National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Educational material for addressing Safe-by-Design in biotechnology: *Cases and guidance*

Case 1: Auxotrophy

Content of the case:

1.1 General information

Biotechnology domain and focus

- contained use;
- industrial biotechnology;
- accidental release of a GMO into the environment.

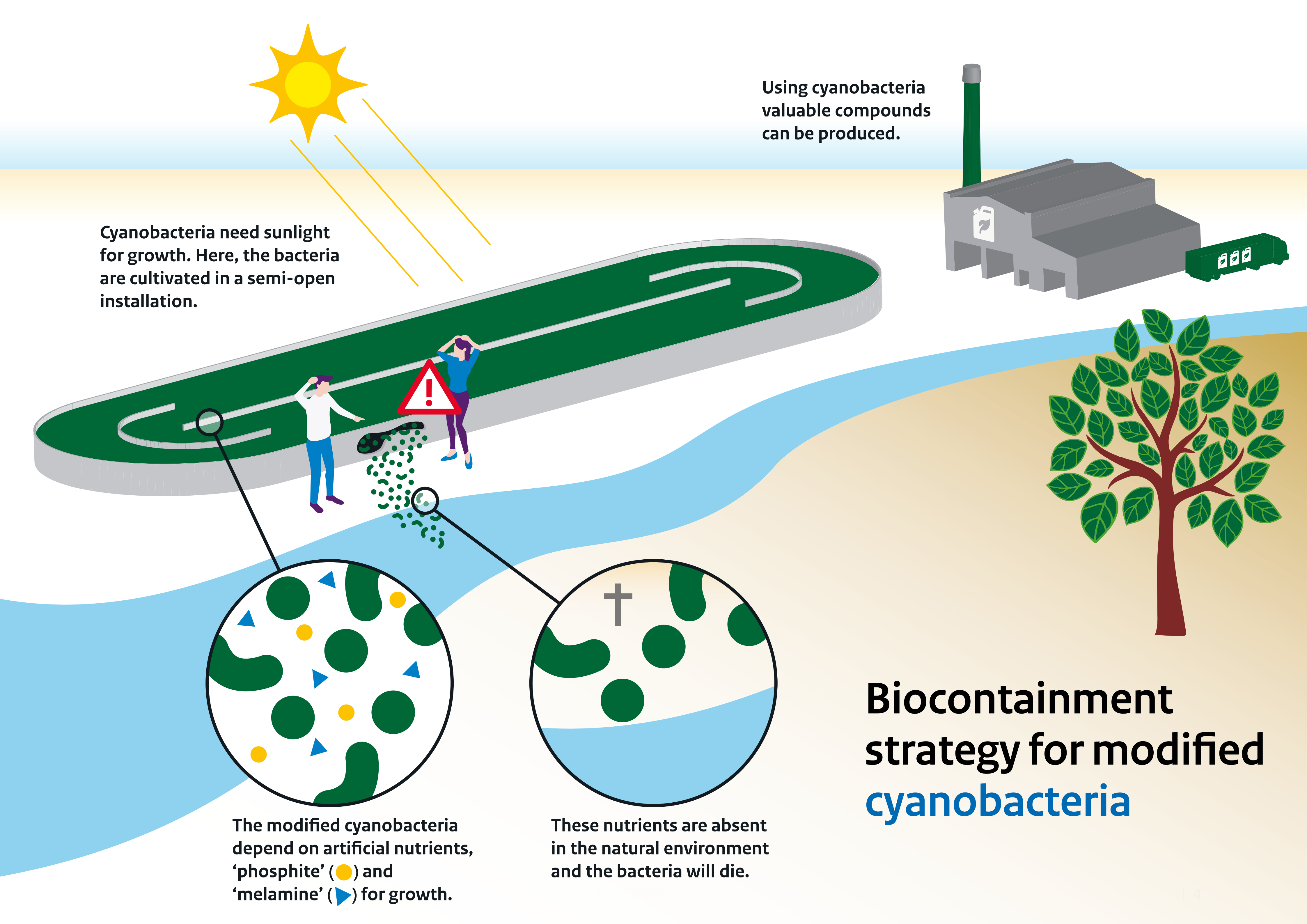
Learning objectives

- understand potential hazards, risks and applicable risk reduction measures;
- list the environmental risks caused by the accidental release of a genetically modified organism (GMO);
- evaluate the added-value of a biocontainment strategy for safety (Safe-by-Design).

Case specific knowledge required

- knowledge of molecular microbiology;
- knowledge of molecular pathway engineering;
- knowledge of large production plants.

Biocontainment strategy for modified cyanobacteria



1.2 Case description

Scientists developed a biocontainment strategy for the cyanobacterium *Synechococcus* by engineering it to depend on nutrients that are not available outside its cultivated environment (the production plant like a photobioreactor or a (semi-)open pond). As a consequence, it is highly likely that it will not be able to thrive if it escapes into the environment. In this particular case, the metabolic pathway of *Synechococcus* was engineered in such a way that it needs non-natural phosphorous and nitrogen sources, i.e. phosphite and melamine, for its growth. In theory, this engineered strain will not survive outside its cultivated environment as both phosphite and melamine are not present in nature.

Origin of case and background information

This case is based on a scientific paper[1] which describes the genetic strategy for designing a biocontainment strategy for cyanobacteria. Proof of principle is at laboratory scale.

Cyanobacteria are phototrophic organisms capable of making sugars out of carbon dioxide and sunlight. There has been much attention given to exploiting these bacteria in industrial production processes to produce biofuels or other valuable compounds like precursors for bioplastics. As their growth is dependent on (sun)light the cyanobacteria are grown in photobioreactors or (semi-)open ponds. Use of genetically modified cyanobacteria in (semi-)open ponds is more cost effective than it is in closed systems, but the possible spread and survival of these GMOs in the environment poses substantial environmental risks. To diminish these risks, a biocontainment strategy that aims to prevent the spread and survival of the GMOs in the environment, might be helpful.

Additional sources

Progress and challenges in engineering cyanobacteria as chassis for light-driven biotechnology
https://www.ncbi.nlm.nih.gov/pubmed/31880868

Ten years of algal biofuel and bioproducts: gains and pains https://link.springer.com/article/10.1007/s00425-018-3066-8

Large scale cultivation of genetically modified microalgae: A new era for environmental risk analysis https://www.sciencedirect.com/science/article/pii/S2211926416305021

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1 https://www.ncbi.nlm.nih.gov/pubmed/31063791

1.3 The group discussion

Theme 1: Potential hazards and risks during the cultivation of GM *Synechococcus*

Background information (for discussion leader)
Synechococcus is an apathogenic microorganism that can safely be handled in a BSL-1 (ML-I) laboratory. Cultivation of genetically modified derivatives of this organism (GMO) on a large scale, e.g. in a photobioreactor or in a semi-open setting, needs additional attention: due to the large cultivation volume, an unintentional or accidental release of the GMO and the consequences for human health and the environment must be considered.

Examples of causes of an unintentional or accidental release:
- failure in system resulting in leakage;
- human error, e.g. operating procedures are not clear;
- uptake and spread of GMO by e.g. birds in case of a (semi-)open pond.

As a result of such a release, the spread of a GMO may cause the following hazards to the environment:
- establishment or dissemination of the GMO in the natural environment,
- spread of genetic material from the GMO to wildtype organisms, so called Horizontal Gene Transfer (HGT).

The risk that a hazard may occur depends on the probability that the hazard may happen. For example, in case of a closed photobioreactor (i.e. there are no open connections with the surrounding environment), the probability of a hazard resulting in a risk to the environment is low. In case of a (semi-)open pond, the probability that a GMO may spread is potential and the identified hazards need to be assessed to determine whether they could lead to a risk to the environment. For example, the hazard HGT can occur if the GMO is able to survive in the environment. It is therefore necessary to collect information on the possibility of the survival of the organism in the environment surrounding the (semi-)open pond, such as:
- Literature research on the biology of wildtype organism.
- Growth experiments of the GMO in water and soil samples collected around the industrial facility.
- Experimental co-cultivation of GMO with wildtype organisms. Is HGT detectable?

Specific to this biocontainment strategy, it is important to consider:
- Elements and activities in the environment surrounding the production plant which are of influence: e.g. industrial complexes releasing phosphor or melamine that could undermine the biocontainment.

Suggestions for the group discussion
The following issues are suggested for discussion:

Hazards
GM *Synechococcus* could be released into the environment because of an unintentional or accidental release from a production plant. Envisage different production plants and discuss:
- How might such an incident happen?
- What kind of hazards could occur?

Risks
How would you assess the probability (e.g. none, low, medium or high) of these hazards occurring and causing risk to the environment?

Risk reduction measures
- In case of a medium or high probability of a risk occurring, what kind of physical measures or working practices would have to be implemented to prevent a release of the GMO into the environment?
- The described biocontainment strategy (see case description) is a risk reduction measure. If this biocontainment strategy is reliable, i.e. the GM *Synechococcus* is unable to survive outside its cultivated environment, what would that mean for the stringency of the physical measures and working practices that would have to be in place?

Biocontainment strategy
In order to use the biocontainment strategy as a risk reduction measure, experimental proof is needed that the GM *Synechococcus* is indeed unable to survive outside its production plant.

Discuss the following issues and determine what proof is needed for the success of this specific biocontainment strategy.
- What data do you need to be able to conclude that the GM *Synechococcus* is unable to survive outside the containment?
- What kind of experiments would you do to demonstrate this?
- Is it possible for the GMO to acquire the necessary genes for using natural N- and P- sources from wildtype organisms?

Theme 2: Expanding the safety perspectives

Background information (for discussion leader)
The group is asked to act as a critical thinker and go beyond the safety aspects discussed under Theme 1.

Suggestions for the group discussion
- Imagine you have designed this biocontainment strategy and are an expert in working with this particular GMO. Your results are, therefore, part of a system: from R&D to future marketing activities. Taking this system perspective into account:
  - What decisions on safety could you, as a scientist, make with regard to e.g. upscaling, the design of the photobioreactor, the site for building a semi-open pond?
  - Where does your influence on decision-making stop? And who else is in charge of taking safety decisions?
  - What kind of safety aspects could be overlooked by others (e.g. by people working in the industrial setting)?
- Regarding the large scale production of GM *Synechococcus* and the fact that many others (people with different skills and knowledge) will handle the GMO.
  - How far do you think their knowledge of safety issues goes? Comparable to you as a scientist?
  - To what extent do you, as a scientist, feel responsible? Are you responsible for the safety of those who are working with the GMM in e.g. industrial processes?
  - If needed, what can you do to help contribute to safety?

1.4 Wrap up

This case illustrates the possible use of a biocontainment strategy for cyanobacteria. In theory, the dependence on artificial nutrients (auxotrophy) for containing of the GMO sounds effective. The robustness of the containment, however, has to be experimentally proven, as some limitations might be foreseen. By broadening the scope in this case from biosafety to Safe-by-Design, insight is gained into the safety issues that could crop up during product development and commercial application. Being aware of these issues in a system approach means thinking about your own role and responsibilities, the success of lab results and the long way to upscaling and commercialisation, and the relevance of early communication with stakeholders.

By taking into account the learning objectives (see Section 1.) the discussion leader can summarise the collective view on the safety aspects of this biocontainment strategy and the additional issues that were discussed in Theme 2.

Options for enriching the learning experience

- Gain insight into possible stakeholder perspectives. For example, by performing a role play in which each group member acts as a different stakeholder.
- Gain insight into the possible surroundings of the production plant and the presence of environmental characteristics and human behaviour that may negatively influence the biocontainment strategy. For example, by asking (sub)group(s) to envisage the production plant and its surroundings using LEGO.

Published by:

National Institute for Public Health and the Environment
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

july 2021

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