Science Last Fortnight

Capturing Carbon Dioxide
Synthetic solution

India is the third largest contributor to the greenhouse gas, carbon dioxide. Our industries are now keen on meeting emission standards and, hence, seek methods for capturing carbon dioxide and other gases.

Now, Gopal Das and team from the IIT Guwahati have devised a chemical method to capture atmospheric carbon dioxide and other pollutants. For this, they chose a tripodal molecule, Tris(2-aminoethyl)amine or tren to make three different receptors, using tris-urea/thiourea, for grasping other small anions.

'These receptors can bind to an array of negatively charged pollutants', says Santanu Kayal, IIT Guwahati.

Indeed, these receptors captured carbon dioxide. But what was the mechanism?

To investigate, the researchers used single crystal X-ray diffraction and deduced the structure and chemical interactions of the receptors with the captured pollutant. On the basis of the structures, the scientists speculate that the amine groups in the urea ring of the receptor bind to the oxygen in carbonate via multiple hydrogen bonds.

Here, the carbon dioxide anion was captivated by a charge-less receptor in a basic environment.

On the other hand, when the team acidified the conditions using hydrofluoric acid and hydrobromic acid, the iodine-containing receptor trapped even hexafluorosilicate and bromide anions on the outer side of its cavity.

Interestingly, here, the pollutant interacts with the positively charged receptor in the presence of water. Thus, by merely varying charge, the same receptor can be used to trap different types of pollutants. These tripodal receptors are specific to anionic pollutants, unlike the case with other pollutant capturing methods.

But is it practical to convert stable pollutants to their anions? Only further studies can help us capture the answers.

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From Fields to Our Bodies
Antibiotic resistant bacteria

Untreated sewage and industrial waste is dumped into rivers. And, downstream, the same water is used for irrigating fields. Micro-organisms from this polluted water are taken up by plants and, when we eat them, they enter our bodies. How worried should we be?

Recently, a team, led by P. Hariprasad, from the IIT Delhi, tried to address this concern. They examined vegetables grown using Yamuna river water. And they found around forty different kinds of endophytic bacteria inside the vegetables. Some of these are native to the plants, while others reach the plant through the water used for irrigation.

Now, we know that the Yamuna in Delhi is quite polluted. The polluted water contains high amounts of heavy metals, and other pollutants. In their struggle to survive such conditions, these bacteria have evolved to be resistant to most of the heavy metals.

More alarming was the resistance they developed to many antibiotics. The researchers found a strong correlation between heavy metal resistance and antibiotic resistance in these bacteria.

The plants do not clear out these bacteria, as they protect the plants from heavy metals and other pollutants. And they promote plant growth. However, when we eat such plants, we also consume the bacteria. Most of these bacteria are not harmful to humans. But, in bacteria, genes providing such resistance can be passed on from one strain to another with ease. So the risk of pathogenic bacteria coming in contact with these bacteria and developing antibiotic resistance is very high.

Active measures to keep a check on river pollution and farmer awareness of the problem are thus required. Let us try to curb the problem before it leads to an outbreak of antibiotic-resistant infections.

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Metarhizium Mystery

Mukund Deshpande was puzzled. His team had collected soils from different parts of Pune and Buldana in Maharashtra, to identify the most potent *Metarhizium* anisopliae fungus strain to kill agricultural insect pests. When his team tested about 60 strains from different agricultural fields, they noted that strains from custard apple fields consistently killed more than 90% of *Helicoverpa armigera*, an insect model used to test biopesticides. This was achieved with less *Metarhizium* spores than usual and in less time.

Image courtsey: Mukund Deshpande

Soils from fields with plants vulnerable to chewing insect pests – such as okra or tomato – yielded *Metarhizium* strains that killed 50% or less of the insects. Why do soils, that too from fields separated by many kilometres, house such potent fungal entomopathogens? Is it just because they are associated with custard apple that is not very prone to attacks by chewing insects?

Mukund set his doctoral student, Ejaj Pathan, at the CSIR-NCL to the task. First, proteomics to check the differences between highly virulent and not so virulent strains. And it turned out that there are many more proteins expressed in highly virulent strains than in those less so. And

some of these extra proteins were associated with insecticidal properties, pointed out existing literature.

The team was excited. So they went in for a full genomic study. And it turned out that the highly virulent strains had genes not seen in other strains. And, what is more, the extra genes are highly related to custard apple genes. So they hypothesised that the genes must have been picked up by the strain from custard apple when it was an endophyte and must have dropped to the soil along with plant debris.

But there was no earlier report on any endophytic relationship of *Metarhizium* with custard apple. So the team looked into it. Indeed, custard apple readily agreed to host *Metarhizium* strains as endophytes.

The team had seen reports of fungi that borrowed the molecular machinery for producing taxol, an anticancer drug, from Taxus trees. If *Metarhizium* is also picking up extra insecticidal traits from its host, custard apple, perhaps the strains from the rhizosphere of insect resistant plants may also yield potent strains?

The team took samples from the rhizosphere of papaya, chili and neem. And, voila, they too killed more than 90% of *Helicoverpa*, the pest that farmers fear.

Though all this took years, Mukund Deshpande, at the CSIR-NCL, is pleased. His contribution will reduce expenses on fungal biopesticides and make them more farmer friendly. Perhaps other researchers will take up the line of thought and search for more potent *Beauveria*, *Nomuraea* and *Lecanicillium* – other entomopathogenic fungi – with endophytic relationships with insect resistant plants.

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Hyaluronic Acid from Bacteria

Medical use of Lactococcus lactis

Hyaluronic acid is an important component of articular cartilage, where it is present as a coat around each cell, in the extracellular matrix in tissues. It has gained popularity among researchers because of its high biocompatibility. Most commercially manufactured hyaluronic acid is from *Streptococcus zooepidemicus*, a known pathogen.

Recently, scientists from the IIT Madras reported harvesting hyaluronic acid from *Lactococcus lactis*, the bacterium that ferments milk sugar to lactic acid. These Gram positive bacteria are commonly used to produce buttermilk and cheese.

The scientists used metabolically engineered *Lactococcus lactis* to produce hyaluronic acid.

Tests showed that the hyaluronic acid derived from the bacterial fermentation of the *Lactococcus lactis* is biocompatible.

They chemically cross-linked the hyaluronic acid on hydrolysed polyethylene terephthalate, commonly known as PET. PET is biocompatible and does not generate immune reactions. So it can be used in implants and scaffolds for bioengineering. The team assessed haemocompatibility and endothelialisation on the surface of implants.

They also tested the potential to load medicines on the modified surface. They found that the rate of drug release depends on the amount of swelling of hyaluronic acid on the modified PET surface.

The scientists claim that the coating of hyaluronic acid derived from *Lactococcus lactis* is a promising strategy to improve haemocompatibility and the attachment of endothelial cells to implant surfaces. They suggest that it is also useful to deliver drugs to specific tissues.

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Dissolvable Needle

For targeted chemotherapy

Doxorubicin and docetaxel are the most commonly used chemo-drugs for breast cancer. Presently, these drugs are administered intravenously. Intravenous delivery causes non-selective toxicity and, thus, has severe side effects. To minimise side effects and to directly target localised tumours, scientists are now turning to targeted delivery systems.

Recently, Shubhmita Bhatnagar and her colleagues at the Hyderabad Campus of BITS, Pilani took a step in this direction. The researchers fabricated and tested polymer-based microneedles for targeted drug delivery to breast cancer cells. The scientists employed the micromolding technique to synthesise microneedles made of polyvinyl alcohol and a polyvinyl pyrrolidone composite. They loaded the two chemo-drugs, singly and in combination, onto the microneedles for transdermal delivery.

'Forcing the drugs across the outermost epidermis depends on the strength of the carrier molecules', says Shubhamita, Hyderabad Campus of BITS, Pilani.

'We used a texture analyser to measure the compression strength of the blank and drug-loaded microneedle array', adds V. V. K. Venuganti, the leader of the team.

The scientists found that the blank microneedle had a strength of 15 Newtons that reduced after loading the drug. However, the needles were strong enough for insertion into skin that requires a force of only 0.089 Newton per needle.

To test the efficacy of both the drug and the delivery system, the researchers used a xenografted breast cancer mouse model. They found that the microneedle delivered 70% of the loaded drug within the grafted cancer.

The drugs administered through the microneedle were as effective in controlling cancer growth as the intratumoral injection. But, in the microneedle delivery system, the toxic effect of the drug was much less than that in the intratumoral injection.

'This was due to the slower release of the drug from the drug-polymer matrix. It is slower than with intratumoral injection', explain the scientists.

The researchers found that during drug release, the microneedle dissolves and diffuses across mouse skin.

'Microneedles are thin and short – less than a few hundred microns. They don't penetrate the dermis, which has nerve endings', says Subhamita Bhatnagar. 'So the application is also painless'.

Notwithstanding these benefits, the microneedle delivery system is

suitable only for a micro-dosing strategy. To increase the chemotherapeutic dose, the size of the microneedle patch has to be increased.

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Indoor Air pollution
Fuel-kitchen setups

Indoor air pollution is a major environmental concern in India, especially in rural areas where more solid fuel is used for cooking than LPG or electricity. Indoor particulate matter concentrations vary based on fuel-kitchen setups and seasons. Thus, the impact of particulate matter on health will also vary based on these factors.

Recently, S. M. Shiva Nagendra and team from the IIT Madras examined particulate matter concentrations of rural households in Telangana. They used a multiple particle path dosimetry model to identify the deposition of particulate matter in the human respiratory tract.

Using biomass for cooking, they report, resulted in higher particulate matter concentrations than those produced by using biomass-LPG and LPG. The researchers found higher particulate matter concentrations of all sizes in indoor kitchens than in separate-enclosed or outdoor kitchens. This, they say, is due to low ventilation and small-size kitchens.

The researchers observed higher particulate matter concentrations of different sizes when biomass was used for cooking. The seasonal indoor air pollution results revealed that particulate matter concentrations were higher during winter than in the monsoon and in summer. The team identified prolonged cooking, bonfires, and low dilution rate of particulate matter in winter as major reasons for this phenomenon. Transitioning from biomass to LPG will reduce health risks, they claim.

Initiatives to provide clean cooking fuel are the need of the hour, according to the researchers. The report drives home the toxic effects of particulate matter on human health and provides insights for designing fuel-kitchen setups.

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Cleaning Toxic Pollutants
Bacillus firmus rescues

Azo dyes, toxic chemicals released from textile, leather and paper/pulp industries, are potential carcinogens. Conventional effluent treatments produce hazardous sludge and consume high amounts of energy. Plant bioremediation studies suggest the involvement of endophytic bacteria. Endophytic bacteria living inside plants may offer solutions for bioremediation.

Last fortnight, scientists from the NMAM Institute of Technology and the Kuvempu University, Karnataka and IISER Pune reported testing *C. asiatica* and associated endophytic bacteria for environmental management. *Centella asiatica* is a plant found in aquatic ecosystems.

Image: Vengolis, via Wikimedia Commons

The researchers isolated an endophytic bacterial strain from tender leaves of *C. asiatica*. They tested the strain for degrading an azo dye. The researchers isolated bacterial strains from pre-treated explants. And they selected a bacterial colony which inhibited other colonies. They isolated its genomic DNA and identified it as *Bacillus firmus*.

The researchers assessed the dye degradation ability of the isolated strain. They also experimented with aerobic decolourisation by the bacteria in flasks containing the dye to be decolourised.

They observed that varying the concentration of inoculum directly influenced degradation percentage. The team got 92% biodegradation in 72 hours.

The azo dye is toxic and creates chromosomal abnormalities in *Allium cepa* cells. The biodegraded metabolite was less toxic.

The results show that endophytic bacteria can help remediate sites contaminated with heavy metals, dyes and other toxicants.

The study opens new avenues for exploring endophytes that help in soil phytoremediation.

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Sewage Blockers
Fat, oil and grease

Fat, oil and grease from industrial waste block sewage. Fermentation using microbes effectively degrades long-chain fatty acids. But the amount of hydrogen produced during the process affects bacterial communities. This reduces sewage degradation.

Recently, Pradip Kumar Chatterjee from the CMER, Durgapur teamed up with researchers in South Korea to look into the matter. They screened anaerobic, hydrogen-producing and spore-forming microorganisms in active digester sludge from a wastewater treatment plant. The sludge was supplemented with various salts and yeast extracts. The researchers adjusted the pH to facilitate better growth of the microorganisms.

For hydrolysing small particulate matter, the team subjected the active digester sludge to 100°C for an hour. The high temperature is lethal to hydrogen consuming and non-spore forming bacteria.

The researchers then isolated organisms that can withstand high temperature and higher amounts of fats, oils and grease from food waste. They grew the consortium in batch fermenters, where the organism and the carbon sources are kept in static phase.

The scientists slowly increased the concentration of the fat, oil and grease mixture to select bacteria that can use higher amounts of long-chain fatty acids. They monitored the converting of long-chain fatty acids to short/mono-chain fatty acids and hydrogen gas. They also kept a tab on changes in the microbial community.

The team found that phylum Firmicutes, Bacteroidetes and Cloacimonetes can oxidise long-chain fatty acids effectively.

Fat, oil and grease are lightweight and float on water. There, they accumulate and block sewage. Degrading such waste using microbes is a promising strategy. Further developments may soon bring a technology applicable in field conditions. Research on using the hydrogen gas released during sewage treatment also needs to be explored.

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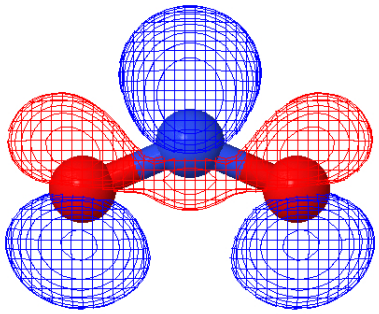
Sensing Nitrogen Dioxide

Aluminium-doped zinc oxide

Nitrogen dioxide from fossil fuels, diesel engines and volcanoes pollutes the air. To combat its adverse effects, a reliable sensor for monitoring nitrogen dioxide is required. Recent research has focused on metal-doped zinc oxide sensors to sense gases.

Recently, M. G. Patil and team from the Dattajirao Kadam Arts, Science, and Commerce College, Maharashtra collaborated with three other institutes in Maharashtra to report synthesising an aluminium-doped zinc oxide sensor with better response.



Nitrogen dioxide image: ChiralJon, via Flickr

To fabricate the sensor, they deposited a layer of aluminium-doped zinc oxide on a flat glass at room temperature using a sol–gel method. The thin film deposited on the zinc oxide was heated to 400°C to improve adhesion.

The researchers observed that the film contained nanocrystalline structures with increased surface to volume ratio. This improved sensing.

To optimise the doping of zinc oxide, they investigated the nitrogen dioxide sensing characteristics of the films at various concentrations of aluminium. They also noted that, with change in temperature, the resistance varied and electrical conductivity increased.

The gas detection involved measuring variations in the resistance. The researchers tested nitrogen dioxide sensing at 100–300°C and at a concentration of 100 parts per million. They find that the sensor made with a 2% aluminium-doped zinc oxide thin film operates optimally at 200°C. The sensor detects nitrogen dioxide gas at concentrations as low as five parts per million. Such sensors will help monitor nitrogen dioxide to reduce environmental problems.

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Parental Perceptions

Outcomes in offspring

All parents have perceptions about their children's executive functions – functions of the brain related to cognitive power and intelligence. Executive functions enable children to plan, focus and organise tasks to achieve goals successfully. And they vary from person to person. How good are parents' perceptions of these functions?

Last fortnight, Karthikeyan Krishnamurthy from the HOTSRC, Chennai in collaboration with researchers in China, the Philippines and the US, reported exploring the matter. The team hypothesised that modernization and urbanization influence parental perceptions about their children's executive functions.

The researchers used quantitative survey research methods. They selected their samples from Shenzhen and Hong Kong in China and Singapore, as these cities exhibit different levels of urbanization and westernization. Samples were selected by stratified sampling with children aged between five and six.

The researchers collected and analysed data from the samples using the Behavior Rating Inventory of Executive Function, an assessment package. They found that parents from Shenzhen had more perceptions than those from Hong Kong and Singapore. Parents from Hong Kong and Singapore had no significant differences in perceptions of children's executive functions. Both cities have been similarly influenced by westernization and urbanization. The team found that parents less influenced by urbanization have more perceptions about their children's executive functions.

From related studies, we see that, compared to urban parents, rural parents more quickly identify developmental delays in children. This may be because the majority from urban areas are nuclear families. In rural areas, on the other hand, most are joint-families, where the child's movements are consciously monitored by elders.

Image: Trey Ratcliff, via Flickr

Children are not born with executive function skills but develop them early under the care giving and support of household members. This early monitoring and identifying of signs and consequent discussions can help children reach their fullest potential. Early intervention appears to play a major role in the development of the child.

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