INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Water is essential to sustain all human related activity. However, water pollution due to different type of contamination limits its use. Among the different contaminating agents that may be present in water, coliforms poses a serious threat to the quality of water as it can lead to several diseases including cholera, dysentery, diarrhoea etc. therefore removal of these microorganisms from water prior to its use is usually carried out by a process commonly referred to as disinfection.

Disinfection is often a chemical unit operation which commonly uses chlorine, hypochlorite, chloramines, chlorine dioxide, bromine and ozone. Among these, chlorine is the most widely used disinfectant for both drinking water and tertiary treatment of wastewaters. Its popularity is due to a high oxidizing potential that provides a minimum level of residual chlorine throughout the distribution system and protects against microbial recontamination. However, it has now been realized that the disinfection by-products due to the chlorination process has both carcinogenic and mutagenic effects on mammals, and therefore, its validity has become questionable. Due to this concern, recent research is focussed towards novel disinfection methods. Compared to the existing chemical methods for disinfection, photosensitization method using photoactive compounds appears more promising due to its easy implementation and low cost. It involves the combination of three non-toxic substance, i.e. a photosensitizer, light and molecular oxygen to achieve the purpose. Although, this technique has been found successful for medical applications, it is not well studied for wastewater applications.

The present work is focused on the effect of different process parameters on inactivation of model microorganisms, viz. *Escherichia coli* and *Enterococcus hirae,* in aqueous solution using two photoactive compounds, viz. methylene blue and sodium anthraquinone-2- sulphonate. To study the effect of parameters, experiments were carried out employing statistically valid full factorial design of experiments. Besides, the effect of municipal wastewater components on photoinactivation was examined. All these experiments were carried out under batch condition.

Initially, the effect of different parameters, viz. concentration of photosensitizer (PS), pH of the bacterial cell suspension and initial cell count, on photo-inactivation of *Escherichia coli* and *Enterococcus hirae* bacteria using methylene blue (MB) and sodium anthraquinone-2- sulphonate (SAQS) was investigated employing full factorial design of experiments. The inactivation efficiency of *E. hirae* using MB ranged between 10.81 and 48.55 %, whereas

in the case of *E. coli* it ranged between10.41-46.44 %. Using SAQS, the inactivation efficiency of *E. hirae* was within 5.26-39.03 %, and in the case of *E. coli* it varied in the range 4.65-37.66 %. Statistical analysis of the photo-inactivation results in the form of analysis of variance (ANOVA) and student't' test revealed significant individual effect of these process parameters. In addition, an increase in dark incubation period with MB or SAQS enhanced the photo-inactivation efficiency against both the microorganisms. Reactive oxygen species measurement and studies of lipid peroxidation assay and protein carbonyl index in these experiments provided a clear insight into the photoinactivation mechanism involved.

The combined effect of the photosensitizers MB and SAQS for inactivating *Enterococcus hirae* and *Escherichia coli* was studied. The photoinactivation efficiency of MB and SAQS in combination and at pH 9.0 varied in the range 32.87% - 49.50% for *E. hirae* and in the range of 27.02% - 37.06% for *E. coli*. Statistical analysis of the photo-inactivation results in the form of analysis of variance (ANOVA) and student't' test revealed significant individual effect of MB on *E. hirae* inactivation but no significant effect on *E. coli,* whereas SAQS had no significant effect on both *E. hirae* and *E. coli* inactivation in this combined study.

In order to study the effect of municipal waste water components on the photoinactivation efficiency due to MB and SAQS on *E. coli* and *E. hirae,* experiments were carried out employing Placket Burman design of experiments. The photoinactivation efficiency due to MB and SAQS was less in presence of wastewater components. Statistical analysis of the photo-inactivation results in the form of analysis of variance (ANOVA) and student't' test showed that presence of synthetic wastewater components have significant effect on inactivation efficiency, with urea having maximum effect. The possible reason for this could be reaction of urea with reactive oxygen species reduces photoinactivation efficiency. In this study SAQS showed minimum inactivation against *E. coli* whereas maximum inactivation is observed with *E. hirae* and MB. Gram –ve bacteria *E. coli* has a lipopolysaccharide membrane which hinders the interaction between photosensitive dyes and the cell membrane and protects the bacteria from inactivation.

The present thesis work has successfully studied the effect of different process parameters and their interaction effect on photoinactivation of *E. coli* and *E. hirae* using photosensitive dyes MB and SAQS. Also the effect of wastewater components on photoinactivation efficiency was examined. Results showed the potential of this technology in photoinactivation of microbes in water and wastewater.