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On The Analysis of Certain Biochemical Parameters of Carps Cultured in Domestic Sewage Oxidation Ponds

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ABSTRACT

Reclamation of waste water for protein production is a concept that is being paid more attention of late, as it is an environmental friendly, economic and experimental interdisciplinary process to produce large quantities of edible fish. Domestic sewage is full of minerals, nutrients and organic matter which suits for aquaculture production without any supplementary feeding and it is also an economically viable biological treatment of domestic waste waters. In the present study changes in tissue glycogen and serum glucose contents of three fishes belonging to the carp family, cultured in secondary domestic waste oxidation ponds have been analysed. Tissue glycogen values in liver, kidney and muscle and serum glucose of *C.carpio, L.rohita* and *C.mrigala* cultivated in secondary sewage ponds were estimated. Tissue glycogen was found highest in liver, followed by muscle and kidney, in *C.carpio* grown in seconadary ponds of domestic sewage, as compared to the other fishes. Serum glucose value was also found highest in *C.carpio* which is a voracious polyphagous feeder. The results of our findings support the fact that there is improved fish growth in sewage ponds which are full of nutrients and minerals, enabling a symbiotic process of reclamation of proteins as well as biological treatment of the sewage. More bio-chemical assessment of fishes grown in sewage ponds are needed to remove the mis concept that sewage cultured fishes are not safe for human consumption

KEY WORDS: SEWAGE, CARP CULTURE TISSUE GLYCOGEN LIVER, KIDNEY MUSCLE.

INTRODUCTION

Water is one of the vital resources for all the lifeforms and it is also the resource which is most adversely affected by human activities, especially after rapid growth in industrialization and urbanization. It is known that the aquatic eco-systems are most delicately balanced and get easily disrupted by various human activities (Chiou et al. 2007). Of various types of human activities, sewage disposal continues to be the most ominous one. Sewage coming from living human quarters consist of biodegradable organic matter and faecal matter. Before disposing into fresh waters, sewage water has to be treated which gave rise to the concept of oxidation or stabilization ponds (Bhatia et al 1970, Hepher and Schroeder (1977) Wu et al. 2013; Kumar et al. 2015). These oxidation ponds are eco-friendly environmental & economical technique to grow fish and prawn (Siddig et al. 2019). Fishes grown in sewage waters exhibit faster growth as sewage is composed of high nutrients, organic and protein contents. It is also observed that these fishes are not harmful for human consumption (Mannacharaju et al. 2020).

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Water reuse or reclamation of waste water for aquaculture from treated municipal effluent waters is a useful technique which is being practiced worldwide. The first recorded scientifically carried out experiment of culturing fish in sewage ponds was done by Mayenne (1933) in Germany. Later on countries like Japan, Taiwan, Indonesia, Phillippins, Hongkong, Malaysia etc., started sewage pond culture of fishes. In India, fish culture practices in fresh water ponds fertilized with domestic sewage started in 1940's, especially in West Bengal, Tamilnadu and other few states. (Modak 1938; Vaas, 1940; Hora 1944; Pillai et at. 1945; Ganapati & Chacko 1950; NEERI, 1985; Saha et al. 1958; Bhatia et al. 1970; Allen et al. 1979; Hepher & Schroeder, 1977; Allen and Carpenter 1977; Jhingran, 1984; Ali 1992; Tripati, 1992; Bunting et al. 2001; Nandeesha, 2002; Datta, 2007; Drechsel et al. 2009; Shakir et al. 2014; Darko and Azanu, 2016; Das et al. 2020).

To find out the physiological and growth aspects of fishes grown in domestic sewage fed ponds, biochemical parameters were studied in the present study. Teleost fishes like *Cyprinus carpio, Labeo rohita* and *Cirrhinus mrigala* were grown in secondary sewage ponds and their tissue glycogen in liver, kidney and muscles and serum glucose were analysed regularly in 3rd month, 6th month, 9th month and 12th month of a year. The data was compared with the data of the biochemical parameters obtained from the same fishes grown in fresh water ponds fed with supplementary feed.

MATERIAL AND METHODS

Study area: The experimental work was made in Shahpura sewage oxidation ponds located at T.T. Nagar, 10 km south-east of Bhopal city (250-17'). There are 8 sewage oxidation ponds constructed in two series of primary and secondary as per specifications of National Environmental Engineering Research Institute (NEERI), Nagpur. Each pond is having an area of 0.4 hectares. The ponds were typical sewage oxidation or stabilization ponds, designated to treat biologically 3 million gallons of domestic sewage per day. Sewage from adjacent areas was collected in a sump near Habibganj Railway Station, from where it was pumped to the oxidation ponds and it was detained for a period of about 15 to 20 days for microbiological transformation. The raw sewage enters the primary pond through 3 inlets and after the detention period the biologically treated effluent goes out from secondary pond through the outlet.

In the present study out of 8 ponds, as mentioned above, four were selected for fish culture. Two being primary, designated as IA & IIIA and two as secondary called as IB & IIIB. Fish seed of *C.carpio, L.rohita* & *C.mrigala* is stocked in all the above ponds but due to high concentration, no fish survived in primary ponds. The fishes grown in secondary ponds (IB & III B) were collected and the biochemical experiments were conducted as per standard procedures. The netting of fishes in secondary sewage ponds and control fresh water pond at regular 3-4 monthly intervals has been done using standard nets. The experiment was conducted for a period of one year.

Estimation of Biochemical Components: Live, mature and healthy *C. carpio, L.rohita* and *C.mrigale* were caught from sewage oxidation ponds and immediately brought for experimentation. After 1-2 hrs of laboratory acclimatization, the fishes were used for blood analysis. By severing caudal peduncle artery blood was collected in the pre oven sterilized centrifuge tubes and subjected for centrifugation for a period of 5 minutes at 3000 rpm. The centrifuge tubes were stored at 40 ^{0}C for serum precipitation.

The caudal peduncle severed fishes were dissected and liver, kidney and muscle were exposed and kept in cold storage for analysis of biochemical parameters. A 5% tissue homogenate was prepared using Potter-Elvehjem homogenizer at 10000 rpm for 10-15 minutes under ice cooled conditions used for biochemical studies. Six species were used separately for each experiment. All the experiments were repeated for 3-4 times and the data were statistically analysed using standard methods (Fischer, 1950; Lewis and Lewis, 1971). Tissue Glycogen: Tissue glycogen was estimated by the glucose oxidase method described by Plummer (1978). All the chemical and reagents used were obtained from Sigma (USA). Tissue glycogen values were expressed in µ gm/g wet weight of the tissue. All the values are mean of 3-4 replicates.Serum glucose: Serum glucose was estimated according to the methods of and Folin and Wu (1929) as cited in Hawk's Physiological Chemistry, Edited by Bernard L. Oser (1965). Serum glucose values were expressed as µg per 1 ml of serum.

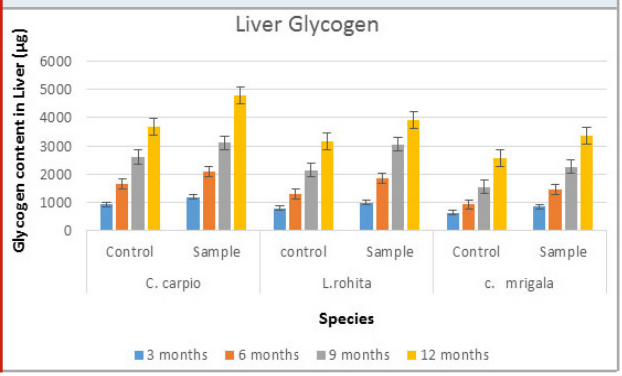
RESULTS AND DISCUSSION

The analysis of tissue glycogen was done from liver, kidney and muscle of the domestic sewage and control pond cultured fishes. It was observed that liver recorded highest content of glycogen followed by muscle and kidney from fishes grown in secondary oxidation ponds. Glycogen content in the liver of *C.carpio*, cultured in oxidation ponds for a period of 3 months exhibited significant greater values from that of *C. carpio* grown in the fresh water control pond for the same period. It was observed that the 3 months *C. carpio* from oxidation pond had 1201.16 µg/glycogen in the liver whereas the liver glycogen content in the fresh water control fish was 932.91 µg only. On the other hand 3 months old *L.rohita* cultured in oxidation ponds along with *C.carpio* showed significantly less glycogen content in

the liver than *C.carpio* and it was found to be 1000.99 µg. Similarly, the fresh water cultured *L.rohita* exhibited 789.46 µg of liver glycogen. Minimum glycogen content was observed in both the experimental and fresh water control *C. mrigala* during the 3 months old stages among the three fishes. The value was 853.13 µg in the fish from the oxidation ponds and 638.98 µg in the fish from the control pond.

During 9 months stage, *C.carpio, L.rohita* and *C.mrigala* cultured in oxidation ponds showed liver glycogen values as 3108.74 µg, 3047.11 µg and 2265.54 µg respectively. On the other hand the same fishes from the fresh water contol pond showed comparatively less liver glycogen values being 2601.79 µg, 2149.30 µg and 1543.67 µg respectively.After 12 months of culture in oxidation ponds, *C.carpio* of oxidation ponds showed maximum glycogen content in its liver i.e., 4791.78 µg whereas the same fish cultured in fresh water showed 36789.60 µg of liver glycogen content. The other fishes *L.rohita* and *C.mrigala* from oxidation ponds exhibited 3903.55 µg and 3362.30 µg of glycogen values in their liver. The same fishes *L.rohita* and *C.mrigala* cultured in control pond showed 3157.84 µg and 2569.81 µg of liver glycogen (Figure 1).

Figure 1: Showing liver and glycogen content of fishes cultured in secondary oxidation ponds for a year along with fishes cultured in fresh water as control

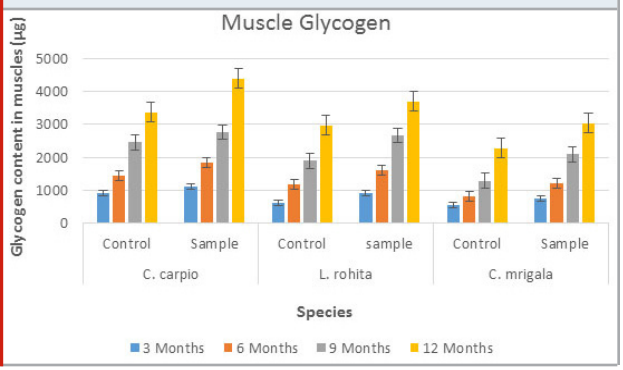


It was observed that *C.carpio* among the three fishes exhibited highest content of glycogen in its liver followed by muscle, *C.carpio* cultured from the oxidation ponds showed 1103.64 µg of glycogen in the muscle during the 3 months stage. On the other hand the fresh water control fish showed 908.28 µg of muscle glycogen content at the same age of 3 months. Similarly, the other fishes *L.rohita* and *C.mrigala* grown in sewage ponds exhibited 913.66 µg and 738.16 µg muscle glycogen whereas the *L.rohita* and *C. mrigala* cultured in fresh water pond showed 616.21 µg and 564.62 µg of muscle glycogen respectively. *C.carpio* cultured in oxidation ponds showed 1843.75 µg of glycogen in the muscle at 6 months stage whereas the 6 month control pond fish showed 1438.31 µg of glycogen in the muscle. Similarly, *L. rohita* grown in secondary sewage ponds during 6 months exhibited 1624.36 µg muscle glycogen and its fresh water control pond counterpart recorded 1182.48 µg glycogen in its muscle. It was found that *C.mrigala* cultured in oxidation pond exhibited 1208.76 µg of muscle glycogen at the stage of 6 month and its control counterpart showed 813.48 µg of muscle glycogen.

During the analysis of 9 month oxidation pond-cultured *C.carpio*, it was observed that 2763.60 µg of glycogen were present in the muscles whereas freshwater grown fish exhibited 2458.11 µg of glycogen in its muscle at the same age of 9 months. Similarly, the other fishes *L.rohita* and *C.mrigala* grown in secondary sewage ponds recorded 2664.87 µg and 2092.50 µg of muscle glycogen during 9 month stage. The fresh water pond cultured *L.rohita* and *C.mrigala* at this stage of 9 months exhibited 1898.74 µg and 1292.16 µg of glycogen in their muscles which is significantly less than the fishes grown in oxidation ponds.

At the stage of 12 months of culture of fishes, it was observed that all the three fishes recorded high amount of muscle glycogen, next to that of liver glycogen. The fishes *C.carpio, L.rohita* and *C.mrigala* cultured in oxidation ponds at the stage of 12 months exhibited 4388.43, 3696.72 µg and 3041.38 µg of muscle glycogen respectively. On the other hand the same fishes *C.carpio, L.rohita* and *C.mrigala* from fresh water pond recorded 3372.57 µg, 2968.36 µg and 2278.64 µg of glycogen content in the muscles respectively during the 12 months of culture (Figure 2).Thus it is evident that the fishes *C. carpio, L.rohita* and *C.mrigala* cultured in sewage oxidation ponds recorded high amounts of liver and muscle glycogen from the initial stages as the sewage ponds are highly nutrient. The normally fed fresh water control pond cultured fishes recorded less glycogen values (Figure 2).

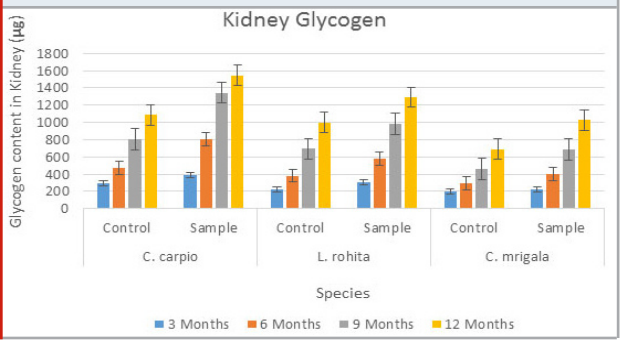
Figure 2: Showing muscle glycogen content of fishes cultured in secondary oxidation ponds for a year along with well-matched controls



During the analysis of glycogen from kidneys it was found that all the three fishes *C. carpio, L. rohita* and *C. mrigala* showed minimum glycogen content in their

kidneys. Data from 3 month old C.carpio cultured in oxidation ponds exhibited 388.68 µg of glycogen in their kidneys whereas the same 3 month old fresh water control cultured fish showed 294.28 µg glycogen in its kidney. Similarly, the other fishes *L.rohita* and *C. mrigala* cultured in oxidation ponds for 3 months showed kidney glycogen of 301.62 µg and 228.58 µg respectively. On the other hand, *L. rohita* and *C. mrigala* cultured in fresh water pond showed 218.81 µg and 196.63 µg of glycogen in their kidneys (Figure 3).

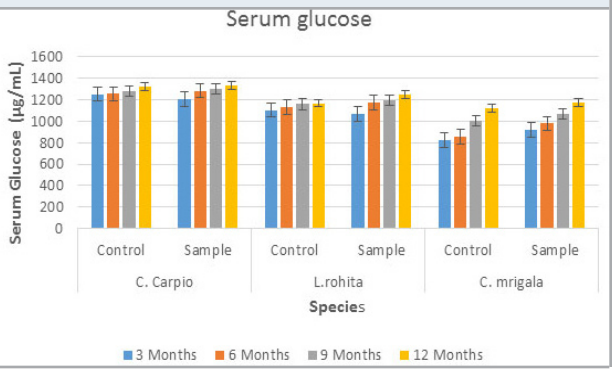
Figure 3: Showing kidney glycogen content of fishes cultured in secondary oxidation ponds for a year along with control fishes



In the next analysis i.e. at 6 months old stage, *C. carpio* from oxidation pond showed 806.71 µg of glycogen in its kidney and the same 6 months old *C. carpio* culture din fresh water showed only of 472.64 µg of kidney glycogen. The other fishes *L. rohita* and *C.mrigala* cultured in oxidation ponds exhibited 580.10 µg and 401.16 µg of glycogen in their kidneys.During 9 months of culture of fishes in oxidation ponds, *C.carpio, L.rohita* and *C.mrigala* exhibited 1345.42 µg, 981.20 µg and 688.30 µg of glycogen contents in their kidneys respectively. On the other hand, the fresh water control fishes *C.carpio, L. rohita* and *C.mrigala* after 9 months of growth exhibited kidney glycogen values of 802.46 µg, 692.66 µg and 461.84 µg respectively. At the end of one year, the analysis of kidney revealed that the experimental *C.carpio* exhibited 1542.93 µg of glycogen in its kidney. On the other hand, the control fresh water 12 months old *C. carpio* recorded 1087.50 µg of kidney glycogen. The other fishes *L.rohita* and *C. mrigala* from oxidation ponds after 12 months of culture exhibited 1292.61 µg and 1026.98 µg of glycogen content in their kidneys and the fresh water *L.rohita* and *C.mrigala* recorded 998.44 µg and 689.10 µg of kidney glycogen (Figure 3).

The analysis of serum glucose of the secondary oxidation and fresh water species of *C.carpio, L.rohita* and *C.mrigala* was also carried out. Except at the initial stage of growth i.e., at 3 months, maximum glucose values were recorded in C.carpio followed by *L.rohita* and *C.mrigala* cultured in oxidation ponds. At the stage of 3 month, *C.carpio* from oxidation pond exhibited 1200.37 µg/ml of serum glucose whereas the control fresh water *C.carpio* exhibited 1248.91 µg/ml of serum glucose during 3 months stage. Similarly, the other fishes *L.rohita* and *C.mrigala* cultured in oxidation ponds, along with *C.carpio* showed serum glucose values of 1062.46 and 918.50 µg/ml during three months culture. On the other hand, 3 month old fresh water cultured *L.rohita* and *C.mrigala* showed 1100.55 and 824.41 µg/ml of serum glucose values. During the next analysis of fishes at 6 months age, high glucose contents were found in all the fishes, cultured in oxidation ponds. *C.carpio* grown in oxidation ponds for 6 months, showed 1280.21 µg/ml of serum glucose whereas *C.carpio* during 6 months growth in fresh water pond showed serum glucose of 1250.19 µg/ml. Similarly, the other fishes *L.rohita* and *C.mrigala* from oxidation ponds during 6 month stage exhibited 1170.83 and 975.25 µg/ml of glucose whereas the 6 months old control fresh water *L.rohita* and *C.mrigala* showed serum glucose of 1128.20 and 855.39 µg/ml respectively.

Figure 4: Showing serum glucose level in fishes cultured in secondary oxidation ponds



C. carpio, L.rohita and *C.mrigala* grown in sewage ponds for 9 months showed serum glucose of 1298.64, 1192,51 and 1061.57 µg/ml respectively. On the other hand, *C.carpio. L.rohita* and *C.mrigala* from fresh water control pond exhibited 1279.15, 1154.10 and 1005.36 µg/ml of glucose respectively.Analysis of fishes after 12 months for serum glucose found that maximum glucose values were recorded in *C.carpio* followed by *L.rohita* and *C.mrigala*. *C.carpio* grown in oxidation ponds during 12 months stage showed 1330.66 µg/ml of glucose content whereas its 12 month old fresh water control counterpart showed glucose content of 1318.80 µg/ml. Similarly the other fishes *L.rohita* and *C.mrigala* from secondary oxidation ponds during one year stage exhibited 1240.53 and 1170.10 µg/ml of glucose content. On the other hand 12 month old fresh water *L.rohita* and *C.mrigala* showed glucose content of 1164.35 and 1120.50 µg/ml. (Figure 4)

Thus throughout the study period high serum glucose values were recorded in *C.carpio* compared to other fishes like *L.rohita* and *C.mrigala* cultured in domestic secondary sewage ponds.

As compared to other two species studied in the present study, *C.carpio* showed highest glycogen levels due to different dietary habits, amount of food consumed, metabolic activities and with high to that environment and physiological tolerance. As *C.carpio* is a polyphagous and a carbohydrate feeder hence it is more active than other fishes. Thus, higher levels of tissue glycogen as detailed in the carps cultured in domestic waste waters is in agreement with the concept that easy availability of nutrient rich food chain enhances the carbohydrate lipid and protein contents of fishes. Liver glycogen represents as the central reserve of fuel for the body tissues and also maintains the level of blood glucose in higher animals including fishes. It is also known that in well-fed animals, glucose is converted to glycogen in the liver and the blood glucose in tissues is maintained by the synthesis of glucose from non-carbohydrate sources i.e., glucogenesis (Plummer, 1978). It has been revealed that a significant relationship exists between dietary state and liver glycogen content (Plummer, 1978; Furukawa et al. 2018).

The reason for higher glycogen levels in liver and muscle may be due to a greater food intake and enhanced glycolytic metabolic pathways found in the experimental carps i.e., fishes grown in domestic sewage oxidation ponds. As very large quantity of nutrient rich food was consumed by the fishes in sewage waste waters, their growth pattern was quite different than that of fresh water fishes which were rather poorly fed, as they were only given supplementary feeding as per standard applications (Jhingran, 1984). The experimental fishes grew 2-3 times faster in growth than the control fishes because of higher food conversion efficiencies associated with higher enhanced metabolic rates. The carps cultured in domestic sewage secondary oxidation ponds utilizes the complete food columns of the eutrophic water and grew fast. There is no doubt that the domestic sewage influent had several important nutrients being many times rich in carbohydrates, fats, proteins and other nutrient stimulators (Mohapatra et al. 2012).

Another reason for higher content of glycogen in liver of all carp fishes was due to the fact that liver being a red tissue is instrumental in all metabolic activities and red muscles also contain hemoglobin, triglycerides, blood capillaries and their capacity to oxidize long chains of saturated fatty acids and acetate is higher than that of white muscles (Shaffi et al. 1977; Chang et al., 2020). Thus the data of the present study that higher content of glycogen was present in the order of liver followed muscle and kidney are in full corroboration with the findings of Plummer (1978); Swaleh et al. (2019); Chang et al. (2020).

In the present investigation serum glucose levels were also estimated for the carps *C.carpio, L.rohita* and *C.mrigala* grown in domestic secondary sewage oxidation ponds and compared with the control ones. The reason for high serum glucose levels of fishes grown in domestic secondary sewage waste waters may be due to their increased feeding habits and their active physiological activities. It is well known that the blood glucose levels in fishes are significantly related with their dietary habits, feeding and metabolic efficiencies. Active swimmers have high glucose levels as compared to less active sluggish forms (Gray and Hall, 1930; Singh and Khanna, 1971; Benchoula et al., 2019). Grey and Hall (1930) reported that a sluggish fish *Lophius piscatorius* had only 5 to 6 mg blood glucose while an active salmon had upto 130 mg. Thus the findings of the present study clearly indicate that activity of the fishes, muscular exercise, food conversion ratios and energy mobility, increase blood glucose levels significantly.

Another reason for the higher serum glucose levels observed in carps cultured in domestic sewage may be due to large amounts of glycogen present in vital tissues of the fish particularly the liver. This was due to very active and increased processes of glycogenolysis, hyperglycemia and hyperlactemia which occurred in the experimental fishes. It is well known that the liver glycogen in fishes maintains the level of glucose in well fed animals and both are maintained and synthesized from non-carbohydrate sources also (Menten, 1927; Grey & Hall, 1930; Chavin and Young, 1970; Plummer, 1978; Chhabria et al., 2020). Thus the high carbohydrate metabolism associated with greater food conversion and enhanced glycolytic pathways clearly support the higher glycogen contents which was responsible for hyperglycemia and hyperlactemia in the fishes grown in domestic sewage waters. It is well known that starvation, under feeding and imbalanced diets cause depletion in glycogen levels in the fresh water cultured control fish tissues through affected carbohydrate metabolism. The blood glucose and tissue glycogen concentrations decrease under such conditions as observed by Kamra (1966); Joshi (1974); Ince & Thorpe (1975), Ghaly et al. (2005). Thus the data of fresh water cultured fishes of the present study are in full agreement with the above findings.

The carps grown in treated domestic secondary waste oxidation ponds do not face any kind of toxicological effect or of any kind of long term physiological stress which could damage the metabolic pathways causing depletion in sensitive biochemical components (Ali et al., 1988; Ali et al. 1991; Ali, 1992). The results of our findings are in corroboration with the very recent studies of Abdelhamid et al. (2015); Bhoi and Patole (2019); Grabicova et al., (2020). They have done experiments on carp fishes and channa cultured in sewage waters and found increased values of glucose and triglycerides.

CONCLUSION

It is concluded that the presence of high glycogen and glucose values in fishes cultured in secondary domestic sewage waters supports their good adjustment to that environment and reveals that physiochemical conditions of that ponds are not showing any negative impact on the biochemical parameters of the fishes grown there. Anyhow more research has to be done on fish culture experiments which are the biological treatment techniques for the purification of municipal or domestic effluent waters and also the health risks associated with the human consumption of fish grown in that water bodies.

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