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Use of Bioassay Test as a Performance Indicator in Effluent Treatment Systems including Role in encouraging shift towards use of Green Chemicals

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Abstract
Industrial effluents as from textile, pharmaceutical, tanneries, electroplating industries etc. contain many organic and inorganic compounds and metals. Determining impact of these pollutants on natural water bodies is a time consuming and uneconomical task if one tries to measure levels of each pollutant in order to determine its impact. Some of the pollutants may be present in trace levels and using instruments as atomic absorption spectrophotometer or gas chromatographs further adds to cost. In addition the limited physical-chemical analysis is of little help in determining the impact of different pollutants in a comprehensive manner. The Central Pollution Control Board, India therefore developed techniques for bio-monitoring of water bodies and notified Biological Water Quality Criteria for comprehensive assessment of health of water bodies. In addition standard was notified for bioassay test so as to understand combine (synergistic or antagonistic) effects on health and aquatic life's as hazardous chemicals and toxic metals may undergo bio-accumulation and magnification. This paper reviews the methodology and utility of Bioassay test- determining 90% survival of fish after 96 hours in 100% effluent, in water pollution control. It is concluded that use of bio-monitoring techniques not only provides a cost effective solution but will also encourage industries to use green chemicals that are biodegradable, non-toxic and does not damage aquatic life.

Keywords: Bioassay, aquatic, bio-monitoring, whole effluent toxicity tests

1. Introduction:

In common parlance bioassay is using a living organism to test for the presence of a compound in a sample. The organism used is sensitive to the compound for which the test is conducted. The presence of absence of compound is detected from the impact of compound on health of organism.

A historical example of bioassay was use of canaries to sense the presence of dangerous gases as methane in mines by miners. Canaries reacted quickly to presence of even small amount of dangerous gases like methane being more sensitive to these than humans. The miners would thus get chance to escape.

Bioassay is also defined as appraisal of the biological activity of a substance by testing its effect on an organism and comparing the result with some agreed standard. The ASTM (American Society for the Testing of Materials) has catalogued over 70 different bioassays.

In the field of prevention and control of water pollution bioassay tests were developed as a tool to evaluate possible harmful effects of effluents discharged into water bodies. These are also called whole effluent toxicity (WET) tests. WET tests are a method of biomonitoring wastewater toxicity. Although quantities of pollutants can be analytically determined in samples, these measurements may fall short of actually identifying toxic discharges. In these tests, carefully chosen organisms are exposed to whole effluent and/or effluent dilutions for a pre-determined time period in order to observe the effluent's effect on the organisms, and thereby, approximate it's potential to effect organisms within the receiving water. Acute tests measure how well organisms survive, while chronic tests measure survival and sub-lethal effects, such as a sample's effect on organism growth & reproduction.

Bio-Monitoring of Water Bodies in India

Not satisfied with chemical methods of assessing the impact of pollution on a river's ecosystem, the Central Pollution Control Board (CPCB) along with Zoological Survey of India (ZSI) under an Indo Dutch collaboration initiated a project to identify aquatic microorganisms which indicate the state of riverine pollution. The studies have shown that 80 percent of pollution of Indian rivers is of bacteriological origin. Thus the routine chemical tests such as those measuring the chemical oxygen demand are inadequate to indicate the profile of pollutants. Therefore whereas chemical methods only help in detecting pollution but bio-monitoring helps to understand its impact on the ecosystem.

The CPCB developed Biological Water Quality Criteria (BWQC) reproduced in Table-I for evaluation of quality of water bodies.

In addition CPCB notified standards for bio-assay test for effluents which lays down that 90% survival of fish after 96 hours in 100% effluent.

Methodology for Bio-Assay Test

Methodology is based on IS (Indian Standard): 6582-1971 R-1992. It is validated to account for variation in standard method which deals with determination of **acute toxicity**; whereas the standards for the discharge of effluent require only compliance to the condition that **"**90 percent (%) survival of fish after 96 hours in 100% effluent" is there or not.

Preparation

- On receipt of sample start test as soon as possible or preserve sample at 4ºC.
- If preserved bring to room temperature before use. Measure pH and other relevant characteristics of the effluent as DO (Dissolved Oxygen), COD (Chemical Oxygen Demand), TSS 9Total Suspended Solids), TDS (Total Dissolved Solids), Conductivity and Turbidity.
- Death of the fish may be caused by deficiency of DO in wastewater. Maintain a DO of minimum 4 mg/l in wastewater during testing by bubbling air. An on line DO meter is used to measure the DO levels in test jars. However, care is to be taken that excessive DO (super-saturation) is avoided. Also avoid violent agitation of the test animals by air supply.
- Filter the effluent if there is excessive turbidity.
- It is also better if temperature of the test effluent is maintained constant during the experiment at 20±2ºC (In winter) or 25±2ºC (In Summer). The test jars thus can be kept in water carrying container fitted with a temperature indicator cum controller (Thermostat) or the temperature can be measure using a calibrated thermometer.
- Individual samples may not be necessarily combined to make composite samples as maximal toxicity of a variable effluent is often required in connection with control of effluent disposal in flowing water rather than knowledge of average toxicity.
- Use fresh water fish adaptable to laboratory conditions of feeding and handling for at least 10 days (In large rectangular glass aquarium tanks say 60-200 liters capacity); The incidence of fish dying in the acclimatizing during a period of 4 days preceding a test shall be less than 10 percent. Preference may be given to use of fish that commonly inhabitate unpolluted waters in the locality if available in suitable size and number. Fish normally 5-7 cm long are used. Fish suitable in the region are- Zebra fish (Brachydanio Rerio)/ Daphnia
- Fish should not be fed for 24/48 hours before they are used in a test. They shall also not be fed during the test.
- If possible use uncontaminated water (upstream of the point of discharge. Filter if excessive suspended matter is present**)** from the same source that is receiving water body. Otherwise may take a sample of these water and test for mineral content. Prepare a water of similar nature in laboratory to simulate natural water conditions particularly to adjust pH, temperature, alkalinity and hardness to match as closely as practicable to those of natural water. Else otherwise report these characteristics of the water used in test report.
- Fish may also be acclimatized for 24-48 hours in a tank where large dilution say 1(sample) :499 (dilution water) may be done.
- In transferring test fish special care shall be taken to handle them without causing injury

Conducting Test and Reporting

- Use 5 to 10 fish for main test. All fish to be introduced to test jars in 30 minutes. Add one fish to one jar (Preferably can use 2 to 2.5 liter beakers/jars, wide mouth and made of glass) containing one liter of 100% effluent. (May use 500 ml or 750 ml if the test effluent available is less in quantity or can add fish together in the jar- It is desirable to maintain a ratio of about 1 g of weight of fish per liter of liquid) Observe for next 96 hours. Record and remove if any fish die during the test. A fish is declared dead if it does not move when touched. If 90% of the fish survive in 100% effluent report results as complies. If more than 10% fish die during this period report as 'Does not comply.'

Interference Extreme volatility, instability and rapid detoxification of the effluent components and excessive oxygen demand can render the routine bio-assay method inapplicable or can cause serious bias in the results. Extreme volatility, instability or detoxification of important constituents is indicated when the recorded average survival time of the test animals in a fresh medium is much less than the survival time in the corresponding older used medium, if adequate DO is present throughout both tests. This may require modification in terms of- RENEWAL OF TEST SOLUTION. This modifications is recommended especially when there are reasons for believing that toxicity of the liquids declines rapidly during the course of a test. Periodic renewal of the liquid tested at 8 hours, 12 hour or 24 hours or at other convenient intervals is necessary to maintain the test conditions. This may be accomplished by transferring the test animals quickly by means of a dip net to a test container with fresh liquid. The bioassay test result sheet is given in Table-2.

Table-I: Biological Water Quality Criteria (BWQC)

S.No.	Taxonomic groups	Range of saprobic score	Range of diversity score	Water quality characteristics	Waer quality class	Indicator colour
1.	Ephemeroptera, Plecoptera, Trichoptera, Hemiptera, Diptera	7 and more	0.2 - 1	Clean	A	Blue
2.	Ephemeroptera, Plecoptera, Trichoptera, Hemiptera, Odonata, Diptera	6 - 7	0.5 - 1	Slight Pollution	B	Light Blue
3.	Ephemeroptera, Plecoptera, Trichoptera, Hemiptera, Odonata, Crustacae, Mollusca, Polychyaeta, Coleoptera, Diptera, Hirudinea, Oligochaeta	3 - 6	03 – 0.9	Moderate Pollution	C	Green
4.	Mollusca, Hemiptera, Coleoptera, Diptera, Oligochaeta	2 – 5	0.4 & less	Heavy Pollution	D	Orange
5.	Dipetra, Oligochaetera or No macro-invertebrates	0 - 2	0 – 0.2	Severe Pollution	E	Red

Table-2: Bio Assay Test Results Sheet

Sample No.		**Quantity:**		
Packing and Marking:				
Date Sample Receipt:	Date Test Start:		Date Test End:	
Preservation Status:				
Visual Observation:				

Dilution Water Used:
Fish used:
Incidence of fish dying in the acclimatizing during a period of 4 days preceding a test:
Incidence of fish dying in the acclimatizing with sample during a period of 24 hours preceding a test:(**DILUTION USED)**

Note: The incidence of fish dying during acclimatizing shall be less than 10 percent

Sample Characteristics			**Dilution Water Characteristics**		
Parameters	**Units**	**Results**	**Parameters**	**Units**	**Results**
pH	-		pH		
Turbidity	NTU		Hardness	mg/l	
Conductivity	µmho/cm		Calcium	mg/l	
DO	mg/l		Magnesium	mg/l	
BOD	mg/l		Chlorides	mg/l	
COD	mg/l		Sulphate	mg/l	
TDS	mg/l		Alkalinity	mg/l	
TSS	mg/l				

Bath Temperature Maintained: _____ ºC

Date:	Time Hr	DO mg/l	**Fish No.**							
			1	2	3	4	5	6	7	8
			Jar No.							
			Remarks							
	0									
	1									
	2									
	4									
	6									
	8									
	12									
	24									
	48									
	96									

Remarks: √- OK; X-Dead; Any other abnormal behavior; DO after 0/8/12/24/48/96 Hours > or < 4mg/l

Any Other Remarks:

_____

Total Fish: _____ Fish Surviving in 100% Sample for 96 Hours: _____ **%** Surviving in 100% Sample for 96 Hours: _____

Results:

_____

Conclusion

The method is validated by testing treated effluents from different sectors as textile, pharmaceuticals including domestic sewage. The effluent from skid mounted units based on Submerged Aerobic Fixed Film Reactors (SAFF) or Moving Bed Bio Reactor (MBBR) technology after passing through Pressure Sand Filter and Activated Carbon Filter is subjected to bio-assay.

Toxicity can be caused by chemical, physical, or biological factors or a combination thereof. Chlorine, a chemical used for disinfection, and ammonia, a by-product of waste, are common causes of toxicity. Solids, pH and issues with dissolved oxygen or ion imbalance may also contribute to toxic responses, as can invertebrates, such as ciliates, and bacteria, especially if they also exacerbate issues with dissolved oxygen.

Further, breakdowns in the treatment process may also allow household and organic chemicals to enter the discharge at toxic levels.

In some cases, the toxicants or cause of toxicity can be found occurring naturally within the environment. It may be the concentration or synergistic effect that makes these factors exceed the tolerance of the test organisms.

The chemical analysis of an effluent cannot by itself predict its potential toxic effect. Many toxic pollutants cannot be detected by commonly available chemical analysis methods. The toxicity of many chemicals is unknown even when they can be detected. Different chemicals combined together in the same effluent can have unknown additive effects even when the toxicity of each individual chemical is well-known. It is here that bio-assay tests come handy and are now being made part of the consents. Obviously this put pressure on chemical manufacturers to develop biodegradable chemicals

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