

## Appendix II

# Decapsulation Procedure for *Artemia* Cysts

### Hydration

1. Hydrate cysts by placing them for 1 h in water (less than  $100\text{ g l}^{-1}$ ), with aeration, at  $25^{\circ}\text{C}$ . This will enable the process to start with spherical cysts, which improves the physical contact with the decapsulation solution.

### Decapsulation

2. Prepare the decapsulation solution with: (a) hypochlorite, (b) an alkaline product, and (c) seawater.
  - (a) The hypochlorite solution can be made up with either liquid bleach,  $\text{NaOCl}$  (fresh product; activity normally = 11–13% w/w), or bleaching powder,  $\text{Ca(OCl)}_2$  (activity normally  $\pm 70\%$ ). Use an amount equivalent to 0.5 g active hypochlorite product per gram of cysts (the activity is normally labelled on the package, otherwise it must be determined by titration).
  - (b) An alkaline product is necessary to keep the  $\text{pH} > 10$ . Use, per gram of cysts:
    - 0.15 g technical grade  $\text{NaOH}$  when using liquid bleach.
    - Either 0.67  $\text{NaCO}_3$  or 0.4 g  $\text{CaO}$  for bleaching powder; dissolve the bleaching powder before adding the alkaline product; use only the supernatants of this solution.
  - (c) Determine the amount of seawater required to end up with a final solution of 14 ml decapsulation solution per gram of cysts.
3. Collect the now hydrated cysts on a  $125\text{ }\mu\text{m}$  mesh sieve, rinse and transfer to the hypochlorite solution.
4. Cool the solution to  $15\text{--}20^{\circ}\text{C}$  (e.g. by placing the decapsulation container in a bath filled with ice water). Add the hydrated cysts and keep them in suspension (e.g. with an aeration tube) for 5–15 min. Check the temperature regularly, since the reaction is exothermic; never exceed  $40^{\circ}\text{C}$  (if necessary, add ice to decapsulation solution). The solution will turn brown–red and release foam. When it turns whitish-yellow it is time to stop the reaction. Check the evolution of the decapsulation process regularly under binoculars.

## Deactivation

5. When cysts turn grey (with powder bleach) or orange (with liquid bleach), or when microscopic examination shows almost complete dissolution of the cyst shell (after 3–15 min), deactivate the hypochlorite with an equivalent amount of 0.1%  $\text{Na}_2\text{S}_2\text{O}_3$  solution.

## Washing

6. Cysts should be removed from the decapsulation suspension and rinsed with water on a 125  $\mu\text{m}$  screen until no chlorine smell is detected anymore. It is crucial not to leave the embryos in the decapsulation solution for longer than strictly necessary, since this will affect their viability. Hypochlorite residues can be detected by putting some decapsulated cysts in a small amount of starch-iodine indicator (starch, KI,  $\text{H}_2\text{SO}_4$  and water). When the reagent turns blue, washing and deactivation have to be continued.

## Use of the Decapsulated Cysts

7. The decapsulated cysts can be incubated immediately for hatching or used directly as food. Alternatively, they can be stored in the refrigerator (0–4°C) for a few days before hatching incubation. For long-term storage cysts need to be dehydrated in saturated brine solution (1 g of dry cysts/10  $\text{ml}^{-1}$  of brine of 300 g  $\text{NaCl l}^{-1}$ ). The brine must be renewed once or twice after 24 h until the salinity of the brine does not drop significantly.