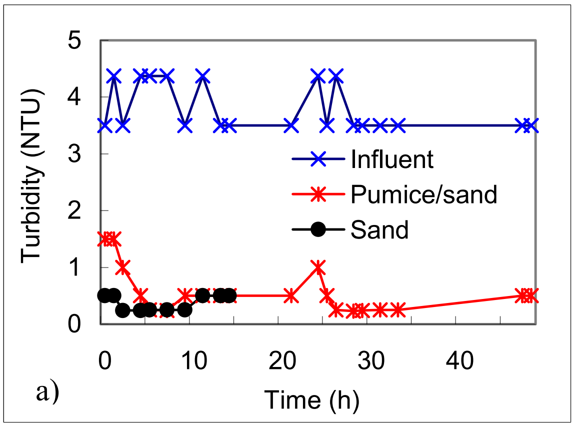
Table 3 Summary of results from the pilot plant column filtration study (phase 2).

Flow rate m/h	No of runs	Influent turbidity (NTU)	Effluent turbidity (NTU)	Headloss (cm)		WPC (m^3)	FRL (h)
				Clean	Terminal		
Dual media (Pumice-sand)							
5.5	7	17.8 ± 3.1	6.04 ± 2.3	11.9 ±1.8	31.4± 14.2	2.92± 1.4	22.1± 10.7
Mono medium (sand from existing filters)							
5.5	7	17.8 ± 3.1	6.44 ± 1.6	12.8 ± 3.1	49.9± 17.5	1.78± 1.5	8.96± 1.8

From observations of scanning electron microscope images, Farizoglu et al. (2003) reported that solids were retained inside the pores of pumice medium. The study also claimed that the retained particles were removed during backwashing and the full storage capacity of the media was restored.

Typical turbidity and headloss curves for phase 1, at loading rate of 5.5 m/h, are shown in Fig. 10. The rate of headloss development in the mono-medium column was very high compared to that of the dual media column due to rapid clogging of the top sand layer. The FRL of the mono-medium and dual media columns were 14 h and 48 h, respectively. At 14 h the headloss in the dual media was only 26 cm, while the corresponding value in the mono-medium column was 84.5 cm. In phase 2 filter runs in both columns were often terminated as a result of turbidity breakthrough. It was not possible to collect useful data on days following heavy rains because the turbidity of the settled water was excessive due to poor pretreatment.

Importance of the dual media system was also demonstrated by the considerable reduction in the velocity and volume of backwash water requirement. The rate (and amount) of backwash water in the dual media was reduced by more than 50%, compared to that of the mono medium column. This would have significant impacts on the operation of the treatment plant by reducing the time and clean water requirement for filter washing. The arrangement of the filter units is such that two filter basins share a common inlet. Due to insufficient pressure in the elevated backwash reservoir only one filter can be washed at a time and the second filter remains out of operation until the first one is cleaned. If dual media were used, instead of the existing sand, it would be possible to wash two filters simultaneously. On average, the percentages of backwash water were 1.9% and 10.5% of the total production, in the dual media and mono-medium columns, respectively. Typical percentage of wash water, for a well performing filter is 1-5% (Cleasby and Logsdon, 1999). Consumption of large amount of clean water for filter washing is not justified for a system, which is already running at a budget deficit.



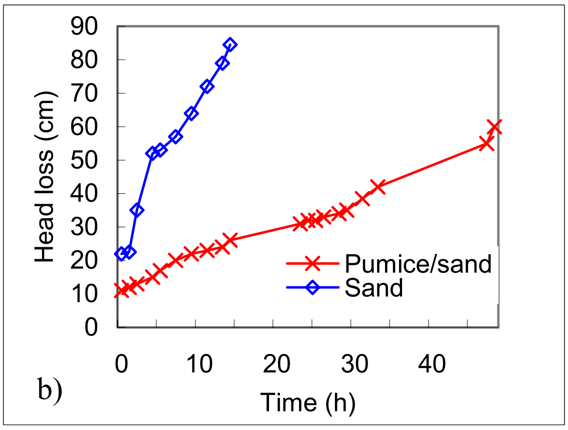


Fig. 10 Typical turbidity and headloss curves from the pilot plant filtration columns at 5.5m/h in phase 1. a) turbidity, and b) headloss.

7 *MORINGA OLEIFERA* FOR SLUDGE CONDITIONING (PAPER III)

This section discusses the use of crude MO extract for conditioning of chemical sludge from a drinking water treatment plant. Comparisons were made with alum and synthetic polyelectrolytes (Praestol 2540 TR, and Praestol 650 TR) based on the dewatering characteristics and floc strength of the conditioned sludge.

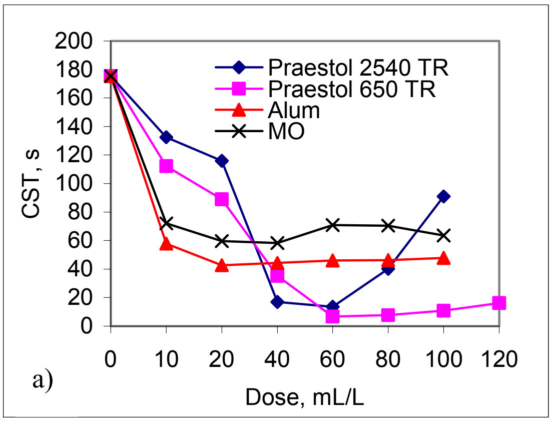
7.1 Capillary suction time (CST) and specific resistance to filtration (SRF)

Well-conditioned sludge is characterized by low values of CST and SRF. Fig. 11 shows that MO, alum and the synthetic polyelectrolytes improved the dewatering characteristics of the sludge as indicated by reduced values of CST and SRF relative to the unconditioned sludge. The effectiveness of the conditioners is basically due to the coagulation/flocculation effect, which enhances the release of water associated with sludge particles. Moreover, clogging of filter pores is reduced when particles are aggregated. Compared to MO, alum showed better conditioning performance. The crude extract was prepared directly from MO seed powder (without oil removal) and the presence of oil may have affected dewatering of the sludge. Oil affects sludge filterability negatively (Ademiluyi 1988; Hwa and Jeyaseelan, 1997). Considering cost of the chemical, sludge volume and disposal, MO would be considered more suitable compared to alum. Dual conditioning, using MO and alum, showed similar performance as alum thus MO can be used as a supplement or alternative conditioner. Dewatering characteristics of sludge conditioned by synthetic polyelectrolytes was better than MO and alum conditioned counterparts. Visual observation revealed that flocs from the polyelectrolyte-conditioned sludge were bigger in size. Large flocs lead to a better sludge permeability, hence improved dewatering (Özacar and Sengil, 2000). The large floc formation by the polyelectrolytes could be attributed to the coagulation mechanism (inter-particle bridging). On the other hand flocs from alum and MO conditioned sludge were smaller in size. This is characteristics of coagulation mechanism by charge neutralization and adsorption or sweep flocculation.

As conditioner dosage increased, CST and SRF decreased until optimum values were reached. The optimum chemical dosages and the corresponding values of CST and SRF are shown in Table 4. Dosages in excess of the optimum values did not improve the dewatering characteristics of the sludge. In the case of Praestol 2540 TR, dosages in excess of the optimum value significantly deteriorated dewatering characteristics of the sludge as indicated by the increased CST and SRF. This is primarily due to the high viscosity of Praestol 2540 TR (600 mPa.s at 0.1% solution).

7.2 Floc strength

Shear strength of flocs affects dewatering characteristics of sludge particularly in the mechanical dewatering systems. Weak flocs easily break down to fine particles and clog filter materials, hence lowered dewatering capacity. The chemical conditioners studied significantly improved the shear strength of the raw sludge. Flocs from MO and alum conditioned sludge were more resistant to breakdown. On the other hand, polyelectrolyte-conditioned flocs were relatively weaker and the CST values, after 100 s stirring time, were similar to that of the unconditioned sludge. It was observed that the optimum dosage for dewatering does not necessarily result in the strongest flocs.



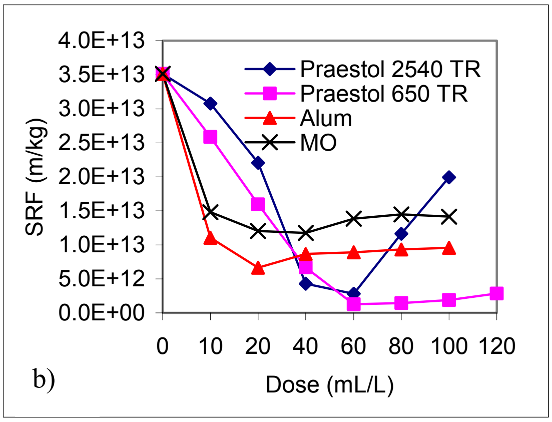


Fig. 11 Dewatering characteristics of alum sludge from drinking water treatment plant: a) CST, and b) SRF. The sludge was conditioned by varying doses of MO, alum and polyelectrolytes.

Table 4 Optimum dosages of conditioner and dewatering parameters for MO, alum and polyelectrolytes conditioned sludge.

Conditioner	Optimum dose, mL/L (kg/t)	CST, s	SRF, * 10^{12} m/kg	Drainage rate for different sludge application depths, mL/min	
				10 cm	20 cm
Unconditioned	0	175.4	35.1	0.05	0.04
MO	40 (125)	59.7	12.1	0.19	0.17
Alum	40 (63)	42.2	6.64	0.21	0.20
Preastol 2540 TR (anionic)	60 (1.8)	13.5	2.82	8.91	7.6
Praestol 650 TR (cationic)	60 (1.8)	6.7	1.27	8.1	7.23

For instance the optimum dosages of the cationic polyelectrolyte (Praestol 650 TR) for maximum dewatering and strong floc formation were 60 and 80 mL/L, respectively.

7.3 Column drainage

The amounts of chemical conditioners required for optimum column drainage maximum CST and SRF reduction were similar. The average drainage rates generally showed declining trends with increasing sludge application depth. There was a marginal decrease in drainage rate when the sludge application depth increased from 10 cm to 20 cm (Table 4). MO and alum increased the drainage rate of raw sludge by 4.2 times. On the other hand, the polyelectrolytes increased the drainage rate by two orders of magnitude and about 90% of the filtrate drained in the first 5 min. Even though there was significant difference in the drainage rates, the total filtrate volume and final cake solids concentration, for all the chemicals were similar. This is because of the fact that sludge conditioners can only improve dewatering rates and not the extent of dewatering (Knocke and Wakeland, 1983). The cake solids concentration for unconditioned sludge, MO-, alum-, Praestol 2540 TR- and Praestol 650 TR-conditioned sludge were 3.2, 4.5, 4.6, 6.8 and 6 %, respectively.

Filtrate turbidity of MO- and alum-conditioned sludge from sand drainage columns ranged from 1.0 to 2.2 NTU. The corresponding values for polyelectrolytes-conditioned sludge ranged from 7.2 to 15.5 NTU. This may be explained by the fact that the polyelectrolytes react rapidly with large sludge particles resulting in poor capture of fine solids.

8 CHARACTERIZATION AND PURIFICATION OF MOCP (PAPER IV-VI)

8.1 Characteristics

MOCP *(Moringa oleifera* coagulant protein) was isolated from the crude extract solution by IEX chromatography and native-PAGE. As a result of the high pI (isoelectric point) of MOCP it was possible to purify it from the crude extract using a simple IEX purification method. Absorbance spectrum at 280 nm and the corresponding coagulation activity of the eluted proteins from water and salt extract samples are shown in Fig. 12. The bound proteins from both extract samples were identified in three peaks (a, b and c) and protein peaks b and c showed coagulation activity. The presence of two coagulant protein peaks may arise from the heterogeneity of one or more active proteins in the extract. Protein peaks b and c were further studied to determine their chemical characteristics as well as coagulation and antimicrobial properties. The non-adsorbed fraction did not have coagulation activity.

Coommassie stained SDS-PAGE results showed that protein peaks b and c had molecular mass distribution less than 6.5 kDa (Fig. 13). Mass spectrometry analysis of the protein also indicated a dominant protein with molecular mass of 4.75 kDa (Paper V). Previous studies have reported that the molecular mass of the protein from MO seed was 6.5-13 kDa (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995). Isoelctric focusing (IEF) experiments indicated that pI of the active proteins were higher than 9.6 (the highest marker used in the study). They showed some variations in their pI values consistent with the elution pattern from the cation exchange resin. Protein peak c, which appeared last in the IEX chromatography showed higher pI value than protein peak b.

In native-PAGE molecules with high surface charge and small size exhibit high mobility and they are extracted from the bottom (or end) of the gel. From native-PAGE experiments two coagulant proteins were isolated. They were spotted close to the end of the gel phase indicating high electrophoretic mobility. This indicates that the coagulant proteins have high surface charge and small size consistent with the IEX, IEF and SDS-PAGE results. Comparison of the coagulant fractions from native-PAGE and IEX showed similar molecular weight distribution (Fig. 13) and coagulation activity.

The coagulant from the seed is believed to consist of a mixture of proteins with similar physical characteristics. The composition (peptide sequence information) of the mixtures was studied from mass spectrometry analysis. A number of peptide sequences were obtained (Table 5), which are similar or identical to the known sequence of a coagulant protein from MO seed (Gassenschmidt et al., 1995). It has been reported that more than one protein family with coagulation activity is present in MO seed (Gassenschmidt et al., 1995). Indeed, ongoing genomics projects around the world continue to demonstrate that plants express many closely related proteins during different developmental stages (Dong et al., 2004) so it is not unexpected that MO produces a range of sequence variants of seed storage proteins with coagulant activity. Further work on the genome and proteome of MO is clearly necessary to identify the exact nature.

Proteins may be denatured during extraction, purification or storage. Extreme temperature is one of the physical factors that denature proteins. MOCP was found to have high thermal resistance and it remained active after 5 h boiling at 95-100ºC.

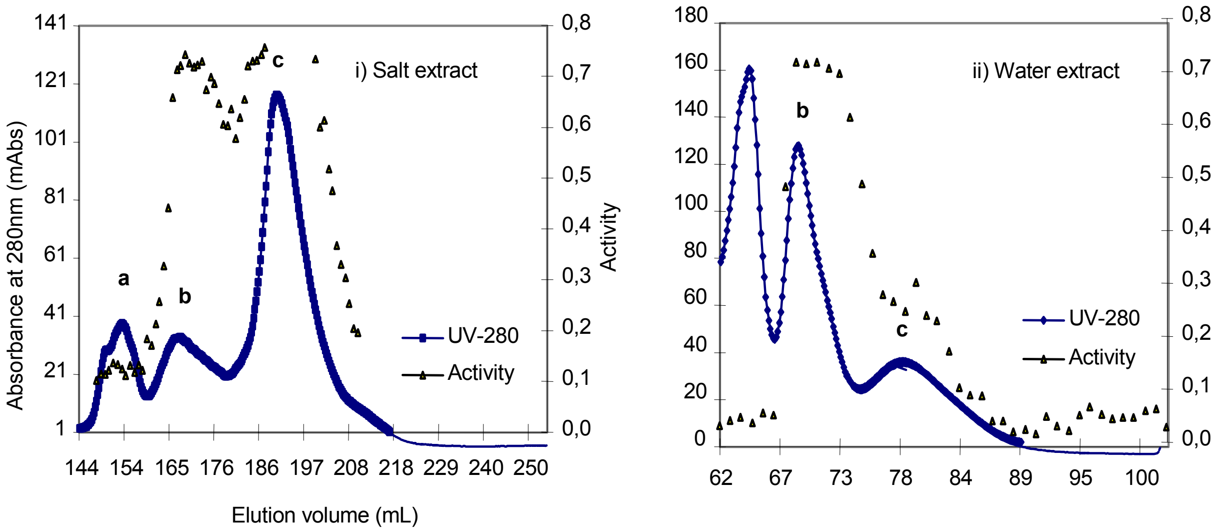


Fig. 12 High-Trap CM fast flow IEX chromatography and coagulation activity for (i) salt extract and (ii) water extract. Ammonium acetate buffer (50 mM, pH 7) was used for equilibration and it was eluted in step gradient with 1 M concentration of the same buffer.

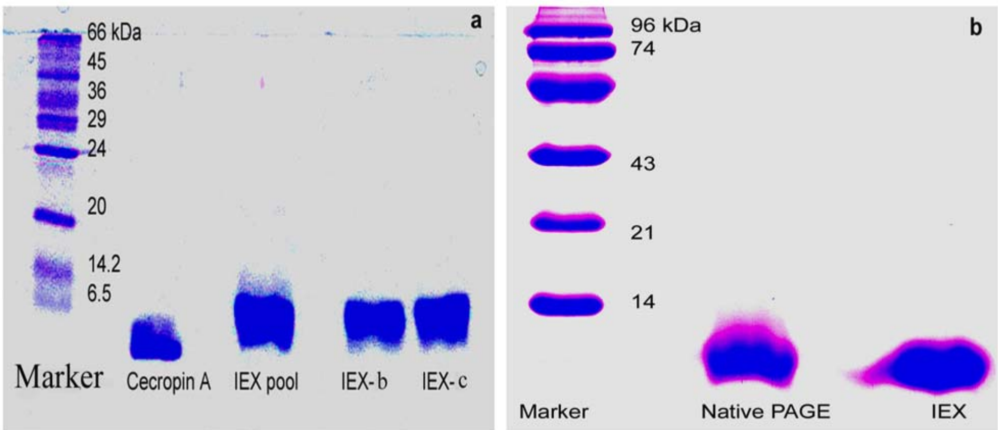


Fig. 13 SDS-PAGE images of the coagulant fractions from IEX and native PAGE. a) molecular mass estimation in comparison with protein markers and cecropin A. The two active proteins (peak-b and peak-c) show similar size b) compares sizes of protein samples from IEX (peak c) and native PAGE.

There was no significant difference in the coagulation activity of the boiled and non-boiled samples. Such a high thermal stability renders it easy to process and handle. Furthermore, boiling could be used to remove lipid from the crude extract.

Table 5 Electrospray ionization-MS/MS determination of the sequences of peptides from the trypsin digested protein.

Observed peptide mass	Observed charge state	Peptide sequence
1268.62	+2	ANPPVQPDFQR
2087.09	+3	QAVQL**TH**QQQGQ**A**GP**L**QVR
2100.18	+2, +3	QAVQL**AH**QQQGQ**V**GP**Q**QVR
2122.12	+2, +3	QAVQL**ET**QQQGQ**V**GP**Q**QVR
2130.28	+2, +3	QAVQL**TH**QQQGQ**V**GP**Q**QVR *

*This is identical to the sequence published by Gassenschmidt et al. 1995.

8.2 Purification

Based on the results of IEX chromatography, MOCP was purified using the batch IEX method. The optimum operating parameters (pH, ionic strength, elution volume and equilibrium constants) were determined and large volume purification was carried out.

8.2.1 Comparison of extraction solutions

Crude extraction of the coagulant material was carried out by water, ammonium acetate buffer or NaCl solution. The protein content and coagulation activity of crude ammonium acetate extract (CAE) and crude water extract (CWE) were similar. On the other hand, the amount of soluble protein in the crude salt extract (CSE) was more than two fold the amount in the CWE or CAE. This may be explained by the salting-in effect of proteins at higher ionic strength. Similarly, the coagulation activity of the CSE was significantly higher than the CWE and CAE. Okuda et al., (1999) reported that CSE showed better coagulation activity at doses 7.4 times lower than CWE.

Apart from the difference in the amount of soluble protein and coagulation activity, there was no characteristics difference (molecular mass and pI) between the proteins extracted by the different methods. In all cases the coagulants were proteins, according to the dye binding method and absorbance at 280 nm. Most of the studies in the literature have used water extraction method and it was reported that the coagulant was a cationic protein (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995). Okuda et al. (2001a), on the other hand, purified an organic molecule (neither protein, polysaccharide nor lipid) from the CSE, which possessed coagulation property. This may indicate that there are different compounds in the seed that possess coagulation property.

The effect of extraction method significantly affects the process of IEX purification. In the case of CSE, large amount of dilution is required to bring the ionic strength to that of the equilibrating buffer. On the other hand CAE can be directly applied to the matrix without the need for ionic strength or pH adjustment. Whenever efficient extraction can be achieved, it would be advantageous to use the same buffer solutions for extracting the protein and equilibrating the matrix.

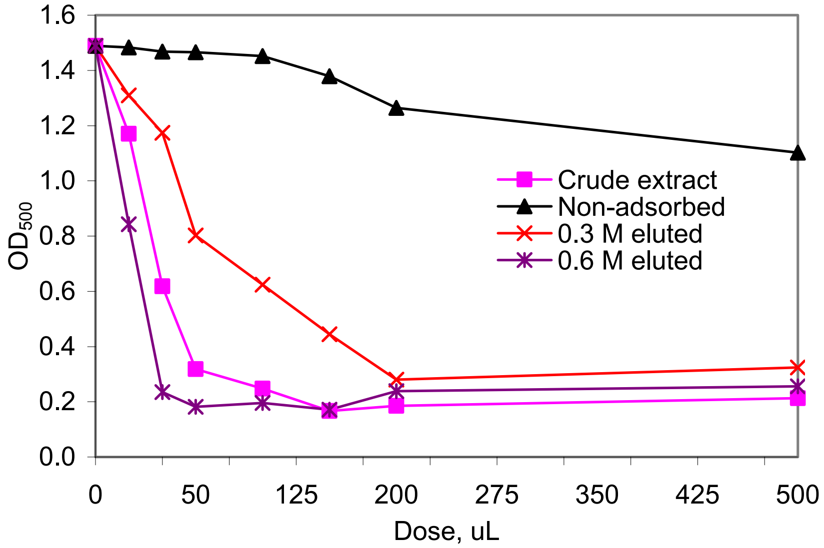


Fig. 14 Coagulation activity of the crude extract and various fractions from IEX purification. Samples were taken from the top part of the suspension after 1h settling and OD_{500} was measured using a UV-Visible spectrophotometer (Cary 50 Bio).

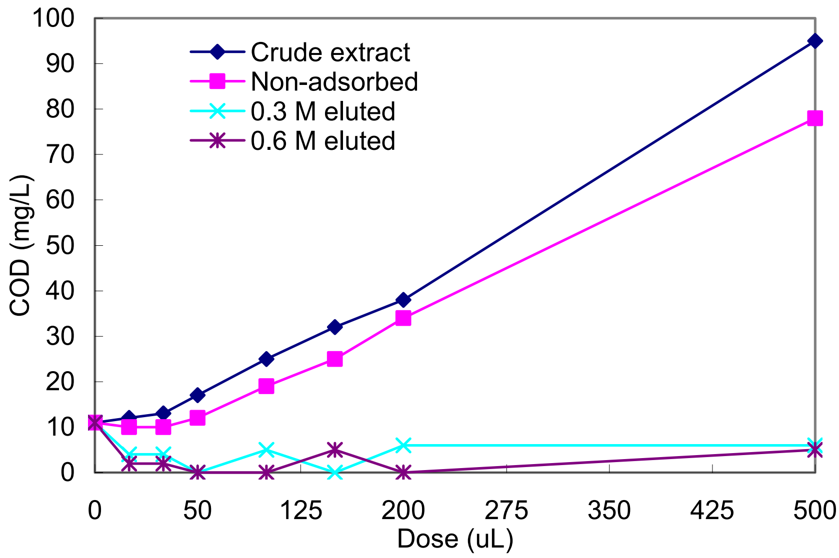


Fig 15 COD values in water samples treated by crude extract and the various fractions from IEX purification. Only the crude extract and the non-adsorbed fraction samples showed significant increase in COD.

8.2.2 Batch IEX purification

Batch IEX purification experiments were carried out to determine optimum values of adsorption and elution parameters that can be used for large-scale purification. The matrix was equilibrated using ammonium acetate buffer (10 mM, pH 6.7). The optimum ionic strength of the NaCl for elution was 0.6 M. According to the IEX chromatography (Fig. 12), the eluted fraction from the matrix comprises of the three protein peaks (a, b and c) out of which peaks b and c were active coagulants. In order to remove the non coagulant adsorbed proteins (that is peak a) multiple step elution was carried out by washing it first with 0.1 M followed by 0.2, 0.25, 0.3 M and 0.6 M NaCl. It was observed that all the non-coagulant adsorbed proteins were removed after 0.3 M elution. Hence high purity MOCP, consisting of only peaks b and c, could be obtained by first washing the matrix with a 0.3 M NaCl followed by 0.6 M NaCl. Such a procedure may be important for purposes where a highly pure protein is needed. For application in water treatment a single elution, by 0.6 M NaCl, would suffice since the non-active adsorbed protein (peak a) would have no significant impact on the water quality as discussed in section 8.2.3.

Kinetic experiments indicated that the rate of protein adsorption to the IEX matrix was rapid in the first 10-20 min followed by a decreased rate. Adsorption equilibrium reached after 90-120 min where maximum adsorption of the coagulant protein was observed. Equilibrium adsorption parameters were estimated from the Langmuir adsorption model given in Equation 3 (Faust and Aly, 1987). Accordingly, maximum adsorption capacity and dissociation constant of the system (based on purified protein) were 68 mg/g and 0.049 mL/g, respectively. Adsorption study was also carried out by adding a constant amount of crude extract protein (instead of purified protein) to varying amounts of matrix volume. The adsorption capacity of the matrix estimated from this procedure was approximately 21 mg/g, which is lower than the adsorption capacity estimated based on purified protein (68 mg/g). This may be attributed to the presence of competition, for adsorption sites, among the different molecules present in the crude extract. Whereas MOCP consists of only small size proteins the crude extract comprises of combination of varieties of organic substances of different size ranges. In the crude extract, large molecules can block access to the adsorption sites in small pores (Karlsson et al., 1989). Small size molecules are known to exhibit higher adsorption per unit weight of adsorbent compared to large molecules (Kilduff et al., 1996).

Based on the optimised parameters, purifications of 1 L and 5 L crude extract samples were carried out. In the case of 1 L purification adsorbed proteins were eluted in two steps (0.3 M followed by 0.6 M NaCl). Coagulation activity test indicated that the crude extract and both the eluted fractions were effective coagulants whereas the non-adsorbed fraction did not have coagulation capacity (Fig. 14). The effective coagulation property of the 0.3 M eluted fraction indicates that, loss of coagulant protein is evident when elution is carried out in two steps. Purification of the 5 L was performed in a similar way (as for the 1 L procedure) but with a single-step elution. In both cases the organic and nutrient loads were removed along with the non-adsorbed fraction (see details in section 8.2.3). This indicated that, for the purpose of water treatment application, two-step elution would not be required. Single-step elution not only simplifies the purification procedure particularly in large-scale production, but also it increases the yield.

Table 6 Nutrient concentrations in the different fractions of IEX purification.

Sample	Average nutrient concentration	
	PO_4 (mg/L)	NO_3 (µg/L)
Crude acetate extract	31	87
Crude water extract oil not removed	30	84
Crude water extract oil removed	21	60
Non-adsorbed	17	44
MOCP	1,8	15

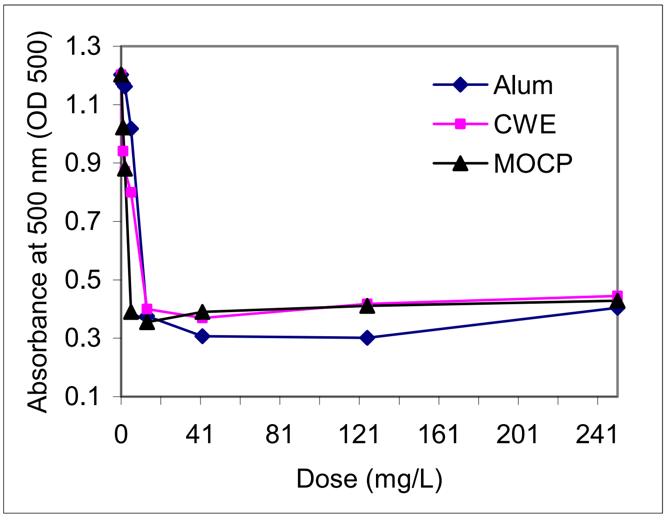


Fig. 16 Coagulation performance of alum, crude (CWE) and purified extracts of MO seed (MOCP) for high turbidity clay suspension.

8.2.3 Impact of purification on organic and nutrient loads

Crude MO extract contains compounds other than proteins such as carbohydrates, lipids and other organic and inorganic molecules that may be released to the water. For example, the average COD value of the crude extract samples, after oil removal, was approximately 12000 mg/L. These render crude MO extract unsuitable for use in large water treatment systems. One of the main purposes of purifying the crude extract is to avoid the release of organic and nutrient loads to the water being treated. In order to check if the purification would achieve this, organic load studies were carried out by measuring the amounts of COD added to the water from the different fractions of the IEX purification process. It was observed that almost all the organic load comes from the non-adsorbed fraction. Fig. 15 shows that both the eluted fractions did not release COD to the water. Therefore a single-step purification method (adsorption followed by 0.6 M elution) would be sufficient to avoid the concerns of organic release from MO. The single-step purification also showed that the concerns of nutrients release from the use of MOCP could be avoided (Table 6). Most of the nutrients from the crude extract remained in the non-adsorbed fraction. Studies by Ndabigengesere et al. (1995) and Okuda et al. (2001a) showed that the purified coagulant protein did not impart organic and nutrient loads to the water but the purification procedures involved a number of steps, which may not be easy to scale-up.

The simple purification method presented in this study will hopefully promote the use of MO in large-scale drinking water treatment. The protein purification can be carried out at treatment plant sites using locally available facilities.

8.3 Coagulation properties

8.3.1 Assay method

A small volume coagulation assay using 1 mL cuvette was developed to facilitate the assessment of coagulation activity of different chemicals by measuring OD_{500}. Coagulation activity was estimated from the difference in OD_{500} at initial and final settling times in relation to a control sample. Compared to the standard jar test analysis, this method is preferable when large number of samples are to be compared. The method is simple and it reduces sample volume and coagulant dosage. Large number of experiments can be carried out simultaneously (versus a maximum of 6 in the standard jar test experiment) thus significantly reducing the time needed for data acquisition. In the case of IEX purification, for example, it was possible to rapidly screen out the coagulant fractions from a very large number of eluent samples. Similarly coagulants from different sources can be easily and rapidly compared. The method is also convenient to perform settling characteristics study of flocs by continuously recording OD_{500}. Measurements of OD_{500} in the 1 mL cuvette may be affected by the attachment of particles to the wall surfaces. In such case reduction in OD_{500} could be measured in a small volume of sample taken from the top part. Using this method relatively higher percentages of reduction in absorbance were observed.

8.3.2 Comparison with other chemicals

Coagulation activity of alum, MOCP and crude MO extract was compared using the assay method described above. The coagulation activity of the three coagulants was similar for high turbidity (250-300 NTU) clay suspension. Typical coagulation performance curves for varying doses of the coagulants are shown in Fig. 16. The optimum dosages required to achieve maximum reduction in absorbance were 15 mg/L for the crude extract and alum, and 5 mg/L for MOCP. At low initial turbidity coagulation performance of alum was better than the crude extract and MOCP. Sutherland et al., (1990) and Muyibi and Evison (1995a) reported that MO was less effective for low turbidity waters. In such cases it may be used as a coagulant aid. Temporal and spatial variations in water quality necessitate the need for onsite studies to check the effectiveness of coagulants for the particular type of water under consideration. The decision whether MO is used as a supplemental or alternative coagulant can then be made.

A study was also made to compare the coagulation effect of cecropin A (a small antimicrobial peptide with molecular mass of 4 kDa and high pI) with MOCP and alum. Cecropin A showed similar coagulation activity as alum and MOCP. This suggests that a number of basic peptides from plants and animal origin could be fruitful sources of coagulants for use in water and wastewater treatment systems.

8.4 Antimicrobial properties

Antimicrobial effects of MOCP were attributed to both flocculation and bactericidal action. Fig. 17 shows the flocculation effect of MOCP on D31, and Bt7 cells.

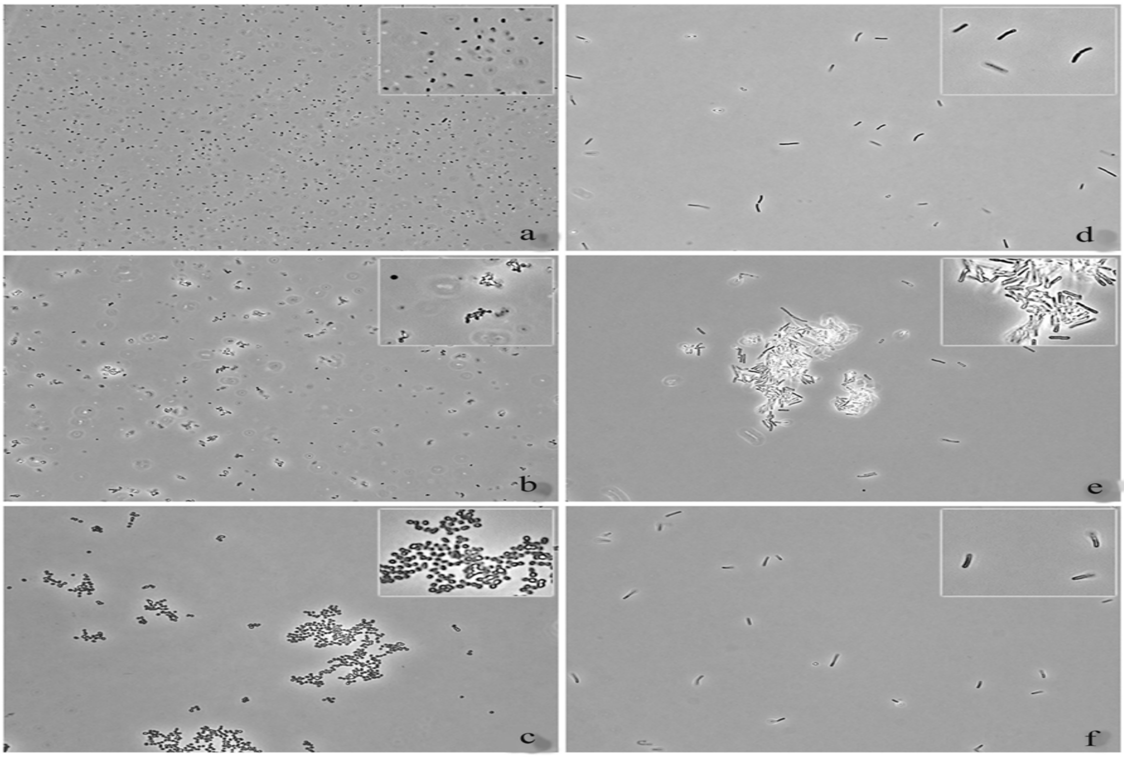


Fig. 17 Flocculation effect of MOCP and cecropin A for D31 and Bt7. Images a, b and c represent control, MOCP dosed and cecropin A dosed samples of D31, respectively. The corresponding images for Bt7 are shown in d, e and f, respectively. Note that cecropin A did not flocculate Bt7 (f).

It also shows that cecropin A could flocculate D31 but not Bt7. Counts of CFU indicated that, MOCP could achieve 1.1 to 4-log reduction of the microorganisms studied. Reductions in *E. coli* (D31) and *B. thuringiensis* were due to both flocculation and bactericidal effect. On the other hand, cell reduction in some microorganisms, such as *E. coli* (K12 and *P. aeruginosa)* occurred without noticeable cell aggregation. This indicates that flocculation was not the only mechanism by which microbial reduction occurred.

Antimicrobial peptides are thought to act by disrupting the cell membrane or by inhibiting essential enzymes (Silvestro et al., 2000; Suarez et al., 2003). So far the mechanism by which MOCP acts on cells is not clear. Close observation under the microscope revealed that cells that have been exposed to MOCP were immobilized, had distorted shapes and in some cases the cytoplasm leaked out while the cells in the control were intact and fast moving. This may indicate that the action may be through attack on the cell wall structure. However, further study is needed to clearly describe the mechanism of action.

MOCP effectively inhibited the growth of *B. thuringiensis* cells, which were resistant to cecropin A. Studies have reported that *B. thuringiensis* inhibits the activity of cecropin A by a protease that degrades the peptide (Dalhammar, 1987). Resistance of MOCP to such protease may have to do with the conformation of the peptide. Thus study of its structure would be important to explain the observations.

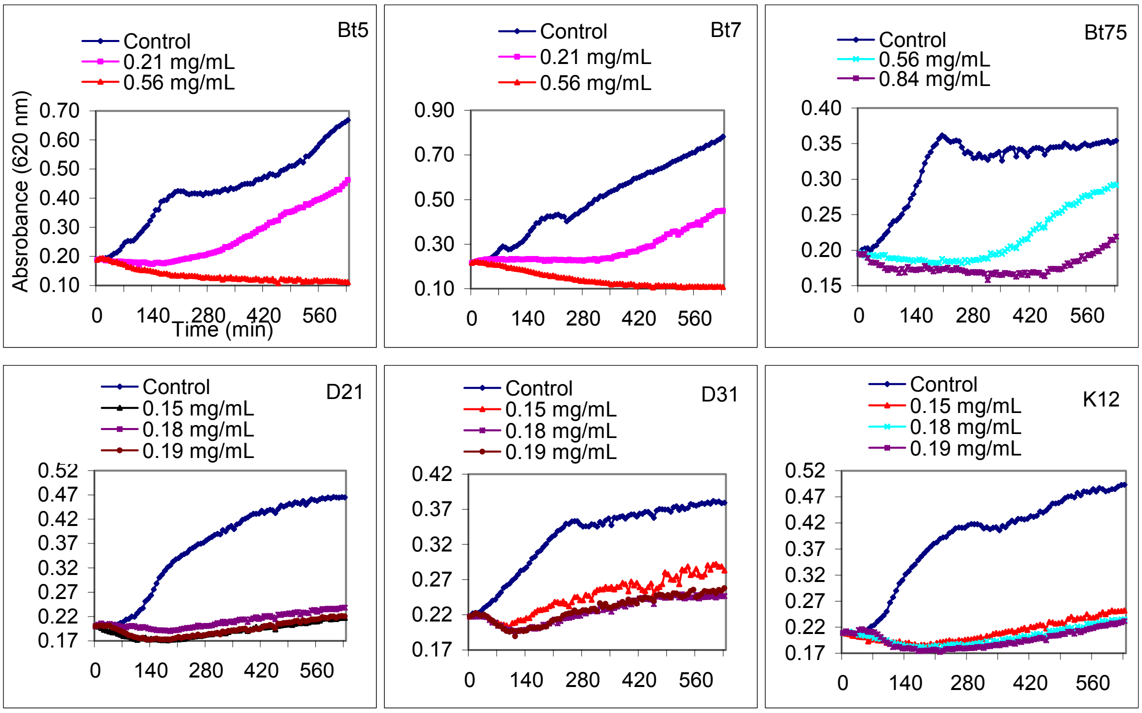


Fig. 18 Growth curves for B. thuringiensis (Bt5, Bt7 and Bt75) and E. coli (D21, D31 and K12) for varying doses of MOCP. MIC is estimated for 90% inhibition.

The results suggest that MOCP may be used against a number of resistant microorganisms in water and wastewater treatment. It may also be useful as an antibacterial drug in pharmaceutical applications. It has been reported that a recombinant MO protein has effective antimicrobial activity on antibiotic resistant pathogenic bacteria (Suarez et al., 2003).

Studies were also conducted by closely monitoring the growth kinetics of different bacterial strains under varying MOCP doses. MOCP showed bacteriostatic and bactericidal effects on gram-negative and gram- positive bacteria. Typical curves for *E. coli* (D21, D31 and K12) and *B. thuringiensis* (Bt5, Bt7 and Bt75) are shown in Fig. 18. The minimum inhibitory concentration (MIC) on *E. coli* strains D21 and K12 was 0.15 mg/mL whereas the MIC on strain D31 was 0.18 mg/mL. The MIC on Bt5, Bt7 and Bt75 were 0.56, 0.56 and 0.84 mg/mL, respectively. One possible explanation for the difference in MOCP activity could be the difference in the cell wall composition of the strains. Moreover, *B. thuringiensis* produce enzymes that could degrade MOCP (Dalhammar, 1987).

Most of the reports on the antimicrobial effect of MO are based on crude extract (Olsen, 1987; Madsen et al., 1987; Eilert et al., 1981) or recombinant form of MO protein (Broin et al., 2002; Suarez et al., 2003). In the present study, investigations were carried out using MOCP directly purified from the seed and it showed effective antimicrobial properties. Whether MOCP and the recombinant forms of the protein have similar effects and mode of action are yet to be studied.

In a study of antimicrobial effects of the crude MO extract, Madsen et al. (1987) observed a secondary bacterial increase due to re-growth during the 24 h period.

The re-growth resulted to even higher microbial concentration than in the untreated water. This may be due to increased organic and nutrient loads from the crude MO seed or due to the application of lower dosages than the MIC. From Fig. 18 it is observed that at dosages less than the MIC, the OD_{620} started to increase after a certain lag phase period. For example, at the dose of 0.21 mg/mL, which is lower than the MIC, the concentration of Bt5 and Bt7 started to increase after 5 h incubation period. At MIC, however, the bacteria did not grow even after 17 h incubation period. Hence the problem of regrowth may be alleviated if the purified protein is used at the proper dosage.

The coagulation and antimicrobial effects of MOCP suggest that it may be used for simultaneous coagulation and disinfection in water treatment. This can have significant implications on the practice of water treatment systems. Further studies are required to assess disinfection capabilities of MOCP on microorganisms found in water and wastewater.

9 CONCLUSIONS

An overview of the water supply situation in Asmara, Eritrea, is given and the problems at the Stretta Vaudetto water treatment plant are discussed. The overall performance of the treatment plant is not satisfactory and the main problems are attributed to inappropriate design and operation of the coagulation and filtration units. Pumice and *Moringa oleifera* (MO) were suggested as alternative natural materials in dual media filtration and coagulation, respectively.

Material characteristics of pumice, from the local area, were in accordance with the requirements for filter materials. In a lab scale column filtration study it showed similar performance as anthracite coal. In a pilot scale study a dual media (pumice-sand) column showed more than a two-fold increase in filter run length and a 50% reduction in the amount of backwash water requirement compared to a mono-medium sand from the treatment plant filters. Conversion of the existing sand filters to dual media would significantly improve performance of the treatment plant in terms of increased hydraulic loading rate and reduced treatment cost.

The MO coagulant protein (MOCP) was isolated both by ion exchange (IEX) chromatography and native polyacrylamide gel electrophoresis and it was identified as a small molecular mass protein with high isoelectric point. More than one amino acid sequence of the MOCP was identified, which indicates that the seed contains an heterogeneous population of coagulant proteins. Based on characterization studies, a simple and inexpensive batch IEX purification of MOCP was developed. Optimum parameters for a single step batch IEX purification were estimated. The purification method can be readily scaled up and carried out using locally available facilities. MOCP did not impart organic and nutrient loads, which are associated with the crude MO extract. The simple purification method developed is thought to promote the use of MO seed for large-scale treatment applications.

MOCP showed similar coagulation and sludge conditioning capabilities as aluminum sulfate. A simple low volume coagulation assay method was developed, which simplified and facilitated coagulation activity study. It was important to compare coagulants from different sources and study settling characteristics of the flocs. In a comparative study it was observed that cecropin A had similar coagulation activity as MOCP and alum. This indicates that other proteins and peptides from plants and animals could be fruitful sources of coagulants. MOCP was not only an active coagulant, but also showed antimicrobial effects on a number of gram-positive and gram-negative bacteria, some of which are antibiotic resistant. The antimicrobial effects of MOCP were attributed to cell flocculation and growth inhibition. The effects on clay suspension and microorganisms suggest that MOCP may be used for simultaneous coagulation and disinfection in water treatment systems.

10 FURTHER STUDIES

This work indicated the possibilities of using alternative natural materials in different water treatment units. In order to appreciate the actual integrated effect and to apply the findings for treatment plant systems pilot- and full-scale studies are required.

Purification of MOCP in large-scale is a work in progress. The processes and procedures for pilot scale are under development.

Existing knowledge of the coagulant from MO seed is far from complete. Existence of heterogeneous coagulant proteins suggests the need to disclose the whole range of active proteins. The highest number of amino acid residues in one of the identified peptide sequence was 19 while Gassenschmidt et al. (1995) identified 60 residues. It is not clear if only the 19 amino acid residue peptides would be sufficient for coagulation and antimicrobial action. In-depth study would be required to identify the active coagulation site in the protein. If it is desired to synthesize the peptide, identification of the minimum number of residues required for its activity is important to reduce the cost and simplify the procedure.

The mechanism of antimicrobial effect of MOCP and the range of microorganisms over which it is effective, are important subjects for further investigations. MOCP was effective on antibiotic resistant microorganisms (Bacillus thuringiensis, paper IV). One possibility could be due to the conformation of the protein. Therefore a study of its structure may be required to explain the mechanism or mode of action.

A study on the removal of other contaminants than turbidity, such as heavy metals and phosphorus can be carried out to assess its wider application.

The study on the use of pumice was primarily based on turbidity removal. Studies on the suitability of pumice for biofiltration can be carried out to exploit the structure and surface texture of the material.

11 ACKNOWLEDGEMENTS

This work would not have come to completion without the support and encouragement of many people.

First of all I wish to sincerely thank my supervisor, Professor Bengt Hultman for accepting me as his student and for his scientific guidance and valuable discussions. He has been continuously encouraging and motivating me during the years of my study. His concern and support in personal matters is very much appreciated.

I am deeply indebted to professor Gunnel Dalhammar, my co-supervisor, and Dr. Gunaratna Rajarao. They have unsparingly and generously shared their time, enthusiasm and knowledge and they introduced me to the fascinating field of biochemistry. Our discussions and their constructive critisms and optimism have always been a great source of inspiration for me and their confidence in me has been very stimulating.

I enjoyed the company of many wonderful colleagues at the department of Land and Water Resources Engineering. In particular, I would like to thank Sachida, Hua, Kristina, Alexandra, Christian, Beata, Luiza, George, Peter and Sally. Special thanks are due to Andrew and Herbert for proof reading the thesis. I would like to give special thanks to Monica and Bertil for sustained assistance and useful discussions in the lab. I am very grateful to Aira for the wonderful assistance in administrative and personal matters.

The group at Applied Environmental Microbiology deserve special thanks for the wonderful hospitality. Special thanks are due to Per and Kay for tirelessly helping and facilitating the lab work.

I enjoyed the splendid company and friendship of my colleagues from University of Asmara. I wish to thank Daniel, Dr. Bereket, Girma, Teclemariam, Futsum, Dr. Semere, Estifanos, Samson, members of SAUA in Uppsala and other friends in Stockholm. Many persons have been involved during my field study in Eritrea. I would like to extend my sincere thanks to Okbay, Mussie, Efrem, Mebrat, Dawit. Special thanks to Lowell Fuglie for supplying moringa seeds from Senegal.

My deep gratitude goes to all my brothers and sisters who have always been supporting and encouraging me throughout my study.

Financial support from SIDA is gratefully acknowledged. I am indebted to the University of Asmara for giving me this opportunity. Special thanks to Dr. Bengt Finnström for the very wonderful administration of the scholarship. His keen interest to help is highly appreciated. Thanks also to professors Gunnar Jacks, Ingmar Grenthe, Olle Wahlberg, and Lennart Nilsson for the useful discussions and encouragements.

Finally I wish to express my gratitude to my beloved wife, Azeb, who unsparingly encouraged and supported me during all these years. It would not have been possible for me to complete my research without her understanding and willingness to take the main responsibility at home. I sincerely appreciate the effort and skill she showed in taking care of our kids.

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