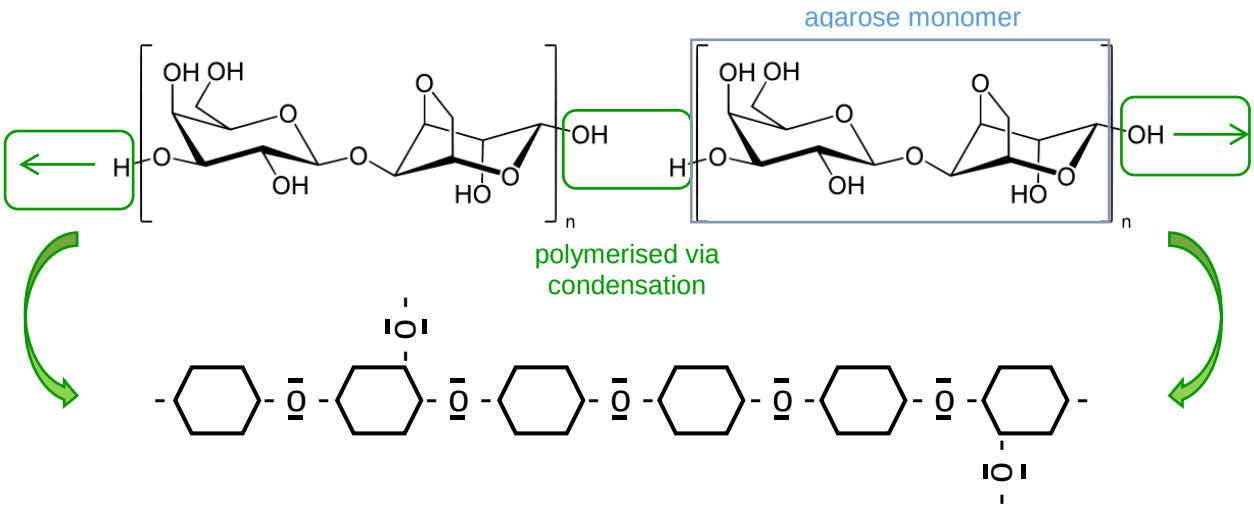
Well advised with Roth.

Why Doesn’t my Agar Gel?

Agar-Agar

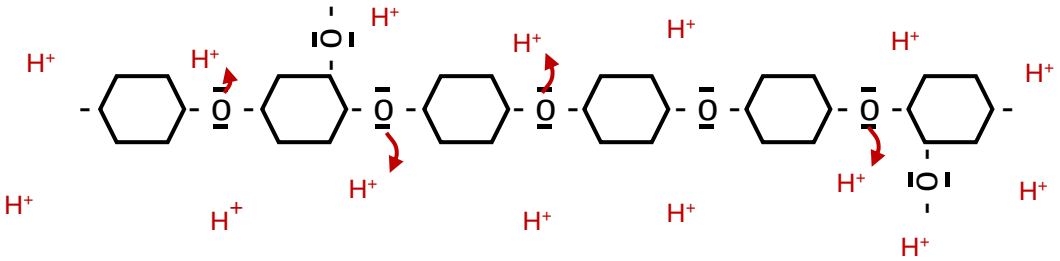
Agar-Agar in fact is a mixture of agarose polysaccharides including residues of impurities stemming from the algae that have been the original material for extraction, with agarose being a dimer of D-Galactose and 3,6-Anhydro-L-galactose (see fig.). After melting and during cooling, agar hardens not by polymerisation, but only via ’interlacing’ of the long, filamentous molecules (starting at a temp. of approx. ≤35 °C). This network is stabilised through hydrogen bonds, electrostatic attraction, and similar weak molecular interactions.



Under Presence of Acid ...

... acidic hydrolysis of the agarose polysaccharides happens (see fig.), resulting in a ‘sugar solution’ which no longer has the ability to harden. This effect is intensified by a) heating during autoclaving (activation energy), b) presence of larger amounts of sugar.

Hence, preparation of sugar containing agar media – which in most cases are meant for cultivation of fungi or yeasts and also have low pH values – often results in soft agar beds even at a pH of 6.0 or little less. Due to the high batch variability of the natural product agar-agar, some batches are even softer than others.



Carl Roth GmbH + Co. KG
Labware · Life Science · Chemicals
Schoemperlenstraße 3–5 · D-76185 Karlsruhe
Phone +49 721 5606-0 · Fax +49 721 5606-149
P.O.Box 100121
76231 Karlsruhe
info@carlroth.com
www.carlroth.com

This effect Typically Happens in the Following Agar Media:

Dichloran Bengal Red Agar (DRBC), Dichloran Glycerol Agar (DG18), Potato Extract Glucose Agar, Malt Extrakt Agar, MRS Agar pH 5.7, Sabouraud Agars, Wort Agar

It Rarely Happens in the Following Agar Media:

Antibiotic Medium Nr. 23, Candida Chromogenic Agar, MRS Agar, Orange Serum Agar

IN Order to Prevent this Effect We Recommend the Following Procedure

Since neither pH value, nor sugar contact may be altered significantly, the agar medium should be autoclaved only very carefully and under reduced heat. We recommend reduction of the autoclaving temperature to 115 °C (118 °C in maximum). Sterilisation phase (core time span of autoclaving) should be reduced to 10 mins. (15 mins. in maximum), since due to heating and cooling, time may be added to the core process.

Background Knowledge

Due to the very high sterilisation impact of heated steam under pressure during autoclaving, not only living bacterial cells but also bacteria spores may be killed very efficiently.

The killing rate is logarithmic. The **decimal reduction time (*D*)**, defined as time span *t* during which (under specified temperature) 90 % of all germs are killed (reduction by factor 10^1, called 10^{-1}), is specific for each bacterial species.

Microbiology generally follows the so called **6D concept**, defining the aim of sterilisation as reduction of the number of living bacteria by factor 10^6 (to 10^{-6}). Therefore, time of sterilisation should be 6fold decimal reduction time ($6D_{temp}$). For sterilisation of foods or clinical instruments, at least 12D concepts are applied, demanding reduction of germs to 10^{-12}.

D-values of vegetative bacteria (*E. coli*, enterobacteria etc.), as well as of yeasts and moulds, generally are very low, ranging mostly under 2 minutes, even at 65 °C (D_{65}), or only in seconds at 121 °C. Heat resistant bacteria often have to be sterilised for hours at 121 °C, prions only can be destroyed at 134 °C for 60 minutes.

Some examples of decimal reduction times at 121 °C

Bacterial Strain	D_{121} (Minutes)	$6D_{121}$ (Minutes)
Bacillus subtilis	0.4-0.8	2.4-4.8
Bacillus cereus	0.03-2.3	0.18-13.8
Bacillus stearothermophilus	2-5	12-30
Clostridium botulinum A and B	0.1-0.2	0.6-1.2
Clostridium sporogenes	0.1-1.5	0.6-9
Clostridium thermosaccharolyticum	ca. 70	ca. 7 h
Escherichia coli	D_{65} - 6 sec.	D_{65} - 36 sec.

s.s. 02/2016