Population growth and industrialisation has led to harmful pollution of our air, freshwater, oceans, and soil. Water quality has worsened as a result of human activity due to mining, fracking and the processing of metals from the likes of steel mills, battery companies and electricity generation. Industrial effluents like petroleum products, polythenes, and heavy metals damage the environment and are a major cause for concern. (1)

Although most of these compounds exist in nature, they often decompose slowly or not at all and can have a severe impact on wildlife, biodiversity, ground water and human health. (1) CRISPR-Cas9 can help provide a solution to address this problem by allowing desired traits to be introduced into and enhanced in microorganisms for bioremediation. (2)

Bioremediation is a cost-effective and practical solution that uses microorganisms and microbial enzymes to detoxify contaminants in soil, water and other environments. (3) Microorganisms are a vital component of plant growth, insect control, soil conservation, nutrient recycling, and pollutant reduction. In order to partake in these key functions microorganisms have evolved to enzymatically break down or adsorb organic and inorganic compounds to produce sources of carbon for energy, synthesise useful metabolites and or lower the toxicity of their environment. (4) By taking advantage of this adaptation, a wide variety of microorganisms can deployed for bioremediation. See table below:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude oil</td>
<td>Aspergillus niger, Candida krusei, and Saccharomyces cerevisiae (8)</td>
</tr>
<tr>
<td>Diesel oil</td>
<td>P. cepacia, B. coagulans, B. cereus, B. cereus A and Serratia ficaria (9)</td>
</tr>
<tr>
<td>Chlorobenzenes</td>
<td>P. putida (GJ31) (10)</td>
</tr>
<tr>
<td>Mercury, nickel and lead</td>
<td>Saccharomyces cerevisiae and, Cunninghamella elegans (11)</td>
</tr>
<tr>
<td>Uranium, copper, nickel,</td>
<td>P. aeruginosa, Aeromonas sp (12)</td>
</tr>
<tr>
<td>chromium</td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>Lysinibacillus sphaericus CBAMS (13)</td>
</tr>
<tr>
<td>Azo dyes effluents</td>
<td>Exiguobacterium indicum, B. cereus, E. aurantiacums and A. baumanii (14)</td>
</tr>
</tbody>
</table>
Some microbes can grow at temperatures as low as −196oF/−126oC and as high as 1200oF/650oC. This adaptability and versatility makes them ideal candidates for remediation [6]. Many genetic traits contribute to the ability of these microbes to function at these temperatures, but other factors are also critical, including bacterial growth rate, high ATP usage, presence of oxygen, soil moisture content, nutrient supply, pH, and competitive species such as protozoa and bacteriophages. All of these factors must be optimized in order to effect the most efficient bioremediation. Enter CRISPR-Cas9.

CRISPR-Cas9 is a remarkable genome editing tool that has been revolutionizing research in virtually every industry and bioremediation is no exception. (2) Through genome editing, a gene can either be introduced, deleted, or up- and down-regulated at a specific site within an organism (7).

CRISPR-Cas9 is an incredibly efficient tool to introduce, “swap” or remove genes - allowing for aggregation of desirable characteristics into a single organism, including specific genes required to break down, adsorb and/or metabolise environmental pollutants. This process alters the native genome in a very precise manner to change the physiological characteristics of an individual microbe.

A report by Martinez-Garcia et al. on the systematic deletion of 11 non-adjacent genomic regions in a Pseudomonas strain is a unique example of genome streamlining in a popular bacterial host with well-defined biodegradation capabilities (5). 300 genes were eliminated from the strain’s genome using homologous recombination after in vivo DNA cleavage. This is an excellent demonstration of how editing a microbe’s genome can enhance a multitude of desirable traits and remove genes that could inhibit the efficiency for remediation. Moreover, due to the higher NADPH/NADP+ ratio, the strains also better tolerated endogenous oxidative stress, a property that provides a crucial advantage for catalysing harsh biodegradation reactions such as aerobic dehalogenation of chlorinated pollutants (5).

“Super” bioremediation solutions can be designed using CRISPR-Cas9 to combine desirable genetic traits from one species, such as Cunninghamella elegans’ ability to chelate lead ions, with Thermus thermophilus’ ability as a carrier to withstand extreme temperatures, creating excellent purpose-specific candidates. (11,15)

CRISPR-Cas9’s versatility allows for the alteration or insertion of virtually any known gene in any organism. This toolkit enables the creation of optimised bio-remediators that have the perfect characteristics to reverse potentially disastrous pollution events such as chemical and oil leaks, safeguarding industrial manufacturing, the water table and the environment.

As the global licensing leader for CRISPR/Cas9, ERS Genomics is the first port of call when developing a commercial or research application using CRISPR/Cas9. This applies whether you’re a new biotech start-up or an established life sciences organisation.

We have already completed more than 100 licence agreements across a range of life science sectors and make patent rights available in more than 80 countries – the most comprehensive collection of proprietary rights to CRISPR/Cas9 available.

Talk to us today to discuss your licensing needs and let our experienced team help you to leverage the power of CRISPR/Cas9.

REFERENCES: