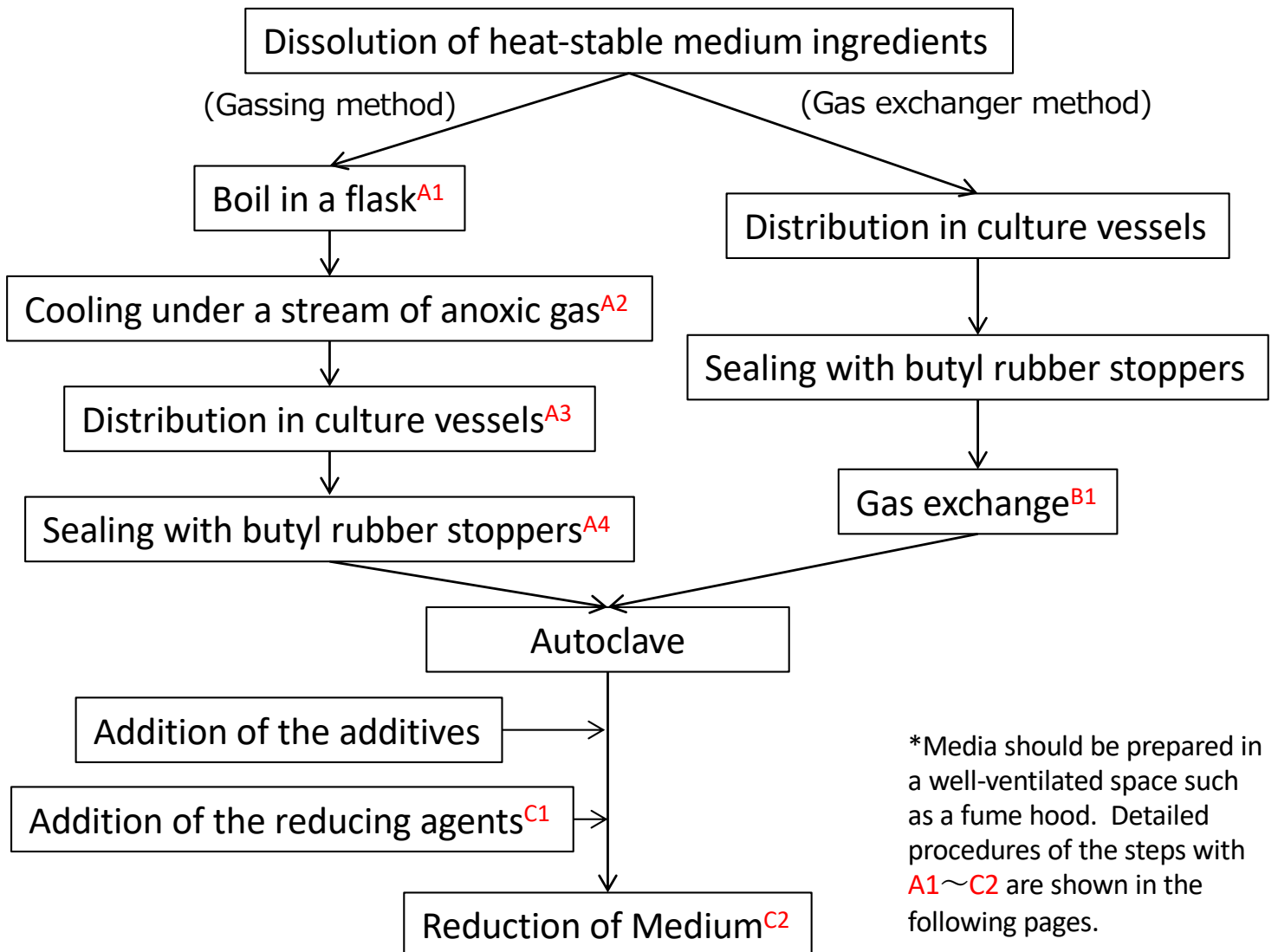


Preparation of liquid media for strictly anaerobes at JCM (1/3)

JCM also provides strictly anaerobic archaea/bacteria such as methanogenic archaea and sulfate-reducing bacteria. This recipe shows how we prepare liquid media for such strictly anaerobic archaea/bacteria.

Flow of the medium preparation

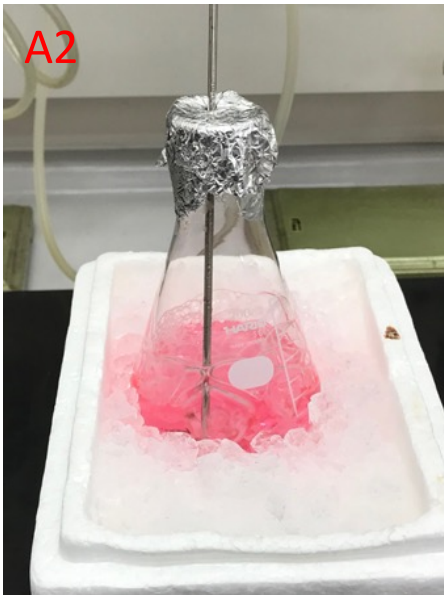


An apparatus for the gassing anoxic gases (in the left hood) and a gas exchanger (gassing manifold) equipped with a vacuum pump (in the right hood)

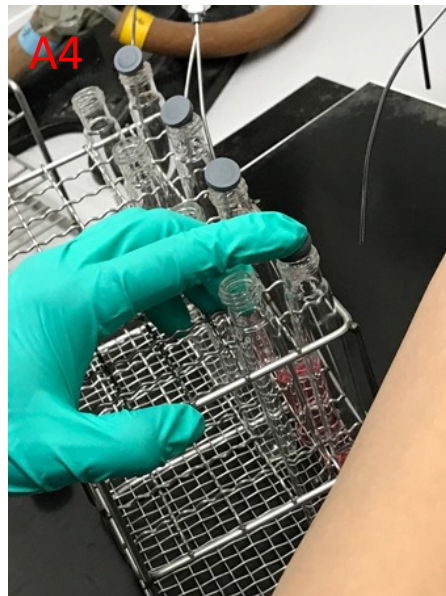
Preparation of liquid media for strictly anaerobes at JCM (2/3)



Prepare a heat-stable solution (not containing the reducing agent, NaHCO_3 , vitamins etc.) in a flask. A few boiling stones may be thrown in it. Put the flask on a burner and bring to a boil. Until then, occasionally, shake the flask slowly.



Bubble anoxic gas through the solution after boiling and during cooling on ice. When the solution is cooled enough, add NaHCO_3 to the solution (alternatively, NaHCO_3 could be added after autoclaving).

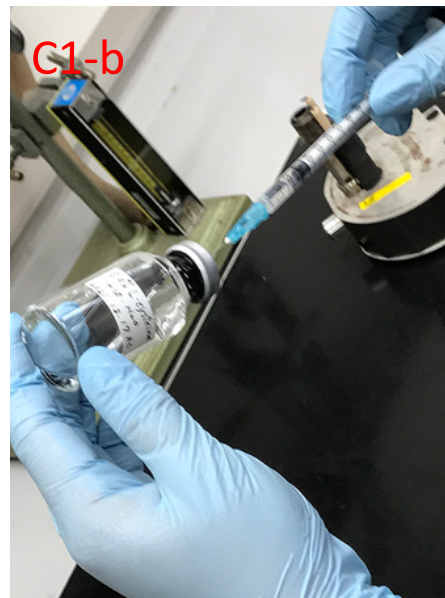
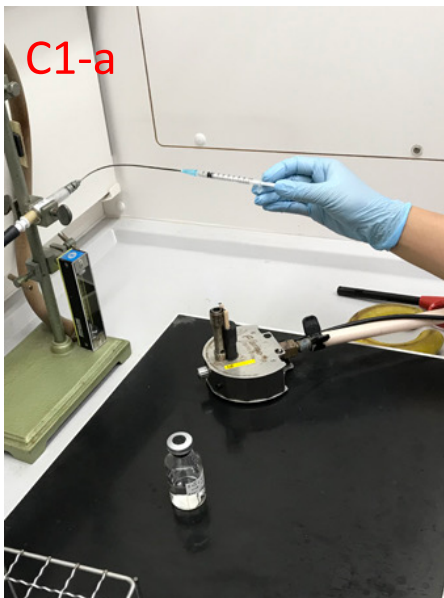


Distribute the solution into culture vessels by a syringe under the anoxic gas (gassing through gassing needles) (A3). Stopper the culture vessels with butyl rubber stoppers while withdraw the gassing needles from culture vessels (A4).

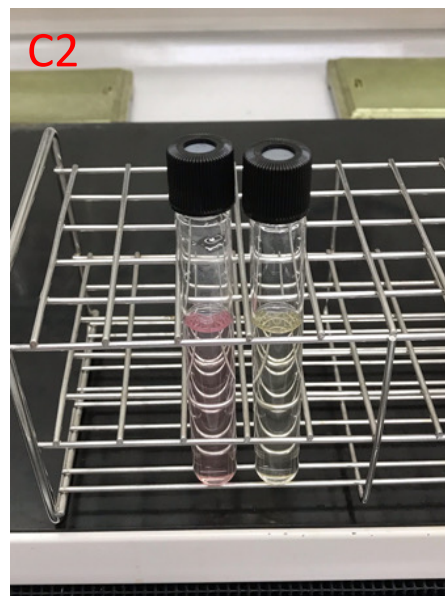
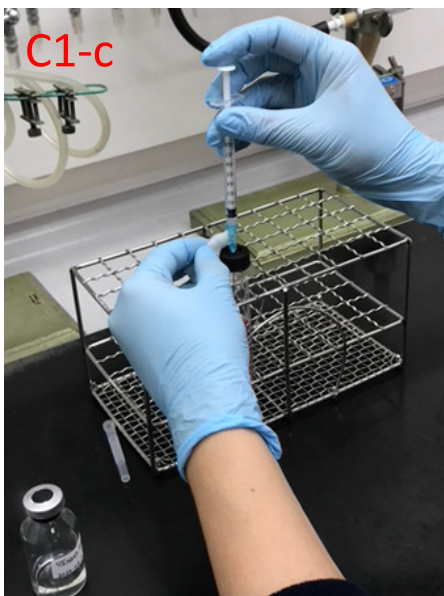
Preparation of liquid media for strictly anaerobes at JCM (3/3)



A gas exchanger (gassing manifold) equipped with anoxic gas cylinders and vacuum pump. It allows to replace gas phases in culture vessels sealed with butyl rubber stoppers by repeating the vacuum-gassing cycles.



Displace the dead air space of a syringe prior to use (C1-a). Then, remove a necessary amount of the reducing agent into the anaerobic syringe (C1-b).



Rapidly, add the reducing agent to the medium in an anaerobic culture vessel (C1-c). When the medium is reduced (left, non-reduced; right, reduced, as judged by the resazurin color change to colorless), the medium is ready to be inoculated (C2).