

## **Abstract**

## Abstract

Based on the fact that amyloids have an important role in cell aggregation and biofilm formation in several bacterial species, the amyloid producing bacterial species were isolated from the flocs of activated sludge and checked for their flocculation activity. One of the isolates designated as CR4 demonstrated 55% flocculation activity as compared to other isolates and was selected for further studies. The isolate CR4 was identified as *Bacillus cereus*, hence called *B. cereus* CR4. The amyloid production in *B. cereus* CR4 was confirmed by several tests such as thioflavin T fluorescence, Congo red birefringence assay, scanning electron microscopy, Transmission electron microscopy, FTIR (Fourier transformed infrared) spectroscopy, CD (Circular dichroism) spectra analysis, SDS (sodium dodecyl sulfate)-agarose gel electrophoresis and by sequencing of the purified protein. The protein sequencing analysis showed the presence of TasA protein that is common amyloid protein among *Bacillus* species. Zeta potential analysis revealed electrostatic mechanism of flocculation caused by charge neutralization of kaolin particles by bioflocculant CR4.

*B. cereus* CR4 demonstrated optimal production of bioflocculant at pH 7 and temperature 37°C. Media optimization for optimum bioflocculant production by Central composite design revealed that bioflocculant production is maximum in presence of 0.25% lactose, 0.5% yeast extract and 0.7% tryptone. Studies on flocculation of kaolin with purified CR4 protein demonstrated 61% flocculation activity at acidic pH 5 and 60µg/ml of amyloid concentration. Optimization of flocculation conditions using central composite design reveals optimum % flocculation of 90% at pH 3 and 72mg/l amyloid concentration respectively. The flocculation studies were further elaborated with biomass harvest of microalgae *Scenedesmus*. The growth of *Scenedesmus* was optimized by Central composite design. Optimization studies demonstrated 3.46g/l of biomass production in presence of 125mg% of NaNO<sub>3</sub> and 2.55mg% of ZnSO<sub>4</sub> respectively. Optimization of conditions for harvest of *Scenedesmus* biomass by flocculation with *B. cereus* CR4 revealed maximum flocculation 82.3% at pH, biomass and Fe<sup>+3</sup> concentration adjusted to 4.0, 0.400 (OD 750nm) and 182

mg% respectively.  $\text{Fe}^{+3}$  was used as coagulant for algal cells. It acts by charge neutralization facilitating flocculation of the algal cells by the bacillus biomass. Once the conditions for optimum flocculation of *Scenedesmus* were optimized the ability of *Scenedesmus* was exploited to remove nutrients from synthetic waste water using batch and continuous reactor. Reactor studies demonstrated that *Scenedesmus* sp. could remove 99% nitrate and 43.9% phosphate in batch reactor while 42.95% nitrate and 20.05% phosphate removal was observed at critical flow rate 4.2 mL/min in a continuous reactor.

Biofilm stimulating agents such as Lactose and Manganese at respective concentration of 0.5%, 60mM demonstrated substantial increase in biofilm and cell surface amyloid production in *B. cereus* CR4. Stress agents such as SDS (0.05%), Ethanol (0.5%), DMSO (1.5%) and NaCl (3%) demonstrated increase in amyloid production and flocculation activity of *B. cereus* CR4. Studies on cell surface hydrophobicity and biofilm type shows that all *Bacillus* species demonstrated strong correlation between presence of TasA amyloid and formation of pellicle, except *B. cereus*. The TasA amyloid had a unique role in providing cell surface hydrophobicity of *Bacillus* cells. The median for % hydrophobicity in presence of TasA was 55.7% while the median in absence of TasA was 29.5%.

Also, the effect of D amino acids in preventing biofilms of *Bacillus* spp was investigated. It was found that combination of DL amino acids such as DL methionine, DL tyrosine, DL leucine, DL tryptophan at concentration of 10nm was effective in preventing the biofilms of most of the *Bacillus* spp under study. The gene responsible for producing protein bioflocculant in *B. cereus* CR4 was TasA identified by CDAG (Curli dependent amyloid generator) system and cloned in *E.coli* curli mutant.