Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hanstveit, A.O., Pullens, M.A.H.L., 1993 BOD and COD of the product L(+) lactic acid according to EC Test Guidelines C.8 and C.9. TNO, report nr. IMW-91-0076-03. GLP, Unpublished.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Purac`Biochem	
1.2.2	Companies with letter of access	No	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Dutch guidelines NEN 6634 and NEN 6633, similar to EC test guidelines C.8 and C.9	x
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch no. ZO 3456	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	79.5-80.5%	x
3.1.4	Further relevant properties	Not applicable	x
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Not reported	x
3.1.7	Specific chemical analysis	Not performed	
3.2	**Reference substance**	No	x
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	Activated sludge from an oxidation ditch. For details, see table A7_1_1_2-2	
3.3.2	Test system	For details see table A7_1_1_2-3	
3.3.3	Test conditions	For details see table A7_1_1_2-4	

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3.3.4	Method of preparation of test solution	Not applicable	
3.3.5	Initial TS concentration	Nominal test concentrations 2.0 and 4.0 mg/L, no chemical analysis performed	x
3.3.6	Duration of test	20 days	
3.3.7	Analytical parameter	Biological and theoretical oxygen demand (BOD and ThOD, mentioned as COD in the study report).	
3.3.8	Sampling	O_2 concentrations were measured after 0, 5, and 20 days.	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	Yes, nitrification control was included by adding 2.5 mg/L allylthiourea to bottles containing 2.0 mg/L lactic acid.	
3.3.11	Controls	BOD: quadruplicate BOD bottles without lactic acid Toxicity: glucose and glutamic acid were added to control bottles and bottles containing 4 mg/L lactic acid. Nitrification: allylthiourea was added to bottles containing 2 mg/L lactic acid.	x
3.3.12	Statistics	The percentage degradation was calculated as (BOD/ThOD)×100	x
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Not presented	
4.1.2	Degradation	After 5 days: 50% After 20 days: 67%	x
4.1.3	Other observations	BOD values in bottles containing glucose and glutamic acid revealed that lactic acid did not inhibit the activity of the inoculum. Addition of allylthiourea resulted in some nitrification.	x
4.1.4	Degradation of TS in abiotic control	COD (ThOD): theoretical oxygen demand was 0.85 mg O_2	x
4.1.5	Degradation of reference substance	Not applicable	x
4.1.6	Intermediates/ degradation products	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	BOD and COD were determined according to the Dutch Guidelines "Water-determination of biological oxygen demand after n days (BODn)" (NEN 6634) and "Water-determination of chemical oxygen demand after n days (CODn)" NEN 6633, similar to EC test guidelines C.8 and C.9. An activated sludge inoculum was used. A control test	x

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		with glucose and glumatic acid as substrate was included in order to assess possible toxic effects of lactic acid on microbiological activity. Furthermore, nitrification control was included by adding allylthiourea.	
5.2	**Results and discussion**	The degradation after 5 days was 50%, after 20 days 67%. No toxic effects were found. Based on these results, lactic acid can be considered readily biodegradable.	x
5.3	**Conclusion**	See pass levels in tables A7_1_1_2-5.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2009/10/14
Materials and Methods	Applicant's version is acceptable apart from the following amendments: 2.1: To be correct, it has to be stated that the mentioned Dutch guidelines NEN 6634 and NEN 6633 are similar to EC test guidelines C.5 and C.6 instead of C.8 and C.9. 3.1.3: The purity of the test substance is 79.5-80% (water is the other constituent of the test substance?). All measured values refer to the purity 79.5-80%. 3.1.4: The measured COD of the test substance is 0.902 mg O_2 mg^{-1} and the theoretical oxygen demand was calculated to be 0.85 mg O_2 mg^{-1}. 3.1.6: In a study according to OECD 209 (cf. Doc III A7.4.1.4_01), an EC_{50} >100 mg L^{-1} was observed for the test substance. 3.2: Following the Dutch guideline, reference substances used in this test are glucose and glutamic acid (aniline is used as reference substance according to OECD 301D). 3.2.1.: Initial concentrations of reference substances in the procedure control are 3 mg L^{-1} glucose and 3 mg L^{-1} glutamic acid. 3.3.3: Composition of the medium was comparable to OECD 301D with two exceptions: (a) instead of $Na_2HPO_4.2H_2O$, $Na_2HPO_4.\underline{7H_2O}$ was used and (b) instead of 0.50 g NH_4Cl, <u>1.7 g</u> NH_4Cl was used for the Phosphate buffer solution. 3.3.5: Concentrations refer to the test substance (purity 79.5-80 %.) 3.3.11: Inoculum blank (4 bottles), procedure control (4 bottles), toxicity control (4 bottles, containing 3 mg L^{-1} glucose, 3 mg L^{-1} glutamic acid and 4 mg L^{-1} test substance), nitrification control (4 bottles, containing 2.5 mg L^{-1} allylthiourea and 2 mg L^{-1} test substance). 3.3.12: COD value of test substance was used for the calculation. 5.1: Refer to comment number 2.1 and 3.3.11.

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Results and discussion	Applicant's version is acceptable apart from the following amendments: 4.1.2: Degradation after 5 days: 48% (concentration 2 mg L^{-1}), 50 % (concentration 4 mg L-1). Degradation after 20 days: 60% (concentration 2 mg L^{-1}), 67 % (concentration 4 mg L^{-1}). The concentrations are based on test substance (80 % purity). The difference of degradation of the two test concentrations is less than 20 %. 4.1.3: In the toxicity test the degradation was 51 % (day 5) and 75 % (day 20). The test substance can be assumed not to be inhibitory. Nitrification control revealed that nitrification took place in the medium. However, this applies to blanks as well as to test substance batches. Therefore the study result is not affected. Oxygen depletion in the inoculum blank was 1.74 mg dissolved oxygen L^{-1} after 20 days. According to OECD 301D, oxygen depletion should not exceed 1.5 mg O_2 L^{-1} after 28 days. The reason for the higher value in the present study presumably is the higher nitrification since more ammonium is added to the mineral medium compared to the OECD mineral medium. The residual concentration of oxygen in the test bottles did not fall below 0.5 mg L^{-1} at any time (except toxicity control after 20 days). 4.1.4: Refer to comment number 3.1.4. 4.1.5: Degradation of the reference substances: 49% (day 5) and 90% (day 20). Even if degradation of the reference substances were not measured after 14 days, it is expected that the validity criterion of OECD 301D (degradation of reference compound reaches pass level after 14 days) is fulfilled. 5.2: Refer to comment number 4.1.2, 4.1.3, and 4.1.5.
Conclusion	Applicant's version is acceptable apart from the following amendments: 5.3: The Dutch guideline NEN 6634 used in the present study is basically comparable to OECD 301D. Deviations are: (a) more ammonium in mineral medium, (b) only 3 sampling points, (c) duration 20 days, and (d) a different reference substance. Because degradation of test substance was above the pass level after 20 days, the short study duration is not a problem. Due to the deviations, the validity criteria of guideline OECD 301D can only be roughly controlled. Nevertheless, the test is regarded as valid (refer to comment number 4.1.2, 4.1.3, and 4.1.5) and acceptable. Lactic acid is readily biodegradable, however the 10-days window criterion is not fulfilled/cannot be assessed.
Reliability	2
Acceptability	Acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*

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Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	Not specified
Strain	Not specified
Source	Oxidation ditch, used to treat domestic sewage
Sampling site	TNO, Delft, the Netherlands
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The original sludge (containing 3.5-4.0 g of solid substance/L) was allowed to settle for 4-8 minutes. 2 mL of the supernatant was used to inoculate.
Pretreatment	Vigorous aeration
Initial cell concentration	Not reported

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	BOD bottles
Number of culture flasks/concentration	4 bottles/concentration
Aeration device	Not reported
Measuring equipment	Oxygen electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	BOD dilution water was prepared from concentrated stock solutions in Milli-Q water, according to the Dutch Guideline "Water-determination of biochemical oxygen demand after n days (BODn)" (NEN 6634)
Additional substrate	Yes, glucose and glutamic acid were added to check the activity of the inoculum and the possible toxicity of the test substance
Test temperature	20 °C
pH	Start: 7.0-7.1 End: 6.6-6.9 End (bottles with glucose): 6.1-6.3
Aeration of dilution water	Yes, dilution water was aerated vigorously before use
Suspended solids concentration	Not reported
Other relevant citeria	Nitrification control was included by adding 2.5 mg/L allythiourea to bottles containing 2 mg/L lactic acid

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	**x**	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		**x**
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	**x**	
Percentage of removal of reference substance reaches pass level by day 14		**x**

5.3.2.1 Criteria for poorly soluble test substances	5.3.2.2	5.3.2.3
5.3.2.4	**5.3.2.5**	**5.3.2.6**
5.3.2.7	**5.3.2.8**	**5.3.2.9**