Science Last Fortnight

Municipal Landfill Leachate
Treating with garbage enzyme

Most municipal waste ends up in landfills, where various physical and biochemical interactions in the solid wastes generate leachates. These pollutant and toxic substances percolate into soil and contaminate groundwater. How do we quickly degrade these leachates?

Sunil Kumar, CSIR-NEERI, and Athar Hussain, Guru Gobind Singh Indraprastha University, Delhi struggled with this question for years, independently of each other. Their students, Aishwarya Rani and Suraj Negi, connected them before they took off to Taiwan for higher studies. Together, the quartet tested the potential of garbage enzymes for treating landfill leachate.

The enzyme is not really an enzyme, but a complex fermented mixture of microorganisms and biomolecules. Typically, you take orange and lemon peels, grind them and mix the product with thrice the amount of jaggery. Add ten times the amount of water and let it ferment. The garbage enzyme has been successfully used to treat domestic wastewater. Could it be used to treat landfill leachate?

The researchers first analysed the enzyme's physico-chemical parameters. And found that it had high total solids. It also had high chemical oxygen demand and low biological oxygen demand. Heavy metals were also found, but in low concentrations.

The team set up a water displacement unit under controlled temperature conditions to collect the methane gas formed and to absorb carbon dioxide. More than 3 litres of methane was generated during 90 days of fermentation.

Sunil Kumar and team collected leachate from the Okhla, Bhalswa and Ghazipur landfill sites in Delhi. They observed high total dissolved solids in the leachate indicating that the leachate had high pollutant content. The leachates also had high concentrations of biodegradable organic matter.

The group assessed the initial leachate pollution index which varied from 25 to 30. Leachate pH from the three sites varied from 7.5 to 9.5. The researchers interpret this as part of leachate ageing. A higher pH also reflects reduced solubility of heavy metals due to the formation of sulphides, carbonates and hydroxides.

The team added different proportions of the enzyme to the leachate for 7–28 days.

After 28 days, the researchers observed a reduction in biological and chemical oxygen demands in all three leachates. In the Bhalswa and Ghazipur landfill leachates, 5% garbage enzyme was adequate to reduce the leachate pollution index by 75%, whereas for Okhla landfill leachate, the 20% mix gave a 60% reduction.

'Further studies are required to identify the actual molecular mechanisms behind landfill leachate detoxification,' says Sunil Kumar, CSIR-NEERI.

108,000 metric tonnes per day is dumped at landfills. Most landfill sites are poorly managed. Since fruit peels are waste and jaggery is easily available, this technique can be a cost-effective method to reduce landfill leachate pollution.

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Sustainable Agriculture
Energy efficiency and yield

Conventional methods of agriculture to increase yields require high energy inputs – multiple tillage operations, fertilisers, water, pesticides – making them unsustainable. So how to reduce inputs of energy and resources and yet increase yield?

Mahesh Gathala from the International Maize and Wheat Improvement Centre collaborated with researchers in India, Bangladesh, Australia, Mexico, and Nepal to identify the best strategies for small landholding farmers in the Eastern Gangetic Plains – a major food-producing region in South Asia. They focused on two districts each in Bangladesh, West Bengal, Bihar and the eastern terai region of Nepal and collaborated with more than 430 farmers to conduct field trials on various options.

Traditionally, two crops are cultivated in the region – a rain fed rice crop followed by irrigated rice, wheat, maize or lentil. Adopting reduced or no-tillage can reduce energy input by 50 to 70 per cent. Retaining stubble and waste biomass in the field improve soil productivity and reduce fertiliser requirements.

The team convinced the farmers to try three other combinations besides the traditional practice of transplanted rice in tilled and puddled fields followed by tilling and sowing of the next crop for the rabi season. In one, for the rabi season, they did not till and used zero-tillage machinery to sow. In the next, they used a zero-tillage machine for kharif also. And, in the last, the rice was transplanted into untilled or minimally tilled, unpuddled fields, followed by machine-tillage in both seasons.

From 2015 to 2017, farmers and scientists collaborated in this massive, experiment. The scientists did not take the first season's data into consideration for analysis since that was a learning period for the farmers.

The researchers then converted data on inputs such as labour, seeds, fuel, time, agrochemicals, fertilisers, etc. to energy equivalents. Emissions from each system were also taken into account in terms of carbon dioxide equivalent. The total energy use of each cropping system could thus be compared. The economics of the yields was also considered.

Reduced or no-tillage with stubble retention emerged winners due to decrease in energy use in terms of fuel and reduced labour. Unpuddled fields reduced irrigation water demand and emissions. These practices will make agriculture more sustainable.

Farmers with small landholdings in the region can now choose agricultural practices based on evidence or follow tradition. At least, those who collaborated in the experiments will draw their

own conclusions and have the confidence to continue on their own.

On a smaller scale, the researchers initiated another experiment: a third crop. They tried rice–wheat–mungbean and rice–wheat–jute and found that there is potential for further intensification of agriculture in the region. 'The results are only indicative and not statistically rigorous. We will follow up with more studies,' says Mahesh Gathala.

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A Promising Natural Dye

Acacia auriculiformis

Acacia auriculiformis, an exotic tree from Australia, is now widely cultivated in India. The tree's extensive root system helps control soil erosion. And fixes nitrogen. The timber too has many uses. Therefore, it is a favourite for afforestation.

A. auriculiformis bark has high lignin content and is used as dyeing agent by the Indonesian batik industry. Sankar Roy Maulik from Visva-Bharati, West Bengal wondered about the plant's potential as a dyeing agent for different kinds of fabrics and the properties of the fabrics after dyeing. He set the problem to his Ph D scholar, Lina Chakraborty, and collaborated with Pintu Pandit, National Institute of Fashion Technology, to support her.

They collected the bark of a locally grown specimen, and dried and powdered it. Mixing 200 grams of bark powder with one litre of water, they heated it to 90°C for one hour with occasional stirring. Then they filtered the extract and made it 1 litre, adding distilled water to compensate for evaporated water.

Next, the researchers screened the extract for phytochemical compounds. The aqueous extract contained saponins, phenols, tannins, terpenoids and glycosides, but no flavonoids.

The researchers dyed cotton, wool, silk and eri fabrics, heating them in the extract at 90°C for about 45 minutes, without using metallic salts or mordants. The fabrics took on a yellow-red colour which increased with increased dye concentration.

The dyed fabrics were then tested for colour fastness, under washing and rubbing as well as exposure to light. The dye showed good colour fastness on protein-based fabrics – wool, eri and silk. But fabric based on cellulosic fibres, such as cotton, did not fare as well.

Does the dyed fabric provide UV protection? The team found that the dye increases ultraviolet protection substantially. The effect was profound in wool, where the UV protection value increased from about 18 to about 95.

The team tested the dyed fabrics for antimicrobial properties. Here silk performed best, reducing bacteria by more than 98%. The other fabrics also had antibacterial properties and showed more than 94% reduction in bacteria when dyed.

'Tannins in the dye may be responsible for colour and UV protection,' says Sankar Roy Maulik. 'And the high antibacterial activity could be due to the synergistic action of tannin and phenolic acids.'

Acacia auriculiformis may find uses in the textile dyeing industry in India, since the process is environment-friendlier than using synthetic dyes. And since metallic salts and mordants are not necessary for dyeing, there is less pollution.

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Water Distribution Mains

Economic benefits of gravity

Pumping can be used to supply water in any topography. But when water input is at a higher elevation than the outlet, gravity can save a lot of energy and pump wear and tear. But is a gravity main always economical?

Last fortnight, Prabhata K. Swamee from the NorthCap University, Gurugram, and Ashok K. Sharma from the Victoria University, Australia provided a technical note to help engineers decide the best system in case of gently sloping terrain.

When slopes are gentle, there is an inverse relationship between pipe diameter and slope difference. So, gravity main might not be as economical as pumping, they say.

The team calculated the cost of pumping and gravity for per unit length of pipe in the main supply. The equation for pumping main has two components: one for the operation and maintenance cost of pumping system and the other for pipes. The resistance factor is considered and an equation is developed for calculating the pipe's optimum diameter. This is independent of ground slope.

For the gravity main equation, pipe diameter and friction slope are considered to calculate the cost of transmitting water through unit length of pipe. This is dependent on the ground slope.

The next step is to calculate the equal cost slope equation, where both pumping and gravity main will have the same cost. For a ground slope less than this equal cost slope, pumping is more economical.

With a few iterations, the team calculated the cost when the slope is an equal cost slope to obtain the optimum diameter.

'We need to consider four factors when choosing a gravity main for supply: Pipe cost and diameter, friction in pipe, and gravitational constant,' says Ashok K. Sharma, Victoria University.

'This input can help water supply system designers select the most economical option for gentle terrains,' says Prabhata K. Swamee, NorthCap University, Gurugram.

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Chitosan Nanoparticles

Intracellular uptake mechanism

Chitosan, from the chitin exoskeletons of marine animals and from fungi, is a versatile biopolymer. It has many positive charges. So it easily attaches to small interfering RNA and can be used to silence the expression of specific genes. Chitosan nanoparticles enter cells very easily, making them good vehicles for delivering drugs into cells to treat even genetic diseases.

But how do the chitosan nanoparticles interact with cells? How do they enter cells? Is it an energy dependent process or is it passive diffusion?

Anomitra Dey, Ratnesh Jain and Prajakta Dandekar from the Institute of Chemical Technology in collaboration with a scientist from Sweden recently reported investigating the

interaction of chitosan with different types of cells using a combination of experimental methods and mathematical analysis.

The team first tagged the chitosan with a fluorescent dye, fluorescein isothiocyanate, to help track the movement of the nanoparticles and their interaction with the cells. Using ionic gelation, they converted the fluorescent chitosan into nanoparticles of about 120 nanometres.

Next, these nanoparticles were added to two cancerous cell lines – HeLa, a cervical cancer cell line, and NCI-H460, a lung carcinoma cell line. The researchers also introduced the nanoparticles in two normal cell lines: a lung epithelial cell line, and primary human dermal fibroblast cells. At the concentrations used for the study, the fluorescent chitosan nanoparticles showed no toxicity to the cells.

The team found that all four cell lines assimilated the nanoparticles in the same quantities. The cell lines retained the nanoparticles upon washing. Since all the cell lines showed similar uptake and retention capacities, the team focused only on HeLa cell lines to explore the impact of fluorescent chitosan nanoparticles at various time intervals.

Using imaging flow cytometry and confocal laser scanning microscopy, they found that the nanoparticles act on the cell surface immediately when introduced. Using Ligand Tracer, machine-enabled tracking of real-time interactions between fluorescent nanoparticles and cells, the team observed that it took up to 2–4 hours for the nanoparticles to enter the cells.

Cells take in molecules by various methods. The process could be mediated by proteins such as clathrin. It could be endocytosis by calveolae or micropinocytosis. It could even be by passive diffusion. What could be the mechanism?

The team investigated.

They blocked the uptake pathways, one by one, and used chlorpromazine to inhibit clathrin, nystatin to inhibit calveolae-mediated endocytosis, amiloride to inhibit micropinocytosis and sodium azide to block passive diffusion. And the cells were then treated with chitosan nanoparticles.

In spite of the blocking, the nanoparticles were able to enter the cells. Blocking endocytosis and micropinocytosis reduced uptake only by 20–40%. Even blocking passive diffusion with sodium azide could achieve a maximum blocking of only 50%.

'This shows that the chitosan nanoparticles can enter via multiple entry pathways,' says Ratnesh Jain.

Lastly, the team employed a Lysotracker deep red dye in the media to track the entrapment of chitosan nanoparticles in the lysosome. They found that the lysosomal entrapment of chitosan occurred after two hours.

The researchers concluded that the uptake of chitosan nanoparticles was due to cell membrane fluidity rather than only mediated by endocytosis.

To test, they subjected the HeLa cells to two different temperatures – 4° and 37° – and then introduced the nanoparticles. They found a higher intake of nanoparticles at 37° degrees than at lower temperatures.

Mathematical analysis of the interactions showed that the positively charged chitosan interacts with the negatively charged surface of the cell membrane and deforms it to enable entry.

'In designing the targeted delivery of drugs or gene-silencing RNAs, we need to consider these aspects of chitosan nanoparticles as payload carriers,' says Ratnesh Jain, Institute of Chemical Technology.

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Evolution of Wars

In-silico model to explain history

Dhritiman Talukdar and Kishore Dutta, researchers from the Handique Girls' College, Guwahati wondered if computer simulations based on historical data can explain the changing frequency of wars and its impact on civilisations. Can computer-based simulations be used to understand how civilizations rise and fall?

To answer the question, the researchers designed a computer-based model to simulate wars. Small-scale primitive human societies were represented by round disc-shaped domains. For the sake of simplicity, they considered only three elements: the entity, infrastructure and culture. Thousand discs of different sizes, representing populations, were placed at random locations on a square Cartesian plane. And three different colours represented differences in culture – commonality in race, language, food, ethnicity, etc.

The computer model allowed the domains to grow linearly with time and at a constant rate. As the radius of the discs increases, the domains belonging to a similar group coalesce when they meet at the point of interaction. When two dissimilar groups meet, they engage in warfare and thus the interaction reduces the population size of both.

The researchers then set some rules in the game. When two dissimilar groups war against each other, the smaller domain is defeated and merges with the larger one. The larger domain, although a winner, may shrink in size depending on the number of casualties. A 'fairness of war' parameter – lying between 0 and 1 – determined the casualties of war. In an ideal 'fair war' scenario, the consequences are the same for both the domains and the fairness parameter is 1. In a completely 'unfair war' scenario, the winner does not suffer any casualty when the parameter is 0.

Having set the rules, the researchers analysed the impact of wars according to the model. Again, in an ideal situation, after many wars, the number of domains must decrease and finally result in a single large surviving domain. But, in reality, surviving civilizations also lose human lives, infrastructure and culture after wars.

So, the scientists analysed the age-size distribution of the domains at different growth rates and in the presence of a stronger group. They varied the different parameters to examine the behaviour of the system.

Interestingly, as per the model, the frequency of wars is greater in the initial period. Though, initially, all 1000 small-scale societies are allowed to evolve at a constant rate, as

time progresses, many groups merge and become bigger, while some disappear. And the number of wars reduces – as it happened in the history of humans on the earth.

The team ran the simulations for 1500 steps to arrive at a single surviving civilisation. And noticed that the decay of any civilisation depended on the initial size, and the fairness factor. Interestingly, as the number of groups reduces to four, the researchers found that the decay rate reduces drastically. The team confirmed this by repeating the simulations, changing initial conditions. In conformity with historical evidence, their model also bears another interesting feature: two neighbouring domains of equal sizes are doomed in a completely fair war.

'Though this is just a simple toy model, it is capable of capturing some historical facts,' says Dhritiman Talukdar, Handique Girls' College, Guwahati.

'Of course, a large number of other factors such as mass migration and epidemics complicate reality. The present model is simple enough to include other domain parameters,' adds Kishore Dutta, his Ph D supervisor.

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Virtual Kills, Real Anxiety

Predicting from online gaming

Research has shown that those who are diagnosed with Internet Gaming Disorder may demonstrate other psychological and behavioural issues such as impulsivity, hostility, emotional distress and low self-esteem. Can behaviour during online games help predict the propensity for such disorders?

A team led by Swati Aggarwal from the Netaji Subhas University of Technology, New Delhi looked into the association between psychological conditions such as attention deficit hyperactive disorder, generalized anxiety disorder and internet gaming disorder and gamer statistics. They took PUBG as a case. Player Unknown's Battlegrounds is an online game where multiple players can engage in virtual battles.

The team sent Asian gamers the link to a survey containing questions related to sociodemographics and gaming information as well as psychometric instruments. Thirty-five who played the game on computers and 19 who played on mobile responded. The team extracted gamer statistics and screenshots of mobile phones during the game.

They used the pre-processed data and the scores obtained from the questionnaires to train various supervised machine learning classifier models. Given the low number of samples, they adopted the leave-one-out to train and test. In each run, one sample is left out and the outcome is tested against it. Thus, using 44 iterations, they strengthened the learning by the machine.

The researchers tried various machine learning strategies: Logistic Regression, KNearest Neighbor, Naive Bayes, Decision Tree and Decision Tree with Adaboost… And found that the Logistic Regression classifier could predict internet gaming disorder with an accuracy of more than 93% and attention deficit disorder with an accuracy of more than 81%. However, the Decision Tree classifier was better at predicting generalised anxiety disorder with an accuracy of nearly 85%.

'Only two out of 44 were females – either because violent games are not popular among females or online gamers are mostly male,' wonders Shubhi Gupta.

'About 20% of teenagers between 15 and 18 years in our study suffer from ADHD. But half of those between 24 and 27 have generalised anxiety disorder,' adds Varshika Gambhir.

'When we left out gender, age or self-esteem, the accuracy dropped significantly, suggesting that these factors play important roles,' says Swati Aggarwal, Netaji Subhas University of Technology.

'Similarly, there are factors such as number of kills and number of wins that emerge as significant,' says Shivin Saluja, her colleague.

'The more the kills and wins, the more the chances of becoming addicted,' says Simrat Pal Singh Satia. 'And the number of hours spent on the game, though not proficiency in the game, is strongly correlated with ADHD.'

There is one question that remains. Does internet gaming cause these disorders or do these disorders predispose you to gaming? Further studies are needed to understand the correlations in terms of causation.

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Biofuels from Biomass

Sesame pinch

A few hundred thousand metric tonnes of sesame seeds are produced in India every year. After harvest, huge amounts of remaining biomass are burned, used as cattle feed or just wasted. Can we convert this biomass into biofuel? What are the best methods?

Researchers from the Gurukula Kangri Vishwavidyalaya, Haridwar, collected the biomass after a sesame harvest. They dried, powdered and sieved it to get particles of 400, 850 and 1300 micrometres.

The biomass contains a lot of lignin and cellulose, besides reducing sugars. The researchers used the crust fungus, *Phanerochaete chrysosporium*, to break the cellulose and lignin down into simpler carbohydrates. And dilute sulphuric acid to break them down to sugars. Then they used yeast, *Saccharomyces cerevisiae,* to ferment the sugars to ethyl alcohol.

'The smaller the size of the biomass, the better the results,' says Vinod Kumar, who led a team of students to conduct the study.

While producing biofuel, the method helps to manage sesame crop residue. It could be applied to other agricultural waste. We need to cling to all straws to achieve the National Biofuel Policy 2018 that targets 20% ethanol blending with petrol by 2030.

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