

REVIEW PAPER

A REVIEW ON GENETICALLY ENGINEERED NATURAL COAGULANT BASED ON *MORINGA OLEIFERA* FOR TURBIDITY REMOVAL

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Abstract: Genetic engineering and engineering in general are two distinct fields that people tend to separate due to conflict of interest between experts. Genetic engineering is the manipulation of an organism genome using biotechnology while engineering is the creation and application of tools and machineries that helps or enhance ability of human to manage this world. Manipulating of the genetic code in terms of producing new materials or increase the production of certain valuable materials of interest is considered as engineering, as it fulfils the meaning of creation. Various coagulants have been employed to reduce water turbidity. Coagulants are divided into three types; as natural inorganics, natural organics and chemical coagulants. The subject of this review is based on *M. oleifera* protein (lectin) as natural coagulant. Several attempts to extract coagulant from seeds of *M. oleifera* are already reported. Researchers are also working on the production of alternatives to *M. oleifera* lectin via genetic engineering approach. The expression of *M. oleifera* lectin from *E. coli* has been reported to provide high and pure lectin with enhanced efficiency in the removal of water turbidity. Future interest in using higher eukaryote system especially *P. pastoris* and genetically modified lectin is expected to increase both production volume and improve coagulation properties. The release and consumption of this lectin raises a few questions about health and environmental safety. This review analyses positive and negative influence of the genetic engineering technique based on various studies done in relation to producing natural organic coagulants, such as lectin.

Keywords: *Genetically modified microorganism, lectin, natural coagulant, protein engineering, turbid water.*

1.0 Introduction

Every year, statistical report on human population showed increasing trend as well as urbanization and industrialization, then, directly affected the global increases in wastewater production (Muller *et al.*, 2014). The wastewater production need to be treated in order to avoid water scarcity. Although water is present in great quantity on

Earth, but the total mass that can be directly utilized by human is about 0.65%, where else are not directly usable by human beings, such oceans and seas (97.2% of the water total mass), and glaciers (2.15%) (Oturán *et al.*, 2014). About more than 4 billion people live in parts of the world where freshwater scarcity directly threatens human water security or river biodiversity.

Apart of increasing demand on freshwater and wastewater production, an estimated one billion people lack access to safe affordable drinking water, 2.7 billion lack accesses to sanitation, and many millions die each year from preventable waterborne diseases. Therefore, it is indispensable to research and develop advance technology efficient and ecologically friendly methods to treat contaminated waters in river or reservoir and reduce or completely eliminate pollutants for clean drinking water.

Traditional water treatment system generally started with coagulation, settling or sedimentation, gravity or granular filtration and finally chemical disinfection (Xua *et al.*, 2014; Bonton *et al.*, 2012). Besides that, there is one addition process for most water treatment plants in Malaysia which known as aeration process before coagulation take place. Aeration brings water and air in close contact in order to oxidizes dissolved metals such as iron, hydrogen sulfide, volatile organic chemicals (VOCs) and remove dissolved gases (such as carbon dioxide). This process is often the first priority process for the treatment plant in Malaysia, because, coagulation, flocculation and precipitation are ineffective for removing many dissolved organic contaminants (Azzouz *et al.*, 2013). In this recent review, the coagulation process will be discussed in broad perspective and the data from other researcher will provided accordingly because coagulation is known as an important unit operation in the water treatment plants.

2.0 Preface to Coagulation Action

Generally, sources of water in this earth surface have both particles that are soluble and suspended especially it happens above water surface. Those particles had been formed whether innately known as natural organic matter (Sharp *et al.*, 2006) or due to human activity like industrial and domestic disposal. Due to this, the contaminations in the water need to be discarded to get source of clean water for use again. Water treatment process involved of coagulation and flocculation process namely the process to separate the suspended solids portion from sources of water. Coagulation happens when coagulant reduces repulsive force of electrical double layers on colloids surface that it is function to push between one and another, furthermore, it cause the particles join to form a larger floc. Those particles also had variety of distinct aspect from the resources, composition charge, particle size, shape and density (Sharp *et al.*, 2006). The factors which involved in deciding efficiency process coagulation includes pH, temperature, concentration of organic matter, ionic strength, total dissolved solids, particle size, distribution of colloidal particles in suspensions, coagulant type and dosage (Sher *et al.*,

2013). Factor of pH will be affected by coagulant usage type and consequently give effect to structure and particles in water. Coagulation happens in two circumstances namely baseline coagulation which involve optimization coagulation condition which the combination between pH and then coagulant dose, while enhance coagulation only focus additional coagulant superfluously and cause depress of the water pH (Bell-Ajy *et al.*, 2000).

There is two main classified of coagulant that used in coagulation process either chemical or natural coagulant bases (Vieira *et al.*, 2010). Chemical coagulant had been describe in the chemical constituent as known are aluminum sulphate, ferrous sulphate, ferric sulphate, ferric chloride, cationic polymer, calcium hydroxide (lime) and sodium aluminate. But, one of the initial and still the most dominantly used, is aluminium sulfate, also known as alum. The alum will alter the charge in the water to have as high as +4 but are typically bivalent (with a charge of +2) and this will make it more effective in neutralizing particle charges than are monovalent ions (Elleuch *et al.*, 2013). Alum has long supplanted those natural clarification aids, which it has most widely used in the wastewater treatment plants (Fatombi *et al.*, 2013). Compare to natural coagulant, this alum is cheaper, ease of handling, mixing properties and storage friendly (Renault *et al.*, 2009). Although is cost saving substance, this alum side effect which it can cause alzheimer disease associated with residual aluminum in treated water, production of large sludge volumes, reducing pH in water and reduce the efficiency of coagulation in cold waters (Vieira *et al.*, 2010).

3.0 Problem on Chemical Coagulant

Most widely used as chemical coagulants also known as alum and polyaluminium chloride (PAC) possessed serious imperfections like Alzheimer's disease and strong carcinogenic properties due to possess high aluminium compound (de Souza *et al.*, 2014; Okuda *et al.*, 2001). Although alum as coagulant in water treatment plant still affordable among developing country likes Malaysia but it is harmful to human health and worst to environment which has remained bottleneck problem, especially the high sludge production that will increase every year which required treatment and disposal. For example, the cost for treat and disposal of sludge in wastewater treatment plants (WWTPs) were estimated about 60% of total cost for entire process and this cost will increasing when the total volume of sludge increase which depend on population development (Wei *et al.*, 2003). Similar situation also occur in drinking water treatment plants which most of the stakeholder not affordable to accommodate the high cost for sludge treatment. There are a lot of cases that the water treatment residues were streamed to the nearby river or canal. This behavior if not been avoid from continuously occur will put the ecosystem of biotic and abiotic in danger situation because the concentration of aluminium as heavy metal will exceed than it natural concentration

exist. Indirectly also, long term using alum will increase country medical cost, reduce soil fertility, reduce animal population and high recovery cost for environmental damage.

Those side effects behind it faster advantages of using this chemical coagulant will actually contribute to the invisible high cost for those long-term negative side effects that people always neglected due to cheaper cost of this material price. The ecosystem damage was estimated to reduce overall life expectancy by up to 7-8 months per person and cost the UK £ 20.5 billions per year by the world health organization (Everett *et al.*, 2010). In other way according to this author, reduce of human capital in term of physical activity cause losses about £ 8 billions a year and large contribution in obesity-related costs approximately £ 2.5 billions per years. Environmental damage that one of the major factors recognizes lower the people attraction to doing outdoor activity. The natural attraction of wild-life area and green space will reduce stress, improving mental health, improving productivity of worker and reducing crimes.

As the chemical coagulants which was found naturally in earth like other chemical the reserve comes to exhaustion. Those chemical also involved in other industrials usage and the problem of material shortage is becoming a bottleneck for the production of chemical coagulants. Then, those chemicals dwindling and perhaps would exhaust someday as other basic material that belonging to non-renewable source. Therefore, it becomes our responsibility as human to find ways to reduce dependence on this source with to search of sustainable technologies.

4.0 *M. oleifera* as Potential Natural Coagulant

Then, now a day, the natural product had been considered as potential coagulants in water treatment especially plant extracts around emerging countries compared to inorganic coagulant where it is believe sometimes more difficult to access (Fatombi *et al.*, 2013). There are a lot of example of plant extract had been reveal possess natural coagulant such as extract from *Cassia alata*, *Opuntia cactaceae*, *Calotropis procera* and *M. oleifera*. Those plants had been reported possess macromolecules or known as secondary metabolite that have coagulation properties that may consider a cost effective water treatment with sustainable development (Fatombi *et al.*, 2013).

The polysaccharides and proteins of various seeds have coagulant properties. The naturally active coagulant occurring in *M. oleifera* was evaluated had compatible performance against the chemical coagulant alum. Previous study mentioned the active compound which responsible on coagulation or known as coagulant agents in wastewater treatment as a cationic protein of 6.5 kDa molecular weight with a 10–11 isoelectric point (Stubbs *et al.*, 1986; Zarate *et al.*, 2008). Those authors also referred that this lectin had coagulant activity and was a natural coagulant for contaminants in water, reducing turbidity and bacterial proliferation.

The biotechnology technique to produce plant secondary metabolites has extensively discovered by many researchers now a day due to high production, efficiently control environment condition, reduce labour cost instead of planted the whole plant, space saving and produce single and high quality product compare to harvest it at field planting. Metabolic engineering has been defined as modifications of metabolic networks in living cells to synthesis desirable chemicals with higher yields and productivity employing DNA technology (Jordan and Goldstein, 1994). In this manner, the microorganism host must be compatible to bring plant genetic code and also successfully expressed the functional targeted protein. There are two types of microorganism hosts that can be used in the gene cloning which are prokaryotic and eukaryotic. In order to produce a protein in bacterial cloning systems, prokaryotic host cannot get the similar post transcriptional and post translational modifications because the prokaryotic and eukaryotic systems work in different manner on the protein modifications.

M. oleifera pods recognized as inexpensive and effective sorbent for the water treatment, beside it is known as non-toxic natural organic polymer. Production coagulant compound of *M. oleifera* via microorganism believed had more sustainable technology compare harvested the pod of the plant at the field.

5.0 Genetically Modified Organism Assessment

P. pastoris had been brought a Biosafety Level 1 (BL-1) designation, and complies with the Organization for Economic Development (OECD) criteria for Good Industrial Large Scale Practice. That designation showed that, *P. pastoris* has been used for the production of over 300 recombinant proteins since the mid-1980's (Codex Alimentarius, 2003). Many human genes have been expressed in *P. pastoris* for pharmaceutical use (Cregg *et al.*, 2000). Additionally, the progenitor strain for SMD 1 168, *P. pastoris* GS115, that has an intact PEP4 gene, has been approved by the FDA as a source of protein for use in broiler feed in concentration up to 10% of the total feed (Gao *et al.*, 2013).

Several questions regarding to the introduced DNA of the genetically modified production microorganism had been ask by approval community on that issues. The first question asks if the expressed enzyme product, which is encoded by the introduced DNA, has a safe history of use (US Food and Drug Administration, 2006). The related answer had been published that showed *M. oleifera* seed content high nutrition value for human consumption which young unripe pods are cooked with curries as a preventive against intestinal worms (Flora and Pachauri, 2011). Then, in the case of *M. oleifera* lectin gene that will introduced from *P. pastoris* that possessed 6.5 kDa as described by (Gassenschmidt *et al.*, 1995) showed no toxicity to human consumption (Araújo *et al.*,

2013; Al-Anizi *et al.*, 2014). As is known, *P. pastoris* is well used as a highly successful system for the production of a variety of heterologous proteins in both laboratory and industry (Cregg *et al.*, 2000).

6.0 *M. oleifera* and Prepossessing Facts

Study on *M. oleifera* covered whole plant part but the most extensively part was seed/leaf due to it possessed coagulant properties. Recent study proof that leaf of *M. oleifera* possesses medical beneficial on human due to huge nutrient content that predicted by native consumer that exposed to this tree previously especially western and sub-Himalayan tracts, India, Pakistan, Asia Minor and Africa. Table 1 shows related study under the keyword *M. oleifera* that had been published on ISI web of knowledge in 2014. The leaves of *M. oleifera* is the most famous part that had been extensively studied by researcher recently and there focus on various therapeutic effects of *M. oleifera* like antioxidant effects, anticancer, anti-inflammatory, antidiabetic and antimicrobial. The anticancer properties in soluble cold *M. oleifera* leaves using distilled water extract (4 degrees C; concentration, 300 μ g/mL) greatly prevent tumor cell growth, promote apoptosis as well as lowered the level of internal reactive oxygen species (ROS) in human lung cancer cells and other several types of cancer cells (Jung, 2014). Several attempted had been made to improve the function of *M. oleifera* leaves that give potential source of protein. It yields high crude protein, about 193–264 g/kg dry mass and true protein (Jelali and Ben Salem, 2014).

While the seed of *M. oleifera* that possess coagulation properties stand at second extensively study which shows 26 articles published on impact factor journal on 2014. In order to reduce heavy metal pollution for save drinking water, sort of study on removal of lead (Pb) and Chromium (Cr) load was suggested that naturally occurring amino acids might be lectin (protein) increased the metal binding (Basra *et al.*, 2014; Meneghel *et al.*, 2014).

On 2014, only three articles that deeply study until molecular level on each article from seedling, seed and leaves. Those studies reveal important gene regulation on certain toward improving plant development. Using modern molecular tool known as real-time PCR making quantify the gene expression under biotic and abiotic agents (Jabeen *et al.*, 2014). That study might be indirectly explain about *M. oleifera* can resist to dry state (Rivas *et al.*, 2013). The genetic diversity reveal that *M. oleifera* was origin from north India and now distribute worldwide (Ganesan *et al.*, 2014). Those authors also suggest the way in order to Improve the oil content on seed which have high impact for commercialization using breeding programme from the genetic diversity data.

Table 1: *M. oleifera* Research That Had Been Published On Isi Web Of Knowledge In 2014

Part of <i>M. oleifera</i>	Number of articles published in 2014	Scope of study
Seed	26	Medical, human nutrition, water purification, genetic, chemical constituent
Flowers	1	Antimicrobial
Root	2	Medical
Stem bark	2	Medical
Leaves	35	Medical, human nutrition, agricultural, genetic, antimicrobial, chemical constituent
Pod (fruit skin)	1	Antimicrobial
Biomass (whole plant)	4	Biofuel
Leaves, flowers and whole pod	5	Medical, chemical constituent
Seedling	3	Chemical constituents, genetic

7.0 Conclusions

A unique and potential lectin in *M. oleifera* seed is demands much on water treatment process that promises environmental friendly and most importantly not harmful to human consumption. Various ecosystems especially source of water worldwide need more intention due to regressively increasing of urban development. Genetically modified lectin is an advanced technology that ensures higher coagulation performance in term of reducing various waste parameters for water treatment process requirement. A critical evaluation of *M. oleifera* usage as a sustainable material for water. Turbidity, metal ions, organic, and biological species from water treatment as essential parameter that can be successfully reduce by *M. oleifera* lectin in order to provide human and ecosystem friendly water. In this review reveals that this biomaterial as known as natural and genetically modified *M. oleifera* lectin is capable of removing pollutants even at lower doses, which makes its application economical. As its sludge is not hazardous, there will be no need to develop any eco-friendly waste management method.

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