INNOVATION PARTNER

BIODEGRADABILITY TESTING OF ONE PRODUCT

CRIQ File No. 640-PX46424

Technical Report

CRIQ
INNOVATION PARTNER

BIODEGRADABILITY TESTING OF ONE PRODUCT

CRIQ File No. 640-PX46424

Technical Report

Mrs. Irina Bogdanova, PhD Sr. Chemist
DIZOLVE GROUP CORPORATION
290 Baig Blvd., Unit B10
Moncton, NB E1E 1C8

Guy Genest
Industrial Advisor

Michel Comeau, R&D Technician
Technical Manager

CHIMISTE
SYLVAIN SAVARD
79-217
QUÉBEC

Sylvain Savard, PhD Chemist
Industrial Eco-Efficiency and
Environment Division

February 25, 2013

Marie-Josée Hardy, director

Centre de recherche industrielle du Québec
333, rue Franquet, Québec (Québec) G1P 4C7
www.criq.qc.ca / infocriq@criq.qc.ca

T 418 652-1550 F 418 652-2202

Québec

The *Centre de recherche industrielle du Québec*'s mission is to contribute to the competitiveness of industrial sectors in Quebec by supporting business innovation.

Enseignement supérieur, Recherche, Science et Technologie
Québec

PRINCIPAL FINANCIAL PARTNER OF CRIQ

BIODEGRADABILITY TESTING OF ONE PRODUCT

Project team

Michel Comeau, R&D Technician, Technical Manager
Sylvain Savard, PhD Chemist, Supervisor in Physical Chemistry
Odette Petitclerc, R&D Technician, Microbiology
Alain Sauvé, R&D Technician, Physical Chemistry

For any information about this project

Technical Manager
Michel Comeau
333, rue Franquet, Québec (Québec) G1P 4C7
Téléphone : 418 659-1550 ext. 2583
Télécopieur : 418 652-2202
Courriel : Michel.Comeau@criq.qc.ca

Industrial Advisor
Guy Genest
333, rue Franquet, Québec (Québec) G1P 4C7
Téléphone : 418 659-1550 ext. 2879
Télécopieur : 418 652-2251
Courriel : Guy.Genest@criq.qc.ca

CRIQ
INNOVATION PARTNER

Québec

TABLE OF CONTENT

Page

1. MANDATE

The objective of this project was to determine the ready biodegradability of a new laundry detergent in accordance with OECD 301D Closed Bottle Test from the Organization for Economic Cooperation and Development as well as the sodium (Na), chloride (Cl-) and sulfate (SO42-) content.

2. DESCRIPTION OF THE WORK

The sample to be tested was received on January 10, 2013 and was registered under CRIQ No. 50041.

2.1 Chemical Oxygen Demand Determination

Before proceeding to the assay, the chemical oxygen demand (COD) of the product to be tested has to be determined in order to know the maximum quantity of oxygen microorganisms may use to oxidize the organic matter of the product. The COD is determined on an aqueous solution of the product according to Standard Methods for the Examination of Water and Wastewater 5220 C (closed reflux). The organic matter is oxidized when the sample is refluxed in a strong acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is determined by titration with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the organic matter that can be oxidized is calculated in terms of oxygen equivalent.

2.2 Microbial Toxicity

Before the biodegradability testing, it is important to determine if the product to be tested is toxic to the microorganisms at the concentration used for the test (usually between 5 to 10 mg O_2/l). The toxicity is estimated by the minimal inhibitory concentration (MIC) method. The product is suspended in nutrient broth at different concentrations and is seeded with microorganisms from the activated sludge used for the biodegradability testing. This solution is incubated at a temperature between 20 and 25 °C for 24 hours. After the incubation period, the solution is visually inspected for growth. A presence of growth indicates the product is not toxic to microorganisms at the concentration tested and the absence of growth shows the product is toxic. A positive and a negative control are added for the validation of the test.

2.3 Biodegradability Testing

The product was diluted in a mineral medium at a concentration of 5 and 10 mg O_2/l (COD equivalent). Two bottles were prepared for each weekly monitoring and inoculated with a suspension of activated sludge.

As a test validation, bottles containing a reference material whose biodegradability is known have been added to the series. Furthermore, bottles containing only mineral medium and inoculum were prepared for the determination of oxygen uptake caused by the inoculums itself for the calculation of the percentage of degradation. Finally, a nitrification inhibitor (2-chloro-6-(trichloromethyl) pyridine) was added to each bottle in order to inhibit the activity of nitrifying bacteria that may be present in the sample of activated sludge.

The concentration of dissolved oxygen in each bottle was measured with a previously calibrated oximeter and, thereafter, the bottles were sealed to prevent the contents from being in contact with air. Finally, all the bottles were placed in an incubator at 20 ± 1 °C. At an interval of 7 days and a maximum of 28 days, two bottles of each series were removed from the incubator to determine the concentration of dissolved oxygen.

The following table describes the experimental conditions of the assay.

TABLE I EXPERIMENTAL CONDITIONS

Test start up	January 23, 2013
Reference compound	Sodium benzoate
Concentration of the reference compound	5 and 10 mg O_2/l
Product to be tested	Dizolve 20X laundry detergent
Concentration of the product to be tested	5 and 10 mg O_2/l
Inoculum	Activated sludge from Valcartier
Inoculum rate	1 ml/300 ml
Incubation temperature	20 ± 1 °C

According to OECD 301D Test Method, a product containing more than one chemical product is considered to be readily biodegradable if decomposed at 60% or more within 28 days. In addition, the test is only valid if the reference material is degraded more than 60% in 14 days.

3. RESULTS

3.1 Chemical Analysis

Table II presents the results of the chemical analysis performed on the sample.

TABLE II CHEMICAL OXYGEN DEMAND

ANALYSIS	No. CRIQ	RESULT
Chemical Oxygen Demand	50041	**1,350,000 mg O_2/kg**

3.2 Microbial Toxicity

The microbial toxicity was determined by the MIC test. The results shown in Table III show that the product is not toxic to the inoculum used for the biodegradability testing.

TABLE III TOXICITY TEST

PRODUCT	OBSERVED GROWTH				
	20 mg O_2/l	10 mg O_2/l	5 mg O_2/l	2.5 mg O_2/l	0 mg O_2/l
Dizolve 20X laundry detergent	+	+	+	+	+
Negative control	-				

3.3 Biodegradability Estimation

Table IV shows the oxygen uptake calculated from measurements that have been made throughout the test while Table V shows the degradation results of the sample. This percentage is obtained by the following calculation:

$$D = \left[\frac{(C_{e(t)} - C_{b(t)})}{C_{th}} \right] \times 100$$

Where D: % of degradation at time t;
- $C_{e(t)}$ Oxygen uptake in the sample bottle at time t;
- $C_{b(t)}$ Oxygen uptake in the blank bottle at time t;
- C_{th} Theoretical O_2 concentration of the sample (5 or 10 mg O_2/l).

TABLE IV OXYGEN UPTAKE VS TIME

PRODUCT	OXYGEN UPTAKE (mg O_2/l)			
	T=7 days	T=14 days	T=21 days	T=28 days
Blank	0.23	0.26	0.11	0.11
	0.18	0.31	0.10	0.12
Mean	***0.21***	***0.29***	***0.11***	***0.11***
Reference compound (5 mg O_2/l)	3.73	4.14	---	---
	3.77	4.12	---	---
Reference compound (10 mg O_2/l)	7.85	8.46	---	---
	7.93	8.52	---	---
Dizolve 20X laundry detergent (5 mg O_2/l)	2.58	3.12	3.05	3.90
	2.54	2.87	2.97	3.82
Dizolve 20X laundry detergent (10 mg O_2/l)	4.90	5.90	6.03	7.06
	5.19	5.90	6.41	7.02

TABLE V BIODEGRADABILITY

PRODUCT	% DEGRADATION			
	T=7	T=14	T=21	T=28
Reference compound (5 mg O_2/l)	70.5	77.1	n.d.	n.d.
	71.3	76.7	n.d.	n.d.
Mean	***70.9***	***76.9***	***n.d.***	***n.d.***
Reference compound (10 mg O_2/l)	76.5	81.8	n.d.	n.d.
	77.3	82.4	n.d.	n.d.
Mean	***76.9***	***82.1***	***n.d.***	***n.d.***
Dizolve 20X laundry detergent (5 mg O_2/l)	47.5	56.7	58.9	75.7
	46.7	51.7	57.3	74.1
Mean	***47.1***	***54.2***	***58.1***	***74.9***
Dizolve 20X laundry detergent (10 mg O_2/l)	47.0	56.2	59.3	69.5
	49.9	56.2	63.1	69.1
Mean	***48.4***	***56.2***	***61.2***	***69.3***

n. d.: not determined

The test is valid because the reference compound had reached the pass level within 14 days.

4. CONCLUSION

To be considered readily biodegradable, a product must reach an oxygen uptake of at least 60% of the theoretical concentration of any reactor in 28 days.

Following the evaluation of the product which was submitted to us, we can conclude that, when tested according to OECD Method 301D:

- The Dizolve 20X laundry detergen is readily biodegradable in 28 days since all the reactors recorded degradation rates above 60% at that time.

CRIQ
INNOVATION PARTNER

Centre de recherche industrielle du Québec
QUÉBEC 333, rue Franquet, Québec (Québec) G1P 4C7 T 418 659-1550 / 1 800 667-2386 F 418 652-2251
MONTRÉAL 1201, boul. Crémazie Est, bur. 1.210, Montréal (Québec) H2M 0A6 T 514 383-1550 / 1 800 667-4570 F 514 383-3250
infocriq@criq.qc.ca **criq.qc.ca**

Québec