Improving DNA Plasmid Production in *Escherichia Coli*

by

ADAM SINGER

(Under the Direction of Mark A. Eiteman)

ABSTRACT

The ability to produce large quantities of plasmid DNA is imperative for wide scale availability of DNA vaccines. Large scale, high yield production relies on the synergy between host strain, plasmid, medium and production scheme. Screening as many variables as quickly and cost effectively as possible is the goal. In this study, *Escherichia coli* strains were transformed with two plasmids and screened for plasmid yield in shake flasks in chemically defined medium supplemented with either glucose or glycerol. High yield candidates were grown in feed batch fermentations at two specific growth rates, $\mu = 0.14\ h^{-1}$ and $\mu = 0.24\ h^{-1}$. As predicted, high production in shake flasks was predictive of high production in fermentations. Using our media and process, we were able to reach volumetric yields of approximately 600 mg/L and specific yields of approximately 17.82 mg/g, regardless of growth rate. We were also able to increase productivity (mg/Lh) over 30%.

INDEX WORDS: *E. coli*, fed-batch, gene therapy, plasmid production

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DEDICATION

To my wife Dana and my daughter Sydney-Rose. Thank you for standing by me.

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CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

INTRODUCTION

A new technology for vaccine development is the use of the plasmid DNA as the antigen. A form of gene therapy, DNA vaccination can be used for the prevention of viral, bacterial, and parasitic diseases, as a therapeutic vaccination for diseases, or for other health conditions such as cancer, Malaria and AIDS (Gregoriadis, 1998; Tuteja, 1999; Mountain, 2000, Barouch et al., 2000; Barouch et al., 2001; Doolan and Hoffman 2001; Lowe et al., 2006; Lowe et al., 2007). The market for gene therapy products is expected to exceed 45 billion dollars by 2010 (Glaser, 1997). Well over 100 DNA vaccine trails have been initiated (Listner et al., 2006). In-fact, as of 2007 16% of all gene therapy clinical trials involved plasmid DNA (http://www.wiley.co.uk/genetherapy/clinical).

Jenner first demonstrated the ability to vaccinate for an infectious disease over 200 years ago. In 1796, Jenner "vaccinated" a young boy with an attenuated strain of Cowpox. Later he injected the boy with the Smallpox virus and observed no infection. Since that time, vaccination has been successful for a variety of infectious diseases. Since initial work using naked DNA as an antigen (Wolf et al., 1990), DNA vaccination has become one of the fastest growing areas of medical research (Leitner et al., 2000). Despite this fact, many of the most harmful diseases such as HIV, malaria, HBV, and tuberculosis cannot be vaccinated against.

In 1996, the FDA released their first major documentation related to the production of DNA vaccines, "The points to consider on plasmid DNA vaccines for preventive infectious disease indications" (FDA). This document provided the guidelines needed to industrialize DNA vaccine production. This document was later revised in 2005 to the "Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications."

DNA VACCINES

Vaccination by naked plasmid DNA is the transfer of genetic material into a host. DNA vaccines can best be described as the expression of exogenous antigen contained on a mammalian expression vector. DNA Vaccination can be used to treat a number of diseases and conditions such as: influenza, HIV, HBV, HCV, CMV, tuberculosis, melanoma, non-Hodgkin's lymphoma, HSV, myocardidis, and various allergens (Taubes, 1997; Gregoriadis, 1998; Tuteja, 1999; Gurunathan et al., 2000; Srivastava and Liu, 2003).

The seminal study on DNA Vaccines showed that exogenous proteins could be expressed from naked DNA plasmid injected into the muscle cells of mice (Wolff et al., 1990). Later, humural and cellular immune responses from injected plasmid encoded antigens were shown (McDonnell and Askari, 1996). Davis et al. (1993) showed that muscle cell uptake led to the expression and extra cellular release of the antigen. The antigen was then taken up by antigen presenting cells (APC). It has also been suggested that APCs will directly take up DNA (Gurunathan et al., 2000; Leitner et al., 2000; Srivastava and Liu, 2003). Johnson et al. (1992) demonstrated the initial concept of direct DNA immunization. Ulmer et al. (1993) showed that mice were protected against influenza A if they had been injected with influenza DNA fragments. Subsequent work throughout the nineties further demonstrated the usefulness of DNA vaccines in combating disease (Robinson, 1993; Liu et al., 1996; Liu et al., 1997; Ulner, 1997). Work continues in the new millennium on some important disease targets as shown in Table 1. Currently, DNA vaccines for diseases such as HIV, Melanoma, and CMV are in phase I and II trials for humans (NIH, 2006).

Additionally, DNA vaccines for animals have received licenses by the USDA and are currently in use for West Nile (Horses, Fort Dodge, 2005), Infectious Haematopoietic Necrosis Virus (Salmon, Aqua Health, 2005) and Melanoma (Dogs, Merial Ltd., 2007).

Table 1. A sample of current DNA vaccine research efforts.

Disease Target	Reference
Intracellular pathogens	Gurunathan et al., 2000 Bergmann-Leitner and Leitner, 2004 Powell, 2004
Cancer treatment	Mountain, 2000 Ferber, 2001 Leitner and Thalnamer, 2003 Berzofsky et al., 2004
HIV	Barouch et al., 2000 Barouch et al., 2001 Sheets et al., 2006
Enteric Pathogens (Viral, Bacterial, Parasites)	Compiled by Herrman, 2006

Although plasmid DNA as a therapeutic compound is a novel technology, certain similarities are shared with currently used vaccination methods. Table 2 compares DNA vaccines with live and killed vaccines (Gurunathan et al., 2000). The advantages and disadvantages of DNA vaccines are reviewed in Table 3.

The production of DNA vaccines requires three steps. Step one involves the creation of the plasmid containing the gene of interest. The plasmid is then transformed into a selected host microorganism that will be used for its production, followed by clonal selection, medium adaptation and small-scale seed production (used to inoculate fermentors). Step two involves the production of the plasmid DNA. Fermentation methods, culture conditions, and fermentation scale-up are refined at this step.

Table 2. A comparison of vaccine methods.

		DNA Vaccine	Live Attenuated	Killed / Subunit
Immune Response				
Humoral	B cells	+++	+++	+++
Cellular	$CD4^+$	+++ Th1 lymphocytes	+/-Th1 lymphocytes	+/-Th1 lymphocytes
	$CD8^+$	++	+++	-
	Antigen Presentation	MHC class I&II	MHC class I&II	MHC class II
Memory	Humoral	+++	+++	+++
	Cellular	++	+++	+/-
Manufacturing				
	Ease of R&D and production	++++	+	++
	Cost	+++	+	+
	Transport/Storage	+++	+	+++
Safety		+++	++	++++

+ = level of effect

Table 3. Advantages and disadvantages of DNA vaccines.

Advantages	Disadvantages
• No harmful organism / infectious agents to work with • No risk of reversion (possible with attenuated vaccines) (Henke, 2002; Moreno and Timón, 2004) • Mimic effect of live attenuated vaccines (Gurunathan, 2000) • Manufacturing/Production, storage, and Quality Control are relatively simple (Mountain, 2000) • Defined product • Good safety	• Only 1 in every 1000 plasmids reach the nucleus and is expressed, inefficient transfection *in vivo* (Ferreira et al., 2000; Mountain, 2000). • Require dosages as high as several mg (Berzofsky et al., 2004; Okonkowski, 2005; Donnelly et al., 2003; Listner et al., 2006) • Short duration of expression in most tissues (Mountain, 2000) • Low immunological potency for self (tumor) antigens (Berzofsky et al., 2004)

In addition, harvest methods must be determined to coincide with the downstream processing methods. Step three involves the downstream processing of the plasmid DNA. Cellular debris along with all impurities (salts, endotoxins, and plasmid isoforms) must be removed leaving only a suitable percentage of supercoiled plasmid for the final product.

PLASMID VECTOR OVERVIEW

The active ingredient injected into a patient for a DNA vaccine is a purified suspension (can be mixed with a number of vaccine adjuvants) of double-stranded plasmid DNA. All plasmids used in DNA vaccination have several common features. Figure 1 is a schematic representation of a typical plasmid employed in the development of a DNA vaccine. The origin of replication (Ori) is a sequence of DNA at which plasmid replication is initiated. Plasmid origins are well-defined sites at which replication starts in both directions until the two replication forks meet. The type of Ori helps determine the maximum copy number of the plasmid per cell. Since the Ori on the vector is specific to bacterial replication, the injected plasmid cannot replicate in a eukaryotic host. A commonly used Ori is derived from the *E. coli* plasmid ColE1 (Carnes, 2005). The pUC derived plasmids, initially described by Vieira and Messing (1982), were derived from pBR322 (Bolivar et al., 1977).

Temperature sensitive mutations can increase yields (copy number) 30-40 times with a temperature increase from 30°C to 42°C (Wong et al., 1982; Lahijani et al., 1996). Boros et al. (1984), and later Lahijani et al., (1996) described a single G→A mutation that caused a 70-fold increase in plasmid yield with a shift from 37°C to 42ºC. The copy number of pUC-derived plasmids can be as high as 500-700 per cell (Minton, 1984; Minton et al., 1988). In addition to increases in copy number, the choice of Ori may increase the yield of supercoiled plasmid DNA, downstream recovery and purification (Shamlou, 2003).

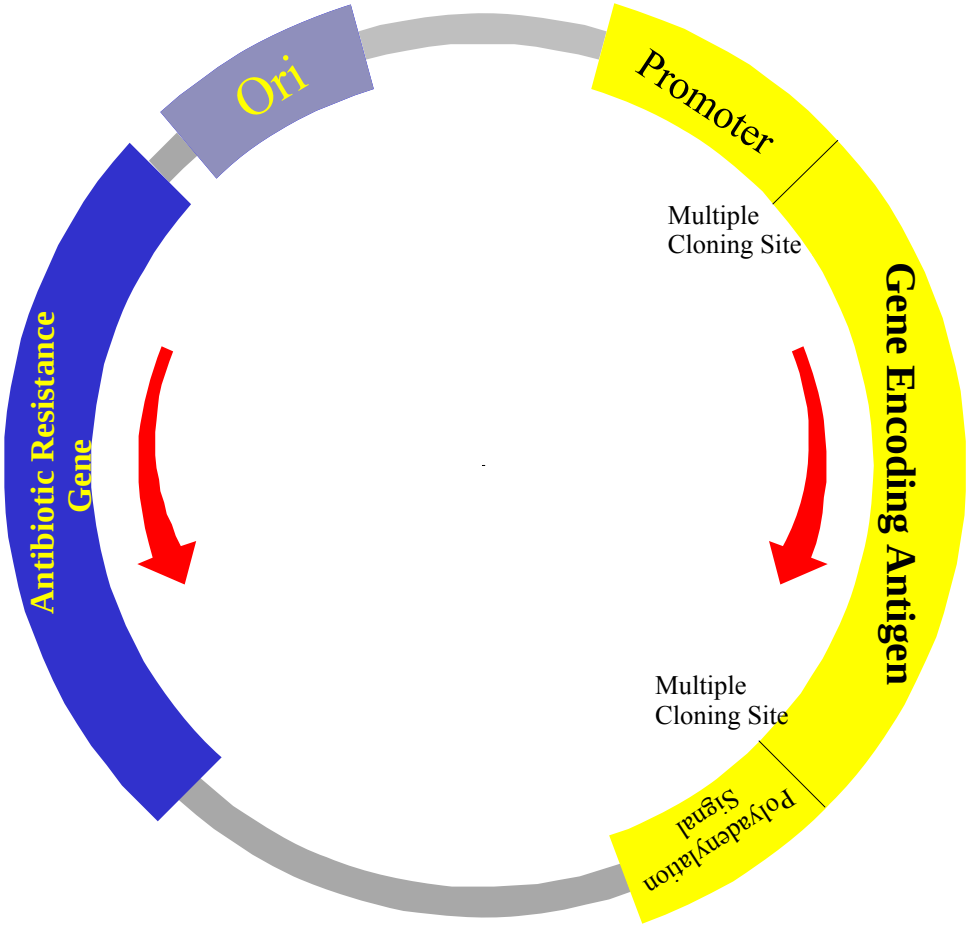

Figure 1. Schematic of the major components shared by most DNA vaccine plasmid vectors. Important features include the origin of replication, the antibiotic marker, the eukaryotic promoter, and the gene of interest.

Supercoiled plasmid DNA is the only form considered therapeutic by the FDA. Linear, nicked and relaxed isoforms are considered impurities and must be removed (FDA, 1996a, 1996b). Currently, the importance of the ratio of supercoiled (SC-pDNA) versus open circular plasmid (OC-pDNA) isoforms on the efficacy of DNA vaccination is only partially understood (Marquet et al., 1997; Middaugh et al., 1998; Bergan et al., 2000; Evans et al., 2000). Cherng et al. (1999) have shown that SC-pDNA has higher transfection efficiency than OC-pDNA. Przybylowski et al. (2007) showed a relationship to stability and percentage of SC-pDNA.

Purification processes strive for a minimum of 90% SC-pDNA per batch. Interaction between the plasmid, bacterial host and production process all contribute to the quality of the final pDNA (O'Kennedy et al., 2003).

An important component of a plasmid is the antibiotic resistance gene. This resistance is critical for maintaining the plasmid during the production process. Ampicillin, kanamycin, and tetracycline are the most commonly used selective markers. Unfortunately, ampicillin and other β-lactam antibiotics must be removed for all clinical work (FDA, 1996a; FDA, 1998) due to hypersensitivity reactions in patients. Kanamycin is currently the most widely used selection agent for DNA vaccines, since it does not present a significant allergic risk (Durland and Eastman, 1998).

The remaining components of the vector allow the gene of interest to transcribe once injected into the patient. Sufficient transcription of the gene of interest requires a strong viral promoter. The most commonly used strong viral promoters are either the cytomegalovirus immediate early gene (CMV-IE), simian virus 40 (sv40), or rous sarcoma virus (rsv) (Manoj and Babiuk, 2004). The CMV-IE promoter is considered the strongest based on in vitro expression studies (Lee et al., 1997). To ensure mRNA stability these promoters are paired with transcription terminators/polyadenylation signals (shown as poly (A) tail in Figure 4) derived from genes such as bovine growth hormone (bgh), sb40 and human β-globin (Tuteja, 1999; Prather et al., 2003). The gene encoding the antigen is cloned after the promoter employing a multiple cloning site within the vector.

Lastly, other components may be included on the vector, such as CpG motifs for immune response, intron sequences for nuclear transport and enhancers (Srivastava and Liu, 2003; Manoj and Babluk, 2004).

HOSTS

A second biological factor involved with DNA vaccine production is the host strain. Although the host and its components are removed during final processing of the plasmid DNA its identity is crucial to plasmid production. The bacterial host is the factory to produce plasmid DNA. In general, K12 derivatives of *E. coli* are the strain of choice. K12 variants are well characterized and exempted by the NIH guidelines for recombinant genetic research (Carnes, 2005). Because the selection of the best bacterial host is critical to the production process, most studies of particular strains have been conducted by specific companies. The results remain unpublished. It is probable that the synergy between plasmid, host and fermentation scheme is the major factor in the success of DNA plasmid production (Durland and Eastman, 1998).

The primary industrial criteria for a host include: high specific (defined as μg plasmid/mg dry cell weight) and volumetric production (defined as mg plasmid/L of culture), minimal production times, homogeneity of plasmid (> 90% SC pDNA), and low endotoxin levels. Currently, strains such as DH5 and its derivatives (DH5α, DH10), XL1-Blue, and JM109 meet the requirements of industry (Carnes, 2005). For example, Merck is developing its HIV vaccines using DH5 (Okonkowski et al., 2005). Additional *E. coli* strains, such as the B strain (BL 21), are also being investigated (Xu et al., 2005). Lastly, the chosen strain/plasmid combination must be in harmony with the production method (medium selection, fermentation, and downstream processing). Certain bacterial genes that affect plasmid production have been identified. These genes are described in Table 4. Some hosts are avoided (e.g. HB101) because they are gal^+ and produce a capsule (colonic acid), making cell lysis and downstream purification more difficult (Carnes, 2005).

PLASMID PRODUCTION

Once an appropriate plasmid has been constructed and the host strain chosen, optimization of plasmid production can commence. The production phase consists of two important components: medium selection and fermentation process development. The interaction between host, plasmid, medium, and fermentation dictates the success of the overall process. Furthermore, production will have a significant effect on the downstream processing of pDNA (O'Kennedy, et al., 2003). As discussed earlier DNA vaccines require dosages at the mg level. Therefore, to reduce costs, yields per fermentation must be maximized. Compounding this issue is the dearth of published papers on successful plasmid production techniques. Success of a production process is measured as:

- Specific yield – plasmid DNA per biomass
- Volumetric yield – plasmid DNA per liter of fermentation

Additionally, any reduction in times to produce these yields can reduce associated costs (yield/time). Indeed, much of the cost associated with DNA vaccines is determined during the production phase.

Medium Selection and Development

Carnes (2005) considered the following factors important in the development of a production media. These include:

- Affect of components on plasmid yield and quality
- Biomass yield
- Lot to lot consistency
- Downstream purification
- Regulatory concerns (current good manufacturing practices)

O'Kennedy et al. (2000) found that use of chemically defined medium (CDM) resulted in higher copy numbers.

Table 4. Major genotypes used in plasmid DNA host strains

Genotype	Consequence
recA	The *recA* family of genes is involved in the complex regulation of the SOS system (an *E. coli* regulatory network that is induced by DNA damage). In addition, proteins expressed by *recA* are also involved in homologous recombination between homologous molecules. r*ecA* mutants are completely deficient in homologous recombination.
endA1	*endA1* encodes the 12-kDa protein which non-specifically cleaves double-stranded DNA (dsDNA) approximately every 400 bp and degrades the DNA into oligonucleotides with an average size of 7bps. *endA* mutants improve yield and quality of the plasmid preparations.
gyrA	The *gyrA* family of genes encodes gyrases (i.e., ATP-dependent topoisomerases) involved in regulation of the chromosomal replication process that remove positive supercoils introduced during replication. Gyrases also have the ability to introduce negative supercoils into a relaxed DNA.
hsdRMS(rk^-mk^+):	The *hsd* family of genes encodes genes involved in protecting the bacterial chromosome from being digested by restriction enzymes (host specificity for DNA). These genes are involved in DNA methylation. Mutation prevents cleavage by an endogenous endonuclease system.
deoR	Mutation involves a gene that encodes a repressor protein. This repressor suppresses a set of genes involved in nucleotide utilization. In the absence of this gene, the regulated genes are expressed constitutively. Cells containing the *deoR* mutation transform at higher efficiency than their non-mutated counterparts.
dam	Encodes an enzyme that methylates A residues in the GATC sequence. Mutations eliminate the presence of methyl-A, which is not normally found in eukaryotes.
mcrA	Restriction system named Modified Cytosine Restriction is directed against DNA methylated at the sequence 5'...Cm^5CGG, which is its only known target (Raleigh and Wilson, 1986)
mcrBC	McrBC requires the presence of two (G/A)mC recognition elements (where mC is 5-hydroxymethylcytosine, N^4-methylcytosine or 5-methylcytosine appropriately spaced in the substrate DNA. DNA cleavage occurs in region between two recognition elements (Dila et al., 1990).
dcm	Encodes an enzyme that methylates the second C residue in the CC(A/T)GG sequence. Mutations eliminate the presence of methyl-C, which is normally founds in eukaryotes, but not in this sequence.
F^-	Conjugative, low copy number F plasmid; when present, it may contaminate the final plasmid preparation.
Prototrophic / minimal auxotroph	Defined media are desired for plasmid production as well as product characterization and cost. Host strains that require as few additional media components as possible are desired. For example, the strain SCS1 is a complete prototroph while DH5α only requires the vitamin thiamine.

Information adapted from Durland and Eastman, 1998.

In addition, they observed that a C:N ratio of 2.78 to 1 (molar ratio of carbon, as carbohydrate, to total nitrogen) resulted in the highest specific plasmid DNA yields (ten fold increase at optimum ratio). CDM supply only the elements needed for cell growth and generally contain the following components:

- Carbon source-glucose or glycerol
- Salts, minerals, and trace metals
- Nitrogen source

High density cell culture requires the correct balance of all these components to support cell growth and avoid inhibition (Lee, 1996; Shiloach and Fass, 2005).

The choice of carbon source plays a key role in cell yield and related acetate production. Since high acetate concentrations can inhibit growth rates and reduce biomass yields, acetate must be controlled (Luli and Strohl, 1990; Majewski and Domach, 1990). The effect of acetate on plasmid DNA production has not been investigated (Xu et al., 2005). Glucose is the most common carbon source, but also generates the most acetate. Xu et al. (2005) found that glycerol produced the highest specific plasmid production while glucose produced the highest volumetric yield. This can be explained since glycerol has been associated with poor cell growth. Determining the optimal medium composition can be a very costly and time-consuming process.

Fermentation Design

Two types of fermentations are used to produce DNA vaccines, batch and fed-batch. Each method has its advantages and disadvantages (summarized in Table 5). A simple schematic of a fed-batch fermentor is shown in Figure 2. The ability to dictate growth rates and grow cells to higher densities makes fed-batch fermentations the production method of choice. The problems related to high growth rates are high acetate production, plasmid instability, and lower percentage of supercoiled plasmid (Carnes, 2005).

As growth rate decreases, the specific yield generally increases (Bentley et al., 1990; Prather et al., 2003). For example, a change in copy number from 15 to 23 per cell was observed with a decrease in growth rate from 1.7 to 0.4 h^{-1} (Lin-Chao and Bremer, 1986). Chen et al. (1997), observed a change in specific yield from 0.7 μg/mg to 1.7 μg/mg associated with a change in growth rate of 0.69 to 0.13 h^{-1}. Seo and Bailey (1986) found that cultures growing at 0.6 – 0.8 h^{-1} contained less plasmid than cultures growing at 0.3-0.4 h^{-1} with a value of 0.2 h^{-1} as the low limit. Rozkov et al. (2006) observed an almost 2-fold increase in specific yields after a switch from batch production (μ_{max}=0.48 h^{-1} and 6 mg/g) to fed-batch (μ = 0.1 h^{-1} and 10 mg/g). Studies have also found that plasmid yields were highest after the culture had entered the stationary phase, a similar condition to the late phase of a fed-batch process (Hecker et al., 1985; Reinikainen et al., 1989).

In addition, super coiling and plasmid stability may be controlled by changes in dissolved oxygen, pH, and temperature (Goldstein and Drlica, 1984; Durland and Eastman, 1998). Additional strategies, such as temperature shock, chemical treatment, and amino acid starvation may have been used to increase specific yields (Shamlou, 2003). For example, a 37°C to 42°C-45°C shift in fermentation temperature (Lahijani et al., 1996), or the use of chloramphenical (Reinikainen et al., 1989).

Table 6 contains a compilation of published yield data along with information about the methods.

Table 5. Comparison between batch and fed-batch fermentations.

	Batch	**Fed-Batch**
Initial Setup	All nutrients are present in excess	All nutrients are present in excess. However, carbon source is at a low initial concentration
Controls	Temperature pH Dissolved oxygen	Temperature pH Dissolved oxygen Feed rate of limiting nutrient (carbon energy source)
Inputs	Air (O_2) Base	Air (O_2) Base Carbon source Any additional nutrients
Growth Rate Control	Will grow at μ_{max} but this value can be selected by lowering temperature or choice of carbon source	Will grow at rate of feed. An exponential feeding rate will provide a constant value for specific growth rate.
Advantages	Simplicity	Precision control of growth rate Higher Dry Cell Weight (DCW) (High Density Cell Culture) Higher plasmid yields
Disadvantages	Difficult to achieve desired low growth rates Can not be used for High Density Cell Culture Metabolic problems are associated with higher growth rates	Complexity

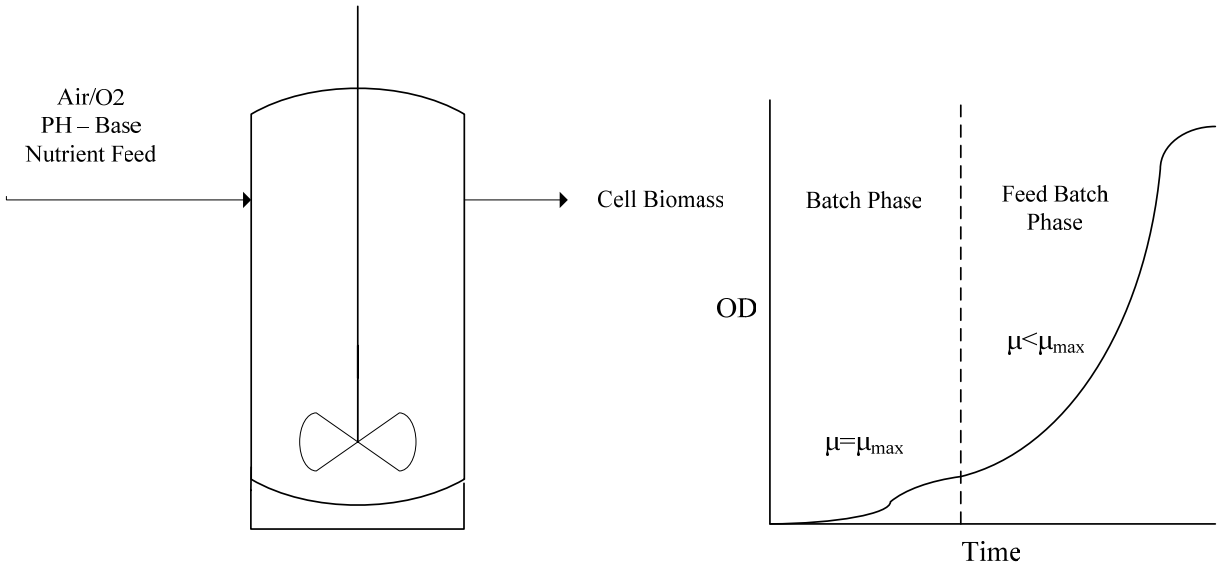

Figure 2. Basic schematic of inputs and outputs for a fed-batch fermentation. This graph is a representation of the growth rates in a fed-batch fermentation. The fermentation starts with growth at $\mu=\mu_{max}$ during the batch phase and transitions growth to $\mu<\mu_{max}$ according to the feed rates.

Table 6. Summary of DNA yield results.

Strain	Volumetric Yield (mg/L)	DCW (g/L)	Specific Yield (mg/g)	Method	Miscellaneous	Reference
DH5	220	~45	~ 4.8	Fed-batch	37°C to 42-45°C temperature shift	Lahijani et al., 1996
DH5α	230	60	3.83	Fed-batch with DO-stat feeding	glycerol and yeast extract $\mu = 0.15\ h^{-1}$	Schmidt et al., 2003
DH10β	82-98	80-105	1.7	Fed-batch DO-stat and pH-stat	$\mu = 0.13\ h^{-1}$	Chen et al., 1997
NR	130-250	70-90	1.8-2.78	Batch	Proprietary medium	Durland and Eastman, 1998
NR	260-430	55-65	6.61	Fed-batch	Proprietary medium High specific yields	Carnes and Williams, 2004
NR	670-1100	~45	~14.8-24.4	Fed-batch	37°C to 42°C temperature shift	Carnes and Williams, 2004
BL21	58.3	5.38	10.8	Shake Flask	Glucose as carbon source	Xu et al., 2005
DH5	634-846	NR	28-34	Fed-batch	Variable process steps	Okonkowski et al., 2005
DH5α	6.96	2.5	12.2	Shake Flask	Semi-defined medium with 1% casamino acids	O'Kennedy et al., 2000
JM109	60	3.5	17.1	Batch	$\mu = 0.33\ h^{-1}$	Wang et al., 2001
DH1	296	NR	10	Fed Batch	$\mu = 0.10\ h^{-1}$	Rozkov et al., 2006
DH5	1200	NR	25-32	Fed Batch	Large scale (2000L)	Listner et al., 2006
DH5α	1500	~33.5	~45	Fed-Batch	37°C to 42°C temperature shift $\mu = 0.12\ h^{-1}$	Carnes et al., 2006

NR = Not reported

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CHAPTER 2

OPTIMIZATION OF DNA PLASMID PRODUCTION BY USE OF DIFFERENT *ESCHERICHIA COLI* HOST STRAINS[1]

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Introduction

The market for gene therapy based medicines is expected to reach 45 U. S. billion dollars by 2010 (Glaser, 1997). Wolf et al. (1990) demonstrated that exogenous proteins could be expressed from plasmid DNA injected into the muscle cells of a mouse, and by 2007, 16% of clinical therapies included the use of "naked" plasmid DNA as the vector (http://www.wiley.co.uk/genetherapy/clinical). Ulmer et al. (1993) were able to protect mice from influenza A by injecting them with influenza DNA fragments. Over 100 human DNA vaccines are currently in clinical trials (Listner et al., 2006), and major human disease targets HIV, cancer, enteric pathogens, malaria and influenza use plasmid DNA vaccines (Tuteja, 2002; Gilbert, 2005; Herrman, 2006; Cantanzaro et al., 2007; Laddy et al., 2007; Lowe et al., 2007, Sheets et al., 2006).

DNA vaccines require milligram dosages to elicit proper immune responses (Donnelly et al., 2003; Berzofsky et al., 2004; Okonkowski, 2005; Listner et al., 2006), and therefore production methods must maximize volumetric and specific yields. Production of large quantities of plasmid DNA depends on many factors including host strain, choice of plasmid, media components, fermentation type/strategy and downstream processing (Durland and Eastman, 1998; Carnes, 2005). Many successful methods use *Escherichia coli* in high-density culture (Shiloach and Fass, 2005), and previous studies have shown that specific growth rate affects yield (Seo and Bailey, 1986; Bentley et al., 1990; Chen et al. 1997; Prather et al., 2003). Fed-batch fermentations routinely achieve cell densities as high as 40 g/L. Rozkov et al. (2006) observed nearly a 2-fold increase in specific yield in a fed-batch process with a low specific growth rate ($\mu = 0.1\ h^{-1}$), compared to a batch process ($\mu_{max}=0.48\ h^{-1}$). Additional strategies such as temperature shock, chemical treatment, and amino acid starvation also can increase specific

yields (Shamlou, 2003). For example, a temperature shift from 37°C to 42°C-45°C resulted in a 70-fold increase in yield (Lahijani et al., 1996), while the use of chloramphenical resulted in a 5-fold increase in plasmid production (Reinikainen et al., 1989).

Most previous studies have used one of only a very few *E. coli* strains such as DH5 (Okonkowski et al., 2005; Listner et al., 2006) or DH5α (Schmidt et al., 2003). For industrial production, a screening procedure that correlates DNA production with fed-batch processes from small, flask scale would save much effort.

The goal of this study was to compare several *E. coli* strains for plasmid DNA production. The strains each were transformed with a high copy plasmid and compared for DNA production in a defined medium using either glycerol or glucose as the carbon source. Select strains were then grown at a 7L fermentor under fed-batch conditions.

Materials and Methods

Strains and Plasmids. Strains used in this study are shown in Table 7. Transformed strains were maintained in chemically defined media (CDM) supplemented with 25% glycerol (v/w) at -70°C. Plasmids used in this study were pLL14 (Merial Ltd, Lyon France), a derivative of pVR1012 (Hartikka et al., 1996; Vical Inc., San Diego, CA) and pUC19 (Norrander et al., 1983). Both plasmids are high copy number, and pLL14 contains a ColE1-like origin and a kanamycin resistance gene while pUC19 contains a ColE1 origin and an ampicillin resistance gene (Yanisch-Perron et al., 1985). Chemical transformation was used following manufacturers' directions or using a chemical transformation kit (Qbiogene, Irvine, CA). Transformed strains on CDM agar plates with 5 g/L glycerol and 100 μg/mL kanamycin or ampicillin were incubated at 37°C for 24 h prior to commencing overnight cultures.

Clonal Selection. After transformation on LB plates containing an antibiotic, five single colonies of each strain were adapted to CDM plates with two passes. Each clone was grown in three mL for 10-12 h at 37°C and 200 rpm. After harvest, DNA was quantified, and the clones with the highest production and best growth characteristics were chosen. Working seed batches were produced and frozen at -70°C with 15% (v/v) glycerol.

Table 7. Strains used in this study.

Strain	Genotype	Source
BL21	B F- *dcm ompT hsdS* (r_B^-, m_B^-) *gal*	Stratagene
DH1	F- λ- *supE44 hsdR17 recA1 gyrA96 relA1 endA1 thi*-1	ATCC 33849
DH5α	F- Φ80*lac*ZΔM15 Δ(*lac*ZYA-*argF*)U169 *recA1 endA1 hsdR17*(r_k^-, m_k^+) *phoA supE44* λ^- *thi-1 gyrA96 relA1*	Invitrogen
JM105	F- Δ(*lac proAB*) *lacI*^{q} *thi repsL endAl slcB15 hadR4 traD36 proAB* Δ(ZM15)	Pharmacia
JM109	e14$^-$(McrA$^-$) r*ecA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) [F' *traD36 proAB lacI*^{q}*ZΔM15*]	Promega
Mach 1	F- Φ80(*lacZ*) ΔM15 Δ*lac*X74 *hsd*R(r_K^- m_K^+) Δ*recA*1398 *endA1 tonA*	Invitrogen
MG1655	F- λ- *ilvG rfb*-50 *rph*-1	ATCC 700926
MC1061	F- Δ*lacX74 rpsL araD139* Δ*(ara leu)7697 galU galK hsdR mcrB thi*	Laboratory Collection
MC4100	F- *araD*139 Δ(*argF-lac*)U169 *rpsL150 relA1 deoC1 rbsR fthD*5301 *fruA*25 λ -	ATCC 35695
NM554	MC1061 *recA13*	Laboratory Collection
SCS1-L	*recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1*	Merial LTD, Lyon France
SCS1-S	*recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1*	Stratagene
SE5000	MC4100 *recA1*	Laboratory Collection

Growth Conditions. Chemically defined medium (CDM) contained (per L): 6.0 g citric acid, 20.0 mg $MnSO_4 \cdot H_2O$, 8.0 mg $CoCl_2 \cdot 6H_2O$, 4.0 mg $ZnSO_4 \cdot 7H_2O$, 3.6 mg $AlCl_3 \cdot 6H_2O$, 3.97 mg $Na_2MoO_4 \cdot 4H_2O$, 3.2 mg $CuSO_4 \cdot 5H_2O$, 1.0 mg H_3BO_3, 800 mg $MgSO_4 \cdot 7H_2O$, 52.8 mg $CaCl_2 \cdot 2H_2O$, 74.0 mg $FeSO_4 \cdot 7H_2O$, 8.0 g KH_2PO_4, 2.4 g $Na_2HPO_4 \cdot 2H_2O$, 750.0 mg $(NH_4)_2SO_4$, 8.0 g $(NH_4)_2HPO_4$, 130.0 mg NH_4Cl, and 10 mg thiamine·HCl. For MC1061 and NM554, the medium also contained 20.0 mg/L leucine. Luria-Bertani (LB) medium contained (per L): 10.0 g bacto-tryptone, 5.0 g yeast extract, and 10.0 g NaCl. The pH was adjusted to 6.8 using 28% NH_4OH, and sterilized for 30 min at 121°C. Kanamycin (Km) or ampicillin (Am) was added at 100 mg/L after sterilization.

Flask cultures were grown at 37°C and 200 rpm in 250 mL baffled flasks containing 30 mL CDM with 5 g/L glycerol or glucose. Cultures were grown to an OD of 1.

Fed-batch fermentations were carried out in a 7 L bioreactor (Applikon Biotechnology, Foster City, CA) with a 3.5 L starting volume. Control was provided by BioexpertXP software, ADI 1030 Bio-controller and an ADI 1035 Bio-console (Applikon). CDM initially contained 5 g/L glucose, 2 mL of antifoam (Antifoam 1500, Dow Corning, Midland, MI), and 100 mg/L Km. Vessels were inoculated with 35 mL of a shake flask culture (5 g/L glycerol and 100 mg/L Km) grown to an OD of 1. Fermentations were carried out at 37°C, a pH of 6.8 (controlled with 15% NH_4OH) and an agitation of 1000 rpm. Dissolved O_2 (DO) was maintained above 20% with aeration at 2.45 L/min (O_2 and air mixed as necessary). Aeration commenced when the DO initially reached 30%. Fed-batch mode was initiated when the initial glucose was exhausted, as indicated by the increase in DO. The feed solution contained 60% glucose (w/v), $MgSO_4 \cdot 7H_2O$ (8.5 g/L) and 1 mL/L antifoam. Feed rates were controlled at exponentially increasing flow rates using a mini-pulse pump (Gilson, Inc, Middleton, WI) and the following control algorithms

adapted from Jones and Anthony (1977) to maintain a constant specific growth rate at either 0.14 h^{-1} or 0.28 h^{-1}:

$$F_0 = \mu\left(\frac{V \cdot s_0}{s_i}\right) \cdot e\left(\mu\left(\frac{t}{3600}\right)\right) \cdot \left(\frac{48000}{T \cdot 60}\right)$$

$$F_i = \left(\frac{F_0}{48}\right) \cdot \left(\frac{s_i}{60}\right) \cdot 0.005$$

Here F_i was the feed flow rate (mL/s), F_0 was the pump head speed (rpm), s_i was the feed substrate concentration (g/L), s_0 was the initial substrate concentration (g/L), μ was the desired specific growth rate (h^{-1}), T was the maximum feed rate of pump (mL/min), t was the process time (min). When the OD reached 60-70, the temperature was raised to 42°C and the feeding rate was reduced by 10% for 4 h ("heat treatment").

Sampling and Analysis. Cell growth was measured as optical density (OD) at 562 nm (Pharmacia LKB Ultraspec III, St. Albans, UK). This measurement was correlated to dry cell weight (DCW). For DCW measurement, 20 mL of cell culture was centrifuged (10 min at 3000 × g), the supernatant decanted, the pellet washed with DI water and the tube centrifuged twice again. The pellet was resuspended in DI water and poured into a weighing boat and dried at 60°C for 24 h**.** DNA samples were obtained from column purifications (Qiagen, Miniprep) for 3 mL of sample having an OD of 1, to ensure the binding capacity of the column was not exceeded. The absorbance was measured at 260, 280 and 320 nm and the DNA concentration was calculated via the spectrophotometer software. Samples that fell in the linear range ($0.1 \leq A_{260} \leq 1.0$) were accepted. Purity was determined by a 260:280 of 1.8 to 2.0. Acetate was measured by liquid chromatography using a refractive index detector as previously described (Eiteman and Chastain, 1997).

Results

DNA Yield Comparison in Shake Flask Cultures. Thirteen strains of *E. coli* were first compared for their production of plasmid DNA in a defined medium in shake flasks. Two different plasmids were compared: pL114 and pUC19. The differences between these plasmids were the selectable markers (kanamycin for pLL14 and ampicillin for pUC19) and the ability to induce plasmid production by increasing incubation temperatures in pLL14. Furthermore, pLL14 contains a single G→A mutation that can increase copy number up to 70-fold (Lahijani et al., 1996). Two different carbon sources were compared: glycerol and glucose. Table 8 shows the results for pL114 with each carbon source, while Table 9 shows analogous results for pUC19. Since these shake flasks were conducted in a batch mode, the strains each grew at their maximum growth rate during the experiment.

Figures 3 and 4 show the distribution of growth rates and DNA yields for pLL14 and pUC19, respectively. Any given strain achieved a higher DNA yield with the pLL14 plasmid compared to the pUC19 plasmid. We observed a slight negative relationship between DNA yield and growth rate. That is, lower specific growth rate favored greater DNA yield, particularly for plasmid pUC19. Moreover, for each plasmid the use of glucose as a carbon source generally resulted in higher growth rate and lower DNA yield than the use of glucose. However, exceptions to these general observations were found for both plasmids.

In order to compare the two carbon sources, for each strain/plasmid we calculated the *growth ratio*: the maximum specific growth rate (μ_{MAX}) observed with glycerol divided by the μ_{MAX} observed with glucose. Similarly, the *yield ratio* was calculated as the DNA yield observed when glycerol was the carbon source divided by the DNA yield observed with glucose. Generally, strains had a higher value of μ_{MAX} with glucose than during growth in glycerol

(growth ratio < 1) and a greater DNA yield when glycerol was the sole carbon source compared to glucose (yield ratio > 1). Figure 5 shows all the yield ratios for both strains as functions of the respective growth ratios. Regardless of plasmid, most tested strains had a *growth ratio* of less than 0.8 and although some strains with low growth ratio showed also a low yield ratio, the highest yield ratios were found at growth ratios between 0.5 and 0.8. Strains with the greatest differences in maximum growth rate for each substrate generally had a greater difference in yield for each substrate.

Of the strains studied, only SCS1-S attained a higher specific growth rate using glycerol than using glucose (for both plasmids). Interestingly, this strain showed very low DNA yield ratio, with the DNA yield observed when the strain grew on glucose being approximately equal to the DNA yield using glycerol. So, this strain behaved differently from all the other strains. For both growth and yield, the results with glucose and glycerol were very similar. Considering the plasmid pLL114, volumetric yields using glycerol ranged from 1 mg/L (MG1655) to 3 mg/L (SCS1-S and SCS1-L), while using glucose yields ranged from 0.5 mg/L (MG1655) to 3 mg/L (SCS1-S). BL21 (2.61 mg/L), SE5000 (2.61 mg/L) and NM554 (2.62 mg/L) were other strains which generated high DNA yields using glycerol as the carbon source. The two strains which consistently generated the lowest DNA (regardless of plasmid or carbon source) were MC4100 and MG1655. Generally, acetate formation from glucose correlated with high growth rate. Although no strain generated both a high acetate concentration and a high DNA yield, there was no correlation between DNA yield and acetate formation for either plasmid (Figure 6).

Table 8. DNA yield, maximum specific growth rate and acetate concentration for strains containing plasmid pLL14. Strains were grown in defined medium in shake flasks to an OD of 1. Standard deviation of yield measurements is shown in parentheses.

Strain	DNA Yield (mg/L)			Maximum Specific Growth Rate (h^{-1})			Acetate (mg/L)	
	Glycerol	Glucose	Ratio	Glycerol	Glucose	Ratio	Glycerol	Glucose
BL21	2.61 (0.02)	2.34 (0.08)	1.11	0.45	0.77	0.59	0	0
DH1	1.76 (0.02)	1.09(0.05)	1.63	0.61	0.90	0.68	0	60
DH5α	2.06 (0.16)	1.42 (0.03)	1.45	0.58	0.99	0.58	0	180
JM105	1.42 (0.09)	0.70 (0.08)	2.02	0.44	0.64	0.68	0	190
JM109	1.97 (0.18)	1.94 (0.06)	1.02	0.51	0.70	0.73	0	190
Mach 1	2.37 (0.16)	1.40 (0.03)	1.69	0.63	0.84	0.75	0	60
MC1061	1.78 (0.07)	1.62 (0.11)	1.10	0.32	0.35	0.91	0	80
MC4100	1.27 (0.05)	0.85 (0.06)	1.50	0.79	0.92	0.86	80	360
MG1655	1.02 (0.07)	0.50 (0.04)	2.03	0.49	0.80	0.61	0	80
NM554	2.62 (0.10)	1.81 (0.04)	1.44	0.39	0.56	0.70	0	210
SCS1-L	3.00 (0.12)	2.06 (0.13)	1.46	0.45	0.90	0.50	0	180
SCS1-S	3.04 (0.09)	3.03 (0.18)	1.00	0.42	0.38	1.11	0	80
SE5000	2.61 (0.20)	1.56 (0.08)	1.67	0.57	1.00	0.57	0	200

Table 9. DNA yield, maximum specific growth rate and acetate concentration for strains containing plasmid pUC19. Strains were grown in defined medium in shake flasks to an OD of 1. Standard deviation of yield measurements is shown in parentheses.

Strain	DNA Yield (mg/L)			Maximum Specific Growth Rate (h^{-1})			Acetate (mg/L)	
	Glycerol	Glucose	Ratio	Glycerol	Glucose	Ratio	Glycerol	Glucose
BL21	0.84 (0.03)	0.78(0.05)	1.07	0.45	0.52	0.87	0	0
DH1	1.22 (0.05)	0.55 (0.06)	2.23	0.47	0.79	0.59	0	60
DH5α	1.30 (0.06)	1.06 (0.09)	1.23	0.46	0.70	0.66	0	0
JM105	1.26 (0.24)	0.91 (0.00)	1.38	0.59	0.82	0.72	0	210
JM109	1.49 (0.06)	0.81 (0.04)	1.84	0.56	0.86	0.65	0	300
MC1061	1.43 (0.09)	0.81 (0.05)	1.75	0.39	0.46	0.85	10	80
MC4100	0.56 (0.05)	0.40 (0.06)	1.40	0.74	1.10	0.67	0	220
MG1655	0.72 (0.03)	0.43 (0.02)	1.68	0.58	0.87	0.67	0	200
NM554	1.61 (0.07)	0.86 (0.10)	1.87	0.33	0.47	0.70	0	80
SCS1-S	1.19 (0.05)	1.35 (0.05)	0.88	0.41	0.40	1.03	0	40
SCS1-L	1.41(0.03)	0.86 (0.08)	1.64	0.34	0.62	0.55	0	0

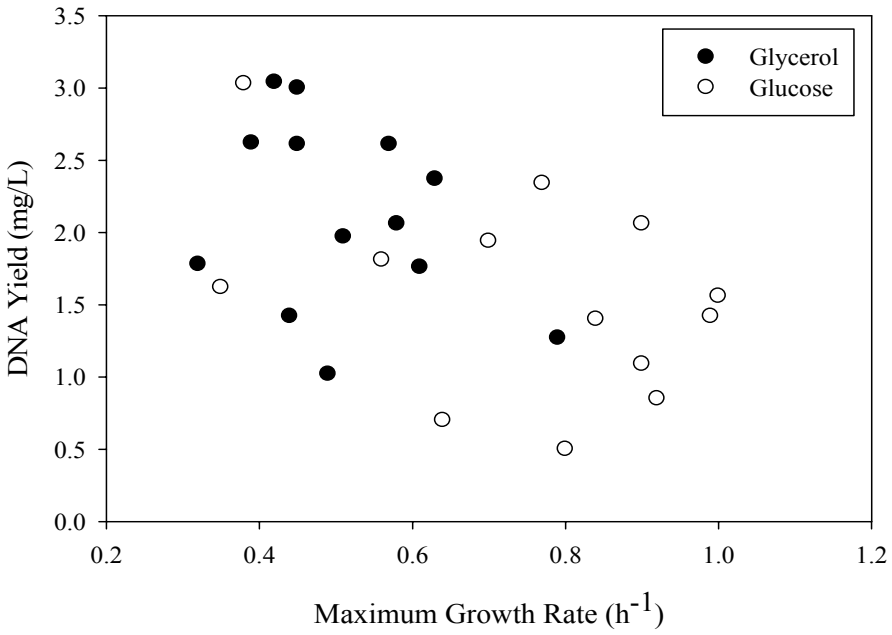

Figure 3. Growth rates and volumetric DNA yields for various *E. coli* strains (listed in Table 8) containing pLL14 strains grown in defined medium containing either glycerol (●) or glucose (○). Strains were grown in shake flask to an OD of 1.

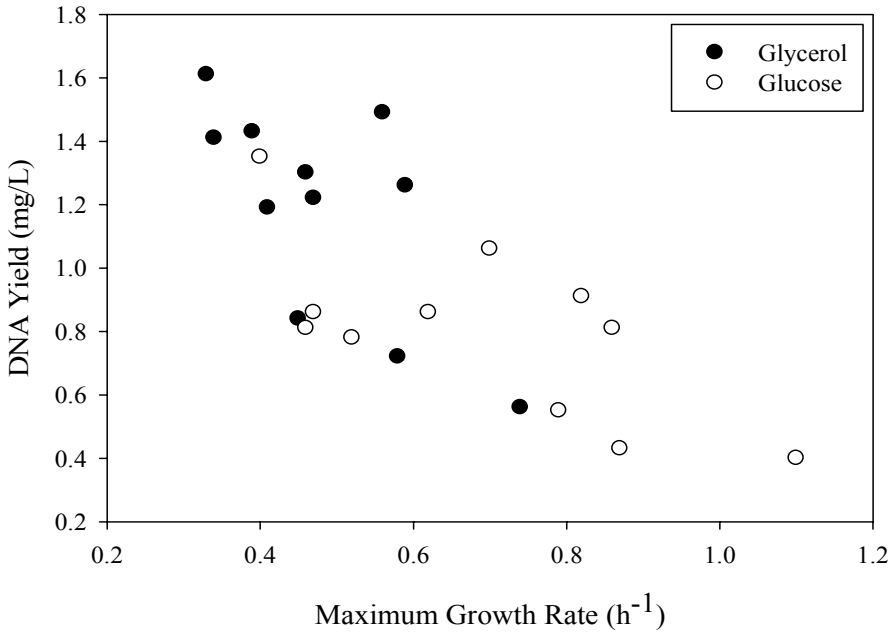

Figure 4. Growth rates and volumetric DNA yields for various *E. coli* strains (listed in Table 9) containing pUC19 strains grown in defined medium containing either glycerol (●) or glucose (○). Strains were grown in shake flask to an OD of 1.

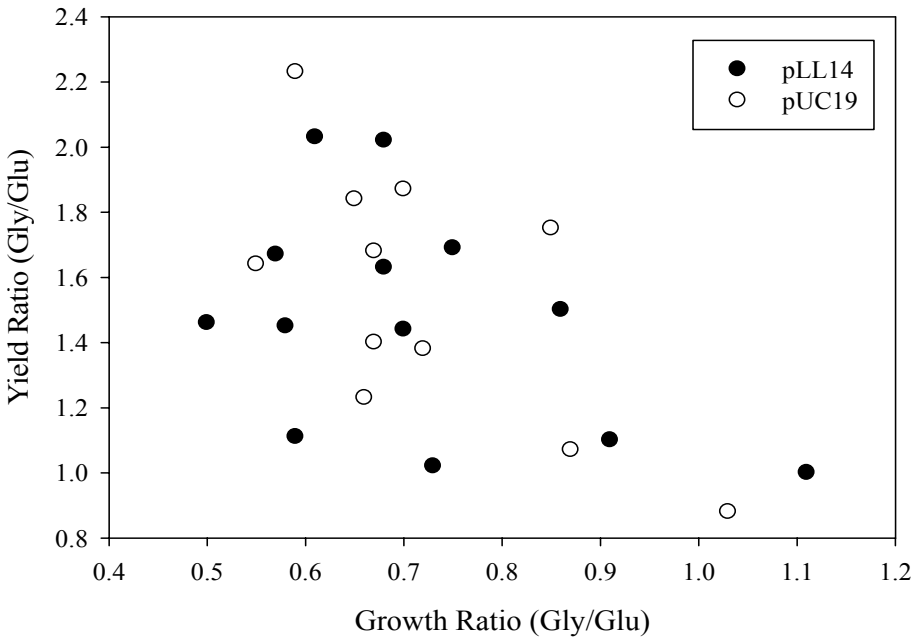

Figure 5. Comparison of DNA yield ratio and specific growth ratio for strains containing either pLL14 (●) or pUC plasmids (○). Strains were grown in defined medium in shake flask to an OD of 1.

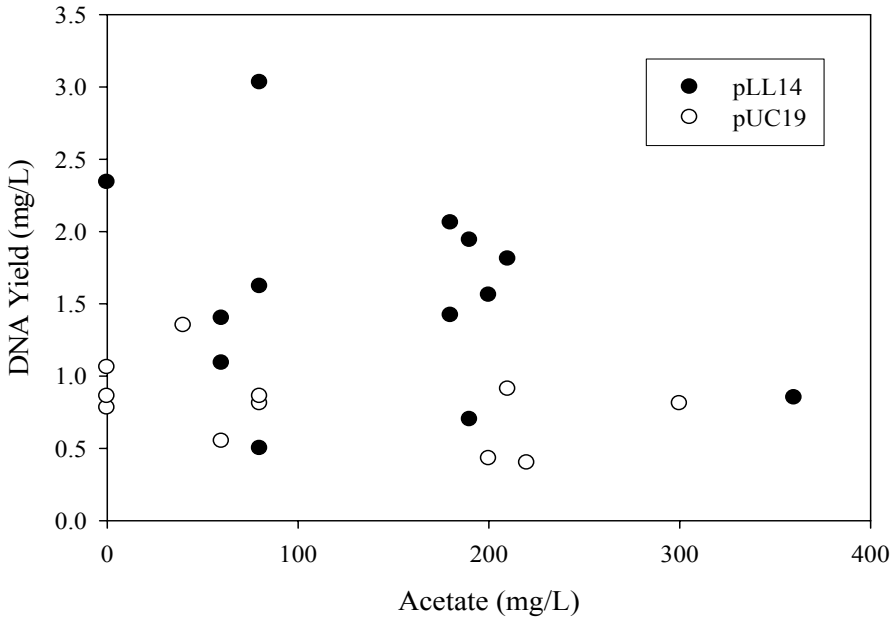

Figure 6. Acetate concentrations and volumetric DNA yields for various *E. coli* strains containing either pLL14 (●) or pUC plasmids (○). Strains were grown in defined medium in shake flask to an OD of 1.

Two sets of isogenic pairs were examined. These pairs include MC4100-SE5000 and MC1061-NM554. Both SE5000 and NM554 are *recA* mutants of their respective pair. In each of the eight cases (two plasmids, two carbon sources, two isogenic pairs), the *recA* mutants yielded more DNA than the control strain (i.e., with the *recA*$^+$ gene). The difference between the pairs was greatest for shake flasks in which glycerol was the carbon source, and for the pLL14 plasmid. The greatest increase (106%) resulting from the *recA* mutation was observed for the MC4100-SE5000 pair with glycerol and the pLL114 plasmid.

Fed-batch fermentations. Three strains of *E. coli* were compared for their production of plasmid DNA in a defined medium in a fed-batch fermentation. Three strains were chosen for the fed-batch process operated at a specific growth rate of 0.14 h^{-1} (SCS1-L, BL21 and MC4100), while two strains were examined at a higher specific growth rate of 0.28 h^{-1} (SCS1-L and MC4100). Each fermentation was conducted using a glucose-limited feed and the strains contained the pLL114 plasmid. Biomass, volumetric and specific DNA yields, and acetate were measured during the course of each fermentation. Table 10 shows the maximum yields for each fermentation prior to and post heat treatment.

Figures 7, 8 and 9 show the biomass, volumetric yield and specific yield for example fermentations of each strain at the specific growth rate of 0.14 h^{-1}, while Figures 10 and 11 show these results for two example fermentations at a specific growth rate of 0.28 h^{-1}..

OD and DNA Yield. At the lower growth rate, OD and volumetric yield increased over time for each strain. SCS1-L produced the greatest volumetric yield (after heat treatment) of 603 mg/L and 7.35 mg/L/OD. BL21 showed the second highest volumetric yield of 519 mg/L and 6.23 mg/L/OD while MC4100 had the poorest volumetric yield of 222 mg/L and 2.78 mg/L/OD. For SCS1-L, the specific yield increased slowly over time to a maximum value of 18.85 mg/g.

For BL21 the specific yields decreased slightly until the heat treatment for a maximum of 15.58 mg/g. MC4100 had relatively constant specific yields even after the heat treatment for a maximum of 7.30 mg/g. Overall SCS1-L produced the highest levels of DNA while MC4100 generated the least quantities For high growth rates, SCS1-L ended the fermentation with a volumetric yield of 611 mg/L and 6.71 mg/L/OD, while MC4100 had a volumetric yield of 353 mg/L and 3.50 mg/L/OD. Specific yields stayed relatively the same until after the heat treatment for SCS1-L for a maximum value of 17.21 mg/g. MC4100 had a gradual increase in specific yield throughout the fermentation to a maximum value of 9.19 mg/g.

As noted in the Materials and Methods section, the temperature was raised to 42ºC for 4 hours immediately after biomass had reached an OD of about 60-70, because such a treatment for the pLL14 plasmid can increase yields (Wong et al., 1982; Lahijani et al., 1996). We calculated whether the heat treatment did indeed improve DNA yield (Table 11). Generally, the heat treatment improved the specific DNA yield. An exception to this rule was MC4100 at low growth rate, in which the heat treatment reduced the DNA yield by 17%. The improvement in DNA yield due to heat treatment was greater for fed-batch processes operating at the higher growth rate.

Two of the strains (SCS1-L and MC4100) we compared for DNA production at two different specific growth rates, 0.14 h^{-1} and 0.28 h^{-1}. Prior to heat treatment, the DNA yield was lower at higher growth rates for both strains (Table 12). For example, the specific DNA yield for SCS1-L was 28% less at a growth rate of 0.28 h^{-1} compared to a growth rate of 0.14 h^{-1}. Because heat treatment resulted in a more significant improvement in DNA yield at higher growth rate as described above, the DNA yield was similar or greater for the two growth rates after heat treatment. Specifically, for SCS1-L the specific DNA yield was only 7% lower at the higher

growth rate compared to the lower growth rate, while for MC4100 the specific DNA yield increased by 35% with an increase in growth rate. A higher growth rate permitted the cells to reach the same OD in less time. Thus, the rate of DNA production as reflected by the volumetric productivity was quite different because of an increase in specific growth rate (Figure 12). For SCS1-L, the volumetric DNA productivity was 48% greater when the fed-batch process was conducted at a growth rate of 0.28 h^{-1} compared to a growth rate of 0.14 h^{-1}. For MC4100, the volumetric DNA productivity was 137% greater when the fed-batch process was conducted at the higher growth rate.

No correlation between acetate formation and DNA yield was observed (Table 10). SCS1-L at both growth rates resulted in very low acetate accumulation. Although BL21 and MC4100 showed similar acetate concentrations at low growth rates (100 mg/L versus 125 mg/L after heat treatment), these two strains generated quite different amounts of DNA. We also did not observe a correlation between growth rate and acetate formation for MC4100 and SCS1-L.

Discussion

Much of the recent work on DNA plasmid production has focused on improved large-scale processes to maximize yield, and some successful approaches are summarized in Table 13. Generally, it is only feasible to examine very few variables for DNA production. Most production schemes limit testing, for example, to media components (O'Kennedy et al., 2000; Xu et al., 2005) or slight changes in growth rate (Chen et al., 1997, Rozkov et al., 2006). Exhaustive studies on variables such as strain, growth rate, and medium components effects on replication of plasmids containing ColE1-like origins of replication have not been published (Wang et al., 2004).

Table 10. Maximum DNA yields during fed-batch fermentations at $\mu = 0.14\ h^{-1}$ and $\mu = 0.28\ h^{-1}$. Standard deviation for triplicate samples are in parentheses.

Run	Strain	μ (h^{-1})	Maximum DNA Yield (pre-heat)				Acetate (mg/L)	Maximum DNA Yield (post-heat)				Acetate (mg/L)
			Volumetric (mg/L)	mg/L/ OD	Specific (mg/g)	OD		Volumetric (mg/L)	mg/L/ OD	Specific (mg/g)	OD	
1	SCS1-L	0.14	439 (22)	6.27	16.10 (0.81)	70	30	603 (7)	7.35	18.85 (0.22)	82	0
2	SCS1-L	0.14	442 (18)	6.23	15.98 (0.66)	71	0	601 (5)	6.99	17.92 (0.15)	86	10
3	SCS1-L	0.28	249 (11)	4.08	10.48 (0.47)	61	0	611 (14)	6.71	17.21 (0.38)	91	0
4	SCS1-L	0.28	297 (40)	4.87	12.56 (1.67)	61	0	602 (21)	6.69	17.13 (0.60)	90	0
1	BL21	0.14	267 (19)	4.94	12.37 (12)	54	20	442 (6)	6.23	15.58 (0.21)	71	100
2	BL21	0.14	283 (26)	4.29	11.00 (1.00)	66	80	519 (16)	5.90	15.12 (0.48)	88	100
1	MC4100	0.14	189 (3)	2.91	7.55 (0.13)	65	10	191 (6)	2.33	6.13 (0.19)	82	150
2	MC4100	0.14	213 (18)	3.28	8.60 (0.71)	65	10	222 (11)	2.78	7.30 (0.36)	80	100
3	MC4100	0.28	186 (9)	2.78	7.29 (0.34)	67	0	353 (8)	3.50	9.19 (0.21)	101	20
4	MC4100	0.28	112 (5)	2.11	5.58 (0.25)	53	0	292 (18)	3.40	8.94 (0.54)	86	20

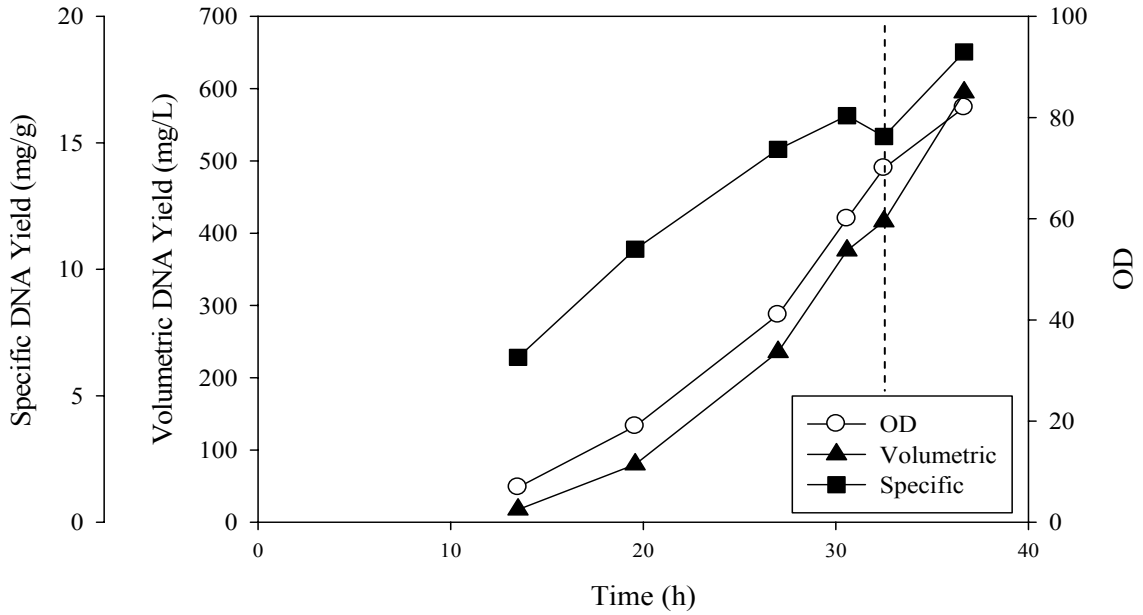

Figure 7. Production of DNA in SCS1-L during a glucose-limited fed-batch process with μ=0.14 h^{-1}: Volumetric DNA yield (▲), specific DNA yield (■), and OD (○). Heat treatment effects are shown to the right of the dashed line.

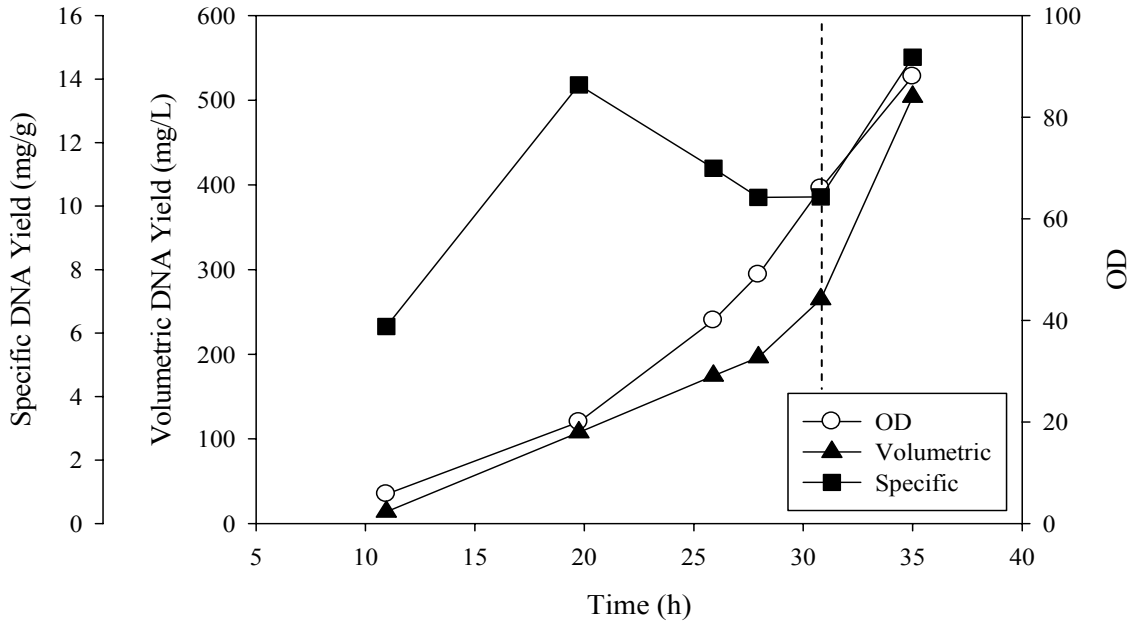

Figure 8. Production of DNA in BL21 during a glucose-limited fed-batch process with μ=0.14 h^{-1}: Volumetric DNA yield (▲), specific DNA yield (■), and OD (○). Heat treatment effects are shown to the right of the dashed line.

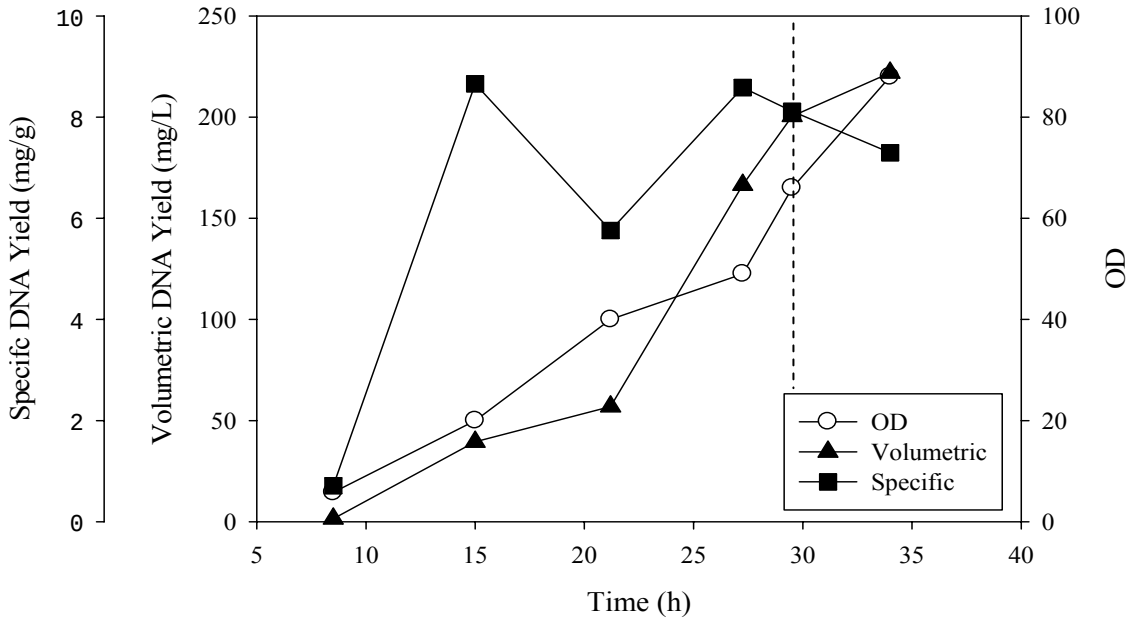

Figure 9. Production of DNA in MC4100 during a glucose-limited fed-batch process with μ=0.14 h^{-1}: Volumetric DNA yield (▲), specific DNA yield (■), and OD (○). Heat treatment effects are shown to the right of the dashed line.

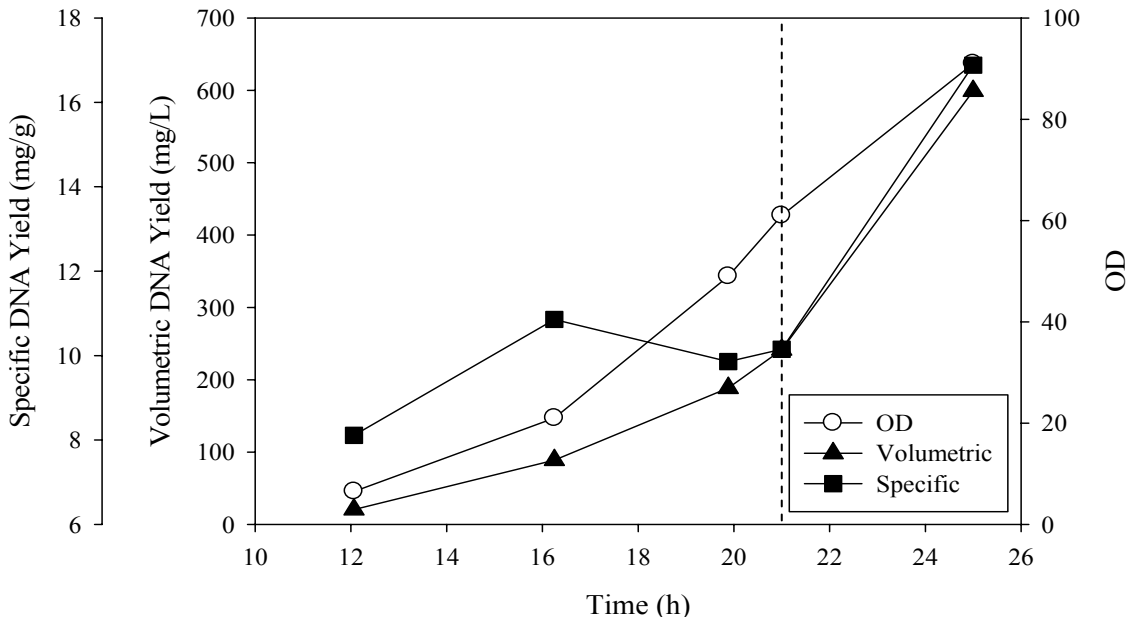

Figure 10. Production of DNA in SCS1-L during a glucose-limited fed-batch process with μ=0.28 h^{-1}: Volumetric DNA yield (▲), specific DNA yield (■), and OD (○). Heat treatment effects are shown to the right of the dashed line.

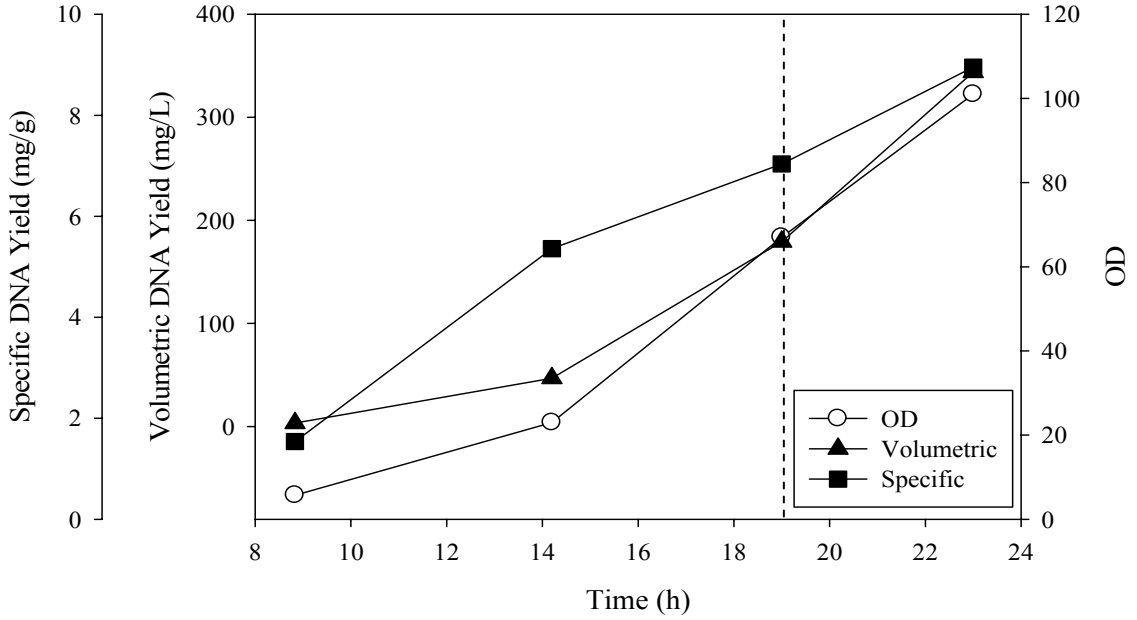

Figure 11. Production of DNA in MC4100 during a glucose-limited fed-batch process with μ=0.28 h^{-1}: Volumetric DNA yield (▲), specific DNA yield (■), and OD (○). Heat treatment effects are shown to the right of the dashed line.

Table 11. The effect of heat treatments on DNA yields during a fed-batch process.

Strain	μ (h^{-1})	Improvement in Specific DNA Yield (%)
SCS1-L	0.14	14.6%
SCS1-L	0.28	50.3%
BL21	0.14	31.7%
MC4100	0.14	-17.0%
MC4100	0.28	43.1%

Table 12. Affect of growth rate on specific DNA yield and volumetric productivity.

Strain	μ (h^{-1})	Average specific DNA yield (before heat treatment) (mg/g)	Average specific DNA yield (after heat treatment) (mg/g)	DNA productivity (after heat treatment) (mg/Lh)
SCS1-L	0.14	16.04	18.39	16.39
	0.28	11.52	17.17	24.26
MC4100	0.14	8.08	6.72	5.98
	0.28	6.44	9.07	14.16

The high cost (manpower, materials, time) associated with commercial-scale process studies encourages the development of a small-scale screening protocol for candidate strains and fermentation conditions, a process similar to the High Throughput Screening used in the pharmaceutical industry.

Shake Flask Screening. One goal of this work was to determine if screening strains at a shake flask scale could correlate to production at a larger fermentation scale. Shake flask experiments indicated that BL21, SCS1-S, and SCS1-L generated the greatest DNA from glucose, while MC4100, JM105, and MG1655 generated the least. Results with the three strains BL21, SCS1-L, and MC4100 in 7 L fermentation studies were consistent with the shake flask results. Although not an exhaustive investigation, the results of the screen conducted at each strain's maximum specific growth rate did correlate with the larger scale studies at controlled growth rates. In addition, the DNA production significantly improved when the process was conducted in a controlled fermentor compared to a shake flask. For example, the DNA yield for SCS1-L during shake flask growth was 2.1 g/L/OD, while for the fed-batch process this yield was around 7 mg/L/OD at lower growth rates after heat treatment.

Growth Rate. Our low specific growth rate fermentations ($\mu = 0.14\ h^{-1}$) were based on the work of previous studies such as Chen (1997) and more recently Rozkov (2006). The results with specific yield and volumetric yield at this growth rate were in line with results obtained in other studies (Table 13), with the specific DNA yield obtained with SCS1-L (18 mg/g) similar to the highest reported values. We also wanted to determine if a higher specific growth rate would impact DNA generation. For this study, we selected one "good" producer (SCS1-L) and one "poor" producer (MC4100).

Table 13. DNA yields obtained in published fermentation studies.

Strain	Volumetric DNA Yield (mg/L)	DCW (g/L)	Specific DNA Yield (mg/g)	Method	Miscellaneous	Reference
DH5	220	~ 45	~ 4.8	Fed-batch	37°C to 42-45°C temperature shift	Lahijani et al., 1996
DH5α	230	60	3.83	Fed-batch with DO-stat feeding	glycerol and yeast extract $\mu = 0.15\ h^{-1}$	Schmidt et al., 2003
NR	130-250	70-90	1.8-2.78	Batch	Proprietary medium	Durland and Eastman, 1998
NR	260-430	55-65	6.61	Fed-batch	Proprietary medium High specific yields	Carnes and Williams, 2004
NR	670-1100	~ 45	~ 14.8-24.4	Fed-batch	37°C to 42°C temperature shift	Carnes and Williams, 2004
DH5	634-846	NR	28-34	Fed-batch	Variable process steps	Okonkowski et al., 2005
DH1	296	NR	10	Fed–batch	$\mu = 0.10\ h^{-1}$	Rozkov et al., 2006
DH5	1200	NR	25-32	Fed-batch	Large scale (2000L)	Listner et al., 2006
DH5α	1500	~ 33.5	~ 45	Fed-batch	37°C to 42°C temperature shift $\mu = 0.12\ h^{-1}$	Carnes et al., 2006

Using the otherwise same fed-batch process, each was grown at $\mu = 0.28\ h^{-1}$. The DNA yields attained prior to the heat treatment were lower at the higher growth rate as expected, approximately 28% lower for SCS1-L and 20% for MC4100. The effect of specific growth rate on plasmid production has been discussed previously. Plasmids containing ColE1-like origins of replication require only proteins from the host strain to replicate (Donoghue and Sharp, 1978). A decrease in cellular activities like those related to growth can account for the up shift in plasmid DNA replication. It is widely accepted in literature that copy numbers of ColE1 type plasmids are inversely proportional to specific growth rate (Seo and Bailey, 1985, 1986; Lin-Chao and Bremer, 1986; Reinikainen et al., 1989; Reinikainen and Virkajärvi, 1989; Prather et al., 2006).

Heat Treatment.

According to previous studies (Wong et al., 1982; Lahijani et al., 1996), a heat treatment can increase both volumetric and specific yields. The single point mutation on the pLL14 affects the negative regulation of replication from our plasmid, especially at high temperatures. In this case the mutation maps upstream of the RNAI promoter. RNAI inhibition alters the initiation of RNA transcription (Fitzwater et al., 1988; Wang et al., 2004). Two proteins *rom/rop* that regulate priming and copy number by interacting with RNAI have also been implicated (Tamm and Polisky, 1985; Wang et al., 2004). Hecker et al. (1985) and Reinikainen et al. (1989) found that plasmid yields were highest after the culture had entered the stationary phase. Heat treatment may mimic the metabolic requirements of a stationary phase due to the drop in growth rate.

At the growth rate of $0.14\ h^{-1}$, a 15% increase in the specific DNA yield was observed for SCS1-L after heat treatment. Surprisingly the specific DNA yield for MC4100 decreased by approximately 17% after heat treatment. For MC4100 this means that biomass increased from

an OD 65 to 81 with no significant improvement in plasmid production. During the 4 hour period of heat treatment, the specific growth rates for both strains were about 0.075 h^{-1} (down from 0.14 h^{-1}). At the higher growth rate of 0.28 h^{-1}, specific yields increased over 40% due to heat treatments for both strains. During the four-hour heat treatment, the specific growth rate was about 0.13 h^{-1}, approximately a 50% decrease (on average for both strains). In studying the affect of growth rate on DNA production, Chen et al. (1997) and Seo and Bailey (1986) observed optimum growth rates of 0.13 h^{-1} and 0.20 h^{-1}, respectively, for their plasmid host combinations. At lower specific growth rates, there is less competition for carbon and nutrients and sufficient time for plasmid production (Chen et al., 1997 and Reinikainen et al., 1989). Additionally, lower specific growth rates allow a coupling of plasmid replication rates with host cell division resulting in improved plasmid segregation (Zabriskie and Arcuri, 1986).

For MC4100, when we compare fermentation 1 (μ=0.14 $h^{-1)}$ with fermentation 3 (μ=0.28 h^{-1}) we see almost no difference in pre-heat volumetric or specific yields. Additionally, given the dramatic improvement in yield after heat treatment at a specific growth rate of μ=0.28 h^{-1} we can only hypothesize that we may have dropped below the optimal growth rate for plasmid DNA production. Of the published papers concerning DNA yields, the lowest published growth rate for a successful fermentation was μ=0.10 h^{-1} (Rozkov et al., 2006).

SCS1-L acted as expected at both growth rates, higher yields at lower specific growth rates prior to heat treatment. However, heat treatments caused a specific yield increase of 14.6% for μ=0.14 h^{-1} as compared to 50.3% for μ=0.28 h^{-1}. There are two hypothetical explanations for these dramatic differences in production. First, as with MC4100 we may have dropped below the optimal growth rate for plasmid DNA production. Second, we may be approaching the maximum possible yields for our process.

For our process, it stands to reason that approximately 600 g/L and 18 mg/g is approaching this limit. A process involving a fast initial growth rate followed by a slow growth stage and/or heat treatment would improve productivity and still produce maximum yields (Chen, 1999 and Schmidt et al., 2003).

Other comments

Although NM554 and SE5000 both showed relatively high yields in shake flasks, particularly using glycerol as a carbon source, these strains grew with difficulty in CDM and were not considered for fed-batch fermentation.

Overall productivity increased at higher specific growth rates as shown in the results section (48% for SCS1-L and 137% for MC4100). However, prior to heat treatment these numbers tell a much different story. Compared to experiments at the lower growth rates before heat treatment, SCS1-L showed a decrease in productivity of 4% and MC4100 had only a slight increase of 17%. Thus, the improved production of plasmid DNA at the higher growth rate was a direct result of the large benefit of heat treatment at higher growth rates.

Using our screening process, we were able to identify both "good" and "poor" candidates for plasmid DNA production. Starting with 5 clones of 13 different strains, we first selected the best clone of each strain and then rated all the strains using shake flasks. The three strains selected for further study using a fed-batch process indicated a direct relationship between the screening in shake flasks and the larger scale process.

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CHAPTER 3

CONCLUSIONS

Improvements in production schemes will be required to meet the growing market for DNA based gene therapies. Molecular genetics and process engineering will have to work hand in hand to attain the highest DNA plasmid yields. Due to the complexity of interactions required to produce plasmid DNA it is important to test as many variables as possible. Although not an exhaustive investigation, we were able to show the efficacy of small scale screening to determine fermentation candidates.

For this study, we looked at host-plasmid interactions and carbon source at the small scale. Using this information we chose promising candidates and grew them at a small fermentation scale. With a streamlined process, we were able to achieve plasmid yields in line with some of the highest published yields.

We can only speculate that "good" and "poor" production of plasmid DNA is related to certain genotypic combinations. In the case of our chosen strains, the genes *recA* and *relA* would be of interest for further study.

Appendix A:

Raw Data From Clonal Selection

pLL14 Clones

Strain	Clone	DNA (ug/ml/OD)	Notes
JM109	1	81	
	2	133	
	3	124	Chosen Clone
	4	99	
	5	103	
MG1655	1	60	
	2	63	
	3	67	
	4	79	Chosen Clone
	5	12	
DH1	1	83	
	2	58	
	3	81	Chosen Clone
	4	46	
	5	72	
MC4100	1	29	
	2	54	Chosen Clone
	3	28	
	4	32	
	5	30	
DH5α	1	103	Chosen Clone
	2	96	
	3	82	
	4	93	
	5	84	
BL21	1	22	
	2	19	
	3	13	
	4	16	
	5	24	Chosen Clone
JM105	1	62	
	2	69	
	3	16	Difficult Lysis
	4	61	
	5	57	
Mach1	1	88	
	2	77	
	3	70	
	4	69	
	5	77	Chosen Clone

Strain	Clone	DNA (ug/ml/OD)	Notes
SCS1 S	1	112	
	2	139	
	3	159	Chosen Clone
	4	119	
	5	132	
SE5000	1	112	Chosen Clone
	2	110	
	3	94	
	4	80	
	5	115	
SCS1 L	1	195	
	2	149	
	3	140	
	4	149	
	5	165	Chosen Clone
MC1061	1	49	
	2	43	
	3	66	
	4	81	
	5	90	Chosen Clone
NM554	1	96	
	2	98	
	3	86	
	4	110	Chosen Clone
	5	88	

pUC19 Clones

Strain	Clone	DNA (ug/ml/OD)	Notes
JM109	1	30	
	2	20	
	3	23	
	4	5	
	5	36	Chosen Clone
MG1655	1	9	
	2	43	
	3	11	
	4	21	Chosen Clone
	5	3	
DH1	1	13	
	2	5	
	3	10	
	4	16	Chosen Clone
	5	13	
MC4100	1	11	
	2	15	
	3	15	
	4	28	Chosen Clone
	5	20	
DH5α	1	8	
	2	25	
	3	20	Chosen Clone
	4	12	
	5	10	
BL21	1	94	
	2	91	
	3	94	Chosen Clone
	4	81	
	5	93	
JM105	1	57	
	2	67	
	3	58	Chosen Clone
	4	52	
	5	76	
Mach1	1	39	
	2	28	
	3	28	
	4	32	
	5	30	Chosen Clone

Strain	Clone	DNA (ug/ml/OD)	Notes
SCS1 S	1	32	
	2	25	
	3	61	Chosen Clone
	4	49	
	5	53	
SCS1 L	1	99	
	2	63	
	3	56	
	4	78	
	5	79	Chosen Clone
MC1061	1	75	
	2	100	
	3	58	
	4	107	Chosen Clone
	5	105	
NM554	1	96	
	2	98	
	3	86	
	4	103	Chosen Clone
	5	88	

Appendix B:

Raw Data From Shake Flasks

pLL14 Shake Flask Data

Strain	Substrate	DNA Yield (µg/mL)			Mean DNA Yield (µg/mL)	mg/L	Std. Dev (µg/mL)	Growth Rate (1/h)	Acetate (mg/L)	Yield Ratio	Growth Ratio
BL21	Glycerol	79	79	78	78	2.61	1	0.45	0		
	Glucose	73	69	69	70	2.34	2	0.77	0	1.11	0.59
DH1	Glycerol	53	53	54	53	1.76	1	0.61	0		
	Glucose	34	32	31	33	1.09	2	0.90	60	1.63	0.68
DH5a	Glycerol	60	67	58	62	2.06	5	0.58	0		
	Glucose	42	42	44	43	1.42	1	0.99	180	1.45	0.58
JM105	Glycerol	45	43	40	43	1.42	3	0.44	0		
	Glucose	23	22	18	21	0.70	2	0.64	190	2.02	0.68
JM109	Glycerol	55	65	57	59	1.97	5	0.51	0		
	Glucose	57	60	58	58	1.94	2	0.70	190	1.02	0.73
Mach 1	Glycerol	73	74	66	71	2.37	5	0.63	0		
	Glucose	43	41	42	42	1.40	1	0.84	60	1.69	0.75
MC4100	Glycerol	38	37	40	38	1.27	1	0.79	80		
	Glucose	25	24	27	25	0.85	2	0.92	360	1.50	0.86
MG1655	Glycerol	32	32	28	31	1.02	2	0.49	0		
	Glucose	14	15	16	15	0.50	1	0.80	80	2.03	0.61
SE5000	Glycerol	76	74	85	78	2.61	6	0.57	0		
	Glucose	47	44	49	47	1.56	2	1.00	200	1.67	0.57
SCS1 S	Glycerol	88	93	92	91	3.04	3	0.42	0		
	Glucose	88	97	88	91	3.03	5	0.38	80	1.00	1.11
SCS1 L	Glycerol	86	91	93	90	3.00	4	0.45	0		
	Glucose	59	66	60	62	2.06	4	0.90	180	1.46	0.50
MC1061	Glycerol	55	54	51	53	1.78	2	0.32	0		
	Glucose	45	50	51	48	1.62	3	0.35	80	1.10	0.91
NM554	Glycerol	76	82	78	79	2.62	3	0.39	0		
	Glucose	53	5.6	54	54	1.81	1	0.56	210	1.44	0.70

pUC19 Shake Flask Data

Strain	Substrate	DNA Yield (μg/mL)			Mean DNA Yield (μg/mL)	mg/L	Std. Dev (μg/mL)	Growth Rate (1/h)	Acetate (mg/L)	Yield Ratio	Growth Ratio
BL21	Glycerol	25	24	26	25	0.84	1	0.45	0		
	Glucose	25	23	22	23	0.78	2	0.52	0	1.07	0.87
DH1	Glycerol	38	37	35	37	1.22	1	0.47	0		
	Glucose	16	15	19	16	0.55	2	0.79	60	2.23	0.59
DH5α	Glycerol	38	38	41	39	1.30	2	0.46	0		
	Glucose	34	29	32	32	1.06	3	0.70	0	1.23	0.66
JM105	Glycerol	41	43	30	38	1.26	7	0.59	0		
	Glucose	28	28	27	27	0.91	0	0.82	210	1.38	0.72
JM109	Glycerol	44	44	47	45	1.49	2	0.56	0		
	Glucose	23	25	25	24	0.81	1	0.86	300	1.84	0.65
MC4100	Glycerol	15	17	18	17	0.56	2	0.74	0		
	Glucose	13	13	10	12	0.40	2	1.10	220	1.40	0.67
MG1655	Glycerol	21	23	21	22	0.72	1	0.58	0		
	Glucose	13	12	14	13	0.43	1	0.87	200	1.68	0.67
SCS1 S	Glycerol	36	37	34	36	1.19	2	0.41	0		
	Glucose	42	40	39	40	1.35	2	0.40	40	0.88	1.03
SCS1 L	Glycerol	43	43	41	42	1.41	1	0.34	0		
	Glucose	26	23	28	26	0.86	3	0.62	0	1.64	0.55
MC1061	Glycerol	43.	45	40	43	1.43	3	0.39	10		
	Glucose	24.	23	25	24	0.81	1	0.46	80	1.75	0.85
	Glycerol	50	48	46	48	1.61	2	0.33	0		
NM554	Glucose	23	25	29	26	0.86	3	0.47	80	1.87	0.70

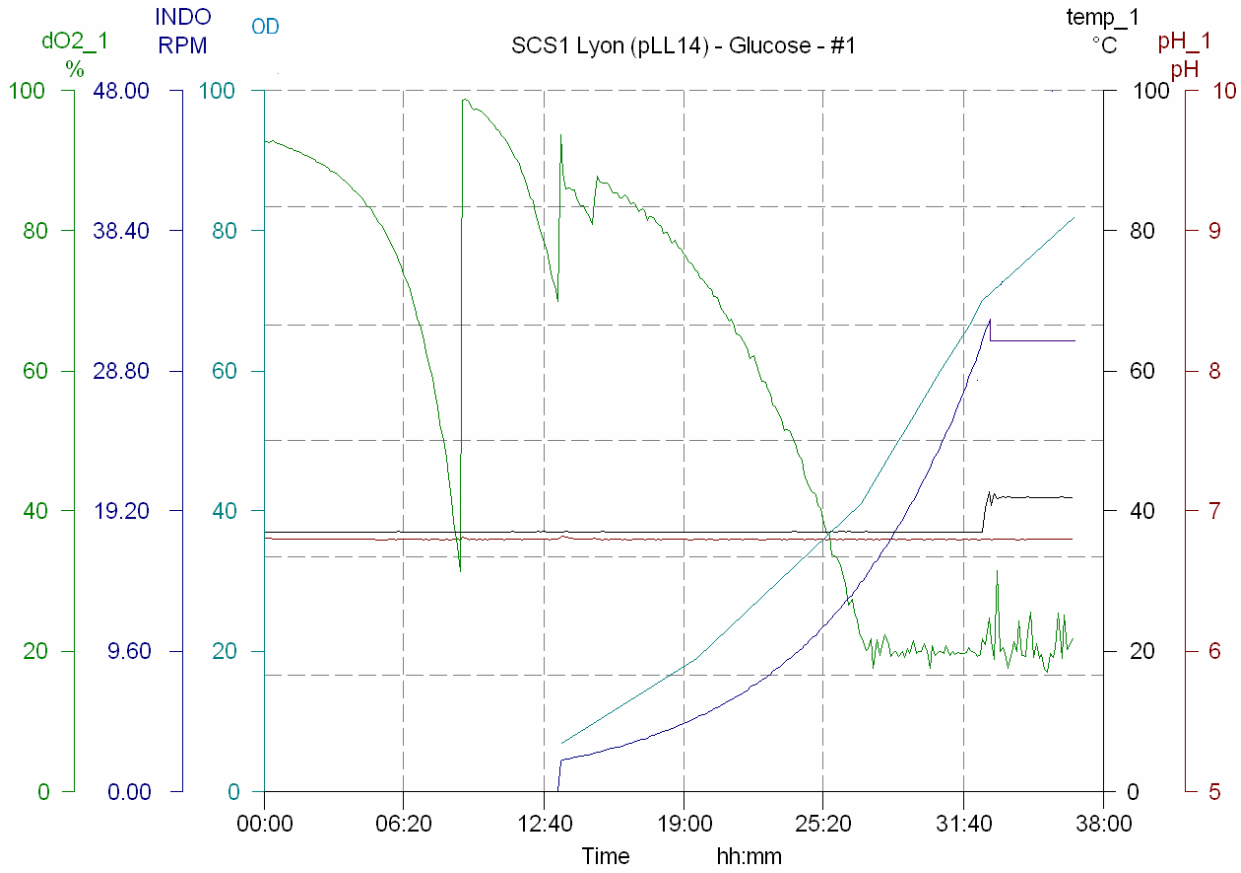
Note: A stable clone of SE5000 and Mach1 with pUC 19 was never created.

Appendix C:

Raw Data From Fermentations

SCS1 Lyon Fermentation #1 April 24, 2007

Strain= SCS1 Lyon	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3564 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4136 ml
Final Dry Cell Weight = 31.98 g/L	
Time Heat treatment started: 32:30 h	Time fed-batch commenced = 13:10 h
Final Specific Yield = 18.6 mg/g	Final Volumetric Yield = 595 mg/L



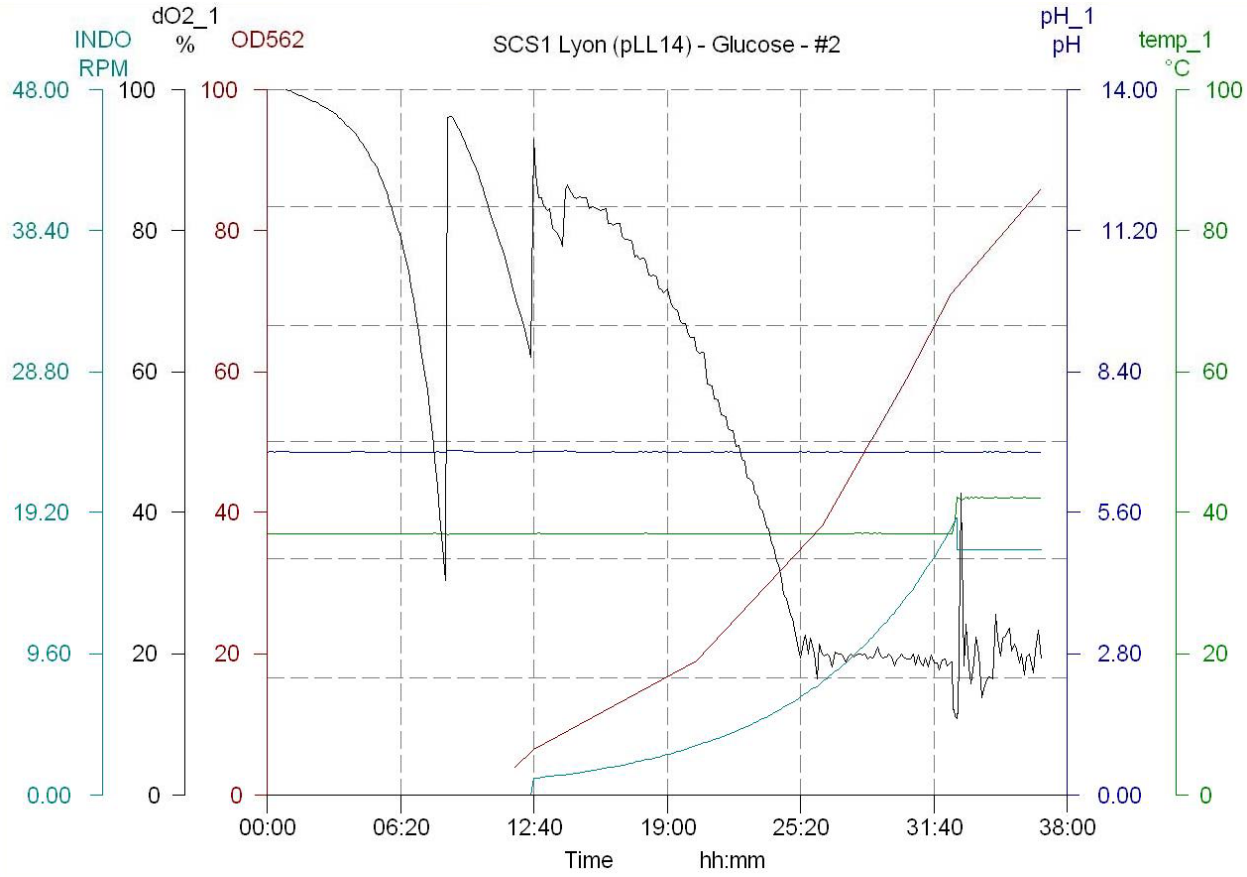
Time	Feed Pump Speed (RPM)	dO2 (%)	temp (°C)	pH	OD 562
0:00:00	0	92.79	36.94	6.80	
1:00:00	0	91.88	36.94	6.80	
2:00:00	0	90.57	37.01	6.80	
3:00:00	0	88.68	37.01	6.80	
4:00:00	0	86.05	37.01	6.80	
5:00:00	0	82.23	36.94	6.80	
6:00:00	0	76.49	37.01	6.80	
7:00:00	0	67.20	36.91	6.80	
8:00:00	0	52.15	37.05	6.80	
9:00:00	0	98.37	36.91	6.81	
10:00:00	0	96.49	36.98	6.80	
11:00:00	0	92.69	37.01	6.80	
12:00:00	0	85.67	37.05	6.80	
13:00:00	0	74.29	37.05	6.80	
13:30:00	2.15	91.55	37.01	6.82	6.9
14:00:00	2.31	85.95	36.98	6.80	
15:00:00	2.65	87.12	36.94	6.80	
16:00:00	3.05	86.05	36.98	6.80	
17:00:00	3.51	83.07	37.05	6.80	
18:00:00	4.04	80.74	36.98	6.80	
19:00:00	4.65	76.58	36.98	6.80	
19:35:00	5.04	73.26	37.05	6.80	19
20:00:00	5.34	72.31	37.01	6.80	
21:00:00	6.15	68.01	36.98	6.80	
22:00:00	7.07	61.65	37.01	6.80	
23:00:00	8.13	55.20	36.98	6.80	
24:00:00	9.36	49.14	37.01	6.80	
25:00:00	10.76	40.03	37.01	6.80	
26:00:00	12.38	32.07	37.01	6.80	
27:00:00	14.24	19.61	36.98	6.80	41
28:00:00	16.38	21.14	36.91	6.80	
29:00:00	18.84	19.80	37.01	6.80	
30:00:00	21.67	20.07	36.98	6.80	
30:35:00	23.52	20.76	37.05	6.80	60
31:00:00	24.93	20.01	37.05	6.80	
32:00:00	28.67	19.57	36.98	6.80	67
32:30:00	30.75	19.43	36.98	6.80	70
33:00:00	32.98	17.72	41.62	6.79	
34:00:00	27.90	19.82	41.91	6.80	
35:00:00	27.90	21.40	41.86	6.80	
36:00:00	27.90	19.54	41.88	6.80	
36:40:00	27.90	23.04	41.88	6.80	82

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	13:30	3565	6.9	2.69	17.23	6.40	250	0
1				2.69	17.85	6.63		
2	19:35	3612	19	7.41	75.00	10.12	280	0
2				7.41	85.00	11.47		
3	27:00	3756	41	15.99	242.47	15.16	0	60
3				15.99	228.77	14.31		
4	30:35	3889	60	23.40	372.00	15.90	0	120
4				23.40	380.00	16.24		
5	32:30	3975	70	27.30	439.53	16.10	30	130
5				27.30	413.95	15.16		
5				27.30	395.35	14.48		
6	36:40	4136	82	31.98	592.00	18.51	0	100
6				31.98	589.33	18.43		
6				31.98	602.67	18.85		

SCS1 Lyon Fermentation #2 May 8, 2007

Strain= SCS1 Lyon	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3570 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4373 ml
Final Dry Cell Weight = 33.54 g/L	
Time Heat treatment started: 32:30 h	Time fed-batch commenced = 12:40 h
Final Specific Yield = 17.8 mg/g	Final Volumetric Yield = 595 mg/L



Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	101.16	0	36.98	6.75	
1:00:00	99.87	0	36.94	6.80	
2:00:00	98.69	0	37.05	6.80	
3:00:00	96.98	0	37.01	6.80	
4:00:00	94.36	0	37.01	6.80	
5:00:00	90.1	0	36.98	6.80	
6:00:00	82.69	0	36.98	6.80	
7:00:00	69.7	0	37.05	6.80	
8:00:00	46.99	0	37.02	6.80	
9:00:00	95	0	36.91	6.83	
10:00:00	88.21	0	36.98	6.80	
11:00:00	78.86	0	37.02	6.80	
11:47:00	70.71	0	37.02	6.80	4
12:00:00	68.61	0	37.02	6.80	
12:40:00	92.97	1.13	36.87	6.81	6.5
13:00:00	84.74	1.18	36.98	6.81	
14:00:00	78.28	1.36	36.98	6.81	
15:00:00	85.02	1.57	37.02	6.79	
16:00:00	83.06	1.8	36.98	6.79	
17:00:00	78.91	2.07	37.05	6.80	
18:00:00	75.09	2.38	37.05	6.80	
19:00:00	71.73	2.74	37.02	6.80	
20:00:00	65.39	3.15	37.02	6.80	
20:25:00	63.06	3.34	37.02	6.80	19
21:00:00	58.07	3.63	37.02	6.79	
22:00:00	51.67	4.17	36.98	6.80	
23:00:00	44.58	4.8	36.98	6.80	
24:00:00	35.16	5.52	37.02	6.80	
25:00:00	23.81	6.35	36.98	6.80	
26:00:00	17.62	7.3	36.98	6.80	
26:20:00	17.71	7.65	36.98	6.80	38
27:00:00	19.92	8.4	37.02	6.80	
28:00:00	19.74	9.66	37.09	6.80	
29:00:00	21.31	11.11	37.02	6.80	
30:00:00	18.58	12.78	37.02	6.80	
30:22:00	18.61	13.46	37.09	6.80	59
31:00:00	21.14	14.7	37.05	6.80	
32:00:00	19.66	16.91	37.02	6.80	
32:30:00	18.67	18.14	37.02	6.80	71
33:00:00	31.9	16.40	41.45	6.80	
34:00:00	9.55	16.40	41.99	6.80	
35:00:00	18.7	16.40	42.03	6.79	
36:00:00	19.69	16.40	42.05	6.80	
36:45:00	19.24	16.40	42.00	6.80	86

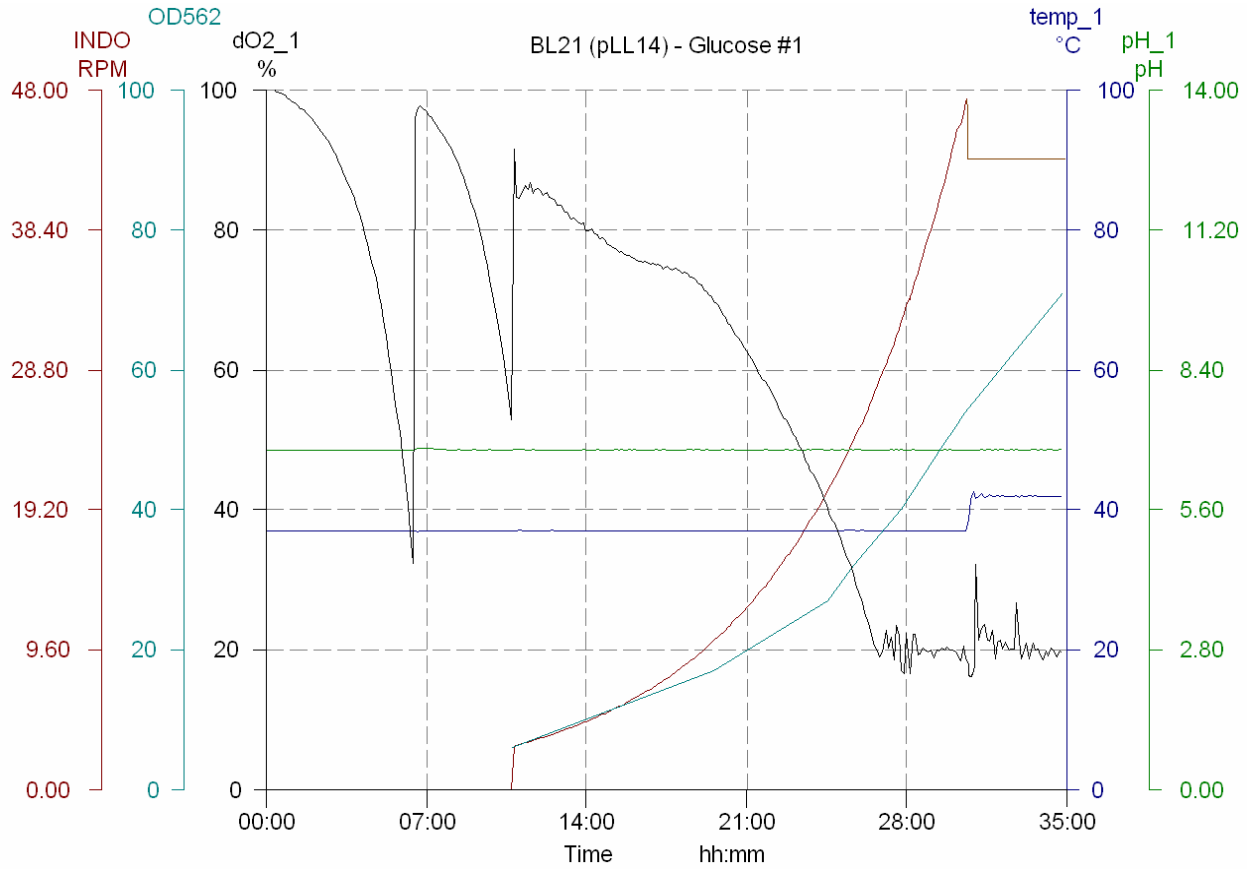
DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	11:47	3575	4	1.56	10.20	6.54	260	0
1				1.56	9.88	6.33		
2	12:40	3570	6.5	2.54	16.91	6.67	0	10
2				2.54	17.78	7.01		
3	20.25	3624	19	7.41	90.38	12.20	0	40
3				7.41	104.30	14.08		
4	26:20	3749	38	14.82	190.51	12.85	0	420
4				14.82	200.13	13.50		
5	30:22	3940	59	23.01	352.75	15.33	0	80
5				23.01	379.22	16.48		
6	32:22	4064	71	27.69	442.38	15.98	0	80
6				27.69	438.57	15.84		
6				27.69	409.05	14.77		
7	36:45	4373	86	33.54	601.14	17.92	10	100
7				33.54	592.57	17.67		
7				33.54	592.00	17.65		

BL21 Fermentation #1 April 24, 2007

Strain= BL21	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3560 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4136 ml
Final Dry Cell Weight = 27.69 g/L	
Time Heat treatment started: 30:30 h	Time fed-batch commenced = 10:48 h
Final Specific Yield = 15.4 mg/g	Final Volumetric Yield = 438 mg/L

Note: Heat treatment was started at 54 when the pump reached its maximum rate (48 rpm). This was due to a slow feed rate of the tubing used.



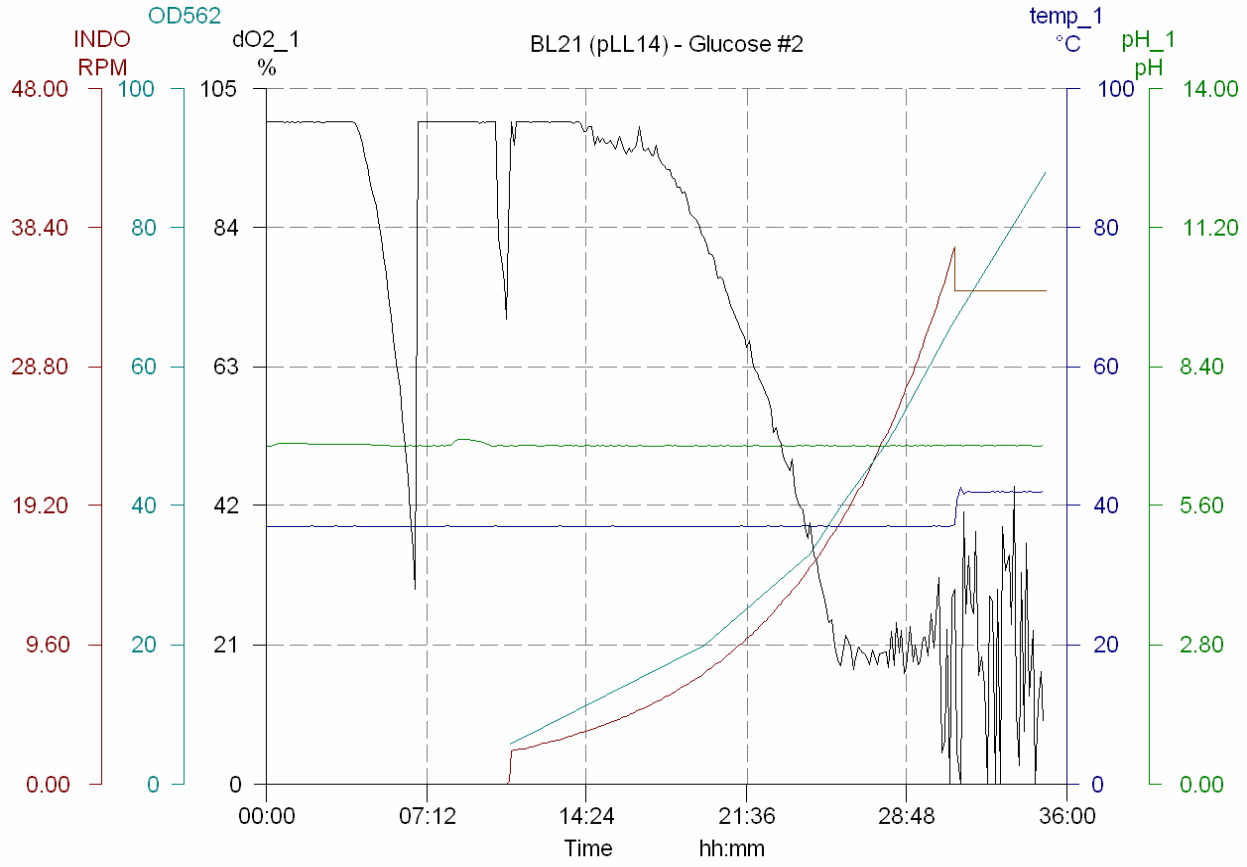
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	100.51	0	36.94	6.80	
1:00:00	98.64	0	36.94	6.80	
2:00:00	95.78	0	36.98	6.80	
3:00:00	91.07	0	37.02	6.80	
4:00:00	82.98	0	37.02	6.80	
5:00:00	69.58	0	37.02	6.80	
6:00:00	46.34	0	37.05	6.80	
7:00:00	96.82	0	36.91	6.82	
8:00:00	92.16	0	36.98	6.80	
9:00:00	84.33	0	36.98	6.80	
10:00:00	69.22	0	37.02	6.80	
10:48:00	86.85	0	36.94	6.81	6.0
11:00:00	83.63	3.07	37.02	6.81	
12:00:00	85.17	3.54	36.98	6.80	
13:00:00	83.04	4.07	36.98	6.79	
14:00:00	79.87	4.68	36.94	6.80	
15:00:00	77.61	5.38	36.98	6.80	
16:00:00	75.99	6.19	37.05	6.80	
17:00:00	75.24	7.12	37.09	6.80	
18:00:00	74.13	8.19	37.02	6.80	
19:00:00	72.11	9.42	37.02	6.80	
19:35:00	69.93	10.23	37.02	6.80	17
20:00:00	67.89	10.84	37.02	6.80	
21:00:00	62.7	12.47	37.02	6.80	
22:00:00	57.27	14.34	37.02	6.79	
23:00:00	51	16.5	37.02	6.79	
24:00:00	44.2	18.98	37.02	6.80	
24:32:00	40.53	20.45	37.02	6.80	27
25:00:00	37.01	21.83	37.02	6.80	
25:40:00	30.78	23.96	37.09	6.80	32
26:00:00	26.73	25.11	37.09	6.80	
27:00:00	21.53	28.88	37.02	6.80	
27:47:00	19.49	32.23	37.02	6.80	40
28:00:00	22.38	33.22	37.05	6.80	
29:00:00	19.97	38.22	37.02	6.80	
30:00:00	19.93	43.96	37.05	6.80	
30:30:00	18.88	46.72	37.02	6.80	54
31:00:00	25.24	43	41.92	6.80	
32:00:00	19.69	43	41.92	6.79	
33:00:00	20.44	43	41.96	6.80	
34:30:00	18.56	43	41.96	6.80	71

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	10:48	3555	6	2.40	6.69	16.06	0	0
1				2.40	6.05	14.53		
2	19:35	3618	17	6.80	12.11	82.33	0	0
2				6.80	15.02	102.17		
3	25:40	3708	32	12.80	10.69	136.81	0	0
3				12.80	10.87	139.15		
4	27:47	3776	40	16.00	12.45	199.20	60	0
4				16.00	10.82	173.07		
5	30:30	3894	54	21.60	12.37	267.14	20	40
5				21.60	11.13	240.36		
6	34:30	4165	71	28.40	15.53	441.19	100	50
6				28.40	15.20	431.67		
6				28.40	15.58	442.38		

BL21 Fermentation #2 May 8, 2007

Strain= BL21	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3575 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4593 ml
Final Dry Cell Weight = 34.32 g/L	
Time Heat treatment started: 30:48 h	Time fed-batch commenced = 10:57 h
Final Specific Yield = 14.7 mg/g	Final Volumetric Yield = 504 mg/L



Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	100	0	36.84	6.79	
1:00:00	99.95	0	36.98	6.84	
2:00:00	99.95	0	37.09	6.83	
3:00:00	99.95	0	36.98	6.83	
4:00:00	100	0	36.98	6.82	
5:00:00	85.61	0	36.98	6.81	
6:00:00	59.75	0	36.98	6.80	
7:00:00	100	0	37.05	6.80	
8:00:00	99.95	0	37.01	6.80	
9:00:00	99.95	0	36.98	6.91	
10:00:00	100	0	37.01	6.82	
10:57:00	100	2.1	36.87	6.80	5.8
11:00:00	100	2.27	36.80	6.81	
12:00:00	99.95	2.61	37.02	6.80	
13:00:00	99.95	3	37.02	6.80	
14:00:00	99.95	3.45	36.94	6.80	
15:00:00	96.88	3.97	37.01	6.80	
16:00:00	96.31	4.57	36.98	6.80	
17:00:00	94.36	5.26	37.12	6.80	
18:00:00	92.82	6.04	37.01	6.80	
19:00:00	87.61	6.95	36.94	6.80	
19:45:00	85.78	7.72	37.02	6.80	20
20:00:00	79.51	8	37.02	6.80	
21:00:00	71.64	9.2	37.05	6.80	
22:00:00	63.59	10.58	37.16	6.80	
23:00:00	52.94	12.17	36.94	6.80	
24:00:00	41.68	14	37.05	6.80	
24:27:00	36.14	14.91	37.05	6.80	33
25:00:00	30.73	16.11	37.09	6.80	
25:54:00	19.39	18.27	37.12	6.80	40
26:00:00	17.05	18.53	37.05	6.80	
27:00:00	18.88	21.31	37.05	6.80	
27:57:00	16.39	24.34	37.16	6.80	49
28:00:00	26.32	24.51	37.05	6.80	
29:00:00	19.42	28.2	37.01	6.80	
30:00:00	20.52	32.43	37.12	6.81	
30:48:00	0.34	36.28	37.09	6.80	66
31:00:00	8.42	37.31	41.90	6.80	
32:01:00	18.95	33.33	41.95	6.80	
33:00:00	0.05	33.33	41.90	6.80	
35:01:00	0.1	33.33	41.81	6.80	88

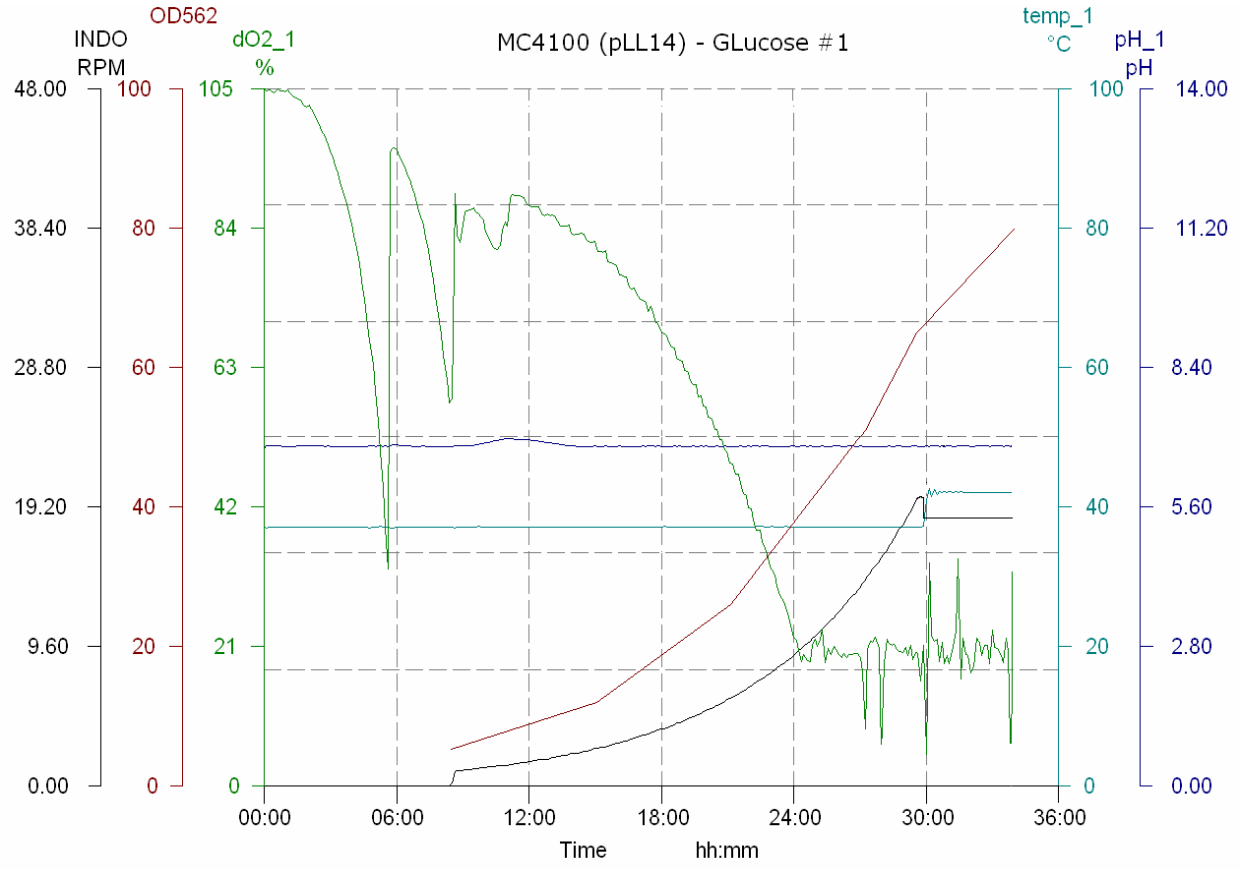
Note: The dO2 probe may have malfunctioned during this run as seen in the graph. However, this did not effect the growth dynamics (as compared to both the expected and to the first BL21 run.

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	10:57	3755	5.8	2.26	14.05	6.21	0	0
1				2.26	14.02	6.20		
2	19:45	3852	20	7.80	105.67	13.55	0	20
2				7.80	109.80	14.08		
3	25:54	4014	40	15.60	173.60	11.13	0	50
3				15.60	175.73	11.26		
4	27:57	4113	49	19.11	206.40	10.80	80	90
4				19.11	186.40	9.75		
5	30:48	4293	66	25.74	283.33	11.01	80	130
5				25.74	246.44	9.57		
6	35:01	4593	88	34.32	519.12	15.13	100	200
6				34.32	506.76	14.77		
6				34.32	486.47	14.17		

MC4100 Fermentation #1 May 7, 2007

Strain = MC4100	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3547
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4480
Final Dry Cell Weight = 30.4 g/L	
Time Heat treatment started: 30:00 h	Time fed-batch commenced = 8:30h
Final Specific Yield = 7.3 mg/g	Final Volumetric Yield = 222 mg/L



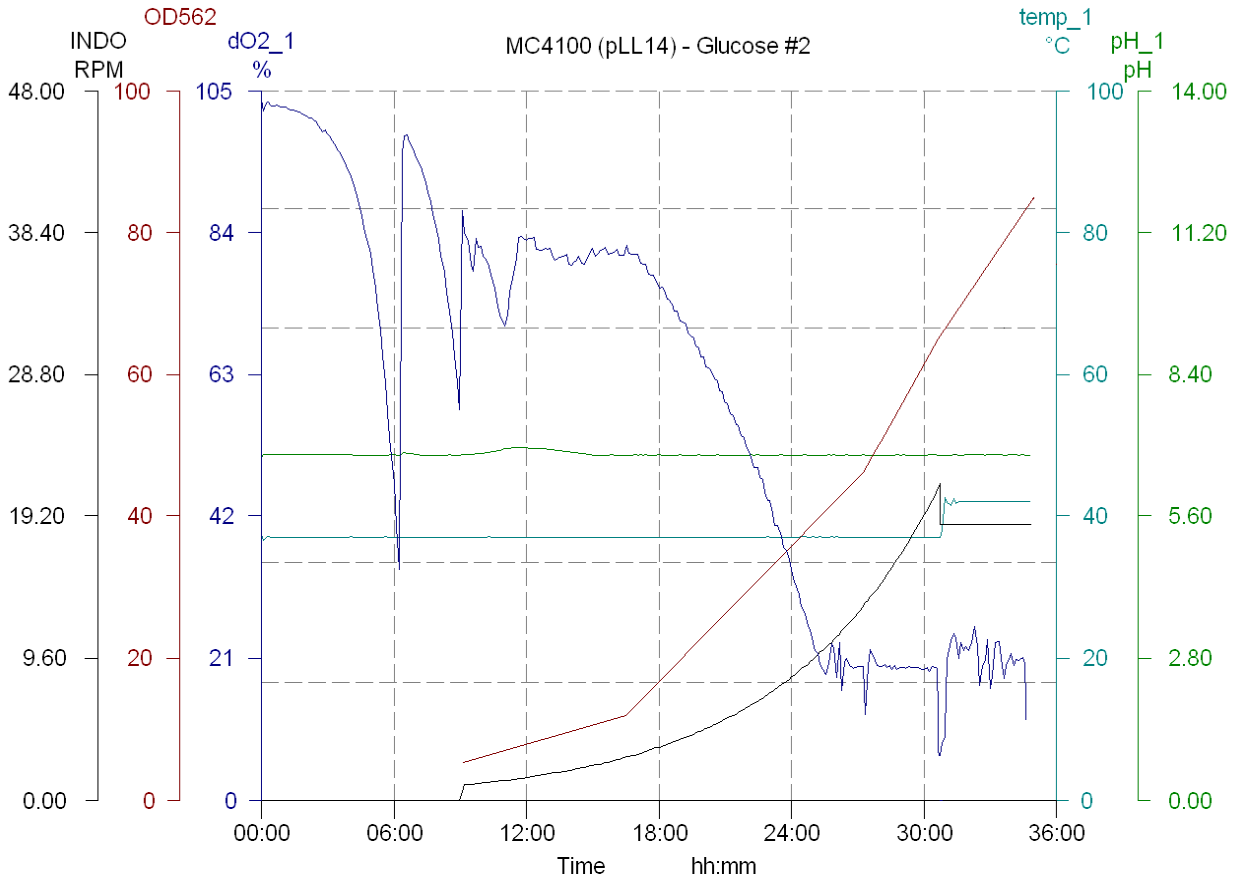
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	105.83	0	6.74	36.94	
1:00:00	104.22	0	6.80	36.98	
2:00:00	102.91	0	6.80	37.05	
3:00:00	95.64	0	6.80	36.98	
4:00:00	84.07	0	6.80	37.02	
5:00:00	60.81	0	6.80	37.02	
6:00:00	95.78	0	6.83	37.05	
7:00:00	87.19	0	6.79	36.94	
8:00:00	68.93	0	6.80	37.02	
8:30:00	54.43	0	6.80	37.05	5.3
9:00:00	83.62	1.1	6.82	36.94	
10:00:00	84.68	1.27	6.87	36.98	
11:00:00	84.02	1.46	6.96	36.98	
12:00:00	87.39	1.68	6.93	37.02	
13:00:00	85.62	1.93	6.87	36.98	
14:00:00	82.98	2.22	6.81	36.98	
15:00:00	81.98	2.55	6.80	37.02	12
16:00:00	78.36	2.94	6.80	36.98	
17:00:00	73.35	3.38	6.80	37.02	
18:00:00	68.26	3.88	6.79	36.98	
19:00:00	63.88	4.47	6.80	37.02	
20:00:00	57.05	5.14	6.80	37.02	
21:00:00	50.27	5.91	6.79	37.02	
21:10:00	48.52	6.05	6.80	37.05	26
22:00:00	41.86	6.80	6.80	36.94	
23:00:00	33.58	7.82	6.80	37.09	
24:00:00	22.33	9.03	6.80	36.98	
25:00:00	20.84	10.35	6.80	37.05	
26:00:00	19.79	11.91	6.79	37.02	
27:00:00	19.35	13.70	6.80	37.02	
27:15:00	19.73	14.18	6.80	37.12	51
28:00:00	23.68	15.75	6.80	37.02	
29:00:00	19.84	18.12	6.79	36.98	
29:36:00	19.82	19.71	6.80	37.02	65
30:00:00	4.69	18.20	6.80	41.02	
31:00:00	14.1	18.20	6.80	41.98	
32:00:00	21.81	18.20	6.81	42.01	
33:00:00	23.41	18.20	6.81	42.01	
34:00:00	37.87	18.20	6.85	41.95	80

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	8:30	3562	5.3	2.01	1.45	0.72	730	0
1				2.01	1.41	0.70		
2	15:00	3605	12	4.56	39.72	8.71	0	0
2				4.56	39.24	8.61		
3	21:10	3699	26	9.88	57.13	5.78	0	70
3				9.88	56.70	5.74		
4	27:15	3970	51	19.38	170.00	8.77	20	90
4				19.38	162.88	8.40		
5	29:36	4145	65	24.70	188.04	7.61	10	150
5				24.70	212.83	8.62		
6	34:00	4480	80	30.40	232.60	7.65	150	110
6				30.40	222.20	7.31		
6				30.40	210.80	6.93		

MC4100 Fermentation #2 May 19, 2007

Strain = MC4100	Feed Batch Growth Rate = 0.14 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3566
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4513
Final Dry Cell Weight = 31.16 g/L	
Time Heat treatment started: 31:00 h	Time fed-batch commenced =09:09 h
Final Specific Yield = 6.18 mg/g	Final Volumetric Yield = 193 mg/L



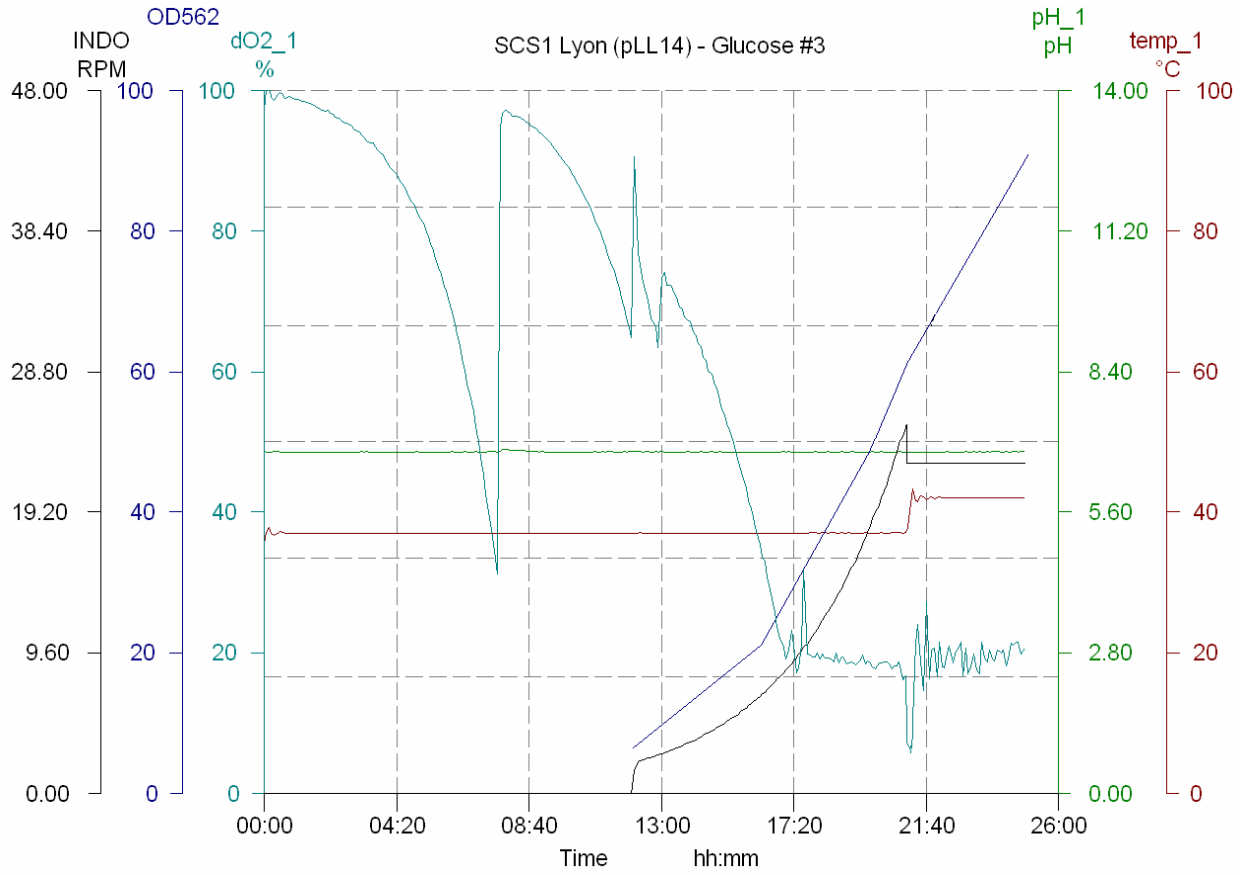
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	103.85	0.00	37.34	6.73	
1:00:00	102.67	0.00	36.98	6.80	
2:00:00	101.39	0.00	36.98	6.80	
3:00:00	98.73	0.00	37.02	6.81	
4:00:00	93.16	0.00	37.02	6.80	
5:00:00	79.45	0.00	37.05	6.80	
6:00:00	46.71	0.00	37.02	6.80	
7:00:00	95.67	0.00	37.02	6.81	
8:00:00	83.65	0.00	36.98	6.79	
9:00:00	57.72	0.00	37.05	6.79	
9:08:00	88.78	0.00	36.91	6.81	5.4
10:00:00	81.14	1.17	36.98	6.83	
11:00:00	70.58	1.35	37.02	6.91	
12:00:00	82.91	1.55	37.05	6.94	
13:00:00	81.68	1.78	37.02	6.90	
14:00:00	79.14	2.05	37.02	6.85	
15:00:00	81.6	2.36	36.98	6.80	
16:00:00	81.38	2.71	36.98	6.80	
16:30:00	82.08	2.91	36.98	6.80	12
17:00:00	80.82	3.12	36.98	6.80	
18:00:00	75.92	3.59	37.02	6.80	
19:00:00	70.77	4.13	36.98	6.80	
20:00:00	65.56	4.75	37.02	6.80	
21:00:00	58.87	5.46	37.02	6.79	
22:00:00	52.33	6.28	36.94	6.80	
23:00:00	44.08	7.23	37.02	6.80	
24:00:00	33.96	8.31	37.02	6.79	
25:00:00	22.57	9.56	37.09	6.80	
26:00:00	20.97	11.00	37.05	6.80	
27:00:00	19.87	12.65	37.02	6.79	
27:15:00	20.11	13.10	37.02	6.80	46
28:00:00	19.47	14.55	37.02	6.80	
29:00:00	20	16.74	37.02	6.80	
30:00:00	19.34	19.25	37.02	6.80	
30:40:00	19.66	18.90	37.05	6.80	65
31:00:00	23.93	18.90	43.24	6.79	
32:00:00	18.83	18.90	41.96	6.79	
33:00:00	16.59	18.90	41.99	6.80	
34:00:00	20.35	18.90	41.98	6.80	
35:00:00	20.1	18.90	41.98	6.80	82

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	9:08	3567	5.4	2.05	2.11	1.03	870	0
1				2.05	2.36	1.15		
2	16:30	3627	12	4.56	28.00	6.14	0	0
2				4.56	31.84	6.98		
3	27:15	3927	46	17.48	112.92	6.46	40	0
3				17.48	110.92	6.35		
4	30:40	4169	65	24.70	191.09	7.74	10	0
4				24.70	186.52	7.55		
5	35:00	4513	82	31.16	199.19	6.39	100	140
5				31.16	190.95	6.13		
5				31.16	187.57	6.02		

SCS1 Lyon Fermentation #3 May 23, 2007

Strain = SCS1 Lyon	Feed Batch Growth Rate = 0.28 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3573 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4368 ml
Final Dry Cell Weight = 35.49 g/L	
Time Heat treatment started: 21:00 h	Time fed-batch commenced = 12:04 h
Final Specific Yield = 16.88 mg/g	Final Volumetric Yield = 599 mg/L



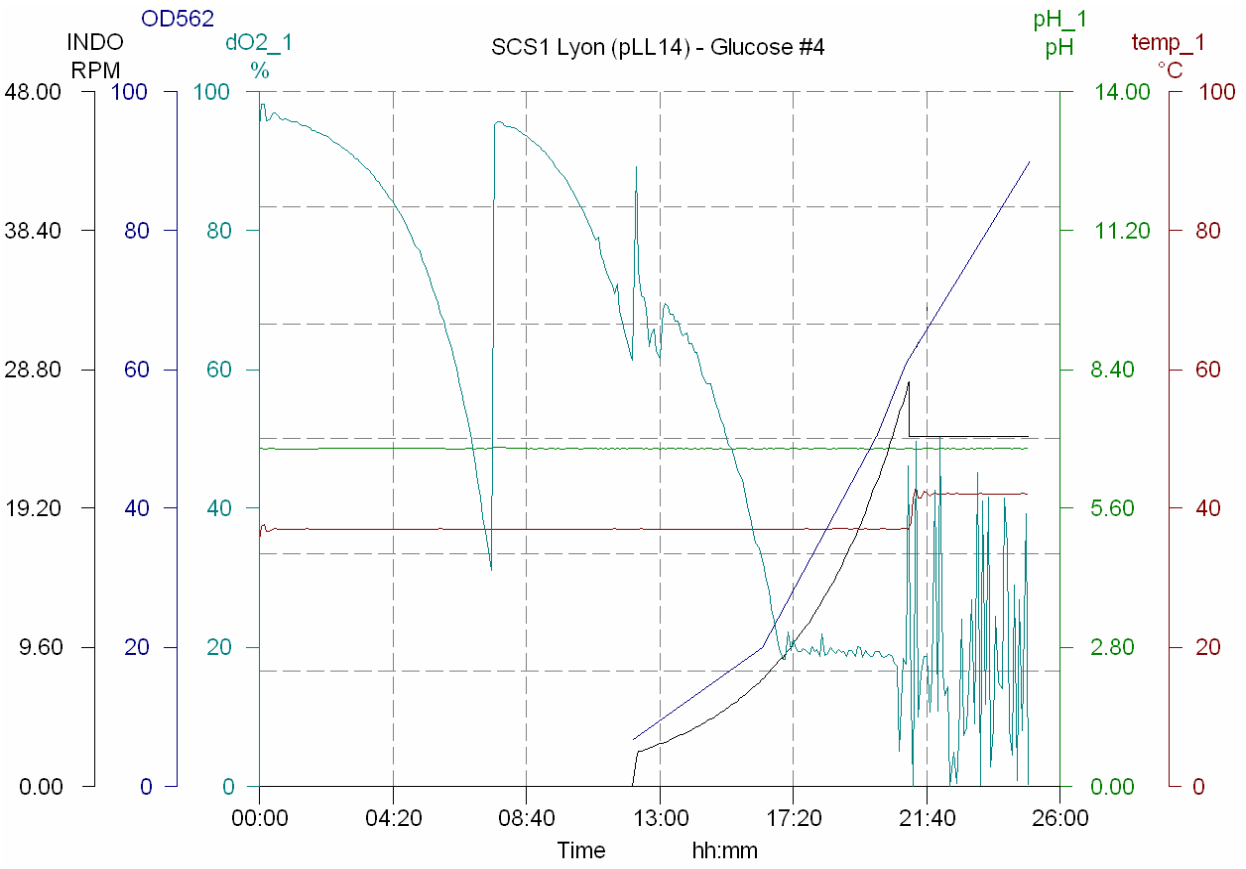
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	97.33	0	35.36	6.8	
1:00:00	98.66	0	36.98	6.8	
3:00:00	94.31	0	36.94	6.8	
4:00:00	90.16	0	37.02	6.8	
5:00:00	83.11	0	37.05	6.8	
6:00:00	70.86	0	36.94	6.8	
7:00:00	50.45	0	37.02	6.8	
8:00:00	96.98	0	37.02	6.84	
9:00:00	94.16	0	37.02	6.8	
10:00:00	88.82	0	37.02	6.8	
11:00:00	79.82	0	37.02	6.8	
12:00:00	65.67	0	37.02	6.8	
12:04:00	64.94	0	37.02	6.8	6.5
13:00:00	73.39	2.69	36.94	6.8	
14:00:00	66.21	3.55	37.05	6.8	
15:00:00	54.66	4.7	36.98	6.8	
16:00:00	39	6.22	37.05	6.8	
16:15:00	35	6.67	37.02	6.79	21
17:00:00	21.07	8.23	36.98	6.8	
18:00:00	19.63	10.89	37.16	6.79	
19:00:00	18.69	14.41	37.05	6.8	
19:53:00	18.59	18.46	37.02	6.8	49
20:00:00	18.97	19.07	37.09	6.79	
21:00:00	18.3	25.12	37.12	6.79	61
22:00:00	23.37	22.50	41.96	6.8	
23:00:00	15.49	22.50	41.99	6.79	
24:00:00	20.6	22.50	41.99	6.8	
25:00:00	22.25	22.50	42.01	6.8	91

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	12:04	3578	6.5	2.54	19.41	7.66	250	0
1				2.54	21.70	8.56		
2	16:15	3645	21	8.19	84.93	10.37	0	0
2				8.19	92.89	11.34		
3	19:53	3847	49	19.11	200.82	10.51	0	0
3				19.11	176.07	9.21		
4	21:00	3943	61	23.79	249.39	10.48	0	0
4				23.79	233.54	9.82		
5	25:00	4368	91	35.49	610.91	17.21	0	80
5				35.49	602.12	16.97		
5				35.49	584.24	16.46		

SCS1 Lyon Fermentation #4 May 24, 2007

Strain = SCS1 Lyon	Feed Batch Growth Rate = 0.28 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3573 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4371 ml
Final Dry Cell Weight = 35.1 g/L	
Time Heat treatment started: 21:00 h	Time fed-batch commenced = 12:09 h
Final Specific Yield = 16.64 mg/g	Final Volumetric Yield = 584 mg/L



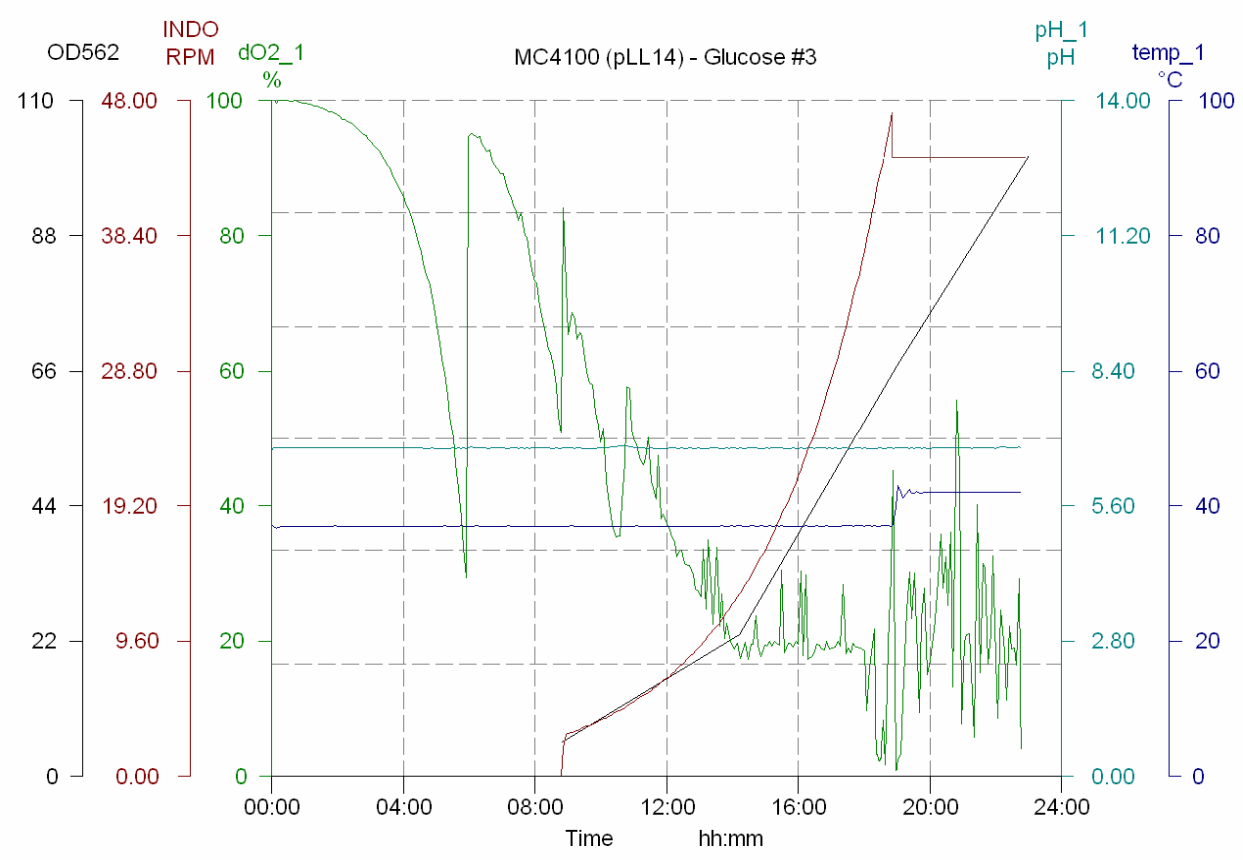
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	94.9	0	35.33	6.8	
1:00:00	95.73	0	36.98	6.8	
2:00:00	93.94	0	36.98	6.8	
3:00:00	90.91	0	37.02	6.8	
4:00:00	86.22	0	37.01	6.81	
5:00:00	78.69	0	36.98	6.81	
6:00:00	67.15	0	36.98	6.8	
7:00:00	46.65	0	37.01	6.8	
8:00:00	95.1	0	36.91	6.82	
9:00:00	92.36	0	37.01	6.8	
10:00:00	86.97	0	36.98	6.8	
11:00:00	79.93	0	37.02	6.8	
12:00:00	63.49	0	37.01	6.8	
12:09:00	61.83	0	36.98	6.8	6.7
13:00:00	61.64	2.92	36.98	6.8	
14:00:00	64.34	3.86	37.09	6.8	
15:00:00	52.93	5.1	37.01	6.8	
16:00:00	46.84	6.75	37.02	6.8	
16:20:00	31.41	7.42	37.02	6.8	20
17:00:00	20.04	8.94	36.98	6.8	
18:00:00	19.33	11.82	37.16	6.8	
19:00:00	19.62	15.65	37.09	6.8	
20:00:00	17.92	20.7	37.02	6.8	50
21:00:00	17.43	27.39	37.12	6.8	61
22:00:00	53.05	25.20	41.97	6.8	
23:00:00	1.31	25.20	42.04	6.8	
24:00:00	14.15	25.20	41.99	6.8	
25:00:00	37.78	25.20	42.09	6.8	90

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	12:09	3578	6.7	2.61	16.98	6.50	200	0
1				2.61	17.84	6.83		
2	16:20	3645	20	7.80	91.00	11.67	0	0
2				7.80	82.73	10.61		
3	20:00	3849	50	19.50	202.50	10.38	0	0
3				19.50	195.50	10.03		
4	21:00	3949	61	23.79	240.41	10.11	0	20
4				23.79	296.50	12.46		
5	25:00	4371	90	35.10	601.52	17.14	0	80
5				35.10	590.30	16.82		
5				35.10	560.61	15.97		

MC4100 Fermentation #3 May 30, 2007

Strain = MC4100	Feed Batch Growth Rate = 0.28 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3550 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4646 ml
Final Dry Cell Weight = 38.38 g/L	
Time Heat treatment started: 19:00 h	Time fed-batch commenced = 8:54 h
Final Specific Yield = 8.95 mg/g	Final Volumetric Yield = 344 mg/L



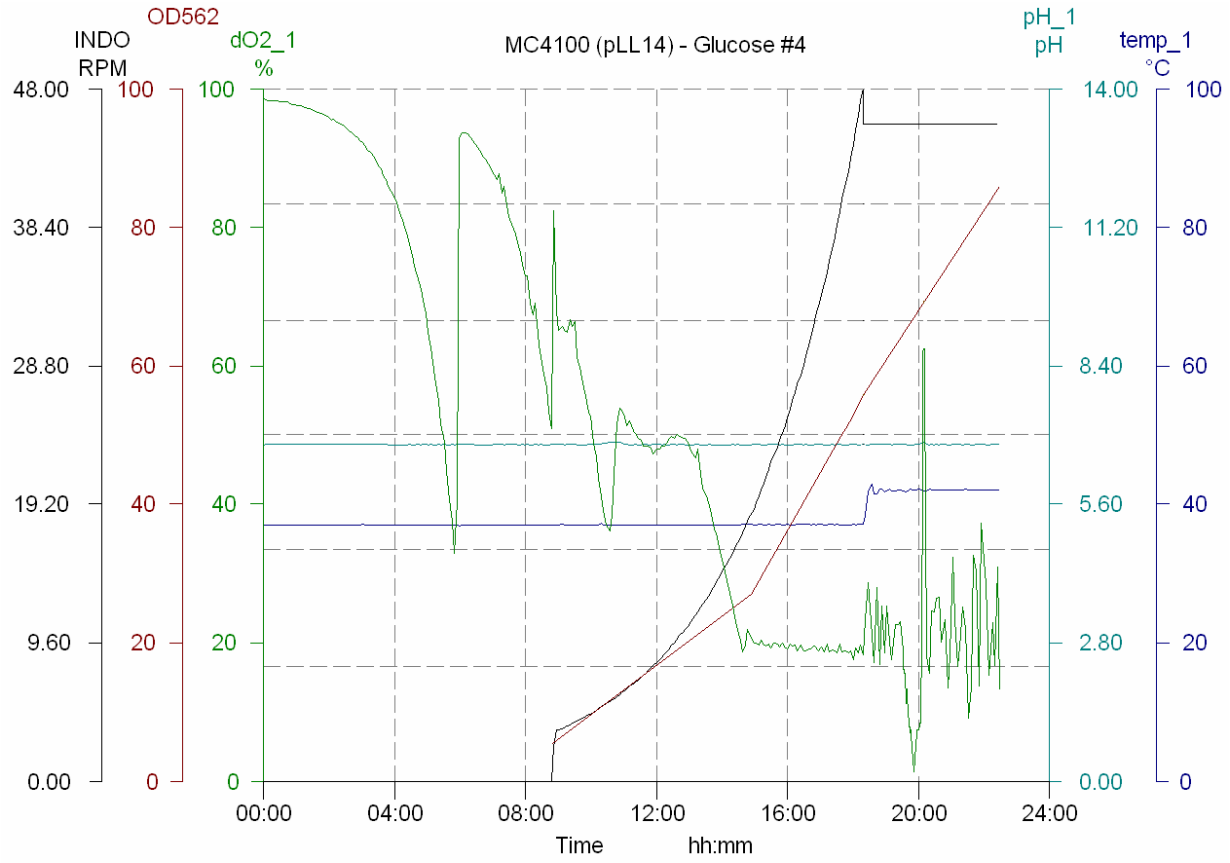
Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	101.02	0	37.12	6.75	
1:00:00	99.65	0	36.98	6.81	
2:00:00	97.87	0	36.98	6.81	
3:00:00	94.21	0	36.98	6.81	
4:00:00	85.86	0	37.02	6.8	
5:00:00	67.76	0	37.05	6.8	
6:00:00	94.66	0	36.84	6.82	
7:00:00	90.03	0	36.98	6.8	
8:00:00	73.28	0	37.02	6.8	
8:50:00	52.86	0	37.02	6.8	5.7
9:00:00	65.76	3	36.98	6.81	
10:00:00	49.42	3.97	36.98	6.81	
11:00:00	50.21	5.25	37.01	6.82	
12:00:00	37.34	6.95	37.02	6.8	
13:00:00	27.65	9.19	36.98	6.8	
14:00:00	19.46	12.17	37.09	6.8	
14:12:00	-0.11	12.87	37.12	6.8	23
15:00:00	18.61	16.1	37.02	6.79	
16:00:00	19.49	21.3	37.05	6.8	
17:00:00	19.67	28.18	37.02	6.8	
18:00:00	18.49	37.29	37.05	6.8	
19:00:00	5.88	49.34	41.71	6.8	67
20:00:00	17.11	65.28	42	6.79	
21:00:00	39.52	86.37	41.97	6.8	
22:00:00	17.25	114.28	42	6.79	
23:00:00	-0.11	144.99	41.68	6.83	101

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	8:50	3555	5.7	2.166	3.42	1.57	980	0
1				2.166	3.28	1.51		
2	14:12	3667	23	8.74	43.85	5.01	0	0
2				8.74	49.92	5.71		
3	19:00	4086	67	25.46	185.56	7.28	0	120
3				25.46	173.11	6.79		
4	23:00	4646	101	38.38	341.96	8.90	20	100
4				38.38	336.76	8.77		
4				38.38	352.84	9.19		

MC4100 Fermentation #4 June 5, 2007

Strain = MC4100	Feed Batch Growth Rate = 0.28 h-1
Batch Carbon Source = 5 g/l Glucose	Starting Volume = 3550 ml
Feed = 600 g/L Glucose + 10 g/l $MgSO_4$ + 1.5 ml/l Antifoam	Final Volume = 4425 ml
Final Dry Cell Weight = 32.68 g/L	
Time Heat treatment started: 18:20 h	Time fed-batch commenced = 8:54 h
Final Specific Yield = 8.90 mg/g	Final Volumetric Yield = 291 mg/L



Time	dO2 (%)	Feed Pump Speed (RPM)	temp (°C)	pH	OD 562
0:00:00	98.64	0	36.87	6.75	
1:00:00	97.7	0	36.94	6.8	
2:00:00	95.86	0	36.98	6.8	
3:00:00	92.24	0	37.05	6.8	
4:00:00	84.26	0	37.01	6.8	
5:00:00	66.44	0	37.01	6.8	
6:00:00	93.04	0	36.76	6.81	
7:00:00	88.04	0	37.01	6.8	
8:00:00	72.94	0	37.02	6.8	
8:49:00	63.25	0	37.01	6.8	5.5
9:00:00	65.54	3.55	36.98	6.8	
10:00:00	52.92	4.7	37.01	6.8	
11:00:00	52.68	6.22	36.98	6.82	
12:00:00	47.87	8.23	37.05	6.8	
13:00:00	48.45	10.89	37.05	6.8	
14:00:00	32.4	14.41	36.98	6.8	
14:54:00	22.4	18.3	37.05	6.8	27
15:00:00	21.3	19.06	37.05	6.79	
16:00:00	19.15	25.22	37.09	6.8	
17:00:00	19.82	33.37	37.09	6.8	
18:00:00	17.53	44.16	37.12	6.8	53
18:20:00	15.03	48	37.1	6.8	56
19:00:00	3.48	43	41.82	6.8	
20:00:00	12.05	43	42.16	6.8	
21:00:00	28.79	43	42.02	6.8	
22:00:00	31.83	43	41.99	6.8	
22:30:00	6.38	43	42	6.8	86

DNA Data

Sample	Time	Volume (ml)	OD	Biomass (gm/L)	Volumetric Yield (mg/L)	Specific Yield (mg/g)	Acetate (mg/L)	Glucose (mg/L)
1	8:49	3553	5.5	2.09	3.11	1.49	660	0
1				2.09	2.98	1.43		
2	14:54	3708	27	10.26	72.34	7.05	0	10
2				10.26	67.48	6.58		
3	18:00	3944	53	20.14	105.44	5.24	0	60
3				20.14	112.44	5.58		
4	22:30	4425	86	32.68	308.00	9.42	20	130
4				32.68	272.57	8.34		
4				32.68	292.29	8.94		

APPENDIX D

Raw Data From Dry Cell Weight Data

Strain	Gross Weight (mg)	OD	Sample Size (mL)	DCW Coefficient
SCS1-L	0.31	42	20	0.37
SCS1-L	0.56	67	20	0.42
SCS1-L	0.31	38	20	0.41
SCS1-L	0.18	26	20	0.35
SCS1-L Average		0.39		
BL21	0.514	70	20	0.37
BL21	0.527	67	20	0.39
BL21	0.465	55	20	0.42
BL21	0.314	40	20	0.39
BL21 - Average		0.39		
MC4100	0.355	46	20.5	0.38
MC4100	0.528	64	20	0.41
MC4100	0.17	26	18	0.36
MC4100	0.359	51	19	0.37
MC4100 - Average		0.38		