Biotechnology Advances 31 (2013) 257–265

Contents lists available at SciVerse ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Redox potential control and applications in microaerobic and anaerobic fermentations

Chen-Guang Liu [a], Chuang Xue [a], Yen-Han Lin [c,*], Feng-Wu Bai [a,b,**]

[a] School of Life Sciences and Biotechnology, Dalian University of Technology, Dalian 116023, China
[b] School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[c] Department of Chemical Engineering, University of Saskatchewan, Saskatoon, Canada SK S7N 5A9

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form 12 November 2012
Accepted 15 November 2012
Available online 22 November 2012

Keywords:
Redox potential
Metabolism
Electron transfer
Product profiles
Ethanol fermentation
Butanol production

ABSTRACT

Many fermentation products are produced under microaerobic or anaerobic conditions, in which oxygen is undetectable by dissolved oxygen probe, presenting a challenge for process monitoring and control. Extracellular redox potentials that can be detected conveniently affect intracellular redox homeostasis and metabolism, and consequently control profiles of fermentation products, which provide an alternative for monitoring and control of these fermentation processes. This article reviews updated progress in the impact of redox potentials on gene expression, protein biosynthesis and metabolism as well as redox potential control strategies for more efficient production of fermentation products, taking ethanol fermentation by the yeast *Saccharomyces* under microaerobic conditions and butanol production by the bacterium *Clostridium* under anaerobic conditions as examples.

Contents

* Corresponding author. Tel.: +1 306 966 4764; fax: +1 306 966 4777.
** Correspondence to: F.-W. Bai, School of Life Sciences and Biotechnology, Dalian University of Technology, Dalian 116023, China. Tel.: +86 411 8470 6308; fax: +86 411 8470 6329.
E-mail addresses: yenhan.lin@usask.ca (Y.-H. Lin), fwbai@dlut.edu.cn (F.-W. Bai).

0734-9750/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biotechadv.2012.11.005

1. Introduction

Fermentations have a long history since human ancestor started to produce flavor food and beverages such as pickle, yogurt, rice wine and so on. Many of these fermentations are anaerobic or microaerobic for desirable flavors, and in the meantime for contamination control since most unwanted microbes propagate quickly under aerobic conditions. More recently, biofuels such as ethanol and butanol produced under microaerobic and anaerobic fermentation conditions respectively have garnered worldwide attention to address concerns on the sustainable supply of crude oil and the impact of over-consumption of petroleum-based products, particularly transportation fuels, on ecological environment.

Unlike aerobic cultures and fermentations in which dissolved oxygen can be monitored online, a big challenge for microaerobic and anaerobic fermentations is lacking of real-time process monitoring technologies, since pH detection that is commonly employed with these fermentation processes reflects proton activity only, and thus is not sensitive to delicate changes of intracellular metabolism. Redox potential, known as oxidation–reduction or oxidoreduction potential (ORP), reflects overall electron transfer and redox balance involved in intracellular metabolism, whose significance in biological systems was predicted nearly one century ago by two prestigious British scientists at the University of Cambridge (Needham and Needham, 1926). Although ORP theory and measurement have been well established with relatively simple chemical reaction systems, investigation on ORP's impact on intracellular metabolism, the core of complex biological systems, and applications of ORP control in bioprocess engineering are still very limited.

Currently, advanced technologies such as nanosensors that can penetrate into individual cells have been developed to measure intracellular ORP directly for deep understanding on intracellular redox balance and impact on cell physiology and metabolism (Auchinvole et al., 2012), which provide basis for engineering strains with preferable redox pathways for more efficient production of desirable fermentation products. On the other hand, extracellular ORP that can be detected conveniently in fermentation broth by an ORP electrode synchronously reflects intracellular ORP status (de Graef et al., 1999). Therefore, as illustrated in Fig. 1, ORP control strategies can be developed and applied for altering intracellular ORP conditions and metabolic profiles.

In this article, update progress in the impact of ORP on intracellular metabolism is reviewed, and strategies for ORP control are discussed at molecular levels and process engineering aspects as well for more efficient production of fermentation products, taking ethanol fermentation

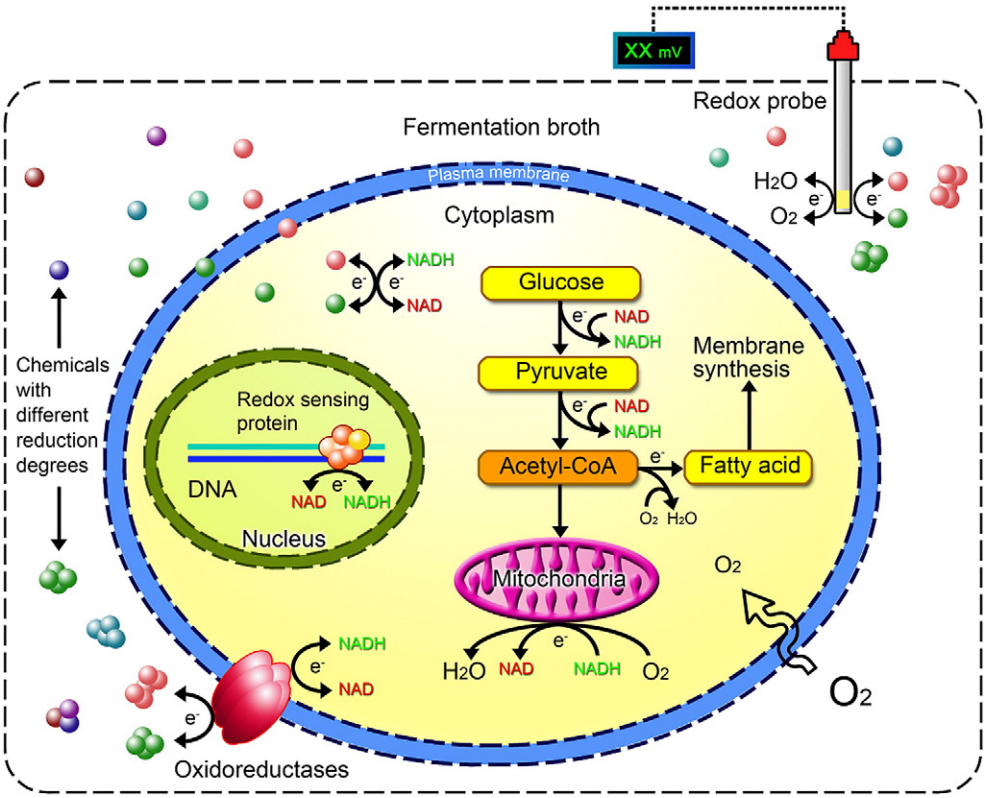

Fig. 1. Interactions between environmental redox potential (ORP) and intracellular metabolism characterized by electron transfer and redox balance. The ORP of fermentation broth is determined by chemicals with different reduction degrees such as dissolved oxygen. Intracellular ORP, dominated by the ratio of NAD(P)H/NAD(P)$^+$, can be affected by metabolism and extracellular ORP level. Some chemicals such as dissolved oxygen can penetrate through the membrane to react with reduced or oxidized metabolites directly, while others that cannot penetrate into cytoplasm affect intracellular metabolism through oxidoreductases located within the membrane (Baker and Lawen, 2000). Gene expression is also affected by ORP through redox sensitive proteins (Pei et al. 2011; Wietzke and Bahl, 2012), which consequently control enzyme synthesis to adjust the intracellular redox balance and metabolism.

by the yeast *Saccharomyces* under microaerobic conditions and butanol production by the bacterium *Clostridium* under anaerobic conditions as examples.

2. Impact of ORP on metabolism

2.1. ORP principles

ORP is the tendency for a compound to acquire electrons. Thus, two compounds are required for an oxidation–reduction reaction to occur, during which the reducing compound donates electrons to the oxidizing compound. Each redox pair has its intrinsic ORP value. The greater is the affinity for electrons, the higher the ORP of a redox pair would be. Table 1 summarizes standard ORP values of major redox pairs involved in intracellular metabolism. Unlike a half galvanic cell reaction, oxidation–reduction reactions occur simultaneously within cells, no matter cultures are aerobic such as antibiotic fermentation, microaerobic or anaerobic for ethanol or butanol production, making ORP detected in extracellular environment an indicator of the net outcome of intracellular metabolism dominated by electron transfer and redox balance.

Compared to pH value that provides information of protons, ORP represents activities of electrons. Therefore, ORP is more sensitive to delicate changes of intracellular metabolism. An ORP electrode system including a sensor composed of inert metal such as platinum, rhodium, iridium or gold and a reference electrode, usually Ag/AgCl, can be used to monitor real-time redox information of fermentation systems with negligible impact on intracellular metabolism.

2.2. Environmental factors affecting ORP

In general, temperature and the ratio of oxidizing to reducing compounds play key roles to modulate ORP in solution. When organisms are involved in cultures or fermentations, more environmental factors such as dissolved oxygen and anaerobic conditions should be taken into account (Kjaergaard, 1977). For microaerobic fermentations such as ethanol production by yeast, dissolved oxygen probe is unable to detect trace oxygen dissolved in fermentation broth. However, as illustrated in Table 1, the standard ORP for O_2/H_2O is the highest among all redox pairs related to intracellular metabolism. Therefore, if electrons are transferred, oxygen must be a prior acceptor, affecting ORP more effectively, even though dissolved oxygen level is much lower than that for other metabolites (Pham et al., 2008). As for anaerobic fermentations, effect of chemicals including nutritional substrates supplemented with medium and metabolites released into fermentation broth by cells becomes significant due to the absence of dissolved oxygen and different degree of reduction presented by these chemicals. (Chen et al., 2012; Nakashimada et al., 2002).

Table 1
Standard ORPs of common redox pairs involved in intracellular metabolism.

Redox pairs	E^0 (mV)	Reference
H^+/H	−420	Murray et al. (2003)
NAD^+/NADH	−320	
$NADP^+$/NADPH	−315	Rodkey and Donovan (1959)
Lipoate; ox/red	−290	Murray et al. (2003)
Acetoacetate/3-hydroxybutyrate	−270	
GSSG/2GSH	−240	Schafer and Buettner (2001)
Pyruvate/lactate	−190	Murray et al. (2003)
Oxaloacetate/malate	−170	
Fumarate/succinate	+30	
Cytochrome b; Fe^{3+}/Fe^{2+}	+80	
Ubiquinone;ox/red	+100	
Cytochrome c1; Fe^{3+}/Fe^{2+}	+220	
Cytochrome a; Fe^{3+}/Fe^{2+}	+290	
Oxygen/water	+820	

2.3. Intracellular ORP homeostasis

Conjugated redox pairs and their homeostasis are fundamentals for intracellular metabolism, since many biological functions of cells are affected by ORP levels through gene expression (Murray et al., 2011; Vemuri et al., 2006) and enzyme synthesis, which consequently affect signal sensing and transduction, and ultimately metabolic profiles (Mason et al., 2006; Riondet et al., 2000), particularly under stress conditions associated with industrial production (Kültz, 2005).

Since techniques to detect intracellular ORP are limited, redox pairs in cytoplasm are commonly used to characterize ORP status. Among various redox pairs, oxidized nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are two major cofactors, which accept electrons from other molecules and become reduced, forming NADH and NADPH, respectively, making the redox pairs NADH/NAD^+ and NADPH/$NADP^+$ overwhelmingly dominant with intracellular metabolism. For example, the bioinformatics database KEGG had included 855 and 1064 redox reactions catalyzed by 106 and 88 enzymes with NAD^+ and $NADP^+$ as cofactors by October 2012. Therefore, intracellular ORP is primarily determined by the ratio of NADH/NAD^+ and to a less extent by the ratio of NADPH/$NADP^+$.

NADH is generated from NAD^+ predominately through catabolism of reducing substrates such as glucose to provide reducing power for various redox reactions, and consequently NAD^+ is regenerated for redox balance. As mentioned, dissolved oxygen is used as the final electron accepter under aerobic and microaerobic culture conditions, but electrons are accepted by intermediate metabolites with desirable fermentation products produced when oxygen is not available under anaerobic conditions. In contrast, NADPH is more important in anabolism as many metabolic pathways involved in the biosynthesis of building blocks such as amino acids, lipids and nucleotides use NADPH as a reducing agent, which is produced most commonly through the pentose phosphate pathway. In addition, interconversion between NADH and NADPH also occurs, such as the ATP-driven conversion of NADH to NADPH mediated by NADH kinases in *S. cerevisiae* (Shi et al., 2005), and the transhydrogen reaction catalyzed by either membrane-bound or souble transhydrogenases in *Escherichia coli* (Sauer et al., 2004).

Redistribution of metabolic flux and improvement of fermentation product yield have thus been investigated by altering intracellular NADH levels. For example, engineering *E. coli* for overexpression of the NAD^+-dependent enzyme formate dehydrogenase to increase NADH availability enhanced ethanol production under anaerobic chemostat culture conditions (Berríos-Rivera et al., 2002), while increasing NADH oxidation in *S. cerevisiae* to reduce NADH levels decreased glycerol production due to alleviation of overflow metabolism (Vemuri et al., 2007). This progress provides insightful understanding on developing ORP control strategies at molecular and process engineering levels as well for more efficient production of desirable fermentation products.

3. ORP control strategies

ORP is determined by a ratio of oxidative to reductive substances within fermentation systems. Therefore, strategies implemented to regulate ORP mainly focus on how to change the ratio, and different strategies including strain modification through metabolic engineering and process engineering have been explored. Table 2 summarizes some metabolites produced under ORP-control conditions.

3.1. Strain modification

Theoretically, ORP can be controlled at molecular levels by altering the expression of genes encoding key enzymes for electron transfer and redox reactions in vivo. Such a strategy has been explored mainly with the model species: *E. coli* and *S. cerevisiae*. Liu et al. (2011d) deleted

Table 2
Examples for enhanced production of metabolites under ORP controlled conditions.

Product	Strain	ORP control strategy*	Result	Reference
Succinic acid	*Corynebacterium crenatum*	3: Substrates 3: $K_3Fe(CN)_6$; Na_2S	More reduced environment benefited succinic acid production.	Chen et al. (2012)
	Actinobacillus succinogenes	2: Electrochemical bioreactor	Succinate production was increased by 20%.	Park and Zeikus (1999)
		3: Glucose Sorbitol Gluconate	The best result was achieved when using highly reduced sorbitol.	Li et al. (2010a)
		3: $K_3Fe(CN)_6$; DTT	The highest succinic acid yield and productivity were observed at −350 mV.	Li et al. (2010b)
	Clostridium thermosuccinogenes	3: Na_2S 4: CO_2; N_2	The succinate yield was the highest and by-products were the least at −275 mV.	Sridhar and Eiteman (1999)
1,3-propanediol	*Klebsiella pneumoniae*	4: Different aeration	Redox potential at −190 mV was preferable.	Du et al. (2006)
		4: N_2 and/or air 3: $NaBH_4$ and NaOH	A mutant is capable of producing more 1,3-propanediol under −280 mV.	Du et al. (2007)
Xylitol	*Candida tropicalis*	4: Agitation	Optimal redox potential level for xylitol production was −100 mV and the shift range for ethanol to xylitol is −50 to −100 mV.	Kastner et al. (2003)
	Candida shehatae Pichia stipitis	4: Agitation	300 mV was a better redox potential level for high xylitol yield.	du Preez et al. (1988)
Hydrogen	*Enterobacter aerogenes*	3: Addition of NADH	Decrease of overall hydrogen. Comparatively, the addition of NAD^+ provided an opposite effect as noted-above.	Zhang et al. (2009)
		3: Different substrates	At low redox potential (such as −540 mV), it favors high H_2 evolution capability.	Nakashimada et al. (2002)
Lactic acid	*E. coli*	1: Knocking out *pflB*	The reduced condition was regarded as effective to improve lactate production and to inhibit formation of other organic acids.	Liu et al. (2011d)
Ethanol	*Z. mobilis S. cerevisiae*	2: Electrochemical bioreactor	*Z. mobilis* was demonstrated to favor reducing circumstance, and *S. cerevisiae* produced more ethanol in high redox potential.	Jeon and Park (2010)
	Saccharomyces cerevisiae	4: Air	The ORP of −150 mV was considered as the best value for VHG ethanol fermentation to achieve faster productivity and higher yield.	Lin et al. (2010)
Butanol	*Clostridium acetobutylicum*	3: Dithiothreitol, cysteine, Na_2S 4: Air	Redox potential control at −290 mV achieved an earlier solventogenesis, and thus solvent production increased by 35% than uncontrolled process.	Wang et al. (2012)
	Clostridium tyrobutyricum E. coli	1: Knocking out *ack* and overexpressing *adhE2*	Butanol titer reached the highest 10 g/L and butanol yield was 27% when using glucose as substrate.	Yu et al. (2011)
		1: Creating NADH and acetyl-CoA driving force	Butanol titer achieved 15 g/L or 30 g/L with *in situ* product recovery	Shen et al. (2011)

* Please refer to Section 3: 1 for strain modification; 2 for energy input; 3 for reagent supplementation; 4 for sparging gases.

pflB and *adhE* in *E. coli* to increase NADH levels, leading to lactate accumulation. Berríos-Rivera et al. (2002) modified *E. coli* to overexpress formate dehydrogenase to increase the ratio of NADH/NAD^+ to lower ORP levels. Singh et al. (2009) tested a genetically modified *E. coli* strain with the genes encoding pyruvate-formate lyase and lactate dehydrogenase deleted, leading to ORP imbalance. Hou et al. (2010) controlled the similar genes of the native NAD^+-dependent formate dehydrogenase in the cytosol or mitochondria of *S. cerevisiae* whose formate metabolism is in vain, which regulated NADH in different parts of the cells through dehydrogenizing formate. Although targeted to one or few key enzymes in whole metabolic net, intracellular ORP control may result in unexpected effect, which limits its applications.

3.2. Process engineering

Although developing robust strains with preferable redox pathways through metabolic engineering tools is an ultimate solution for more efficient production of various fermentation products, the progress is slow and time-consuming, and the reported successful story is very limited due to the intrinsic complexity of biological systems. In contrast, process engineering can bring immediate effect on techno-economic performance of industrial production.

3.2.1. Energy input

Bioelectrical reactors (BERs) equipped with electrodes can control ORP of fermentation systems directly through electron flow supplied by energy input in the form of electric current. The efficacy of BERs depends on interactions between microorganism(s) and the surface of working electrode, but underlying mechanism remains to be elucidated (Fleming, 2010; Thrash and Coates, 2008). Therefore, potentials at the working electrode and abiotic reactions occurring at the electrode surface are two key factors. ORP control through such an electrochemical strategy has been explored with different microbial systems including pure cultures for ethanol fermentation by *Zymomonas mobilis* (Jeon et al., 2009a) and *S. cerevisiae* (Na et al., 2007) and butanol production by *C. acetobutylicum* (Peguin and Soucaille, 1996) as well as mixed cultures for methane and hydrogen production from organic wastes (Jeon et al., 2009b; Wang et al., 2011) and biodegradation of recalcitrant pollutants (Mu et al., 2010).

BERs are only suitable for culture and fermentation at small scale for laboratory research or production of value-added products due to significant electrical energy consumption and limit surface area provided by the working electrode for redox reactions to occur. To address concern on electrical energy consumption, microbial fuel cell can be integrated into a BER system, which was explored recently in biohydrogen production by Wang et al. (2011), but the reliability of such a complicated system and significant capital investment on production facilities impede its application at large scale for industrial production.

3.2.2. Reagent supplementation

Chemicals with different degrees of reduction such as potassium ferricyanide, methyl viologen and neutral red are redox reagents, which can be supplemented into fermentation systems as electron carriers affecting environmental ORP and intracellular metabolism (Bagramyan et al., 2000; Girbal et al., 1995; Peguin and Soucaille, 1996). On the other hand, cystine/cysteine, oxidized/reduced glutathione and NADH/NAD^+ are major biological redox pairs, which not only affect ORP status, but as intermediates also directly involve in intracellular metabolism. Thus, these redox reagents or precursors like vitamin nicotinic acid can be supplemented.

Unlike BERs which require special bioreactor configurations, redox reagents can be supplemented conveniently with any bioreactors and fermentation systems, but there are two obvious disadvantages with this strategy: a) supplemented chemicals potentially interfere with physiological functions of cells, and b) some chemicals, particularly

biochemical reagents, are too costly to be used from the viewpoint of industrial application for bulk commodity production.

3.2.3. Sparging gases

Redox-active gases such as oxygen, hydrogen and carbon monoxide can be sparged into fermentation systems to control ORP. While dissolved oxygen elevates ORP, hydrogen decreases it. In addition, inert gases nitrogen and helium can also be sparged into fermentation systems to strip off dissolved oxygen and hydrogen, and thus affect ORP indirectly.

Pham et al. (2008) sparged oxygen, hydrogen and helium, respectively, during ethanol fermentation by *S. cerevisiae* to create different ORP conditions to study their impact on yeast physiology and metabolism, which provides insight for more efficient ethanol production through ORP control. As for anaerobic fermentations such as butanol fermentation by strictly anaerobic clostridia, carbon monoxide that inhibits hydrogenases, and consequently H_2 production can be sparged to direct more reducing power to solvent production (Meyer et al., 1986). In contrast, nitrogen sparging can improve fermentative hydrogen production by stripping off hydrogen and alleviate its inhibition in H_2-producing enzymes (Kim et al., 2006; Mizuno et al., 2000).

Compared with BERs and supplementation of redox reagents, sparging gases is more economically competitive for ORP control at large scale for industrial processes, if low cost gas is available. Carbon monoxide and nitrogen gases are too expensive for industrial applications in bulk commodity production, and air seems to be the only selection for this purpose.

4. ORP control under microaerobic conditions: ethanol fermentation

Fuel ethanol is a major biofuel, which is now commercially produced worldwide from sugar- and starch-based feedstocks. Comparing to other parameters such as temperature, pH and medium composition, ORP has less influence on ethanol fermentation under regular fermentation conditions with medium containing sugars no more than 250 g/L and ethanol concentration in fermentation broth less than 13% (v/v), due to the intrinsic redox balance associated with the glycolytic pathway and downstream reduction of acetaldehyde to ethanol, as illustrated in Fig. 2, and good tolerance of *S. cerevisiae* to ethanol inhibition.

Very high gravity (VHG) fermentation that uses medium containing sugars in excess of 250 g/L to increase ethanol titer significantly in fermentation broth not only saves energy consumption for ethanol distillation, but also for distillage treatment, since the discharge can be reduced substantially (Bai et al., 2008). However, when ethanol fermentation is carried out under VHG conditions, severe stresses, particularly inhibition of elevated ethanol concentration, detrimentally affect viability of yeast cells, and stuck fermentation with prolonged fermentation time and more sugars remained unfermented frequently occurs (Ingledew, 1999). Yeast cells respond to these stresses with biosynthesis of more protective metabolites such as glycerol, and thus detract NADH from ethanol production, resulting in an imbalance of redox, making ORP control more effective.

4.1. ORP profile of VHG ethanol fermentation

As illustrated in Fig. 3, the ORP profile of VHG ethanol fermentation by *S. cerevisiae* resembles a bathtub (Lin et al., 2010). When yeast cells were inoculated into fresh medium, dissolved oxygen as electron acceptor was consumed quickly through respiration to generate ATP for growth, and a drastic decrease of ORP was thus observed. With gradual depletion of dissolved oxygen, yeast metabolism switched from aerobic growth to anaerobic ethanol fermentation, and in the meantime many pathways such as enhanced glycerol biosynthesis were activated to respond to stresses, which correspondingly consumed reducing equivalent NAD(P)H. Moreover, oversupply of reducing substrates such as glucose further decreased ORP. As sugars consumed, severe stresses slowed down yeast growth and ethanol fermentation, ORP started to increase due to the lysis of yeast cells and corresponding release of oxidative metabolites into the environment.

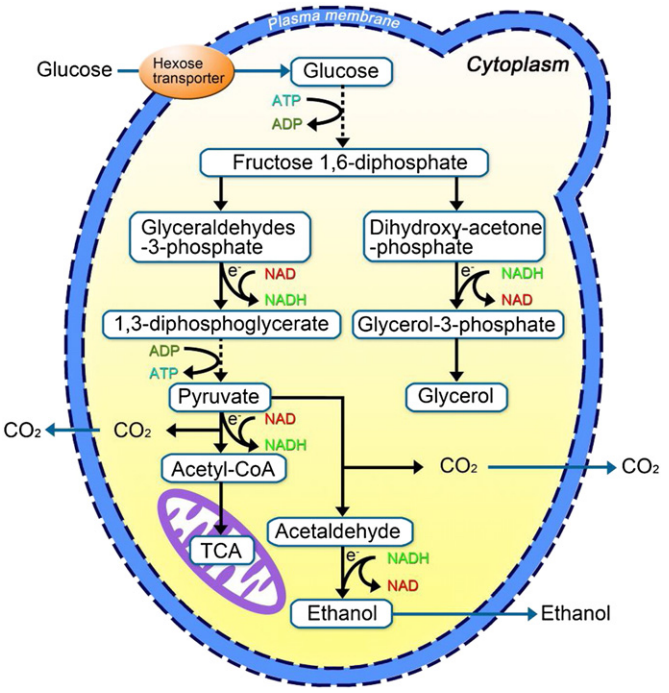

Fig. 2. Redox balance in ethanol fermentation by the yeast *Saccharomyces*. Ethanol fermentation is a redox neutral process. However, biosynthesis of protective metabolites such as glycerol under VHG fermentation conditions distracts NADH, which consequently affects the redox balance, making ORP control more efficient for ethanol production.

4.2. ORP control strategies

Unlike butanol fermentation in which metabolic engineering has been intensively explored on strain modifications to direct carbon flux and electron flow for more efficient butanol production, similar report on ethanol fermentation with *S. cerevisiae* is very limited, which has been focused mainly on reducing glycerol production to increase ethanol yield from sugars, and pentose fermentation for ethanol production from lignocellulosic biomass. Recently, bioprocess engineering strategies were developed to improve yeast viability and ethanol production under VHG fermentation conditions (Liu et al., 2011a,b,c).

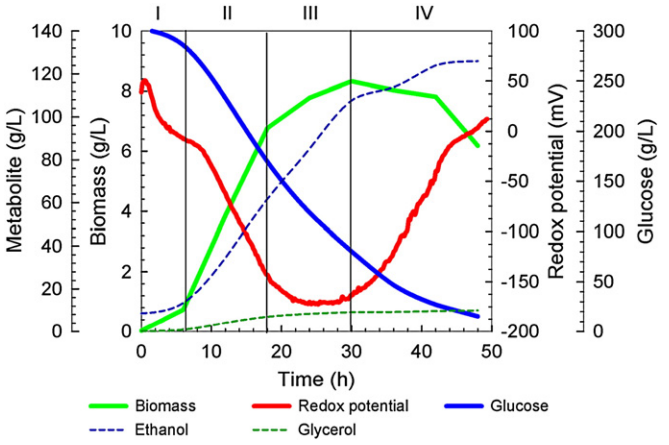

Fig. 3. Time-course of VHG ethanol fermentation by *S. cerevisiae* (Data adapted from Lin et al., 2010). Four regions were divided to indicate correlation between ORP and yeast growth: lag phase (I), log phase (II), stationary phase (III), and death phase (IV).

4.2.1. Strain modification

Glycerol as the main by-product competes for carbon flux and reducing power with ethanol production, since glycerol synthesis requires NADH which is also essential for acetaldehyde reduction to ethanol. Metabolic engineering stratagies were thus developed to alter the internal redox balance and redistribute carbon flux by deleting and/or overexpressing genes encoding the key enzymes.

Guo et al. (2009) deleted *GPD1* or *GPD2* encoding glycerol-3-phosphate dehydrogenases in an industrial yeast. Thus decrease of glycerol (4.29% for *gpd1*Δ and 7.95% for *gpd2*Δ) and increase of ethanol (6.83% for *gpd1*Δ and 7.41% for *gpd2*Δ) were observed. However, lower biomass density compromised ethanol productivity. To solve the problem of redox imbalance caused by deleting *GPD1* or *GPD2*, these researchers expressed two heterologous genes related to glyceraldehyde-3-phosphate dehydrogenase from *Bacillus cereus* and *Kluyveromyces lactis* in the *gpd2*Δ yeast strain (Guo et al., 2011), which enhanced ethanol production with less glycerol produced, and in the meantime growth of the modified strain was not significantly affected.

Pentose such as xylose is the second most abundant sugar in lignocellulosic biomass, which needs to be converted efficiently for bioethanol to be economically competitive as a biofuel. However, *S. cerevisiae* is unable to ferment pentose unless metabolically modified. In enginnered *S. cerev*isiae strains, both NADPH-dependent xylose reductase (XR) and NAD^+-dependent xylitol dehydrogenase (XDH) from the xylose-metabolizing yeast *Pichia stipitis* are required to convert xylose sequentially, which inevitably causes an imblance of redox cofactors that leads to an accumulation of xylitol, and compromises ethanol production rate as well as yield.

Hou et al. (2009) overexpressed *POS5* encoding the native NADH kinase in the cytosol to convert accumulated NADH into NADPH and overcome the redox imblance, which enhanced ethanol production under aerobic culture conditions rather than anaerobic fermentation conditions, but growth of the modified yeast was reduced due to increased energy dissipation. Verho et al. (2003) overexpressed gene *GDP1* encoding $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase, and in the meantime deleted *ZWF1* encoding glucose-6-phosphate dehydrogenase in a xylose-metabolizing *S. cerevisiae* strain. Ethanol yield was increased by 50%, and xylitol accumulation was reduced, correspondingly, due to the reconstruction of redox reactions. Progress in engineering *S. cerev*isiae for better redox balance to improve xylose fermentation was reviewed recently by Matsushika et al. (2009).

4.2.2. Bioprocess engineering

Aeration control is a common method to regulate redox potential in ethanol fermentation (Lin et al., 2010; Liu et al., 2011a; Yu et al., 2007), which needs a feedback system including an ORP probe and a controller to control aeration duration and rate once ORP level is lower than setting values. The ORP level of −150 mV was observed to be optimal, leading to the highest ethanol yield and better ethanol productivity under VHG fermentation conditions (Lin et al., 2010; Yu et al., 2007). Besides, Liu et al. (2011a) developed glucose feeding scheme to provide reducing power in time to maintain the ORP level. However, limited improvement on ethanol yield was observed, since high cell viability could not be maintained with this scheme. In order to overcome this drawback, a continuous process combining with programmed aeration was fabricated, which stimulated growth of yeast cells to replenish the loss of their viability (Liu et al., 2011a).

5. ORP control under anaerobic conditions: acetone–butanol–ethanol fermentation

Acetone–butanol–ethanol (ABE) is produced by strictly anaerobic bacterium *Clostridium* spp. at a ratio of approximately 3:6:1 (Jones and Woods, 1986). Compared to ethanol, butanol contains more energy, and is less volatile and hydroscopic and more compatible with existing infrastructure, which is regarded as an advanced biofuel (Dürre, 2008). However, fermentative production of butanol is very costly. On the one hand, major byproducts acetone and ethanol significantly compromise butanol yield, which correspondingly increase feedstock consumption. On the other hand, extremely low butanol titer in fermentation broth due to butanol toxicity in cells makes butanol recovery very energy-intensive (Keasling and Chou, 2008).

5.1. Impact of ORP on butanol production

ABE fermentation is a biphasic process characterized by acidogenesis and solventogenesis (Lee et al., 2008), which is predominantly controlled by electron transfer and redox balance, as illustrated in Fig. 4. Acetic and butyric acids are produced and built up as the major intermediates during the acidogenic phase concomitant with generation of ATP, which, together with NADH generated in the glycolytic pathway, supports biosynthesis for cell growth. Subsequently, cell growth slows down till cessation occurs at stationary phase due to the toxicity of accumulated acetate and butyrate, and solvent production is then initiated by the uptake of acidic intermediates and continued glucose consumption. The fermentation ends with sporogenesis and cell autolysis due to butanol toxicity. Therefore, ORP control would be an effective strategy for ABE fermentation, which was highlighted by the recent progress in global transcriptional regulation of *C. acetobutylicum* exposed to artificial electron carriers such as methyl viologen (Hönicke et al., 2012), and application of ORP control for ABE fermentation to switch to solventogenesis earlier (Wang et al., 2012).

5.1.1. Acidogenesis

Two moles each of pyruvate, ATP and NADH are produced from one mole glucose consumed through the glycolytic pathway in the acidogenic phase. The cleavage of pyruvate by pyruvate-ferredoxin oxidoreductase results in the formation of acetyl-CoA and CO_2 concomitant with the reduction of ferredoxin, an important electron carrier in ABE fermentation, during which electrons are released and accepted by protons for hydrogen production catalyzed by hydrogenases. Stoichiometrically, two moles acetate or one mole butyrate could be produced from one mole glucose consumed, yielding four or three moles of ATP, correspondingly, to support cell growth, and in the meantime generating two moles of NADH for solvent production later in the solventogenic phase (Jones and Woods, 1986).

The key enzyme in the electron transfer system is NADH ferredoxin oxidoreductase, which catalyzes the oxidation of NADH. Meanwhile, another enzyme, NADPH ferredoxin oxidoreductase controls production of NADPH from reduced ferredoxin to support biosynthesis associated with cell growth as well as butyraldehyde reduction catalyzed by the NADPH-dependent butanol dehydrogenase later in the solventogetic phase. An update review on key enzymes for butanol production is available (Gheshlaghi et al., 2009).

When acids accumulate to sufficiently high levels, cells cannot maintain the pH gradient across membranes, and a dramatic decrease in growth occurs (Huang et al., 2010). Therefore, the shift to solvent production in clostridia is an adaptive response to toxic effect of acidic metabolites through their reassimilation and induced expression of genes for stress response (Grimmler et al., 2011; Grupe and Gottschalk, 1992).

5.1.2. Solventogenesis

As intracellular ATP is consumed by biosynthesis, solventogenesis is initiated to consume NAD(P)H accumulated during the acidogenesis (Grupe and Gottschalk, 1992). Acetyl-CoA and butyryl-CoA function as key intermediates for ethanol and butanol production from acetate and butyrate. Meanwhile, acetoacetyl-CoA not only serves as the bridge between acetyl-CoA and butyryl-CoA, but also is the substrate for acetone production catalyzed by acetoacetyl-CoA:acetate/butyrate: CoA transferase, which also catalyzes the conversion of acetate and butyrate

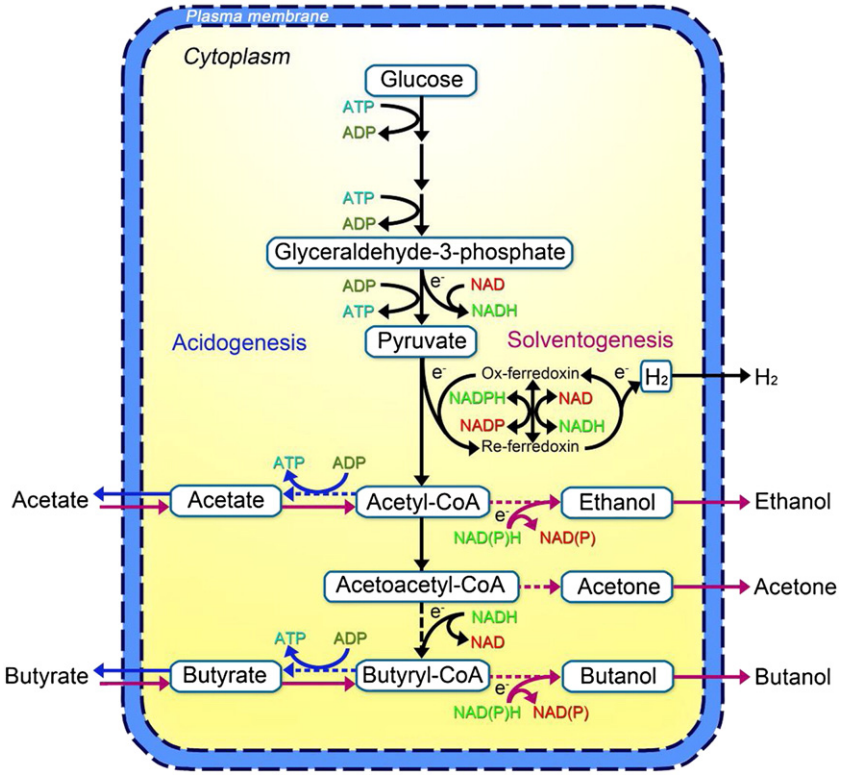

Fig. 4. Major redox reactions in acetone–butanol–ethanol fermentation by the bacterium *Clostridium*. Blue and purple arrows for acidogenesis and solventogenesis, respectively.

accumulated in the acidogenic phase back to acetyl-CoA and butyryl-CoA. The reduction of acetyl-CoA and butyryl-CoA to acetylaldehyde and butyraldehyde is catalyzed by acetaldehyde dehydrogenase and butyraldehyde dehydrogenase, respectively, followed by the further reduction of acetylaldehyde and butyraldehyde to ethanol and butanol by ethanol dehydrogenase and butanol dehydrogenase (Gheshlaghi et al., 2009). These reduction processes consume NAD(P)H to maintain intracellular redox balance.

5.2. ORP control strategies

Manipulating intracellular electron transfer and redox balance can direct clostridial metabolism towards more efficient butanol production. Two strategies can be applied for this objective: strain modification and bioprocess engineering.

5.2.1. Strain modification

Unlike well established model strains such as *E. coli* and *Bacillus subtilis*, metabolic engineering of clostridia is more challenging. Although tools for gene transformation, overexpression and knockout as well as transcriptome analysis have been developed for clostridia (Papoutsakis, 2008), success in metabolic engineering pathways for electron transfer and redox balance of the species to optimize product composition and improve butanol ratio and titer is still limited (Lütke-eversloh and Bahl, 2011).

Since electron flow is primarily regulated at the ferredoxin/hydrogenase node in clostridia, an antisense RNA strategy was thus developed to depress the expression of *hupC* encoding a hydrogen-uptake hydrogenase in *C. saccharoperbutylacetonicum*, and an increase in acetone production was observed, but butanol production was compromised (Nakayama et al., 2008), indicating that targeting the hydrogenase and hydrogen production might be a promising metabolic engineering strategy.

Engineering butyric acid-producing *C. tyrobutyricum* to overexpress alcohol dehydrogenase from *C. acetobutylicum* for butanol production was also reported, and 10.0 g/L butanol was produced after inactivating a few other genes (Yu et al., 2011). Recently, the model strain *C. acetobutylicum* ATCC 824 was engineered with the secondary alcohol dehydrogenase (*adh*) to reduce acetone into isopropanol with NADPH/$NADP^+$ as cofactor, and thus isopropanol–butanol–ethanol (IBE), a mixture that is more suitable for fuels use was produced (Collas et al., 2012; Lee et al., 2012). Similar strategy was applied immediately in engineering the robust butanol-tolerant industrial strain *C. acetobutylicum* Rh8 (Dai et al., 2012). The progress not only improves the quality of the mixture as biofuels, but most importantly provides a platform for modifying redox balance of clostridia to optimize their product profiles.

With the development of the ClosTron technology, the targeted mutagenesis of the ABE pathway in clostridia can be performed more effectively, resulting in an in-depth understanding of underlying biological mechanisms and the improvement of butanol production (Heap et al., 2007). Cooksley et al. (2012) developed mutants defective in alcohol/aldehyde dehydrogenases 1 and 2 (*adhE1* and *adhE2*), butanol dehydrogenases A and B (*hydA* and *hydB*), a uncharacterized putative alcohol dehydrogenase (CAP0059) and other key enzymes in order to understand their impact on the ABE biosynthesis, and to locate potential targets for further metabolic manipulation to improve butanol titer.

In addition to engineering targeted enzymes and pathways, global manipulation of the butanol biosynthetic pathway could also be explored. For example, the redox-sensing protein Rex, a transcriptional regulator in *C. acetobutylicum* encoded by the gene *CAC2713* and its role in the solventogenic shift were investigated, and experimental results illustrated that Rex controls the expression of genes in butanol biosynthetic pathways in response to the intracellular NADH/NAD^+ shift, and thus a Rex-negative mutant produced more ethanol and butanol with less hydrogen and acetone as by-products (Wietzke and Bahl, 2012). These findings provide insights for engineering redox balance at a global level to enhance butanol production.

Although genetic manipulation of clostridia has been tapped with unremitting effort, solvent production by the microorganisms

is still not economically competitive, particularly when butanol is produced as biofuel at a large scale. Synthetic biology that can build up new biological entities such as molecules, pathways, regulatory network, organisms and systems for more efficient production of targeted products provides a new tool to address challenges associated with engineering or modification of native strains. Bond-Watts et al. (2011) reported a significant progress in construction of the butanol production pathway by incorporating two plasmids into *E. coli*. The first plasmid (pBAD33-Bu1) carried three genes encoding PhaA (acetoacetyl-CoA thiolase/synthase) and PhaB (3-hydroxybutyryl-CoA dehydrogenase) from *Ralstonia eutrophus* and Crt (crotonase) from *C. acetobutylicum* as a single operon driven by the arabinose promoter to produce crotonyl-CoA from acetyl-CoA, and the second plasmid contained an operon comprising the genes *ccr* and *adhE2* encoding crotonyl-CoA reductase and butyraldehyde/butanol dehydrogenase, respectively, which was driven by a T7*lac* promoter for producing secretory butanol from crotonyl-CoA. After optimization of the synthetic pathway, replacing *ccr* with *ter* encoding a crotonyl-CoA specific *trans*-enoyl-CoA reductase in *Euglena gracilis* that does not catalyze the reverse oxidation of butyryl-CoA to crotonyl-CoA in the presence of NAD^+ or $NADP^+$, the butanol titer was increased to 2.95 g/L. However, the redox imbalance nature requires an additional oxidation reaction to generate reducing equivalents to direct the synthetic butanol pathway. Almost simultaneously, an elegant solution to this problem was resolved by constructing a modified butanol pathway in *E. Coli* for more metabolic flux towards butanol synthesis driven by excess NADH, and a butanol concentration of 15 g/L was achieved (Shen et al., 2011).

5.2.2. Bioprocess engineering

While genetic modification of clostridia is tedious and slow, bioprocess engineering strategy can be explored with immediate impact on ABE fermentation in case it is economically competitive for industrial application.

When using more reduced substrate mannitol instead of glucose, a high butanol titer of 16.0 g/L was obtained by an engineered butyric acid producing mutant *C. tyrobutyricum* (Yu et al., 2011). Similarly, more alcohols and less hydrogen were produced using the mixture of glucose and glycerol in the culture of *C. acetobutylicum* ATCC 824 (Vasconcelos et al., 1994). Other studies on addition of artificial electron carriers such as methyl viologen and neutral red also caused significant carbon flow shift from acids to alcohols production accompanied by decreased hydrogen evolution (Girbal et al., 1995; Peguin and Soucaille, 1996). These results indicate that reducing substrates provide more reducing power, and in turn drive more metabolic flux toward butanol production.

In addition to increasing reducing equivalents by reduced chemicals which are more expensive than sugars from industrial feedstocks like corn-starch, inhibition of hydrogenases by sparging CO or elevating hydrogen partial pressure was attempted to manipulate redox balance and improve butanol production. For example, butanol concentration was increased to 7.8 g/L by sparging a mixture of 85% N_2 and 15% CO, compared to 4.8 g/L butanol produced by sparging pure N_2 (Kim et al., 1984), and elevated H_2 partial pressure also significantly increased butanol titer and ratio (Yerushalmi et al., 1985). These results indicate that CO or H_2 may inhibit hydrogenase activity and electron transfer from reduced ferredoxin to $NAD(P)^+$, and consequently improve NAD(P)H pool and alcohol production.

Recently, Wang et al. (2012) demonstrated that controlling ORP of ABE fermentation by *C. acetobutylicum* DSM 1731 at −290 mV initiated solvent production earlier, and solvent productivity was increased by 35% compared with the control without ORP control, although the ratio of butanol in the solvent did not increase significantly. Moreover, they sparged small amount of air into the ABE fermentation system to control ORP, which not only provides an insightful understanding on the metabolism of the strictly anaerobic species, but also an economically competitive method for enhancing butanol production.

6. Conclusions

Most fermentation products produced under microaerobic or anaerobic conditions are bulk commodities. Although these fermentations have been well established with long-term production practices, cost saving is an endless effort, particularly for the production of biofuels and bio-based chemicals such as ethanol and butanol at large scales to address global concern on sustainable development. Since redox reactions and homeostasis are basis for intracellular metabolism, ORP status controls profiles of metabolites, which subsequently provides an effective strategy to direct more materials and energy to the pathways for efficient production of desirable products. With advanced technologies that can detect intracellular ORP levels and analyze gene expression and protein biosynthesis caused by ORP shifts, mechanism underlying this phenomenon can be elucidated at molecular and bioprocess engineering levels, and more robust strains and optimized processes can be developed, correspondingly, which have been highlighted in ethanol and butanol fermentations under microaerobic and anaerobic conditions.

Acknowledgements

CGL, CX and FWB appreciate support in time and funding from the Natural Sciences Foundation of China (20276038), the National High-Tech R & D Program of China (2012AA021205) and China Postdoctoral Science Foundation (2012M510809). YHL thanks research support received from the Natural Sciences and Engineering Research Council of Canada.

References

Auchinvole CAR, Richardson P, McGuinnes C, Mallikarjun V, Donaldson K, McNab H, et al. Monitoring intracellular redox potential changes using SERS nanosensors. ACS Nano 2012;6:888–96.
Bagramyan K, Galstyan A, Trchounian A. Redox potential is a determinant in the *Escherichia coli* anaerobic fermentative growth and survival: effects of impermeable oxigent. Bioelectrochemistry 2000;51:151–6.
Bai FW, Anderson WA, Moo-Young M. Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnol Adv 2008;26:89-105.
Baker MA, Lawen A. Plasma membrane NADH-oxidoreductase system: A critical review of the structural and functional data. Antioxid Redox Signaling 2000;2:197–212.
Berríos-Rivera SJ, Bennett GN, San K-Y. The effect of increasing NADH availability on the redistribution of metabolic fluxes in *Escherichia coli* chemostat cultures. Metab Eng 2002;4:230–7.
Bond-Watts BB, Bellerose RJ, Chang MCY. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. Nat Chem Biol 2011;7:222–7.
Chen X, Jiang S, Zheng Z, Pan L, Luo S. Effects of culture redox potential on succinic acid production by *Corynebacterium crenatum* under anaerobic conditions. Process Biochem 2012;47:1250–5.
Collas F, Kuit W, Clement B, Marchal R, Lopez-Contreras AM, Monot F. Simultaneous production of isopropanol, butanol, ethanol and 2,3-butanediol by *Clostridium acetobutylicum* ATCC824 engineered strains. AMB Express 2012;2:45.
Cooksley CM, Zhang Y, Wang H, Redl S, Winzer K, Minton NP. Targeted mutagenesis of the *Clostridium acetobutylicum* acetone–butanol–ethanol fermentation pathway. Metab Eng 2012;14:630–41.
Dai Z, Dong H, Zhu Y, Zhang Y, Li Y, Ma Y. Introducing a single secondary alcohol dehydrogenase into butanol-tolerant *Clostridium acetobutylicum* Rh8 switches ABE fermentation to high level IBE fermentation. Biotechnol Biofuels 2012;5:44.
de Graef MR, Alexeeva S, Snoep JL, de Mattos MJT. The steady-state internal redox state (NADH/NAD) reflects the external redox state and is correlated with catabolic adaptation in *Escherichia coli*. J Bacteriol 1999;181:2351–7.
du Preez JC, van Driessel B, Prior BA. The relation between redox potential and D-xylose fermentation by *Candida shehatae* and *Pichia stipitis*. Biotechnol Lett 1988;10:901–6.
Du C, Yan H, Zhang Y, Li Y, Cao Z. Use of oxidoreduction potential as an indicator to regulate 1,3-propanediol fermentation by *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 2006;69:554–63.
Du C, Zhang Y, Li Y, Cao Z. Novel redox potential-based screening strategy for rapid isolation of *Klebsiella pneumoniae* mutants with enhanced 1,3-propanediol-producing capability. Appl Environ Microbiol 2007;73:4515–21.
Dürre P. Fermentative butanol production bulk chemical and biofuel. Ann N Y Acad Sci 2008;1125:353–62.
Fleming JT. Electronic interfacing with living cells. Adv Biochem Eng Biotechnol 2010;117:155–78.
Gheshlaghi R, Scharer JM, Moo-Young M, Chou CP. Metabolic pathways of clostridia for producing butanol. Biotechnol Adv 2009;27:764–81.
Girbal L, Vasconcelos I, Saint-Amans S, Soucaille P. How neutral red modified carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH. FEMS Microbiol Rev 1995;16:151–62.

Grimmler C, Janssen H, Kraube D, Fischer RJ, Bahl H, Dürre P, et al. Genome-wide gene expression analysis of the switch between acidogenesis and solventogenesis in continuous cultures of *Clostridium acetobutylicum*. J Mol Microbiol Biotechnol 2011;20:1-15.
Grupe H, Gottschalk G. Physiological events in *Clostridium acetobutylicum* during the shift from acidogenesis to solventogenesis in continuous culture and presentation of a model for shift induction. Appl Environ Microbiol 1992;58:3896–902.
Guo ZP, Zhang L, Ding ZY, Wang ZX, Shi GY. Interruption of glycerol pathway in industrial alcoholic yeasts to improve the ethanol production. Appl Microbiol Biotechnol 2009;82:287–92.
Guo ZP, Zhang L, Ding ZY, Wang ZX, Shi GY. Improving ethanol productivity by modification of glycolytic redox factor generation in glycerol-3-phosphate dehydrogenase mutants of an industrial ethanol yeast. J Ind Microbiol Biotechnol 2011;38:935–43.
Heap JT, Pennington OJ, Cartman ST, Carter GP, Minton NP. The ClosTron: a universal gene knock-out system for the genus *Clostridium*. J Microbiol Methods 2007;70:452–64.
Hönicke D, Janssen H, Grimmler C, Ehrenreich A, Lütke-Eversloh T. Global transcriptional changes of *Clostridium acetobutylicum* cultures with increased butanol:acetone ratios. N Biotechnol 2012;29:485–93.
Hou J, Vemuri G, Bao X, Olsson L. Impact of overexpressing NADH kinase on glucose and xylose metabolism in recombinant xylose-utilizing *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 2009;82:909–19.
Hou J, Scalcinati G, Oldiges M, Vemuri GN. Metabolic impact of increased NADH availability in *Saccharomyces cerevisiae*. Appl Environ Microbiol 2010;76:851–9.
Huang H, Liu H, Gan YR. Genetic modification of critical enzymes and involved genes in butanol biosynthesis from biomass. Biotechnol Adv 2010;28:651–7.
Ingledew WM. Alcohol production by *Saccharomyces cerevisiae*: a yeast primer. In: Jacques K, Lyons TP, Kelsall DR, editors. The alcohol textbook. Nottingham University Press; 1999. p. 49–87.
Jeon BY, Park DH. Improvement of ethanol production by electrochemical redox combination of *Zymomonas mobilis* and *Saccharomyces cerevisiae*. J Microbiol Biotechnol 2010;20:94-100.
Jeon BY, Hwang TS, Park DH. Electrochemical and biochemical analysis of ethanol fermentation of *Zymomonas mobilis* KCCM11336. J Microbiol Biotechnol 2009a;19:666–74.
Jeon BY, Kim SY, Park YK, Park DH. Enrichment of hydrogenotrophic methanogens in coupling with methane production using electrochemical bioreactor. J Microbiol Biotechnol 2009b;19:1665–71.
Jones DT, Woods DR. Acetone–butanol fermentation revisited. Microbiol Rev 1986;50:484–524.
Kastner JR, Eiteman MA, Lee SA. Effect of redox potential on stationary-phase xylitol fermentations using *Candida tropicalis*. Appl Microbiol Biotechnol 2003;63:96-100.
Keasling JD, Chou H. Metabolic engineering delivers next-generation biofuels. Nat Biotechnol 2008;26:298–9.
Kim BH, Bellows P, Datta R, Zeikus JG. Control of carbon and electron flow in *Clostridium acetobutylicum* fermentations: utilization of carbon monoxide to inhibit hydrogen production and to enhance butanol yields. Appl Environ Microbiol 1984;48:764–70.
Kim DH, Han SK, Kim SH, Shin HS. Effect of gas sparging on continuous fermentative hydrogen production. Int J Hydrogen Energy 2006;31:2158–69.
Kjaergaard L. The redox potential: Its use and control in biotechnology. Adv Biochem Eng 1977;7:131–50.
Kültz D. Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 2005;67:225–57.
Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by clostridia. Biotechnol Bioeng 2008;101:209–28.
Lee J, Jang YS, Choi SJ, Im JA, Song H, Cho JH, et al. Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for isopropanol–butanol–ethanol fermentation. Appl Environ Microbiol 2012;78:1416–23.
Li J, Jiang M, Chen K, Shang L, Wei P, Ying H, et al. Enhanced production of succinic acid by *Actinobacillus succinogenes* with reductive carbon source. Process Biochem 2010a;45:980–5.
Li J, Jiang M, Chen K, Ye Q, Shang L, Wei P, et al. Effect of redox potential regulation on succinic acid production by *Actinobacillus succinogenes*. Bioprocess Biosyst Eng 2010b;33:911–20.
Lin YH, Chien WS, Duan KJ. Correlations between reduction–oxidation potential profiles and growth patterns of *Saccharomyces cerevisiae* during very-high-gravity fermentation. Process Biochem 2010;45:765–70.
Liu CG, Lin YH, Bai FW. Development of redox potential-controlled schemes for very-high-gravity ethanol fermentation. J Biotechnol 2011a;153:42–7.
Liu CG, Lin YH, Bai FW. A kinetic growth model for *Saccharomyces cerevisiae* grown under redox potential-controlled very-high-gravity environment. Biochem Eng J 2011b;56:63–8.
Liu CG, Lin YH, Bai FW. Ageing vessel configuration for continuous redox potential-controlled very-high-gravity fermentation. J Biosci Bioeng 2011c;111:61–6.
Liu H, Kang J, Qi Q, Chen G. Production of lactate in *Escherichia coli* by redox regulation genetically and physiologically. Appl Biochem Biotechnol 2011d;164:162–9.
Lütke-Eversloh T, Bahl H. Metabolic engineering of *Clostridium acetobutylicum*: recent advances to improve butanol production. Curr Opin Biotechnol 2011;22:1-14.
Mason JT, Kim SK, Knaff DB, Wood MJ. Thermodynamic basis for redox regulation of the Yap1 singnal transduction pathway. Biochemistry 2006;45:13409–17.
Matsushika A, Inoue H, Kodaki T, Sawayama S. Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: current state and perspectives. Appl Microbiol Biotechnol 2009;84:37–53.
Meyer CL, Roos JW, Papoutsakis ET. Carbon monoxide gasing leads to alcohol production and butyrate uptake without acetone formation in continuous cultures of *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 1986;24:159–67.
Mizuno O, Dinsdale R, Hawkes FR, Hawkes DL, Noike T. Enhancement of hydrogen production from glucose by nitrogen gas sparging. Bioresour Technol 2000;73:59–65.
Mu Y, Radjenovic J, Shen J, Rozendal RA, Rabaey K, Keller J. Dehalogenation of iodinated X-ray contrast media in a bioelectrochemical system. Environ Sci Technol 2010;45:782–8.
Murray R, Granner D, Mayes P, Rodwell V. Harper's illustrated biochemistry. 26 ed. New York: McGraw-Hill Companies; 2003. p. 87.
Murray DB, Haynes K, Tomita M. Redox regulation in respiring *Saccharomyces cerevisiae*. Biochim Biophys Acta 2011;1810:945–58.
Na BK, Hwang TS, Lee SH, Ahn DH, Park DH. Effect of electrochemical redox reaction on growth and metabolism of *Saccharomyces cerevisiae* as an environmental factor. J Microbiol Biotechnol 2007;17:445–53.
Nakashimada Y, Rachman MA, Kakizono T, Nishio N. Hydrogen production of *Enterobacter aerogenes* altered by extracellular and intracellular redox states. Int J Hydrogen Energy 2002;27:1399–405.
Nakayama SI, Kosaka T, Hirakawa H, Matsuura K, Yoshino S, Furukawa K. Metabolic engineering for solvent productivity by downregulation of the hydrogenase gene cluster hupCBA in *Clostridium saccharoperbutylacetonicum* strain N1-4. Appl Microbiol Biotechnol 2008;78:483–93.
Needham J, Needham DM. The oxidation–reduction potential of protoplasm: a review. Protoplasma 1926;1:255–94.
Papoutsakis ET. Engineering solventogenic clostridia. Curr Opin Biotechnol 2008;19:420–9.
Park DH, Zeikus JG. Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. J Bacteriol 1999;181:2403–10.
Peguin S, Soucaille P. Modulation of metabolism of *Clostridium acetobutylicum* grown in chemostat culture in a three-electrode potentiostatic system with methyl viologen as electron carrier. Biotechnol Bioeng 1996;51:342–8.
Pei J, Zhou Q, Jing Q, Li L, Dai C, Li H, et al. The mechanism for regulating ethanol fermentation by redox levels in Thermoanaerobacter ethanolicus. Metab Eng 2011;13:186–93.
Pham TH, Mauvais G, Vergoignan C, De Coninck J, Dumont F, Lherminier J, et al. Gaseous environments modify physiology in the brewing yeast *Saccharomyces cerevisiae* during batch alcoholic fermentation. J Appl Microbiol 2008;105:858–74.
Riondet C, Cachon R, Waché Y, Alcaraz G, Diviès C. Extracellular oxidoreduction potential modifies carbon and electron flow in *Escherichia coli*. J Bacteriol 2000;182:620–6.
Rodkey FL, Donovan JA. Oxidation–reduction potentials of the triphosphopyridine nucleotide system. J Biol Chem 1959;234:677–80.
Sauer U, Canonaco F, Heri S, Perrenoud A, Fischer E. The soluble and membrane-bound transhydrogenases UdhA and PntAB have divergent functions in NADPH metabolism of *Escherichia coli*. J Biol Chem 2004;279:6613–9.
Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free RadicBiol Med 2001;30:1191–212.
Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC. Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. Appl Environ Microbiol 2011;77:2905–15.
Shi F, Kawai S, Mori S, Kono E, Murata K. Identification of ATP-NADH kinases isozymes and their contribution to supply of NADP(H) in *Saccharomyces cerevisiae*. FEBS J 2005;272:3337–49.
Singh A, Lynch MD, Gill RT. Genes restoring redox balance in fermentation-deficient *E. coli* NZN111. Metab Eng 2009;11:347–54.
Sridhar J, Eiteman M. Influence of redox potential on product distribution in *Clostridium thermosuccinogenes*. Appl Biochem Biotechnol 1999;82:91-101.
Thrash JC, Coates JD. Review: direct and indirect electrical stimulation of microbial metabolism. Environ Sci Technol 2008;42:3921–31.
Vasconcelos I, Girbal L, Soucaille P. Regulation of carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH on mixtures of glucose and glycerol. J Bacteriol 1994;176:1443–50.
Vemuri GN, Altman E, Sangurdekar DP, Khodursky AB, Eiteman MA. Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. Appl Environ Microbiol 2006;72:3653–61.
Vemuri GN, Eiteman MA, McEwen JE, Olsson L, Nielsen J. Increasing NADH oxidation reduces overflow metabolism in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 2007;104:2402–7.
Verho R, Londesborough J, Penttila M, Richard P. Engineering redox cofactor regeneration for improved pentose fermentation in *Saccharomyces cerevisiae*. Appl Environ Microbiol 2003;69:5892–7.
Wang AJ, Cheng HY, Liang B, Ren NQ, Cui D, Lin N, et al. Efficient reduction of nitrobenzene to aniline with a biocatalyzed cathode. Environ Sci Technol 2011;45:10186–93.
Wang S, Zhu Y, Zhang Y, Li Y. Controlling the oxidoreduction potential of the culture of *Clostridium acetobutylicum* leads to an earlier initiation of solventogenesis, thus increasing solvent productivity. Appl Microbiol Biotechnol 2012;93:1021–30.
Wietzke M, Bahl H. The redox-sensing protein Rex, a transcriptional regulator of solventogenesis in *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 2012;96:749–61.
Yerushalmi L, Volesky B, Szczesny T. Effect of increased hydrogen partial pressure on the acetone–butanol fermentation by *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 1985;22:103–7.
Yu Y, Wang YH, Chu J, Zhuang YP, Zhang SL. The influence of controlling redox potential on ethanol production by *Saccharomyces cerevisiae*. Chin J Biotechnol 2007;23:878–84.
Yu M, Zhang Y, Tang IC, Yang ST. Metabolic engineering of *Clostridium tyrobutyricum* for n-butanol production. Metab Eng 2011;13:373–82.
Zhang C, Ma K, Xing X. Regulation of hydrogen production by *Enterobacter aerogenes* by external NADH and NAD^+. Int J Hydrogen Energy 2009;34:1226–32.