5. DISCUSSION

5.1 Physicochemical parameter of water sample

Physico-chemical parameters were analyzed in ground water samples collected from Rajasthan and Tamil Nadu. From the over all analysis, the concentration of TS, TSS, BOD, sodium and potassium were high in Dharmapuri town ground water samples and the amount of DO, COD, calcium, magnesium, sulphate, phosphate, total nitrogen, ammonium and nitrate were found to be high in ground water samples of Ottapatti village. From the above said parameters, nitrate (90 - 110 mg.l⁻¹), total nitrogen (229 - 813 mg.l⁻¹) and phosphate (102 - 341 mg.l⁻¹) contamination was very high in both Dharmapuri and Ottapatti water samples of Tamil Nadu. The high nitrate, phosphate and nitrogen contamination in ground water may be due to enormous application of nitrogenous fertilizers, organic manures, human and animal wastes and industrial effluents (Hem, 1992; Kross et al., 1992). Nitrate may also be generated in to ground water through conversion from atmospheric nitrogen by bacteria (Fetter, 1993) or through the oxidation of the more reduced species of nitrogen, nitrite and ammonia (Postma et al. 1991).

Nitrate contamination in ground water collected from Kar village of Rajasthan, seems to be very high (460 mg.l⁻¹). The presence of nitrate in these areas may be mainly due to poor vegetation and also due to the nature of the soil. Hallberg and Keeney (1993) reported natural sources, contribute a high concentration of nitrate to the ground water. Natural, mature forests conserve nitrogen but human disturbances can lead to nitrate pollution of the ground water. However, while this is a potential problem for ground water, forests represent a very small source of nitrogen compared to agriculture.

5.2 Bacteriological parameters in water samples and soil

In the present study, the bacterial count was higher in soil because soil contains all the nutrients that support the growth of microorganisms. There was a reduction in the
microbial population of ground water when compared to soil. The genera of *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Micrococcus*, *Moroxella*, *Acinetobacter* and the members of Enterobacteriaceae were identified from both the samples of ground water and soil collected from Rajasthan and Tamil Nadu. Among the bacterial species *Bacillus* spp. were found to be higher in both water and soil samples. *Bacillus* spp. is ubiquitous spore forming resistant bacterial species and may tolerate higher concentration of nitrate contaminated samples.

5.3 Nitrate reducing heterotrophic bacteria

The different heterotrophic bacteria were isolated from water and soil samples collected from Jodhpur districts in Rajasthan and Dharmapuri districts in Tamil Nadu. A total of 52 isolates belonging to different genera (*Bacillus*, *Micrococcus*, *Alcaligenes*, *Moroxella*, *Pseudomonas*, *Corynebacterium*, *Acinetobacter* and members of Enterobacteriaceae) were identified. These heterotrophic bacteria were tested for their ability to reduce nitrate. The results showed that among the 52 isolates, 8 bacterial isolates (two of *Pseudomonas* sp. and six *Bacillus* sp.) were selected as nitrate reducers and screened for nitrate reduction. Among 8 isolates, *Pseudomonas* sp. (RS - 7) was found to be efficient in nitrate reduction. Doudoroff and Palleroni (1974) selected a denitrifying bacterium, *Pseudomonas aeruginosa* for nitrate removal because of its commercial availability, denitrification abilities, and physical versatility. It is commonly found in terrestrial soil and can grow in a variety of low nutrient conditions. Shanthi (2003) have isolated different heterotrophic denitrifying bacteria such as *Alcaligenes*, *Micrococcus*, *Bacillus*, *Corynebacterium*, *Pseudomonas* from lake water and sediment. Patureau et al. (2000) isolated many aerobic denitrifiers from various environments such as ponds, canals and soils or activated sludges and they belong to different genera *Paracoccus*, *Thiobacillus*, *Enterobacter*, *Comamonas* and *Sphingomonas*. Bacteria capable of denitrification are easily isolated from aquatic, sediment, and soil
Acinetobacter sp., Aeromonas sp., Pseudomonas sp., Shewanella putrefaciens, Alcaligenes sp. and Achromobacter were isolated from a hydrogenotrophic reactor and estuarine environments for the denitrification of drinking water (Selenka and Dressler, 1990; Liessens et al., 1992). MacFarlance and Herbert, (1982) reported Aeromonas is one of the most prevalent nitrate-respiring organisms in estuarine environments.

5.4 Nitrate removal

Physical, chemical and biological processes have been developed for nitrate removal from water. Although reverse osmosis, electrolysis and ion exchange are very effective in removing nitrate from contaminated water, they also result in concentrated waste brine, which must still be disposed of (Kesseru et al., 2002). The other methods like ion exchange membrane bioreactor system, catalytic reduction, distillation, chemical denitrification and constructed wetland remediation currently have limited potential for full scale operation and/or their complex process on plant scale and the process is considered too expensive (Kapoor and Viraraghavan, 1997).

Sison et al. (1995; 1996) have studied a denitrification process by using granular activated carbon (GAC) column and dynamic mode of external carbon addition. This process is suitable for water or wastewater containing NO\textsubscript{3} but insufficient organic matters. It was demonstrated that about 87\% of nitrate was removed by adding the organic matters once a day with continuous inflow of 20 mg.l\textsuperscript{-1} of nitrate. By using activated carbon also they can reduce the nitrate from synthetic solution and ground water but it creates disposal problem of solid waste. Due to these limitations for removal of nitrate from industrial waste water and ground water, the most versatile and widely used technology is biological denitrification (Mateju et al., 1992; Kesseru et al., 2002). Ground water with high nitrate content poses a serious threat to the environment and living beings. Microorganisms play a major role in the reduction of inorganic compounds.
Hence biological treatment methods can be followed, which is eco-friendly and uses microorganisms and/or plants to convert the inorganic to some of its byproducts. Here aerobic microbial treatment combined with chemical treatment was attempted to remove nitrate from synthetic nitrate solution and ground water.

5.4.1 Nitrate removal by aquatic plants

The various terrestrial plants like *Phleum pretense* (Friedrich *et al.*, 1977), *Beta*, *Borago*, *Chenopodium* and *Menyanthes* (Salisbury and Ross, 1978), *Borago* (Redden *et al.*, 1995), *Barley* (Kronzucker *et al.*, 1999), *Beta vulgaris* (Santamaria *et al.*, 1999), *Switch grass* (Sanderson *et al.*, 2001), *Duckweed* and *Bluegrass* (Jiang *et al.*, 2001) and *Lemna minor* L. (Smith *et al.*, 2004) were used for nitrate removal in aqueous solution and nitrate contaminated soil. However there were very limited reports on the nitrate removal using aquatic plants. Hence an attempt was made to remove nitrate from synthetic and ground water by aquatic plants (water hyacinth, water lettuce and *Salvinia*).

Aquatic plants are used because they can absorb and accumulate the nutrients from water for a limited period. Young plants are more efficient in the removal of nutrients than the old plants and hence regular harvesting of old plants is essential. If not harvested at proper time, nutrients from the plants are reaching back to the water and old plants after death cause an anaerobic condition in water. Mitchell, (1978) have selected aquatic plant species for wetland remediation based on the some criteria such as rapid and relatively constant growth rate, ease of propagation, capacity for absorption of pollutants, tolerance of hyper eutrophic conditions and ease of harvesting and potential usefulness of harvested material.

The aquatic plants like water hyacinth, water lettuce and *Salvinia* were used to study their efficiency in nitrate removal. In aqueous solution, water hyacinth was efficient in removing nitrate to a maximum in all the concentration (100, 200, 300, 400...
and 500 mg. l\(^{-1}\)) when compared to water lettuce and salvinia. Report by Larson et al. (2001) on hydroponic experiments with switch grass starting at 273 mg. l\(^{-1}\) nitrate the grass removed nitrate to undetectable levels within 6 days. The floating aquatic plant Hornwort was capable of removing nitrate from an initial concentration of 350 to 75 mg. l\(^{-1}\) in 9 days. It removed on an average about 2 mg. l\(^{-1}\) nitrate per day per gm of plant. One acre of hyacinth can remove 1394.5 kg. N/year (Rogers and Devis, 1972). In 100 and 200 mg. l\(^{-1}\) concentration of nitrate, water hyacinth removed maximum level of nitrate whereas in higher concentration (above 300 mg. l\(^{-1}\)) the amount of removal was very less. About 64% and 80% nitrate uptake was recorded at 100 and 200 mg. l\(^{-1}\) of nitrate respectively by water hyacinth. But water hyacinth will take more duration (10 days) for nitrate uptake and water hyacinth can increase water loss through evaporation at a far greater rate. In 500 mg. l\(^{-1}\) nitrate concentration, the plants were not able to remove nitrate and it may be due to presence of higher concentration of nitrate which do not support the uptake. Only about 63.2% (from 460 to 166 mg. l\(^{-1}\)) nitrate was utilized by water hyacinth in water samples collected from Rajasthan.

5.4.2 Nitrate Reduction by Heterotrophic Bacteria

Nitrate, the most abundant form of nitrogen in the biosphere after atmospheric dinitrogen, is the N source used most widely by living organisms, including higher plants, algae, fungi and bacteria (Guerrero et al., 1981; Stewart, 1988). Assimilatory nitrate utilization involves the reduction of NO\(_3^-\) to NO\(_2^-\) in a two-electron reaction mediated by nitrate reductase, and further reduction of NO\(_2^-\) to NH\(_3\) involves six electrons in a reaction mediated by nitrite reductase (Guerrero et al., 1981). Some facultative anaerobes are also able to use NO\(_3^-\) as an alternative electron acceptor in respiratory chains (Zumft, 1992). This inorganic form of nitrogen is a pollutant and its elimination is of high priority to environmental protection agencies. Its presence at relatively low concentrations usually does not limit its uptake, as most organisms have active NC\(_3^-\) uptake systems.
(Fossing et al., 1995). Indeed complex microbial communities in biological treatment plants are able to cope with low concentrations of nitrate (< 50 mg.l⁻¹) in a similar manner to those found in natural environments (Fossing et al., 1995).

Most denitrifying bacteria, however, are heterotrophic. They require an organic carbon source for cell growth and nitrate reduction. The best characterized heterotrophic bacteria are the genus *Pseudomonas*, capable of nitrate and nitrite reduction with succinic acid, ethanol and acetic acid as carbon sources (Kesseru et al., 2002). In the present study, the bacterial strains *Pseudomonas* sp. (RS 7), *Bacillus* sp. (RS 11), *Pseudomonas* sp. (RW 21), *Bacillus* sp. (DS 1), *Bacillus* sp. (DS 5), *Bacillus* sp. (DS 7), *Bacillus* sp. (DS 9) and *Bacillus* sp. (DW 6) were screened for nitrate reduction and *Pseudomonas* sp. (RS 7) was selected as best nitrate reducers. Tolerance to nitrate in these strains was assayed by determining the growth after the exposure of cultures to different NO₃⁻ concentrations.

There was no inhibition in the growth of all the strains of bacteria at higher concentration (< 500 mg.l⁻¹), which means that some bacterial strains have the ability to tolerate high NO₃⁻ concentrations. In this assay, the strain *Pseudomonas* sp. (RS 7) showed resistant to higher concentration, growth rate and nitrate removal than the other strains. This makes *Pseudomonas* sp. an excellent strain for the removal of nitrate from ground water having high amount of nitrate (Kornaros et al., 1996; Kesseru et al., 2002).

### 5.4.2.1 Effect of carbon sources on nitrate removal

The studies on nitrate reduction from synthetic medium supplemented with different concentration (1 %, 2 % and 3 %) of carbon sources with 100 to 500 mg.l⁻¹ of nitrate by *Pseudomonas* sp. (RS 7) was carried out under aerobic conditions. From the results of the above study conducted with different and various concentration of carbon sources (glucose, starch and cellulose) inoculated with *Pseudomonas* sp. (RS 7) the following criteria were drawn. The selected species *Pseudomonas* sp. (RS 7) was capable
of removing maximum concentration of nitrate in all the concentrations of nitrate in synthetic medium with 1% starch as carbon source when compared to glucose and cellulose. The removal of nitrate was decreased when the concentration of starch (2% and 3%) increased. From the above results, it was inferred that, due to higher organic load the growth was affected. Hence, starch at 1% concentration was used as carbon source for nitrate removal in the water samples collected from Rajasthan and Tamil Nadu.

In the present study, about 86% (500 to 71 mg.l\(^{-1}\)) of nitrate removal was achieved in MSM supplemented with 1% starch by *Pseudomonas* sp. (RS 7) after 72 hrs at an optimum temperature of 30°C. The nitrate reduction was high at high concentration (<500 mg.l\(^{-1}\)) of nitrate supplemented with 1% starch and this may be due to the fact that nitrate at 500 mg.l\(^{-1}\) may act as source of nitrogen for growth and energy. The studies of Smith (1983) showed that the concentration above 6000 mg.l\(^{-1}\) inhibits the cell growth and thus reduces the rate of nitrate reduction. To quantify nitrate reduction kinetics of bacterial isolate three different carbon sources were tested separately along with control without carbon source. The results showed that the carbon sources influenced the growth and higher level of nitrate reduction under aerobic condition. Methanol, ethanol and acetic acid were commonly used as organic substrates to provide the reducing power for nitrate elimination (Bockle *et al*., 1986; Dahab and Young, 1988; Liessens *et al*., 1993; Bohler *et al*., 1994). Mateju *et al*., (1992) indicated that 3.0 kg of methanol was required to remove 1 kg of nitrate nitrogen rather than the stoichiometric amount of 2.47 kg. The results of this study demonstrated that all the bacterial isolates could utilize all the four carbon sources to achieve a high level of nitrate reduction (Kesseru *et al*., 2002). Shanthi (2003) achieved nitrate removal in synthetic wastewater with 50 mg.l\(^{-1}\) of nitrate amended with various carbon sources such as glucose, sucrose, cellulose and acetic acid by microbial consortium under aerobic and anaerobic conditions. The results revealed
acetic acid as the suitable carbon sources by bacterial consortium that reduced about 99.26 % of nitrate in synthetic wastewater.

It was observed that the carbon sources influenced the pH of medium during nitrate reduction under aerobic condition. There was very negligible pH change in synthetic medium with carbon sources during nitrate reduction under aerobic condition. The pH change was shown to be a clear indication of progress of nitrate reduction reaction, including the ability to discriminate between the negligible pH effects of nitrate reduction to nitrite and the pH increase was associated with the reduction of nitrite to non-ionic nitrogen products (Glass and Silverstein, 1998).

During nitrate reduction, accumulation of significant amount of nitrite and ammonium was observed in media, both with and without carbon sources. The accumulation of nitrite in bacterial culture may be in principle due to either assimilatory or dissimilatory nitrate reduction or due to heterotrophic nitrification. However it is unlikely that nitrite accumulation results from nitrate assimilation since nitrate and nitrite reduction should highly coupled during assimilation. Under anoxic conditions, many species of bacteria are able to couple the generation of a transmembrane proton electrochemical gradient to the reduction of nitrate to nitrite, catalyzed by a respiratory nitrate reductase. The nitrite so generated can be further reduced either to ammonia, or to nitric oxide, nitrous oxide, and dinitrogen (Cole, 1988; Kuenen and Robertson, 1987). The nitrite reduction rate under aerobic conditions was very low and results from previous work with A. faecalis (Otte et al., 1999) indicated that the observed N2O production may not be due to reduction of NO2-, but may be a by product of heterotrophic nitrification. Low NO3- supply and a non-limiting supply of fermentable substrate favours rapid reduction to NH4+, high NO3- and limited energy source appear to permit reduction only to NO2-. Recently, Kornaros et al. (1996) have shown that nitrite
was accumulated in the cultures of *Pseudomonas denitrificans*, ATCC 13867 when nitrate was present at higher concentrations.

Many investigators (Tiedje, 1981; Wilderer *et al.*, 1987; Veylovsec *et al.*, 1994; Kornaros *et al.*, 1996; Rijn *et al.*, 1996) have observed that nitrite can inhibit denitrification, especially at high concentration. Accumulation of extracellular nitrite has been reported during denitrification in pure cultures implying that, under some conditions, denitrifying bacteria transport the nitrite intermediate out of the cell and later take the extracellular nitrite back in to the cell for complete denitrification.

The reduction of nitrate from synthetic and ground water with carbon sources by bacterial isolates was found high in aerobic conditions. There has been considerable speculation concerning the physiological role and the ecological implication of the co-respiration of nitrate and oxygen (Lloyd, 1993). The bacterial isolate *Pseudomonas* sp. (RS 7) used for this study was able to respire nitrate in the presence of oxygen. Recently, a member of the genus *Comamonas* has been isolated from an up flow anaerobic filter (where conditions are not strictly anoxic) and is able to respire nitrate in the presence of oxygen. It was suggested that the co-respiration of nitrate and oxygen might offer a physiological advantage in environment subjected to fluctuating oxygen availability.

Nitrate respiration has been observed under oxic conditions in a number of bacteria, including *E.coli*, *P.aeruginosa*, and *Paracoccus denitrificans* LMD 32.5 (Lloyd *et al.*, 1987; Davies *et al.*, 1989; Brons and Zehnder, 1990; Robertson and Kuenen, 1990; Ludwig *et al.*, 1993; Thomas *et al.*, 1994).

In aerobic condition the predominant fate of \( \text{NO}_3^- \) reduction is generally believed to be reduction to \( \text{N}_2\text{O} \) and \( \text{N}_2 \) by bacterial respiration (denitrification). In our experiments nitrate reduction has led to the formation of nitrite and ammonium which means the bacterial genera in our study may have catalyzed an alternative reduction
pathway, dissimilatory reduction of nitrate to NH$_4^+$ (Payne, 1973; Scott and DeMoss, 1976). There is no fundamental argument why denitrification cannot occur under oxic condition. However, only during the past few years this activity received some attention (Berks et al., 1995; Robertson et al., 1995; Gupta, 1997; Patureau et al., 1998).

### 5.4.2.2 Effect of various temperature on nitrate removal

The temperature effect on the denitrification rate is another important feature in the design of a denitrification process (Orhon et al., 2000; Carrera, 2003). Konishi (1969) have reported incubation near 30°C generally, but not always favor denitrification. Thermophilic and psychrophilic denitrifying bacteria are known to have different temperature optima than do the mesophiles. High temperature optima reported were possibly a reflection of nitrobiological reactions (Bremmer and Shaw, 1958). Carrera (2003) have studied nitrate removal by activated sludge process in aerobic bioreactor at various temperatures (6, 8, 10, 15, 20 and 25°C). In this study he observed maximum nitrate removal at 25°C (79 to 86 %) after 24 hrs. In the present study, about 85.8 % (from 500 to 71 mg.L$^{-1}$) of nitrate reduction was recorded at 30°C whereas the percent removal was less when the study was carried out at lower temperatures. Because the lower and higher temperature might have affected the bacterial growth in synthetic medium containing nitrates.

### 5.4.2.3 Nitrate reduction by Pseudomonas sp. (RS 7) from ground water samples

Nitrate removal was also carried out in water samples by *Pseudomonas* sp. (RS 7) collected from Rajasthan and Tamil Nadu. In Tamil Nadu water samples, the nitrate level was reduced below the permissible limits (< 45 mg.L$^{-1}$) after bacterial treatment whereas, in Rajasthan water sample the nitrate reduction level was about 50.2 and 61.6 mg.L$^{-1}$ by treatment II (sample + starch + bacteria) and III (sample + starch – MSM + bacteria) respectively. This might have been due to the initial higher concentration of nitrate in the
water collected from Rajasthan. During removal of nitrate, the growth of *Pseudomonas* sp. (RS 7) in Tamil Nadu water containing 110 mg.l⁻¹ of nitrate with 1% starch was 98 x 10⁴ CFU.ml⁻¹ at an optimum temperature of 30°C after 72 hrs whereas in Rajasthan water (460 mg.l⁻¹ of nitrate) the growth was found to be 72.5 x 10⁴ CFU.ml⁻¹. The higher nitrate concentration influenced the bacterial growth as well as nitrate reduction when compared to lower (<500 mg.l⁻¹) concentrations.

Driscoll and Bisogni (1978) used *Thiobacillus denitrificans* to reduce nitrate concentration from 24 to 1 mg.l⁻¹ in packed bed reactors using elemental sulfur or sulfide as an electron source. Lewandowski et al. (1987) encapsulated autotrophic denitrifiers in calcium alginate beads containing sulfur and calcium carbonate to evaluate autotrophic in a completely mixed batch reactor. Here nitrate concentrations were reduced from 27 to 6 mg.l⁻¹ in seven hours. Kurt et al. (1987) studied hydrogenotrophic denitrification using a fluidized bed sand reactor. The optimum pH for nitrate removal was 7.5. Nitrite accumulated when the pH was above 9. A residence time of 4.5 hrs was required for complete denitrification of water that contained 25 mg.l⁻¹ of nitrate nitrogen. Clifford and Liu, (1993b) developed a combined process using ion exchange and a sequencing batch reactor (SBR) for the biological denitrification of 0.5 N sodium chloride spent regenerant solution containing up to 835 mg.l⁻¹ of nitrate nitrogen. Complete denitrification of spent 0.5N NaCl brine was achieved in 20 hrs using a methanol to nitrate nitrogen ratio of 2.2. More than 95 % denitrification was achieved in 8 hrs using a methanol to nitrate nitrogen ratio of 2.7.

### 5.4.2.4 Nitrate reduction by inorganic coagulants

During bacterial treatment supplemented with 1% starch, the 100 % efficiency in the removal of nitrate was not attained. Hence attempt was made to remove the remaining concentration of nitrate using chemical coagulants (Alum, Lime and PAC). Using lime at
150 mg.l\(^{-1}\), the removal was 99.32% followed by PAC (98.93%) from the bacterial treated synthetic medium containing 70 mg.l\(^{-1}\) of nitrate. Whereas in the case of alum at 150 mg.l\(^{-1}\) only 88% (from 70 to 8 mg.l\(^{-1}\)) of nitrate removal was noticed.

In ground water sample collected from Kar village of Rajasthan about 93.8% (from 50.2 to 3.1 mg.l\(^{-1}\)) and 90.24% (from 50.2 to 4.9 mg.l\(^{-1}\)) of nitrate removal was observed in the bacterial treated sample (Treatment II) containing 50.2 mg. l\(^{-1}\) of nitrate using lime and PAC at 150 mg. l\(^{-1}\) level respectively. Whereas in the case of alum at 150 mg. l\(^{-1}\) only 74.3% (from 50.2 to 12.9 mg.l\(^{-1}\)) removal was noticed. The maximum removal (93.8%) was recorded at 150 mg. l\(^{-1}\) of lime followed by 100 mg.l\(^{-1}\) (92.3%). Very negligible amount of nitrate reduction was recorded when the coagulant dosage was increased above 200 mg.l\(^{-1}\). About 81% of nitrate was removed by this coagulant at 150 mg.l\(^{-1}\) in the bacterial treated sample (Treatment III) containing 61.6 mg. l\(^{-1}\) of nitrate. In treatment I the percentage of nitrate removal was very less when compared to Treatment II and III using alum, lime and PAC at 150 mg.l\(^{-1}\) because of less bacterial growth during nitrate reduction that interfered the precipitation of bacterial cells and nitrate.

According to World Health Organization the permissible limit of nitrate in drinking water is 45 mg.l\(^{-1}\) of nitrate (Lunkad, 1994). In the present study using bacterial inoculum, the nitrate was not reduced below the permissible limit. Subsequent chemical treatment with lime and PAC at 150 mg.l\(^{-1}\) brought down the concentration of nitrate below the permissible level. Hence the bacterial treatment followed by coagulant (lime and PAC) treatment may be the effective method or treating nitrate contaminated water. The use of bacterial cultures before chemical treatment was not only useful in reducing nitrate but also the bacterial biomass was useful for the attachment of chemical coagulants and quicker precipitation. Grabow et al. (1978) and Dzinkiewicz et al. (1989)
have reported that lime acted as an effective precipitant for phosphate and many trace metals and bacteria and as a coagulant for the removal of suspended and colloidal materials in municipal wastewater. Coagulation process removed 74 - 99.4% of *E. coli* and Coliforms (Bitton, 1994). Under laboratory conditions, coagulation and flocculation was effective in removing 90 - 99% of viruses from water (Bitton, 1980). Removal of protozoan cysts by coagulation and sedimentation may also exceed 90% (Bitton, 1994).

5.5 Sand filtration

Coagulation reduces bacterial cells and particles from water to the flocculated materials and hence sand filtration was carried out to remove turbidity, coagulated particles and to purify the water samples. In the present investigation, the bacterial count of treatment I and III at the end of 72 hrs was comparatively less than that of treatment II. So sand filtration was used for the removal of bacterial cells. About 81% (73 to $1 \times 10^4$ CFU.ml$^{-1}$) of the bacterial cells were eliminated in Rajasthan water samples amended with 1% starch (treatment II) after sand filtration, the efficiency of which depends on the filter medium, concentration and type of solids to be filtered out, and the operation of the filter. Similarly in Tamil Nadu water sample (treatment II) about 83.5% (98 to 17 CFU.ml$^{-1}$) of bacterial cells was eliminated by sand filtration. Cleasby et al. (1984) and Bellamy et al. (1985) have used slow sand filtration for removal of bacteria and viruses. *Salmonella* spp., *Shigella* and *Giardia* cysts were removed using rapid sand filtration method (Bitton, 1980).

5.6 Physicochemical parameters of treated water samples

The treated ground water samples of both Tamil Nadu and Rajathan were analyzed for certain physicochemical parameters. The examination of the treated water samples collected from Rajasthan showed lesser concentration of all the parameters than that of the untreated samples. In Tamil Nadu water, all the parameters, after treatment
showed less compared to that of untreated water samples except total solids, total suspended solids, nitrite and ammonium. The results clearly have revealed that water treated with bacteria and then with coagulants have efficiently reduced the physico-chemical values that are of significant and importance concerning health. The treated water samples meet the criteria value of USEPA standards (APHA, 1998).

5.7 Disinfection of heterotrophic bacteria

After bacterial and coagulant treatment followed by sand filtration the samples (samples treated with lime at 150 mg.l\(^{-1}\)) were disinfected by various concentration of residual chlorine. The bacterial populations were completely eliminated (from 14 \(\times\) 10\(^4\) to 0 CFU.ml\(^{-1}\)) at 0.5 mg.l\(^{-1}\) chlorine concentration after 105 min. In the case of 0.1 mg.l\(^{-1}\) of chlorine the population reduced only about 21.43 % (from 14 to 11 \(\times\) 10\(^3\) CFU.ml\(^{-1}\)). This may be due to lower concentration of chlorine that will not effectively kill all bacteria. Bitton (1994) have reported that chlorine is generally quite efficient in inactivate pathogenic and indicator bacteria. Water treatment with 1 mg.l\(^{-1}\) or less for about 30 min is generally efficient in significantly reducing numbers of bacteria. Wagenet and Lemley (1998) reported that chlorination was effective against many pathogenic bacteria. Savithamani (2002) reported 100 % elimination of *Aeromonas hydrophila* by chlorination at a dosage of 0.5 mg.l\(^{-1}\) for 150 min. But at normal dosage rates, it does not kill all viruses, cysts or worms. Likewise in the present study, the populations were gradually eliminated when the chlorine concentration was increased from 0.2 to 0.5 mg.l\(^{-1}\). Similarly in Tamil Nadu water samples, the entire bacterial population was removed (from 17 to 0 \(\times\) 10\(^4\) CFU.ml\(^{-1}\)) when samples were treated with 0.5 mg.l\(^{-1}\) of chlorine.

By boiling, the bacterial cells were completely reduced in both Tamil Nadu and Rajasthan water samples. Similarly complete elimination was achieved in both the samples by using membrane filter technique. The samples exposed to UV radiation up to
50 min completely eliminated the entire bacterial population. Among the disinfectant method, boiling, UV treatment and membrane filter method proved to be very effective. Whereas in chlorination, at normal dosage there was a very negligible removal of microorganisms. UV disinfectant is particularly efficient against viruses, which are major agents of water borne diseases in ground water (Craun, 1986). Wolfe (1990) and Rice and Hoff (1981) have studied disinfection of water with ultra violet radiation at a range of 380 to 5500 μW - s/cm² for bacteria, 3600 to 8000 μW - s/cm² for viruses and 35000 to 82000 μW - s/cm² for protozoan cysts. The use of ultraviolet (UV) light to disinfect water of water borne pathogens capitalizes on the germicidal properties of a narrow range of the UV spectrum. The UV wavelength ranging from 240 to 280 nanometer is an optimum dosage to deactivate, or effectively kill, microorganisms by damaging their DNA so as to prevent the DNA, and the organisms, from replication (Harm, 1980).