Development of a naturally derived coagulant for water and wastewater treatment

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Abstract Seeds of the pan tropical tree, Moringa oleifera Lam. (M. oleifera) contain water soluble, positively charged proteins that act as an effective coagulant (molecular weight 13 kDa and isoelectric pH 10–11). Treatment studies are reviewed using the extracts of the crushed seed kernel and of the presscake (solids residue remaining after oil extraction). M. oleifera seed was incorporated into a pilot scale treatment system as a primary coagulant to clarify river water of 400 NTU in the wet season. Over 90% turbidity removal was achieved in the sedimentation stage at a dosage of 100 mgL⁻¹. The filtrate turbidity was maintained well below 5 NTU. Subsequent trials were conducted in an adjacent works operating at 60 m³h⁻¹. Treatment performance was comparable to that of using aluminium sulphate. Inlet turbidities of 270–380 NTU were consistently reduced to below 4 NTU. A comprehensive study was undertaken to evaluate the potential of using M. oleifera coagulant within a contact flocculation filtration (CFF) pilot rig. A wide range of operating conditions was evaluated in order to establish the useful “working envelope” for this process combination (filtration rates 5–20 mh⁻¹, initial turbidities 20–50 NTU). Principal results are presented. As a coagulant within chemically enhanced primary sedimentation (CEPS) of a mixed wastewater, M. oleifera dosed at 150 mgL⁻¹ gave additional removals (compared to a plain sedimentation control) of 40% biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and in excess of 80% for suspended solids (SS). Subsequent laboratory work coupled an upflow anaerobic sludge blanket reactor (UASB) to CEPS. M. oleifera coagulant in the CEPS pre-treatment unit beneficially increased the ratio of soluble COD to volatile SS by a factor of ten compared to plain sedimentation and by a factor of three when dosing ferric chloride. The UASB yielded more biogas and gave enhanced COD removal. Recent work on the extraction and purification of the active coagulant components is reviewed and the prospects for commercialisation are considered.

Keywords Developing countries; Moringa oleifera; natural coagulant; water/wastewater treatment

Introduction

In developing countries, river water drawn for human consumption and use can be highly turbid, particularly in the rainy season. River silt is churned into suspension and run-off from fields and other surfaces carry solid material, bacteria and other microorganisms into the river. It is of paramount importance to remove as much of this suspended matter as possible prior to a disinfection stage and human consumption. This can generally only be achieved by the addition of coagulants to the raw water, within a controlled treatment sequence. In many developing countries, proprietary chemical coagulants, such as aluminium sulphate and synthetic polyelectrolytes are either not available locally or are imported using foreign exchange. If available, chemical supplies are subject to the vagaries of purchase, allocation, distribution and transportation, resulting in inconsistent use. A viable alternative is the use of crushed seed of the M. oleifera tree as a natural coagulant.

The M. oleifera tree is a native of Northern India, which now grows widely throughout the tropics. English vernacular names include “drumstick” (shape of the pods) and “horseradish” (taste of the roots). It may be propagated from seeds or cuttings, it grows rapidly and well even in poor soils requiring minimal horticultural attention and is able to survive long periods of drought. Extended and multiple harvests in a single year are evident in...
many parts of the world. Large-scale cultivation of the tree is confined to India for the production of green, immature pods as a vegetable. There are several development projects promoting the small-scale production of fresh and dried leaves as a source of human and animal nutrition and the extraction of the high quality edible oil contained within the seed kernel. The latter is achieved using manual or motorised screw presses in the rural community setting. Other diverse products and uses of this multi-purpose tree include use of the oil in cosmetic products including soaps, the leaf extract used as a foliar spray to stimulate plant growth, use of all the plant parts in a variety of traditional medicines, and incorporation of the tree within live fencing, etc.

For treatment application, the seedpods are allowed to dry naturally on the tree prior to harvesting. The mature seeds are readily removed from the pods, easily shelled and then may be crushed and sieved using traditional techniques such as those employed for the production of maize flour. The crushed seed powder, when mixed with water, yields water soluble proteins that possess a net positive charge (molecular weight 13 kDa and isoelectric pH 10–11). Dosing solutions are generally prepared as 1–3% solutions and are filtered prior to application to the untreated water (Sutherland et al., 1990).

The seeds contain up to 40% by weight of oil. The fatty acid profile shows an oleic acid content of 73% confirming that the oil is similar to olive oil and thus of high edible quality and market value. Edible oils are an essential component of human nutritional requirements. In developing countries, a few, large-scale urban-based companies usually dominate the production and marketing of edible oils. Rural supplies of the finished products are erratic with increased prices due to additional transport costs. Bench scale testing at Leicester confirmed that the press cake (solids residue remaining after oil extraction) still contains the active, water-soluble proteins. Significantly, two potentially valuable products may be derived from the seed.

*Moringa* derived coagulants offer several advantages over conventional coagulants such as aluminium sulphate:

- activity is maintained over a wide range of influent pH values – no pH correction is required;
- natural alkalinity of the raw water is unchanged following coagulation – no addition of alkalinity is required;
- sludge production is greatly reduced (by a factor of up to five) and is essentially organic in nature with no aluminium residuals (Ndabigengesere and Narasiah, 1998).

**Evaluation of the use of Moringa oleifera coagulant**

**River water treatment at pilot scale**

It is now regarded as axiomatic that both water and wastewater technology for developing countries must be no more complex than strictly necessary and be robust and inexpensive to install and maintain. A prototype treatment works was designed founded on this philosophy. The pilot plant was constructed within the grounds of the Thyolo Water Treatment Works controlled by the Malawi Government. The pilot plant, with a design flow rate of 1 m³h⁻¹, consisted of: a header tank, where *M. oleifera* seed solutions were introduced into the turbulent jet of incoming water and mixed hydraulically; an 18 minute flocculation period provided within gravel bed flocculators; plain horizontal sedimentation and rapid gravity filtration. All the units were locally fabricated in sheet steel.

The system was successfully commissioned during the rainy season with the source river exhibiting turbidity levels in excess of 400 NTU throughout the study period. In general, solids removal within the plant was consistently above 90% following the gravel bed flocculation stage and plain horizontal flow sedimentation. Subsequent rapid gravity sand filtration gave final, treated water turbidity generally well below 5 NTU. *M. oleifera* seed
dose ranged from 75–250 mgL−1 depending on the initial raw water turbidity (Folkard et al., 1993).

**River water treatment at full scale**

During the following wet season the main Thyolo works was operated using *M. oleifera* solution as coagulant. The works comprised upflow contact clarifiers followed by rapid gravity filters and chlorination. The clarifiers were in a state of some disrepair with the impeller drives and chemical feed pumps inoperative. Under normal operation, alum solution is introduced into the incoming flow of 60 m³h⁻¹ by simple gravity feed at a declining rate. Comparable treatment performance with alum was achieved. During a 7.5 hour test run with the main works flow at 60 m³h⁻¹, the inlet turbidity of 325 NTU was reduced to below 2 NTU following filtration with a seed dose of 75 mgL⁻¹. This was the first time that *M. oleifera* had been successfully used as a primary coagulant at such a scale with the treated water entering supply (Sutherland et al., 1994). *M. oleifera* seed for the full-scale trials was purchased from enthusiastic, local villagers. This was viewed as a temporary yet very welcome new source of cash income in what is a poor rural community of Southern Malawi. The tree is widely cultivated in this area, being highly prized as a source of fresh, green vegetable.

**M. oleifera coagulant within contact flocculation filtration (CFF)**

A comprehensive study was undertaken to evaluate the potential of using *M. oleifera* coagulant within a contact flocculation filtration (CFF) pilot plant rig. CFF is defined as the high rate filtration process for relatively low turbidity raw waters (<50 NTU) wherein the coagulant is dosed immediately prior to entry onto the sand bed. Flocculation and subsequent deposition occurs entirely within the filter bed. A wide range of operating conditions was evaluated in order to establish the useful “working envelope” of operational parameters for this single stage process. Previous studies had shown that at the low turbidity experienced by the River Nswadzi in the dry season, the effectiveness of *M. oleifera* coagulant is reduced. Flocs that formed were small, compact and light giving reduced settling velocities. This is considered to be a result of the fundamental nature of the coagulation and flocculation involved. The relatively low molecular weight of the active proteins indicates that charge neutralisation and floc formation are brought about by the patch mechanism, as opposed to the bridging mechanism (Gregory, 1977).

The field installation of the pilot CFF rig and full experimental details are given elsewhere (McConnachie et al., 1999). However, for prevailing raw water turbidities of <50 NTU the single stage treatment of CFF gives a consistent filtrate turbidity <1 NTU for filtration rates up to 10 mh⁻¹. Moreover, the *M. oleifera* seed dose required to achieve this is relatively low (<25 mgL⁻¹) and the filter run times are appropriate for effective plant operation.

**M. oleifera coagulant for wastewater treatment applications**

As a coagulant within chemically enhanced primary sedimentation (CEPS) of a mixed domestic/industrial wastewater, *M. oleifera* dosed at 150 mgL⁻¹ gave additional removals (compared to a plain sedimentation control) of 40% for biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and in excess of 80% for suspended solids (SS) (Folkard et al., 1999).

Subsequent laboratory work at the University of Ghent coupled an upflow anaerobic sludge blanket reactor (UASB) to CEPS (Kalogo et al., 2000). The UASB process relies on the propensity of anaerobic biomass to aggregate into dense flocs or granules over time. Mixing is achieved by pumping influent wastewater from an entry at the base upwards
through the sludge blanket. Above the blanket, finer particles flocculate in the upper settlement zones and settle back as sludge in the blanket thus preventing washout of biomass.

The biogas, which has poor solubility in water, is separated at the top of the reactor. Domestic wastewater treatment in UASB reactors has proved particularly effective in tropical regions of the world. Effective removal of organic matter and suspended solids is evident at reduced excess sludge volume compared to aerobic treatment. The system is compact, requires minimal energy inputs and does not require support media normally associated with anaerobic systems (de Sousa and Foresti, 1996).

UASB is characterised by a very high mean cell retention time (MCRT) and a relatively low hydraulic retention time (HRT). *M. oleifera* coagulant in the CEPS pre-treatment unit beneficially increased the ratio of soluble COD to volatile SS by a factor of ten compared to plain sedimentation and three when dosing ferric chloride as coagulant. The UASB yielded more biogas and gave 71% removal of total COD at 2 hours HRT. This compared with 54% removal of total COD at the same retention time when ferric chloride was used.

The loading capacity of an anaerobic wastewater treatment system is essentially determined by the amount of active biomass retained in the reactor. In UASB reactors, the microbial aggregates must combine two important characteristics, namely a high biodegradation activity and excellent settling properties, favoured by the formation of granular sludge particles. One of the main problems in the application of this treatment process so far has been the extensively long start-up periods needed for the development of granules (up to six months).

In a subsequent study, a water extract of *M. oleifera* seeds was used to enhance the start-up of a self-inoculated UASB reactor treating raw domestic wastewater (Kalogo et al., 2001). Two reactors labelled “control” and “test” were started without special inoculums. Both reactors were fed continuously for 22 weeks with domestic wastewater with an average total COD of 320 mgL⁻¹ and SS of 165 mgL⁻¹. The reactors operated during the entire experimental period at 29°C and at a HRT of 4 hours. The “test” reactor received 2 mL of a 2.5% (w/v) *M. oleifera* seed stock solution per litre of influent wastewater.

The “test” reactor gave the following enhanced performance advantages over the “control”:
- shortened the biological start-up period by 20%;
- increased the acidogenic and methanogenic activity by factors of 2.4 and 2.2, respectively;
- increased the specific biogas production by a factor of 1.6;
- favoured fast growth of the sludge bed;
- allowed the aggregation of coccoid bacteria and the growth of microbial nuclei – the precursors of anaerobic granulation.

**Alternative applications of *M. oleifera* seed coagulants: the food sector**

There are a number of food production processes where solid/liquid separation is an essential stage to achieve the final product quality. One such example is the production of sugar from sugar cane. In the production of organic sugar, the use of synthetic polyelectrolytes to remove extraneous solids suspended in the cane juice is not permitted. Coagulant derived from natural plant materials are used, e.g. the bark of *Triumfetta lappula* and gum from *Lannea coromandelica*. A laboratory study was conducted at the Mauritius Sugar Research Institute to evaluate the efficacy of applying *M. oleifera* seed coagulant to clarify limed cane juice (Wong Sak Hoi and Tse Chi Shum, 1999). In one test series, *M. oleifera* dosed at 0.48% gave a 37% increase in turbidity removal compared to a proprietary coagulant (Superfloc A2130). Other tests were conducted with the addition of bentonite in small quantities as a weighting agent to the *M. oleifera* flocs. The authors conclude that the
quantities of *M. oleifera* seed which would be required for the daily production of cane juice are favourable compared to alternative natural coagulants in use.

**Extraction and purification of the active components from *M. oleifera* seed**

Treating water with water extracts of *M. oleifera* seeds has one identified disadvantage. The coagulant-inactive seed material, which is also water-soluble, leads to elevated dissolved organic material in the treated water (nitrates, orthophosphates, etc.). If chlorination is adopted for final disinfection of the clarified water then the potential for the formation of disinfection by-products (DBP) is increased. DBPs such as chloroform are suspected carcinogens and are strictly regulated in Europe and in the United States. Residual organic matter may also exert a chlorine demand at the treatment works and be utilised by microorganisms as substrate for re-growth in the distribution system. Therefore, there has been much recent research work on the extraction and purification of only the coagulant-active proteins from within the seed kernel. Protein extraction and purification from *M. oleifera* seed has been reported at laboratory scale only. The aim of these studies was to secure only a few milligrams of pure protein for the characterisation of coagulant activity and structure.

Extraction of the proteins using 1M sodium chloride solution gave enhanced coagulation at significantly reduced dosage compared to water extracted material – 95% turbidity reduction at 4 ml L⁻¹, compared to 78% reduction at 32 ml L⁻¹ for a prepared test water comprising kaolin in water of initial turbidity 50 NTU (the dosage being expressed as volume of 1% stock seed solution; Okuda *et al.*, 1999). The improvement in extraction is attributed to the “salting-in” mechanism whereby increased ionic strength gives increased protein solubility. The extraction of seed proteins in other salts gave similar improvements.

The research group achieved purification of the active components using the following sequence of stages (Okuda *et al.*, 2001).

1. Extraction of the seed material in 1M NaCl solution.
2. Dialysis with molecular cut-off 12–14 kDa (precipitate forms by the “salting out” mechanism).
3. Centrifugation to recover the precipitate.
4. Precipitate washed with deionised water.
5. Removal of lipids by homogenisation in cold acetone.
6. Recovery of precipitate by centrifugation followed by rinsing with cold acetone.
7. Precipitate dissolved in ammonium buffer and centrifuged to remove insoluble matter.
8. Pass solution through an anion exchange column and elute with NaCl solution.

The purified material was deemed to be an organic polyelectrolyte of molecular weight around 3 kDa, but not to be a protein, polysaccharide or lipid. The authors claim that the “specific coagulation efficiency” of this active material is up to 34 times more than that of a water extract of seed, that it is effective for low turbidity waters, and that no increase in residual organic carbon is evident following application.

**Towards commercial production of a protein extract coagulant**

There are many unresolved issues relating to the commercial viability of the scale-up of protein separation, including:

- applicability and technical feasibility of scale-up of the laboratory unit processes that comprise the sequence of separation;
- quality control in production and acceptable levels of residual impurities;
- final desired form of the protein products – concentrated solutions/dried products;
- stability of protein products in storage and in transit;
- ease of preparation of dosing solutions for specific treatment applications;
• regulatory aspects – particularly stringent with respect to drinking water treatment and food processing;
• cost of production and the overall economic case – from cultivation, seed purchase, harvesting, oil extraction and sales to the placing of products in the various commercial markets.

Existing markets for water and wastewater chemicals are highly competitive with producers supplying large quantities at relatively low margins.

Conclusions

The promotion and development of *M. oleifera* as a natural coagulant offers many advantages to many countries of the developing world: sustainable, appropriate, effective and robust water treatment; effective enhancement of wastewater treatment processes; decreased reliance on the importation and distribution of treatment chemicals; the creation of a new cash crop for farmers and employment opportunities. Many technical and economic issues remain to be resolved with regard to the extraction and purification of the active components from within the seed kernel. The problem of securing a guaranteed supply of seed in sufficient quantity remains central to further industrial scale development. However, there could be relatively small, niche or speciality markets that could be developed where *M. oleifera* seed coagulant (in some finished form) offers specific technical advantages, e.g. for UASB reactor start-up and operation, or in the clarification of sugar cane juice.

References