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PAPER

Surfactant-induced coagulation of agarose from aqueous extract of *Gracilaria dura* seaweed as an energy-efficient alternative to the conventional freeze–thaw process†

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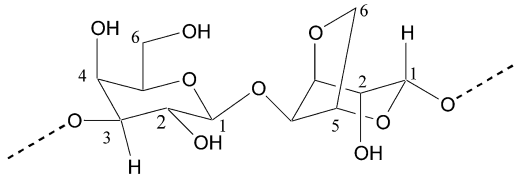
Surfactant-induced coagulation of agarose from alkali-treated *Gracilaria dura* seaweed extract (SE) is reported. The new approach, which was suitable for linear galactans with low sulphate content is an alternative to the traditional energy intensive process of "freeze–thaw" cycles employed for product isolation from the extract. Only nonionic surfactants were effective, and detailed studies were undertaken with octyl phenol ethoxylate (Triton X-100). The coagulated product was successively washed with water and water–isopropyl alcohol (IPA) to yield a fine powder of agarose in 13–15% yield (with respect to dry biomass). The product exhibited excellent properties [sulphate content: 0.2% w/w; degree of electro-endosmosis: 0.13; gel strength: 2200 g cm^{-2} (1% gel, w/v); and gelling temperature: 35 ± 1 °C] essential for demanding molecular biology applications, and the desired gel electrophoretic separation of DNA and RNA was demonstrated. It was further confirmed that there was no degradation of nucleic acids in the gel. The agarose-depleted extract, along with water used for washings, was subjected to reverse osmosis for recovering the surfactant in concentrated form for its subsequent reuse. Energy savings from the improved process were assessed.

Introduction

Agarose is a purified linear galactan hydrocolloid that is isolated from agar or agar-bearing marine algae prepared by the purification of agar. Agar may be depicted by the structural formula shown below. The structure comprises alternating D-galactose sub-units (G) and 3,6-anhydro-L-galactopyranose sub-units (A) linked by α-(1 → 3) and β-(1 → 4) glycosidic bonds. A small fraction of the hydroxyl groups at the 4 position of G and/or the 2 position of A is present in sulphated form.[1,2] Various grades of agarose are reported with sulphate content ranging from 0.10% to 0.35% (w/w). Agarose forms a gel matrix in aqueous medium that is ideal for the diffusion and electro-kinetic movement of biopolymers. This makes it suitable for applications in molecular biology, electrophoresis and cell culture. Agarose is commonly prepared from superior quality agar or agar-bearing marine algae such as *Gelidium* spp., *Gracilaria* spp., *Acanthopeltis* spp., *Ceramium* spp., *Pterocladia* spp., and *Campylaephora* spp.[1] The red seaweed *Gracilaria dura* found in Indian seawater has been reported recently as a promising bioresource for agar preparation.[1]



The process of agarose preparation involves (i) alkali pretreatment of the seaweed followed by autoclaving, (ii) subjecting the aqueous extract to several cycles of freeze–thaw to isolate the product, and (iii) further purifying the product through solvent/chemical treatment and/or chromatography to eliminate residual impurities.[1,3–6] The energy intensive nature of the process, expensive purification steps and long batch time are the prime reasons behind the high cost of the product.

The present work emanated from a desire to explore alternative techniques of isolating agarose from seaweed extract. The rheological properties of agar sol and gel in the presence of various cationic, anionic and nonionic surfactants have been

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