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ACCLIMATION OF ACTIVATED SLUDGE TO MONO-SUBSTITUTED DERIVATIVES OF PHENOL AND BENZOIC ACID

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Successful adaptation of sludge to 24 out of 35 phenolic and benzoic acid derivatives was achieved by incubating domestic activated sludge samples with increasing amounts of the various compounds tested. In addition, with manometric techniques it was found that sludges which had adapted to one compound were able to oxidize other compounds to which they had not been previously exposed. However, no strict relationship between the nature and position of substituents in the aromatic ring and the effectivness of the processes of acclimation was found.

In biological waste treatment processes, an important consideration is the capacity of the microbial population to adapt, within limits, to changing conditions established in the ecosystem.

The term adaptation or acclimation, as commonly applied to activated sludge, denotes the adjustments made by the microbial community in response to changes in the environment, disregarding the mechanisms implicated in the process. The recognized adaptive mechanisms include mutation, enzyme adaptation, modification of cell structure, and changes in the composition of the population through multiplication, death, or shifts in predominance (*1*).

Laboratory studies have shown that the time necessary to obtain total acclimation of an activated sludge system is highly dependent on the complexity of the substrate tested. MCKINNEY et al. (*2*) found that it took four weeks to adapt activated sludge to metabolize various aromatic compounds. GAUDY (*3*) found that the adaptation of activated sludge to sorbitol was complete in three days. Other acclimation experiments, using pure and mixed cultures (*1*, *4–8*), have been done to obtain cultures able to metabolize compounds resistant to degradation but usually found in soils and sewage in appreciable amounts. For ex-

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ample, many derivatives of phenol and benzoic acid are frequently found in industrial wastes, domestic sewage and soil as products of the biodegradation of pesticides, fungicides and herbicides (*9*, *10*).

In industrial waste treatment plants, activated sludges from domestic sewage treatment plants are frequently used as inoculato initiate the biological treatment process. Thus, it appeared useful to investigate some relevant features of the process of acclimation of domestic activated sludge to selected mono-substituted derivatives of phenol and benzoic acid that are usually present in industrial waste.

MATERIALS AND METHODS

Samples. Activated sludge was obtained from the secondary settling tank of a domestic sewage treatment plant in El Pardo, Madrid, Spain.

Artificial waste medium (AWM). The medium (*11*) was composed of (mg/*l* of distilled water): glucose, 1,000; potassium phosphate (dibasic), 1,070; potassium phosphate (monobasic), 527; ammonium sulfate, 500; magnesium sulphate, 100; manganesium sulphate, 10; calcium chloride, 7.5 and ferric chloride, 0.5. The pH was adjusted to 7.0–7.2.

Aromatic compounds. The substances tested (Merck, Darmstadt, Germany) were benzoate, phenol, catechol, resorcinol, hydroquinone, and *ortho-*, *meta-*, and *para*-isomers of methylbenzoate, aminobenzoate, chlorobenzoate, nitrobenzoate, hydroxybenzoate, aminophenol, cresol, nitrophenol, chlorophenol, and phthalates. Each was made up as a solution in distilled water and neutralized to pH 7.0 with 1 N NaOH. In all experiments the compounds tested were used in concentrations expressed as ppm (mg/*l*) of chemical oxygen demand (COD).

Determinations. Glucose was determined according to SOMOGYI (*12*). Chemical oxygen demand was determined as described elsewhere (*13*).

Acclimation of activated sludge. Sludges were acclimated by a modification of the procedure described by MC. KINNEY et al. (*2*). One liter of AWM was inoculated with 200 ml of sludge and the mixture incubated at room temperature (15–20°) with aeration. Every 48 hr aeration was stopped, the floc allowed to settle for 1 hr, and 800 ml of supernatant fluid removed for chemical analysis. Then, 800 ml of fresh medium containing an appropiate concentration of the aromatic compound being tested was added to each bottle and aeration resumed.

The acclimation procedure consisted of replacing at 2 days intervals 10% of the glucose with 10% of the aromatic compound under assay maintaining always a final concentration of 1,000 ppm COD of carbon source. This procedure was repeated until the sludges received only aromatic compounds as the sole carbon source. An unadapted control sludge was treated similarly, except that only glucose was added throughout the experiment.

Manometric techniques. Activated sludges acclimated to a particular aromatic compound were tested for their ability to oxidize other aromatic substances

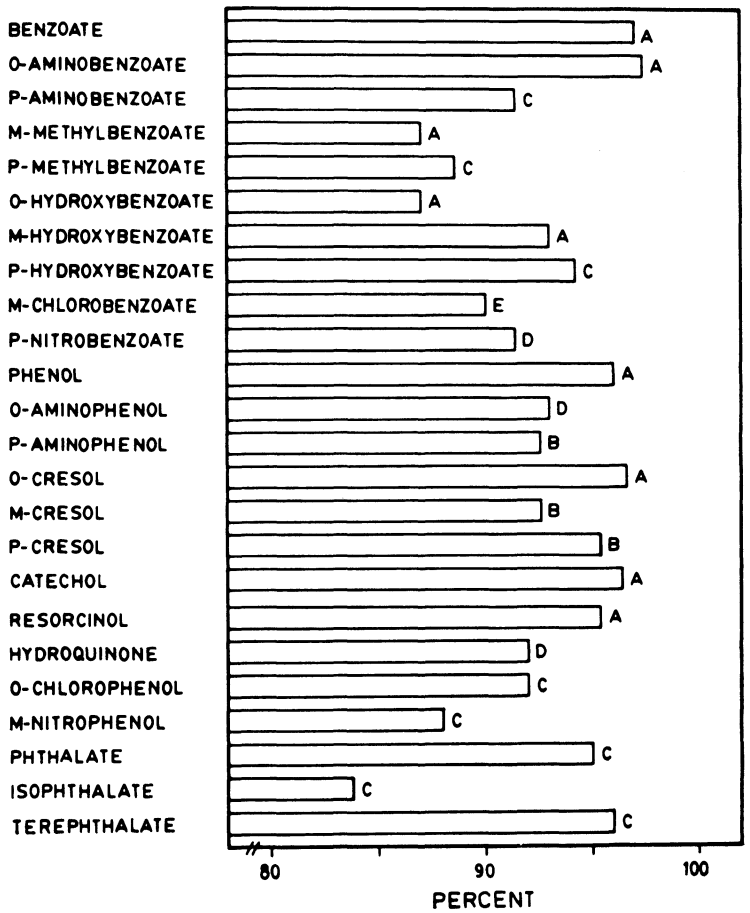

Fig. 1. Percent consumption of selected aromatic compounds by activated sludges at the end of the acclimation period. Days required to complete the acclimation process were: A, 20; B, 22; C, 24; D, 26; and E, 30.

Table 1. Influence of the substituent position in the aromatic compound on the acclimation of activated sludge.

Aromatic substrate[a]	Substituents[b]						Substituent position
	NH_2	OH	CH_3	NO_2	Cl	COOH	
Benzoate							
	+	+	−	−	−	+	*ortho*
	−	+	+	−	+	+	*meta*
	+	+	+	+	−	+	*para*
Phenol							
	+	+	+	−	+		*ortho*
	−	+	+	+	−		*meta*
	+	+	+	−	−		*para*

[a] Acclimation to benzoate and phenol was successful (see text).

[b] Symbols: (+) acclimation successful; (−) no acclimation.

Table 2. Ability of acclimated sludges to

Activated sludge adapted to No.	Compounds	Q_{O_2}[d] of adapted 0	1	2	3	4	5	6
0	glucose[a]	22.9	9.1	—[c]	—	—	—	—
1	benzoate	12.4	137.1	20.0	—	—	1.0	46.6
2	*o*-aminobenzoate	16.6	27.2	49.5	—	—	5.1	10.0
4	*p*-aminobenzoate	5.9	—	—	—	58.9	—	—
6	*m*-methylbenzoate	4.7	209.9	36.2	—	—	9.8	99.5
7	*p*-methylbenzoate	12.8	151.7	10.2	—	—	3.7	95.9
8	*o*-hydroxybenzoate	6.9	131.1	6.5	—	—	2.6	93.3
9	*m*-hydroxybenzoate	19.7	115.2	10.8	—	—	11.9	70.3
10	*p*-hydroxybenzoate	23.4	6.1	—	—	—	—	—
12	*m*-chlorobenzoate	4.3	27.6	2.5	—	—	—	—
16	*p*-nitrobenzoate	14.9	2.0	—	—	—	—	—
17	phenol	28.0	40.1	5.4	—	1.3	2.0	20.2
18	*o*-aminophenol	18.9	—	—	—	—	—	—
20	*p*-aminophenol	4.7	—	—	—	—	—	—
21	*o*-cresol	23.2	9.8	—	—	—	—	12.6
22	*m*-cresol	21.5	13.3	1.9	2.8	1.9	2.7	8.0
23	*p*-cresol	17.9	6.5	2.0	2.5	1.7	3.1	6.7
24	catechol	16.6	27.9	—	—	—	—	—
25	resorcinol	23.9	3.3	—	—	—	1.7	2.3
26	hydroquinone	24.1	17.7	2.3	—	1.4	1.5	2.2
27	*o*-chlorophenol	7.0	1.0	1.3	1.1	1.1	1.0	—
31	*m*-nitrophenol	5.0	1.1	—	—	—	4.9	7.9
33	phthalate	6.2	11.0	4.1	1.8	2.7	2.8	5.0
34	isophthalate	10.9	6.5	—	—	1.2	2.4	2.8
35	terephthalate	13.2	3.3	1.2	2.7	1.6	1.9	3.8

[a] Glucose was used as the control for acclimation (see text).

[b] Numbers correspond to those assigned to the compounds listed in this table.

[c] Symbol: (—) Inability to oxidize.

[d] Q_{O_2} = μl O_2 uptake/mg dry weight of sludge/hr.

to which the sludge had not been previously exposed.

Acclimated activated sludge was harvested by centrifugation and the supernatant fluid discarded. The solids were washed twice with 0.8% NaCl, and finally suspended in 150 ml of the same saline solution to obtain a concentration ranging from 10–14 mg $\times$ ml^{-1} of dry weight of solids; 0.5-ml samples of this suspension were used for determining oxygen uptake by manometry (*14*). Samples were incubated at 30° in the presence of 1,000 ppm of CDO of the aromatic compound being tested. Incubation was continued up to 4 hr, with readings taken at 30-min intervals. In each case, a control was run to measure the endogenous respiration of the activated sludge.

oxidize selected aromatic compounds.

activated sludges tested against[b]									
7	8	9	10	11	12	13	14	15	16
—	—	3.2	1.6	—	—	—	—	—	—
25.2	31.3	47.4	3.2	2.8	1.1	—	—	—	4.3
—	9.1	40.8	13.4	3.7	—	—	4.6	—	—
—	—	1.2	26.3	—	1.3	1.8	2.2	—	3.8
50.7	67.3	8.7	12.5	22.9	—	—	—	—	—
102.5	7.9	5.8	7.4	8.8	—	2.6	4.1	—	1.1
5.9	167.9	5.5	9.1	1.2	1.3	—	—	—	—
13.0	13.4	289.6	10.7	15.4	—	—	4.4	—	—
—	—	7.6	64.6	—	—	—	1.0	—	—
—	1.6	5.2	4.3	—	8.9	—	—	—	—
—	—	3.8	14.2	—	—	2.3	1.2	—	5.1
12.8	4.8	5.0	5.8	1.6	3.3	6.5	3.9	1.4	4.3
—	—	—	1.8	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
8.3	2.6	4.9	9.3	—	—	—	—	—	—
2.1	7.6	8.9	19.0	6.8	—	—	—	—	1.2
1.0	1.3	13.5	58.8	—	—	—	—	—	—
—	—	1.9	2.0	—	—	7.7	2.7	—	5.5
4.2	—	8.0	9.3	8.2	—	5.1	1.3	—	—
1.8	2.0	5.2	10.2	—	1.9	1.6	—	—	1.0
2.8	2.0	1.0	2.7	—	—	—	—	—	—
2.8	13.0	1.2	—	1.3	1.1	—	2.2	—	3.6
4.7	4.8	8.9	11.5	—	—	—	—	2.3	—
1.1	1.8	8.8	24.0	—	—	—	—	—	—
1.0	1.8	6.4	7.2	1.3	1.5	—	—	—	1.3

RESULTS AND DISCUSSION

Acclimation of activated sludge

The sludge was successfully adapted to 24 of the 35 aromatic compounds tested. In most cases at the end of the acclimation procedure the activated sludge could degrade more than 85% of the added aromatic compound, with the exception of isophthalic acid (Fig. 1). However, the time required for adapting the sludge to the different aromatic compounds differed. *m*-Chlorobenzoate, *p*-nitrobenzoate, *o*-aminophenol and hydroquinone required the longer adaptation times (Fig. 1).

Under the experimental conditions used, it was not possible to adapt activated sludge to the following compounds: *m*-aminobenzoate, *meta* and *p*-chlorophenol, *o*-methylbenzoate, *ortho*- and *p*-chlorobenzoate, *ortho*- and *m*-

Table 2.

No.	Activated sludge adapted to Compounds	Q_{O_2}[d] of adapted 17	18	19	20	21	22	23
0	glucose[a]	—[c]	1.3	—	2.2	—	—	—
1	benzoate	—	—	—	2.5	1.3	—	2.2
2	*o*-aminobenzoate	14.5	—	—	1.0	—	1.6	4.7
4	*p*-aminobenzoate	1.2	8.9	—	3.3	3.6	3.2	1.9
6	*m*-methylbenzoate	3.1	2.1	—	2.5	2.4	2.6	1.2
7	*p*-methylbenzoate	2.9	12.0	—	2.1	4.8	6.8	7.4
8	*o*-hydroxybenzoate	1.0	2.5	—	3.6	1.6	—	—
9	*m*-hydroxybenzoate	—	—	—	—	—	—	—
10	*p*-hydroxybenzoate	—	4.6	—	2.6	—	1.6	3.0
12	*m*-chlorobenzoate	—	—	—	1.2	—	—	—
16	*p*-nitrobenzoate	—	6.7	—	6.1	1.8	2.3	7.7
17	phenol	10.6	14.3	—	3.6	6.2	6.3	5.3
18	*o*-aminophenol	—	14.5	—	—	—	—	—
20	*p*-aminophenol	—	2.4	—	2.6	—	—	—
21	*o*-cresol	21.8	—	—	—	31.5	9.6	14.0
22	*m*-cresol	16.4	4.8	2.9	6.1	20.4	24.5	22.1
23	*p*-cresol	—	1.8	—	24.2	—	2.9	33.8
24	catechol	3.7	4.1	—	6.6	4.1	—	6.3
25	resorcinol	4.9	2.3	—	6.3	1.6	—	1.2
26	hydroquinone	10.5	3.4	1.3	6.6	1.9	3.9	5.6
27	*o*-chlorophenol	13.4	—	—	1.9	—	—	6.2
31	*m*-nitrophenol	—	1.4	—	7.1	—	—	5.0
33	phthalate	—	5.1	—	5.4	—	—	1.3
34	isophthalate	2.7	—	—	2.6	—	1.1	1.0
35	terephthalate	—	—	—	1.1	—	—	—

nitrobenzoate, *ortho*- and *p*-nitrophenol, and *m*-aminophenol.

Furthermore, metabolism of the mono-substituted derivatives of phenol and benzoic acid was markedly influenced by the nature and position of the substituents (Table 1).

Oxidative capacity of adapted sludge

The oxidative capacity of each adapted sludge against glucose and the 35 aromatic compounds under assay was determined. The acclimated sludges were capable of oxidizing a greater number of benzoic acid derivatives than phenolic derivatives (Table 2).

In general, sludges adapted to benzoic derivatives oxidized these derivatives more efficiently than sludges adapted to phenolic derivatives which were less efficient in oxidizing phenolic compounds. Also, it should be pointed out that the compounds least oxidized by adapted sludges included *m*-nitrobenzoate, *meta*- and *p*-chlorophenol, *meta*- and *p*-nitrophenol, and *m*-aminophenol.

The stabilization of the sludge to phenolic and benzoic acid derivatives

(Continued)

activated sludges tested against[b]											
24	25	26	27	28	29	30	31	32	33	34	35
2.2	1.9	—	—	—	—	—	1.8	—	1.3	2.9	1.4
66.0	3.4	—	—	—	—	1.5	—	—	—	—	—
37.2	23.6	47.1	3.1	—	—	3.0	—	—	—	—	—
4.6	3.9	1.1	2.7	—	—	—	—	—	1.0	—	—
33.6	4.3	—	—	—	—	1.5	—	—	3.1	6.7	4.9
26.7	7.8	—	3.3	—	—	—	—	—	—	4.9	—
66.5	—	—	—	—	—	4.7	—	—	—	1.2	—
36.2	5.5	—	—	—	—	—	—	—	—	—	—
7.8	—	—	1.4	—	—	—	—	—	—	—	—
20.4	1.0	1.5	1.2	—	5.1	—	—	—	1.4	2.6	3.1
4.2	4.6	8.0	1.9	—	—	—	—	—	—	—	—
37.0	9.6	19.6	3.5	—	—	—	—	—	—	4.5	1.7
1.6	1.0	6.2	—	—	—	—	—	—	3.6	3.0	1.8
1.0	—	1.1	—	—	—	—	—	—	—	—	—
40.5	7.0	—	—	—	—	—	—	—	4.3	—	1.2
44.4	2.1	—	1.2	—	—	1.0	—	—	—	—	—
12.6	—	—	—	—	—	—	—	—	1.9	1.1	2.3
44.4	4.7	—	2.3	—	—	—	—	—	3.7	6.7	—
13.2	20.4	—	—	—	—	—	—	—	1.3	5.8	—
18.6	10.9	30.3	2.1	—	—	2.7	—	—	1.0	1.7	1.7
2.8	—	—	2.1	1.7	—	—	—	—	—	2.3	—
7.9	—	6.3	1.6	3.6	—	3.2	7.8	5.8	3.4	5.8	1.7
4.7	—	1.9	1.8	—	—	1.4	—	—	40.4	—	1.4
2.0	—	—	—	—	—	—	—	—	—	27.1	3.0
1.0	1.3	—	—	—	—	—	—	—	—	1.5	4.3

depends on the chemical structure of the compounds. ALEXANDER and LUSTIGMAN (*15*) observed that the presence of carboxyl and hydroxyl groups were the most favorable and the sulfonate and nitro substituents were the least favorable for microbial degradation of the benzene rings containing only a single substituent. They also found that the meta isomer was more resistant to microbial attack than the *ortho* and *para* isomers, the latter being degraded at a faster rate.

However, and in agreement with our results (*16*), found that in the degradation of monosubstituted benzoates and phenols by wastewater, there was no relation between their oxidation and the position of the substituents.

We found that different samples of activated sludges from the same treatment plant did not always behave similarly with respect to their capacity to adapt to a particular organic compound. Consequently, we do not consider it suitable to establish general criteria of biodegradability. From a practical point of view, for treatment plants receiving wastewater contaminated with organic compounds that are resistant to degradation it is necessary to perform studies of biodegradability to establish the maximum concentration that the microbial populations of each

biological treatment system can tolerate.

We observed that sludge acclimated to a particular aromatic compound was able to oxidize over 85% of the compound when it was used as the sole carbon source at a final concentration of 1,000 ppm. In contrast, it must be emphasized that the microbial populations of a nonacclimated sludge did not grow and, in many cases, was inactivated by the presence of 100–200 ppm of the aromatic compound used as sole carbon source (*ortho* and *p*-chlorobenzoate, *ortho* and *m*-nitrobenzoate, *meta* and *p*-chlorophenol, etc.). From a sludge that could not be adapted to *o*-nitrobenzoate and was inactivated by 100–200 ppm of that compound, a culture was obtained after treatment with N, N-nitrosoguanidine that was able to oxidize up to 1,000 ppm of *o*-nitrobenzoate provided as the sole carbon source (LUND and CABRERA, unpublished data).

A non-acclimated sludge was, in effect, incapable of oxidizing the 35 aromatic compounds tested, whereas sludges adapted to a given aromatic compound could also oxidize compounds of similar structure, possibly through the same metabolic pathway. Similar results have been reported by PRAKASAM and DONDERO (*1*).

All of the 24 acclimated sludge samples included in this study oxidized glucose and catechol, 21 of them *meta* and *p*-hydroxybenzoate, and 20 benzoate and *p*-aminophenol. Furthermore, sludge adapted to catechol could oxidize 15 other aromatic compounds. The sludge adapted to *m*-hydroxybenzoate was able to oxidize 11 compounds, and the one adapted to *p*-hydroxybenzoate, 9 compounds. The benzoate adapted sludge oxidized 16 compounds and that acclimated to *p*-aminophenol, 3 compounds. It is possible that co-metabolism carried out by different microorganisms present in the activated sludge was the mechanism responsible for the variations observed in the results discussed above.

Although it was not possible to acclimate sludges to several of the aromatic compounds tested, it is noteworthy that many of the acclimated sludges obtained were able to oxidize such compounds. For example sludges adapted to *m*-methylbenzoate, *m*-hydroxybenzoate and resorcinol, were able to oxidize *o*-chlorobenzoate (Table 2). These results may have important practical applications; adaptation of sludge to an aromatic compound that is easily degraded could be used to obtain sludge able to degrade resistant compounds, specially those that frequently occur in industrial wastewater.

Clearly, studies of acclimation can be useful for chemical industries producing newly synthesized organic chemical products. In such cases, biodegradability of new compounds entering sewage treatment plants can be ensured prior to marketing of the compound.

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