What is the Advantage for Bacteria to Use Polycyclic Aromatic Hydrocarbons as Carbon Source under Aerobic Condition?

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ABSTRACT

The environment varies hugely and widely in terms of merely carbon source so there is the environment that possesses a mixture of carbon sources or the environment that possesses a single type of carbon source. Some bacteria have choices to use some kind of carbon for their growth. This mechanism is the carbon catabolite repression (CCR), and the preference order for utilization of carbon sources is usually organic acids, glucose, and aromatic substrates in bacteria with diauxic growth although not all bacteria in an environmental setting experience the diauxic growth. For some bacteria in the second case, they use polycyclic aromatic hydrocarbons (PAHs) as their sole carbon and energy source, and these specially PAH-degrading bacteria do not utilize sugars. Therefore, an interesting question is what advantage for bacteria to use PAHs as sole carbon and energy source? This question is relatively easy to answer for the bacteria that have choices to decide their preference order on carbon sources because we can compare their metabolic pathways to find the advantage, but is not easy to answer for the bacteria that are specialized to utilize PAHs because we have yet to know why the other metabolic pathways do not function in these bacteria. In this review, we attempt to answer this question by reviewing literature in terms of oxygen consumption, number of reactions to generate pyruvate and ATP consumption with reference to glycolysis of glucose. Our analyses demonstrate the advantage of utilization of typical PAHs over glucose under aerobic condition.

Introduction

The environment varies hugely and widely in terms of merely carbon source so there is the environment that possesses a mixture of carbon sources or the environment that possesses a single type of carbon source. Yet, bacteria are not selected in a particular environment just based on availability or supply of carbon substrates. For some bacteria in the first case, they have choices to use which carbon for their growth. This mechanism is the carbon catabolite repression (CCR), i.e. a bacterium uses its preferable carbon and inhibits transportation and metabolism of non-preferred carbons until the amount of preferable carbon is not sufficient [1,2]. This leads to the phenomenon of diauxic growth, i.e.

the growth of bacteria has two log-phases although not all bacteria in an environmental setting experience the diauxic growth. The diauxic growth was observed in *Bacillus subtilis* for sucrose-dextran [3]; in *Escherichia coli* for glucose-lactose [4], glucose-melibiose [5] and glucose-glycerol [6]; in *Lactobacillus casei* for glucose-maltose [7] and glucose-ribose [7].

Although CCR is mainly related to the glycolysis and citric acid cycle, bacteria also evolved alternative metabolic pathways such as sugar metabolism via the keto-deoxy-phosphogluconate pathway (Entner–Doudoroff pathway) in *Pseudomonas* [8] because *Pseudomonas* lacks phosphofructokinase for glycolysis [9] and the

pentose phosphate pathway [10]. Therefore, bacteria are able to live on various carbon sources. Indeed, *Pseudomonas aeuroginosa* PP4 and *Pseudomonas sp*. C5pp utilize glucose in their first log-phase growth,but utilize aromatics in their second log-phase growth [11]. Further development shows that preference order for utilization of carbon sources in *Pseudomonas* is organic acids, glucose and aromatic substrates [12]. Intriguingly, not all species from *Pseudomonas* follow this preference order. For example, *P. putida* CSV86 utilizes aromatic substrates such as naphthalene, benzyl alcohol, benzoate, phenylacetic acid and phenylpropanoids (veratraldehyde, ferulic acid, vanillin and vanillic acid) prior to glucose [11-15]. This preference is very suggestive to biodegradation and bioremediation in environments [16], because pollution of aromatic compounds, especially, pollution of polycyclic aromatic hydrocarbons (PAHs), is harmful to humans and environments [17,18]. Accordingly, PAHs can be metabolized prior to other carbon sources. For bacteria, their uptake of PAHs requires

1) High-affinity systems,

2) Adhesion of PAHs to the solid substrate, and

3) Excretion of biosurfactant [19].

Although these requirements are relevant to uptake of PAHs, they are likely to be an indicator of CCR mechanism, which at first represses and then begins the synthesis of the enzymes for the transportation of less favorable carbon sources. In reality, *P. putida* CSV86 significantly expressed its *ben* locus in the first log phase and its *glc* locus in the second log phase when growing on glucose and benzoate [15]. Still, 3-day and <1 day lag phases in growth of *Mycobacterium sp.* strain CH1 with pyrene and phenanthrene or fluoranthene were observed [20]. Moreover, there are specialist PAH-degrading bacteria do not utilize sugars compared to generalist hydrocarbon-degrading organisms like *Pseudomonas*. An interesting question is what advantage for bacteria to use PAHs as sole carbon and energy source over other carbon sources? This question is relatively easy to answer for the bacteria that have choices to decide their preference order on carbon sources because we can compare their metabolic pathways to find the advantage, but is not easy to answer for the bacteria that are specialized to utilize PAHs because we have yet to know why the other metabolic pathways do not function in these bacteria. Indeed, it is hard to know how many carbon compounds in a certain environment are under the bacterial choices although pentose and hexose are the main choice in bacterial experiments. At this stage, we actually can only direct our attention to a small part of carbon sources, i.e. glucose as first step to approach to this topic. In this review, we attempt to answer this question by reviewing literature in terms of oxygen consumption, number of reactions to generate pyruvate and ATP consumption with reference to glycolysis of glucose.

Aromatic Catabolism in General

Although at least 660 PAH structures have been documented [21], it is unknown how many PAHs bacteria can utilize. A review compiled 27 aromatic compounds, which can be utilized by around 40 bacterial species [22]. Thus, our knowledge on metabolism of aromatic compounds is limited to a small number of aromatic compounds including several typical PAHs. In fact, PAH bioremediation is dependent upon aerobic conditions [23]. Although aromatic compounds can serve as carbon source for bacteria, not all bacteria use aromatic compounds under aerobic condition. For example, *P. aeruginosa* is a facultative anaerobe living with partial or total oxygen depletion, under which it uses nitrate or nitrite as a terminal electron acceptor. When lacking of nitrate and nitrite, *P. aeruginosa* ferments arginine and pyruvate by substrate-level phosphorylation under anaerobic condition [24]. Utilization of aromatic compounds in bacteria not only means that a different preference order governed by CCR but also suggests the existence of additional mechanisms. For the latter case, *Sinorhizobium*, *Rhizobium* and *Bradyrhizobium* from α-proteobacteria [25,26], and *Pseudomonas* from γ-proteobacteria [27] are in favor of acetate as well as intermediates in the citric acid cycle such as succinate rather than common carbon sources such as glucose, fructose or lactose. For *Pseudomonads*, the underlying mechanism is the involvement of catabolite repression control (Crc) protein [28-33]. For *Rhizobacteria*, the underlying mechanism is the involvement of inducer accumulation [26]. Indeed, different mechanisms lead to different metabolic pathways.

Thus an interesting question is whether the existence of oxygen could evolutionarily lead the different mechanisms to converge into a few pathways, which become less diverging? This rationale could be plausible because the oxygen environment should play an extremely important role on the bacterial evolution and give aerobic bacteria the energetic advantage over anaerobic bacteria. Really, the metabolism of PAHs in fungi and anaerobes are usually nonspecific [34], but many pathways converge to catecholic intermediates in aerobic microorganisms [35,36]. In this case, we just need to pay our attention to the limited number of pathways and some parts of a pathway, which are very classic in PAH studies. To a broader sense, technical revolution and globalization led many indigenous languages to extinction because they lose their local advantage and languages converge into few languages. Another example should be that the environmental changes led to extinction of megafauna.

Starting and Ending Points for Comparison

In order to find out the advantage of utilization of PAHs over glucose in bacteria, we should compare catabolism and metabolism of PAHs with glucose. The uptake of a molecule of glucose by bacteria through phosphoenolpyruvate(PEP):carbohydrate phosphotransferase system consumes an ATP [1]. On the other hand, it is still not clear which transport systems are responsible for uptake of aromatic compounds, so we have no ways to know whether the uptake of PAHs consumes ATP. Hence, our starting point for comparison should began from the time, when glucose and PAHs enter into bacterial cells because we have yet to know the exact mechanisms of uptake of aromatic compounds through

bacterial membrane. Glucose can go through three catabolic pathways in bacteria:

    i.   the glycolysis, which produces pyruvate;

    ii.   the Entner–Doudoroff pathway, which produces pyruvate [8]; and

    iii.   the pentose phosphate pathway, which is mainly related to anabolism rather than catabolism [10].

For the last two pathways, their initial steps are the same from β-D-glucose 6-phosphate to 6-phospho-D-gluconate whereas the first two pathways can meet at KDPG (Figure 1), and then both glycolysis and Entner–Doudoroff pathway produce pyruvate. Importantly, these reactions do not need oxygen no matter of whether bacteria are under aerobic or anaerobic conditions. Thereafter, pyruvate goes through the citric acid cycle [37] requiring oxygen, i.e. bacteria must be under aerobic condition. In Figure 1, we can see an advantage in *Pseudomonas*, because it lacks phosphofructokinase for glycolysis [9] and the pentose phosphate pathway [10], so glucose can only go through the Entner–Doudoroff pathway, which has fewer reactions than glycolysis although produces fewer ATP. To our knowledge, it is not clear whether a bacterium prefers more reactions with big production of ATP or fewer reactions with small production of ATP. However, Figure 1 does show a species advantage for *Pseudomonas*. Naphthalene can also be metabolized to pyruvate before reaching catechol and gentisate in Figure 2, and both catechol and gentisate can further be catabolized to pyruvate [38-42]. Therefore, the ending point for comparison should be pyruvate, not only because it is the end product of glycolysis but also because it is reachable to PAHs. Hereafter, pyruvate can go to citric acid cycle under aerobic condition or go to fermentation under anaerobic condition. On the other hand, the comparison would be arbitrary because the intermediates in the citric acid cycle are different leading to the difficulty in comparison.
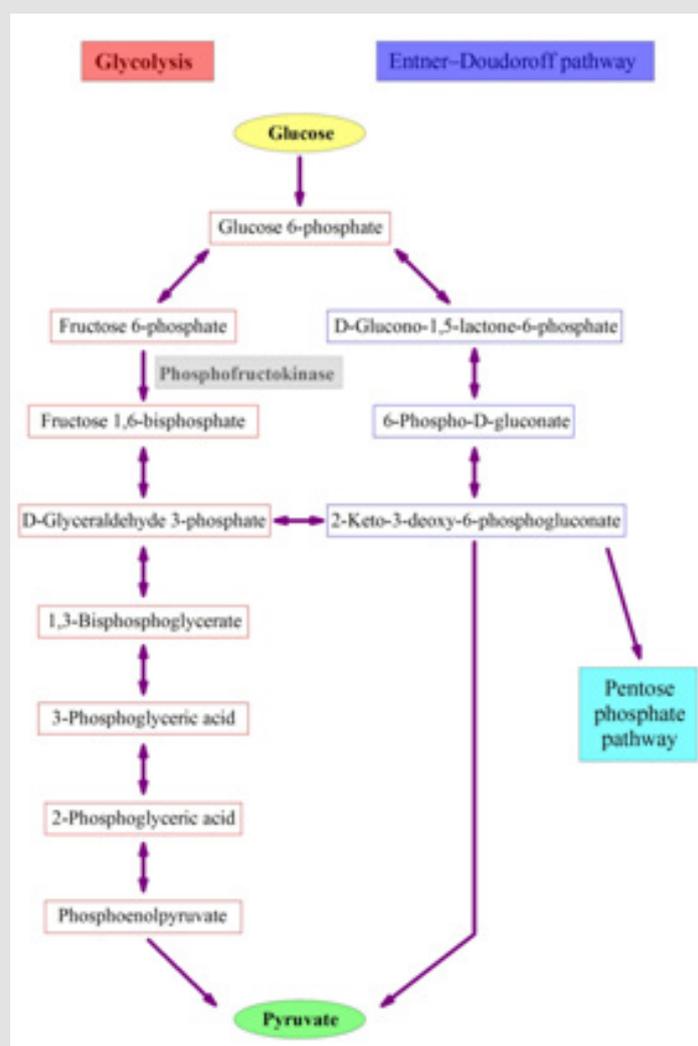

Figure 1: Three pathways for glucose metabolism before reaching pyruvate.
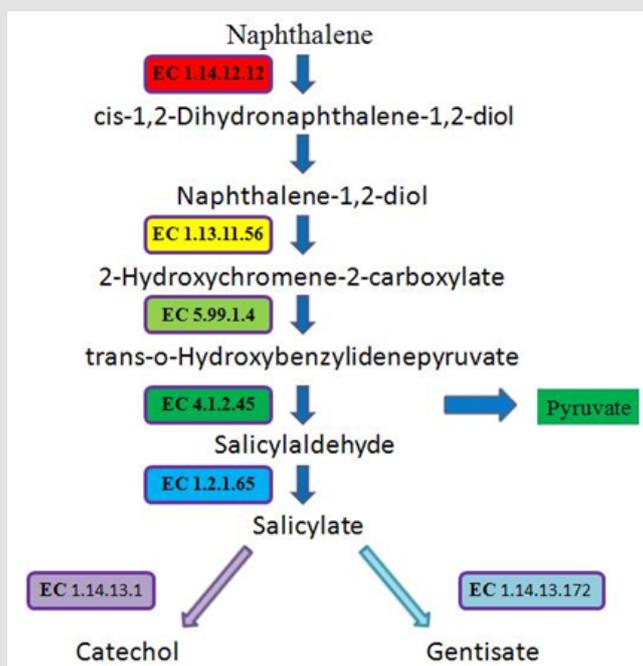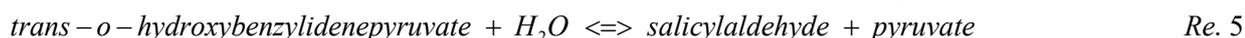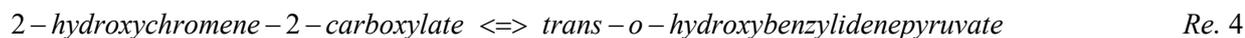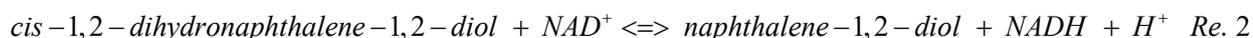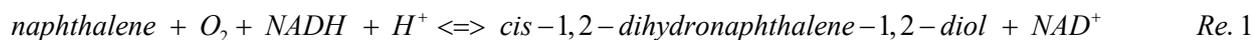
Figure 2: Catabolic pathways for naphthalene.

Again, if a bacterium prefers fewer reactions, then the choice of naphthalene has just five reactions to generate pyruvate whereas glycolysis needs ten reactions to generate pyruvate. For humans, the metabolism of PAHs aims to reduce their toxicity, whereas it is not clear whether bacteria have the same aim. However, it would be preferable to have the biological precursors such as pyruvate and acetyl coenzyme A, and fewer toxic intermediates such as o-quinones as metabolites [43-46]. This is the second reason to choose pyruvate as an ending point for comparison. We agree that the starting and ending points for comparison are debatable because there are many intermediate chemicals in degradation pathways. This is the dilemma between generalization and individuation: if we detail each intermediate chemical for comparison we lose the generalization, otherwise the comparison looks speculative and hypothetic. This is also the dilemma between simplification and sophistication: we cannot find the rules without simplification, but the rules always include exceptions. For this review, we had to make a compromise as an initial step towards future researches.

From PAHs to Salicylate

Although pyruvate is set as the ending point for comparison, salicylate is an important intermediate in Figure 2. In fact, the catabolic pathway of typical PAHs usually reaches to salicylate. As shown in Figure 2, 2-ring naphthalene reaches to salicylate through six reactions as follows [38,47,48].

$$naphthalene \ + \ O_2 + NADH \ + \ H^+ <=> \ cis-1,2-dihydronaphthalene-1,2-diol \ + \ NAD^+ \qquad Re.\ 1$$

$$cis-1,2-dihydronaphthalene-1,2-diol \ + \ NAD^+ <=> \ naphthalene-1,2-diol \ + \ NADH \ + \ H^+ \quad Re.\ 2$$

$$naphthalene-1,2-diol \ + \ O_2 <=> \ 2-hydroxychromene-2-carboxylate \qquad Re.\ 3$$

$$2-hydroxychromene-2-carboxylate \ <=> \ trans-o-hydroxybenzylidenepyruvate \qquad Re.\ 4$$

$$trans-o-hydroxybenzylidenepyruvate \ + \ H_2O \ <=> \ salicylaldehyde \ + \ pyruvate \qquad Re.\ 5$$

$$salicylaldehyde \ + \ NAD^+ + \ H_2O \ <=> \ salicylate \ + \ NADH \ + \ H^+ \qquad Re.\ 6$$

For net consumption and production, the degradation from naphthalene to salicylate consumes NAD^+, 2 O_2 and 2 H_2O, but produces NADH, H^+ and pyruvate. This pathway was called the upper catabolic pathway in *P. putida* G7 [38]. Comparing Figure 1 with Figure 2 and above six reactions, we can immediately find the advantage of utilization of naphthalene over glucose in terms of oxygen consumption, number of reactions to generate pyruvate and ATP consumption.

1)   The pathway from naphthalene to salicylate consumes 2 oxygen molecules, whereas glycolysis does not need oxygen.

2) Although salicylate is not the final product for naphthalene, pyruvate is already generated and the number of reactions to produce pyruvate is just 5, whereas this requires ten reactions in glycolysis.

3) No ATP is needed for naphthalene, but ATP is needed in glycolysis, which though produces ATP eventually. Following this line of thought, we can look at other typical PAHs.

Fluorene has 3 rings, but only two are benzene rings. Although fluorene has three major catabolic pathways [42,49-53], it has a pathway to reach salicylate [51,52]. Anthracene has three benzene rings. Of its four catabolic pathways, there is a pathway to reach salicylate [38,54-56]. Figure 3 shows the net consumption and generation of molecules from typical PAHs to salicylate. However, this figure does not include typical 3-ring phenanthrene, 4-ring pyrene and 5-ring benzo[α]pyrene, because they do not go through the pathways to salicylate. As can be seen in Figure 3, these PAHs already produce pyruvate before catabolizing to salicylate. On the other hand, the catabolism of salicylate will further produce pyruvate. This should be an advantage for bacteria to utilize PAHs. All these pathways from three PAHs to salicylate begin with naphthalene 1,2-dioxygenase (EC 1.14.12.12), which requires the first oxygen. Then the second oxygen is required for naphthalene with 1,2-dihydroxynaphthalene dioxygenase (EC 1.13.11.56), for fluorene with 3,4-dihydroxyfluorene 4,4a-dioxygenase (EC 1.13.11.-), and for anthracene with anthracene-1,2-diol 1,2-dioxygenase meta-cleavage (EC 1.13.11.-).
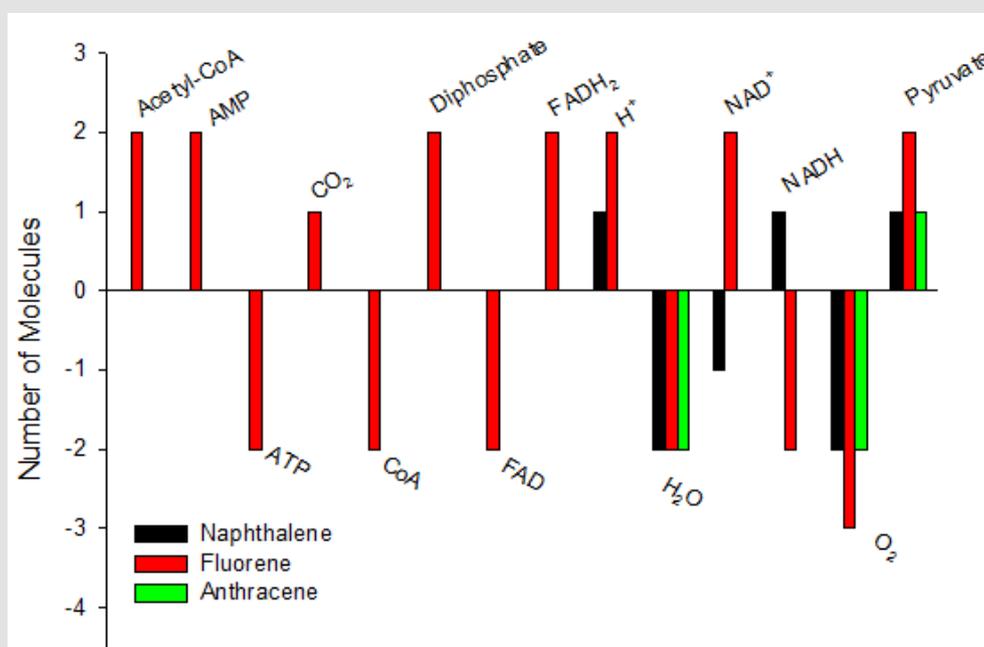

Figure 3: Net consumption and generation of molecules from naphthalene, fluorene and anthracene to salicylate.

These two oxygen-requiring reactions sufficiently take the advantage under aerobic condition. By clear contrast, glycolysis does not take this advantage under aerobic condition. Both fluorene and anthracene need only four reactions to generate pyruvate, but do not need ATP. Moreover, the glycolysis is a balanced reaction, so its reacted products can stay in bacteria without going to further reactions. By contrast, the pathways from PAHs to salicylate generate the products, which require further reactions such as diphosphate and NAD^+. This prerequisite provides the base for further reactions. Actually, pyruvate is required for the uptake of glucose through phosphoenolpyruvate(PEP):carbohydrate phosphotransferase system [1], whereas the uptake of PAHs does not go through this system, thus the pyruvate generated by PAHs can be used for other purposes.

From PAHs to Phthalate

Compared with the pathways from PAHs to salicylate, the pathways from PAHs to phthalate appear to be widespread and efficient because fluorene, anthracene, phenanthrene and pyrene have the pathways to phthalate (Figure 4). Although phenanthrene and pyrene can reach salicylate, they have to go through naphthalene [57-60]. The pathways from PAHs to phthalate are more efficient because they have more reactions to use oxygen. Fluorene, anthracene and phenanthrene have three oxygen-requiring reactions, and pyrene has five oxygen-requiring reactions. Clearly, these reactions take fully the advantage in aerobic condition. For fluorene, the first oxygen-requiring reaction again needs naphthalene 1,2-dioxygenase (EC 1.14.12.12), which in fact serves as monooxygenase adds a single oxygen atom at

fluorene and the other oxygen atom combines with 2 H to become H_2O. The second oxygen-requiring reaction needs 9-fluorenone-3,4-dioxygenase (EC 1.14.12.-), and the third oxygen-requiring

reaction needs 2,3-dihydroxy-2'-carboxybiphenyl 1,2-dioxygenase (EC 1.13.-.-). Although this pathway looks efficient, only 7.4% initial fluorene went through it in *Arthrobacter sp.* strain F101 [52].
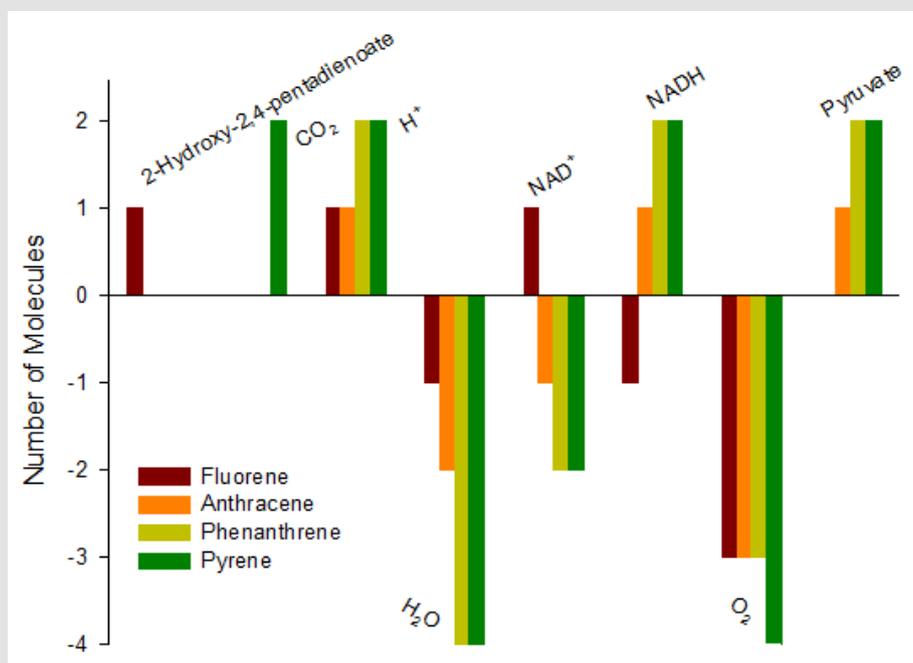

Figure 4: Net consumption and generation of molecules from fluorene, anthracene, phenanthrene and pyrene to phthalate.

For anthracene, the first oxygen-requiring reaction again needs naphthalene 1,2-dioxygenase (EC 1.14.12.12), the second oxygen-requiring reaction needs anthracene-1,2-diol 1,2-dioxygenase (EC 1.13.11.-), and the third oxygen-requiring reaction needs 3-hydroxy-2-naphthoate 2,3-dioxygenase (EC 1.13.11.-). In fact, this pathway is more complicated than the pathway to salicylate; however, it consumes one more oxygen molecules than the pathway to salicylate. Thus it is not clear whether a bacterium deliberately does so. For phenanthrene, the first oxygen-requiring reaction needs phenathrene dioxygenase (EC 1.13.11.-), the second oxygen-requiring reaction needs extradiol dioxygenase (EC 1.13.11.-), and the third oxygen-requiring reaction needs 1-hydroxy-2-naphthoate dioxygenase (EC 1.13.11.38). Although this pathway consumes three oxygen molecules, phenanthrene can pass around 1-hydroxy-2-naphthoate dioxygenase (EC 1.13.11.38) to naphthalene degradation to salicylate, which requires total four oxygen molecules. For pyrene, the first oxygen-requiring reaction needs pyrene dioxygenase (EC 1.14.-.-), the second oxygen-requiring reaction needs 4,5-dihydroxypyrene dioxygenase (EC 1.13.11.-), and the third oxygen-requiring reaction needs phenanthrene-4-carboxylate dioxygenase (EC 1.14.12.-), from here the reactions go to the phenanthrene pathway to consume additional two oxygen molecules to reach phthalate requiring a total five oxygen molecules, or pass around 1-hydroxy-2-naphthoate dioxygenase (EC 1.13.11.38) to naphthalene degradation to reach salicylate

requiring total six oxygen molecules. In comparison with glycolysis, a lot of oxygen molecules are consumed in the pathways from PAHs to phthalate, so PAHs catabolism takes fully the advantage of aerobic condition.

In terms of the number of reactions to generate pyruvate, anthracene and phenanthrene need four and five reactions, respectively, while pyrene needs 9 and 12 reactions to generate its first and second pyruvates. The pathways to phthalate also produce pyruvate except for fluorene. Moreover, they do not require as many reactions as glycolysis to generate pyruvate. This is also an advantage of PAHs over glucose. In terms of ATP, these four typical PAHs do not consume ATP to reach phthalate. Comparing Figure 4 with Figure 3, it is clear that the pathways from PAHs to phthalate are simpler than the pathways from PAHs to salicylate because these pathways do not require complicated molecules, for example, CoA in fluorene. This is very meaningful because CoA can be used to form benzoyl-CoA or methyl-benzoyl-CoA, which then can be used by bacteria under anaerobic conditions [61].

Other Pathways

In addition to salicylate and phthalate, there are several minor pathways to generate different products. For fluorene, it goes to 3-chromanone [50,52,62], which requires 2 H^+, 2 NADH, 2 H_2O and 3 O_2, but produces 2 e^-, 2 NAD^+, CO_2 and pyruvate. Actually, this pathway is very efficient because it has five reactions but needs three

oxygen molecules, and produces pyruvate in the third reaction. The first oxygen molecule is added together two H^+ by a dioxygenase. Actually, the first step in this pathway and the pathway to salicylate is the oxidation to dihydrodiol products. For anthracene, it goes to 9,10-anthraquinone, which requires O_2 and 2 e^-, but produces 2 H^+. Although this pathway requires oxygen, it does not produce pyruvate, and stops at 9,10-anthraquinone. In another pathway, anthracene goes to 3-(2-carboxyvinyl)naphthalene-2-carboxylic acid, which requires two oxygen molecules but does not generate pyruvate, and 3-(2-carboxyvinyl)naphthalene-2-carboxylic acid is the end product. Anyway, these two pathways have yet to find their further catabolic pathways so far [63] although the difference between the pathway to 9,10-anthraquinone and the pathway to salicylate is the difference between anthracene-cis-1,2-dihydrodiol and anthracene-9,10-dihydrodiol. For pyrene, 6,6-dihydroxy-2,2-biphenyl dicarboxylic acid implies the dioxygenation on 4,5 positions and 9,10 positions, and the cleavage on both central rings [64].

The abovementioned dihydroxylated intermediates may then be catabolized by either an ortho-cleavage pathway or a meta-cleavage pathway [65]. For phenanthrene, two minor pathways going to 9-phenanthrol and 1-methoxy-phenanthrene, which are still 3-ring compounds and have yet to find their further catabolic pathways so far [63]. However, the initial step in these two pathways is an addition of single oxygen atom by a hydroxylase serving as mono-oxygenase. Benzo[**α**]pyrene is also a typical PAH. Because of its five benzene rings, but few bacteria can catabolize it such as *Mycobacterium sp.* [66-68], *Sphingomonas paucimobilis* [69] and *Stenotrophomonas maltophilia* [70-72]. For benzo[**α**]pyrene, its initial step in the aerobic catabolism is also the oxidation to a dihydrodiol at almost all double carbon bond position: benzo[**α**]

pyrene-cis-4,5-dihydrodiol, benzo[**α**]pyrene-cis-7,8-dihydrodiol, benzo[**α**]pyrene-cis-9,10-dihydrodiol and benzo[**α**]pyrene-cis/trans-11,12-dihydrodiol. Accordingly, 4-ring benz[**α**]anthracene can also be catabolized in a similar manner [68,73]. But it is still not much known on the further catabolic mechanism for 5-ring to 4-ring PAHs [74-77].

Final Step to Pyruvate

After reaching salicylate from PAHs, salicylate can be catabolized to catechol, consuming H^+, NADH and O_2, and producing CO_2, H_2O and NAD^+. From salicylate to pyruvate and acetaldehyde in naphthalene catabolism was called the lower catabolic pathway through the catechol meta-cleavage pathway [38,41,47,48]. Thereafter, catechol can produce pyruvate as consuming 2 H_2O, CoA, NAD^+ and O_2, and producing formate, H^+, NADH and pyruvate. Nevertheless, catechol consumes O_2, H_2O, acetyl-CoA and succinyl-CoA but produces CoA and succinate, which are carbon source for *Sinorhizobium, Rhizobium, Bradyrhizobium* [25,26] and *Pseudomonas* [27]. For phthalate, it can produce 2 pyruvates and formate consuming 2 H_2O and 2 O_2, and generating CO_2. In fact, phthalate is very important because many pathways channel to it. For instance, the catabolism of fluoranthene in M. vanbaalenii PYR-1 is initiated by mono- and di-oxygenation reactions in four pathways routing to phthalate, which then goes to **β**-ketoadipate pathway to become intermediates in citric acid cycle [78]. For gentisate, it can go to pyruvate and fumarate consuming O_2 and H_2O. Table 1 summarizes the pathways from glucose to pyruvate and from typical PAHs to pyruvate. As can be seen in this table, the low-molecular-weight PAHs do not have too many reactions compared with glucose to be catabolized to pyruvate. However, PAHs do not generate ATP during their catabolism to pyruvate.

Table 1: Comparison of pathways from glucose to pyruvate and from typical PAHs to pyruvate (the pathways were obtained by combining of reactions from KEGG [63] together).

Pathways	Number of Reactions	Via
glucose + 2 NAD^+ + 2 ADP + 2 P_i == > 2 pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O	10	
naphthalene + 4 O_2 + 3 H_2O == > 2 pyruvate + acetaldehyde + formate + CO_2	11	salicylate catechol
naphthalene +4 O_2 +2 H_2O == > 2 pyruvate + fumarate	10	gentisate
2 fluorene + 2 ATP + 2 CoA + 2 FAD + 6 H_2O + 4 NADH + 10 O_2 == > 2 acetaldehyde + 2 acetyl-CoA + 2 AMP + 2 diphosphate + 2 $FADH_2$ + 2 formate + 3 CO_2 + 4 NAD^+ + 4 pyruvate	13	naphthalene salicylate catechol
2 fluorene + 2 H^+ + 2 NADH + 8 H_2O + 7 O_2 == > 4 e^- + 2 NAD^+ + 2 acetaldehyde + 2 CO_2 + 2 formate + 4 pyruvate	10	3-chromanone
fluorene + 3 H_2O + NADH + 5 O_2 == > H^+ + NAD^+ + CO_2 + formate + 2 pyruvate + 2-hydroxy-2,4-pentadienoate	13	phthalate
anthracene + H^+ + 3 H_2O + NADH + 4 O_2 == > acetaldehyde + CO_2 + formate + NAD^+ + 2 pyruvate	11	salicylate catechol
anthracene + 4 H_2O + NAD^+ + 5 O_2 == > CO_2 + formate + NADH + 3 pyruvate	14	phthalate
phenanthrene + 4 H_2O + 6 O_2 == > acetaldehyde + 2 CO_2 + formate + 3 pyruvate	16	catechol
phenanthrene + 6 H_2O + 2 NAD^+ + 5 O_2 == > CO_2 + formate + 2 H^+ + 2 NADH + 4 pyruvate	16	phthalate
pyrene + 6 H_2O + 2 NAD^+ + 7 O_2 == > 3 CO_2 + formate + 2 H^+ + 2 NADH + 4 pyruvate	20	phthalate

The metabolic network for 10 PAHs in *M. vanbaalenii* PYR-1 includes 183 metabolites and 224 chemical reactions [46]. Although typical PAHs can go through two major pathways to pyruvates, i.e. catechol and phthalate, it seems that phthalate may be preferable. For example, although phenanthrene can go through catechol pathway to pyruvate, i.e. a part of pathway of catabolim of naphthalene, bacteria using ortho-cleavage mechanism cannot grow up with naphthalene as sole carbon source [79,80]. Nevertheless, the generation of energy is not the only determinant factor to decide the PAH catabolic pathways. For example, the catabolism of fluoranthene in *M. vanbaalenii* PYR-1 can go C-1,2, C-7,8 and C-8,9 dioxygenation pathways but not in the C-2,3 dioxygenation pathway [81]. An important aspect is whether bacteria deliberate to channel PAHs towards more productivity and less toxicity, which was considered to be related to pleiotropic and epistatic functional responsibility in enzymes [81]. Furthermore, there are bacteria that can utilize PAH as sole carbon and energy source, such genera are *Cycloclasticus* [82,83], *Porticoccus hydrocarbonoclasticus* [84,85], *Neptunomonas* [86], *Algiphilus* [87,88].

These bacteria are marine bacteria, which draw much attention recently. However, the marine environments are quite different from the environments, where our familiar bacteria live. In fact, most of our current knowledge on carbon metabolism and PAH degradation comes from bacteria living in the land environments. Therefore, it is still difficult to apply our analysis to the marine bacteria with respect to each metabolic pathway. Anyway, these marine bacteria might have already evolved to the stage that they can solely use PAHs without utilization of sugar, which on the other side demonstrates the advantage of utilization of PAHs over other carbon sources. An important issue that is not clear to us is whether an enzymatic reaction can be clarified as endothermic or exothermic although we certainly know that these enzymatic reactions either absorbing of heat or releasing of heat. This is because it is often mentioned that an enzyme reduces the activation energy for a chemical reaction, but without indicating whether the reaction is endothermic or exothermic.

Conclusion

It is not clear whether each reaction in PAHs catabolism is endothermic or exothermic, thus we have no way to determine the advantage from viewpoint of heat consumption and generation. In this review, the analyses were conducted according to three comparison criteria, oxygen consumption, number of reactions to generate pyruvate and ATP consumption with reference to glycolysis of glucose. Our review demonstrates the advantages of utilization of PAHs over glucose under aerobic condition. We have yet to address the advantage of cometabolic biodegradation, which was shown as early as 70s in last century [89], and whether there is a possible advantage under anaerobic condition. However, it is unlikely to find such advantage of utilization of PAHs over glucose under anaerobic condition.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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