Supplementary material 1. List of multiple-choice question stems related to the ESTOPA methodology, including temporal consistency and classification according to the revised Bloom’s taxonomy.

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| **Code** | **Bloom classification** | **Question** |
| 2021/2022\_1.Q1. | B3 | You are studying a new bioreactor designed to remove CO from industrial exhaust. If you decide to maintain high oxygen tension in the reactor, what biological process is being favored, and how should you adjust the temperature to maximize the rate of CO removal? |
| 2021/2022\_1.Q2. | B3 | A team of engineers has modified a crop's symbiotic bacteria to lock the NifA protein in its active conformation, regardless of oxygen presence. If these modified bacteria are grown in a field with excess nitrogen, how will nitrogenase activity be affected and why? |
| 2021/2022\_1.Q3. | B3 | Which of the following sampling points would you choose to have, a priori, higher probabilities of studying denitrification? |
| 2021/2022\_1.Q4. | B3 | A new compound is added to a microbial culture. Initial analysis shows a 2% decrease in the compound after 48 hours, with no change in biomass. Based on these results, propose a test to confirm if the primary limiting factor is the compound's structure or the available electron acceptor, and justify your choice. |
| 2021/2022\_2.Q1. | B3 | In which of the following environments would you take samples to isolate denitrifying microorganisms? |
| 2021/2022\_2.Q2. | B5 | Which of the following strategies is best, from a biochemical and biotechnological point of view, for the elimination of a recently created anthropogenic compound? |
| 2021/2022\_2.Q3. | B3 | A new compound is added to a microbial culture. Initial analysis shows a 2% decrease in the compound after 48 hours, with no change in biomass. Based on these results, propose a test to confirm if the primary limiting factor is the compound's structure or the available electron acceptor, and justify your choice. |
| 2022/2023\_1.Q1. | B3 | Under which of the following conditions would you expect to have greater success in isolating CO2-fixing bacterial species? |
| 2022/2023\_1.Q2. | B3 | Against which of the following enzymes would you design qRT-PCR primers to determine if you have a type II ICL- methylotrophic organism? |
| 2022/2023\_1.Q3. | B3 | If the aim is to isolate nitrifying organisms, which of the following conditions would favor it? |
| 2022/2023\_1.Q4. | B3 | Under what conditions could it be verified whether an organism is respiring Fe3+? |
| 2022/2023\_1.Q5. | B3 | A soil sample shows high levels of arsenate contamination. To design a cost-effective bioremediation strategy, you choose a bacterium that induces the ArsB transporter, what of the next would be necessary steps in the culture medium (nutrients, pH, aeration) to maximize the activity of this specific transporter? |
| 2022/2023\_1.Q6. | B4 | What could you study if you add cyanide and ammonium to a medium for a cyanotrophic organism? |
| 2022/2023\_2.Q1. | B3 | A deep-sea hydrothermal vent sample is used to inoculate a bioreactor with H2​, CO2​, and formate at 85∘C. Predict what of the next primary metabolic products would be synthetized? |
| 2022/2023\_2.Q2. | B4 | Which of the following analytes would you analyze to elucidate what type of nitrate metabolism is being carried out? |
| 2022/2023\_2.Q3. | B3 | Under what conditions could denitrification be studied without confusing the fate of the substrate(s) used? |
| 2022/2023\_2.Q4. | B5 | Which of the following forms would be the most ecological way to raise the amount of free orthophosphate in soils so that it is available for crops? |
| 2022/2023\_2.Q5. | B5 | Which of the following options would be the quickest and cheapest way to determine how 2,4-dinitrophenol is being biodegraded in a new organism? |
| 2023/2024\_1.Q1. | B5 | When trying to enrich a culture in organisms that carry out the degradation of a solid structure, the culture medium evaporated, and the organisms were left without liquid medium; what should be done with that sample? |
| 2023/2024\_1.Q2. | B3 | Under which of the following conditions would you expect to have greater success in isolating CO2​-fixing bacterial species? |
| 2023/2024\_1.Q3. | B3 | Where would you sample to obtain NOB strains with high-affinity NXR? |
| 2023/2024\_1.Q4. | B3 | You isolate a chemolithoautotroph that uses Fe(II). If this organism is placed in an environment where the available Fe(II) concentration suddenly triples, describe two non-obvious ways the cell would adjust its electron transport chain or its gene expression to manage the increased electron flow. |
| 2023/2024\_1.Q5. | B3 | How would you verify that a bacterium is capable of oxidizing Fe2+? |
| 2023/2024\_1.Q6. | B3 | Under what conditions could it be verified whether an organism is respiring Fe3+? |
| 2023/2024\_1.Q7. | B3 | An organism is known to express the ArsB transporter in the presence of arsenate. However, a researcher suspects that there are other inducible transporters that ArsB alone does not reveal. What is the most robust methodological reason to utilize a proteomics approach (as opposed to studying only ArsB via qPCR) in this case? |
| 2023/2024\_1.Q8. | B3 | What aspect would you select to show a solution when there is a chemical limitation in biodegradation due to lack of nutrients? |
| 2023/2024\_1.Q9. | B5 | Choose the option that best reflects an effective strategy to address the biodegradation of recalcitrant xenobiotic compounds: |
| 2023/2024\_1.Q10. | B4 | What could you study if you add cyanide and ammonium to a medium for a cyanotrophic organism? |
| 2023/2024\_1.Q11. | B5 | We know that over the years plastic consumption has increased and pollution in rivers and seas is abysmal, what strategy would be most effective biochemically speaking to improve plastic biodegradation? |
| 2023/2024\_2.Q1. | B3 | Based on what was seen in class, what would you do to culture Asgard archaea? |
| 2023/2024\_2.Q2. | B3 | You are managing a large-scale anaerobic digestor. To specifically increase the population of type II methanogens, you adjust the temperature and the H2​ concentration. Using their known metabolic limitations (e.g., cofactors or energy source affinity), what of the next specific adjustments you would make to the medium (beyond H2​) to maximize their growth? |
| 2023/2024\_2.Q3. | B3 | Under which of the following conditions would you expect to have greater success in isolating CO2​-fixing bacterial species? |
| 2023/2024\_2.Q4. | B3 | Against which of the following enzymes would you design PCR primers to determine if you have a bacterium capable of performing aerobic methane respiration? |
| 2023/2024\_2.Q5. | B4 | In a culture with AOB and NOB, how would you expect to find nitrite throughout the study of a growth and consumption curve using ammonium as a nitrogen source? |
| 2023/2024\_2.Q6. | B5 | Which of the following conditions should we compare to study denitrification only in an experiment where we could also study nitrate assimilation? |
| 2023/2024\_2.Q7. | B3 | Under what conditions could it be verified whether an organism is respiring Fe3+? |
| 2023/2024\_2.Q8. | B3 | A consortium of bacteria is degrading toluene. If you genetically silence the expression of the meta-cleavage pathway enzyme, what would you use as control condition in a differential expression analysis? |
| 2023/2024\_2.Q9. | B5 | What is the best strategy to follow to find microorganisms/enzymes that degrade recalcitrant plastics? |
| 2024/2025\_1.Q1. | B3 | Based on what was seen in class, what would you do to culture Asgard archaea? |
| 2024/2025\_1.Q2. | B3 | To scale up the production of type II methanogens in a biogas plant, you propose increasing the substrate concentration. If you find that the yield unexpectedly plateaus despite the increased substrate, what is the most likely rate-limiting cofactor for type II methanogens, and how would you adjust the medium to solve the problem? |
| 2024/2025\_1.Q3. | B3 | Under which of the following conditions would you expect to have greater success in isolating CO2​-fixing bacterial species? |
| 2024/2025\_1.Q4. | B3 | Against which of the following enzymes would you design PCR primers to determine if you have a bacterium capable of performing aerobic methane respiration? |
| 2024/2025\_1.Q5. | B4 | In a culture with AOB and NOB, how would you expect to find nitrite throughout the study of a growth and consumption curve using ammonium as a nitrogen source? |
| 2024/2025\_1.Q6. | B5 | Which of the following conditions should we compare to study denitrification only in an experiment where we could also study nitrate assimilation? |
| 2024/2025\_1.Q7. | B3 | Under what conditions could it be verified whether an organism is respiring Fe3+? |
| 2024/2025\_1.Q8. | B3 | A newly synthesized organochlorine pesticide is introduced into an ecosystem, how would you design an experiment to determine if some microorganisms are capable of fully degrade this compound? |

***Question repetition details by code***

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| Question Code | Question topic | Repeated in academic years |
| 2021/2022\_1.Q4. | Compound metabolism, low growth | 2021/2022\_2.Q3. |
| 2022/2023\_1.Q1. | Isolation of CO2​ fixers | 2023/2024\_1.Q2., 2023/2024\_2.Q3., 2024/2025\_1.Q3. |
| 2022/2023\_1.Q4. | Fe3+ respiration verification | 2023/2024\_1.Q6., 2023/2024\_2.Q7., 2024/2025\_1.Q7. |
| 2022/2023\_1.Q6. | Cyanide and ammonium in cyanotrophic organism | 2023/2024\_1.Q10. |
| 2023/2024\_2.Q1. | Culturing Asgard archaea | 2024/2025\_1.Q1. |
| 2023/2024\_2.Q2. | Production of type II methanogens (optimized medium) | 2024/2025\_1.Q2. |
| 2023/2024\_2.Q4. | PCR for aerobic methane respiration | 2024/2025\_1.Q4. |
| 2023/2024\_2.Q5. | Nitrite levels with AOB and NOB (growth curve) | 2024/2025\_1.Q5. |
| 2023/2024\_2.Q6. | Conditions to study denitrification only | 2024/2025\_1.Q6. |
| 2021/2022\_1.Q4. | Compound metabolism, low growth | 2021/2022\_2.Q3. |