sharp, distinct zones around the alginate lyase producing microbial colonies within 2–3 min. Gram's iodine method was found to be better than the CPC method for the clarity of visualization and ease of measurement of zone size. The alginate-lyase-activity region obtained using Gram's iodine method was larger and sharper than the CPC method.

2.2.3 Production of Alginate by *Pseudomonas*

In the presence of *in vivo* conditions, nonpathogenic *Pseudomonas* creates an alginate layer, which prevents the permeation of heavy metal ions into the cell and protects against heavy metal toxicity; this type of alginate secretion could be increased in the presence of NaCl and ethanol in fluorescent *Pseudomonas* species. It is thus possible to deduce that osmolarity and dehydration experienced by the algal cell may be metabolic cues to kick-start production of alginate polysaccharide [15].

2.2.4 Production of Alginate by *Azotobacter* spp.

Azotobacter vinelandii continues to produce significant amounts of acetylated alginate steadily, despite variations of the *in vitro* and *in vivo* conditions. Alginate, an excess metabolite by-product, has a barrier role preventing permeation of toxic heavy metal ions, and conserves cell conditions internally when subjected to environmental and chemical degradative stress. *A. vinelandii* growing diazotrophically in the presence of varying partial pressures of available oxygen, in a fermenter under high shear stress, still forms alginate capsules [16]. When *A. vinelandii* is subjected to higher dissolved oxygen concentration (DO), more compact alginate capsules are formed at higher density; the reason deduced is the increased need for a diffusion barrier to oxygen to seclude the oxygen-sensitive enzyme, nitrogenase, by surrounding the cell with a denser, more impermeable alginate layer.

2.2.5 Influence of Medium Components

2.2.5.1 Effect of Nutrients on Bacterial Alginate Production

Numerous trials have been attempted with changes in the fermentation medium of *Azotobacter* to achieve optimum alginate production. While maintaining the nitrogen level in the culture medium at a fixed level, both favorable and detrimental effects were studied in detail. For *Azotobacter* spp., the accumulated nitrogen may retard the production of alginate [14]. In contrast, peptone used in the medium will alter the alginate production