RespirTek™
CONSULTING LABORATORY

OECD 301B Ready/Ultimate Biodegradability Assessment

Date of Final Report: June 24, 2021

Total Number of Pages: 14

Report Prepared For:
Minus Works LLC
200-3 Blydenburgh Rd.
Islandia, NY 11749
516-527-7882

Report Prepared By:
RespirTek, Inc.
12450 Shortcut Rd. Bldg F
Biloxi, MS 39532
228-392-7977

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Study Summary

The Test Substance, P1 – 2 gel packs, was evaluated for ready and ultimate biodegradability in an aqueous medium, when exposed to an inoculum source according to the procedures detailed in the OECD 301B methodology.

Based on the test method employed, the maximum biodegradability of the test material is as follows:

Test Substance	Percent Biodegradation	Classification
P1 – 2 gel packs	100%	Ready

This value is the highest observed during the 25-day test for each test substance.

Project ID: MIN-2951
Date: June 24, 2021
Quality Assurance Unit Statement

The purpose of the Quality Assurance Unit is to monitor the conduct and reporting of laboratory studies. Enclosed is the final report data for project ID MIN-2951. All analyses were conducted following procedures set forth by the ISO/IEC 17025:2017 accreditation program standards. A copy of RespirTek's ISO/IEC 17025:2017 certification and scope are attached at the end of this report. Quality assurance systems and quality control criteria have been reviewed for the data collected, either internally or externally by one of RespirTek's affiliate laboratories, and the data review generated the following response:

QA/QC criteria met for all analyses

Emmy Radich, Ph.D.
Chemical & Biomolecular Engineering
Technical Director
RespirTek, Inc.
Phone: (228) 392-7977
Fax: (228) 396-3984
www.respirtek.com

Brenda Faz, M.S.
Environmental Management
Quality Manager
RespirTek, Inc.
Phone: (228) 392-7977
Fax: (228) 396-3984
www.respirtek.com

PJLA Testing
Accreditation #69085

Client: MIN-2951

Test Product(s): P1 – 2 gel packs

Test Method: OECD 301B - CO_2 Evolution Test

Report Date: June 24, 2021

1.0 Laboratory

Testing as presented in this report was conducted by RespirTek, Inc (RespirTek). The testing facility is located at 12450 Shortcut Rd., Bldg F, Biloxi, MS 39532.

2.0 Sample Receipt

Sample receipt was recorded on May 3, 2021 at the RespirTek testing facility. One package was received from UPS and contained the product for testing. The sample was securely wrapped. The test material was labeled as below and given the following laboratory identification:

- **P1 – 2 gel packs (MIN-TC1)**

The sample was received at ambient temperature in good condition with no evidence of damage or contamination. No temperature preservation was required. A Sample Submittal From, Control 032, and MSDS was included within the package.

3.0 Summary of Method

The OECD 301B biodegradability testing monitors the degree of activity of microorganisms exposed to a material that is being tested for a biodegradable status. In the test, if the microorganisms recognize the material as a food source, then an increase in biological activity is observed through data collection specifically designed to assess biological conversion of organic carbon to inorganic carbon. If the material is not a recognizable food source or is toxic or inhibitory, then there is no measurable increase in biological activity or, in some cases, there is a marked decrease in activity relative to a biodegradable control.

4.0 Project Preparation

Prior to test setup the appropriate number of 5 L Pyrex reactor bottles were washed and rinsed with tap water. The bottles were then rinsed three times with distilled water (DI H_2O) and allowed to dry. The mineral salt stock solutions for the project were prepared in media bottles using the appropriate chemicals and DI H_2O. The chemicals were weighed out using an analytical balance, and the DI H_2O was measured out using several 1000 mL or 100 mL volumetric flasks. Individual solutions were made up as follows:

Solution 1: The following compounds were added to 1000 mL of DI H_2O:
- 8.50 g of KH_2PO_4
- 21.75 g of K_2HPO_4
- 33.40 g of $Na_2HPO_4 \bullet 2\ H_2O$
- 0.50 g of NH_4Cl

The pH of the solution was then adjusted to 7.4.

Solution 2: 36.40 g of $CaCl_2 \bullet 2\ H_2O$ was added to 1000 mL of DI H_2O.

Solution 3: 22.50 g of $MgSO_4 \bullet 7\ H_2O$ was added to 1000 mL of DI H_2O.

Solution 4: The following compounds were added to 1000 mL of DI H_2O:
- 0.25 g of $FeCl_3 \bullet 6\ H_2O$
- 1 drop of concentrated HCl

All mineral salt stock solutions were kept in cold storage at 4°C chiller until used. A record of all chemical lot numbers and expiration dates are maintained in the laboratory Quality Standards Log.

5.0 Inoculum Collection and Conditioning

The Inoculum was collected from the Escatawpa, Mississippi POTW on May 14, 2021. This inoculum was immediately taken to the lab, homogenized, then placed into a 6 L Erlenmeyer flask. A Teflon stir bar was then added to the flask. The inoculum was placed on a magnetic stir plate. A CO_2-free aeration system, which uses a CO_2 scrubber system consisting of NaOH, was used to purge the inoculum. The inoculum continued stirring and aerating, uninterrupted, throughout the 6-day conditioning period.

6.0 Procedure

On May 20, 2021, a mineral stock solution was made up, as follows, according to OECD method 301B specifications:

DI water:	97,713 mL
Solution 1:	990 mL
Solution 2:	99 mL
Solution 3:	99 mL
Solution 4:	99 mL
For a total of:	99 L

Then, 2400 mL of the homogenized mineral stock solution was added to each 5 L reactor bottle. A Teflon stir bar was added to each reactor, which was then placed on a stir plate and connected to a CO_2-free air tank obtained from AirGas. Air flow to the system was confirmed using a Restek ProFlow 6000 Flowmeter to ensure air flows were within the 30-100 mL/min range that is stated within the method. The remaining nutrient solution was connected to a CO_2 scrubber system overnight.

A Total Suspended Solids test was performed on the inoculum using a Hach Lange DR5000. The test was performed on a 1:10 dilution of inoculum to DI H_20 in triplicate. The average TSS was calculated to be 3,588 mg/L.

The 301B method requires 30 mg of TSS to be added per liter of nutrient solution for a total of 3 L of nutrient-biomass solution. Therefore 25.1 mL of inoculum was added to each reactor bottle already containing the mineral medium.

The nutrient-biomass solution (2400 mL nutrient solution + 25.1 mL Inoculum) remained in the 5 L reactor bottles on a stir plate and hooked to the CO_2 scrubber system for 24 hrs.

Total Organic Carbon Analysis

On May 20, 2021, RespirTek, Inc. performed an analysis of the reference material to obtain a Total Organic Carbon (TOC) value.

On May 6, 2021, RespirTek, Inc. performed an analysis of the test material to obtain a Total Organic Carbon (TOC) value.

The TOC concentration value obtained during the preparation of the reference and test material is tabulated below:

Sample ID	TOC mg/L
Sodium Benzoate (PC)	344.6
P1 – 2 gel packs (MIN-TC1)	264.1

Using the percent TOC values, appropriate test chemical and positive control additions were made to obtain a final reactor TOC value of 10 mg/L for both the PC and TC.

The total amount of product to be added to nutrient-inoculum solution was added to enough mineral stock solution (the remaining solution that scrubbed overnight) to obtain a final total reactor composition of 3 L.

- **P1 – 2 gel packs (MIN-TC1):** 113.6 mL MIN-TC1 + 2400 mL CO_2 Free Mineral Stock Solution+ 25.1 mL biomass + 461.3 mL DI water.

- **Sodium Benzoate (PC):** 87.1 mL PC Sodium Benzoate Stock Solution + 2400 mL CO_2 Free Mineral Stock Solution + 25.1 mL biomass + 487.8 mL DI water.

- **Blank (B)**: 2400 mL CO_2 Free Mineral Stock Solution + 25.1 mL biomass + 574.9 mL DI water only.

All reactors were delivered CO_2-free air by passing compressed air through several soda lime and 1N NaOH scrubbers. The reactors were then continually stirred, kept in diffused light and allowed to vent into a three-series 0.05N NaOH scrubber solution. Each scrubber solution was analyzed for TIC (Total Inorganic Carbon) concentrations periodically throughout the extent of the test to determine concentrations of CO_2 produced by each reactor. Scrubber solutions were periodically refreshed to ensure adequate absorption of CO_2 was maintained. TIC analyses were performed on a Shimadzu TOC-V CSH instrument, which was calibrated prior to test initiation and periodically throughout the duration of the test. Test reactors were set up in duplicate for statistical validation of results, and a total of 8 sampling events was executed.

It was noted after the setup, that the TOC value achieved for the test material was not correct and adjustments were made based on the correct TOC value.

7.0 Results and Conclusions

Based on the testing conducted in accordance with the specified method above, test product, P1 – 2 gel packs, achieved 100% biodegradation. The product met method requirements for a *Ready Biodegradability* testing classification.

8.0 Records

Original raw data are archived at RespirTek, Inc. A copy of the final report and any report amendments are archived at RespirTek, Inc. The original final report, and any protocol amendments or deviations, is forwarded to the client.

All used and unused test substance shall be disposed of by RespirTek 6 months following test termination.

9.0 Confidentiality

Per corporate policy, confidentially shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between RespirTek and the Client.

Project Number: MIN-2951
Final Report Date: June 24, 2021
Project Initiation Date: May 20, 2021
Test Method: OECD 301B CO_2 Evolution Test

Test Chemical	Biodegradation (%)	Classification
P1 – 2 gel packs	100.0	Ready

Prepared for Minus Works LLC

Prepared by RespirTek, Inc.
12450 Shortcut Road
Building F
Biloxi, MS 39532

The enclosed data relates only to those samples received by the laboratory.

MIN-2951 Data and Calculations

Test Compound P1 – 2 gel packs: TC1
Positive Control Sodium Benzoate: PC

Analytical	
Positive TOC	10.00
TC1-1TOC	13.65
TC1-1 TOC	13.65
Positive ThCO2	110.00
TC1-1 ThCO2	150.15
TC1-1 ThCO2	150.15

Day 0 is a reference point for graphical illustrations only. No samples were collected on Day 0.

TIC (mg/L)

	Day 0	Day 1	Day 5	Day 7	Day 9	Day 13	Day 15	Day 19	Day 25
Blank-1	0.0	10.4	20.4	13.4	13.6	14.0	8.1	9.3	11.4
Blank-2	0.0	10.4	20.2	13.0	12.0	13.8	8.2	9.1	10.3
PC-1	0.0	38.1	82.9	37.4	28.5	25.5	13.0	14.5	14.0
PC-2	0.0	31.3	76.0	28.2	18.0	20.9	12.7	12.4	13.9
TC1-1	0.0	32.0	103.3	56.5	30.3	31.0	15.5	17.2	18.5
TC1-2	0.0	34.7	102.1	52.8	32.2	30.1	15.0	18.0	17.9

Total CO2 (mg)

	Day 0	Day 1	Day 5	Day 7	Day 9	Day 13	Day 15	Day 19	Day 25
Blank-1	N/A	7.6	14.9	9.9	10.0	10.3	6.0	6.8	8.4
Blank-2	N/A	7.7	14.8	9.5	8.8	10.1	6.0	6.6	7.6
PC-1	N/A	28.0	60.8	27.4	20.9	18.7	9.5	10.7	10.3
PC-2	N/A	23.0	55.8	20.7	13.2	15.4	9.3	9.1	10.2
TC1-1	N/A	23.5	75.8	41.5	22.2	22.7	11.4	12.6	13.6
TC1-2	N/A	25.5	74.9	38.8	23.6	22.1	11.0	13.2	13.1

Cumulative CO2 (mg)***

	Day 0	Day 1	Day 5	Day 7	Day 9	Day 13	Day 15	Day 19	Day 25
Blank-1	N/A	7.6	22.6	32.4	42.4	52.7	58.7	65.5	73.9
Blank-2	N/A	7.7	22.5	32.0	40.8	50.9	56.9	63.5	71.1
PC-1	N/A	28.0	88.8	116.3	137.2	156.0	165.5	176.1	186.4
PC-2	N/A	23.0	78.8	99.5	112.7	128.0	137.4	146.5	156.6
TC1-1	N/A	23.5	99.3	140.8	163.0	185.7	197.1	209.7	223.2
TC1-2	N/A	25.5	100.4	139.2	162.8	184.9	195.9	209.1	222.2

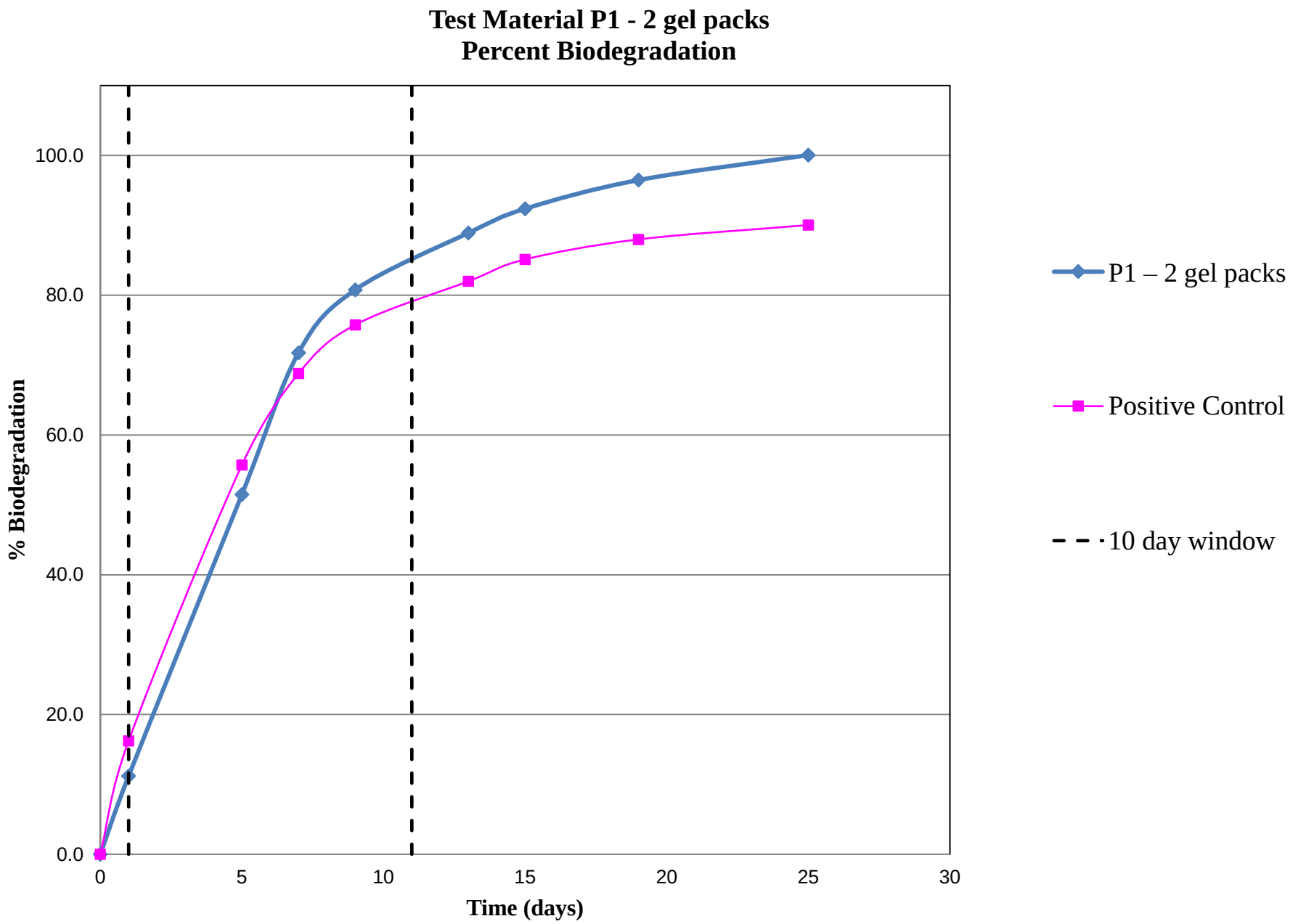
Percent Biodegradation (%)

	Day 0	Day 1	Day 5	Day 7	Day 9	Day 13	Day 15	Day 19	Day 25
PC-1	N/A	18.5	60.3	76.4	86.9	94.7	97.9	101.5	103.6
PC-2	N/A	14.0	51.2	61.2	64.6	69.3	72.4	74.5	76.5
TC1-1	N/A	10.5	51.1	72.3	80.8	89.2	92.8	96.7	100.4
TC1-2	N/A	11.9	51.9	71.2	80.7	88.7	92.0	96.3	99.7
PC Average	**0.00**	**16.2**	**55.7**	**68.8**	**75.8**	**82.0**	**85.1**	**88.0**	**90.0**
TC1 Average	**0.00**	**11.2**	**51.5**	**71.8**	**80.8**	**88.9**	**92.4**	**96.5**	**100.0**

Standard Deviation

	Day 0	Day 1	Day 5	Day 7	Day 9	Day 13	Day 15	Day 19	Day 25
PC	N/A	4.8	4.9	6.5	7.5	3.3	0.2	1.5	0.1
TC1	N/A	1.9	0.8	2.6	1.4	0.6	0.4	0.6	0.4
Blank	N/A	0.0	0.1	0.3	1.1	0.2	0.0	0.2	0.8

Sample Days	**0**	**1**	**5**	**7**	**9**	**13**	**15**	**19**	**25**


Test Material P1 - 2 gel packs
Percent Biodegradation
% Biodegradation
100.0
80.0
60.0
40.0
20.0
0.0
0
5
10
15
20
25
30
Time (days)
P1 – 2 gel packs
Positive Control
10 day window

PERRY JOHNSON LABORATORY ACCREDITATION, INC.

Certificate of Accreditation

Perry Johnson Laboratory Accreditation, Inc. has assessed the Laboratory of:

RespirTek, Inc.

12450 Shortcut Road, Building F, Biloxi, MS 39532

(Hereinafter called the Organization) and hereby declares that Organization is accredited in accordance with the recognized International Standard:

ISO/IEC 17025:2017

This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system
(as outlined by the joint ISO-ILAC-IAF Communiqué dated April 2017):

Biological, Chemical and Environmental Testing

(As detailed in the supplement)

Accreditation claims for such testing and/or calibration services shall only be made from addresses referenced within this certificate. This Accreditation is granted subject to the system rules governing the Accreditation referred to above, and the Organization hereby covenants with the Accreditation body's duty to observe and comply with the said rules.

For PJLA:

Tracy Szerszen
President/Operations Manager

Perry Johnson Laboratory Accreditation, Inc. (PJLA)
755 W. Big Beaver, Suite 1325
Troy, Michigan 48084

Initial Accreditation Date:	*Issue Date:*	*Expiration Date:*
September 16, 2011	March 04, 2020	June 30, 2022

Accreditation No.:	*Certificate No.:*
69085	L20-130

The validity of this certificate is maintained through ongoing assessments based on a continuous accreditation cycle. The validity of this certificate should be confirmed through the PJLA website: www.pjlabs.com

Certificate of Accreditation: Supplement

Respirtek, Inc.
12450 Shortcut Road, Building F, Biloxi, MS 39532
Contact Name: Jude Martin Phone: 228-392-7977

Accreditation is granted to the facility to perform the following testing:

FIELD OF TEST	ITEMS, MATERIALS OR PRODUCTS TESTED	SPECIFIC TESTS OR PROPERTIES MEASURED	SPECIFICATION, STANDARD METHOD OR TECHNIQUE USED	RANGE (WHERE APPROPRIATE) AND DETECTION LIMIT
Environmental Biological [F]	Plastic Material	Aerobic Biodegradation	ISO 14855	% Biodegradation
			ASTM D5338	
			ISO 14852	
		Oxobiodegradation & Biodegradation	ASTM D6954	
		Compostability	ASTM D6400	
		Anaerobic Biodegradability	ISO 15985	
	Chemical	Aquatic Aerobic Biodegradation	OECD 301A	
			OECD 301B	
			OECD 301C	
			OECD 301D	
			OECD 301E	
	Water/Soil Samples	Treatability/Toxicity Testing	Internally developed protocols-microcosm studies	
		HPC	SM 9215B	
Biological [F]	Chemical Compounds	Aquatic Aerobic Biodegradation	OECD 301F	
			ASTM D5210 ASTM D5988	CO_2 Gas D. L. = 1% CH_4 Gas D.L. = 0.1 %
			OECD 311 OECD 302B	
			ASTM D5511	
			ASTM D5864	
			ASTM D5271	
			OECD 310	
			ISO 14593	
	Aqueous Sample	TOC	SM5310B	
			ISO 14593	
			ISO 9439	
			ISO 15985	
Chemical [F]	Aqueous Samples	Biological Oxygen Demand	Standard Methods 5210 D	I.D.L = 1 mg/L
		Total Organic/Inorganic Carbon	Standard Methods 5310 C	M.D.L = 0.5 mg/L

1. The presence of a superscript F means that the laboratory performs testing of the indicated parameter at its fixed location. Example: Outside MicrometerF would mean that the laboratory performs this testing at its fixed location.