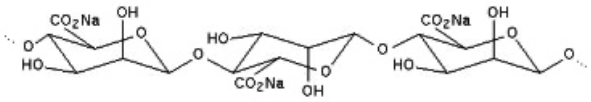
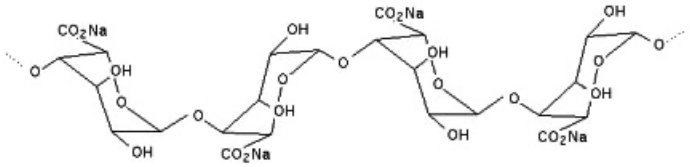
Applied Biochemistry

Immobilization of yeast cells in an alginate gel and fermentation test

Alginate
Alginate is a linear co-polymer of β-D-Mannuronic acid and α-L-Guluronic acid produced by numerous species (algae, microorganisms and plants). The ratio between the two acidic sugars can vary according to the producing species and the growth conditions so in the polymer it is possible to distinguish mannuronic acid-rich or guluronic acid-rich blocks.

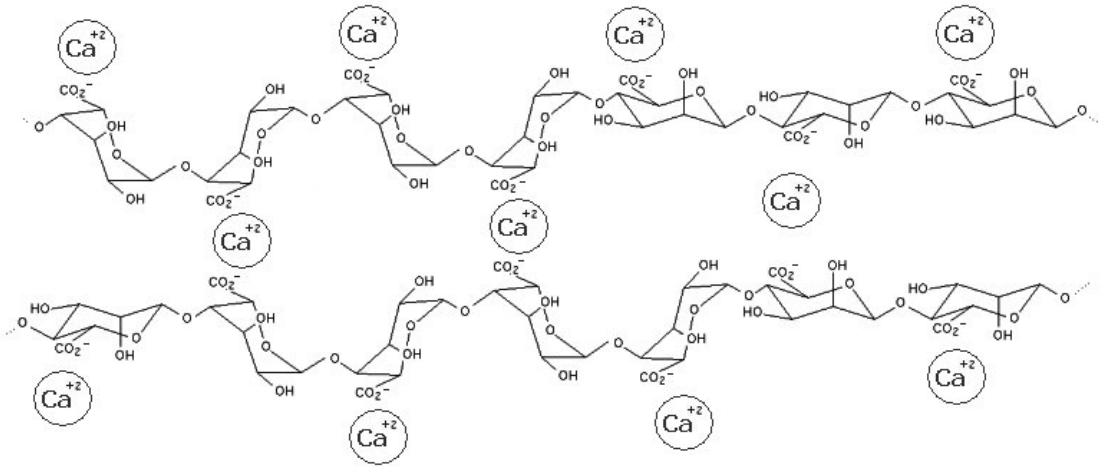


Sodium salt of poly-mannuronic acid



Sodium salt of poly-guluronic acid

The presence of acidic sugars confers the ability to bind divalent cations, in particular calcium ions. These ions can form cross-links interacting with carboxyl groups of non-adjacent sugars belonging to different molecules. The resulting product is a gel with a more or less rigid structure that is able to entrap molecules of various size. Diffusion of entrapped molecules is a function of molecule size and degree of cross-linking of the polymer, which is influenced by alginate concentration and composition, and calcium concentration.



Calcium alginate

Rigid blocks of poly-guluronic acid can bind calcium more efficiently than poly-mannuronic acid-rich blocks, although formation of cross-links depends on the distribution of the different blocks of sugars in the polymer. Alginates rich in poly-glucuronic acid form more rigid gels that are employed in animal feedstock, while alginate used in the pharmaceutic and food industry has a low content of guluronic acid.

Materials:

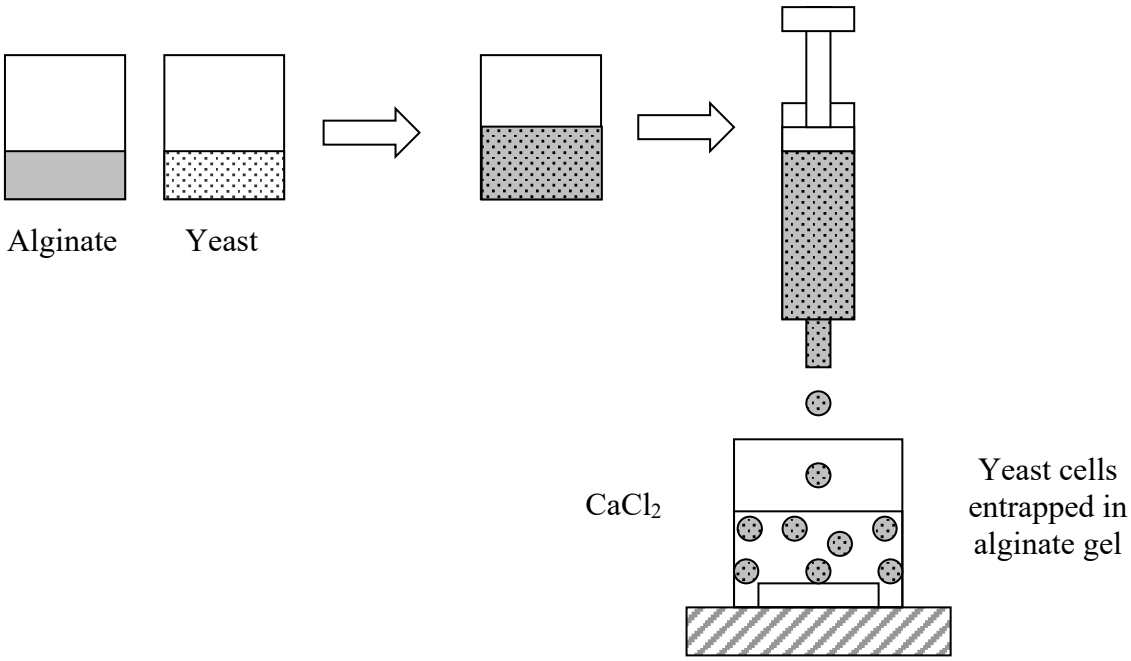
- Sodium Alginate 4%
- $CaCl_2$ 2%
- Yeast, dough or lyophilized
- Water
- Bromothymol blue (BTB) solution + glucose 5%
- Ethanol 70% + bromophenol blue

Equipment:

- Micropipettes p1000
- Balance
- Vortex
- Filter paper
- Beakers to collect $CaCl_2$
- Spoons
- Falcon
- Empty Petri plates

Procedure:

1. Resuspend 1 g yeast in 10 ml water
2. Prepare a 5% (w/v) suspension of yeast cells
3. Mix equal amounts of yeast suspension and alginate (es. 200 ul + 200 ul)
4. Drip the mixture with a syringe or a micropipette in the $CaCl_2$ solution
5. Drain the beads with the paper filter
6. Transfer the beads in the 2 ml tube containing 1 ml BTB + glucose 5% and wait (10-15 minutes, depending on amount and vitality of yeast)
7. Yeast cells will start to consume glucose and produce CO_2, this will lower pH and the colour of BTB will turn from dark blue to yellow
8. To fix the cells and stop fermentation, change the BTB solution with a solution of ethanol 70%


Alginate
Yeast
$CaCl_2$
Yeast cells entrapped in alginate gel