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Functional State Metabolism in *E. coli* Fed-batch Cultivation Processes

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Abstract: *This paper presents an overview of a biochemical correspondence to defined functional state based on specific metabolic mechanisms. For E. coli fed-batch cultivation processes a set of functional states is considered. As a result through proposed functional states the changes in metabolic pathways can be described accurately.*

Keywords: *Functional states, Metabolism, Glycolysis, Escherichia coli.*

Introduction

Various approaches have been employed to describe nonlinear behavior of bioprocesses [8]. Some of them are directly based on the divide-and-conquer strategy [1, 5]. These, so-called local approaches can often give a simplified and transparent nonlinear model or control representation. In addition, the local approaches have computational advantages, lend itself to adaptation and learning algorithms and allow direct incorporation of high-level and qualitative plant knowledge into the model. These advantages have proven to be very appealing for industrial and practical applications. Intuitively appealing nature of the framework is demonstrated by Murray-Smith and Johansen [5] with applications of local methods, and in particular – functional state modelling approach [10], to problems in the process industries, biomedical applications and autonomous systems.

This paper illustrates further the concept of functional state modelling approach in connection with the *Escherichia coli* fed-batch cultivation processes. The *E. coli* growth process is divided into several functional states according Halme investigations [9, 10]. In each functional state the bacteria metabolism is dominated by certain metabolic pathways.

Description of functional state metabolism

Based on known analogies between metabolic mechanisms of yeast and *E. coli* the whole bacteria growth process can be divided into five functional states in batch and fed-batch cultures [6]:

- *First acetate production state (FS I)*
- *Mixed oxidative state (FS II)*
- *Complete sugar oxidative state (FS III)*
- *Acetate consumption state (FS IV)*

- *Second acetate production state (FS V)*

The common part of each functional state is glucolysis. The glucose catabolism provides energy for the cells and intermediate metabolites. The pathway of glycolysis can be seen as consisting of two separate phases. The first is the chemical priming phase requiring energy in the form of adenosine triphosphate (ATP), and the second is considered the energy-yielding phase. In the first phase, two equivalents of ATP are used to convert glucose to fructose-1,6-bisphosphate (F-1,6-BP). In the second phase F-1,6-BP is degraded to pyruvate, with the production of four equivalents of ATP and two equivalents of nicotinamide adenine dinucleotide (NADH). The glycolysis pathway is presented in Fig. 1 [4].

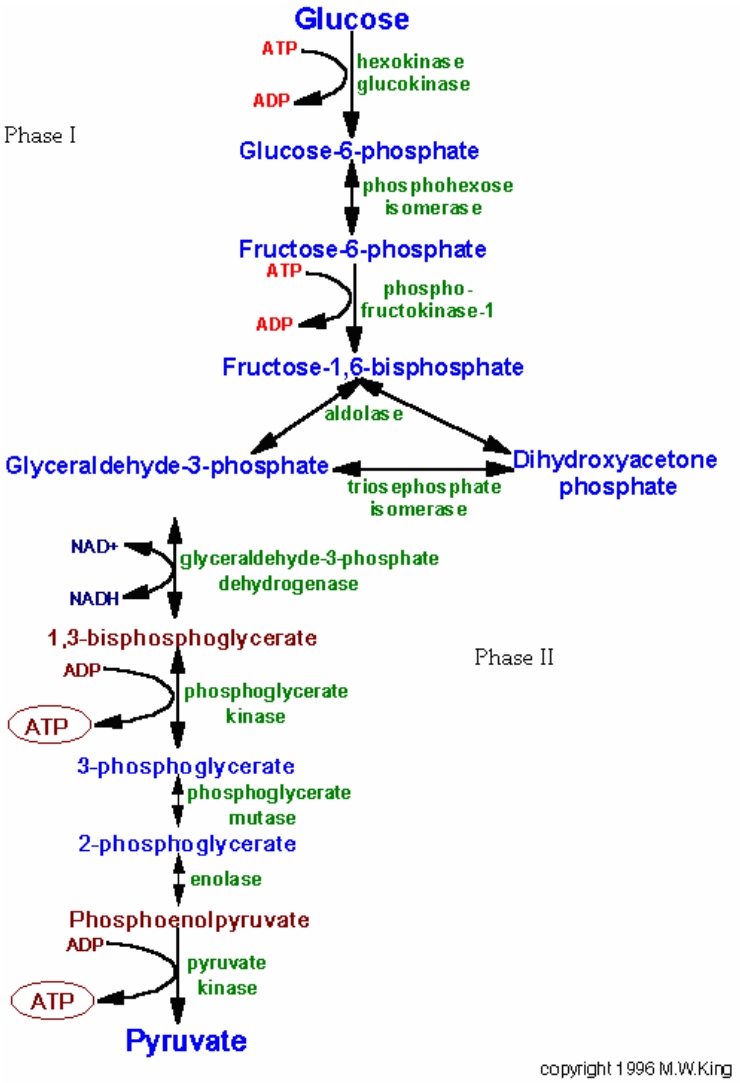

Fig. 1 Glycolysis pathway

Each functional state is characterized with specific biochemical processes depending on current cultivation condition. Considering an *E. coli* fed-batch cultivation process these particularities are described below.

First acetate production state (FS I)

The *E. coli* growth process is defined to be in this state when the glucose concentration (S) is above the critical level (S_{crit}), there is sufficient dissolved oxygen (pO_2) and acetate (A) is produced. This acetate excretion is the result of a metabolic "overflow" mechanism occurring when the carbon (glucose) flux into the central metabolic pathways exceeds the biosynthetic demands and the energy generation capacity of the cell [2]. This by-product induces a stress response even at extremely low concentrations, hinders growth, and reduces the production of recombinant proteins. Overflow metabolism has been attributed to an enzymatic limitation in the tricarboxylic acid (TCA) cycle. In *E. coli* the complete oxidation of 1 mol of glucose in glycolysis and the TCA cycle generates 10 mol of NAD(P)H and 2 mol of flavin adenine dinucleotide ($FADH_2$) [7]:

$$\text{Glucose} + 8NAD^+ + 2NADP^+ + 2FAD + 4ADP + 4P_i \rightarrow$$
$$\rightarrow 6CO_2 + 8NADH + 2NADPH + 2FADH_2 + 4ATP + 10H^+$$

NAD^+ is a very important cofactor found in cells. NADH is the reduced form of NAD^+, and NAD^+ is the oxidized form of NADH. The chemical structure of NAD^+ is presented in Fig. 2.

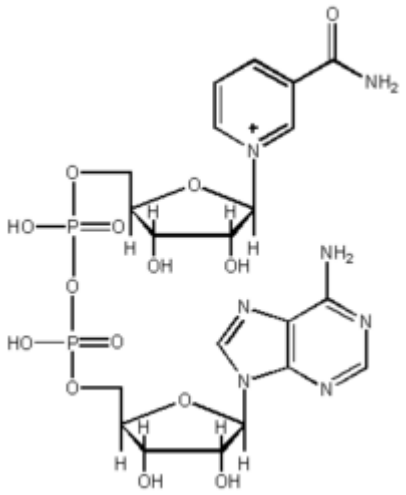

Fig. 2 Chemical structure of NAD^+

It transports electrons in redox reactions. During this process NAD picks up a pair of electrons and a proton and is thus reduced to NADH, releasing one proton (H^+).

$$MH_2 + NAD^+ \rightarrow NADH + H^+ + M: + \text{energy},$$

where M is a metabolite.

Two hydrogen atoms (a hydride ion and a proton H^+) are removed from the metabolite. The proton is released into solution. From the hydride electron pair, one electron is transferred to the positively-charged nitrogen, and the other one attaches to the carbon atom opposite to the nitrogen (Fig. 3).

The reducing potential stored in NADH can be converted to ATP through the aerobic electro transport chain or used for anaerobic metabolism. In *E. coli* electro transport chain is localized in the mesosome. ATP is the universal energy currency of cells, and the contribution of NADH to the synthesis of ATP under aerobic conditions is substantial. However, under

certain conditions (e.g. hypoxia) the aerobic regeneration of oxidized NAD^+ is unable to meet the cell's immediate demand for ATP. In contrast, glycolysis does not require oxygen, but it does require the anaerobic regeneration of NAD^+, thus fermentation is the oxidation of NADH to NAD^+ in the absence of oxygen.

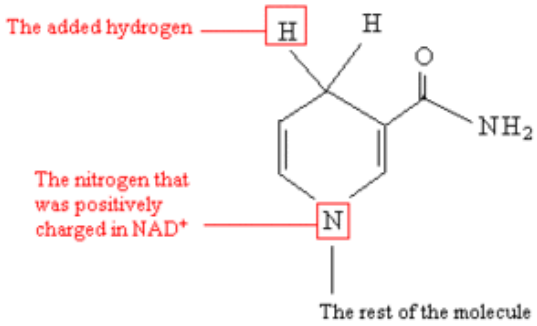

Fig. 3 NAD^+ reduction

If the rate of dissolved oxygen utilization is sufficiently high, the reduced cofactors generated by glucose consumption are reoxidized in the electron transport chain, which serves the dual purpose of maintaining an optimal redox environment and generating energy by oxidative phosphorylation. If the rate of glucose consumption is greater than the capacity to reoxidize the reduced equivalents metabolic intermediates accumulate to maintain the redox balance [7].

Mixed oxidative state (FS II)

The process enters this state when the sugar concentration decreases to be equal to or below the critical level and there is sufficient dissolved oxygen. Both sugar and produced acetate are cometabolised through the oxidative pathways in the state. In this case the glucose level is lower which leads to lower consumption rate. And thus the bacteria *E. coli* are able to reoxidize the reduced metabolic intermediates that were accumulated during the first functional state. In this functional state accumulated acetate is also metabolized. This is achieved with the help of the TCA cycle. This metabolic cycle is shown in the Fig. 4 [2].

Complete sugar oxidative state (FS III)

The process is defined to be in this state when there is no acetate available, the sugar concentration is not higher than the critical level and the dissolved oxygen is above its critical level (pO_{2crit}). In this state, sugar is completely oxidized to water and carbon dioxide. Continuing from second functional state we assume that acetate is depleted faster by the bacteria or removed from the media and the only carbon source left in the media is glucose. But the concentration of glucose is not higher than the critical level. There is sufficient quantity of dissolved oxygen in the media which helps with the reoxidizing of the metabolic intermediates in the cell (NADH and FADH). This means that there will be no acetate formation and the pyruvate will enter directly the TCA cycle and will be completely oxidized to water, carbon dioxide and several molecules of NADH and FADH.

Acetate consumption state (FS IV)

The process is defined to be in this state when acetate is available but no sugar is in the broth, and the dissolved oxygen concentration is above the critical level. Acetate is the only carbon source for *E. coli* growth. This state can start from functional state one when the glucose in the media is depleted and the only carbon state for the *E. coli* growth is acetate. In this functional state dissolved oxygen level is also above the critical level which again gives a

chance for reoxidizing of metabolic intermediates. Since the acetate is the only carbon source for the bacteria growth it is introduced in the TCA cycle and completely metabolized to water and carbon dioxide. Before that the acetate is converted back to acetyl-CoA [3] (Fig. 5).

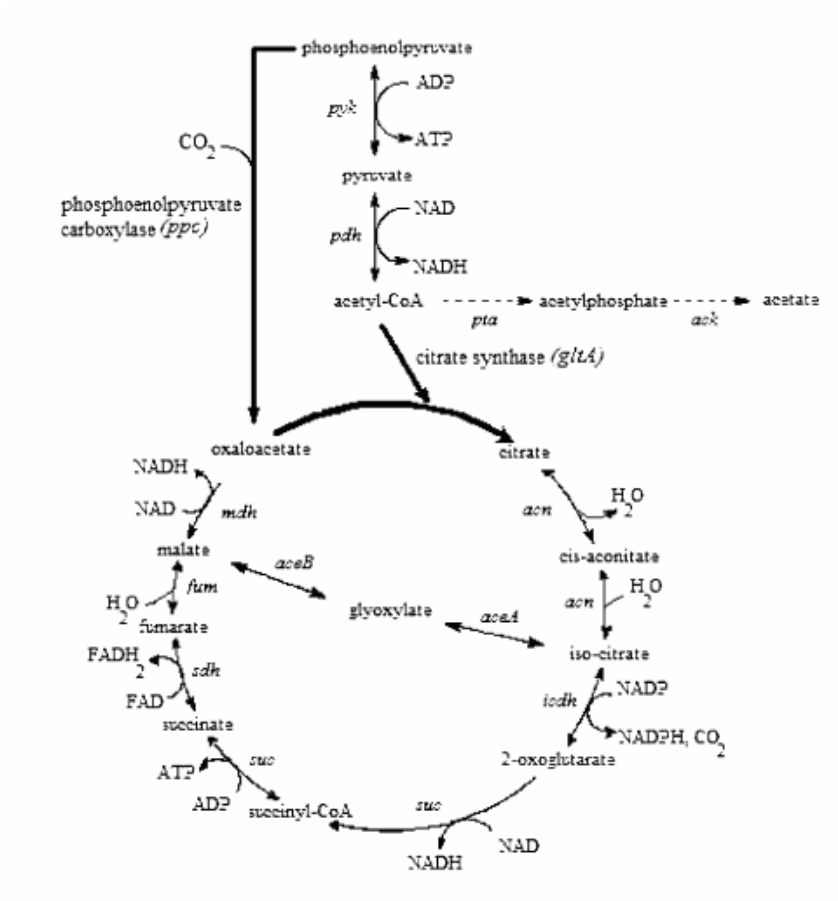

Fig. 4 TCA cycle

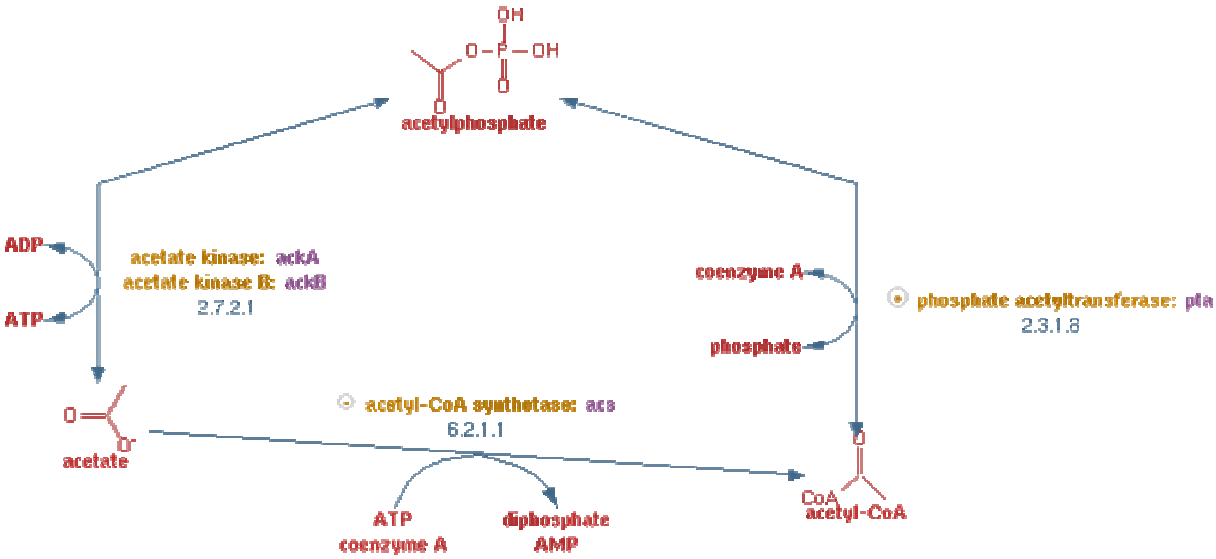

Fig. 5 Acetate utilization

Second acetate production state (FS V)

The conditions for this state are both sugar and dissolved oxygen concentrations to be below the corresponding critical levels. When the dissolved oxygen becomes the limiting factor for *E. coli* growth, acetate is produced. This happens because the level of dissolved oxygen is too low and metabolic intermediates can not be reoxidized. This means that the process of glucose metabolism will stop at the stage of acetyl-coA formation by the pyruvate dehidrogenase complex. And from there the acetyl-coA will be oxidized to acetate which will be stored or excreted from the cell. This acetate will be used after that for consumption by the cell if dissolved oxygen concentration becomes above the critical minimum.

An *E. coli* growth process switches from one functional state to another when the metabolic conditions are changed. The functional state diagram of the process can be illustrated as it is shown in Fig. 6.

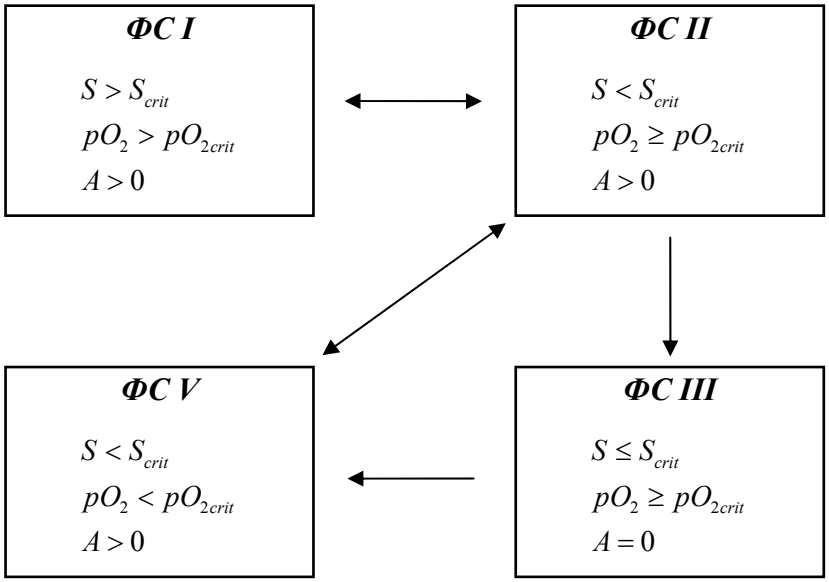

Fig. 6 Interrelationships and rules for recognition of functional states

Fig. 6 illustrates the interrelationships of the different functional states during fed-batch *E. coli* cultivation. These interrelationships will be used as rules for recognition of functional states during cultivation process. The *FS IV* is not considered here because that functional state appears only in batch cultivation processes.

In principle *FS I* can appear in all types *E. coli* growth processes (batch, fed-batch and continuous cultivations). With the decreasing level of the glucose concentration the process switches from *FS I* to *FS II.* At this point the process can be easily switched back to *FS I* with the addition of glucose or it can continue on different pathway. If the level of glucose is kept close to the critical point and the level of acetate is low, the acetate will be depleted fast. This will set the process to *FS III.* From there the system can switch to *FS V* where the level of dissolved oxygen and glucose are below their critical points. Starting directly from *FS II* reducing the levels of dissolved oxygen and glucose the process can switch to *FS V* and vice versa using the same pathway. It should be noted that the cultivation process could be only in one functional state at given time. However, a certain functional state can appear in the process more than once during one run. Depending on the goals of cultivation process only some of functional states can appear.

Conclusion

In this paper a set of functional states in *E. coli* fed-batch cultivation processes is considered. A biochemical correspondence to defined functional state is presented based on specific metabolic mechanisms. The changes in metabolic pathways can be accurately described through proposed functional states.

The mechanisms of biochemical processes in each functional state of *E. coli* fed-batch cultivations can be described by mathematical models. Using such local mathematical models is achieved detailed description of process dynamics. Moreover this approach leads to the simplifying of the process modelling. The next step of this work will be development of mathematical models corresponding to definite functional state and to the process behavior.

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