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Managing wastewater using plastic eating bacteria – A sustainable solution for sewage fed fisheries

Jaydev Misra[a] and Priyadarshini Mallick*[b]

[a]Department of Economics, Dhruba Chand Halder College, Dakshin Barasat-743 372, South 24-Parganas, West Bengal, India

[b]Department of Microbiology, Asutosh College, Kolkata-700 026, India

E-mail: priyadm1@yahoo.com

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Affluent like plastics has made the water management issues of sewage fed fisheries much more difficult as well as cost-ineffective. However, possible impact of introduction of bacteria in managing plastics in wastewater for sustainable sewage fed fisheries operation has not been yet documented. Present study is an attempt to investigate whether *Ideonella sakaiensis* can be used to eradicate problems with plastics in sewage water so that fisheries operation can be continued in a sustainable way. The objective is to find the best suitable degradation procedure of various kinds of plastic involving biological means as well as other sources of natural means too. Bacterial and fungal species were also widely employed in these degradation processes. Several strains of *Ideonella sakaiensis* were used in developing the desired process. It was observed that, the hydrocarbon, present in plastics, can be degraded by organisms which can also use it as proper sources of carbon and these organisms can be employed. The outcomes are established by the changes in weight differences, tensile strength and reduction in the viscous properties in most of the instances while in few cases, molecular weight distribution, and fragility was also noticed. Thus it can be also concluded that HDPE plastics shows more resistance to soil conditions than that of the LDPE plastics. Further, *Ideonella sakaiensis* as a species does not pose any threat to the growth and cultivation of fishes. Thus in near future, plastics causing pollution in wastewater can be treated using this special variety of bacteria for improvement of fish cultivation.

Keywords: *Ideonella sakaiensis*, bioremediation, surfactant modified silica gel, wastewater.

Introduction

Plastic is all around us involved in food packaging, encasing electronic gadgets, carry bags, etc. 'Plasticós' is the Greek word from where the term 'plastic' has been brought to the fore and it means 'to mould', as most of the plastics are more or less moldable and initially soft during their original production. Plastics are synthetic polymeric units made up of further smaller organic monomeric units joined into longer chains due to the presence of covalent bonds formed between them during a polymerization reaction. Several of the monomers used to obtain plastics are derived from crude oil, making them a limited resource[1].

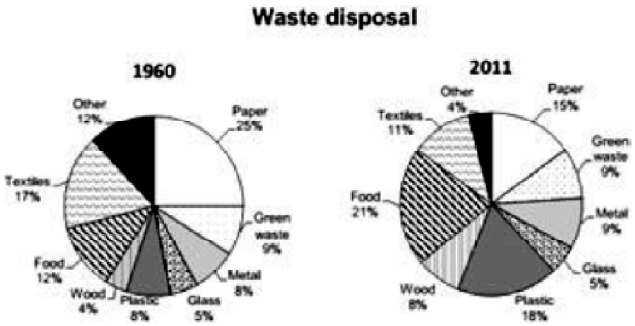

Fig. 1. Pie-charts comparing different types of waste disposed off in India in 1960 and 2011 respectively.

The various types of plastic which are commonly used are polyethene, polyvinyl chloride (PVC), polyethylene (PE), polystyrene, polypropylene (polypropene), polyurethane and polyethylene terephthalate (PET). PET and polyurethane are

formed from two different types of monomeric units that are connected together into a separate chain, while the other types mentioned are derived from the source alkene monomer in which during the polymerization reaction, the double bonds unwrap in order to form a linear hydrocarbon chain[2,3]. The applications for plastics are numerous in today's world. Only 10% of all the plastics produced are recycled. Globally, we create approximately 57 million tonnes of plastic waste per year, 15 million tonnes of it end up in our surroundings, particularly in the ocean-beds. In 2014, it was observed that one fourth of the plastic wastes within the European Union were recycled: among which 33 per cent ended up being disposed of as landfill, while the rest being burnt up as sources of energy recovery. During this time frame, around 64 per cent of all packaging waste was recycled (like cardboard and other packaging materials), but no less than 40 per cent of plastics that were exploited for packaging was further recycled.

Considering a general example, a fruit core when thrown into the grime, can be consumed by a worm, which eventually releases nutritious waste for plantations to feed on it. But plastics, rubber, Styrofoam or aluminum does not follow this natural cycle that allows the growth pattern in our ecosystem. While an apple on recycling into new material takes around two months time, on the other hand a plastic materials like bottle or bowl can take around 400 years to decompose. Plastic fragments inhibit the normal cycles of our ecosystem, and it is difficult to totally remove such durable waste without causing more damage to the environment. While plastic seems cost-effective, lightweight, and disposable, but it doesn't disappear after being thrown away. Instead, most of the thrown away part gets accumulated in the ocean beds.

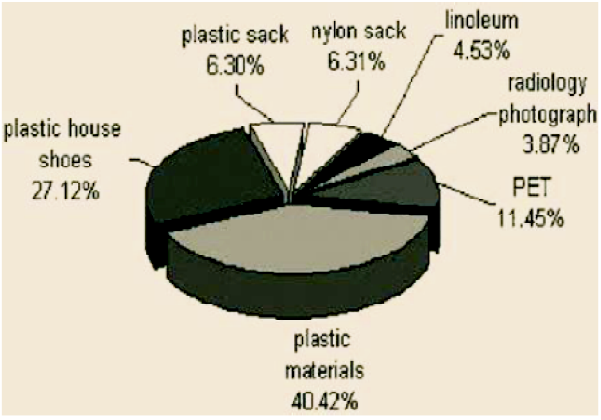

Fig. 2. Percentage of plastic components in polluting debris.

The easiest ways that marine debris travels from land to water is by being swept with the help of storm drains during rains. Waterways like rivers, estuaries are also responsible in washing these trashes into the bay. Thus the believed amount of used plastic on land gets collected on ocean-beds. Needless to say, the plastics in the ocean proves hazardous to the marine life because of its choking hazard and toxic nature. Several kind of marine species like mammals, sea turtles and crustaceans are commonly exposed to entanglement encounters, which leads them to death. Plastics, especially polyvinyl chloride (PVC), are toxic for human health as well as the environment. PVC is known to release dioxins, mercury and phthalates, which results in life-long health damages, such as cancer and spoils the immune as well as reproductive structure. Even in considerably favorable atmosphere, such as in bacteria enriched soil or in warm temperatures, plastic bags decompose only 50 per cent after a period of 389 days. Biodegradable plastics are known to take approximately 3 years to degrade in underwater, because these biodegrading states of affairs apparently diverge from what has been observe on land. It is also observed that, UV light is not capable of breaking down heavier plastics if they sink. Though there are another ways to minimize disasters, sea garbage continues to amplify; making cleaning programs inadequate[4].

A study observed that people consider biodegradable plastic to be alright and believe that it can degrade easily. Educationally changing the mindset of treating plastics as a temporary item and behaving in a similar fashion would help solve some percentage of plastics from inflowing the ocean. At the end, finding a approach to completely prevent plastic littering, and amalgamating recycling into social behavior should be the main objective. Meanwhile, new thoughts for eradicating the bulky plastic deposition either by reducing carbon dioxide discharge, or exploiting fewer material or energy to degrade plastics have emerged.

Plastics disposed off as debris, either as landfill or released into the environment, can remain for a period of 450 years[5,6]. A research team from Japan, in 2016, came up with their findings of a particular bacterial strain that could degrade PET. On the basis of samples collected from 250 sites

that were contaminated with PET fragments, including mud from a PET recycling plant, they observed that something present in these samples was able to degrade PET film at a rate of 0.13 mg on day 1, and the activity of a particular strain of bacteria is entirely responsible for this degradation process. This bacteria is known as *Ideonella sakaiensis*[7].

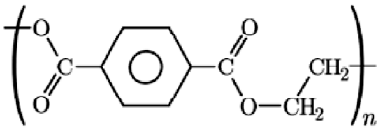

Fig. 3. Chemical structure of PET.

The research team discovered that this particular bacterium has two notable enzymes which can facilitate the process of degradation. The foremost enzyme is secreted on outer surface of the cell and responsible to break the PET polymer into MHET (mono-hydroxyethyl terephthalate). Scientists have termed this enyme as PETase. Minimal percentage of MHET were observed in the enzyme medium, indicating that the bacterium is able to speedily break it down, in order to exploit it as a resource of carbon for its expansion. The second enzyme, that is, MHET hydrolase thereafter hydrolyses the MHET soon it is captivated within the cell (Fig. 4), breaking it down into terephthalic acid and ethylene glycol, which are the unique monomeric units from where PET is actually prepared. However, this entire enzymatic process is incredibly time-consuming since it takes around one and half months for a single small piece of PET film to go through complete degradation. The scientists also found that the bacteria affixes itself to the PET surface by means of thin tubes and by this process the cell is capable to transport the PETase enzyme to the surface of the substrate[8].

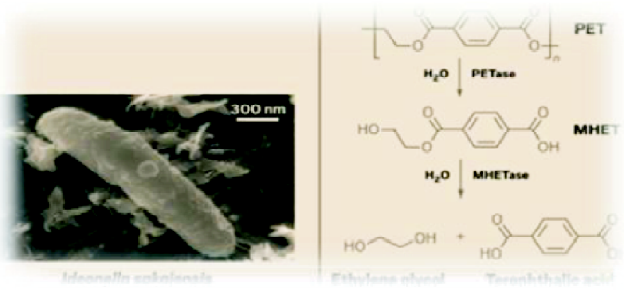

Fig. 4. Structure and composition of *Ideonella sakaiensis.*

The intriguing characteristic of this novel bacterium is its ability to eat this type of plastic that was previously considered to be one of the most infamously resistant materials. *Ideonella sakaiensis* is an aerobic, non-spore forming, Gram-negative, rod-shaped bacterium. PETases falls under the esterase class of enzymes which can catalyze the hydrolysis of polyethylene terephthalate (PET) plastic to monomeric mono-2-hydroxyethyl terephthalate (MHET). But PETases can't catalyze the hydrolysis of aliphatic polyesters. Actually, if PET plastics are anaerobically heated, it decomposes into terephthalic acid (TA), gas and oil[9].

Materials and methods

The objective is to find the best suitable degradation procedure of various kinds of plastic involving biological means as well as other sources of natural means too. The plastics taken into consideration are polyethylenes, polyhydroxyalkanoates, polyesters, polyvinyl chloride, polylcaprolactone, polyvinyl alcohol, polyurethane, polylactic acid, nylon and polyethylene, polyester-polyurethane, etc. Bacterial and fungal species were also significantly employed in these degradation processes. Different strains of *Ideonella sakaiensis* had been used in developing the desired process.

Sewage sludge samples were collected once every month during early morning from a depth of 0.5m from the surface of the targeted water bodies from four different sites for one year (July 18-June 19) and stored in sterilized glass culture bottles. The containers were placed in an ice-box immediately after samples are collected. After collection of samples, lithogenic objects and foreign debris if present are removed manually. Samples from particular sites were made composite by mixing them using a mechanical homogenizer. Different water quality parameters (like temperature, pH, turbidity, alkalinity, dissolved oxygen, dissolved carbon dioxide, free ammonia, hydrogen sulphide, phosphate, nitrate, alkalinity, chloride, suspended solids, etc.) were estimated by standard methods as stated by APHA21. Water quality assessment was done on the basis of average values of the existing physico-chemical components during the mentioned study period (2018-2019). In order to identify the harmful effects of sewage water on aquatic life after introduction of *Ideonella sakaiensis*, biochemical oxygen demand (BOD), dissolved oxygen content (DO_2), carbon dioxide content, ammonia and

various sulphur concentration values have also been estimated before and after introduction of *Ideonella sakaiensis*. Certain methods have been implemented to reach the aim and objective of the study. They are:

(1) Soil burial treatment: identical pieces of cellulose which are blended as PVC films (dimensions: 5×2.5 cm each) were buried in the soil for a stretch of 3 months and inoculated in presence of collected sludge of sewage for the isolation of these microbial strains having the capability to stay and degrade the polymeric film. Fig. 5, shows the demonstrative view of the above mentioned process[10].

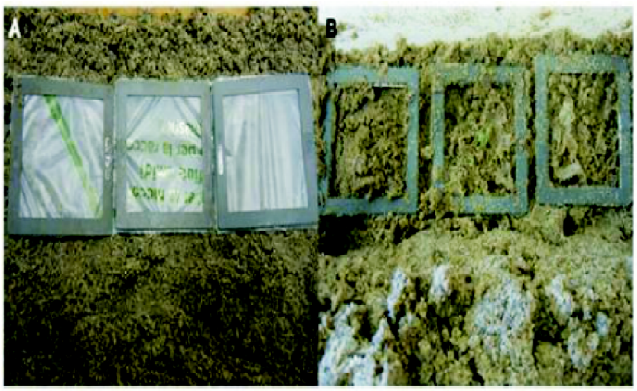

Fig. 5. Picture showing soil burial treatment in a domestic garden.

(2) Shake flask experiment: The PVC films blended with cellulose had been further incubated with the isolated microbes obtained from the soil burial experiment in shaking condition[11]. The desired mineral salt media (MSM) used per 1000 mL contained in distilled water were as per the measurements depicted in Table 1.

The well blended cellulose with PVC film (3 pieces) in MSM (90 mL) were inoculated exactly with 10 mL of the collected spore suspension ($10\pm2.1\times10^6$ spores mL^{-1}) and incubated at 30°C for a period of 12 weeks. At an interval of every month, those samples of polymer had been observed and estimated both manually and visually with the help of an infrared spectroscopy measured on Bio-Rad Merlin FTIR (Instrument specification: Excaliber Series FTS 3000 MX, USA). From Fig. 6, we get a vivid interior view of the shaking incubator performing the experiment.

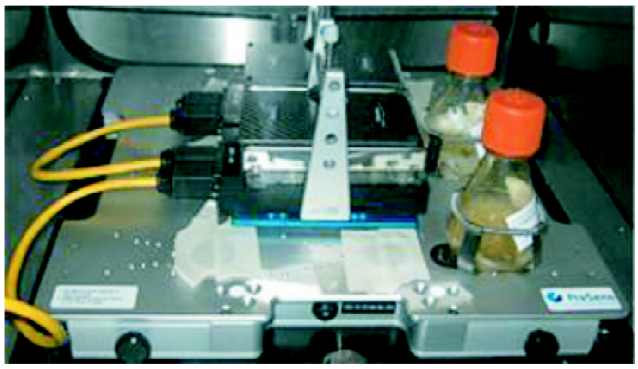

Fig. 6. Interior view of the shaking incubator.

(3) Sturm test biodegradation: CO_2 evolved from the cellulose blended PVC biodegradation was obtained and further determined by a process called the sturm test. In order to perform this process, the polymers pieces were inserted to the existing culture bottles containing MSM (amounting to

Fig. 7. Pictorial view of sturm test biodegradation being performed in laboratory.

Table 1. Amount of chemicals involved in the Shake Flask Experiment

Ingredients	Amt. (g)	Ingredients	Amt. (g)	Ingredients	Amt.
K_2HPO_4	1	KH_2PO_4	0.2	Boric acid	0.005 mg
NaCl	1	$CaCl_2.2H_2O$	0.2	$(NH_4)_2SO$	1 g
$MgSO_4.7H_2O$	0.5	$MnSO_4.H_2O$	0.001	$ZnSO_4.7H_2O$	0.001 g
$CuSO_4.5H_2O$	0.001	$FeSO_4.7H_2O$	0.01		

285 mL) without providing them with any proper carbon source. The spore suspensions obtained from the specific strain of *Ideonella sakaiensis* (2.9×10^6 spores mL^{-1}) was employed as inoculums of 5% (v/v) in performing the sturm test and control bottles (without plastic) were employed. Perfectly maintained sterilized air was supplied to maintain aerobic conditions and the reaction bottles had been stirred thoroughly on a magnetic stirrer. Exactly after a month, gravimetric analysis of CO_2 production was performed by collecting the obtained gas in adsorption bottles already containing a solution of KOH (1 *M*). The precipitates obtained after this titration using barium chloride solution (1 *M*) and then the control was filtered, weighed and calculated to estimate the CO_2 production per liter[12].

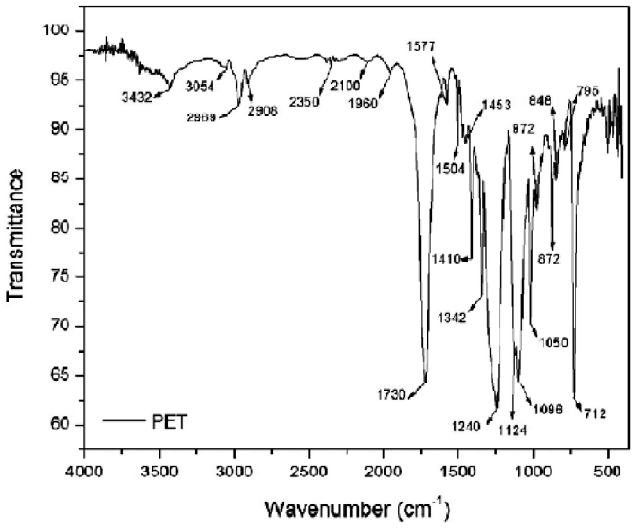

Fig. 8. FT-IR spectrum of the PET sample after 3 months.

Fourier Transform Infrared Spectroscopy (FTIR) was employed to attain an infrared spectrum of amalgamation of the PET polymer samples and the graphical data was obtained containing wavelength (cm^{-1}) against transmission.

(4) Fungal treatment: Finally this method was done where certain strains of the species *Basidiomycetes* which were previously known to degrade plastics were employed and made to traverse the same path as *Ideonella sakaiensis*. These fungal cultures were then prescreened with the use enrichment media containing petridishes. Surface-sterilized (3 per cent H_2O_2 for two minutes) polyester filaments were gently added on agar medium surfaces and inoculation with those fungal strains was done simultaneously[13]. Other degradation tests were also carried out using liquid media, where identified fungal strains were obtained in a 250 ml Erlenmeyer flask containing 15 ml of the desired culture media in which pieces of polyester or co-polyester films are immersed for a considerable period of time. Before inserting the content into the nutrient medium, polymer pieces were surface sterilized at 3 per cent of H_2O_2 for two minutes and stored in sterile conditions. Two different culture media namely, nitrogen limited medium and nutrient rich glucose malt extract medium[15]. Needless to say that, the species *Basidiomycetes* are not as efficient as *Ideonella sakaiensis* since they take a longer time for PET degradation and being a fungal strain *Basidiomycetes* itself acts as a contaminant to the samples. Thus proving that *Ideonella sakaiensis* is currently the best possible way out in the category of microbes in order to perform plastic consumption (Muller and Pantke, 1992).

Results and discussion

In order to find out whether introduction of *Ideonella sakaiensis* in PET samples of sewage water can degrade PET significantly using the above mentioned treatments, several samples of PET have been collected from targeted sewage water in East Calcutta wetlands. A total of 150 samples have been collected purposively by forming sample group in such a way that weight of each group increases gradually by 5 μg/ml. In this way, 12 different sample groups has been formed each of different sample size. Treatment procedures using *Ideonella sakaiensis* have been applied to

Table 2. Decrease in weight (μg/ml) in observed samples in a continuous period of 3 months

[Mean weight of PET samples (μg/ml)]

Sample name	Sample size	Duration (Weeks) 0	4	8	12
1	14	5	0.45	0.23	0.02
2	12	10	2.4	1.25	0.08
3	14	15	3.3	1.58	0.19
4	12	20	4.2	2.1	0.28
5	16	25	5.0	2.57	0.35
6	10	30	5.9	3.0	0.44
7	11	35	7.0	3.52	.51
8	14	40	8.1	4.03	0.62
9	09	45	9.0	4.5	0.75
10	14	50	10	5.3	1.07
11	11	55	11	5.35	1.25
12	13	60	12.5	6	1.75

notice the differences in weight of PET samples. Mean results of such weight has been shown in Table 2. It is quite evident that mean weight of PET sample was decreasing gradually over the weeks for almost all the sample groups. However, it was observed that degradation process is quicker for PET sample of lower weights.

ANOVA results, depicted in Table 3, was used to determine whether there are any statistically significant differences between the means of weight of PET (μg/ml) collected from sewage water on introduction with the particular bacterium. The results had been found statistically significant as *F*-value is 162 and *p*-value is 0.00001. Low *p*-value clearly indicates clear evidence against the null hypothesis that sample means are some way or other equal. Moreover, Post-hoc test has found median value as 0.156 which means the significance value of the Levene statistic could not produce a noteworthy result, which signifies that the requirement of homogeneity of variance has been fulfilled, and the above test can be recognized to be robust.

Table 3. One-way ANOVA results for significant difference among duration and PET sample

Source	SS	df	MS	
Between-weeks	38974.1604	3	12991.3868	
Within-weeks	18809.9383	236	79.7031	$F = 162.9972$
Total	57784.0986	239		

NOTE: *p*-value is 0.00001. *p*-value is significant at $p < 0.05$.

Another aspect like the impact of introducing *Ideonella sakaiensis* into sewage water for degradation of plastics laid upon the growth of fish needs to be focused. In case of a fish, metabolism is providing energy to empower it so that it can perform all required body procedures or sustaining the various organs needed to function appropriately. They successfully obtain their required energy from oxidation of those complex molecules which are consumed by them. It develops toxicity in fishes if they consume debris containing disposable plastics from the water bodies where they dwell. The products of digestion are then absorbed into the body of the organism where oxidation processes occur which release the energy formed as a product. Fishes are unable to monitor and regulate their body temperature, so they are entirely influenced by the temperature surrounding them. Warm water accelerates fish metabolism, eventually feeding and respiration also increases, and there is a general increase in the movement of fish. In cold water, fish tends to becomes lethargic and behave inactive. It has been observed that the fish metabolism shows highest activity at 30°C approximately. *Ideonella sakaiensis*, helps to maintain the water temperature around the above mentioned ideal temperature by not emitting any such compounds which would cause a deviation. On the other hand, *Ideonella sakaiensis* has the capability to metabolize itself with very minimal amount of dissolved oxygen from water, thus it does not cause any significant change in the ratio of dissolved oxygen in water bodies where it has been introduced. It is evident that, more oxygen the fish consumes, the more energy it uses, causing the metabolism to be high. In endothermic animals, the process of regulating body temperature increases the metabolic rate. Fishes breathe through their respiratory organs known as gills. Small holes in this membranous gill structure allow tiny oxygen molecules in the water to penetrate into the fish's body. Situations often arise when plastic molecules penetrate through these gills and cause respiratory choking or several other organelle entanglement cases in fishes posing threat to their survival. In this case, *Ideonella sakaiensis* acts as a savior who helps to reduce the amount of plastic content in water bodies. Respiration rates will typically increase as dissolved oxygen concentration decreases. Soon water temperature increases, dissolved oxygen available for the fish to breathe from the water decreases. Fish health in overall can affect human health on a large scale because we tend to consume those edible fishes time and again.

Conclusion

Plastics are a major threat to the environment and specifically it is posing threat to aqua-marine life. The entire globe is inclined to sort out a convenient process to degrade the polymer and come up with a healthier alternative that is "a biodegradable plastic". Most plastics resist degradation and while very few shows properties of degradation to certain extent. Some of them exist for an enormous period of time as a persistent organic pollutant (POP). The objective is to find the best suitable degradation procedure of various kinds of plastic involving biological means as well as other sources of natural means too. The sample plastics are polyethylenes, polyesters, polyhydroxyalkanoates, polylactic acid, polylcaprolactone, polyurethane, polyvinyl chloride, polyvinyl alcohol, nylon and polyethylene, polyester-polyurethane,

etc. Bacterial and fungal species were also extensively employed in these degradation processes. Several strains of *Ideonella sakaiensis* had been used in developing the desired process. Finally it was concluded that, the hydrocarbon degrading organisms present in plastics and exploit them as proper sources of carbon should be employed. The results are confirmed by the alterations in weight, tensile strengths and reduction in the viscous properties in most cases while in few cases, molecular weight distribution, and fragility was also noticed. Thus it can be also concluded that HDPE plastics are displaying more resistance to soil circumstances than LDPE plastics. It was observed that the degradation of gum enzyme was also a helpful procedure. The unadulterated culture biodegradation assay demonstrates the capability to recognize that particular fraction of the degradation which is due to chemical degradation and what can contribute straight to the biologically degradation procedures. By carrying these experiments, plastic degradation can be made more cost-effective, less time consuming and needless to say that once these methods are incorporated to treat the challenges and threats imposed by plastics, the acute problem of water pollution and disproportion to aquatic life would majorly get solved. There are several cases and instances that edible fishes along with larger varieties of non-edible fishes get choked due to inhalation or entanglement of plastic molecules present as debris in water bodies. In this case, *Ideonella sakaiensis* solves a major part of this particular type of problem too. On the other hand, biochemical pathways followed by this particular microorganism to survive in water bodies does not emit any such toxic compound which can inhibit fish growth or pose any challenge to the normal survival of aquamarine structure. Thus in near future, plastics causing pollution can be treated using this special variety of bacteria for improvement of fish cultivation in sewage water.

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