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Involvement of the TCA cycle in the anaerobic metabolism of polyphosphate accumulating organisms (PAOs)

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ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form
3 December 2008
Accepted 7 December 2008
Published online 24 December 2008

Keywords:
Accumulibacter
Polyphosphate accumulating organisms
Anaerobic
Glycogen
Tricarboxylic acid cycle
Metabolic models

ABSTRACT

For decades, glycolysis has been generally accepted to supply the reducing power for the anaerobic conversion of volatile fatty acids (VFAs) to polyhydroxyalkanoates (PHAs) by polyphosphate accumulating organisms (PAOs). However, the importance of the tricarboxylic acid (TCA) cycle has also been raised since 1980s. The aim of this study is to demonstrate the involvement of the TCA cycle in the anaerobic metabolism of PAOs. To achieve this goal, the glycogen pool of an activated sludge highly enriched in *Candidatus* Accumulibacter Phosphatis (hereafter referred to as *Accumulibacter*), a putative PAO was reduced substantially through starving the sludge under intermittent anaerobic and aerobic conditions. After the starvation, acetate added was still taken up anaerobically and stored as PHA, with negligible glycogen degradation. The metabolic models proposed by Pereira, Hesselmann and Yagci, which predict the formation of reducing power through glycolysis and the full or partial TCA cycle, were used to estimate the carbon fluxes. The results demonstrate that *Accumulibacter* can use both glycogen and acetate to generate reducing power anaerobically. The anaerobic production of reducing power from acetate is likely through the full TCA cycle. The proportion of TCA cycle involvement depends on the availability of degradable glycogen.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is the most economical and sustainable process for removing phosphorus from wastewater. EBPR is achieved by recycling polyphosphate accumulating organisms (PAOs) through alternating anaerobic and aerobic conditions. When the wastewater enters the anaerobic phase, PAOs accumulate carbon sources as polyhydroxyalkanoates (PHAs). The energy to store this polymer is obtained from the hydrolysis of energy rich internal phosphorus chain called polyphosphate (polyP), and also from breakdown of glycogen. Since polyP is broken down to orthophosphate for energy supply, the liquid phase phosphate concentration in the anaerobic phase increases. The anaerobic phase needs to be followed by an aerobic or anoxic phase. During this phase the stored PHA is consumed, generating energy for growth, for uptake of orthophosphate from the liquid phase and for replenishment of the glycogen pool.

When comparing the current EBPR metabolic models, the main difference is the source of reducing power required for PHA synthesis. Initially two hypotheses were proposed to explain the origin of these reducing equivalents. Comeau et al. (1986) proposed that TCA cycle was the source of the reducing power necessary to reduce acetyl-CoA to poly-beta-hydroxybutyrate (PHB) (Fig. 1A). On the other hand, Mino et al. (1987) concluded that the consumption of the intracellular carbohydrate, namely glycogen, supplied the reducing power

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doi:10.1016/j.watres.2008.12.008

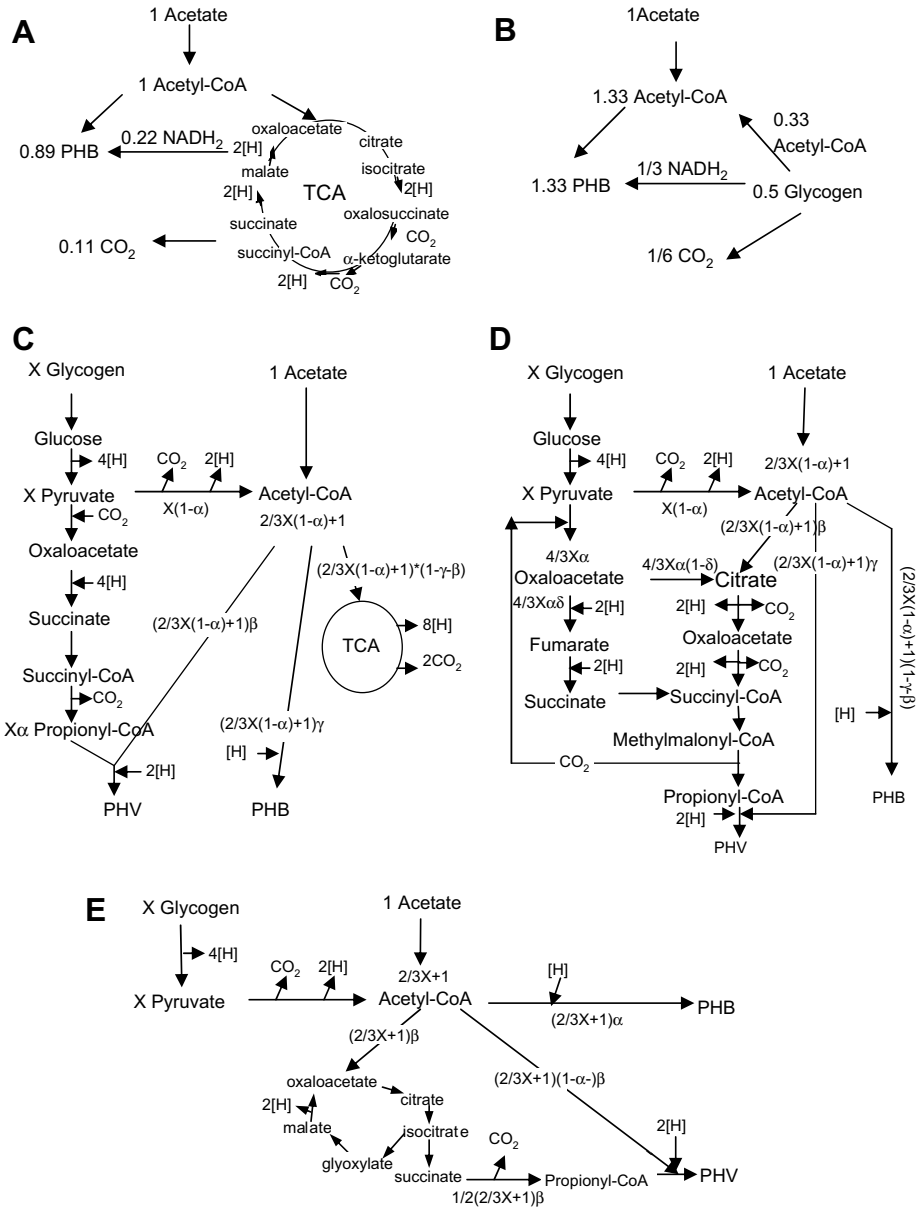

Fig. 1 – Schematic diagrams for the conversion of acetate and glycogen into PHA according to: Comeau et al. (1986) (A), Mino et al. (1987) (B) (adapted from Filipe and Daigger, 1998), Pereira et al. (1996) (C), Hesselmann et al. (2000) (D) with carbon fluxes worked out by Pijuan et al. (2008), and Yagci et al. (2003) (E) with carbon fluxes worked out in this study. In (C), X = glycogen degradation/VFA uptake (Cmol/Cmol); α = pyruvate fraction proceeding through propionyl-CoA; γ = acetyl-CoA fraction used for PHB production; β = acetyl-CoA fraction used for PHV production; In (D), X = glycogen degradation/VFA uptake (Cmol/Cmol); α = pyruvate fraction proceeding through oxaloacetate; β = acetyl-CoA converted to propionyl-CoA through the oxidative pathway of the TCA cycle and methylmalonyl-CoA pathway; γ = acetyl-CoA used for PHV production; δ = oxaloacetate converted to propionyl-CoA through the reductive pathway of the TCA cycle and methylmalonyl-CoA pathway; In (E), X = glycogen degradation/VFA uptake (Cmol/Cmol); α = acetyl-CoA fraction used for PHB production; β = acetyl-CoA fraction proceeding through glyoxylate pathway.

(Fig. 1B). For decades, glycolysis has been generally accepted to supply the reducing power for the anaerobic conversion of volatile fatty acids (VFAs) to PHAs by PAOs.

The involvement of glycogen in the EBPR process was later confirmed by Pereira et al. (1996) and Maurer et al. (1997) using nuclear magnetic resonance (NMR). However, Pereira et al. (1996) also suggested that TCA cycle was functional anaerobically since labelled CO_2, derived directly from labelled acetate, was detected during the anaerobic phase (Fig. 1C). This was in agreement with an observation made earlier by Bordacs and Chiesa (1989). One problem with this study was that the sludge used likely contained a substantial amount of glycogen accumulating organisms (GAOs), as indicated by the low phosphorus content (2–3%) of the sludge,