

## Science Last Fortnight

### Removing Dyes Disinfecting water

To safely reuse wastewater, first pollutants are removed and then water is disinfected. To remove pollutants, plant-based biomass and nanomaterials are widely explored due to their high adsorption efficiency. Disinfection, using chlorinated chemicals, can form carcinogenic by-products. Can this also be overcome by plant-based products?

Recently, Bhandari, National Chemical Laboratory, Pune in collaboration with Bhattacharya, Central University of Jharkhand, Ranchi came up with bio-nanocomposites to remove dyes and disinfect water. The team used a base of iron oxide magnetic nanoparticles with the paste of unripe pods of amaltas, *Cassia fistula*, as biomass. They chemically synthesised the iron oxide nanoparticles.

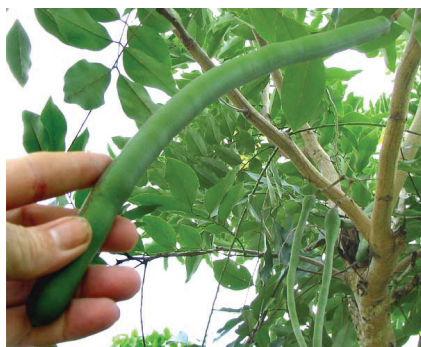


Image: Jim Conrad, via Wikimedia Commons

To make the bio-nanocomposite disinfectant, the team used a paste of aloe vera, which is antibacterial and antioxidant. They digested the mixture and calcinated it. The researchers observed well-developed pores in the nanocomposites produced.

The team treated the nanocomposites with two dye solutions and estimated the per cent reduction, using a spectrophotometer. To increase dye adsorption, they also used ultrasound to increase cavitation. They observed that the nanoparticle composite removed Congo Red by 54% and Methyl Blue by 38%. Under ultrasound, dye removal increased to 80–100%.

Then they used octanol instead of aloe vera and compared dye removal.

Due to high porosity, both nanocomposites showed more than 60% Congo Red dye reduction. For Methyl Blue, the aloe vera modified nanocomposites showed 84% reduction and the octanol modified nanocomposite showed only 68%.

'The difference may be due to the ability of the biomass derived nanocomposite to bind to pollutant species more strongly. Significantly, ultrasound cavitation did not affect dye adsorption in the octanol modified nanocomposite', says Vinay Bhandari, NCL, Pune.

'The aloe vera nanocomposites could achieve a ninety per cent reduction in the bacterial count of *E. coli* cells in an hour while the octanol modified nanocomposite showed 96% bacterial cell reduction', says Arnab Bhattacharya, Central University of Jharkhand. The difference in charge between the nanocomposites and the adsorbed bacterial cells may be the mechanism of action. However, the aloe vera nanocomposites reduced the cost tenfold.

Dye removal and disinfection can be achieved with low amounts of nanoparticles in bio-nanoconjugates.

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### Monitoring Cortisol Sensing in saliva

Cortisol, a steroid hormone, helps control blood sugar levels, regulate metabolism, reduce inflammation, and it assists in memory formulation. It also helps control blood pressure. Monitoring cortisol can provide clues about reproductive cycles for artificial insemination and pregnancy planning as well as for the early diagnosis of hormonal disorders. Enzyme immunoassay, the method routinely used, typically costs about Rs 3500–9500, depending on whether blood, urine or saliva is tested and the number of times tested per day.

Recently, scientists from the CSIR-Indian Institute of Chemical Technology, Hyderabad, the CSIR-Central Electrochemical Research Institute, Karaikudi, the California State University and the Florida International

University reported a new sensor for analysing salivary cortisol. The scientists used metalloporphyrins and multi-walled carbon nanotube nanocomposites to create the sensor. Metalloporphyrins are a crucial part of organic catalysts, widely distributed in nature. They act as enzyme mimics, capable of catalysing cortisol hydroxylation.

The scientists tested three different metal dependent porphyrins – copper, nickel and ruthenium – for sensitivity to cortisol. They did computational modelling studies and binding energy calculations to find out a suitable metalloporphyrin for cortisol detection. Copper porphyrin turned out to be the best.

The scientists then fabricated the electrochemical sensor using screen-printed multi-walled carbon nanotube electrodes modified by metalloporphyrins. They tested the sensor on various concentrations of cortisol. And found that the detection limit is as low as 50 femtomolars, or one quadrillionth of a mole!

The scientists used the screen printed cortisol sensor to detect variations in salivary cortisol levels in young adult women. They compared the results with the commercial enzyme and found it accurate.

'The system does not require any specialised antibodies or enzyme labels for cortisol detection. So the cost of the cortisol assay comes down a hundred fold', says Pandiaraj Manickam, CSIR-CECRI, Karaikudi.

This low-cost sensor, capable of detecting minor variations in cortisol levels, is poised to change the scenario of mass monitoring of health conditions even in a highly populated country like India.

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### West Nile Fever Potential vaccine

The West Nile virus, transmitted by mosquitoes, causes a neurological disease – West Nile fever – in humans and animals. Although an effective veterinary vaccine against the virus is

available, no human vaccine has been approved for commercial use. A recombinant protein of the West Nile viral envelope was shown to induce protective immunity by producing neutralising antibodies against the virus. However, the production of the protein was done only at laboratory scale. For vaccination purposes, bulk amounts are required.

Is it possible to produce the protein at industrial scale while retaining the conformation of the neutralising epitopes of the protein? Nagesh K. Tripathi and co-workers from the Defence Research Development and Establishment, Gwalior decided to tackle the problem. They cloned the gene for viral envelope domain III protein in an expression vector and produced it in *E. coli* cells. The team optimised growth conditions for large-scale production of the viral envelope protein using batch and fed-batch cultures of *E. coli* in a bioreactor. They could get a protein yield of 5.81 mg/l.

However, the process of protein isolation from these cells involves urea. This chemical denatures the protein, and affects the functionality of the protein. To address this, the team used chromatography techniques to isolate the protein in its pure form while ensuring proper refolding.

The next challenge was to check if the purified protein triggers the production of antibodies, which can neutralise the West Nile virus. They used the Western blotting technique to show that the protein was immunoreactive. They also showed that rabbits immunised with this protein could produce the specific antibodies.

Yet, another important question remained to be answered: can the antibodies against the purified and refolded protein neutralise the virus? The team used the Plaque Reduction Neutralisation Test, a standard assay, to answer this question. They showed that the antibodies produced against the protein could neutralise virus replication *in vitro*.

The team established that the West Nile virus envelope domain III protein was immuno-reactive and can neutralise the virus. Further animal stud-

ies now need to be done to show that the protein can protect against the virus infection, before clinical trials in humans.

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### **Pinning Down Parkinson's** *Clinching the genetic environment*

About six million suffer from Parkinson's disease. The challenge is compounded by an aging population at risk for the disease. Despite significant research, we have no clear picture of the molecular origin of the disease. What we know is that, in Parkinson's, addition of a methyl group to DNA plays a role. To find patterns of methylation in DNA data, various computing methods have been used. The K-Nearest Neighbour classification using the sequential forward algorithm and logistic regression were found to be partially useful, but not very dependable.

Last fortnight, Aishwarya Kakade, Baby Kumari and Pankaj Singh Dholaniya, from the University of Hyderabad, came up with an algorithm to understand methylation. The team had at their disposal data samples from DiseaseMeth, an online repository of human methylation studies. The researchers combined statistical tools to link variables in the data.

The researchers then used the links to construct an algorithm to detect markers associated with Parkinson's. To improve the model's efficacy, they used Random Forest, a machine learning algorithm, for evaluating features selected through logistic regression. Using different samples, the researchers pinned certain features, identified from DNA methylation data, to genes associated with Parkinson's disease.

The Random Forest algorithm offers the lowest percentage error in identifying the condition. The method identified some genes that lead to symptoms of Parkinson's, including a gene that codes for the enzyme responsible for the degradation of dopamine, another that interferes with dopamine synthesis and a third that is involved in intracellular transport.

With the alarming rate at which Parkinson's disease strikes, every bit

of understanding about its underpinnings is important. Besides bringing us closer to the understanding of the molecular mechanisms behind Parkinson's, the techniques pave the way for finding methylation patterns in other degenerative conditions.

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### **Targeted Drug Delivery** *Moringa gum carrier*

Orally administered drugs degrade substantially in the upper gastrointestinal tract before reaching the colon. This reduces the efficacy of the drug. And increasing drug dosage to achieve therapeutic levels in the colon produces side effects.

Last fortnight, Baljit Singh and Ajay Kumar from the Himachal Pradesh University, Shimla came up with a solution. They selected levofloxacin, a broad spectrum antibiotic used for colon targeted treatments, as model. As drug carrier, they used a stem gum extract of moringa, a medicinal tree. Enzymes of the digestive tract cannot digest the polysaccharide gum but microbes inhabiting the colon can. This helps retard drug release in the stomach and small intestine. Then, it is released in the colon by the action of gut microbes.



Image: J. M. Garg via Wikimedia Commons

For loading the drug onto the polymer, the team made a homogeneous mixture of the gum with acrylamide, a hydrophilic monomer, stirring it at high speed. This homogenised mixture was treated with gamma radiation to induce copolymerisation. The researchers put the polymer in a solution of the drug for 24 hours. The dried polymer was now loaded with the drug.

The retention of the drug in the colon depends on the polymer-drug

matrix adhering to intestinal mucus. The researchers used goat large intestine as model, and a texture analyser to test the mucoadhesive property. They found that grafting acrylamide onto moringa gum increased the mucoadhesive property.

'The average surface roughness of the matrix was about twenty nanometres. Surface roughness increases the mucoadhesion of the polymer matrix on the mucosal membrane', says Ajay Kumar, Himachal Pradesh University.

The bioavailability of the drug depends on its retention period in the colon and the rate of drug release from the drug carrier. The researchers checked the kinetics of drug release and found faster initial release without burst effect – desirable kinetics for colon-specific treatment.

'The copolymer matrix had a porous network structure, which controls swelling and drug release', says Baljit Singh.

But is the drug carrier safe? The researchers performed thrombogenicity and haemolysis tests. They found that the drug carrier was non-thrombogenic and had a low haemolytic index – safe for biomedical application.

The moringa gum–acrylamide carrier matrix delivers levofloxacin effectively in the colon. 'We can also control the rate of drug release in the colon by customising copolymerisation', says Baljit Singh.

'Moringa gum is not only safe but is also antioxidant', adds Ajay Kumar, his colleague. The drug-carrier matrix showed high antioxidant activity as indicated by tests for free radical scavenging.

Acrylamide, however, is a mild nerve irritant. A safer alternative needs to be explored.

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### Countering Cotton Necrosis *Bacteria versus virus*

The tobacco streak virus attacks a variety of crops, including cotton. 63% yield loss, due to cotton necrosis, has been reported in India. This disease is usually transmitted by vectors. The usual disease management

practice involves killing vectors with chemicals. This, however, also eliminates non-target beneficial organisms.

Another approach is to improve the resistance of the plants against the virus. Soil bacteria are known to synthesise bio-agents that induce a defence mechanism in plants. This has been exploited for managing plant fungal diseases. Researchers from the Tamil Nadu Agricultural University, Coimbatore asked if soil bacteria can demonstrate the same potential against tobacco streak virus in cotton.

The researchers isolated rhizospheric and endophytic bacteria from both tobacco streak virus infected and symptomless cotton plants. They then enumerated the microorganisms in soil samples. They isolated and characterised the bacteria using the 16S rRNA gene. The phylogenetic tree showed a variety of bacillus species. The team observed that healthy plants had more microbial diversity than infected plants. Do some of these bacteria confer resistance against the virus?



Cotton necrosis can reduce the yield by more than 60% in such healthy fields.  
Image: Sarangib via Pixabay

The team chose cowpea as indicator plant. They inoculated the isolated bacillus strains and tobacco streak virus, in a buttermilk base, on cowpea plants. Buttermilk, as inoculum base, exhibits antibacterial and antiviral activity.

The team also observed that buttermilk alone reduced the viral titre. But adding bacterial formulations to buttermilk enhanced the antiviral effect and promoted plant growth.

The plants were inoculated with bacteria and virus simultaneously, with a gap of 24 hours before and after. Simultaneous treatment showed better antiviral effects.

Plants treated with *B. amyloliquefaciens* and *B. licheniformis* had just two or three lesions per leaf. In the control, there were about twenty-five lesions per square centimetre. Enzyme-linked immuno assay of leaf samples treated with *B. amyloliquefaciens* showed four times less virus titre than seen in the control.

The scientists then conducted field experiments with a high yielding cotton hybrid. When *B. amyloliquefaciens* was applied, disease incidence was 21.67% while untreated control showed 45% incidence. Plants treated with the bacteria also showed better growth than control.

The study brings hope that the tobacco streak virus, which affects many crops, may soon be controlled with bacteria.

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### Reducing Chemical Fertilisers *Biopriming seeds*

In the last few decades, it has become clear that it is not sustainable to use chemical fertilisers in agriculture. However, chemical fertilisers are still being used to keep up with the demand for agricultural products. The amount of chemical fertilisers used can be reduced by pre-treating seeds with beneficial microorganisms. But, which organisms to use and by how much will they reduce the need for chemicals?

Last fortnight, scientists from the BHU, the ICAR-Indian Agricultural Research Institute, New Delhi and the Vivekananda Parvatiya Krishi Anusandhan Sansthan, Uttarakhand reported their experiments with three microbes *Trichoderma viride*, a phosphate solubiliser, *Frateuria aurentia*, a potassium solubiliser, and *Glomus intraradices*, a phosphorus solubiliser. They tested combinations on seeds of baby corn.

The team surface sterilised baby corn seeds and treated them with each of the three microbes and in combinations. They sowed two seeds per pot. As control, they grew baby corn seeds without microbes and chemical fertilisers.

In a second set, they grew the seeds with 100% of the recommended dose



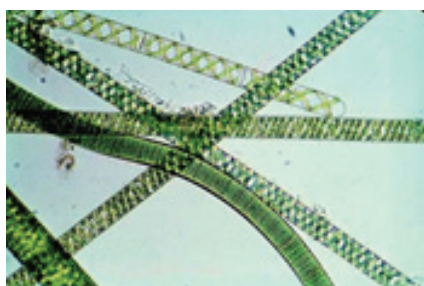
of chemical fertilisers. In a third set, they grew the seeds with each microbe in combination with 75% of the recommended dose of chemical fertilisers. All pots had soil with low nitrogen, phosphorus and potassium content.

The team harvested the plants after 20, 40 and 60 days. The researchers observed higher leaf area, increased root length, fresh and dried weight and chlorophyll content in corn seeds bioprimed by *Trichoderma viride* and *Glomus intraradices* with a reduced dose of chemical fertilisers than in the control and with other combinations of microbes.

Yadav and team advise biopriming with *Trichoderma viride* and *Glomus intraradices* to reduce the dose of fertiliser by 25% for sustainable productivity of baby corn.

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### Spirulina Biodiesel



*Spirulina platensis* filaments  
Image: M. Elser, via Microbe Wiki

Spirulina, a blue-green microalga, is a fast growing and lipid carrying single-celled organism. They have long been cultivated as nutritional supplement and processes of cultivation are now optimised. Genetic-manipulation can easily enhance lipid and fat accumulation for biodiesel production. This biodiesel could be blended with petroleum–diesel without reducing engine efficiency. But, what is the best blend of biodiesel for an internal combustion engine?

Last fortnight, Upendra Rajak and Tikendra Nath Verma from the National Institute of Technology, Manipur reported investigating the problem. The team compared three options: only petroleum diesel; a

blend of 80% petro-diesel with 20% spirulina, and 100% spirulina bio-diesel. Then, they checked the effects on performance, combustion and exhaust emission rate at different engine speeds and 100% load condition.

They found that the engine performed better with a blend of 20% biodiesel and 80% petro-diesel at an engine speed of 1500 rpm than with diesel alone. This proportion gives 20% of the benefit of 100% use.

Then, the team computed the efficiency level of the engine by evaluating the physicochemical properties of the steady-state condition of the engine. They tested engine performance for thermal competence, exhaust gas temperature, ignition delay, rate of heat release and specific fuel consumption. They also measured the emission rate for soot formation, particulate matter, carbon-dioxide, oxides of nitrogen and smoke released. They compared the data with petro-diesel standards.

The 20% biodiesel blend had less emission and the engine performed better. They tested the 20% biodiesel blend as per standard specifications. And, the diesel engine performed favourably.

Currently, India imports 82% of its fuel needs. To reduce imports, we need to switch to alternative sources of biofuel. Given the renewable, biodegradable, eco-friendly and cost-effective properties of microalgae, spirulina could serve as viable substitute, reducing the load on conventional fuel for compression ignition engines. 'And diesel fuel could be replaced with 20% biofuel for the diesel engine', says T. N. Verma, National Institute of Technology, Manipur.

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### Detecting Melamine

#### *Cadmium selenide nano-sensor*

Melamine is a white compound, used to inflate estimated protein levels in milk powder, wheat gluten and even chicken feed. This is a health hazard. So scientists have been looking for

a specific and sensitive method to detect melamine.



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Last fortnight, scientists from the CSIR-CSIO, Chandigarh and the AcSIR, New Delhi synthesised an efficient nano-sensor for melamine. Melamine is positively charged. So, the scientists prepared a negatively charged quantum dot based nano-sensor to induce selectivity for melamine.

The sensor is based on changes in the nanosensor's fluorescence in the presence of melamine. The intensity of fluorescence decreased with the increase in melamine content. The researchers attribute the quenching in the fluorescence to the bonding between the melamine and the quantum dots.

The team also tested the sensitivity and selectivity of the quantum dots towards other common constituents of milk such as amino acids, proteins and sugars and found that these compounds did not interfere with the results. 'Miniaturisation of the technique as fluorescent reader will make it even simpler', says Suman Singh, CSIO, Chandigarh.

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