

INNOVATIVE SOLUTIONS TO REDUCE THE ENVIRONMENTAL FOOTPRINT OF OLIGONUCLEOTIDES

As interest in oligonucleotide therapeutics begins to accelerate, manufacturers and researchers are working to identify scalable processes that address the environmental burdens of oligonucleotide manufacturing. Ajinomoto Bio-Pharma Services, an industry leader in developing high quality custom oligonucleotides, recently hosted a webinar titled, *Sustainable Approaches to Oligonucleotide Manufacturing*. Yuki Shinohara, an AJIPHASE® team member, outlined the various sustainability concerns around oligonucleotide production and what developers can do to combat them.

One alternative solution is liquid-phase synthesis, which serves as a substitute for solid-phase synthesis and allows for increased manufacturing volume, reduces solvent consumption, and emits significantly less CO₂ than solid-phase. A second solution is utilizing an enzymatic method for manufacturing siRNA, which simplifies the purification process, reduces solvent consumption, and significantly improves the emission factor. Below, Yuki addresses webinar attendee questions about sustainability, oligonucleotide manufacturing, and both alternative processes.

Q: How do you calculate the emission factor of raw materials?

Shinohara: It is quite difficult to calculate accurately. We can estimate the rough value by considering only the solvents that are used during the synthetic route, because the impact of the solvent is much larger than the reagent. In other words, the emission factor of raw materials is calculated from the total amount of solvent used in each step of the synthetic route. In this way, we can estimate the emission factor of raw materials. In general, an emission factor is calculated by dividing the quantity of CO₂ emission (kg) by the quantity of product produced (kg).

Q: How do you go about accurately calculating the emission factor for an amidite?

Shinohara: An amidite is a chemically synthesized form of DNA/RNA used to produce oligonucleotides. Though relatively small amounts of amidites are used in oligonucleotide production, they have a major impact on CO₂ emission. Producing amidites that are monomers of an oligonucleotide entails several synthetic steps, and thereby increases the emission factor. That said, emission factor can be estimated based on several hypotheses.

Emission factor also depends on the amidite type. For example, you normally conduct at least five to 20 chemical reactions from raw materials to reach the final amidite product, especially when using locked amidites such as locked nucleic acid (LNA) and bridged nucleic acid (BNA), which require significant chemical transformation. At each step you must consume organic solvents, reagents, and energy. Amidites are not raw materials but rather, building blocks with large emission factors.

Q: How long does it take to elongate an oligonucleotide using liquid-phase synthesis and does the period of elongation affect the emission factor?

Shinohara: It usually takes one day per one mer elongation at any scale. We ignore the impact of

the period of elongation because the energy, such as light or mortar, has less effect on the emission factor than the amount of organic solvents and monomers used.

Q: Do you have any plans to further attempt to reduce CO₂ in these approaches?

Shinohara: Yes. Recently, we tried to change the hazardous solvent in the elongation process to a less hazardous solvent. We already found a good candidate and are now confirming the side effects due to solvent change. If this goes well, the liquid-phase synthesis process may be able to reduce CO₂ emissions even further than what is shown in the webinar.

Q: Could you share the track record of the production volume when using this technology?

Shinohara: Typically, we have a track record of more than 100kg per batch. AJIPHASE® is a green technology that uses less solvents and reagents than the conventional technology while still providing high-yield batch sizes. This advanced technology allows Aji Bio-Pharma to manufacture commercial quantities of various middle molecules under good manufacturing practice (GMP), without sacrificing purity or quality. In this presentation, I focused on the sustainability aspect of the AJIPHASE® process, but AJIPHASE technology was originally developed to achieve mass production with high purity, which cannot be done with solid-phase synthesis.