

## Screening the Growth Inhibition Mechanism of Sulfate-Reducing Bacteria by Chitosan/Lignosulfonate Nanocomposite in Seawater Media

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**Abstract :** Sulfate-reducing bacteria (SRBs) induced biofilm formation is a global industrial concern due to its role in the development of microbial-induced corrosion (MIC). Herein, we have developed a biodegradable chitosan/lignosulfonate nanocomposite (CS@LS) as an efficient green biocide for the inhibition of SRBs biofilms. We investigated in detail the inhibition mechanism of SRBs by CS@LS in seawater media. Stable CS@LS-1:1 with 150-200 nm average size and zeta potential of + 34.25 mV was synthesized. The biocidal performance of CS@LS was evaluated by sulfate reduction profiles coupled with analysis of extracted extracellular polymeric substances (EPS) and lactate dehydrogenase (LDH) release assays. As the nanocomposite concentration was increased from 50 to 500 µg/mL, the specific sulfate reduction rate (SSRR) decreased from 0.278 to 0.036 g-sulfate/g-VSS\*day showing a relative sulfate reduction inhibition of 86.64% as compared to that of control. Similarly, the specific organic uptake rate (SOUR) decreased from 0.082 to 0.039 0.036 g-TOC/g-VSS\*day giving a relative co-substrate oxidation inhibition of 52.19% as compared to that of control. The SRBs spiked with 500 µg/mL CS@LS showed a reduction in cell viability to  $1.5 \times 10^6$  MPN/mL. To assess the biosafety of the nanocomposite on the marine biota, the 72-hours acute toxicity assays using the zebrafish embryo model revealed that the LC50 for the CS@LS was 103.3 µg/mL. Thus, CS@LS can be classified as environmentally friendly. The nanocomposite showed long-term stability and excellent antibacterial properties against SRBs growth and is thus potentially useful for combating the problems of biofilm growth in harsh marine and aquatic environments.

**Keywords :** green biocides, chitosan/lignosulfonate nanocomposite, SRBs, toxicity

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