Science Last Fortnight

Cyanide-Degrading Bacteria

Containing water contamination

Cyanides are used in pesticides, fumigants, electroplating and mining. But they are lethal. Cyanides prevent cells from using oxygen and can cause death. So, degrading cyanide from industrial effluents is a challenge.

Most physical and chemical methods available for remediating cyanide contamination lead to hazardous by-products. So, biological degradation using microbes is safer. And inexpensive. Using microorganisms that grow naturally in cyanide-contaminated substrates is a simple trick. But what are the best sources for such microbes?

Recently, Manikandan from the Thiruvalluvar University, Vellore and collaborators from South Africa used cyanide-degrading bacteria from different cyanide-contaminated sources. They collected mining and thiocyanate-containing wastewater samples and spread them on agar plates. Once bacterial colonies bloomed, they selected a *Pseudomonas aeruginosa* strain which grew well under cyanide for further testing.

The researchers compared the bacteria's free cyanide-degrading efficiency. Cyanide-degrading bacteria from thiocyanate wastewater showed higher degrading capacity than isolates from mining wastewater.

The team used a spectrometer to measure bacterial density and free cyanide. And found that bacterial density increased while free cyanide concentration decreased. X-ray diffraction analyses showed that the reduction in free cyanide concentration was linked with potassium chloride's disappearance.

The microorganism is equipped with enzymes that can break the notoriously strong bond between cyanide's carbon and nitrogen atoms. It uses the atoms as food and the by-products are safe. However, *P. aeruginosa* can be pathogenic, especially to those whose immunity is compromised. So there is a need to evaluate the efficiency of enzymes involved in degrading cyanide under field conditions, say the scientists.

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Trichoderma from Wastewater

Bioremediating chromium

Large coastlines and two annual monsoons make most of India quite humid. Humidity and high temperatures lead to the corrosion of metal surfaces. To overcome the problem, a thin layer of chromium can help. This has led to the development of electroplating industries. However, electroplating industries release wastewater containing chromium. Hexavalent chromium is highly toxic to living organisms, more so than trivalent chromium.

Conventional technologies to remove chromium contamination are not efficient. So scientists are searching for microbes that can minimize chromium contamination.

Wastewater from electroplating industries is the most probable site to find such organisms. So Vinay Kumar and S. K. Dwivedi from the Babasaheb Bhimarao Ambedkar University, Lucknow took wastewater samples from an electroplating industrial area at Ballabhgarh, Faridabad. They cultured fungi from the wastewater and tested their ability to remediate chromium contamination. Last fortnight they reported isolating a fungus, *Trichoderma lixii*, which has a high level of heavy metal tolerance.

They exposed the fungi to different concentrations of heavy metals and found that the fungus had strong tolerance to chromium. It could survive chromium concentrations of up to 1000 milligrams per litre, beyond which its ability to grow ceased, perhaps due to oxidative stress. At 50 milligrams per litre, the fungus could reduce more than 99% of the chromium – higher efficiency than reported earlier using other organisms. Though the *T. lixii* strain was tolerant of a pH range from 5.0 to 8.5 and a temperature range of 20°C to 35°C, the optimum performance of the fungus was at pH 6.5 and 28°C.

The fungus could be used to remediate other heavy metals such as arsenic, nickel, zinc and copper. But the fungal strain had poor tolerance towards lead and cadmium.

Using a scanning electron microscope to assess cell structure, the researchers found that the fungus accumulates chromium by adsorption. They confirmed the accumulation of chromium in the cell wall of the fungus using high throughput spectroscopic techniques.

Can the wastewater treated by the fungus be used for agriculture? The researchers checked. They grew mung and chickpea using the treated wastewater. Hundred per cent germination and good growth – better than seen with distilled water – showed that it is safe.

Thus we now have one more candidate, *T. lixii*, the fungus isolated from electroplating, to remediate chromium contaminated water.

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Preventing Pollination

Sprinkler irrigation in pumpkin

Pumpkin is usually sown after the monsoon. But the crop requires greater soil moisture to produce flowers and fruits. Traditionally, farmers use water-filled earthen pots to irrigate pumpkin plants in the morning when anthesis begins. This activity requires labour. So when water pumps became available, many tried pumping water into the fields. But flooding harms the trailers instead of just moistening the soil. So when sprinkler irrigation became available, farmers started using the technology to maintain soil moisture and to save water.

Last year, Pooja and Aneha, scholars working with P. A. Sinu at the Central University of Kerala, noticed that overhead sprinklers tend to fill the flowers with water. Because of their cup shape, the flowers retain this water and the reproductive parts get completely immersed. The researchers hypothesised that this might reduce the chances of pollination.

In the next season, Sinu and his team were ready with an experimental

design to test the hypothesis. The team selected two pumpkin fields for the test. Pumpkin is a monoecious plant and produces male and female flowers separately, in the same plant. So it depends on bees and ants to carry pollen to female flowers.

Over 6 months, the team observed 40 male flowers: 20 each of waterless and water-filled flowers. Pumpkin usually has fewer female flowers. So the researchers could observe only 31 female flowers, 16 water-filled and 15 without water. They recorded pollinator visits and time spent on male and female flowers in waterless and water-filled condition every 15 minutes. And they also recorded fruit set in the female flowers.

Image: P. A. Sinu, Central University of Kerala

The team found that pollinators are equally likely to visit both water-filled and empty flowers. But time spent by pollinators on selected flowers is drastically reduced in water-filled condition. Once they are filled with water, the flowers stay as is until they close. This prevents the pollinators from entering the flower's reproductive parts and the interaction between pollinators and flowers is interrupted. Eventually, the water-filled flowers did not develop fruits. In waterless condition, however, 40 per cent of selected female flowers developed fruits.

'Based on our results, we suggest that farmers switch from sprinkler irrigation to drip irrigation during the flowering phase to mitigate yield loss in pumpkin cultivation', says Sinu Palatty, Central University of Kerala.

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Treating Leukemia

Sensing drug resistance

Leukemia, the uncontrolled cell proliferation of myeloid and lymphoid cells from bone marrow, leads to abnormalities in blood, and can be treated with doxorubicin. But there are cases of remission. The cancer cells start pumping out doxorubicin. So doxorubicin cannot reach the target site in the cells to stop the enzyme that causes DNA damage.

Scientists have identified a glycoprotein embedded in the plasma membrane, the P-glycoprotein, which transports doxorubicin from the inside to the outside of the cell. Researchers at the Jamia Millia Islamia University and the Delhi University have now developed a sensor that can detect this cancer drug resistance marker.

The trick is seemingly simple: develop antibodies against P-glycoprotein. But here is when it becomes complex. How do you know whether the antibody–antigen reaction has taken place?

The researchers thought of multiwalled carbon nanotubes. Attach the antibodies to carbon nanotubes and, if the antigen–antibody reaction takes place due to changes in the electronic configuration, the electrical conductivity of the nanotubes will change.

Now the problem is to fix the nanotubes on a flexible substrate made of PET, so that it can be used for testing.

Last fortnight, the researchers reported fabricating a highly sensitive and advanced flexible electrochemical immunosensor for chronic myeloid leukemia detection. The process of producing such sensors is simple, in theory. But, in practice, it involves dealing with non-visible components. Cajoling P-glycoprotein macromolecules to attach to multiwalled carbon nanotubes, making sure that all the nanotubes are vertically aligned and to attach the immunosensor to the only visible component – a small strip of PET – are all complicated processes.

But the product is not too complicated for application in clinical settings. If there are ten cancer cells per millilitre of blood, the device can detect and inform the physician of remission of cancer and the need to change treatment.

However, to get there, the entrepreneurial spirit has to collaborate with the scientific spirit. Biomedical entrepreneurs, are you listening?

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Thermally Stable Surfactant

From marine bacteria

Try removing oil from your hands using water. It does not work because oil and water do not mix. But if you were to use soap, oil will readily come off. Soap reduces the surface tension between water and oil, thus allowing the two to mix.

Soap is the most common example of a surfactant. Various other synthetic surfactants are produced commercially for use as detergents, for making food emulsions, and in pharmaceuticals. But using synthetic surfactants gives rise to the waste management problem. So scientists turned to biosurfactants that are biodegradable. However, finding a stable and cheap biosurfactant is a huge challenge.

Last fortnight, a team from the Nation Chemical Laboratory (NCL), Pune and Pune University, reported isolating a thermostable biosurfactant with excellent surfactant properties from marine bacteria.

The team collected sediments from the intertidal region of mangroves at Chorao Island, Goa and cultured the microorganisms. They screened the forty colonies isolated, and screened them for biosurfactant production, using the hemolysis test: if biosurfactants are produced then streaking the bacteria on agar plates containing sheep blood will lead to lysis of the blood cells. A strain belonging to *Bacillus velezensis* showed the best activity.

The team removed all the bacterial strain's cells and used the biosurfactant available in the supernatant for testing its efficacy. Results from the oil displacement and micelle formation assays and surface tension activity encouraged the scientists to take the next step.

They purified the biosurfactants and identified two active compounds. On testing, one of the compounds turned out to be toxic to the micro-crustacean, *Artemia salina*. But the other compound was not toxic and was easily biodegradable.

The biosurfactants were found to be stable for up to 160°C and over a pH range of 6–12.

The biosurfactant could reduce the surface tension of water by almost

half. In agriculture, these biosurfactants can be used to increase the wettability of plant surfaces, reducing the use of pesticides which do not spread very easily on plants. The biosurfactants would also be useful in waste-management, for cleaning lubricants and used oil, absorbed in soil or liquid waste sites.

Unlike the complex methods of production of other surfactants, these biosurfactants are produced in a medium of yeast extract and tap water – easy to adopt for production on an industrial scale, says Syed G. Dastager, NCL Pune.

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Wheat Flour Discs

Cook to transform shapes

Engineering food materials for predetermined shape after cooking is work under progress. Food items will have low size for transportation and storage. But get into the desired shape when the end user cooks the product.

Recently, Vidhi Gupta and R. Mahendran from the Indian Institute of Food Processing Technology, Thanjavur reported a technique to get complex 3D shapes by cooking planar 2D shapes made from wheat flour.

They heated a wheat flour suspension in distilled water, constantly stirring it till it reached 90°C. Using a micropipette, the gelatinized suspension was dripped slowly over food grade substrate. The disc, when dried, separates from the substrate. Since water from the top surfaces evaporates faster, it becomes denser than the lower part. The lower part will be more porous. This stress gradient leads to change in shape when the disc is boiled in water.

Now the problem was to programme the shape change by creating constraints. The researchers used ethyl cellulose for this. A solution of ethyl cellulose is applied on specific parts of the disc and allowed to evaporate. The pattern and coverage of the ethyl cellulose film that is left behind determines the 3D shape, when the disc is cooked.

The researchers studied bending angle, height and end-to-end curve distance by varying ethyl cellulose concentration. They found that the xerogel with a circle of ethyl cellulose near the perimeter of the disc was transformed into a saddle shape. Three arcs of ethyl cellulose gave an even more complex 3D shape.

Wheat xerodiscs are inexpensive to manufacture. The 2D discs reduce packaging, storage and shipping costs. And the shape transformation while cooking can be controlled by the constraints programmed during manufacturing, says R. Mahendran, Indian Institute of Food Processing Technology, Thanjavur.

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Plywood Industries

Strategy for zero discharge

Plywood – a thin veneer of wood glued on other wood products – is much cheaper than flat planks of timber of the same dimensions, but can serve the same purposes. So it has become ubiquitous in modern life. But wastewater from the plywood industry contains glue, resins and preservatives, contaminating the environment.

Last fortnight, researchers from the Guru Jambheshwar University of Science and Technology and the Bhagat Phool Singh Mahila Viswavidyalaya, Haryana came up with a strategy to achieve zero liquid discharge: lime.

Lime and limestone can help precipitate and aggregate suspended impurities, quickly and efficiently. But what is the optimum amount of lime needed to make the wastewater suitable for reuse?

Rajesh Kumar Lohchab's Ph D scholars went to a plywood factory in Nainital District. The factory pumps out 130 litres of wastewater from its melamine urea formaldehyde resin unit and 310 litres from the phenol formaldehyde resin unit. Both discharges contained large amounts of woody particles. The researchers collected the wastewater after letting it sediment and tested the effect of various concentrations of lime, ranging from half a gram to two and a half grams per litre of wastewater.

They found that an optimum dose of one and a half grams of lime per litre was sufficient to treat liquid wastewater and remove suspended solids. They analysed the total dissolved, suspended and volatile solids as well as the total nitrogen, phenols and chemical oxygen demand using standard methods. Lime treatment could remove 40% of the waste in the water. It could remove both forms of resins.

They used this treated wastewater for making resin. The adhesions of piles as well as the glue shear, static bending and nail holding strengths of the plywood were assessed by the team. They found the characteristics of the resin within prescribed limits of plywood manufacturing. And the mechanical and physical properties of the plywood were greater than standard prescribed values.

Lime and limestone are cost-effective, easily available, and environment-friendly. And using lime-treated wastewater for preparing resin can help achieve zero liquid discharge in the plywood industry, says Rajesh Kumar Lohchab, Guru Jambheshwar University of Science and Technology, Haryana.

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Alternative Lubricants

Mustard and coconut oil blends

Mineral oils are widely used as machine lubricants. They cannot be degraded completely and cause environmental problems. And, due to a shortage of supply, their costs are high. Vegetable oils are better and cheaper alternatives. But the cold flow property and the ability to reduce friction are different in different vegetable oils. So the approach is to blend them to get the optimum lubricant properties.

Recently, Sajeeb and Rajendrakumar from the NIT Calicut evaluated different blends of coconut oil and mustard oil as lubricants. Coconut has the lowest coefficient of friction, but its cold flow property is poor. Mustard oil is useful to reduce wear and tear due to higher amounts of oleic fatty acids and has better cold flow properties than coconut oil. What is the optimum ratio for blending the two oils into a more perfect lubricant?

The researchers prepared five different blends – 10% to 50% of mustard oil in coconut oil – and compared the properties with mineral oil SAE20W40. The blend with 50% mustard oil had the best properties.

The team investigated the fatty acid composition of the blends and found that the proportion of oleic acid, linoleic acid and erucic acid increases with the addition of mustard oil. In terms of the viscosity, the blends had better lubricant properties. They attributed this to the moderate amount of saturated and unsaturated fatty acids in them.

Mustard oil has more stability than coconut oil and blending improves thermal stability. The researchers found that vegetable oils have better stability than mineral oils.

The scientists found that the blends have lower saponification values. Saponification results in foaming and affects performance. The researchers thus propose that blending coconut oil and mustard oil in a 1 : 1 ratio improves the quality of the lubricant.

They also compared the costs of mineral oils and the blends and estimated savings of Rs 52.5/litre by replacing mineral oils with the blend. The team says that savings would be much higher if waste disposal of mineral oils is also considered.

When compared to mineral oil, the vegetable oil blend is environment friendly and biodegradable. Other additives may improve the properties further, say the researchers.

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Sustainable Manufacturing

Managing defects

Quality management systems in manufacturing industries focus on minimizing defects in products. Minimising defects increases profitability.

Recently, industries have come under pressure to make their production process and products environment-friendly. Quality management systems are therefore taking into account this bottom line of sustainable manufacturing. But besides economic and environmental aspects, there is a third bottom line in the concept of sustainability – social aspects. Is it possible for quality management systems to align with all three aspects of sustainable manufacturing?

Ankur Goyal and Rajat Agrawal from IIT-Roorkee in collaboration with C. R. Saha from the Bharat Heavy Electrical Limited, Haridwar have now come up with an integrated framework that effectively links quality management and sustainability.

They conducted a preliminary survey with about 150 people from more than 20 different manufacturing and service industries using a single question. The majority believed that the lower the number of defects in the products, the greater the quality.

On the contrary, real life examples show that even one defect in a product could have severe economic, social and environmental impacts. This was puzzling.

So the researchers reviewed literature in the domain of sustainability and quality management – a systematic review of more than 120 research papers, reviews, conference proceedings and reports, published between 2000 and 2018. Though much literature focused on reducing the number of defects because of the economic dimension and some examined the environmental dimension, there was almost nothing regarding the negative impacts of defects in all three aspects of sustainability.

So the team developed a model where the impact of the defect is also taken into account. A small error at the design stage can have a major negative consequence whereas a small defect at the manufacturing stage has a much lower negative consequence. So instead of merely counting the number of defects, the team developed a weighted measure, based on Six Sigma principles. They modified the existing calculation of 'Defects Per Million Opportunities' to connect sustainability and quality management. Thus they developed a framework of quality management with weighted defects incorporating sustainable aspects.

But will the new framework be useful in manufacturing industries?

The team took up a case study of an electrical product manufacturing process: stress grading on the surface of a high voltage insulated component.

Through group discussions and brainstorming with all stakeholders involved in the process, first they understood the issues involved in stress grading on the product's insulated surface. Then, with the help of knowledgeable and experienced members of stress grading, they came up with the list of possible defects in stress grading with respect to sustainability. Each possible defect was given weightage. The calculations with the weights showed that the quality index, Defects Per Million Opportunities, increased in magnitude.

But shifting focus from the mere number of defects to the negative social, economic and environmental impact helped the company. They eliminated the most impactful defects by implementing the newly appropriated Six Sigma management system for sustainability.

The researchers could thus validate their proposal to include sustainability in the quality management process. However, the proposal to integrate quality management with sustainability poses a problem: revising weights whenever there is a change in product or production culture.

Such management practices can be extended to the whole manufacturing process and even to service sectors such as hospitals, say the researchers.

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