Investigation of food additives readily biodegradability in respirometric tests

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Abstract

The biodegradability of fourteen (14) food additives, belonging to different groups such as artificial sweeteners, preservatives and coloring agents, was evaluated using the OECD 301F protocol (manometric respirometry test). According to the results, eight out of fourteen compounds, namely Aspartame, Cyclamate Na, Saccharin, Erythritol, Potassium Sorbate, Benzoic Acid, Sodium Ascorbate and Xylitol are characterized as readily biodegradable with biodegradation rates of 83.6 ± 11.4%, 91.5 ± 5.8%, 76.4 ± 10.3%, 70.6 ± 9.0%, 99.3 ± 7.9%, 97.5 ± 4.0%, 83.8 ± 6.1% and 68.9 ± 2.6%, respectively, during the 28-d experiment. On the other hand, Alitame, Curcumin, Ponceau 4R, Allura Red, Sunset Yellow and Azorubine did not meet the strict definition of ready biodegradability. Further biodegradations tests are required for these compounds in order to investigate their biodegradation potential under different experimental conditions.

Keywords: food additives; wastewater; activated sludge; OECD protocol

1. Introduction

1.1. Food Additives

Food additives are chemical substances that are used during food preparation for preservation, sweetening, colouring and flavoring. They can be derived from plants, animals, minerals, or they can be synthetic.

So far, several monitoring studies have reported the occurrence of different food additives in wastewater and aquatic environment at concentrations up to a few dozens μg L^{-1} for municipal wastewater (Thomaidi et al., 2015), and some μg L^{-1} for surface water and ground water (Yang et al., 2018). Despite the wide use of food additives, there is limited information for their readily biodegradability in the environment as well as in wastewater treatment systems. On the other hand, OECD ready biodegradability tests (OECD, 1993) have been widely applied for obtaining information for the accessibility of various organic compounds to microbial degradation (Stasinakis et al., 2008) and play an important role in the EU environmental classification of chemicals (EC, 1993), as well as in environmental risk assessment (EC, 2003). Among different OECD protocols, OECD 301 F has extensively used for estimating the biodegradability of different synthetic chemicals. In this test, the consumed BOD is measured for 28 d and it is compared to the theoretical oxygen demand (ThOD) of the added target compound. In cases that a BOD value ≥ 60% of ThOD is measured during the experiment and this threshold level is reached in a 10-d window, the target compound can be characterized as readily biodegradable and it is considered that it will be rapidly biodegraded in the environment.

The main objective of this study was to evaluate the ready biodegradability of six artificial or natural sweeteners, three food preservatives and five food colorings by activated sludge. Biodegradation percentages of the target compounds were calculated in 28-days manometric respirometry tests using OECD protocol 301F and activated sludge as inoculum.

2. Materials and Methods

2.1. Activated sludge inoculums

Activated sludge was collected from the University Campus Sewage Treatment Plant (Mytilene, Lesvos). The inoculum diluted to a concentration of 3-5 g l^{-1} dry matter and aerated for 5 days. This starved activated sludge suspension was further diluted to the inoculum concentration given in OECD protocol.

2.2. Biodegradation experiments

Manometric respirometry tests were carried out in the Sensomat system (AQUALYTIC® ZN, Tintometer GmbH, Germany). Owing to microbial activity, oxygen is taken from the gas phase of the hermetically sealed reaction vessels, while carbon dioxide released from respiration is absorbed by KOH in a small tube and the resulting reduction in air pressure inside the closed system is measured. Biodegradation experiments were performed in 6 experimental cycles. Biodegradation of each target compound was studied in triplicate flasks. Initially, appropriate volumes of the mineral medium consisted of KH_2PO_4, K_2HPO_4, $Na_2HPO_4 \times 12H_2O$, NH_4Cl, $MgSO_4 \times 7\ H_2O$, $CaCl_2$ and $FeCl_3 \times 6\ H_2O$ were added to each flask. The concentrations of the target compounds was 50 mg l^{-1} while the inoculum concentration was 30 mg l^{-1} dry matter. To prevent nitrification, thiourea was added in all flasks at concentration of 10 mg l^{-1}. In each experimental cycle, two Biotic Control was used to check BOD due to endogenous respiration, while differen Positive Control

(10 ml of $CH_3COONa \times H_2O$) was used to check inoculum viability. All the experiments were carried out in thermostatically controlled conditions (20 ^oC), while the cultivation medium was stirred to maintain biomass in suspension. To calculate biodegradation percentage at the end of each test (28th day), the amount of oxygen taken up by the microbial population in manometric respirometry test (corrected for uptake by Biotic Control, run in parallel) was expressed as percent ThOD (OECD (1993).

3. Results

The majority of sweeteners were biodegraded at percentage higher than 60% by the end of the experiment (28 days), while this threshold level was reached in a 10-d window. As a result, Aspartame, Cyclamate Na, Saccharin and Erythritol can be characterized as readily biodegradable compounds with biodegradation rates 83.6 ± 11.4%, 91.5 ± 5.8%, 76.4 ± 10.3%, 70.6 ± 9.0% (Table 1). In addition the preservatives, Potassium Sorbate, Benzoic Acid, Sodium Ascorbate showed biodegradation rate equal to 99.3 ± 7.9%, 97.5 ± 4.0% and 83.8 ± 6.1%, respectively and they can also characterized as readily biodegradable compounds. The definition of ready biodegradability was also met by the natural sweetener Xylitol (Table 1).

Table 1. Degradation (%) and classification of studied food additives according to OECD 301F manometric respirometry test (duration: 28 d)

Compound	Degradation (%)	Classification for readily biodegradability
Aspartame	83.6 ± 11.4	yes
Cyclamate Na	91.5 ± 5.8	yes
Saccharin	76.4 ± 10.3	yes
Erythritol	70.6 ± 9.0	yes
Potassium Sorbate	99.3 ± 7.9	yes
Benzoic Acid	97.5 ± 4.0	yes
Sodium Ascorbate	83.8 ± 6.1	yes
Alitame	9.5 ± 1.2	no
Xylitol	68.9 ± 2.6%	no
Curcumin	54.1 ± 2.3	no
Ponceau 4R	22.3 ± 4.1	no
Allura Red	9.4 ± 0.0	no
Sunset Yellow	0.0 ± 0.0	no
Azorubine	0.0 ± 0.0	no

On the contrary, the sweetener Alitame as well as all coloring agents cannot be characterized as readily biodegradable compounds. Specifically, the ratio of BOD to ThOD for Alitame was only 11.4 ± 1.4% during the experiment. Concerning coloring agents, Curcumin, Sunset Yellow, Allura Red, Azorubine and Ponceau 4R showed low biodegradation rates ranging from zero to 54.1 ± 2.3% (Table 1). Further biodegradations tests are required for these compounds in order to investigate their biodegradation potential under different experimental conditions (anoxic, anaerobic) or using different inoculum.

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