ELSEVIER

FEMS Microbiology Letters 170 (1999) 291–300

FEMS MICROBIOLOGY LETTERS

MiniReview

Biodegradation of aromatic compounds by microalgae

Kirk T. Semple *, Ronald B. Cain [1], Stefan Schmidt [2]

Department of Environmental Science, Institute of Environmental and Natural Sciences, Lancaster University, Lancaster LA1 4YQ, UK

Received 16 May 1998; accepted 5 November 1998

Abstract

The microbial degradation of aromatic pollutants has been well characterized over a period of more than 30 years. The microbes of most interest have been bacteria and fungi. Only relatively recently has the question of how algae figure in the catabolism of these compounds attracted a degree of interest. The aim of this review is to highlight the biodegradative capabilities of microalgae on aromatic compounds, ranging from simple monocyclic to more complex polycyclic pollutants. This paper will briefly encompass studies which have investigated the growth on and the oxidation of these compounds by algae, as well as a more detailed characterization of the catabolic sequences involved in the transformation of these compounds.

Keywords: Microalga; Biodegradation; Aromatic compound; Mineralization; Ring cleavage

1. Introduction

Organic pollutants in the aquatic environment are subject to biodegradation by a range of naturally occurring microorganisms, but studies have concentrated, almost exclusively, on the role of bacteria [1] and fungi [2] in the degradative processes. Algae remain the poor relations of the environmental microbiologist, in spite of their ubiquitous distribution, their central role in the fixation and turnover of carbon and other nutrient elements and recognition of their heterotrophic abilities [3]. Information on the relationship between algal heterotrophy and the biodegradation of xenobiotic compounds is far less than the information accumulated concerning bacteria and fungi. Some information on the interactions between pesticides and eukaryotic algae was compiled by Kobayashi and Rittman [4], showing that not only were algae capable of bioaccumulating pesticides, but they were also capable of biotransforming some of these environmental pollutants (Table 1).

The aim of this paper is to review the research encompassing the degradation of aromatic compounds by algae, ranging from whole cell heterotrophic studies to detailed studies of catabolic pathways

* Corresponding author. Tel.: +44 (1524) 594534; Fax: +44 (1524) 593985; E-mail: k.semple@lancaster.ac.uk

[1] Present address: Environmental Microbiology Laboratory, Department of Biological and Nutritional Science, The University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.

[2] Present address: Institut für Allgemeine Botanik der Universität Hamburg, Abteilung Mikrobiologie, D-22609 Hamburg, Germany.

0378-1097/99/$19.00
PII: S0378-1097(98)00544-8

Table 1
The bioaccumulation and biotransformation of selected pesticides by eukaryotic algae (adapted from [4])

Alga	Bioaccumulation	Biotransformation
Chlamydomonas spp.	Mirex	Lindane, naphthalene, phenol
Chlorella spp.	Toxaphene, methoxychlor	Lindane, chlordimeform
Chlorococcum sp.	Mirex	
Cylindrotheca sp.	DDT	
Dunaliella sp.	Mirex	DDT, naphthalene
Euglena gracilis	DDT, parathion	Phenol
Scenedesmus obliquus	DDT, parathion	Naphthalene sulfonic acids
Selenastrum capricornutum	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene, naphthalene, 2,6-dinitrotoluene, phenanthrene, di-*n*-butylphthalate, pyrene	Benzo[*a*]pyrene

of common aromatic pollutants found in freshwater ecosystems receiving industrial wastewaters.

2. Algal degradation of aromatic compounds

2.1. Heterotrophic studies

Some species of algae are capable of heterotrophic growth on organic carbon sources [5]. One way of investigating the biodegradation of organic pollutants by algae is to encourage the cells to grow in the presence of the pollutant. To this effect, Walker et al. [6] performed experiments with the achlorophyllous alga *Prototheca zopfii*, which was found to degrade petroleum hydrocarbons found in Louisiana crude and motor oils. Interestingly, in the crude oil, 38–60% of the saturated aliphatic hydrocarbons and 12–41% of the aromatic compounds were degraded, whereas in the motor oil, 10–23% of the saturated aliphatic hydrocarbons and 10–26% of the aromatic compounds were degraded. This suggested that the alga was capable of degrading different oils to varying levels. Another interesting observation was made by Jacobson and Alexander [7], in which non-axenic cultures of *Chlamydomonas* sp., grown in the light and in the dark on acetate, were found to dehalogenate 4-chloro-3,5-dinitrobenzoic acid and produce a metabolite which was identified as 2-hydroxymuconic semialdehyde, indicating *meta* cleavage. In the absence of the alga, the bacteria were unable to achieve this. Jinqi and Houtian [8] investigated the degradation of azo dyes by *Chlorella vulgaris* and *C. pyrenoidosa* and found that certain dyes, such as Eriochrome blueSE and blackT, could be decolorized and actually used as carbon and nitrogen sources, but this was dependent on the chemical structure of the dyes. The degradation was found to be an inducible catabolic process. They also found that the algae degraded aniline, a potential degradation product of the azo dye breakdown. In another study, *Ochromonas danica*, a nutritionally versatile chrysophyte, grew heterotrophically on phenol or *p*-cresol as the sole source of carbon up to concentrations of 4 mM [9]. Confirmation of the removal of phenol and the absence of accumulating metabolites was achieved using DOC (dissolved organic carbon) analysis which showed the phenol-carbon was removed at rates similar to that for the disappearance of the phenol measured by HPLC [10].

As polycyclic aromatic hydrocarbons (PAHs) are known to produce carcinogenic and mutagenic effects [11] and are therefore considered to be priority pollutants in the environment, their modes of degradation have been studied in some detail and have been elegantly reviewed by Cerniglia [11]. Naphthalene has proved interesting to algal researchers, who found that this compound was toxic to *Chlamydomonas angulosa*, a chlorophyte: 60–98% of cells were killed (lethality was determined by monitoring mobility) in open and closed flasks containing saturated aqueous solutions of naphthalene within 1 day in the light. [12,13]. However, after a lag phase of up to 3 days, the alga began to multiply at similar rates to that of the control (no naphthalene), with final growth yields being similar between the test and control incubations. It was noted that the algal cells were able to remove naphthalene from the growth medium by accumulation within the cells, but were unable to metabolize the pollutant. Cerniglia et al.

Table 2
Decrease of some diesel exhaust fraction compounds remaining in incubation media after 3 days of growth with adapted and non-adapted cells of *Chlamydomonas reinhardtii* (adapted from [18])

Compound	Concentration of compounds in blanks (μg μl^{-1})	Concentration of compounds in incubation media (μg μl^{-1})		Percentage decrease
		Non-adapted cells	Adapted cells	
9H-Fluorene-9-one	6.91	6.72	0.32	95.2
Phenanthrene	33.3	30.1	21.4	28.9
4-Methylbenzo[*c*]cinnoline	6.76	6.3	6.03	4.3
1-Methylanthracene	1.15	1.36	0.62	54.3
2- Methylanthracene	20.5	20.5	16.7	18.5
1,2-Dimethyl phenanthrene	5.1	4.81	0.71	85.2
3,4-Dimethyl phenanthrene	10.15	9.88	6.83	30.9

[14–16] showed that both cyanobacteria (blue-green algae) and eukaryotic microalgae were capable of biotransforming naphthalene to four major metabolites, 1-naphthol, 4-hydrox-4-tetralone, *cis*-naphthalene dihydrodiol and *trans*-naphthalene dihydrodiol at concentrations which were non-toxic (Fig. 1). The formation of *cis*-naphthalene dihydrodiol was the first demonstration in a eukaryotic cell. However, the total degradation of naphthalene was not great, ranging from 1 to 1.9%. In another study, Cerniglia et al. [17] characterized the initial degradation of naphthalene in diatoms. Once again, 1-naphthol was found to be a major metabolite, but only 0.7–1.4% of the aromatic compound was degraded. Liebe and Fock [18] found that *Chlamydomonas reinhardtii*, after having been suitably adapted, was able to remove some of the iso-octane-extracted PAHs from diesel particulate exhaust. The extent of removal ranged from 4 to 95%, although the mechanisms for this are unclear (Table 2). However, there were no significant differences in growth between adapted cells in fresh media with or without the extract. It is therefore unlikely that the PAH derivatives were consumed as nutrients. Luther and Soeder [19], Luther and Shaaban [20] and Luther [21] were able to confirm that the alga, *Scenedesmus obliquus*, was able to utilize naphthalene sulfonic acids as a source of sulfur for the production of biomass, releasing the desulfonated carbon ring into the medium. However, desulfonation is dependent on the number (1-NS > 1,5-NDS > 1,3,6-NTS) and position (1,6-NDS > 2,6-NDS > 2,7-NDS > 1,5-NDS) of the substituents on the carbon structure [21]. In the case of 1-naphthalene sulfonate, 1-naphthol is liberated along with sulfite. Furthermore, Luther [21] found that the chlorophyte alga also utilized nitro and amino substituents from aminonaphthalenes and amino- and nitrobenzoates as nitrogen sources. Luther [21] noted that bacteria then metabolized the products of desulfonation. Bacteria require long periods of adaption to degrade naphthalene monosulfonic acids [19]; however, *S. obliquus* can desulfonate these compounds in a matter of hours in sulfur-deficient medium. This suggests that an algal-bacterial consortium may accelerate the degradation of these compounds. In addition, chlorobenzoates were dehalogenated and the chloride accumulated in the cells. Diaryl ethers such as halogenated diphenyl ethers are important environmental pollutants. Therefore, the bacterial degradation of these and structurally related compounds attracted considerable interest [22,23]. However, by using the diphenyl ether herbicide diclofop methyl, Wolfaardt et al. [24] demonstrated the significant contribution of a *Chlorococcum* sp. present in an algal-bacterial consortium to the rate of $^{14}CO_2$ production from ^{14}C-labelled diclofop methyl. These examples demonstrate that algae are indeed capable of contributing to the degradation of environmental pollutants, either by directly transforming the pollutant in question or by enhancing the degradation potential of the microbial community present.

2.2. Respirometric studies

By definition, algae are obligate aerobes, and it is logical to hypothesize that the degradation of organic compounds will require oxygen to proceed. The

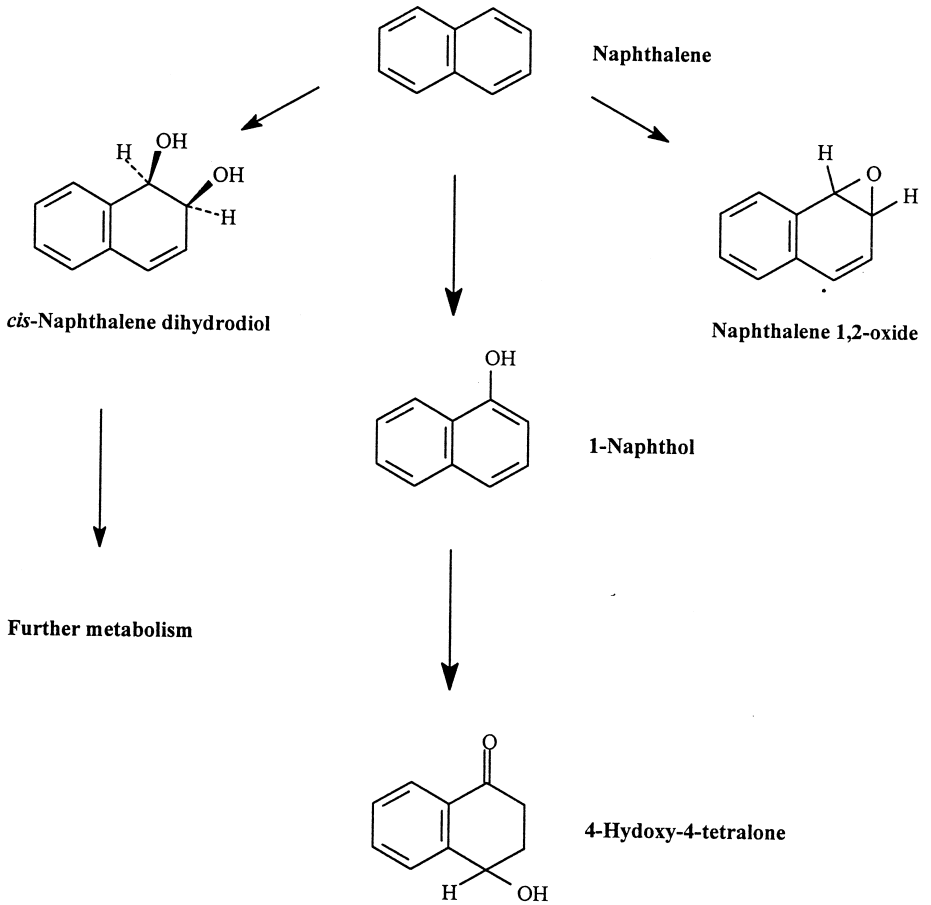

Fig. 1. The proposed biotransformation of naphthalene by algae (adapted from [15]).

measurement of respiration (i.e. O_2 utilization and CO_2 production) can be an extremely useful tool in the elucidation of the aerobic degradation of pollutants. Semple [25] found that the metabolism of phenolics by *O. danica* was obligatorily aerobic. There was no detectable phenol turnover under anaerobic conditions (O_2-free nitrogen) until air was admitted when phenol removal commenced. Warburg manometric studies revealed that the amount of oxygen consumed per mol of phenol, catechol, *p*-cresol, 4-methylcatechol and 3,4-xylenol was some 65% of the theoretical oxygen demand for complete oxidation to CO_2 [26].

The use of ^{14}C radiolabelled pollutants is yet another powerful method by which the degradation of organic substrates may be quantified. In one of the first ^{14}C studies involving algae, Craigie et al. [27] showed that several eukaryotic marine algae produced $^{14}CO_2$ from [*ring*-^{14}C]phloroglucinol. After preconditioning the algae with non-radiolabelled phloroglucinol for 35 days, enhanced liberation of $^{14}CO_2$ from the aromatic ring was measured over a 16-h incubation period in the dark. However, the levels of mineralization were not great, ranging from 1.65 to 5.54% $^{14}CO_2$ released. It is not clear, in this study, what the incubation conditions were in terms of cell densities at the beginning of these incubations as this may determine the overall extent of mineralization. In a detailed screening study, Vose et al. [28] incubated 22 species of unicellular marine algae with [*ring*-^{14}C]DL-phenylalanine in the light and found nine species to produce $^{14}CO_2$ ranging from 0.05% to 1.5%. However, only four of the species liberated $^{14}CO_2$ in the dark with amounts rang-

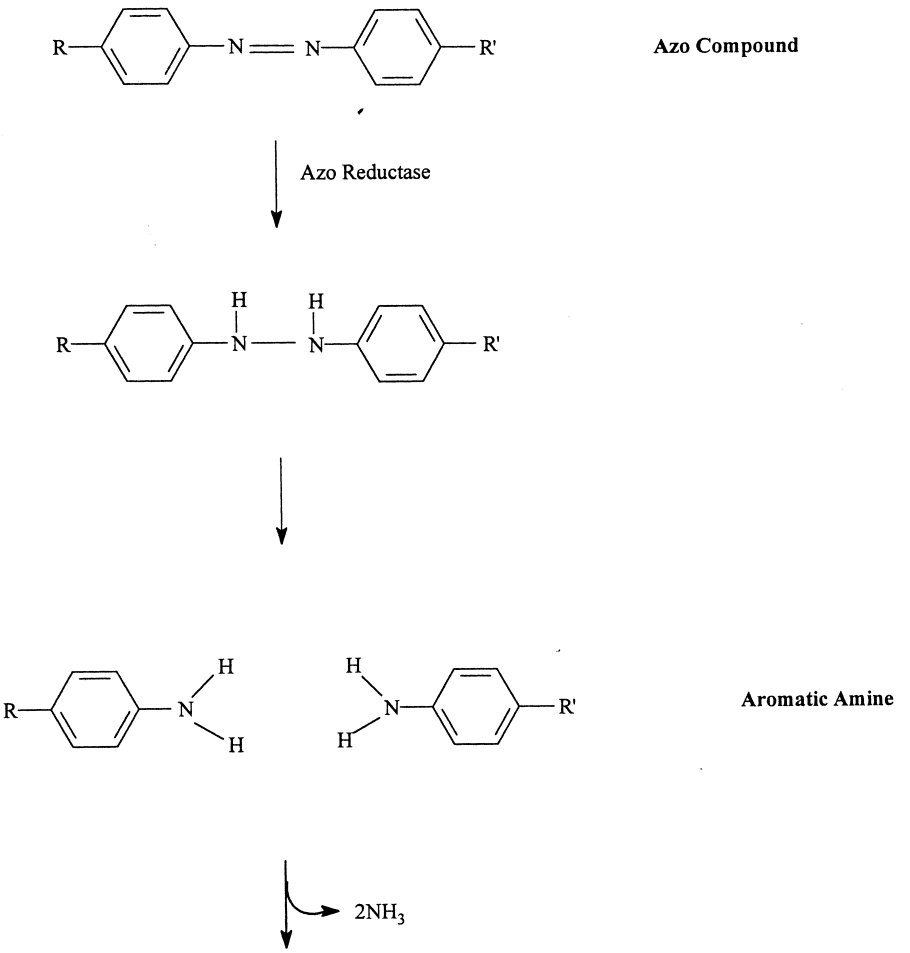

Fig. 2. Proposed degradation of azo dyes by eukaryotic algae (adapted from [8]).

ing from 0.12% to 0.46%. The authors point out that the metabolism of DL-phenylalanine cannot be related to the evolution of $^{14}CO_2$ as the algae were expected to produce a variety of incompletely oxidized products. In fact, the authors found that an additional ^{14}C-labelled volatile metabolite was produced in preliminary studies, indicating that it was not phenolic in nature.

Later, Ellis [29] incubated six species of freshwater algae with [UL-^{14}C]catechol and [UL-^{14}C]phenol. With no prior exposure to the aromatic compounds, all six species produced $^{14}CO_2$ from [UL-^{14}C]-catechol but only four produced $^{14}CO_2$ from the [UL-^{14}C]phenol. Strangely, prior exposure to 0.1 mM phenol proved to be inhibitory to the algae with only two of the algae able to mineralize the phenol, but all six of the algae exposed to 0.1 mM catechol were still able to mineralize the aromatic alcohol. It is significant, however, that the amount of $^{14}CO_2$ evolved in these experiments ranged from 0.1% to 7.7% with no prior exposure and from 0.5% to 5.5% with prior exposure. Ellis [29] suggested that algae are capable of assisting in the catabolism of phenols in the aqueous environment provided the

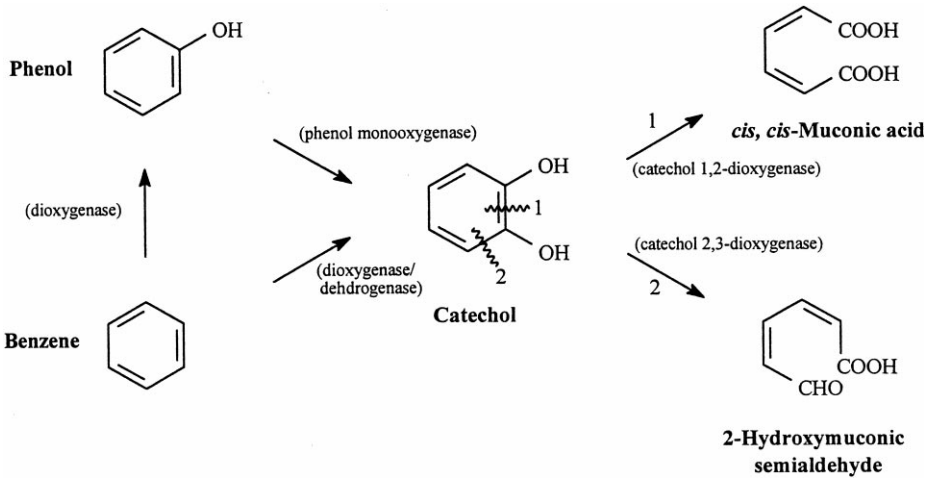

Fig. 3. Reactions involving the metabolism of the aromatic nucleus (adapted from [1]).

concentration does not become too high. Unequivocal proof that an alga can mineralize phenol was provided in a study showing *O. danica* was able to metabolize [UL-^{14}C]phenol completely, with approximately 65% of the ^{14}C label appearing as $^{14}CO_2$, approximately 10–12% of ^{14}C remaining in the aqueous medium. Analysis of the algal biomass showed that ^{14}C label was incorporated significantly into the cellular protein, nucleic acid and lipid [26]. In a recent study [30], three species of *Chlorella* were found to degrade pentachlorophenol. *Chlorella* VT1 was found to mineralize up to 13.8% of [UL-^{14}C]PCP to $^{14}CO_2$ over an 11-day incubation in a light/dark cycle. Mineralization of the chlorophenol was further enhanced by the addition of glucose. Approximately 10–20% of the ^{14}C label was associated with the algal biomass with the remaining ^{14}C label accounted for in the growth medium. The mechanisms for the degradative pathway have yet to be elucidated.

2.3. Enzymology of aromatic catabolic pathways

There are few examples of algae degrading aromatic compounds and, until recently, no catabolic pathways for the degradation of aromatic compounds had been described. Both Cerniglia et al. [16] and Jinqi and Houtian [8] have shown the removal of pollutants and, in the former case, the accumulation of catabolic intermediates (Figs. 1 and 2, respectively) and these authors have made suggestions as to the processes occurring. More detailed studies were carried out by Lindquist and Warshawsky [31,32], Warshawsky et al. [33–35] and Schoeny et al. [36], who examined the effects of the chlorophyte alga, *Selenastrum capricornutum*, on benzo[*a*]pyrene. They found that the alga used a dioxygenase system to oxidize the compound to *cis*-dihydrodiols which were then converted to sulfate ester and α- and β-glucoside conjugates. The presence of this ring hydroxylating dioxygenase system is of particular importance as this mechanism is typically found only in bacteria and not in eukaryotes, where *trans*-dihydrodiols typically originate from epoxidation by the action of cytochrome P-450 monooxygenases and epoxide hydrolases on the PAH molecule.

The enzymology of the degradation of phenol and its methylated homologues by *O. danica* has also been investigated [26]. Specific activities for the hydroxylation of phenol to catechol involving a putative phenol hydroxylase could not be found in cell-free extracts. These activities were elucidated using whole cells with the alga having the highest specificity for phenol, then *p*-cresol, *o*-cresol and finally *m*-cresol. Catabolism of phenol to catechol was confirmed by the isolation of the product from incubations in which the catechol 2,3-dioxygenase (catechol:oxygen 2,3-oxidoreductase; EC 1.13.11.2) was inhibited with 3-chlorocatechol [26], which was shown earlier to suicide inhibit the enzyme [37].

Aromatic rings can be ring opened by either *ortho*

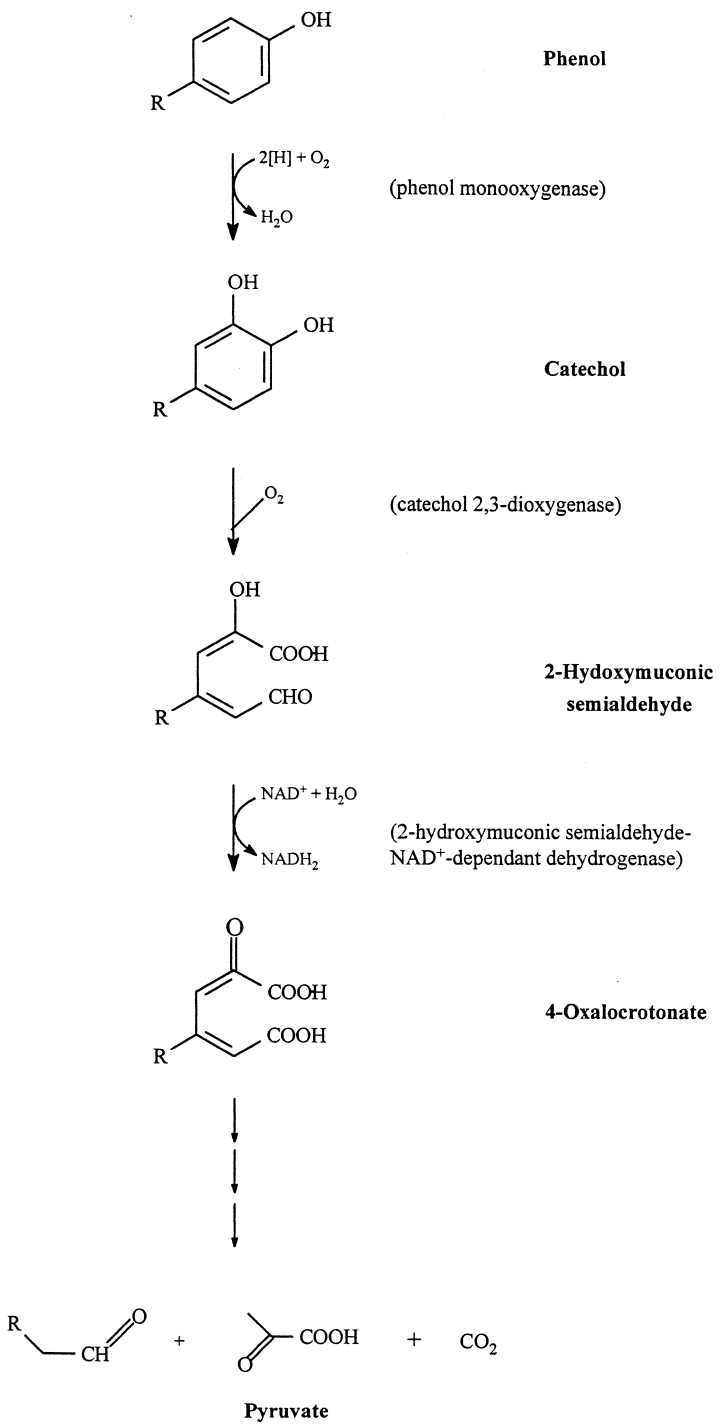

Fig. 4. Characterized steps in the catabolism of phenol, via the *meta* cleavage pathway, by *Ochromonas danica* (adapted from [26]).

cleavage or *meta* cleavage after the formation of a 1,2-dihydroxybenzoidal moiety (Fig. 3). Enzymes of both *ortho* and *meta* cleavage pathways which are commonly found in bacteria [38,39] were assayed spectrophotometrically using extracts of phenol-induced cells. Axenic cultures of *O. danica* were found to cleave catechol in the 2-3 position resulting in the formation of 2-hydroxymuconic semialdehyde. The algal extracts also oxygenated 3-methylcatechol, 4-methylcatechol, 4-bromocatechol and 4-fluorocatechol to the corresponding ring cleavage products, which showed the expected shifts in spectral peaks under acid and alkaline conditions. Inhibition studies using 4-isopropylcatechol [40] and 3-chlorocatechol [37] gave results corresponding to those of bacterial studies, showing irreversible and suicide inhibitions, respectively. Specific activities in these extracts for catechol 1,2-dioxygenase (catechol:oxygen 1,2-oxidoreductase; EC 1.13.11.1), the enzyme initiating the *ortho* pathway, were found to be negligible. Metabolism of 2-hydroxymuconic semialdehyde was catalyzed predominantly by an NAD^+-dependent dehydrogenase (2-hydroxymuconate semialdehyde: NAD^+ oxidoreductase; EC 1.2.1.-). Prolonged incubation of the muconic semialdehyde products with cell-free extracts resulted in the production of pyruvate, confirmed by its reduction to L-lactate with NADH and crystalline lactate dehydrogenase and by TLC detection of its 2,4-dinitrophenylhydrazone [26]. Fig. 4 shows the catabolic pathway for phenol characterized in *O. danica* [26].

3. Conclusion

This minireview has shown that eukaryotic algae are capable of biotransforming and biodegrading aromatic pollutants commonly found in natural and wastewaters. Furthermore, these organisms are able to enhance the degradation potential of the microbiota present and therefore contribute to the elimination of pollutants from the respective ecosystem. Traditionally, plant ecology has regarded microalgae as an essential part of the aquatic food web by providing reduced C and N in aquatic ecosystems [41]. In addition, many of these organisms were found to be useful indicators of environmental pollution and therefore applied in toxicity testing [42]. At present, however, the respective importance of microalgae for the catabolism of pollutants entering their ecosystem is not known.

As it is extremely rare to find a single microorganism that is capable of completely degrading a pollutant or a mixture of xenobiotics under environmental conditions [43], the combined action of microalgae and other microorganisms might be a rather important process for the elimination of these undesired compounds from the environment. The degradation of pollutants under these conditions usually involves the combined actions of two or more microorganisms [44]. In the case of algae, it is clear that although the complete degradation of aromatic pollutants is rare, these organisms are capable of carrying out biotransformations on aromatic pollutants, such as the hydroxylation of naphthalene and benzo[*a*]pyrene to their hydroxylated intermediates. These initial biotransformations may be extremely important in the overall degradation of pollutants in the environment as they may change the physical and chemical nature of the pollutants by decreasing K_{ow} values or making them more susceptible to attack by other microorganisms after the removal of a substituent group. This has been highlighted by Meulenberg et al. [45] where partially oxidized PAHs became more bioavailable and therefore more biodegradable in soil systems. However, it is not clear whether the production of phenolic structures such as 1-naphthol by microalgae leads to the known oxidative coupling [46] of these metabolites under environmental conditions. An additional process that might significantly eliminate pollutants from the water phase is the uptake of these compounds by microalgae [47], which, if the compounds are to be retained in the cells, will decrease immediate toxic effects. As a matter of fact, we do need much more information on algal-based processes before we will be able to reliably predict the short- and long-term impact of pollutant stress on, for example, freshwater systems. It would be very interesting to find out if algal specialists reported to degrade, or at least to detoxify, xenobiotics such as herbicides, entering their particular environment, are able to protect the phytoplankton present from the adverse effects of these compounds. In addition, the mechanisms underlying the enhanced degradation of pollutants by algal-bacterial interaction need to be elucidated.

What is generally unclear are the biochemical mechanisms which these microalgae employ to catabolize these pollutants, and the relative contribution of these algae to overall metabolism of these compounds in the hydroecosphere.

References

[1] Dagley, S. (1978) Microbial catabolism, the carbon cycle and environmental pollution. Naturwissenschaften 65, 85–95.

[2] Middlehoven, W.J. (1993) Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeast-like fungi. Antonie van Leeuwenhoek 63, 125–144.

[3] Semple, K.T. and Cain, R.B. (1995) Metabolism of phenols by *Ochromonas danica*. FEMS Microbiol. Lett. 133, 253–257.

[4] Kobayashi, H. and Rittman, B.E. (1982) Microbial removal of hazardous organic compounds. Environ. Sci. Technol. 16, 170A–183A.

[5] Neilson, A. and Lewin, R. (1974) Uptake and utilization of organic carbon by algae. Phycology 13, 229–257.

[6] Walker, J.D., Colwell, R.R. and Petrakis, L. (1975) Degradation of petroleum by an alga, *Prototheca zopfii*. Appl. Microbiol. 30, 79–81.

[7] Jacobson, S.N. and Alexander, M. (1981) Enhancement of the microbial dehalogenation of a model chlorinated compound. Appl. Environ. Microbiol. 42, 1062–1066.

[8] Jinqi, I. and Houtian, O. (1992) Degradation of azo dyes by algae. Environ. Pollut. 75, 273–278.

[9] Semple, K.T. (1997) Biodegradation of phenols by a eukaryotic alga. Res. Microbiol. 148, 365–367.

[10] Semple, K.T. and Cain, R.B. (1997) Biodegradation of phenol and its methylated homologues by *Ochromonas danica*. FEMS Microbiol. Lett. 152, 133–139.

[11] Cerniglia, C.E. (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3, 351–368.

[12] Soto, C., Hellebust, J.A., Hutchinson, T.C. and Sawa, T. (1975) Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa*. 1. Growth. Can. J. Bot. 53, 109–117.

[13] Soto, C., Hellebust, J.A. and Hutchinson, T.C. (1975) Effect of naphthalene and crude oil extracts on the green flagellate *Chlamydomonas angulosa*. 2. Photosynthesis and the uptake and release of naphthalene. Can. J. Bot. 53, 118–126.

[14] Cerniglia, C.E., Gibson, D.T. and van Baalen, C. (1979) Algal oxidation of aromatic hydrocarbons: formation of 1-naphthol from naphthalene by *Agmenellum quadruplicatum*, strain PR-6. Biochem. Biophys. Res. Commun. 88, 50–58.

[15] Cerniglia, C.E., van Baalen, C. and Gibson, D.T. (1980) Metabolism of naphthalene by the cyanobacterium *Oscillatoria* sp., strain JCM. J. Gen. Microbiol. 116, 485–494.

[16] Cerniglia, C.E., Gibson, D.T. and van Baalen, C. (1980) Oxidation of naphthalene by cyanobacteria and microalgae. J. Gen. Microbiol. 116, 495–500.

[17] Cerniglia, C.E., Gibson, D.T. and van Baalen, C. (1982) Naphthalene metabolism by diatoms from the Kachemak Bay region of Alaska. J. Gen. Microbiol. 128, 987–990.

[18] Liebe, B. and Fock, H.P. (1992) Growth and adaption of the green alga *Chlamydomonas reinhardtii* on diesel exhaust particle extracts. J. Gen. Microbiol. 138, 973–978.

[19] Luther, M. and Soeder, C.J. (1987) Some naphthalene sulphonic acids as sulphur sources for the green microalga, *Scenedesmus obliquus*. Chemosphere 16, 1565–1578.

[20] Luther, M. and Shaaban, M.M. (1990) Abbau von 1,5-Naphthalindisulfonsäure (1,5-NDS) durch eine Algen-Bakterien-Mischkultur. Forum Mikrobiol. 1/2, 42.

[21] Luther, M. (1990) Degradation of different substituted aromatic compounds as nutrient sources by the green alga *Scenedesmus obliquus*. Dechema Biotechnol. Conf. 4, 613–615.

[22] Schmidt, S., Fortnagel, P. and Wittich, R.M. (1993) Biodegradation and transformation of 4,4′- and 2,4-dihalodiphenyl ethers by *Sphingomonas* sp. strain SS33. Appl. Environ. Microbiol. 59, 3931–3933.

[23] Bünz, P.V. and Schmidt, S. (1997) The microbial degradation of halogenated diaryl ethers. Biotechnol. Adv. 15, 621–632.

[24] Wolfaardt, G.M., Lawrence, J.R., Robarts, R.D. and Caldwell, D.E. (1994) The role of interactions, sessile growth, and nutrient amendments on the degradative efficiency of a microbial consortium. Can. J. Microbiol. 40, 331–340.

[25] Semple, K.T. (1994) Ph.D. Thesis, The University of Newcastle, Newcastle upon Tyne.

[26] Semple, K.T. and Cain, R.B. (1996) Biodegradation of phenolics by *Ochromonas danica*. Appl. Environ. Microbiol. 62, 1265–1273.

[27] Craigie, J.S., McLachlan, J. and Towers, G.H.N. (1965) A note on the fission of an aromatic ring by algae. Can. J. Bot. 43, 1589–1590.

[28] Vose, J.R., Cheng, J.Y., Antia, N.J. and Towers, G.H.N. (1971) The catabolic fission of the aromatic ring of phenylalanine by marine planktonic algae. Can. J. Bot. 49, 259–261.

[29] Ellis, B.E. (1977) Degradation of phenolic compounds by freshwater algae. Plant Sci. Lett. 8, 213–216.

[30] Tikoo, V., Scragg, A.H. and Shales, S.W. (1997) Degradation of pentachlorophenol by microalgae. J. Chem. Technol. Biotechnol. 68, 425–431.

[31] Lindquist, B. and Warshawsky, D. (1985) Stereospecificity in algal oxidation of the carcinogen benzo(a)pyrene. Experientia 41, 767–769.

[32] Lindquist, B and Warshawsky, D. (1985) Identification of the 11,12-dihydro-11,12-dihydroxybenzo(a)pyrene as a major metabolite by the green alga, *Selenastrum capricornutum.* Biochem. Biophys. Res. Commun. 130, 71–75.

[33] Warshawsky, D., Radike, M., Jayasimhulu, K. and Cody, T. (1988) Metabolism of benzo(a)pyrene by a dioxygenase system of the fresh green alga *Selenastrum capricornutum*. Biochem. Biophys. Res. Commun. 152, 540–544.

[34] Warshawsky, D., Keenan, T.H., Reilman, R., Cody, T.E. and Radike, M.J. (1990) Conjugation of benzo(a)pyrene by freshwater green alga *Selenastrum capricornutum*. Chem.-Biol. Interact. 74, 93–105.

[35] Warshawsky, D., Cody, T., Radike, M., Reilman, R., Schumann, B., LaDow, K. and Schneider, J. (1995) Biotransfor-

mation of benzo[a]pyrene and other polycyclic aromatic hydrocarbons and heterocyclic analogs by several green algae and other algal species under gold and white light. Chem.-Biol. Interact. 97, 131–148.

[36] Schoeny, R., Cody, T., Warshawsky, D. and Radike, M. (1988) Metabolism of mutagenic polycyclic aromatic hydrocarbons by photosynthetic algal species. Mutat. Res. 197, 289–302.

[37] Bartels, I., Knackmuss, H.-J. and Reineke, W. (1984) Suicide inactivation of catechol 2,3-dioxygenase from *Pseudomonas putida* mt-2 by 3-halocatechols. Appl. Environ. Microbiol. 47, 500–505.

[38] Dagley, S., Evans, W.C. and Ribbons, D.W. (1960) New pathways in the oxidative metabolism of aromatic compounds by microorganisms. Nature 188, 560–561.

[39] Bayly, R.C., Dagley, S. and Gibson, D.T. (1966) The metabolism of cresols by species of *Pseudomonas*. Biochem. J. 101, 293–301.

[40] Bird, A.J. and Cain, R.B. (1974) Microbial degradation of alkylbenzene sulphonates. Metabolism of homologues of short alkyl-chain length by an *Alcaligenes* sp. Biochem. J. 140, 121–134.

[41] Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H. (Eds.) (1983) Physiological Plant Ecology IV. Ecosystem Processes: Mineral Cycling, Productivity and Man's Influence. Springer Verlag, Berlin.

[42] Nyholm, N. and Källquist, T. (1989) Methods for growth inhibition toxicity tests with freshwater algae. Environ. Toxicol. Chem. 8, 689–703.

[43] Alexander, M. (1994) Biodegradation and Bioremediation. Academic Press, San Diego, CA.

[44] McEldowney, S., Hardman, D.J. and Waite, S. (1993) Pollution: Ecology and Biotreatment. Longman Scientific and Technical, New York.

[45] Meulenberg, R., Rijnaarts, H.H.M., Doddema, H.J. and Field, J.A. (1997) Partially oxidized polycyclic aromatic hydrocarbons show an increased bioavailability and biodegradability. FEMS Microbiol. Lett. 152, 45–49.

[46] Bollag, J.-M. and Loll, M.J. (1983) Incorporation of xenobiotics into soil humus. Experientia 39, 1221–1231.

[47] Ware, G.W. and Roan, C.C. (1970) Interaction of pesticides with aquatic microorganisms and plankton. Residue Rev. 33, 15–45.