Industry Day Theme # Sustainable Medical Technologies (SMT)

Effect of dissolved oxygen on recombinant protein production

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Abstract

Microaerobic fermentation has been shown to improve lactose transport and recombinant protein production in *Escherichia coli*. Mechanistic correlation between lactose and dissolved oxygen has been studied and it has been demonstrated that *E. coli* can switch its genetic machinery upon fluctuations in dissolved oxygen levels and thereby impact lactose transport and resulting in product formation. Continuous induction of lactose in microaerobic fermentation led 3.3 fold improvement in product titre of rLTNF oligomer and 2 fold improvement in product titre of rSymlin oligomer as compared to traditional aerobic fermentation. Transcriptome profiling indicated that ribosome synthesis, lactose transport, and amino acid synthesis genes were upregulated during microaerobic fermentation.

Introduction

- *Escherichia coli* is a commonly used host used for production of recombinant proteins and enzymes due to its well understood genome, metabolome, and proteome as well as the wide availability of tools for its genetic manipulation.
- Recombinant protein expression using inducer in *E. coli* has been extensively studied by researchers, particularly at high cell density cultivation using fed batch operation. Induction of recombinant proteins using natural inducers such as lactose, arabinose, and rhamnose have been examined by several researchers and a few alternative induction strategies have be eproposed, including “auto-induction” and “continuous induction”.
- In this work, we aim to demonstrate a novel application involving growth of *E. coli* cells to high cell density for biomass formation in the aerobic phase and gradually shifting the cells to microaerobic phase, for enhancing production of recombinant lethal toxin neutralizing factor (rLTNF) and recombinant pramlintide (rSymlin) oligomers.
- Transcriptomic profiling of metabolic changes due to aerobic process and microaerobic process during protein formation phase has been performed and the results provide a deeper understanding of protein production in E. coli BL21 (DE3) strains with lactose based promoter expression systems.

Materials and Methods

- Primary culture was used for inoculation in the bioreactor. Fed-batch experiments were performed in a 1.3 L bioreactor (Eppendorf, USA) with an initial volume of 0.3 litre. A DO probe was calibrated using 0% pO2 with 100% nitrogen and 100% pO2 with 100% air for the experiments. The dissolved oxygen concentration was controlled at 30% of saturation for aerobic fermentation and at 0-5% for microaerobic fermentation, first by changing the agitation speed between 300 and 900 rpm, after which the supplied air was enriched with pure oxygen up to 40% for aerobic fermentation and minimal use of pure oxygen for microaerobic fermentation.
- Transcriptomic profiling: After 2 hours of lactose induction, aerobic and microaerobic fermentation samples were collected and washed with 0.9% sodium chloride and stored in a -20˚C freezer. Samples were collected at three time points (4h,6h,8h) post-induction for transcriptome analysis at Genotypic Technology Pvt. Ltd., Bangalore, India.

Result

Table 1. A comparison of key process attributes in microaerobic and aerobic fermentation.

Recombinant Proteins	Fermentation mode	Max. Biomass (g/L)	Max. Product (g/L)	Lactose consumption (%)	$Y_{p/s}$ (g-product/ g-lactose)	Specific product formation rate (g-product/g-lactose/h)
rLTNF	Aerobic	38.94	2.82	88.60	0.029	0.0003
	Microaerobic	31.29	9.38	98.31	0.068	0.0052
rSymlin	Aerobic	23.55	2.94	93.70	0.021	0.0010
	Microaerobic	26.34	6.01	98.91	0.045	0.0032

Table 2. Fold change in expression of various genes related to lactose metabolism in microaerobic condition in comparison to aerobic condition.

Gene ID	4 h	6 h	8 h	Gene description
setB	-1.5	+1.6	+3.6	Sugar efflux transporter
ptsG	+2.9	-3.5	-3.1	PTS system glucose-specific EIICB component (EIICB-Glc) (EII-Glc)
lacZ	0.63	-2.6	-0.94	Beta-galactosidase
lacY	0.18	-2.7	-0.88	Lactose permease
lacA	0.27	-1.3	-0.85	Galactoside O-acetyltransferase
galK	+1.8	+1.47	0.6	Galactokinase
galT	- 1.3	+1.2	+1.4	Galactose-1-phosphate uridylyltransferase
galM	-1.1	0.5	0.6	Galactose 1-epimerase

Table 3. Fold change in expression of various genes related to stress response in microaerobic condition in comparison to aerobic condition

Gene ID	4 h	6 h	8 h	Gene description
fimC	-1	+4.2	+1.3	Type 1 fimbriae periplasmic chaperone
ibpB	-1	+2.3	3.1	Small heat shock protein homodimer
soxS	-1	0.1	0.6	DNA binding transcriptional dual regulator
cbpM	-1	-0.07	-0.02	Chaperone modulator
traJ	-2	+2.2	+2	Plasmid transfer ATPase
pspA	-1.9	+1.7	+3.3	Phage shock protein A
rspB	-1	+1.5	-0.2	Starvation sensing protein
soxR	-1.2	0.1	0.3	Redox-sensitive transcriptional activator
cspG	-2.4	+3.1	+5.3	Cold shock-like protein
hscA	-1.1	-2.4	-2.4	Iron sulphur cluster biosynthesis chaperone
uspF	-1.6	-1	-1	Universal stress protein F
asr	-1.1	-1	-1.3	Acid shock protein
pspC	-2	+1.2	2.3	Envelope stress response membrane protein
pspB	-2.2	+1.2	0.9	Envelope stress response membrane protein
ybgP	-1.6	-1	-1	Uncharacterized fimbrial chaperone
cspD	-0.02	-1.5	-1.5	Cold shock-like protein
glsB	0.21	-1.2	-2.1	GlsB/YeaQ/YmgE family stress response membrane protein
hrsA	-0.4	-1.1	-1.3	Heat-responsive suppressor
yciH	+1.1	-2.4	-0.6	Stress response translation initiation inhibitor
phoH	-0.2	-2.4	-0.6	Phosphate starvation protein
rcsF	-0.6	-1.1	-1	Rcs stress response system protein
ibaG	0.04	-0.2	-1.1	Acid stress protein
acrR	0.2	-1.2	-2.1	HTH-type transcriptional regulator (Potential acrAB operon repressor)
clpB	0.3	-0.9	-1.6	Chaperone protei
bhsA	-1.9	+2.6	+1.9	Multiple stress resistance protein (Copper-induced outer membrane component)
SfmC	-0.4	0.5	+1.05	Probable fimbrial chaperone
spy	-0.9	+2.5	+2.6	ATP independent periplasmic chaperone
ibpA	-0.6	0.76	+1.1	Small heat shock protein

Conclusions

Recombinant protein production can be significantly improved by controlling oxygen upon lactose induction. As a result, flux generated from lactose gets directed towards protein production machinery such as promoter induction, ribosome, ATP recycling and amino acid biosynthesis. A 3.3 fold higher titer for rLTNF fermentation and 2.0 fold higher for rSymlin fermentation was observed. Also transcriptome profiling of recombinant protein induced cultures found that novel transporter *setB* was upregulated.

Industrial Significance

Lactose uptake significantly improved during microaerobic condition so maintaining dissolved oxygen less than 10% improved recombinant protein production where lactose used as a inducer

Technology Readiness Level: Ready

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