SUMMARY

6. SUMMARY

The thesis embodies investigations on the 'microbial ecology of the aquaculture sewage treatment system fed with domestic sewage' established at Matagajpur (Lat 20° 11′ 06″ 20° 11′ 45″ N, Long 85° 50′ 52″ 85° 51′ 35″ E), Cuttack, Orissa, India with a view to characterise its treatment potential, considering both the structural and functional aspects, along with comparative studies on the bacterial populations in water and sludge/sediment media

Aquaculture Sewage Treatment Plant (ASTP) is a 1 MLD treatment plant established near River Kathajori at Cuttack treating 1 million litre sewage daily generated from Cuttack city The distance covered by the effluent from the collection point to the treatment site is about 3 Kms The design of the duckweed-fish culture complex comprises a network of 18 duckweed ponds of 0 02 ha each (25 m x 8 m x 1 0 m), six ponds in each row followed by two fish ponds of 0 1 ha each (50 m x 20 m x 1 5 m). The duckweeds used were *Spirodela polyrrhiza*, *Lemna minor*, *Wolffia minor* and water fern *Azolla caroliniana* The fish species used in fish ponds were *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix* and *Cyprinus carpio*

The objectives of the study were to characterise the bacterial communities of Aquaculture Sewage Treatment Plant (ASTP), to characterise the bacterial flora and pathogens associated with fish grown in this system, to evaluate the treatment efficiencies of aquaculture-based sewage treatment system consisting of duckweeds and fish with reference to microbial ecology of the system The yard experiments on nutrient uptake rates of duckweeds and bioassay study contributed to design of the Aquaculture Sewage Treatment Plant (ASTP) at Matagajpur, Cuttack, Orissa, India The aspects of study included the processes of treatment, meteorological conditions, hydrography, sludge/sediment nutrient status, plankton and bacterial communities in water, sludge and fish organs of the aquaculture-based sewage treatment system Weekly samples on different aspects were collected during November, 1995 to April, 1997 and analysed at the laboratory of Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Orissa, India

The salient observations made were as follows

1 Yard experiments conducted on 'Nutrient uptake rate of duckweeds from domestic sewage' showed reduction in the levels of nutrients and BOD_5 to the extent of 70 58-98 25% and 93 33% respectively by *Wolffia* sp at 0 05 g/l inoculum density The reduction levels of 73 38-95 86%, 91 99% and 80 00% in respect of nutrients, BOD_5 and COD respectively were observed by *Lemna* sp at 0 025 g/l inoculum density Application of *Spirodela* sp with 0 3 g/l density reduced 31 52-99 31% of nutrients, 71 4% BOD_5 and 55 55% COD Reduction levels by water fern *Azolla* at 0 2 g/l inoculum density were 65 69-90 84% of nutrients, 98 57% of BOD_5 and 67 5% of COD

2 Bioassay study revealed that LC_{50} values of raw domestic sewage with *Cirrhinus mrigala* is 42%

3 The seasonal variations of rainfall, relative humidity and atmospheric temperature of ASTP were conspicuous and the ranges were 0-334 3 mm, 19%-100% and 9 1°C-43 5°C respectively during November, 1995-April, 1997

4 The studies on water quality showed maximal values at sewage source and minimal levels at the outlet excluding pH and dissolved oxygen The water temperature fluctuated in the range of 17 0-33 5°C in different treatment ponds The ranges of dissolved oxygen content were 0-7 mg/l at source, 0 8-13 0 mg/l in duckweed pond complex, 2 0-11 8 mg/l in fish ponds and 3 2-10 0 mg/l at the outlet The variations were found to be significant between treatment ponds and periods ($p<0\ 05$) The increase was upto 422 20% through duckweed pond complex, 92 95% through fish pond and 611 11% through the whole treatment system

5 The water pH levels in the treatment system were in the ranges of 6 2-8 6, 6 8-8 4, 6 7-8 3, 6 9-8 4, 6 8-8 6 and 6 1-8 4 in source, duckweed pond 1, duckweeds pond 2, duckweed pond 3, fish ponds and outlet respectively Significant variations were noticed between treatment ponds and periods ($p<0\ 05$) Increase in pH values

from source to outlet through duckweed pond complex and fish pond was significant

6 The total alkalinity levels varied in the ranges of 76-324 mg/l, 80-304 mg/l, 72-288 mg/l, 80-292 mg/l, 76-284 mg/l and 68-180 mg/l at source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively Variations between the treatment ponds and periods were found to be statistically significant ($p<0.05$) There was a distinct reduction in the values from source to outlet during the treatment period Reduction upto 49 17%, 46 66% and 49 33% respectively through duckweed pond complex, fish ponds and the whole treatment system were recorded

7 Wide variations in total hardness contents at source, duckweed pond 1, duckweeds pond 2, duckweed pond 3, fish ponds and outlet, *viz*, 60-340 mg/l, 40-340 mg/l, 52-298 mg/l, 68-312 mg/l, 56-320 mg/l and 40-228 mg/l respectively were recorded respectively Significant variations between treatment ponds and periods were recorded ($p<0.05$) Reduction upto 57 6% by duckweed culture, 26 5% by fish culture and 74 0% by the whole treatment system were observed

8 The mean ammonium-nitrogen levels varied over ranges of 54 07-338 39 µg-at N/l at source, 34 90-311 90 µg-at N/l in duckweed pond 1, 24 58-361 82 µg-at N/l, in duckweed pond 2, 19 58-261 93 µg-at N/l in duckweed pond 3, 8 20-191 84 µg-at N/l in fish ponds and 8 10-102 54 µg-at N/l at outlet Significant variations were recorded between treatment ponds and periods during the period of investigation Reduction levels upto 97 02%, 32 77% and 96.00% through duckweed pond complex, fish ponds and the whole treatment system were noticed

9 The levels of nitrite-nitrogen in the treatment system varied within 0.54-58.2 µg-at N/l at source, 0 45-52 72 µg-at N/l in duckweed pond 1, 0 56-57 0 µg-at N/l in duckweed pond 2, 0 19-57 39 µg-at N/l in duckweed pond 3, 0.53-30.79 µg-at N/l in fish ponds and 0 21-10 86 µg-at N/l at outlet A clear reduction in nitrite-nitrogen content was found to be statistically significant ($p<0.05$). Reduction

levels of upto 84 95%, 86 84% and 91 78% were recorded through duckweed pond complex, fish ponds and the whole treatment system

10 The mean nitrate-nitrogen contents were in the ranges of 3 44-118 48 µg-at N/l at source, 1 98-71 43 µg-at N/l in duckweed pond complex, 1 79-49 14 µg-at N/l in fish ponds and 1 45-19 76 µg-at N/l at the outlet respectively Considerable reductions were found from source to fish pond (15 53-92 98%), from duckweed pond complex to fish ponds (1 66-58 15%) and from source to outlet (25 86-94 92%) The levels varied significantly between treatments and periods ($p<0$ 05)

11 The phosphate-phosphorus content of water varied over ranges of 0 4-6 60 mg/l, 0 26-6 10 mg/l, 0 24-5 25 mg/l, 0 04-4 78 mg/l, 0 18-4 24 mg/l and 0 04-2.30 mg/l at source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively Reduction levels of 48 0-92 0% in duckweed pond complex, 11 0-73 5% in fish ponds and 61 0-94 5% in the whole treatment system were recorded The treatment system showed significant variations between treatments and periods ($p<0$ 05)

12 The fluctuations in mean levels of chloride in water were 21 83-70 79 mg/l at source, 21 17-63 51 mg/l in duckweed pond 1, 17 20-58 22 mg/l in duckweed pond 2, 21 17-64 84 mg/l in duckweed pond 3, 22 82-52 92 mg/l in fish ponds and 15 21-43 66 mg/l at the outlet The variations were statistically significant between the treatment ponds and periods ($p<0$ 05) Reduction upto 48 6% through duckweed ponds, 58 6% through fish ponds and 62 3% through the whole treatment system were observed

13 The mean biochemical oxygen demand varied over 52-123 mg/l at source, 40-82 mg/l in duckweed pond 1, 41-72 mg/l in duckweed pond 2, 26-77 mg/l in duckweed pond 3, 24-43 mg/l in fish ponds and 11-44 mg/l at outlet There were significant variations between treatment ponds with respect to periods ($p<0$ 05) The BOD_5 levels reduced upto 48 05-85 52% from source to outlet

14 The mean fluctuations in COD levels varied within minimum and maximum values of 106-184 mg/l at source, 90-156 mg/l in duckweed pond 1, 83-172 mg/l

in duckweed pond 2, 63-121 mg/l in duckweed pond 3, 37-103 mg l^{-1} in fish ponds and 22-94 mg/l at outlet Gradual reduction from source to fish ponds, fish ponds to outlet and source to outlet was observed which reached a maximum value of 72 06%, 37 5% and 84 5% respectively The variations between the treatment ponds with respect to periods were found to be statistically significant ($p<0$ 05)

15 The mean values of dissolved organic matter varied within 9 20-17.06 mg/l, 7.06-15 20 mg/l, 5 73-13 20 mg/l, 5 86-12 80 mg/l, 4 53-9 86 mg/l and 4 13-8 80 mg/l at source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds respectively Variations between the treatment ponds and periods were found to statistically significant ($p<0$ 05) A decreasing trend from source to duckweed pond complex, duckweed pond complex to fish ponds and fish ponds to outlet was evident Reduction upto 59 0%, 33 8% and 62 0% through duckweed pond complex, fish pond and through the whole treatment system was discernible

16 The pH levels of sludge samples in source, duckweed pond complex and fish ponds were varied over 6 8-8 1, 5 4-7 2 and 5 9-7 3 The monthly fluctuations in specific conductivity were 0 246-0 927, 0 174-0 988, 0 134-0 711, 0 172-0 722 and 0 171-0 800 millimho/cm in source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively The organic carbon contents varied in the ranges of 1 11-4 7%, 0 73-3 57%, 0 61-2 31%, 0.48-1.44% and 0 33-0 96% at the corresponding sites Sludge/sediment total nitrogen varied within 0 019-0 288%, 0 022-1 27%, 0 022-0 84%, 0 027-0 117% and 0.017-0 1001% in source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively The available phosphorus content registered values in the ranges of 1 52-4 40, 0 32-3 52, 0 64-2 48, 0 32-3 20 and 0 48-2 88 mg/100g sludge and sediment of source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively The variations in specific conductivity, organic carbon and available phosphorus were found to be statistically significant between treatments and periods ($p<0$ 05)

17 The total plankton counts varied within 403-7444/l, 606-10,579/l, 674-10,496/l and 652-33,276/l in duckweed pond 1, duckweed pond 2, duckweed pond 3 and fish ponds respectively The monthly variations of total plankton counts in surface waters were significant ($p<0.01$) While phytoplankton mostly comprised of blue green algae, zooplankton was dominated by rotifers The levels of phytoplankton and zooplankton were found to be significant between treatment ponds and periods ($p<0.05$) The generic representation of phytoplankton was *Anabaena* spp , *Oscillatoria* spp , *Microcystis* spp , *Phormidium* spp , *Arthrospira* spp , *Spirulina* spp , *Coelastrum* spp , *Pediastrum* spp , *Chlamydomonas* spp , *Scenedesmus* spp , *Closterium* spp , *Cosmarium* spp , *Spirogyra* spp , *Euglena spp* , *Phacus* spp , *Navicula* spp , *Melosira* spp , *Cyclotella* spp , *Synedra* spp , *Pinnularia* spp and *Peridinium* spp Genera of zooplankton encountered in the aquaculture-based treatment system were *Cyclops* spp , *Diaptomus* spp., *Daphnia* spp , *Moina* spp , *Bosmina* spp , *Diphanosoma* spp , *Filinia* spp., *Brachionus* spp., *Keratella* spp , *Asplanchna* spp , *Polyarthra* spp and nauplius larvae The study brought out the significance of this community through the treatment and it suggests the important role played by duckweeds and fish to control eutrophication of wastewater and species diversity

18 The comprehensive investigation provided information on various bacterial communities hitherto not analysed in the aquaculture-based sewage treatment system and also characterised the system in its entirety In addition, the study identified several aspects for further detailed research, *viz* , the role of other microbes like yeasts and molds in nutrient cycling, decomposition of organic matter, mineralisation, heterotrophic food chain, community interactions, etc The heterotrophic bacterial populations considerably varied and greatly reduced in the water and sludge medium from source (0 92-27 00 x 10^3/ml, 0 72-78 20 x 10^5/g) to outlet (0 20-6 00 x 10^3/ml) commensurate with their organic content and presented significant monthly variations ($p<0.05$)

19 The pathogenic bacteria like total coliforms, varied within 36-1350 x 10^2 MPN/100 ml, 29 75-391 0 x 10^5/g in water and sludge medium respectively, while

faecal coliform varied from 2 3-460 x 10^2/100 ml, 0 63-127 5 x 10^5/100 g, faecal streptococci varied from 0 08-2 80 x 10^2/ml, 1 7-42 5 x 10^5/g and *Salmonella* varied from 0 1-20 2 x 10^2/ml, 4 25-54 4 x 10^3/g at source and significantly reduced through the duckweed pond complex and fish ponds They reduced upto 99 99%, 100% and 100% in this system

20 The bacterial populations involved in the nitrogen cycle, *viz* aerobic nitrogen fixers in water and sludge were 0 05-8 0 x 10^3/ml, 0 20-23 8 x 10^3/g; nitrifiers-I 45-140/ml, 16 5-140 x 10^3/g, nitrifiers-II 45-140/ml, 15-140 x 10^3/g and denitrifiers 2 5-140/ml and 9 5-140 x 10^3/g being in moderate ranges, reduced upto 98 49%, 94 64%, 100% and 99 18% respectively, and significantly varied in the treatment system ($p<0$ 05)

21 The populations and fluctuations of amylolytic bacterial communities in water and sludge were considerable (0 01-9 0 x 10^3/ml, 0 25-31 45 x 10^5/g dry weight, 0.2-4 05 x 10^3/ml, 0 16-57 4 x 10^5/g dry weight, 0 02-3 02 x 10^3/ml, 0 08-48 0 x 10^5 /g dry weight, 0 01-2.12 x 10^3/ml, 0 08-24 8 x 10^5/g dry weight, 0 01-1 06 x 10^3/ml, 0 07-11 9 x 10^5/g dry weight and 0 01-0.42 x 10^3/ml) in source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish ponds and outlet respectively, in the aquaculture-based treatment system

22 Aerobic cellulolytic bacteria varied from 0-140/ml and 0-140 x 10^3/g in water and sludge medium and gradually reduced through duckweed pond complex and fish ponds The counts in sludge/sediment medium were quite high in comparison to water medium for all the bacterial communities

23 The mean counts of inorganic phosphorus solubilising bacteria varied within 0.16-2 45 CFUs x 10^3/ml, 0 11-1 05 CFUs x 10^3/ml, 0 07-0 98 CFUs x 10^3/ml, 0.06-0 44 CFUs x 10^3/ml, 0-1 07 CFUs x 10^3/ml and 0-0 24 CFUs x 10^3/ml in source, duckweed pond 1, duckweed pond 2, duckweed pond 3, fish pond and outlet. They reduced considerably through the duckweed pond complex and fish ponds. The counts in water and sludge media were found to be significantly varying in the system ($p<0$ 05)

24 Various factors were found to influence the bacterial population levels, the important ones being the unsuitable environment created by duckweed culture, such as dissolved oxygen, nitrogen, phosphorus, etc In turn, the activities of different bacterial communities were demonstrated to influence the hydrobiological conditions With information new to Indian Sewage treatment system research, the study showed the influence of duckweed and fish culture in sewage on bacterial activity

25 A survey of fish raised in ASTP and bacterial flora of fish organs was conducted during the investigation period The results obtained in case of fish showed that in healthy and clean fish, no bacteria were recovered from the muscles and low populations were enumerated in digestive tracts, gills and skins at populations as high as 14 0 x 10^3/g, 2 5 x 10^3/g and 0 91x 10^3/g, respectively, and no *Salmonella* were seen in fish reared in domestic sewage The high bacterial populations in the gut content of these fishes in the present findings were due to the nature of food ingested, the habitat and also the occurrence of natural flora in the gut Significantly higher bacterial counts were found in gut of mrigal than silver carp, catla and rohu in the present findings

26 Among 450 heterotrophic bacterial strains isolated in the treatment system, bacterial genera identified were *Pseudomonas* spp , *Achromobacter* spp , *Escherichia coli*, *Micrococcus* spp , *Bacillus* spp , *Lactobacillus* spp , *Arthrobacter* spp , *Corynebacterium* spp , *Klebsiella* spp , *Aeromonas* spp , *Citrobacter* spp , *Serratia* spp , *Flavobacterium* spp , *Proteus* spp , *Alcaligens* spp , *Enterobacter* spp , *Staphylococcus* spp , *Streptococcus* spp and *Clostridium* spp Among these 75-80% of the bacteria were found to be *Pseudomonas* spp , *Bacillus* spp., *Flavobacterium* spp. and *Proteus* spp

27 Giving a general picture of the population and distribution of bacterial communities in waters of different treatment ponds, the study showed that a combination of factors along with duckweeds influenced the bacterial regime, without any particular parameter having a determining effect