Formulating Biologicals for Agriculture

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Whilst biological products provide a sustainable alternative to chemical pesticides, how do we overcome the formulation challenges they present?

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the seed enhancement company

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Introduction – the opportunity

Whilst chemical products remain widely used in agriculture, there is a clear shift towards more sustainable alternatives – one of which is biologicals.

The reasons for this shift are well known - long-standing chemical approaches to pesticides and soil enrichment come with environmental trade-offs. Environmental laws and consumer pressure raise the cost of using such products. The European Commission is aiming for a 50% reduction of chemical pesticides and hazardous pesticides by 2030, and there are already crops for which the list of useable chemical products is very limited.

Farmers are therefore looking for alternatives. To meet this need, agrochemical companies are looking for new approaches, doing environmental cost-benefit analyses of their existing portfolio, and considering replacement strategies. Biologicals are being widely explored as alternatives to some chemical products, alongside wider crop and pest management strategies, by agrochemical companies as well as startups and academics.

Whilst there are sound long-term economic and regulatory reasons for this, there is also a moral imperative. Many of us in agriculture and chemicals have sustainability goals (including Croda) - promising to care for human health, ecosystems, and the environment. We cannot stand by these credibly if we are not supporting alternatives to chemical products.

Biologicals are effectively products with biological (as opposed to chemical) active ingredients. They comprise a broad spectrum from natural extracts, to microbes, to peptides and proteins, all of which – when delivered correctly - can modify aspects of plant ecosystems in beneficial ways, from stimulating plant growth to killing pests.

What unites these areas is that, instead of delivering conventional chemicals into the environment, we are delivering living organisms or large complex molecules. That changes the game when it comes to formulation. These actives – which are more complex than chemical actives - need not just to be housed and released at the right time, but also kept alive or intact until they are ready for delivery.

These are products designed by nature, not in a lab, so adapting them to suit our needs is a significant challenge. But it is also a big opportunity for the chemicals sector to spur innovation that we expect to eventually lead to better products that deliver more sustainable agriculture.

In this paper we discuss the challenges of formulating and delivering biologicals. It is our hope that this will (1) provide guidance to companies and academics who have discovered useful biological actives, and are thinking about how to turn them into useful products, and (2) help companies across the agriculture value chain – including those interested in partnering with such companies – to understand the process and challenges, and ask the right questions of their suppliers to ensure they get efficacious biologicals with adequate shelf life.

What do we mean by biologicals?

The term can be broad. For the purposes of this paper we will discuss microorganisms (eg fungi, bacteria) or large molecules (eg peptides, proteins, RNA) that can be formulated into products and applied to crops to deliver beneficial results.

These include:

Biofertilisers:

formulations of micro-organisms or molecules that are applied to seeds, plants or soil, to promote growth by making nutrients more available for the plants.

Biostimulants:

microorganisms or molecules that improve plant responses to stress and/or enhance yield quantity and/or quality.

Biopesticides and biocontrol agents:

(micro)organisms or large molecules that kill or debilitate pests.

Microorganisms, whilst still a relatively new area, are the more mature market with a number of practical learnings to share around formulation challenges, and will form the bulk of our discussions. Large molecules are at a much earlier stage when it comes to commercially viable agricultural products but we will consider some of the challenges throughout and discuss specific challenges in the context of RNA interference in the final section.

For clarity, in this paper we are talking about biological ingredients that need to be formulated. We are not talking about the use of beneficial insects, which are sometimes described as biologicals.

Why is it hard to get biological formulation right?

Some biologicals perform similar functions to existing chemical treatments and use similar means of delivery. Others operate quite differently. Either way, there is a need to formulate the active biological ingredient into a product that is delivered to a seed or a crop. Such a process involves carefully combining ingredients with the actives, which may include:

- Rheology agents to create good structure and avoid phase separation
- Dispersants to provide good dispersion of bio particles inside the formulation
- Emulsifiers to ensure that oil in any formulation is evenly dispersed when diluted in water
- Wetting agents to help wet powdered biologicals into the formulation and/or spread the formulation on the treatment surface
- Protective agents such as UV stabilizers to reduce biological degradation

Formulation is well-established and sophisticated for chemical actives, but when the active ingredient is biological, there are different challenges to formulation, chief among them keeping the biological component alive (in the case of microorganisms) or avoiding it being broken down (in the case of large molecules) for example by naturally occurring enzymes in the field.

These are technically hard and inherently complex problems. Biologicals can be harmed by harsh chemicals used in the production process, or by other ingredients in the formulation. They can be damaged by too much water, heat or UV in manufacture or in use.

Whilst a chemical active will likely behave the same way everywhere, a biological's efficacy may be affected by environmental conditions, soil type, the natural microbial population, and even the way it was grown (for example fungi grown under optimal food and light designed to maximise yield may be less biologically robust than those grown in harsher conditions). Different biologicals and different protective formulations may be needed in different environments.

This complexity of interactions and challenges with survival can lead to inconsistent performance which – if not addressed both through formulation, testing data, and transparent labelling - undermines the trust of farmers.

The challenge of biological products

The focus of this paper is formulation and delivery. The creation of new biological actives is a whole topic in itself, for the purpose of this paper, we are asking: 'once an active has been identified and mapped to a target – how do you formulate it into a useful product that remains stable during storage and delivery?'

To illustrate the challenges and solutions, we will look at microbial formulation challenges in seed treatments and foliar applications, before discussing some specific challenges of formulating new RNAi products, one of the most exciting areas of large molecule research.

Seed

Microbes can be applied to seeds as enhancements which have a beneficial effect on the seed once it is planted.

In Northern Europe, production usually starts in October to create seeds due for planting in April/May, so the biological usually needs to stay alive for six months, and ideally 18 so any unsold seeds are still viable the following season (other regions have different start points with similar timescales). There may be some exceptions for shorter-lived microorganisms if they have particular benefits that would justify the added cost of a quick run from factory to farm.

Seed treatment formulations usually involve integrating the active biological, and the supporting formulation ingredients, into a liquid coating that will stick to the seed. This formulation is designed to make sure the active microbe stays alive until it is needed and remains effective once activated.

The first goal is not to kill it when formulating. Many common ingredients can be toxic to biologicals, so there is a need to carefully select and test the right ingredients for the formulation.

The microbe needs to be inactive when applied on the seed and remain inactive until the seed is planted in the soil, which means storing the treated seed in the right conditions for both seed and microbe.

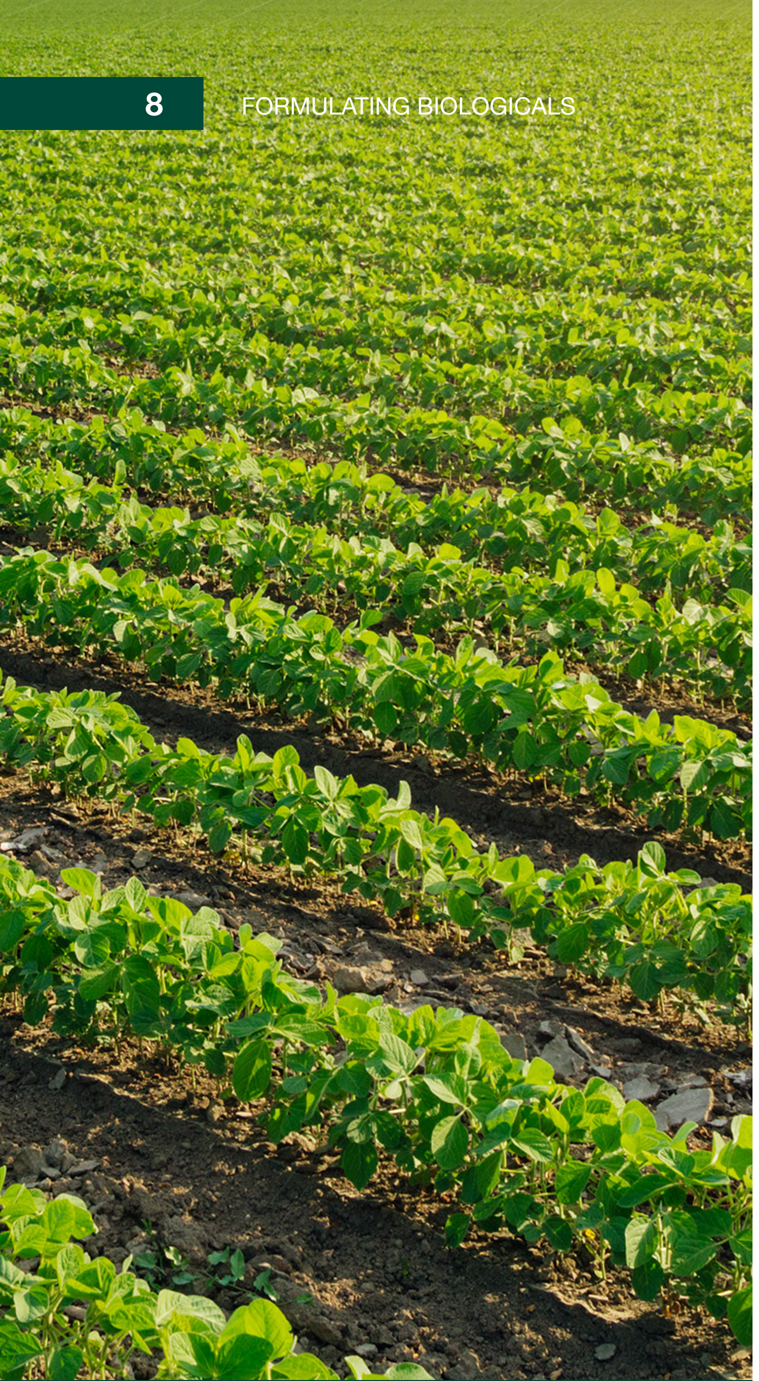
Keeping the microbe dry and safe means applying it onto a seed with the help of a film that will encase it, until it reaches the farm at which point the microbe will be released and can then start growing.

Most seeds are treated using a thin film coating, a mature process widely used in industry and the most cost-effective way of treating seeds at scale, so will always be preferred where possible as it can be easily deployed with existing infrastructure.

But thin films may not be suitable if the microbe is too big or if seeds are not going to be stored in dry conditions. In those cases other options may be better, such as pelleting or encrustment which incorporate the ingredients into much thicker layers and offer much more protection from external elements, or allow ingredients that may interfere with each other to be kept apart.

There are also some newer techniques under investigation, such as biopriming, whereby microorganisms create a biofilm – a protective layer – under certain conditions. We may be able to stimulate this effect during formulation.

The appropriate formulation will be different for every microbe, depending upon the microbe parameters, the seed it is applied to, the application method, and what it needs protecting from. We should start with what is best for the microbe, but we must also consider that industry may find change hard.



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Formulation means choosing a list of ingredients that serve all these needs, but are not toxic to the biological actives.

Foliar

Microbial foliar products face similar but slightly different formulation challenges to seeds.

These products must be formulated into sprays which are retained on a canopy, limiting loss from wind, rain and sun. That will mean formulations that enhance rapid uptake by the leaf, and protection aids eg UV protection. Formulation means choosing a list of ingredients that serve all these needs, but are not toxic to the biological actives, either on their own or when combined.

The need for the final product to be a liquid spray, means we do not have the option of a thick protective coating to safely wrap up the microbes to shield them from moisture and other harmful elements, as we do with seeds. However, many can be formulated into soluble powders and can be vacuum sealed, creating a safe, dry environment where it is relatively easy to keep microbes alive in an inactive state until they are ready to dissolve.

As with seed treatments, these products also need to be stored for six months more, so, with the exception of premium products that may justify investment in refrigeration – most microbes must be selected based on their ability to survive at ambient temperatures.

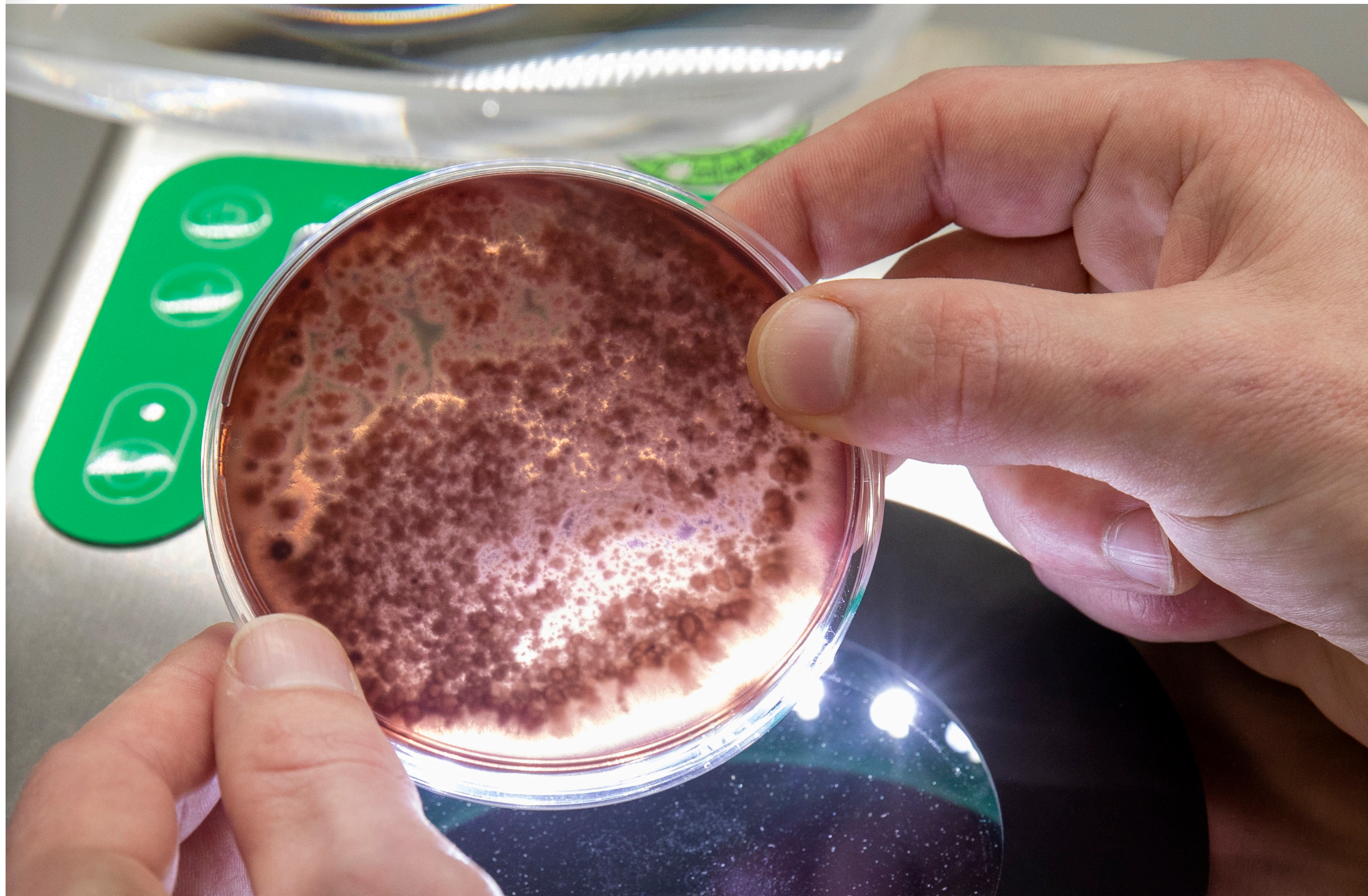
RNAi

The big disruptive innovation in large molecule biologicals is RNA interference (RNAi). This involves delivering RNA into a plant or insect cells, to exploit natural processes in order to silence a gene without altering the plant’s genetics. This is known as ‘SIGS’ or spray-induced gene silencing. That may be for example silencing a plant gene that expresses herbicide resistance, or disrupting the production of a protein that a pest needs to survive.

This has the advantage that it is highly tailored to a single target, and leaves everything else unharmed – unlike current pesticides, which can cause collateral damage.

This is a new technology and there are as yet no commercially available solutions. Promising RNAi products are in development, but so far RNAi-based actives are “naked” – fine in a lab but unstable in the environment and hard to pass critical barriers in targets.

As these develop, making them viable will depend upon formulating them into products that can be delivered into agricultural environments. The challenges are threefold: (1) getting the RNAi into the relevant cell, (2) keeping it intact and in place (eg on a leaf) between being applied and being absorbed, and (3) keeping it intact in storage (avoiding microbial degradation) until it is ready to use.



challenge

1 Getting RNAi into cells

Once applied, the RNA needs to get through cuticles, cell walls and membranes to do its job. This may mean seeping into a plant, or entering an insect that has eaten it off a leaf.

Academic literature shows evidence we can get RNA into plant cells but there is nothing yet commercial in agriculture. The Covid mRNA vaccines used lipid nanoparticles (LNP), tiny balls of fat that encase the RNA strands and deliver them safely into cells. Polyethylene polyplexes are another potential route.

These are relatively mature technology in pharma, but are expensive. However, agriculture does not have the same purity requirements as pharma. This gives us much more flexibility to draw on pharma research but experiment with lower-cost versions of these delivery mechanisms.

challenge

2 Keeping it in place

To be practical for crops, we are mostly looking at formulating these into sprays.

That means the RNA need to stick to the leaf long enough to do their job (eg be eaten by an insect). This is of added importance - many sprays see up to 30% runoff into soil, and this should be avoided for an expensive technology. It also means protecting it from UV, and enzymes that exist in plants and the wider natural ecosystem, both of which will degrade the RNA.

This should be simpler than transfection. Existing technologies for rain fastness on leaf and UV stabilisers can be incorporated into the formulation. But these need to be carefully considered and tested to ensure they themselves do not have unwanted effects on the RNA.

challenge

3 Storage

We've all heard of the complex vaccine cold chains that are needed to keep mRNA vaccines at very low temperatures. That is unlikely to be a practical way to store agricultural products for a year or more.

Fortunately, the agricultural industry has much greater flexibility to add stabilising and protective additives. We can also make changes to the molecule, for example increasing the number of base pairs in the RNA payload, so that it will take much longer to degrade. Beyond formulation itself, local production hubs may also make these technologies more viable.

RNAi is a cutting-edge technology and solutions are still being explored. There are no ready-made answers and research is still needed to work out how to formulate these into deliverable products. For now the goal is to prove delivery concepts, but ultimately costs of raw materials will need to come down to allow mass manufacturing. At Croda, we are keen to join industry conversations around how to do this.

Testing to validate new biological products

When deciding the best formulation, experience can be instructive. But because of the complexity of biologicals, it is impossible to be completely certain of the effects of combining lots of ingredients. Therefore everything needs to be tested.

Firstly, each component of the formulation needs to be tested separately to assess biocompatibility – ie how it impacts the biological active, and at what concentration it might become toxic.

This includes looking at component purity – low purity may mean harmful residues, so increasing purity can reduce harm or increase the potential concentration of a useful ingredient. Higher purity can sometimes double survival rates.

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Then we need to test everything together in the formulation. Some ingredients are fine on their own but create negative effects on survival of the microbes when we blend them. If we rigorously test all ingredients separately, we can have high certainty that the blend will not cause harmful effects, but that is not 100%, so we need to test the final formulation too.

For microorganism-based products, there are three tests we apply over a period of time, under simulated real-world conditions (specific tests for RNAi and other large molecule biologicals will be developed over time, but the immediate focus for these is on design and delivery, so these are not discussed here).

1. **Colony forming unit method:**
   This is a common technique used for both bacteria and fungi, which measures colonies that grow on plates to determine how much of the microbe is alive.
2. **Conidia germination method:**
   Used in fungi to measure its growth as an indicator of its viability by determining the percentage of viable conidia (spores) over time - an indication of the number of cells that remain viable enough to proliferate and form small colonies.
3. **Conidia vigor:**
   A measure of the speed at which a fungi microbes germinate. This is done by processing of images obtained via an optical microscope to determine the conidia vigor over time. This is an important addition to the above tests since some ingredients do not kill the microbe but do slow it down, affecting performance when applied in the field. This is a new method developed and patented by Croda.

These tests are usually done over 180 days (but may be done over years) to provide data and direction about the way the formulation will behave. This is partly about confirming the microorganism will survive, but mostly about understanding the conditions needed for survival in order to benefit from the product, so recommendations for storage and planting can be made.

This early testing can provide a very strong indication of robustness, shelf life and optimal storage and planting conditions. Seed and crop companies will subsequently need to test the formulations under their own factory, greenhouse and field conditions to create absolute certainty. This will likely involve evaluating the efficacy of the products (success at controlling pests, supporting growth etc).

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Conclusion: Getting formulation right

In all of the cases discussed above, we are talking about integrating biologicals – of varying complexity and fragility – into a product that will sit on a shelf in uncontrolled conditions for months and then be introduced into a complex real-world ecosystem.

Formulating biologicals into a product that will overcome these challenges is critical to ensuring the end user – the farmer – gets the value they paid for, and so embraces this sustainable technology.

Doing that means exploring the full range of formulation options. It means looking at libraries of biodegradable chemicals with different properties to explore which are best, and in some cases (especially for RNAi) exploring new delivery approaches.

It means drawing on expertise in formulation to put these parts together in a way that protects and enhances the biological, and does not cause it harm, whilst also being sustainable, scalable and cost-effective.

And it means rigorous testing of the formulation to ensure it does what we want it to in the wide variety of conditions it is likely to be deployed into.

This paper provides a view of the challenges that need to be considered when formulating biologicals. Some can be solved through established technologies and knowledge, others are emerging as challenges that will need a broad range of expertise to solve.

In all cases we welcome industry conversations to address these together.

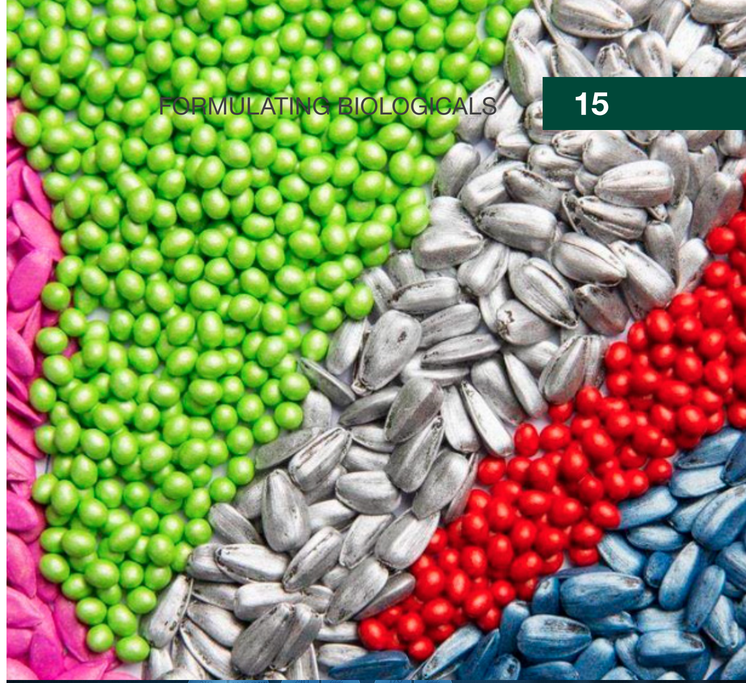
How Croda can help

Croda invites discussion and collaboration with industry and academia to explore and address the challenges of formulating biologicals into viable products.

We have answers to many common questions in this area. We bring a strong understanding of formulating biological and chemical products for delivery into agricultural settings. We have a vast library of speciality chemicals that can help design sustainable formulations and delivery systems for new products.

We have advanced testing facilities for understanding how formulations impact biological actives over time, and identifying optimal storage and deployment recommendations to maximise lifetime and efficacy.

But this is an evolving area and we are also on a journey, adding new knowledge and capabilities all the time. We want to hear from people which biologicals they are struggling to formulate, and from people developing formulation solutions, so we can all come together to solve formulation challenges.



> **We bring a strong understanding of formulating biological and chemical products for delivery into agricultural settings.**

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