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Indirect Measurement of the Biodegradability by the Utilization of Conductometric Probes and Self Developed Interface

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Abstract **– It was constructed self-developed interface allowed continual indirect measurement of evolved carbon dioxide, by the by the utilization of conductivity probes. This continual data record reduced demands on the human operators in the ready biodegradability assessment; while the indirect measurement of produced carbon dioxide was not assessed by the titration but conductivity measurement of absorption solution - barium hydroxide. This measurement was allowed because change in conductivity of barium hydroxide was in the linear correlation to reacted carbon dioxide. For the verification of the interface operation; while the range of the method was simultaneously determined; it was tested different concentrations of standard chemical (glucose), with the different amounts of inoculum to meet the condition of typical standardized test for both the ready and inherent biodegradability (OECD 301 B and OECD 302 B).**

Keywords **– Biodegradability, Carbon dioxide, Conductivity, Interface.**

I. INTRODUCTION

Degradation processes can be both abiotic (e.g. photochemical reactions and hydrolysis) and biotic [1]. Biodegradation by natural populations of microorganisms is the major route by which oil products are removed from soil and water compartments [2]. During these ultimate biodegradation processes the organic matter is converted into CO_2, H_2O, inorganic salts, microbial biomass and organic metabolites [3].Methods for measuring biodegradability can be divided into two principal groups: direct measurement of parent compound concentrations and indirect measurement of parent compound bioconversion, such as CO_2 production, decrease in DOC, cumulative oxygen consumption (BOD), and decrease in COD (chemical oxygen demand). The indirect measurement of biodegradation by using summary parameters, such as BOD, COD or DOC is often easy and can be automated but it may be necessary to determine physical chemical elimination processes, such as adsorption to biomass or stripping processes, to differentiate biodegradation from abiotic elimination [4].

Because of the possible misinterpretation of the data obtained in the inherent biodegradability tests (e.g. OECD 302 B test [5]) when assessing the biodegradability of poorly soluble or adsorbing substances, the industry recognize mostly the following tests such as OECD 301 B [6], ASTM 5864-11 [7] and ASTM 6139-11[8] which belongs to the ready biodegradability tests (OECD 301 B is similar to C.4-C Reach method [9] or ISO 9439 [10]). Useful schematic views of these tests provide authors in [11]. Inherent biodegradability tests (e.g. OECD 302 B) are primarily focus on the measurement of the biodegradability from the water phase by assessing of summary parameter such as COD or DOC, ready biodegradability tests (e.g. OECD 301 A-F) are primarily focus on the indirect activity of the microbial consortium during the biodegradation process. As these are usually respiration, ready biodegradability tests measure consumed oxygen in closed systems or produced carbon dioxide. Other differences in these two groups of test are in the amount of used tested sample and inoculum. While ready biodegradability tests which, are useful also for poorly soluble or adsorbing substances, usually use lower concentration of inoculum and lower concentrations of tested sample, inherent biodegradability use higher concentrations of tested samples and higher amounts of inoculum.Anyway it is important to note that there are different volume uses in these tests so total amount of inoculum and used tested chemical in lower limit of OECD 302 B is similar to those used in OECD 301 B.

The pass levels for the ready biodegradability are 70 % removal of DOC and 60 % of the theoretical oxygen demand or theoretical carbon dioxide. This is because some of the carbon from the chemical is incorporated into new biomass; the percentage of CO_2 produced is lower than the percentage of carbon being used. Tests such as OECD 301 B, ASTM 5864-11 are based on the passing the carbon dioxide free air through the bioreactor and trapping the carbon dioxide produced by the inoculum during the biodegradation process in the system of absorption bottles. Carbon dioxide reacts with the solution placed in the absorption bottles (obtained mostly solution of barium hydroxide) and creates insoluble precipitate. The amount of carbon dioxide is calculated from the residual hydroxide or as inorganic carbon. Titration methods utilized for the measurement of the residual hydroxide are discontinuous and need some practices. As it is allowed (e.g. ISO STN EN 9439) to measure evolved carbon dioxide directly by sensors, these may be high in investments [1]-[6].

Norr*et al.*, 2001 developed and defined a modified test system in [12]based on the combination of both OECD 302 B and OECD 301 B. They used concentrations of

tested substances and amounts of inoculum as they are allowed in inherent tests such as OECD 302 B is, but they measure the biodegradability as it is define in ready biodegradability tests (consumption of oxygen and production of carbon dioxide) while they used a closed apparatus. Oxygen was measured indirectly by the change in the pressure of temperature controlled environment, and evolved carbon dioxide was measured by the change in conductivity of absorption solution. Three years later, Strotmann *et al.*, 2004 in [4]released their study on a multicomponent biodegradation test system. This method used the direct linear relationship between the CO_2 production and the change of conductivity in a well-specified, calibrated system. As the carbon dioxide absorption solution was used KOH solution; while the biogenously evolved CO_2 produced insoluble precipitate K_2CO_3.

The main purposes of this study wereconstruction of apparatus enabling the biodegradability measurement according to [12], establishment of a conductivity-based online CO_2 evolution test with self-developed interface, checking its functionality and the evaluation of limited conditions.

II. Material and Methods

A. Principles of biodegradability measurement in constructed apparatus

For the measuring 10 closed apparatus were constructed; while it allowed the biodegradability measurements of eight different samples together with the blank and control simultaneously. One apparatus is obtained of two bottles (1.8 l); the air part is pumped from one to the other by the peristaltic pump. The first bottle is the reaction bottle where the biodegradation of tested substance occurred by the microorganism's activity. Carbon dioxide produced by the inoculum is than absorbed in the second bottle (absorption bottle) contained 1 liter of 0.0175mol/l $Ba(OH)_2$ which is normally conductive and achieve conductivity about 7.6±0.1mS/cm. Conductivity of the solution decreases when CO_2 react with the barium hydroxide; while creates an insoluble precipitate according to (1):

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O \qquad (1)$$

The amount of carbon dioxide (as mg C) produced by the inoculum after its reaction with the absorption solution was calculated from the calibration curve. Conductivity was recorded in all of 10 apparatuses simultaneously. Apparatus is shown in the Fig 1.

The air used in the test was not cleaned from the presence of CO_2 as it is accomplished in a standardized test, but the same level occurred also in blank. During the test, the absorption solution was replaced when it reached the level of conductivity below 1.5 mS/cm. The apparatus was opened, so that inoculum was also supplied by the fresh oxygen. It has to be note that the apparatus for the blank was not opened too.

Fig.1. Apparatus constructed for the indirect biodegradability measurement.

The percentage degradation of the test substance was calculated according to (2):

$$D = 100. (m_{CO_2\ test} - m_{CO_2\ blank}) / ThOC \qquad (2)$$

Where, ThOC is the carbon input by the application of the test substance in mg C and m_{CO_2} is the amount of evolved carbon dioxide (in mg C), calculated from the calibration curve according to recorded conductivity of absorption solution.

B. Calibration

Measurements for the calibration curve (the change in the conductivity after defined amount of carbon dioxide react with the barium hydroxide) were realized by injecting of defined volume of 1.0 mol/l Na_2CO_3 through the rubber septum by the syringe into the 2.0mol/lHCl solution with volume of 50 ml according to (3) as it was described in [12]. The absorption bottle during calibration contained 1 liter of 0.02 mol/l $Ba(OH)_2$. Air volume was arranged to the same portion as it was during the test.

$$Na_2CO_3 + 2HCl \rightarrow 2NaCl + CO_2 + H_2O \qquad (3)$$

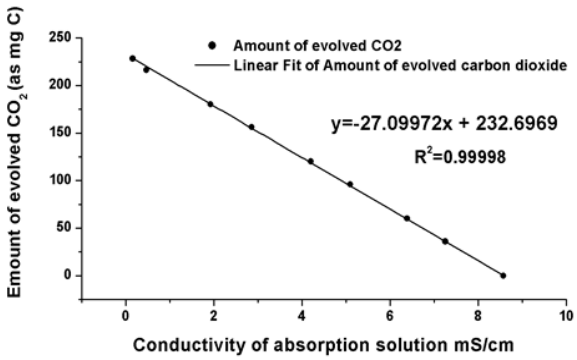

Fig.2. Calibration curve.

Calibration curve (Fig. 2) shows high linearity with the correlation coefficient at 99.998 %.

C. Conductivity measurement, self-developed interface

The conductivity measurements were executed by Vernier CON-BTA conductivity probes connected to self-developed interface. The developed interface is a 12 channel microprocessor controlled data acquisition and processing device with RS-485 communication interface. All of the 12 channels are isolated analog inputs with the range from 0 to 5 V. The input range of the interface was

proposed with respect to the range of the conductivity probe output signal which is 0 to 5 V. The block diagram of the developed interface is shown in Fig. 3.The analog voltages from the probes are measured and converted to digital signal with a 13 bit A/D converter. The digital signal is then shifted through the galvanic isolator to the microcontroller unit (MCU). A low power and high performance 8-bit microcontroller from Microchip is used as a controller.

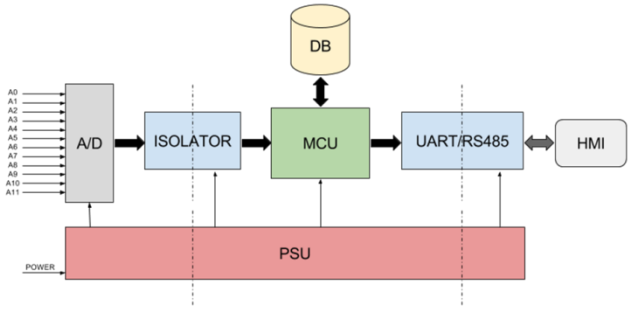

Fig.3. Measuring interface block diagram.

The single MCU controls the whole interface, processes the measured signal and stores it in the DB which is a 1 Mbit Serial SRAM. The SRAM is battery backed and thus it is a non-volatile memory. The power supply unit provides an isolated and proper voltage and current source to each parts of the developed interface. As a HMI a standard PC with USB port is used, which is connected to the communication network through an RS-485/USB converter. Between the data acquisition devices and HMI and Master-Slave communication take place wherein the HMI is the Master node. A GUI application was also implemented to the HMI which allows the online monitoring of the measured values and historical data export to xls. In this preliminary test the conductivity probes were set to measure in the range of 0-20 000µS/cm and the time capture was set up to 60 minutes.

D. Conditions

Comparison of selected parameters commonly used in modified and standardized biodegradability tests are described in the Table1. For our experiments we used two different additions of activated sludge and these were set up to 0.03 g/l and 0.1 g/l of suspended solids. The applicability of the test were assessed by the measurement of the glucose biodegradability in the range 10-200mgC/l to meets both criteria from OECD 301B and OECD 302B. All experiments were realized in triplicates.

III. RESULTS

At the Fig.4and 5 there are displayed recorded values of absorption solution conductivity from all apparatuses, while the Fig. 4 is for the used concentration of inoculum 0.03 g/l and the Fig. 5is for the used concentration of inoculum 0.1 g/l of suspended solids.

The conductivity decreases as it was expected according to the amount of inoculum and concentration of glucose. The lowest decrease was recorded in the case of blank apparatus and also in the case where was added 7.5 mg C of glucose. Both the record for blank and glucose (7.5 mg C) fluctuates; while the range for the probes was set up to the widest range (0-20mS/cm). Lapses recorded in the line for the glucose 150 mg C (Fig. 4) between 4-6 days were caused by the obstructing the pipes in the apparatus. After reparation all accumulated carbon dioxide react immediately with the absorption solution; while the conductivity decrease a lot in a short period of time. Breaks recorded during the 13th day (Fig. 4) and 10th day (Fig. 5) in glucose addition 150 mg C was caused by the replacing the absorption solution at the end of its capacity for the fresh one.

The decrease of the conductivity in blank may be explained by the endogenous respiration of microorganisms.

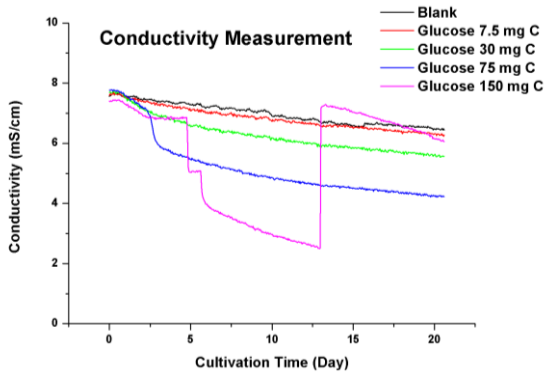

Fig.4. Conductivity kinetics during the test (activated sludge addition – 0.03 g/l of suspended solids).

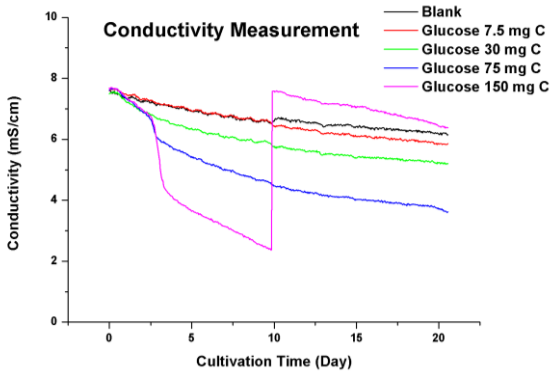

Fig.5. Conductivity kinetics during the test (activated sludge addition – 0.1 g/l of suspended solids).

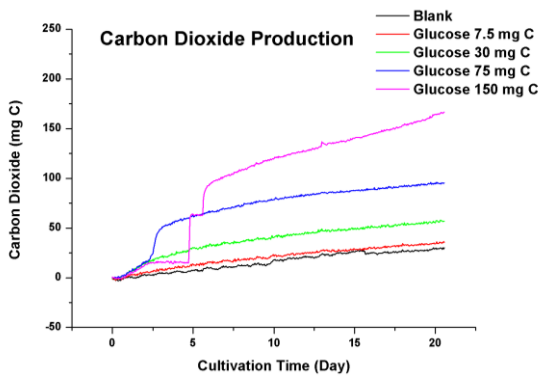

Fig.6. Evolved carbon dioxide, (activated sludge addition – 0.03 g/l of suspended solids).

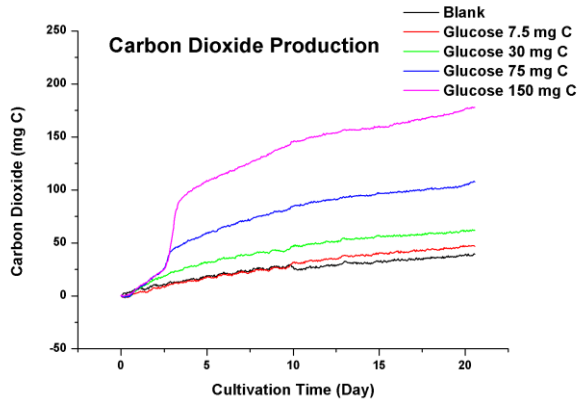

Fig.7. Evolved carbon dioxide,
(activated sludge addition – 0.10 g/l of suspended solids).

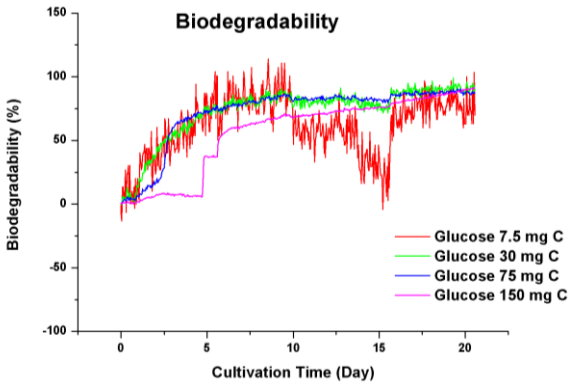

Fig.8. Biodegradability,
(activated sludge addition – 0.03 g/l of suspended solids).

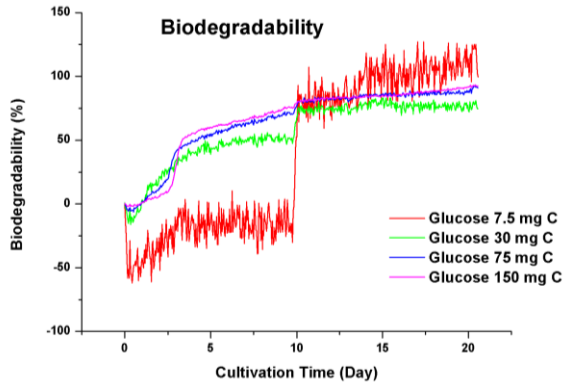

Fig.9. Biodegradability,
(activated sludge addition – 0.1 g/l of suspended solids).

Calculated amounts of evolved carbon dioxides are displayed in the Fig. 6 and 7. Estimated biodegradability rates are shown in the Fig. 8 and 9. According to (2) the estimated value of the biodegradability depends on the recorded amount of carbon dioxide in the blank. As it seemed from the record displayed on the Fig. 4 and 5, conductivity fluctuates and displayed unexpected leaps during the 10th and 16th day in the case of activated sludge 0.03 g/l and during the 10th day in the case of activated sludge 0.1 g/l of SS. These laps show in the lowest measured concentration of tested chemical (7.5 mg C of glucose) undesired leaps in the biodegradability curves. However, wrong range of probes gives rise to unacceptable noise. Moreover; calculated evolved carbon dioxide from the decrease in the conductivity of absorption solution was lover in the case of 7.5 mg C glucose than in the blank and these cause negative values of the biodegradability. As the concentration of the tested substances rises the noise in the estimated biodegradability curves decrease.

The biodegradation was in the 10-days window above 60 % for glucose 30-75 mg C (activated sludge 0.03 g/l) and 30-150 mg C (activated sludge 0.1 g/l). The total amount of carbon dioxide in the blank was in the allowed limit – below 50 mg C at the end of the test in both addition of activated sludge.

IV. Conclusion

For avoiding the fluctuations in recorded conductivities of blank or in the cases of small concentrations of tested chemicals; which cause serious problems in final outputs; it is recommended to use appropriate range for utilized probes, e.g. for our purposes, when tested blank or low concentrations of tested chemicals(lower than 10 mg C) it is recommended to use the range 0-2 mS/cm. It is also desirable to change the concentration of the absorption solution in these apparatuses to set in the range of probes. From these preliminary experiments result; with complying listed recommendations; that the biodegradability of some chemicals may be indirectly assessed, by the measuring the conductivity of the absorption solution when the concentration of tested chemical is higher than 100 mg C/l.

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Table 1 - Parameters according to standardized biodegradability tests.

	Parameter	Volume [l]	Concentration of Inoculum g/lof SS	Concentration of Test substance
Authors in [12] *	CO_2 continual - conductivity probe	0.75	**0.3**	**108-302 mg/l**
ISO 9439 *1	CO_2 discontinue titrating method or direct measurement of CO_2	3	**0.03**	**10-40 mg/l of TOC**
ASTN 5864-11	CO_2	3	**0.03**	**10-20 mg C/l**
ASTM 6139-11 *1	discontinue titrating method	1-3	**0.03**	
OECD 301 B *1	CO_2 discontinue titrating method	3	**0.03**	**10-20 mg/l of DOC/TOC**
OECD 302 B *2	COD or DOC from the water phase	1	**0.1-1**	**50-400 mg/l of DOC**
This study *1	CO_2 continual - conductivity probe	0.75	**0.03** **0.1**	**7.5-150 mg C/l**

Note: * modified method, *1 - Ready biodegradability test, *2 - Inherent biodegradability test