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Digestive Enzymes Profile in Fish exposed to Recycled Sugar Factory Effluent

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ABSTRACT

This study highlights the impact of recycled sugar factory effluent on the digestive enzymes of fish. The need for this task arises from the trend of releasing the untreated effluent into the aquatic body. The bioassays conducted separately with the raw and recycled sugarfactory effluent against the fish,*Catla catla.* This study exposed that quantum of decrease in the digestive enzyme level in the fish exposed to the highest concentration of raw sugar factory effluent is more when compared to that of fish treated in the recycled sugar factory effluent. The reuse of recycled sugar factory effluent is highly recommended for aquaculture through this study.

1. Introduction

Wastewater aquaculture practices may provide two advantages to the aquafarmers via, solving the problem of scarcity of water and the waste could be made use for creating wealth in water bodies (Gill and Pant, 1981). Wastewater can be used as a nutrient and water resource.

Many methods have been practiced in different industries to detoxify the pollutants. Normally, the membrane reactor is used (Kumaraguru and Beamish, 1983). The performance and operation of the membrane bioreactor (MBR) evaluated in terms of p characteristics and variability (COD, colour, total N and P, microbiological counts), membrane specific flux and other operational parameters (sludge growth and yield (Kondal *et al*., 1984). The use of this recycled industrial wastewater is not practiced on a large scale for aquaculture in India. Hence this project has attempted to explore the possibility of using recycled wastewater for the fish growth.

The National co-operation sugar mills Ltd, is located at Alanganallur about 10km northeast of Madurai city. . The mill effluents are being treated through a recycled process and discharged into the aquatic body. Hence an anxiety develops to know the quality of the recycled water on one end and to make use of the recycled waste water for the sustainable fish growth. Further, the efficiency of using the recycled wastewater for fish culture is tested through the analysis of enzyme profile of the fish. Moreover this is confirmed through the comparison of Invertase and Amylase content of the fish exposed to raw and recycled sugar factory effluent water and to compare this result with the control.

2. Materials and Methods

Healthy fingerlings of *Catla catla* collected from a local fish farm near Madurai and transported to the laboratory in closed polythene bags filled with oxygen. During transportation, care taken to reduce any hyperactivity and physical injuries to the fish. Immediately after reaching at the laboratory, disinfective dip treatment with 0.1% $KMnO_4$ to the fish as a precaution. They acclimatized to lab condition of aquatic vegetation, for a period of one month. Only non- chlorinated ground water was used. During this period, the fish fed with the pellet diet having 38% crude protein prepared according to standard method (Hardy, 1980)

2.1. Fish Feed

The feed for the test animals prepared with the constituent peanut oil-cake, rice bran, fish meal, dry fish and tapioca flour in the ration 3:2:2:2:1. The biochemical composition of the feed tested using standard methods (APHA, 1975) and presented in Table 1. The energy value of the formulated feed was estimated using an Oxygen bomb calorimeter.

2.4. Collection and Characterization of Effluent:

Raw and recycled effluent from the sugar factory effluent collected from the main points of discharge at Alanganallur, Madurai. The collected effluents were transported immediately to the laboratory and the physical and chemical characteristics of raw and recycled sugar factory effluent were estimated (Table 2) using Standard Methods (APHA et al., 1992).

2.4.1. Dilution procedure

The undiluted sugar factory industry effluent collected from the discharge point of the industry was considered as a 100 % solution. From this, the selected effluent concentrations for the experiments were obtained by diluting it with clean non-chlorinated ground water. The physical and chemical characteristics of the ground water used for dilution were also analyzed.

The test media prepared by using non- chlorinated ground water for dilution. A single stock sample of raw and recycle sugar factory effluent collected from the discharge point of the industry was subjected to physico- chemical analysis. The sample kept in sealed can for the duration of one month and physicochemical analysis done periodically to check the stability of the sample. The single effluent sample used to carry out all the experiments.

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2.5. Studies on Digestive Enzyme Activity:

2.5.1. Experimental Design

To find out the effect of various sub lethal concentration of toxicants on selected aspects of biochemical constituents in different of *Catla catla* the test animals exposed to raw and recycled sugar factory industrial effluent along with control maintained. Rearing of fishes as a group (10 fishes in each group) carried out in rectangular glass aquaria containing 10 liter of the medium. During exposure, fishes fed ad-libitum with prepared pellet food once in a day for 2 hours (08.00 to 10.00 hours). The food remains collected using a siphon immediately after the feeding session. In all the experiments, the medium changed every day. The experiments conducted for one month. After the experimental period, both the control and experimental fishes from each test medium sacrificed. The the gut dissected out and washed in ice cold fish ringer solution. The adhering tissues and the the gut contents carefully removed and stored in a refrigerator at – 30^0C for digestive enzyme studies.

2.5.2. Preparation of Enzyme extract:

The method followed for the preparation of the enzyme extract was that Christopher (1983). At the end of one month, fish subjected to heat and dissected out to separate the the gut and the removed the gut washed with ice cold fish ringer solution (Burnstock, 1958). The adhering tissues and the the gut contents carefully removed. The the gut weighed to mg. accuracy care taken to remove the adhering water in the the gut tissues using blotting paper. The the gut homogenized for 3minutes at 0^0C using a chilled tissue grinder. The the gut suspended in ice- cold buffer and diluted twenty fold relative to the weight of the the gut. The homogenate centrifuged for 15minutes at 1200rpm at 4^0C in a refrigerated centrifuge and the supernatant used as enzyme source.

2.5.3. Assay of digestive enzyme activity:

2.5.3.1. Assay of Amylase activity:

An amylase and Invertase activity determined using dinitrosalicylic acid reagent (DNSA) for free aldehyde groups to amylase glucose formed out of starch and sucrose digestion respectively (Ishaaya and Swirsky, 1970). The DNSA reagent prepared using the procedure of Noelting and Enfield, 1948. 1g of DNSA dissolved in 20ml of 2N NaoH and 50ml of water with the aid of a magnetic stirrer. Potassium sodium tartarate (30g) added and magnetic stirring continued until a clear solution obtained. Distilled water added to bring the final volume to 100ml. This DNSA reagent, when stored in a dark was suitable for use for at least 3 months. The amylase reaction mixture consisted of 2ml of 2% freshly prepared starch solution, 1ml of 0.01 M Phosphate buffer (PH 6.8) and 0.25ml of enzyme extract. After 60minutes incubation at 37^0 C for 5 minutes absorbency of the sample measured at 50 NM in Erma colorimeter against a blank in which the enzyme extract replaced by dematerialized water. The amylase activity expressed in terms of the weight of the reducing sugar (maltose) produced by the enzymatic action per unit weight of the the gut using glucose as standard.

2.5.3.2. Assay of Invertase activity:

The invertase reaction mixture consisted of 2.0ml of 1% sucrose solution. 2ml of 0.1M Phosphate buffer (PH 6.8) after 10 minutes incubation at 37^0C, 0.25 ml of enzyme solution added to the mixture. This mixture was again incubated for 60 minutes in a water bath maintained at 37^0C and subsequently the activity terminated by adding 1.5 ml of DNSA reagent. The reaction mixture maintained at 100^0C for 10 minutes and then cooled. The absorbency of the sample measured at 550nm in spectronic-20 against a blank in which the enzyme extract replaced by dematerialized water. The invertase activity expressed in terms of the enzyme action per unit weight of the gut using glucose as standard.

3. Results and Discussion

3.1. Survival Test:

The Lc 50 value for *Catla catla* against Raw Sugar Factory Effluent is duration dependent (Table 4). The sub lethal values derived from the LC 50 96h value used in the experiment. However there is no mortality shown by fish exposed to recycled sugar factory effluent and in the control group. 4.1. Survival Test

The 96h LC50 value for *Catla catla* was 10% , that corroborates with the findings of Lc 50 value, 12% in C*yprinus carpio* (Stoner and Livingstone, 1978); for *Labeorohita* as 19% (Ramakrishnan *et al.,* 2005). The 96hr LC50 value of *Catla catla* in different effluents has been reported; 14% in a dye factory effluent (Navaraj, 1988), 18% in a textile mill effluent (Haniffa and Jassentha, 1988) and 16% in paper mill effluent for (Nanda et al., 2000) have also been reported. This clearly indicates that *Catla catla* is more sensitive to the effluent.

3.2. Amylase activity

The Amylase activity in all the selected tissues of fish exposed to the raw sugar factory effluents decreased over that of the control.

The amylase activity in the gut of *Catla catla* exposed to control water 145.10mg maltose/reaction decreased to 133.26, 121.05, 112.70, 99.27 and 90.01 mg maltose/reaction in the selected 2%, 4%, 6%,8% and 10% sub lethal concentrations of raw sugar factory effluent respectively (Table 5; Fig.6). This decrease from control was 8%, 16%, 22%, 31% and 37 respectively in 2%, 4%, 6%, 8% and 10% sub lethal concentration of effluent respectively.

The amylase activity in the gut of *Catla catla* exposed to control water 148.1mg maltose/reaction was decreased to 147.2, 147.0, 146.8, 146.6 and 146.4 1mg maltose/reaction in the selected 2%, 4%, 6%, 8% and 10% sub lethal concentrations of recycled sugar factory effluent respectively (Table). This decrease from control was 0.6%, 0.7%, 0.8%, 1%, and 1% respectively in 2%, 4%, 6%, 8% and 10% sub lethal concentrations of effluent respectively.

Two- way ANOVA test indicated a significant decrease in amylase activity in fish exposed to different concentrations of sugar factory effluent when compared to control fish.

Amylase activity of fish indicated significant differences between the control and highest sub lethal concentration in the raw sugar factory effluent (Table 5a). But there is an insignificant difference exists between fish reared in control and recycled sugar factory effluent. However, there is a significant difference is found in amylase activity between fish reared in recycled and raw sugar factory effluent.

This result highlights the lower amylase activity of fish exposed to raw sugar factory effluent medium when compared to those of the other test media.

3.3. Invertase Activity

The Invertase activity in fish exposed to the raw sugar factory effluents decreased over that of the control (Table 6: Fig.7).

Table 1: Proximate Composition (Mean ± SD) of the feed

Ingredient	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber	Ash	Calories
Peanut Oilcake	10.47	35.42	9.50	25.40	-	12.16	10.8
Rice Bran	10.65	08.62	6.00	38.60	9.15	14.20	9.5
Fish Meal	2.50	25.40	18.10	32.30	7.50	11.10	18.8
Dry Fish	1.90	28.12	03.10	-	-	-	3.2
Topioca flour	00.80	03.80	02.60	45.80	-	1.75	2.4

Total = 34.1 KJ.g-1

Table 2: Physico- chemical characteristics of raw and recycled sugar factory effluent

Parameter	ISI limit	Raw Effluent	Recycled Effluent
Colour	Colourless	Yellowish brown	Colourless
Suspended solids	100	1392	420
Dissolved solids	2100	3970	280
pH	5.5-9.0	5.5	6.2
BOD	30	1540	960
Dissolved oxygen	6.0	Nil	5.2
Dissolved chlorides	600	289	220
Dissolved sulphates	2200	2550	1700
Dissolved calcium	7.5	805	280
Dissolved nitrates	300	40	30
Dissolved nitrites	300	35	0.55
Alkalinity	482	430	145

The value expressed in (ppm) except pH

Table 3: Physico – Chemical Characteristics of ground water used for dilution

Parameter	Value
Temperature	28.5^0C
PH	7.0
Dissolved oxygen	6.3
Organic nitrogen	0.7
Total hardness	2.9
Calcium	3.6
Chloride	4.7
Magnesium	2.9
Sodium	3.4

Except pH all value are expressed in $g.1^{-1}$

Table 4: The LC50 value of *Catla catla* at different duration in Sugar factory effluent

Hours	LC 50 value (%)
24	30
48	24
72	16
96	12

Table 5. Amylase activity in Fish exposed to Sugar Factory Effluent *Catla catla* (Mean ± SD)

Test Concentration	Raw effluent	Recycled Effluent
Control	145.1±0.034	148.1±0.034
2	133.26±0.030	147.2±0.1
4	121.05±0.036	147.0±0.15
6	112.70±0.034	146.8±0.05
8	99.27±0.051	146.6±0.1
10	90.01±0.06	146.4±0.05

Table 5a. Two -way ANOVA amylase activity in tissues of fish exposed to different test media for different concentrations.

Test media	$F_{(5,17)}$ Value
Raw effluent	6976*
Recycled effluent	2.89*

Table value of $F_{(5,17)}$ = 3.10 P value > 0.05

Table 6. Invertase activity in *Catla catla exposed to different types of Sugar Factory effluent* (Mean ± SD)

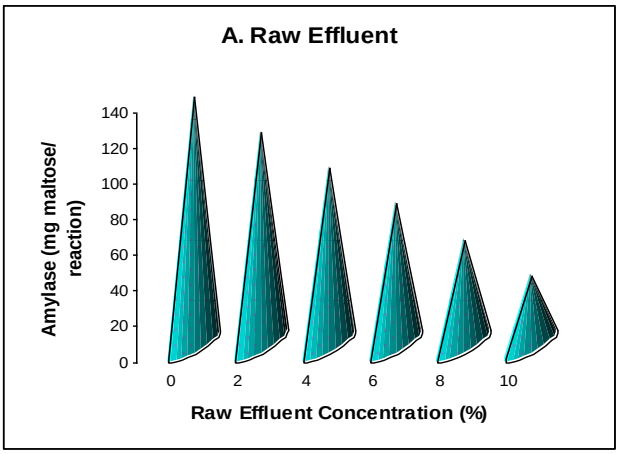
TestConcentration(%)	Raw Effluent	Recycled Effluent
0	130.00 ±0.08	135.00 ±4.0
2	125.98±0.03	134.98±0.1
4	115.90±0.06	134.7±0.05
6	102.62±0.034	134.5±0.05
8	93.04±0.045	133.9±0.05
10	79.29±0.026	133.7±0.05

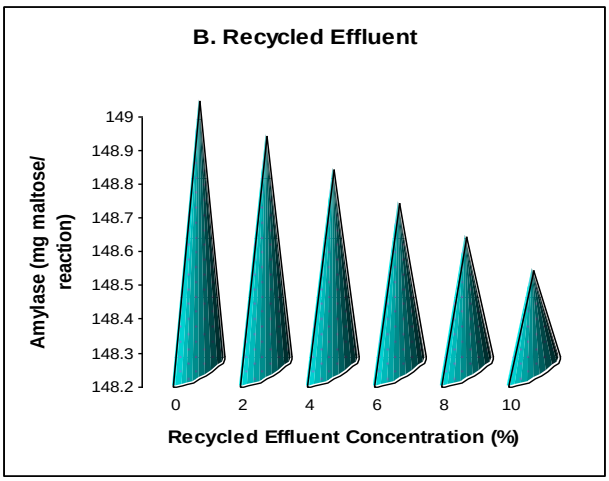
Table 6a . Two -way ANOVA invertase activity in tissues of fish exposed to different test media for different concentrations

Test media	$F_{(5,17)}$ Value
Raw effluent	4272*
Recycled effluent	3.01*

Table value of $F_{(5,17)} = 3.10$ p value > 0.05

Fig.6. Amylase activity of *Catla catla* exposed to different test medium





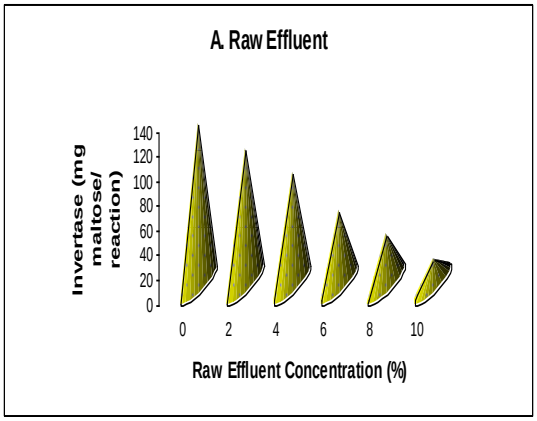
The invertase activity in the gut of *Catla catla* exposed to control water 130.00 mg maltose/reaction decreased to 125.98, 115.90, 102.62, 93.04 and 79.29 mg maltose/reaction in the selected 2%, 4%, 6%, 8% and 10% sub lethal concentrations of raw sugar factory effluent respectively. This decrease from the control was 3%, 10%, 21%, 28% and 39% respectively in 2%, 4%, 6%, 8% and 10% sub lethal concentration of effluent respectively.

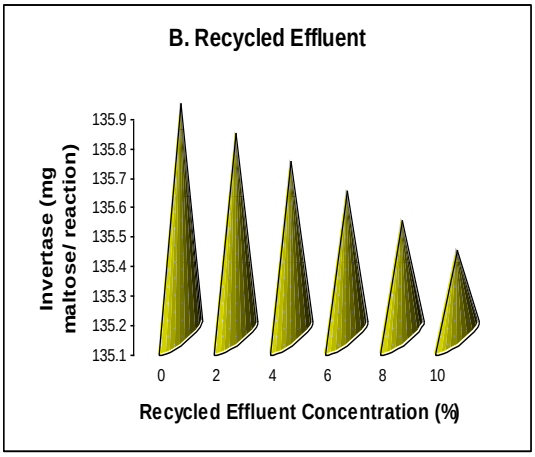
The invertase activity in the gut of C*atla catla* exposed to control water 135.00 mg.maltose/ reaction decreased to 134.98, 134.7, 134.5, 133.9 and 133.7 in the selected 2%, 4%, 6%, 8% and 10% sub lethal concentrations of recycled sugar factory effluent respectively. This decrease from control was 3%, 1%, 1%, 0.8%, and 0.9% respectively in 2%, 4%, 6%, 8% and 10% sub lethal concentration of effluent respectively.

Two - way ANOVA test indicated a significant decrease in invertase activity in fish exposed to different concentrations of raw sugar factory effluent when compared to those of other tested media (Table 6a). With regard to Invertase activity, a significant difference between the fish exposed to control and highest sub lethal concentration in the raw sugar factory effluent. But there is an insignificant difference exists between fish reared in control and recycled sugar factory effluent. However, there is a significant difference is found between the fish reared in recycled and raw sugar factory effluent.

This result highlights the lower invertase activity of fish exposed to raw sugar factory effluent medium when compared to those of the other test media.

Fig. 7. Invertase activity of *Catla catla* exposed to different test medium





Digestive enzymes are very sensitive macromolecules and are easily affected even by smaller changes in the external (or) internal medium. The growth of the fish depends on the digestibility of food (Bhattacharya and Mukherjee, 1976). The enzymes present in the the gut, control the digestibility and the activity and the activity of such enzymes was altered due to density stress. Amylase and invertase which have been reported to occur in the digestive tract of fishes are believed to be essential for digestion and utilization of starch, sucrose and protease respectively. In the present investigation, the activities of digestive enzymes viz, amylase and invertase altered under the influence of higher density stress (Verma *et al.,* 1974).

The activity of the enzymes may increase or decrease depending upon the type of enzymes, density stress and the length of time elapsed between stress and assay.

Thus the present study has revealed that sugar factory effluent significantly affects the activity of two major digestive enzymes viz., amylase and invertase in *Catla catla.*

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