

# IMPACTS OF SYSTEM OPERATION AND THE BIOCHEMISTRY OF ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL ON THE OCCURRENCE OF ANAEROBIC STABILIZATION

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## Introduction

The existence of anaerobic stabilization of COD (AnS) resulting in 8.4% to 27.3% of unaccounted for COD (Table 1) has been demonstrated through experimental work (Randall *et al.*, 1984; Randall *et al.*, 1992; Wable and Randall, 1994). Evaluation of operational data from existing plants (Barker and Dold, 1995 and 1996) has also indicated the definite presence of anaerobic stabilization (10% to 25%) at plants that include anaerobic zones as part of their operation.

By exploring the biochemical reactions taking place in EBPR process, particularly the involvement of the storage mechanisms, namely PHA, poly-P and glycogen storage, the potential mechanisms of the anaerobic stabilization of COD in EBPR systems were explored. EBPR sludges cultivated in two separate UCT systems operated at 20°C and 5°C were used for examination of the anabolic and catabolic reactions taking place during the anaerobic and aerobic stages of EBPR

## Materials and Methods

One A/O configuration and two UCT configuration BNR lab-scale systems were operated at 20±1°C and 5±1°C. The mean cell residence time (MCRT) of 10 days was used throughout the study at 20°C, while the MCRT of the 5°C system was adjusted as needed during the acclimation period and then maintained at 20 days as explained in Erdal *et al.* (2003 b). Acetate was the sole VFA source in the synthetic feed. The plants were monitored for 1.5 years through measurement of soluble and total COD, MLSS, MLVSS, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, PO<sub>4</sub><sup>-3</sup>-P, *in-situ* OUR, PHB,

PHV, and glycogen on samples taken from the influent, each of the reactors, the recycle lines, and the system effluent. Mixed liquor samples collected for glycogen, PHB and PHV, and for enzyme assays were instantly frozen in liquid N<sub>2</sub> and processed and Erdal *et al.* (submitted for publication). To determine the extent of gaseous losses during an anaerobic-aerobic cycle, gas release (CO<sub>2</sub>, H<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S) was measured using an HP gas chromatograph equipped with Bondapak column.

### Results and Discussion

At both temperatures, glycogen metabolism was shown to be essential for EBPR. Enzyme assays performed on biomass samples indicated that the reducing equivalents for PHA synthesis were obtained through the EMP pathway, and the branched TCA pathway was operative during anaerobic metabolism at 20°C because an excess of NADH is produced during glycolysis. Low temperatures were shown to slow down glycogen metabolism significantly, thereby providing a competitive advantage to poly-P metabolism. The anaerobic COD stabilization, or the “COD loss” from the system can be calculated using Equation 1.

$$\% \text{CODBalance} = \frac{\text{CODoutput}}{\text{CODinput}} = \frac{M_{\text{TotalCOD, eff}} + M_{\text{TotalCOD, WAS}} - M_{\text{TotalCOD, oxid}}}{Q_{\text{inf}} \cdot C_{\text{TotalCOD inf}}} \quad (1)$$

At 20°C, the anaerobic stabilization in both systems varied, the UCT system showing higher AnS. The system maintained at cold temperature did not display any COD imbalance (Figure 1). These values were calculated from *in-situ* OUR and system performance data collected in a strictly similar fashion and at statistically independent time intervals during the 1.5 year operation of the three systems.

Gas release from the systems operated at 20°C and 5°C was non-detectable as measured during four batch tests performed on different days. Based on enzyme assay results and carbon balances, biochemical pathways for anaerobic and aerobic EBPR metabolism were constructed for the two EBPR populations cultivated at 20°C and 5°C. Detailed mass balance calculations performed on

electron equivalents (NADH/NAD<sup>+</sup> couples), available energy (ATP/ADP), carbon and oxygen performed on EBPR systems based on the pathways elucidated as part of this study (Figures 2 and 3) revealed that EBPR populations balance excess NADH generated via EMP pathway by using branched TCA cycle under anaerobic conditions, and tend to conserve carbons under aerobic conditions by selecting glyoxylate shunt at warmer temperatures. Slowed EMP pathway reactions do not generate excess NADH at cold temperatures, and branched TCA is therefore replaced by glyoxylate shunt under anaerobic conditions. Through these balancing and internal storage reactions (taking place differently under different environmental conditions) it was revealed that COD was not actually lost from the systems as initially proposed, yet stored and utilized more efficiently without resulting in oxygen consumption (i.e. not measured as part of OUR) as theoretical calculations would dictate. These findings are significant in providing answers to the long going debate over anaerobic COD stabilization(AnS), and are expected to impact the mathematical modeling of the EBPR systems, and minimizing the over-sizing of the aeration systems and associated unnecessary financial burden for utilities and POTW owners.

#### **References:**

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Table 1. COD/oxygen utilization mass balance results for Conventional AS and EBPR systems (Randall *et al.*, 1992)

Phase	System	TOR <sub>predicted</sub> , g/day	TOR <sub>actual</sub> , g/day	AnS/COD <sub>s</sub> , %
IA	Conventional	23.9	25.0	18.3
	EBPR	20.2	16.5	
IB	Conventional	31.9	27.4	8.4
	EBPR	19.9	18.2	
IC	Conventional	43.3	42.4	10.0
	EBPR	36.2	32.6	
IIA	Conventional	26.1	25.6	11.6
	EBPR	22.0	19.4	
IIB	Conventional	34.9	34.1	24.6
	EBPR	28.7	21.7	
IIC	Conventional	33.8	34.2	27.3
	EBPR	29.5	21.4	

TOR : Total Oxygen Requirement  
CODs : Stabilized COD

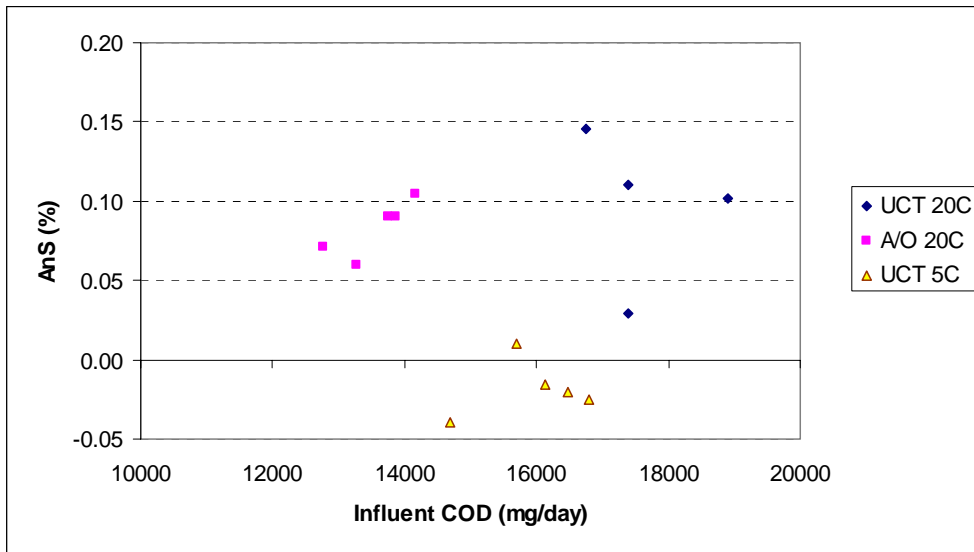


Figure 1. Anaerobic Stabilization Calculated for Three Different EBPR Operational Conditions

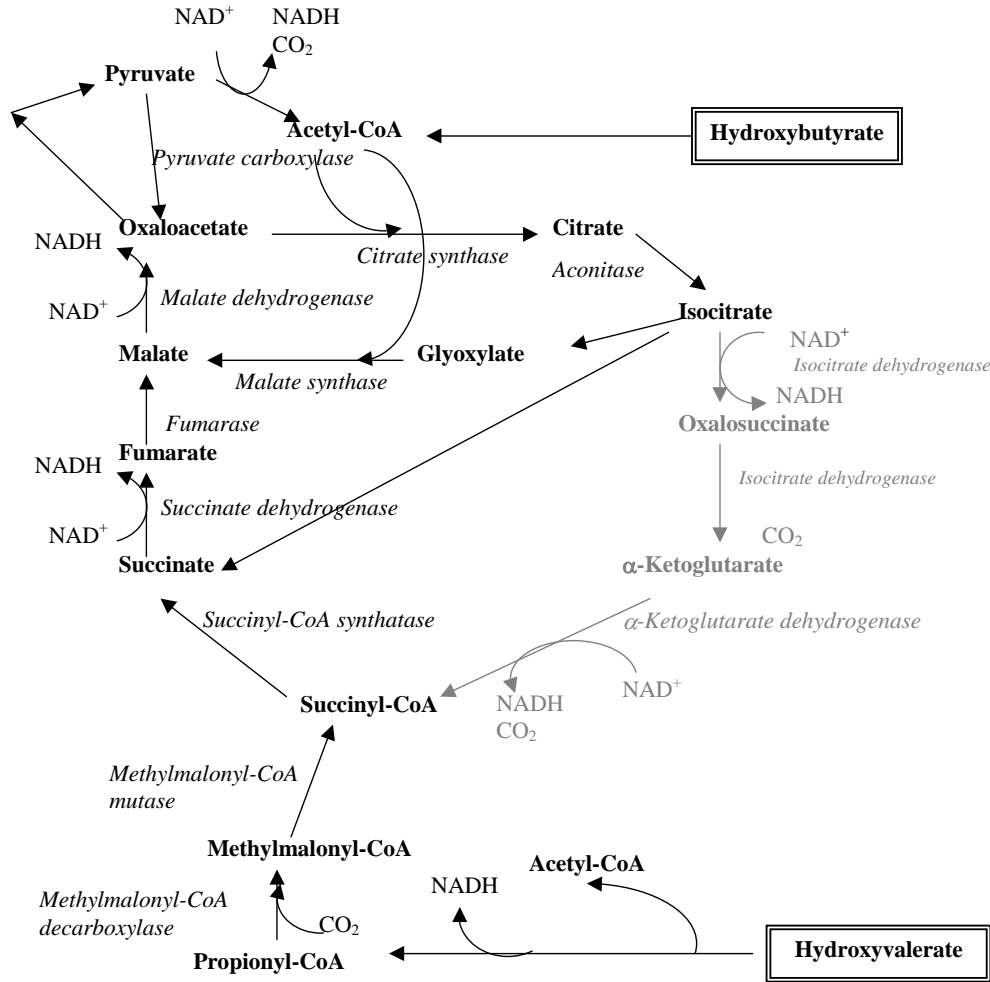


Figure 2. Aerobic metabolism of EBPR at 20°C leading to conservation of carbons for internal storage.

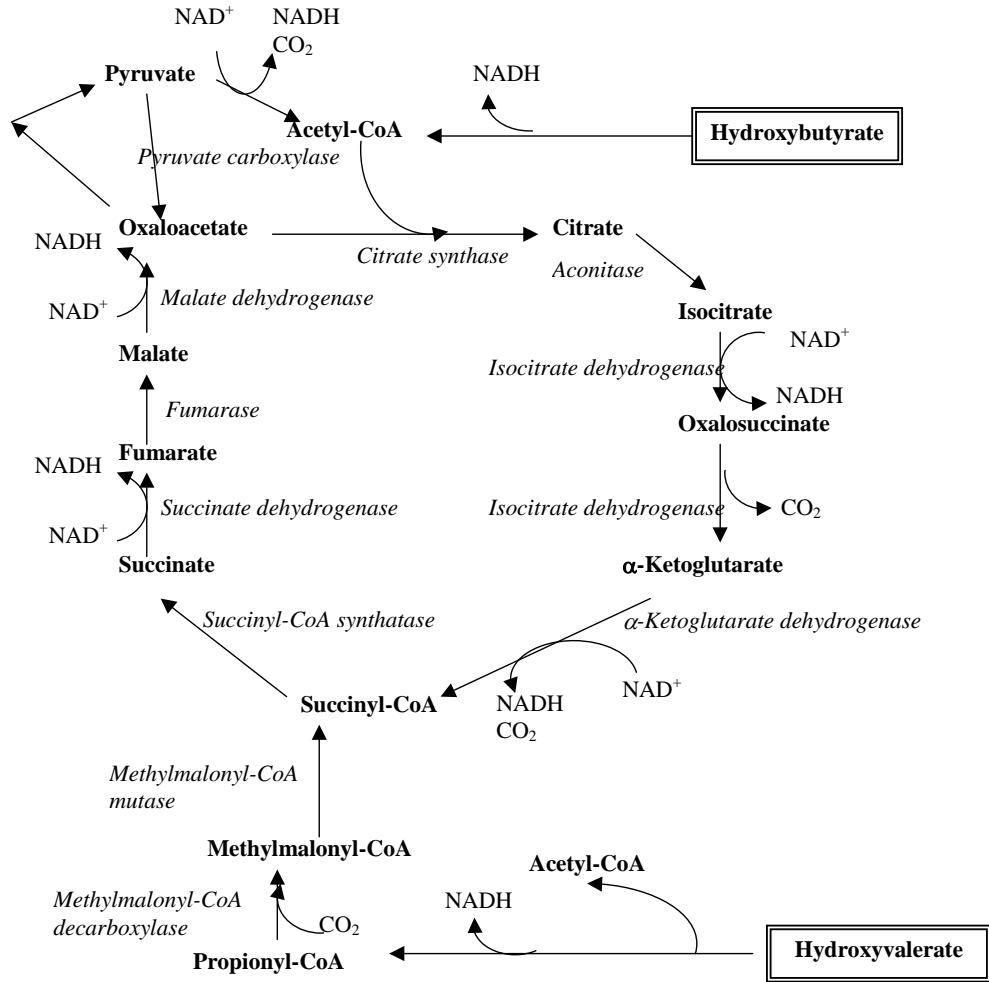


Figure 3. Aerobic metabolism of EBPR at 5°C leading to release of carbons and decreased glycogen storage.