EVALUATION STUDIES OF HEADSPACE BOD TECHNIQUE

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CERTIFICATE

This is to certify that the thesis entitled **"*Evaluation Studies Of Headspace BOD Technique*"**, is an authentic record of my own work carried out as requirements for the award of degree of Master of Technology in Environmental Science & Technology from Thapar University, Patiala, under the guidance of **Dr.A.S.Reddy** (Associate Professor, Biotech. & Env. Sciences) and **Dr.Shukhrishpal Kaur** (Research scientist,) during January to June 2012

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Introduction

1. 1 Introduction/background information

Organic matter is one of the important pollution parameter for water and wastewater. It includes a wide variety of organic substances, such as, biodegradable organic matter, non-biodegradable or recalcitrant organic matter, toxic organic matter, volatile organic compounds, etc. Of all the types, biodegradable organic matter is considered as the most important.

Characterization of wastewaters is very important in the selection of appropriate effluent treatment systems and in the design of the constituent treatment units of such system. Since biodegradable organic matter is an important parameter (organic pollution parameter), such characterization requires inclusion of biodegradable organic matter as one of the parameters of characterization. A multitude of treatment units, including biological treatment units, are used for treating wastewaters (prior to discharge) and reducing their biodegradable organic matter concentrations to desired levels. Efficient and effective operation of such treatment units usually requires monitoring of their input and output streams for biodegradable organic matter concentration.

Organic matter of water and wastewater sample is highly heterogeneous. It can be suspended, colloidal and dissolve forms. It can be composed of carbohydrate, Fats, Protein etc. and all these in term include wide variety of substances. Hence, it is almost impossible to have a single direct method for measuring the organic matter concentration. Indirect methods are thus restarted for the measurement. The indirect methods are divisible into two categories. (1) Method based on the measurement of the samples oxygen demand. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) test are included in this category. (2) Method based on the measurement of the samples organic carbon concentration. Total organic carbon test (TOC) tests are included in these categories.

BOD of the sample is defined as the amount of oxygen requirement by the microorganism to oxidize the organic matter by aerobic microbial decomposition to stable inorganic forms at some standard time and temperature.

Microbial oxidation of biodegradable organic matter in water bodies causes depletion of dissolved oxygen (DO). When the organic matter concentration is high, this oxidation can also cause reduction in the pH and pE of the water. Inability of the natural dissolved oxygen replenishment mechanisms to meet the oxygen demands of the microbial oxidation processes is responsible for this. DO depletion, and reduced pH and pE can adversely affect aquatic life of water bodies. Through regulating biodegradable organic matter discharge into water bodies, DO levels can be maintained sufficiently high and ecological health of water bodies can be protected. Such regulation of discharges for maintaining desired DO levels in water bodies demand monitoring of effluent discharges and of water bodies for biodegradable organic matter concentration.

1.2 Biochemical Oxygen Demand (BOD) Concept

Oxidation of Organic Matter and Theoretical Oxygen Demand (ThOD)

Oxidation of organic matter in the presence of oxygen or oxygen supplying oxidizing agent results in the generation of carbon dioxide, water and other inorganic end products (such as ammonia, hydrogen sulfide etc., depending on the elemental composition of the organic matter). Through stoichiometric calculations, it is possible to theoretically estimate the amount of oxygen demanded, by the sample, for the complete oxidation of the organic matter, present in it, into inorganic end products. Oxygen demand stoichiometrically estimated for a sample is known as its Theoretical Oxygen Demand (ThOD).

Stoichiometric equation for the oxidation of organic matter can be written as

$$C_nH_aO_bN_c + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3c}{4}\right)O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3c}{2}\right)H_2O + cNH_3$$

For glucose it can be written as

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

This stoichiometric equation indicates that six moles or 192 grams of oxygen is required for the complete oxidation of one mole or 180 grams of glucose into inorganic end products (water and carbon dioxide). From the above equation, one can deduce that a sample having ‘x’ mg/l of glucose theoretically has 1.067x mg/l of theoretical oxygen demand. Organic matter of a sample is rarely completely oxidized. Hence, oxygen demand actually experienced in the water bodies or measured by BOD or COD tests for water and wastewater samples is usually lesser than the ThOD estimated.

In both BOD & COD tests, organic matter of the sample is not completely oxidized. Hence, BOD and COD values of a sample are always lesser than the ThOD.

Fate of organic matter present in the sample

In the BOD test, microorganisms (bacteria) utilize biodegradable fraction of organic matter of the sample as food. The microorganisms used may not be efficient in utilizing the organic matter specially when its concentration drops below certain threshold value. Because of this reason, some fraction of biodegradable organic matter along with the non-biodegradable organic matter is left behind in the sample as residue. Utilization of the organic matter by the microorganisms may include the following two steps: biosorption and bio-oxidation. When organic polymers are biosorbed, they are first hydrolyzed by extra-cellular enzymes and then taken inside the cell for bio-oxidation.

Bio-oxidation of organic matter may involve the following two routes:

1. Aerobic oxidation (respiration) of some fraction of the taken up organic matter into inorganic end products (CO_2, H_2O, etc.) and generation of metabolic energy required by the microorganisms

2. Utilization of rest of the organic matter as building blocks and synthesis of cellular material or new microbial biomass. This synthesis utilizes the metabolic energy generated during the aerobic oxidation

Due to the utilization of organic matter, during the initial period of incubation, the sample's biodegradable organic matter concentration decreases. Simultaneously, due to the synthesis of new microbial biomass, microbial biomass concentration of the incubated sample increases. This initial period of incubation during which organic matter concentration decreases and microbial biomass concentration increases, is often called as a synthesis phase. Increase in the biomass concentration cannot continue for long. As the organic matter concentration decreases and biomass concentration increases, the former gradually becomes limiting and the microorganisms will be subjected to starving. Under such starving conditions, the microorganisms are forced to utilize their own cellular material as food and oxidize it into inorganic end products. Because of this reason, concentration of the microbial biomass, after reaching a peak value, will start gradually declining. Figure-1 shows the dynamics of the organic matter concentration and the microbial biomass concentration of an incubated sample. This phase of incubation, during which

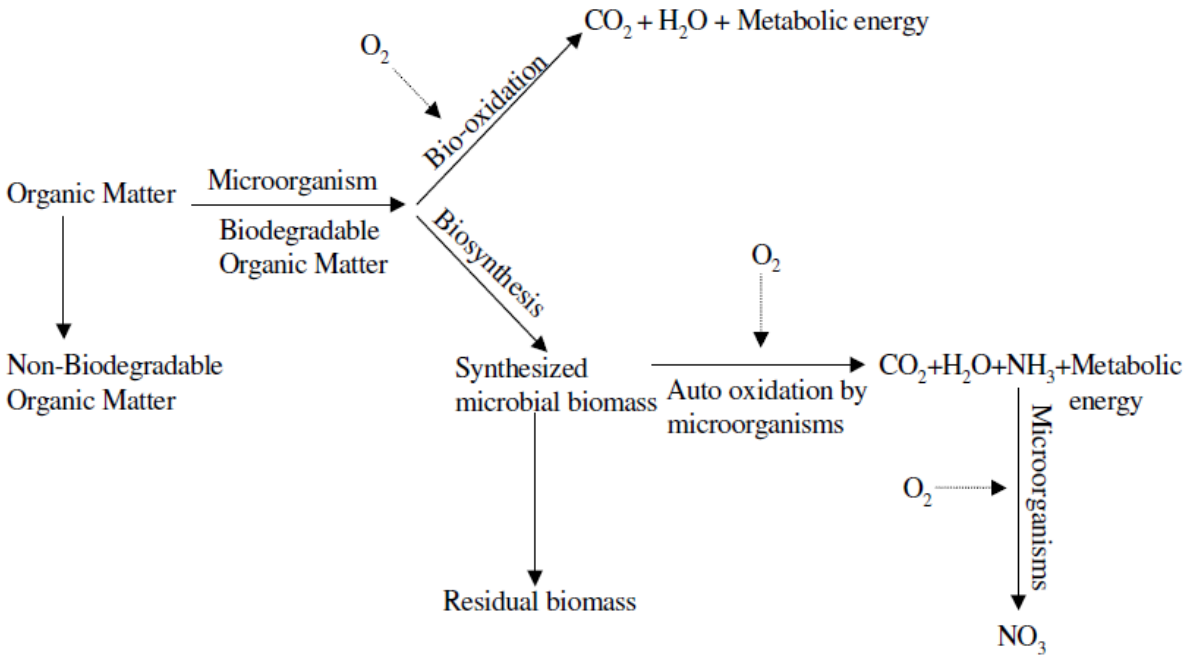
concentration of the microbial biomass declines, is known as auto-oxidation/ auto-lysis/ decay phase. All the cellular material synthesized during the synthesis phase may not get completely auto-oxidized even when the sample is incubated for indefinite period. Some fraction of it will be left behind in the sample as residual biomass.

Both synthesis and auto-oxidation phases of microbial growth consume oxygen. During the synthesis phase, oxidation of organic matter into inorganic end products, and, during the auto-oxidation phase, lysis of microbial biomass into inorganic end products both require oxygen. In BOD test, oxygen utilized in both the synthesis and auto-oxidation phases is together measured and recorded as BOD of the sample for the period of incubation of the sample. This BOD value is commonly denoted as 'BOD_t' (Oxygen demanded by the sample during the't' period of incubation).

Microorganisms utilize only biodegradable organic matter of the sample. Further, a significant fraction of the newly synthesized microbial biomass is not auto-oxidized even when the sample is incubated for indefinite period. Work done on glucose solution of 300 mg/l has indicated that its oxygen demand, when incubated for over 20 days, is 250 to 285 mg/l (just about 85% of the ThOD, which is 320 mg/l). This indicates that, though biologically assimilable, all the glucose present is not getting oxidized into inorganic end products. That is, the fraction of organic matter oxidized in the BOD test is generally lesser than that oxidized in the COD test. Hence, BOD is generally lesser than COD and always lesser than ThOD. However, for certain industrial effluents (dairy effluents), BOD can be greater than COD. These effluents have significant levels of nitrogen containing heterocyclic compounds which resist oxidation in the COD test. But these will easily get bio-oxidized in the BOD test.

Elemental composition of microbial biomass can be indicated by the approximate formula $C_5H_7O_2N$. During auto-oxidation, nitrogen present in the microbial biomass is released as ammonia. The released ammonical nitrogen can be oxidized by certain class of microorganisms (nitrifying bacteria: *Nitrosomonas* and *Nitrobactor*) into nitrate. This oxidation process (usually known as nitrification) also demands oxygen. Hence, in the BOD test, oxygen consumption by the sample during incubation may actually include three components, namely, oxygen consumption during the synthesis phase; oxygen consumption during auto-oxidation phase; and

oxygen consumption in the nitrification of the ammonia produced. Oxygen consumption for the nitrification is usually known as nitrogenous BOD (N-BOD), and the rest of the oxygen consumption is known as carbonaceous BOD (C-BOD).



Fate of the biodegradable organic matter, during incubation in the BOD test

Nitrogenous BOD exertion is not very significant during the first few days of incubation. Auto-oxidation of cellular material (microbial biomass) and bio-oxidation of nitrogen containing organic matter produce ammonia. This is then nitrified first into nitrite and then into nitrate by nitrifying bacteria. Population size of these bacteria in the incubated sample becomes large enough to affect nitrification usually after 5 to 7 days of incubation. Nitrification rate is also affected by DO concentration of the incubated sample. DO levels of above 2 mg/l favor the nitrification process. If the interest is to measure only the C-BOD of a sample, one should avoid nitrogenous BOD exertion. For this one can limit the incubation period to less than 5 to 7 days, or use nitrification inhibitors, such as methylene blue, thio-urea and allyl-thio-urea, 2-chloro-6-(tri-chloro-methyl) pyridine (TCMP), etc.

1.3 Alternative schemes for measuring BOD of a sample

The first laboratory procedure for the determination of biochemical oxygen demand (BOD) is believed to have been done by Frankland in 1870, but the first standardized test was not published in standard methods until 1917 (O'Brein and Clarck, 1962, Young and Clark, 1965). Although the test has been refined over the years, the basic approach of using a dilution technique has remained essentially unchanged (Logon and Wagenseller, 1993). The main reason for this is the low solubility of oxygen. The BOD procedure is based on the dilution of wastewater so that the final dissolve oxygen concentration in bottle is not depleted below 1-2 mg/l (Logon and Wagenseller, 1993). These dilutions reduce the concentration of substrate and microorganisms in sample, there by decreasing the overall kinetic rates. Thus, BOD tests are run for 5 days, even though wastewater treatment in the activated sludge unit is accomplished on time scale of hours.

The present day BOD_5 test suffers from many limitations (Schroeder, 1977, Glrady and Lim 1980, Metcalf and Eddy, Inc, 1991). Both the time period of 5 days and dilution basis disconnected the test from treatment process conditions, make it different to relate the BOD_5 to characteristic treatment process. Another problem with this test is that it is labour intensive and time taking. The most serious limitation is that in case of operation problems adverse conditions will be unknown for 5 days until the outcome of the BOD test is known.

Many alternative technique has been proposed to the conventional BOD test, but none have been adopted for the routine use in waste treatments. Among these tests were 8 hrs. (Bush, 1958, Hiser and Bush, 1964, Mullis and Schroedar, 1971) and two days (Zehnpfennig and Nicholas, 1953) test based on observations of pleateaus in oxygen and substrate use, but these tests required additional measurement of Chemical Oxygen demand (COD) or dissolve organic carbon (DOC) concentration Logan and Pantnaik, (1997). Various workers from time to time also developed BOD sensors useful for rapid and reliable BOD estimation in industrial wastewater (Restogi et. al, 2002 and Riedel, 2009, Namour et. a1, 2010, and Yoshida et. al, 2000). Other techniques were manometric methods that had the advantage of continuous monitoring samples without the dilution. The Warberg apparatus among the most successful alternative manometric method to the BOD test (Gellman and Heukelekian, 1951). In this

process diluted wastewater is placed in vials that are shaken and incubate at controlled temperatures. KOH is placed in a center well to strip out bacteriologically produced CO_2 so that oxygen utilization can be related to change in pressure sealed flask (Logon and Wagenseller, 1993). But warberg apparatus was not widely used due to high cost, need for trained operators and troublesome operation.

Use of electrolytic devices has become more wide spread for research purposes. Although these units work very well their expense and relatively sophisticated operation have resulted limited use in wastewater treatment plants.

Respirometers are being increasingly used for specialized applications and newest edition of standard method (1995) contains a proposed respirometric method. In general, a BOD_5 will be exterted in a respirometric BOD (RBOD test) test in~ 2~3 days providing an estimate of the BOD_5 in less than 5 days (Logon and Patnaik, 1997). Although many modern respirometers are reliable, their high per sample cost and relatively sophisticated operation have limited their routine use in wastewater treatment plant.

1.4 Headspace BOD tests (HBOD): A non dilution RBOD type test, called the HBOD was proposed by Logon and Wageneseller, 1993. HBOD and RBOD test are similar in that both are based on replenishing DO in the liquid sample from a gas phase or headspace, sealed in with the liquid sample. In the HBOD test, however wastewater sealed in small gas tight test tubes and the whole tubes is agitated on a laboratory shakers. In the original HBOD test oxygen consumption was evaluated by measuring the DO of a sample of pouring it into small (10 ml) sample holder. This transfer procedure was messy and risked re-aeration of sample before DO measurement (Logon and Patnaik, 1997). But later this method was improved by calculating oxygen demand from the decrease in oxygen concentration in gas phase or headspace of the sealed HBOD tube by gas chromatograph also called as dry measurement technique (Logon and Patnaik, 1997 and Logon and Kohler, 2001). The main disadvantage of HBOD is the lack of a simple and direct method to measure oxygen concentration in the gas phase and the sample has to transferred from HBOD tube to another vessel for DO measurement.

In present study it is observed that the DO level in the liquid phase has been assumed to be in dynamic equilibrium with the oxygen level in the headspace gas phase. DO level in the liquid phase (incubated sample) is measured and used in the estimation of the oxygen level in the gas phase and in the BOD measurement of the sample.

Chapter: 2

Literature Review

Bruce et.al.(1993) suggested headspace biochemical oxygen demand(HBOD) test having three main advantages: the test does not require sample dilution, oxygen demand determined with in a shorter period of time(24-36 h) that can be used to predict 5- day BOD value and experimental conditions used in the HBOD test, more accurately reproduce the hydrodynamic and culture conditions.

Sohn et.al.(1995) compared a multi-staged bioreactor system consisting of *Trichosporon cutaneum* immobilized in Ca-alginate bead and nylon column for the estimation of BOD of waste water with conventional 5-day method. A linear relationship was observed between the DO decrease and concentration below 90 ppm glucose and 90 ppm glutamic acid (5-day BOD, 130 ppm)

Murakami et.al.(1999) discovered a surface photo voltage (SPV), sensitive to surface pH and it was applied to the fabrication of an organic pollution sensor. Japanese Industrial Standard designated *Trichosporon cutaneum* as BOD sensor and employed it as immobilized biocatalyst. They made the comparison between 5 day BOD test (BOD5) and sensor used in measurement of BOD(BODs).Response time was 25 min, and microbial membrane can be used for 14 weeks.

Yoshida et.al. (2001) demonstrated the advantages of a mediator type biosensor, which does not require air-supply equipment for onsite measurements, and made a fully disposable sensor tip for a portable device. The tip consisted two electrode systems with *Pseudomonas flurescence* immobilized on a cellulose acetate membrane and is packed in a polyester film to prevent it from drying out. Examination of real samples from three different sources showed that BOD as determined by the sensor correlates well with the conventional 5- day BOD method.

Trosok et.al. (2001) studied the two Yeast strain (SPT1 and STP20) for mediated microbial biosensor for waste water BOD measurement. These two strains were immobilized on glassy carbon electrode to form microbial biosensor for estimation of biochemical oxygen demand.

Rastogi et.al. (2002) developed and characterized a novel immobilized microbial membrane for rapid determination of biochemical oxygen demand load in industrial waste waters. They

discussed the preparation of novel immobilized microbial membrane used in conjugation with an apt transducer.

Chee et.al. (2004) developed a photocatalytic biosensor of flow system using semiconductor TiO2 to evaluate biochemical oxygen demand (BOD) levels in river water.

Pasco et.al. (2004) described the development of a ferricyanide-mediated rapid biochemical oxygen demand method using an immobilized *Proteus vulgaris* biocomponent. They are developing a rapid microbial technique, MICREDOX® for measuring BOD by eliminating oxygen and, instead quantifying an equivalent biochemical co-substrate demand, the co-substrate being a redox mediator.

Paul et.al.(2005) developed a bio-electrochemical device and a process for quick and rapid estimation of biological oxygen demand.The device comprises of microbes, immobilized electrode,multimeter and a laptop workstation installed with required software vials.

Kwok et.al.(2005)developed an optical biosensor for multi-sample determination of biochemical oxygen demand. The biosensor monitors the dissolved oxygen(DO) concentration in water through an oxygen sensing film immobilized on the bottom of glass sample vial

Lin et.al. (2006) studied a novel BOD optical fiber biosensor based on co-immobilized micro-organisms in ormosils matrix. Measurements were taken for 3 min followed by 10 min. recovery time in 10mg/L glucose/glutamate (GGA) BOD standard solution and the range of determination was from 0.2 to 40mg/L GGA. The effect of temperature, pH and sodium chloride concentration on the BOD sensing film were studied.

Hudson et.al.(2007) determined the fluorescence intensities of trptophan-like, tyrosin like and humic-like material using excitation- emission-matrices (EEMS) for a wide range of sample.Fluorescence intensities reported in arbitrary fluorecsecnce units (AFU) wre correlated with standard 5- day BOD values which were used as an indicator of the amount of biodegradable organic matter present.

Hideaki et.al.(2007) studied a new chemiluminescense biochemical oxygen demand(BOD_{CL}) determining method by employing redox reaction between quionone and Baker's yeast.The measurement was carried out by utilizing luminal cheiluminiescence (CL) reaction catalyzed by

ferricynide with oxidized quionone of menadion, and Sacchomyces *cerevisiae* using a bathch type luminometer.

Chen et.al.(2007) developed a novel biochemical oxygen demand (BOD) sensing method employing a ferricyanide (FC) mediator immobilized in an ion-exchangeable polysiloxane.

Hoque et.al.(2008) described respirometric and titrimetric techniques for monitoring aerobic biodegradation of surfactant. This study described the results of work with this biosensor to investigate the aerobic biodegradation of a surfactant, one of the emerging organic contaminants in wastewater which have complex chemical formulae and high molecular weights.

Dhall et.al.(2008) developed an amperometric biosensor for determination of biochemical oxygen demand in waste water to overcome the time consuming monitoring procedures. The response time of the BOD sensor was only 90 min. being independent of the concentration, and lower detection limit was 1mg/l. The obtained BOD values showed correlation with that of the conventional method for BOD determination (BOD5) with a deviation of±10%.

Dogan et.al (2008) used artificial neural networks (ANNs) to predict and forecast water resources' variables. This study investigated the abilities of an artificial neural networks' model to improve the accuracy of the biological oxygen demand(BOD) estimation.Comparison results revealed that the ANN model gave reasonable estimation for BOD prediction.

Iranpous & Zermeno(2008) at Los Angeles/Glendale water Reclamation Plant(California) investigated, whether or not it would be feasible to use the measured value of biochemical oxygen demand of wastewater obtained by an online instrument.A comparison was made between the plant influent BOD values obtained by BIOX-1010 online monitor from the end of August (2000), to late January,2001 and the individual and average values obtained for the same period using the standard BOD5, 20^{o}C test, to determine the effectiveness of BIOX-1010 to identify shock loads and their duration. The results were satisfactory, so the instruments was used to trigger a shock-load warning alarm since late September.

Yang et.al. (2009) described simultaneous determination of chemical oxygen demand and biological oxy gen demand (BOD5) in wastewater by Near- Infrared spectrometry. By statistical

significance test, the results of determination were compared with those of standard methods with no significant difference at 0.05 level.

Roppola et.al. (2009) characterized the organic fraction of pulp and paper mill wastewater with manometric respirometric biochemical oxygen demand method and automatic chemical oxygen demand analyses.

Setdar et.al. (2009) developed a BOD sensor based on an immobilized *Pseudomonas Syringae* in highly porous micro-cellular polymer (MCP) in combination with a dissolved oxygen electrode.The sensor showed the detection linearity over the range 5-100 mg L^{-1} BOD5 (r2>0.99) at a flow rate of 0.6 mL/min.They concluded that the use of molded MCP disk containing microbial activity offers better stability and lifetime for commercial use in environmental monitoring.

Namour et.al. (2010) reviewed the sensors developed in past two decades for measuring total organic matter (TOM) and biodegradable matter (BOD) in water. They reported the state of the art of the most significant technologies.

Mark et.al. (2010) incorporated activated slugde as the biocatalyst in the fast, ferricyanide-mediated biochemical oxygen demand (FM-BOD) bioassay. Following a 24 h starving period, the return activated sludge and mixed liquor sludges reported the highest oxidative degradation of a standard glucose/glutamic acid (GGA) mixture and the return activated sludge also recorded the lowest endogenous FM respiration rate. They obtained a dynamic working range upto 170 mg BOD5 L-1 and 300mg BOD L-1 for OECD standard solution and GGA respectively. Its considered as an improvement upon the BOD5 standard assay and most other rapid BOD techniques. OECD standard is formulated as a synthetic sewage analogue.

Gatti et.al.(2010) evaluated the information provided by respirometric and physical chemical methods to assess the biodegradable matter fraction of wastewater. The respirometric analysis yielded a low value of Ss component than the one using the physical-chemical method. The respirometric analysis can-not measure the total content of slowly biodegradable organic matter Xs, this method is only capable of determining the readily hydrolyzed biodegradable substrate.

Francisco et.al. (2011) studied the performance of a waste water bench-scale ultrafiltration membrane bioreactor (MBR) treatment plant using pure oxygen to supply the aerobic conditions for 95 days. The results showed the capacity of the MBR system to remove organic material under a hydraulic retention time of 12 h a sludge retention time of 39.91 days.

Zhang Z.& Zhu J.(2006) developed laboratory-scale experiment that reveal the temporal characteristics of solids, biochemical oxygen demand (BOD5) and volatile fatty acids (VFAs) in the aerated liquid swine manure for minimizing odor generation potential during 190- day storage.

Nakamura et.al.,(2007) design a Biosensors that is a new biochemical oxygen demand (BOD) sensing method employing a double-mediator (DM) system coupled with ferricyanide and a lipophilic mediator, menadione and the eukaryote *Saccharomyces cerevisiae*has been developed and sensor responses to 14 pure organic substances were compared with the conventional BOD_5 method and other biosensor methods.

Tan and Wu (1999) installed a BOD biosensor with a biofilm containing immobilized thermally killed cells of a Bioseed microbial culture gave BOD sensing sensitivity comparable with that of a biosensor using the same population of the immobilized living cells of the same culture. The thermally killed cell sensor showed better response and storage stability but slightly longer response and recovery times than the living cell sensor

Oota S. et.al., (2010) designed a In flow injection analysis system for BOD sensor system and it was constructed by combining an immobilized microbial reactor with an electrochemical flow cell of three electrodes configuration, has been developed to estimate BOD ,It was demonstrated consequently that the mediated sensing was realized by employing phosphate buffer containing potassium hexacyanoferrate as the carrier. The output current was found to yield a peak with a sample injection, and to result from reoxidation of reduced mediator at the electrode. By employing the peak area as the sensor response, the effects of flow rate and pH of the carrier on the sensitivity were investigated.

Logan et.al.,(1997) was developed a Headspace BOD (HBOD) test that avoided the need to dilute wastewater samples. A disadvantage of the original HBOD test was that the sample had to be transferred from the HBOD tube to another vessel for a dissolved oxygen measurement from the study demonstrates that it is possible to conduct HBOD tests using a gas chromatograph to measure oxygen utilization in the sealed tube and that a 3-day HBOD provides a reliable estimate of the BOD_5. The $HBOD_3$ values measured for primary and secondary clarifier effluents from an activated sludge plant were 114 and 23 mg/L; BOD_5 measurements were 116 and 24 mg/L. Changes in headspace volumes did not significantly change the HBODs.

Hickey W.C and Nagels W.J (2003) were developed a new BOD analysis technique by modifications to an electrolytic respirometric system which facilitate precise measurements of BOD progressions for low substrate concentration ($BOD_5 < 20$ g m^{-3}) as found in natural waters receiving organic enrichment system

Nataraja M. et.al.,(2006) were found UV absorbance measurements a useful analytical tool for wastewater treatment , allowing them to quickly monitor for changes in the BOD_5 during the treatment process and to quickly estimate the BOD_5 when determining what dilutions to use in the standard BOD_5 test

Zhao H et.al.,(2004) developed a novel rapid methodology for the determination of chemical oxygen demand (COD) based on photoelectrochemical oxidative degradation principle (PECOD) was proposed and experimentally validated. With this new method, the extent of degradation of dissolved organic matter in a water sample is measured simply by directly quantifying the extent of electron transfer at a TiO_2 nanoporous film electrode during an exhaustive photoelectrocatalytic degradation of organic matter in a thin layer photoelectrochemical cell.

Zhang S. et.al.,(2006) employed a specially designed thin-layer photoelectrochemical cell that incorporates a highly effective nanoparticulate TiO_2 photoanode. This approach overcomes many problems associated with the conventional COD determination techniques such as long analysis time, consumption of expensive and toxic reagents, production of secondary toxic waste, and poor reproducibility. The effect of important experimental parameters on the analytical signal generation was systematically investigated, and the optimum conditions were obtained. The

method was successfully applied to determine the COD of real samples from various industrial wastewaters**.**

Yu H. et.al.,(2009) were developed a simple, environmentally friendly and continuous flow method for the determination of COD based on a flow injection analysis (FIA) system, in which a BDD electrode was employed as the detecting element. The structure and the electrochemical behavior of BDD were investigated by a scanning electron microscope, Raman spectroscopy, and cyclic voltammetry, respectively.

Pang L.H et.al.,(2007) developed a microplate-based biosensor consisting of an organically modified silica (ORMOSIL) oxygen sensing film for high-throughput determination of BOD in wastewater. The ORMOSIL oxygen sensing film was prepared by reacting tetramethoxysilane with dimethyldimethoxysilane in the presence of the oxygen-sensitive dye tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride. The bacterium *Stenotrophomonas maltophilia* was loaded into the ORMOSIL/PVA composite (deposited on the top of the oxygen sensing film) and used to metabolize the organic compounds in wastewater. This BOD biosensor was found to be able to determine the BOD values of wastewater samples within 20 min by monitoring the dissolved oxygen concentrations. Moreover, the BOD values determined by the BOD biosensor were in good agreement with those obtained by the conventional BOD_5 method.

Rustum et.al.,(2008) developed a Kohonen self-organizing map (KSOM)-based software sensors for the rapid prediction of BOD_5. The findings indicate that the KSOM-based BOD_5 estimates were in good agreement with those measured using the conventional bioassay method. This offers significant potential for more timely intervention and cost savings during problem diagnosis in water and wastewater treatment processes

Kara S. et.al.,(2009) developed a BOD sensor, based on an immobilized ***Pseudomonas syringae*** in highly porous micro-cellular polymer (MCP) in combination with a dissolved oxygen electrode for the analysis of biodegradable organic compounds in aqueous samples.

Celina M. et.al.,(2011) developed a methods for short-term BOD analysis (BOD_{st}) based on ferricyanide mediator reduction have succeeded in overcoming some problems associated with

the standard BOD test analysis (BOD_5) such as long-term incubations (5 days), the need to dilute samples and low reproducibility.

Engin O.G. et.al.,(2005) developed a technique in order to determine the boundaries of legal standards, reliable and efficient odour measurement methods need to be defined. An electronic nose was used for the purpose of characterising sewage odours. Samples collected at different locations of a wastewater treatment plant were classified using an Artificial Neural Network (ANN) trained with a back-propagation algorithm.The same method was used to determine the relation between sewage sample odours and their related Biochemical Oxygen Demand (BOD) values.

Min et. al.,(2004) observed the main disadvantage of the HBOD and other respirometric tests has been the lack of a simple and direct method to measure oxygen concentrations in the gas phase. The recent commercial production of a new type of fiber optic oxygen probe, however, provides a method to eliminate this disadvantage. This fiber optic probe, referred to here as the HBOD probe , was tested to see if it could be used in HBOD tests.

Young et.al.,(2003) developed a new methods for determination of BOD by completion of reaction within few hours instead of 5days by net cumulative oxygen uptake is correlated to the dilution BOD_5 and is calibrated against an organic standard such as glucose-glutamic acid..

Chee J.G et.al., (2000) developed an optical fiber biosensor for the evaluation of low Biochemical Oxygen Demand .The response time of the sensor was 15 min, and the optimal BOD response was observed at 30°C, pH 7.0. A linear relationship was obtained between the output voltage and BOD_5 values, and the range of determination was 1–10 mg 1^{-1} BOD.

Liu L.et.al., (2009) developed a organic inorganic hybrid material, which is composed of silica and the grafting copolymer of poly (vinyl alcohol) and 4-vinylpyridine (PVA-g-P(4-VP)), was employed to immobilize *Trichosporon cutaneum* strain 2.570 cells. Cells entrapped into the hybrid material were found to keep a long-term viability.

Sakaguchi T.et.al., (2006) developed a BOD monitoring system based on a bio-chip which immobilized luminous bacterium in micrometer-order holes were arrayed and fabricated by micro-machine techniques.

Chapter : 3

Materials & Method

3.1 Materials of HBOD :- Glassware(Specially designed air tight, 200ml capacity HBOD Flask with headspace,
magnetic stirrer, magnet, Volumetric flask, Measuring cylinder).

Reagent:-

a. Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75g K_2HPO_4, 33.4g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl in about 500mL distilled water and dilute to 1L
b. Magnesium sulfate solution: Dissolve 27.5 g $MgSO_4.7H_2O$ in distilled water and dilute to 1L.
c. Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1L
d. Ferric chloride solution: Dissolve 0.25 g $FeCl_3.6H_2O$ in distilled water and dilute to 1L.
e. Acid and alkali solution: 1N, for neutralization of caustic of acidic waste samples.
   Acid- Slowly and while stirring, add 28 mL conc. Sulfuric acid to distilled water. Dilute to 1L
   Alkai- Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1L
f. Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000mL distilled water. Solution is not stable, prepare daily
g. Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103^oC for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1L. Prepare fresh

3.2 Warberg's Respirometer (Headspace BOD Test)

In conventional BOD test, samples are prepared without any air (headspace) in the sample. Because dissolved oxygen in the liquid cannot be replenished, the amount of oxygen consumed is directly proportional to the change in dissolved oxygen in solution. In the HBOD test, however, by sealing a known volume of air into container, the oxygen in the air can be used to replenish the oxygen in the liquid phase, extending the measurable range of oxygen demand.

Because the sample bottle is sealed and air-tight during the test, it is not necessary to know the pressure in the vessel.

The basis of the test is that the amount of oxygen in the gas phase can be related to the concentration in the liquid phase via Henry's Law, or

$$P = Hc \qquad (1)$$

Where p is the partial pressure of oxygen[atm], equal to the product of the total pressure and the mole fraction of oxygen in the air; H is Henry's law constant [atm-mg/L]. The amount of oxygen consumed can be obtained from a mass balance the total moles in both the gas and liquid phases, assuming the two phases are in equilibrium,

At the start of the HBOD test, the moles of oxygen in the gas phase, m_g can be calculated from the ideal gas law as :

$$m_g = \frac{P_i V_g}{RT10^3}$$

Where V_g is the volume of air [mL] in the container, R the universal gas constant [0.082 atm/mol.^{o}K]. The moles of oxygen initially in the liquid phase are:

$$m_l = \frac{c_i V_l}{M10^6} \qquad (3)$$

Where M is the molecular weight of oxygen, and V_l the volume of the liquid phase. Combining equations 2 and 3, the total moles of oxygen at the beginning of the test are:

$$m_i = (m_l + m_g) = \frac{P_i V_g}{RT10^3} + \frac{c_i V_l}{M10^6} \qquad (4)$$

The total moles of oxygen present in a sealed container can be determined as above at the end of a HBOD test. Using equation 1 and 2, the total moles of oxygen in the gas and liquid phases at the end of the test, m_f are:

$$m_f = \frac{Hc_f V_g}{RT10^3} + \frac{c_f V_l}{M10^6} \quad (5)$$

Where c_f is the concentration of oxygen in the liquid phase in equilibrium with the gas phase at the end of the test.

The total headspace biochemical oxygen demand, HBOD, can be obtained from the difference in oxygen in the liquid measured for a sealed container before and after incubation, as:

$$\text{HBOD} = (m_i - m_f)\frac{M10^6}{V_l} \quad (6)$$

Where m_i and m_f are the initial and final total moles of oxygen in the system. Using equation 4 and 5 in equation 6, the HBOD can be calculated as:

$$\text{HBOD} = (V_g [M10]^{-3})/(V_l RT)\ (P_i - [Hc]_i(f)) + (c_i - c_f \quad (7)$$

This equation can be simplified using the Henry's Law constant obtained in equation 1. From Standard Methods (1975), the saturation concentration of oxygen, C_{sat}, is known for different pressures and temperatures. Therefore, using $H = P_i/C_{sat}$ in equation 7, we have:

$$\text{HBOD} = (V_g [MP_i 10]^{-3})/((V - V_g RT)\ (1 - C_f/C_{sat}) + (C_i - C_f) \quad (8)$$

Where, $V = V_i + V_g$ is the total volume of the container. It is not important that the sample is saturated with oxygen at the start of a test because oxygen in the container headspace will provide ample oxygen for the liquid phase. If it is suspected that the saturation concentration of oxygen in the sample is substantially different from that calculated using Standard Method (1975), C_{sat} can easily be obtained after the HBOD test is over by aerating the wastewater for 20 to 30 min.

2.3 Head Space BOD (HBOD) Test:

In present study, DO is not saturated by providing aeration prior to the incubation but head space is used to replenish the DO in the liquid phase during incubation. DO is measured from liquid phase using wrinkle method. DO level in the liquid phase has been assumed to be in equilibrium with oxygen level in the headspace gas phase. DO levels in the liquid phase of incubated sample has been measured and used in the estimation of the oxygen level in the gas phase and in the BOD measurement of sample.

Glucose glutamic acid is used as standard solution. pH was adjust to 7.2 with phosphate buffer prior to incubation. Standard solution was used in the range of 25%, 50%, 75% and 100% and incubated for 48 and 60 hours with continuous stirring on magnetic stirrer at 28± 2^{o}C.

BOD bottles were not used in the present study but special glassware (long neck flasks of 200ml capacity with head space) are designed and articulated.

BOD of the incubated sample was calculated according to the following formulae,

$$BODmg/l = 32000\frac{Vg \times Ppi}{Vl \times RT}\left(1-\frac{Cf}{Csat}\right)+(Ci-Cf)$$

Where,

Vg- volume of gas in liter

Vl- incubated volume in liter.

Ppi- 0.21dimensionless ratio.

R-Universal gas constant 0.821 atm/mol.k

T- incubation temperature in Kelvin

Ci & Cf initial and final volume of DO(dissolved Oxygen) respectively.

Chapter: 4

Result & Discussion

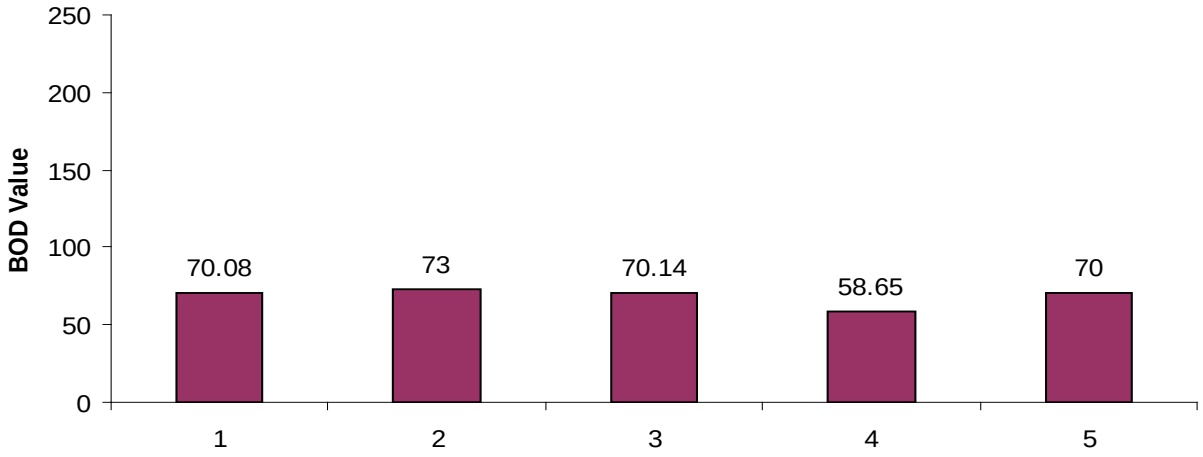


Fig 1 : *BOD exertion of 25% standard conc. for 48 hrs

*** Standard solution: Glucose, Glutamic acid**

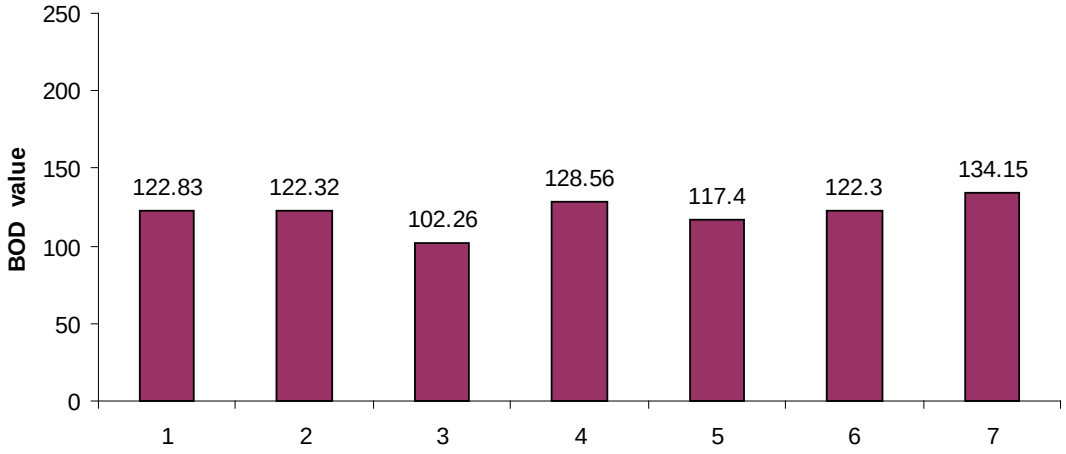


Fig 2 : BOD exertion of 50% Standard Conc. for 48 hrs

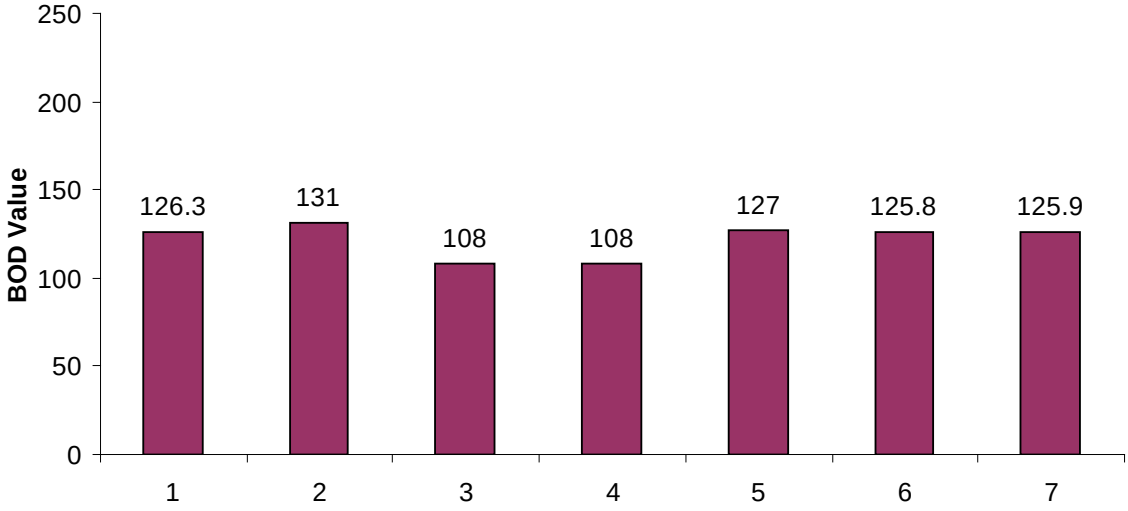


Fig 3 : BOD exertion of 50% standard conc. for 48 hr

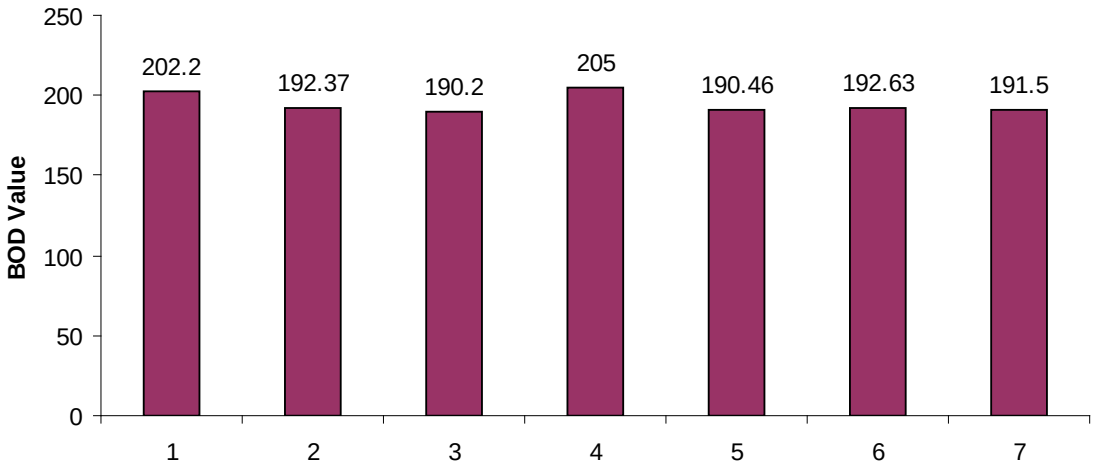


Fig 4 : BOD exertion of 100% standard conc. for 48 hrs

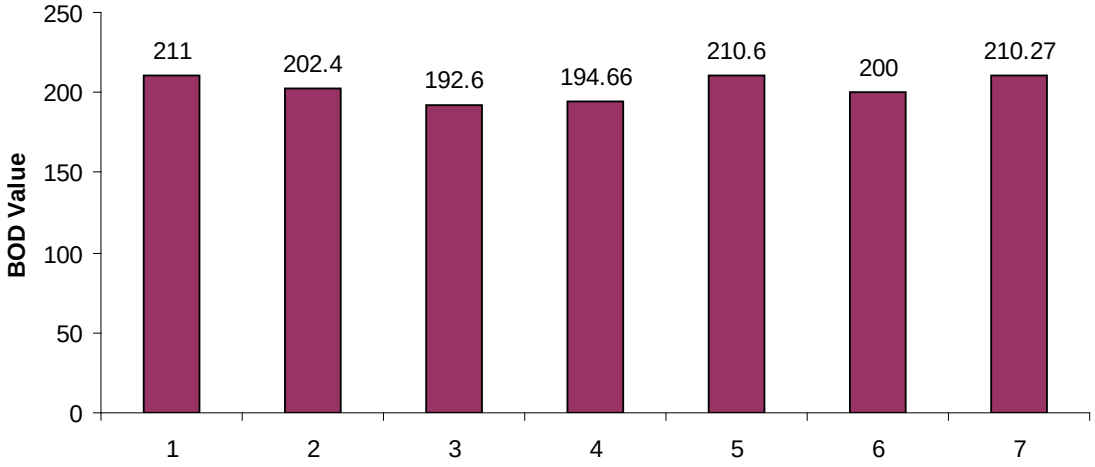


Fig 5 : BOD exertion of 100% standard conc. for 48 hrs

No.of days	**BOD Value for 100 %**
3	205.83
4	205.3
5	247.6

Table 1: BOD exertion of 100% standard conc. For 3, 4 and 5 days.

Fig: 6. Headspace BOD experimental setup

Fig. 1, 2, 4 depicts that after 48 hours incubation that results are BOD value are similar to the values of BOD5 for 25%, 50% and 100% standard (Glucose glutamic acid) concentrations respectively.

Fig.1 shows results of 25% standard concentration after 48 hours incubation. Organic matter content are approximately 68 in 25% standard concentration. Organic matter contents of 50% and 100 % standard concentration are approximately 120 and 195 respectively. Fig. 2 and 4 similar results were obtained after repeated these experiment at least five to sevan times.

Table 1 shows that after increasing the incubation period for 3, 4 and 5 days BOD exertion for 100% standard concentration was 205.83, 205.3, 247.6 respectively which shows the stability of experiment.

The HBOD test can provide rapid and reliable estimates of oxygen demands of non diluted wastewater.

Liquid based HBOD test results can also be erratic when the samples stand still too long before DO analysis. When a sample is not mixed, the consumption of oxygen in the wastewater by the microorganisms can result in liquid phase DO not being in equilibrium with the gas phase concentration. Thus the DO measured in the liquid may not reflect the oxygen consumption in the both liquid and gas phases. Since small changes in the DO can create large changes in the final HBOD, this additional consumption of DO could over estimate the final HBOD in a liquid-based test. Mixing the sample before liquid analysis is recommended in the liquid-based test to restore equilibrium conditions between the gas and liquid phases before DO measurement, but there is no easy method to verify that equilibrium between the gas and liquid phases has been reached resulting in a sample that could be over- or understurated with DO. In contrast, there is relatively little change in final HBOD .Thus, once a HBOD tube is no longer mixed, the gas phase concentration of oxygen is constant and produces no further change in the calculated HBOD.

From a practical viewpoint, one of the most obvious advantages of the HBOD test may be that it can provide more rapid estimates of wastewater oxygen demands than a BOD test (3 versus 5 days). For some wastewater treatment plants, this would allow a more rapid detection of changes the plant or discharge BOD5 values and provide an earlier warning of violations that

could lead to reduced fines. In a system where there are other operational problems, the effect of nutrient additions or combination of in-house wastewater streams to reduce toxic levels of chemicals could be easily be evaluated on full-strength wastewaters. At many sites , respirometers have been purchased to address such problems, but respirometer are quit expensive on a per bottle basis. In contrast, the number of samples analyzed using the HBOD method can easily be increased at little or no additional expenses.

Conclusion:

Characterization of waste water is very important in the selection of appropriate effluent treatment systems and in the design of constituent treatment units of such systems. Since biodegradable organic matter is an important parameter, such characterization requires inclusion of biodegradable organic matter as one of the parameters of characterization. From the review of literature it is observed that conventional biochemical oxygen demand BOD test requires a series of sample dilution because usually wastewater contains high concentration of organic matter, while concentration beyond 7mg/l can-not be tested by this method. Dilution of the sample is the main cause for introduction of error into the BOD measurement by considering these factors other alternative techniques have been developed like respirometric technique, bio-sensor methods and headspace methods. Respirometric BOD test procedures are now included in the standard methods as proposed method. Respirometric methods are routinely used in Europe.

Various types of biosensors have been developed. But relationship between the bio-sensor response and oxygen demand concentration of the sample is linear only within a certain concentration range and when the concentration range is very low decrease in current output or response of the bio-sensor is not significant.

A few years ago a headspace BOD (HBOD) test was developed that avoided the need to diluted wastewater samples. The main advantage of HBOD test is that the test can be performed more easily than the BOD test because no sample dilutions are necessary, the oxygen demand can be determined within a shorter period of time (24- 36 hours) can provide an accurate prediction of the 5 day value and the experimental conditions used in the HBOD test more accurately reproduce the hydro-dynamic and culture conditions typical of wastewater treatment bioreactor.

Biochemical oxygen demand is an international regulatory environmental index for monitoring organic pollutants in wastewater and the current legislated standard tests for BOD monitoring requires 5 day to complete BOD 5 test but there is still need to develop techniques for rapid and accurate measurements of biochemical oxygen demand.

Conventional BOD test is time taking and involves large errors. The sample dilution needed introduces error into the BOD measurement. Warberg's respirometer and Headspace BOD

technique, which need no or minimal sample dilution, suffer from the complications associated with the measurement of pressure drop or oxygen concentration in the gas phase. A variant to the Head space BOD test, which requires DO measurement in the liquid phase (rather than pressure drop or oxygen concentration measurement in the gas phase), has been conceptualized and evaluated in the present study.

DO level in the liquid phase has been assumed to be in dynamic equilibrium with the oxygen level in the head space gas phase. DO level in the liquid phase (incubated sample) has been measured and used in the estimation of the oxygen level in the gas phase and in the BOD measurement of the sample.

This method required no sample dilution (if the BOD is in the range of 15-400). Further, the prepared samples needed no aeration prior to incubation. Measurement of initial DO of the prepared sample can also be avoided without introducing any significant error in the BOD measurement. The evaluation studies indicated that BOD measurement can be more accurate and has greater reproducibility. Results are indicating that concentrated samples require more than 2 days incubation period.

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