INSTITUTE OF CHEMICAL TECHNOLOGY, MUMBAI

1,3-PropaneDiol

Biochemical Engineering Project Report

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December 2, 2025

Abstract: This report brings together fragmented approaches from multiple studies to design a coherent process for producing 1,3-propanediol from glycerol using engineered E. coli. It integrates metabolic pathway insights, fermentation scale-up, and purification strategies.By reconciling methods originating from different research goals and scales, it develops a unified, practical design workflow.

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Chapter 1

Introduction

1.1 Overview

This report focuses on the biotechnological production of **1,3-propanediol (1,3-PDO)** using a genetically engineered *Escherichia coli* system. 1,3-PDO ($C_3H_8O_2$) is a three-carbon diol that serves as a versatile building block in polymer chemistry, particularly in the synthesis of polytrimethylene terephthalate (PTT)[1]. Its attractive properties, such as low toxicity, high miscibility with water and alcohols, and suitability for polycondensation reactions, make it an important industrial chemical.

1.2 Motivation

1.2.1 Sustainability

The biotechnological route to PDO production is attractive due to its renewable feedstocks and lower carbon footprint compared to petrochemical synthesis routes from acrolein[2] or using ethylene oxide as done by Shell[2]. This supports global sustainability goals and the transition toward green chemistry.

1.2.2 Industry

PDO serves as a monomer for the bio-based polymer PTT (commercially known as *Sorona*®), used in textile fibers, coatings, and engineering plastics. Its market is growing steadily as industries move towards sustainable materials [3].

1.3 Scope of Study

In this report, I have tried to study and understand several important aspects of chemical and biochemical engineering, such as:

- **Kinetic modeling** to analyze fermentation reaction behavior.
- **Reactor design** considerations for biological systems under aerobic and anaerobic conditions.
- **Downstream separation and purification** techniques used to recover and refine the final product.

The overall aim was to study the available the biological pathways involved in production with process design and industrial feasibility.

Chapter 2

Literature Review

2.1 Historical Background

The interest in biotechnological production of 1,3-PDO began with the discovery that certain microorganisms such as *Klebsiella pneumoniae* and *Clostridium butyricum* could ferment glycerol to 1,3-PDO through the action of dehydratase and dehydrogenase enzymes. However, these natural producers are often pathogenic or require strict anaerobic conditions, which complicate scale-up and industrial implementation [3][4], [5].

2.2 Industrial Pathways

2.2.1 Glycerol-based Production

Glycerol, a by-product of biodiesel production, can be converted into 1,3-PDO via two enzymatic steps:

$$\text{Glycerol} \xrightarrow{\text{Dehydratase}} \text{3-Hydroxypropionaldehyde} + H_2O \tag{2.1}$$

$$\text{3-Hydroxypropionaldehyde} + \text{NADH} + H^+ \xrightarrow{\text{Dehydrogenase}} 1,3-\text{Propanediol} + \text{NAD}^+ \tag{2.2}$$

This process has been successfully employed in China by companies such as Zhangjiagang Glory Biomaterial and Shenghong Group with capacities around 20–65 kt per year [3].

2.2.2 Sugar-based Production using Engineered Strains

Recent advances in metabolic engineering have enabled the production of PDO directly from glucose. In engineered *E. coli*, glucose is first converted to dihydroxyacetone phosphate (DHAP), which is then reduced to glycerol through heterologous genes from *Saccharomyces cerevisiae*. Subsequently, introduced genes from *K. pneumoniae* convert glycerol to 3-hydroxypropionaldehyde, which is then reduced to PDO by an *E. coli* enzyme [3].

2.3 Comparison of Hosts

2.3.1 Natural Producers

Klebsiella pneumoniae, *Citrobacter freundii*, and *Clostridium butyricum* have been widely studied as natural PDO producers, achieving high yields but facing biosafety limitations.

2.3.2 Engineered Hosts

E. coli has emerged as the preferred production host due to:

- Ease of genetic manipulation.
- Ability to utilize inexpensive carbohydrate feedstocks.
- Availability of well-characterized industrial fermentation systems.

Chapter 3

Biochemistry

3.1 Metabolic Pathway Overview

The engineered *E. coli* pathway integrates native glycolytic intermediates with heterologous genes to produce PDO. The simplified route from glucose is as follows:

1. Glucose → Dihydroxyacetone phosphate (DHAP)
2. DHAP → Glycerol (via *S. cerevisiae* genes)
3. Glycerol → 3-Hydroxypropionaldehyde (via *K. pneumoniae dhaB* operon)
4. 3-Hydroxypropionaldehyde → 1,3-Propanediol (via native *E. coli* dehydrogenase)[6]

3.2 Key Enzymes and Cofactors

- **Glycerol dehydratase (DhaB)** — B_{12}-dependent enzyme catalyzing glycerol dehydration.[6]
- **1,3-PDO dehydrogenase (DhaT)** — NADH-dependent reduction of 3-HPA to PDO.
- **Cofactors:** NADH/NAD^+ redox balance critically affects flux toward PDO.[7]

3.3 Engineering Considerations

3.3.1 Cofactor Balance

Maintaining NADH regeneration through glycolysis and side pathways is essential. Co-utilization of glucose as a reducing equivalent donor supports higher PDO yields.

3.3.2 Pathway Optimization

Typical genetic engineering steps include:

- Overexpressing heterologous *dha* genes.
- Knocking out competing glycerol pathways.
- Balancing promoter strengths and ribosome binding sites.

Chapter 4

Microbial Host Engineering

4.1 Background and reaction pathway

The canonical biological route to 1,3-propanediol (PDO) proceeds in two enzymatic steps: glycerol is dehydrated to 3-hydroxypropionaldehyde (3-HPA) and then 3-HPA is reduced to PDO. In many native PDO producers (e.g. *Klebsiella*, *Citrobacter*, *Clostridium*) these steps are catalyzed by a B_{12}-dependent dehydratase[8] and a linked NAD(P)H-dependent oxidoreductase (DhaT). The patent provides the pathway summary and the stoichiometries (Equations 4.1–4.3) used here[6].

$$\text{Glycerol} \xrightarrow{\text{dehydratase}} \text{3-HPA} + H_2O \tag{4.1}$$

$$\text{3-HPA} + \text{NADH} + H^+ \xrightarrow{\text{DhaT / ADH}} \text{1,3-PDO} + NAD^+ \tag{4.2}$$

$$\text{Glycerol} + NAD^+ \xrightarrow{\text{glycerol dehydrogenase}} \text{DHA} + \text{NADH} + H^+ \tag{4.3}$$

Equations 4.1–4.3 show the key redox link: PDO formation consumes reducing equivalents (NADH) while alternative glycerol oxidation (to DHA) produces NADH. Balancing these fluxes and cofactors is central to designing an efficient glucose→PDO route[6].

4.2 Key genetic elements (dha regulon and host activities)

The patent documents the **dha regulon** (genes typically from *Klebsiella*) used to provide glycerol→3-HPA capability in *E. coli* and lists the core genes: `dhaR`, `orfY`, `dhaT`, `orfX`, `orfW`, `dhaB1`, `dhaB2`, `dhaB3`, `orf7`. These encode regulatory, dehydratase subunits, and reactivation factors required for a functional glycerol dehydratase[6].

Crucially, the patent shows that **E. coli** encodes an endogenous, non-specific oxidoreductase activity capable of converting 3-HPA to PDO (not the canonical DhaT). The authors identify this native activity biochemically and link it to a specific open reading frame (noted in the patent as `yohD`/SEQ ID NO:57-58) that encodes a putative oxidoreductase (alcohol dehydrogenase family). Experimental work (gel activity staining, peptide sequencing, gene disruption) supports that this native enzyme provides the required reduction of 3-HPA in dhaT-minus strains.

4.3 Genetic modifications

[6] Based on the patent's examples the strain design combines the following deliberate genetic edits and plasmid additions:

1. **Introduce exogenous pathway genes (dha regulon subunits)**: `dhaB1, dhaB2, dhaB3` (dehydratase subunits) plus `orf7`, `orfX` (reactivation factors) and regulators to enable glycerol → 3-HPA activity in *E. coli*. Purpose: supply the rate-limiting dehydration step absent in wild-type *E. coli*[6]–[8]. native *E. coli* lacks a B_{12}-dependent glycerol dehydratase complex and reactivation machinery; this activity is essential to convert glycerol → 3-HPA[6].

2. **Removing `dhaT` (the canonical PDO oxidoreductase)**: experiments in the patent show that processes containing `dhaT` often accumulate aldehydes (3-HPA and related species) and experience higher cell lysis/ lower viability, likely because of imbalanced redox/reaction coupling and side reactions. Unexpectedly, eliminating `dhaT` results in lower accumulation of toxic intermediates, improved cell viability, and overall higher PDO titre. *This is a key counter-intuitive design choice that the patent highlights*[6].

3. **Introduce glycerol synthesis module**: genes encoding glycerol-3-phosphate dehydrogenase (G3PDH) and glycerol-3-phosphatase (G3P phosphatase) to convert glycolytic intermediates (DHAP) into glycerol in the same host, generates an internal glycerol pool from DHAP, coupling glycolytic flux to PDO formation. This creates a net route glucose → glycerol → 3-HPA → PDO, enabling single-organism conversion[6].

4. **Targeted knockouts in host competing pathways**: inactivate endogenous genes such as glycerol kinase (`glpK`), glycerol dehydrogenase (`gldA`/`dhaD`) and triosephosphate isomerase (`tpi`). Purpose: reduce competing consumption of glycerol/DHA or redirect carbon flux into the glycerol production branch and PDO pathway. Examples KLP23 / RJ8 strains in the patent use combinations of such inactivations[6], [8].

5. **Plasmids / expression cassettes**: the patent examples use plasmids (e.g. pKP32, pDT29, pAH48, pKP32) to carry pathway genes or glycerol modules and to tune expression; choice of plasmid alters observed titer and viability in fermentations.

4.4 Final Strain

The production strain used in the study[9] would be used for kinetic modeling. The strain is a genetically modified *E. coli* K-12 derivative[9].

4.5 Heterologous genes introduced

- **DAR1 (G3PDH) and GPP2 (G3P phosphatase)** - genes from *S. cerevisiae* introduced to convert dihydroxyacetone phosphate (DHAP) into glycerol (glycerol-3-phosphate →

glycerol). This supplies the glycerol precursor in a glucose-based route[10].

- **dhaB1, dhaB2, dhaB3 (glycerol dehydratase) and reactivation factors dhaBX / orfX** - from *Klebsiella pneumoniae*. These perform the dehydration: glycerol → 3-hydroxypropionaldehyde (3-HPA). The reactivation factors maintain dehydratase activity[8], [9].
- **yqhD (endogenous oxidoreductase)** - an E. coli enzyme (or other non-specific alcohol dehydrogenase activity) reduces 3-HPA to 1,3-PDO. In some constructs, deliberate deletion of dhaT (a dedicated 1,3-PDO dehydrogenase) was shown to actually improve yields because the endogenous enzyme gave lower harmful intermediate accumulation. See the patent examples for discussion[7], [8].

4.6 Deletions and host modifications

- **glpK, gldA deletions:** prevent produced glycerol from re-entering central carbon metabolism (avoid loss of carbon back into growth or by-product pathways)[8], [9].
- **arcA disruption:** removes oxygen-regulated repression that can trigger unwanted by-product formation under oxygen limitation. This supports high respiration and avoids acetate overflow[9].
- **PTS removal + galP + glk introduction:** replacing the phosphotransferase system (PTS) for glucose uptake with a galP (permease) + glk (glucokinase) system improves substrate uptake rate and often increases yield (reduces PEP drain)[9].
- **gapA downregulation:** tuning glycolytic flux to favour glycerol formation and redox balance for PDO production[9].

Chapter 5

Process design

A complete process chapter for producing 1,3-propanediol (1,3-PDO) from glycerol: seed-train scale-up (10× steps), fed-batch scale-up into a bubble-column reactor (geometry and aeration based on Bisgaard), fermentation operating strategy (strain design and staging based on Nakamura, Tang), pH/temperature guidance (Dharmadi), and a literature-informed downstream separation train (Przystałowska)[7]–[9], [11], [12].

5.1 Design assumptions

- Reactor geometry anchored to Bisgaard: diameter $D = 5.30$ m, choose initial liquid height $H = 3D = 3 \times 5.30 = 15.9$ m which till the end of process becomes $H = 5D = 5 \times 5.30 \approx 26$ m[9].
- Inoculum policy: target inoculum = 10% (v/v) of reactor initial liquid (Bisgaard practice for industrial seeding which I am extrapolating as discussed in class by Prof. VGG). 10× volume steps until the final inoculum is prepared; the last step is adjusted to match the 10% reactor inoculum volume. [9].
- Strain: engineered *E. coli* carrying glycerol → 1,3-PDO pathway with dhaB (B12-independent when applicable)[7], [8].
- Feedstock: crude biodiesel glycerol. [12]

5.2 Detailed calculations

Below are explicit calculation steps (formulas and evaluated numbers). keep these in your design chapter for traceability.

5.2.1 Reactor volume from geometry

Given:

$$D = 5.30 \text{ m}, \qquad H = 3D = 3 \times 5.30 = 15.9 \text{ m}.$$

Cross-sectional area:

$$A = \pi \left(\frac{D}{2}\right)^2 = \pi \left(\frac{5.30}{2}\right)^2 \approx 22.067 \text{ m}^2.$$

Volume:

$$V_{\text{reactor,init}} = A \times H = \pi \left(\frac{D}{2}\right)^2 H = \pi (2.65)^2 \times 15.9. \approx 350.783 \text{ m}^3.$$

[9].

5.2.2 Inoculum target (10% v/v)

$$V_{\text{inoc}} = 0.10 \times V_{\text{reactor,init}} = 0.10 \times 350.783 \text{ m}^3 \approx 35.0783 \text{ m}^3 \approx 35078.3 \text{ L}.$$

5.2.3 Seed-train (10× progression)

A strict 10× chain starting from common lab volumes gives:

$$0.1 \text{ L} \rightarrow 1 \text{ L} \rightarrow 10 \text{ L} \rightarrow 100 \text{ L} \rightarrow 1000 \text{ L} \rightarrow 10000 \text{ L} \rightarrow 35078 \text{ L (overshoot)}.$$

To hit the exact inoculum target and to avoid the overshppting adjust the last inoculum.

5.2.4 Biomass in final inoculum

Bisgaard reports biomass concentration based on optical density at 550 nm, using the conversion

$$1 \text{ OD}_{550} \equiv 3.0 \text{ g dry weight per L}$$

[9].

5.2.5 Aeration and superficial gas velocity

Bisgaard reports representative mixing-test air flow points:

$$Q_{\text{air,ref}} = 4.75 \text{ Nm}^3 \text{ s}^{-1}$$

[9]

Convert to hourly volumetric flow:

$$Q_{\text{air,ref}(m^3/h)} = 4.75 \frac{m^3}{s} \times 3600 \frac{s}{h} = 4.75 \times 3600 = 17100 \, m^3 \, h^{-1}.$$

Cross-sectional area (from above) $A \approx 22.067 \text{ m}^2$. Superficial gas velocity:

$$U_{\text{sg}} = \frac{Q_{\text{air,ref}}}{A} = \frac{4.75 \text{ m}^3/\text{s}}{22.067 \text{ m}^2} \approx 0.2153 \text{ m.s}^{-1}.$$

[9].

5.3 Seed-train schedule

5.4 Fermentation strategy

1. **Seed phases:** aerobic cultivation (staged, see volumes above). Typical setpoints: T = 30–37 C, pH near 7.0 (control with NH_4OH) and DO greater than 30% saturation during biomass build [7], [9].

Step	Volume (L)	Purpose / notes
1	0.1	Lab flask inoculum
2	1	intermediate
3	10	intermediate
4	100	intermediate
5	1000	intermediate
6	10000	intermediate
7	35078	Final inoculum (10% v/v of reactor)

Table 5.1: Seed Train Steps

2. **Main reactor fill and growth:** fill the bubble-column to the initial liquid height $H = 15.9$ m and inoculate with the prepared inoculum (10% v/v). Operate an aerobic fed-batch growth phase.[9].

3. **Transition to production:** two valid choices depending on the strain:

   - *Aerobic engineered 1,3-PDO route:* maintain aerobic conditions and feed glycerol (or feed glycerol co-substrate) while operating DO at strain-appropriate setpoint (e.g., DO > 20%) [8].
   - *Tang two-stage glycerol conversion:* switch from aerobic growth to a production stage by (i) replacing/perfusing medium with glycerol feed, (ii) applying the thermal induction (example: 30°C → 42°C in Tang) and (iii) adjusting oxygen to microaerobic values if required by the glycerol pathway regulation [7].

4. **pH control during glycerol conversion:** glycerol fermentation and by-product distribution are pH sensitive; maintain pH in the approximately 6.0–7.0 window [11].

5.5 Feeding strategy

- **Growth:** glucose/dextrose feed (or glycerol if preferred) under DO-based or biomass-growth feedback.
- **Production:** feed glycerol in a controlled fed-batch profile to avoid glycerol inhibition [9], [11].

5.6 Downstream Train

- **Product/byproducts:** 1,3-PDO (target product) plus byproducts such as acetate, ethanol, succinate and others depending on oxygenation and genetics.[11]–[13].
- **Downstream (recommended staged train):**

  1. Cell removal: membrane filtration or High Speed Centrifuge.

2. Volatile removal: low-temperature distillation/vacuum stripping to remove ethanol and light volatiles.
3. Acid/byproduct removal: pH adjustment and liquid-liquid extraction or ion exchange to remove succinate/acetate (reviewed options in Przystałowska) [12].
4. PDO concentration/polishing: evaporation and distillation to reach commercial purity; final polishing ion exchange.

Chapter 6

Reactor & Kinetics

6.1 Bioreactor and process description

I model the same system studied in the paper by Bisgaard et al. (2022): a bubble column of diameter $D = 5.3\,\mathrm{m}$ and nominal total volume $V \approx 600\,\mathrm{m}^3$ operated as a fed-batch producing 1,3-propanediol with a recombinant *E. coli* strain. The process ran for 32 hr, the gassed liquid height increased during the feed, and air was supplied through bottom spargers with a time-varying gas flow profile. The paper measured DO and pressure at a probe 5.85 m from the bottom and used flow-following sensors to generate axial velocity fields[9].

image

6.2 Hydrodynamics and transformations

6.2.1 Axial position from pressure (Pascal's law)

From the sensor pressure $P(t)$ we can calculate the axial position $z(t)$ using

$$z(t) \;=\; \frac{P_{\max} - P(t)}{\rho_f(t)\, g}, \tag{1}$$

where $P_{\max}$ is the maximum measured pressure (bottom of the vessel), $\rho_f(t)$ is the instantaneous fluid density and g is gravity. The paper describes compensation of headspace and volume changes before this transform.[9].

6.2.2 Gas hold-up correlation

The study used the an't Riet & Tramper (1991) style correlation for a heterogeneous regime as reported in the paper:

$$\varepsilon \;=\; v_s^{0.25} \;+\; 0.45\,(g\, v_s\, D)^{1/3}, \tag{2}$$

where ε is the gas hold-up (gas fraction), v_s is the superficial gas velocity ($\mathrm{m\,s^{-1}}$), g is gravity and D the column diameter.

6.2.3 Fluid density of the gas-liquid dispersion

Given the gas hold-up $\varepsilon$the dispersion (bulk) density as the liquid-gas volumetric average:

$$\rho_f \;=\; (1-\varepsilon)\,\rho_l \;+\; \varepsilon\,\rho_g. \tag{3}$$

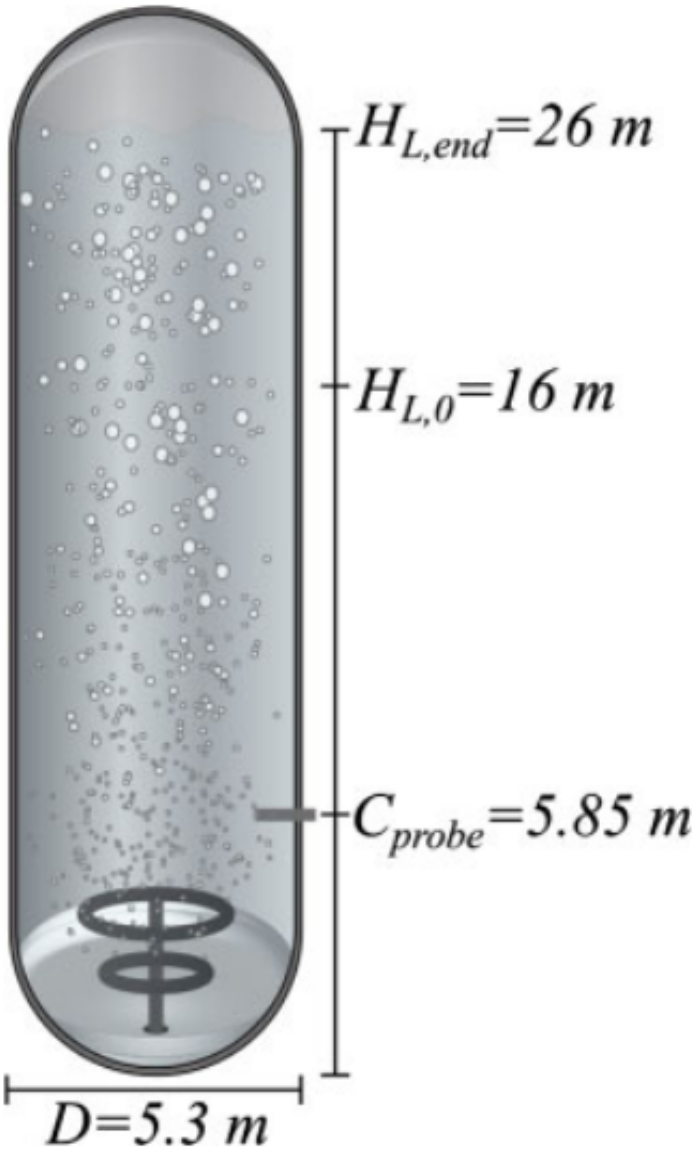

Figure 6.1: Illustration of Bubble Column Bioreactor

6.2.4 k_L a model

Bisgaard et al. adopted a linear relation (Guske & Miller, 2009) and used the simple relation:

$$k_L a(j,k) \;=\; 0.288\, v_s(j,k), \tag{5}$$

6.3 Compartmental model

6.3.1 Model layout

Partition the column axially into K axial compartments (index $k = 1,\ldots,K$), where each compartment is assumed ideally mixed. Between compartments there are bidirectional axial flows $Q_{k\leftrightarrow k+1}(t)$ derived from the Lagrangian sensor velocities; volumes $V_k(t)$ change with the liquid height and are updated at discrete update steps $j = 1,\ldots,J$.

6.3.2 governing differential equations

The paper presents the following standard per-volume ODEs for a single ideal compartment (their Equations 3-6).:

Biomass:

$$\frac{dC_{x,k}}{dt} \;=\; \mu_k C_{x,k}\,. \tag{6}$$

Substrate:

$$\frac{dC_{s,k}}{dt} \;=\; -r_{s,k} C_{x,k} \;+\; F_{s,j,k=K}. \tag{7}$$

Feed F_s is added to the top compartment $k = K$ according to the profile and is updated at each compartment update step image

Product (PDO):

$$\frac{dC_{p,k}}{dt} \;=\; r_{p,k} C_{x,k} \tag{8}$$

image

Dissolved oxygen (DO):

$$\frac{dC_{o,k}}{dt} \;=\; k_L a(j,k)\left(C_o^*(j) - C_{o,k}\right) \;-\; r_{o,k} C_{x,k} \tag{9}$$

where $C_o^*(j)$ is the local saturation concentration in the compartments for the update step j. image

```
# compute derivatives and instantaneous specific rates (finite
    differences)

```

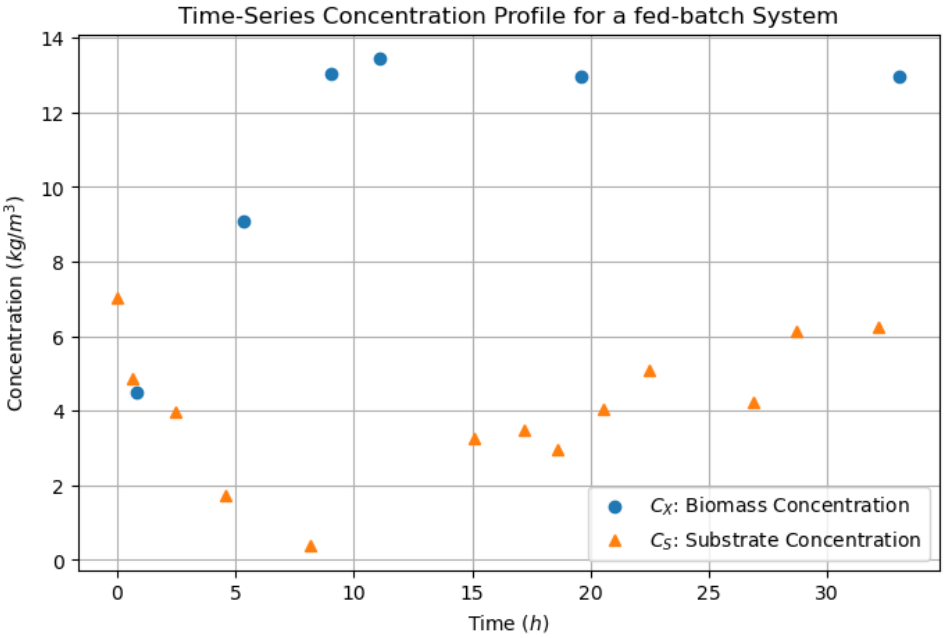

Figure 6.2: Biomass & Substrate Concentration wrt Time

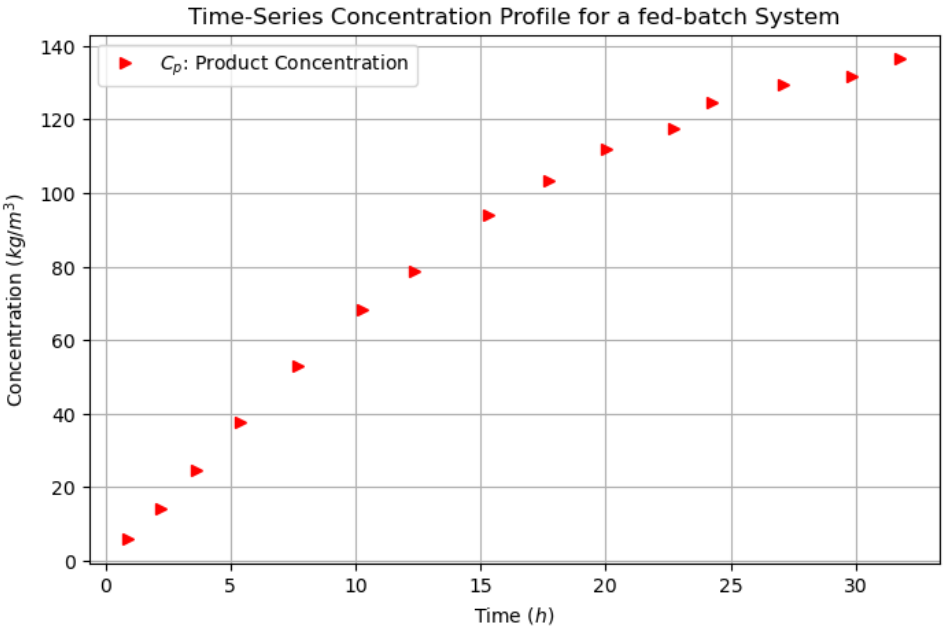

Figure 6.3: Concentration of Product v/s Time

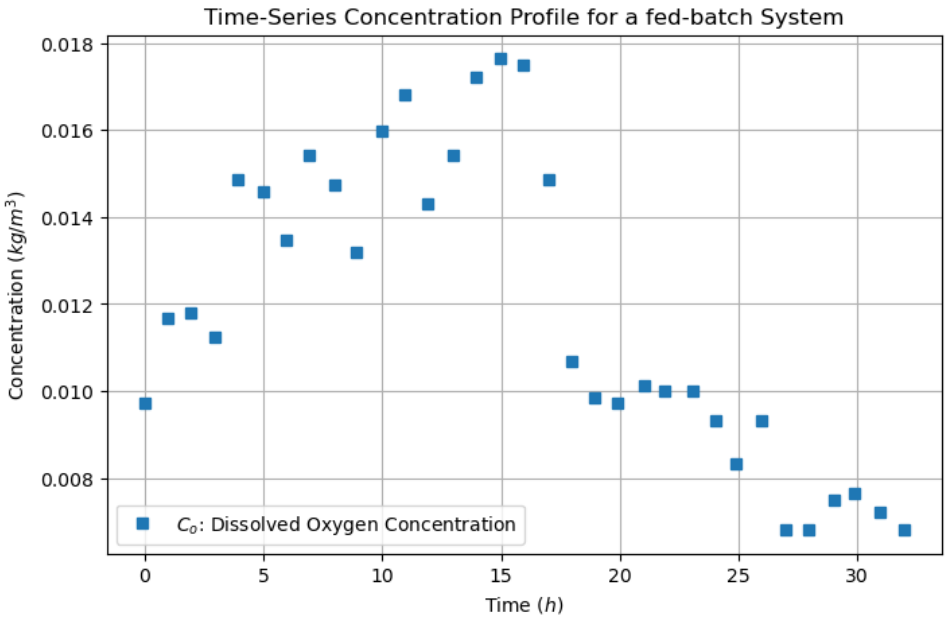

Figure 6.4: Concentration of Dissolved Oxygen v/s Time

```
dt = np.gradient(t)  # time step array (h)
dCxdt = np.gradient(Cx, t)
dCsdt = np.gradient(Cs, t)
dCpdt = np.gradient(Cp, t)
dCodt = np.gradient(Co, t)

# avoid division by zero
eps = 1e-12
rp_inst = dCpdt / (Cx + eps)          # specific product formation rate (1/h)
rs_inst = -dCsdt / (Cx + eps)         # specific substrate uptake (mass substrate / (mass biomass * h))
mu_inst = dCxdt / (Cx + eps)          # instantaneous specific growth rate (1/h)
```

Listing 6.1: Simple Monod kinetics simulation in Python

6.4 Kinetic rates

6.4.1 Growth rate (Monod with product inhibition)

Bisgaard et al. used a Monod form with oxygen limitation and product inhibition:

$$\mu_k = \mu_{\max} \frac{C_{s,k}}{C_{s,k}+K_s} \frac{C_{o,k}}{C_{o,k}+K_o} \left(1 - \frac{C_{p,k}}{K_p}\right), \tag{10}$$

where K_s is the half-saturation for substrate, K_o for oxygen, and K_p the product inhibition concentration.

```
# fit mu params: mu_max, Ks, Ko, Kp using dX/dt = mu(Cs,Co,Cp) * X

def mu_model(cs, co, cp, params):
    mu_max, Ks, Ko, Kp = params
    term_s = cs / (cs + Ks + 1e-12)
    term_o = co / (co + Ko + 1e-12)
    term_p = np.maximum(0.0, (1 - cp / (Kp + 1e-12)))
    return mu_max * term_s * term_o * term_p

def simulate_Cx(t, mu_max, Ks, Ko, Kp):
    C0 = Cx[0]
    params = [mu_max, Ks, Ko, Kp]

    def rhs(C, tt):
        cs = np.interp(tt, t, Cs)
        co = np.interp(tt, t, Co)
        cp = np.interp(tt, t, Cp)
        mu = mu_model(cs, co, cp, params)
        return mu * C

    Csim = odeint(lambda C, tt: rhs(C, tt), C0, t).flatten()
    return Csim

# initial guesses and bounds
p0 = [4, 5, 1, 20.0]
bounds = ([1e-6, 1e-6, 1e-6, 1e-6], [5.0, 1e3, 1.0, 1e5])

# fit model to experimental biomass data
popt, pcov = curve_fit(simulate_Cx, t, Cx, p0=p0, bounds=bounds, maxfev=2000)
```

Listing 6.2: Simple Monod kinetics simulation in Python

6.4.2 Product formation

Specific product formation is given by:

$$r_{p,k} = Y_{pX}\,\mu_k + r_{pX}, \tag{11}$$

6.4.3 Substrate and oxygen uptake rates

As in the paper:

$$r_{s,k} = \frac{\mu_k}{Y_{xs}} + \frac{r_{p,k}}{Y_{ps}} + r_{m,s}, \tag{13}$$

$$r_{o,k} = \frac{r_{s,k}}{Y_{so}} + r_{m,o}, \tag{14}$$

where the Y's are stoichiometric yields and $r_{m,\cdot}$ are maintenance terms.

```
dX_total = Cx[-1] - Cx[0]
dP_total = Cp[-1] - Cp[0]
dS_total = Cs[0] - Cs[-1]    # substrate consumed (positive)

Yxs_global = dX_total / dS_total if abs(dS_total) > 1e-12 else np
    .nan
Yps_global = dP_total / dS_total if abs(dS_total) > 1e-12 else np
    .nan
Ypx_global = dP_total / dX_total if abs(dX_total) > 1e-12 else np
    .nan
```

Listing 6.3: Simple Monod kinetics simulation in Python

6.4.4 Fitted parameters

I fitted the kinetic and transfer parameters to the experimental data. Table 6.1 lists the fitted parameter values using `curve_fit` from `scipy.optimize`, though Bisgaard et al. (2022) uses `LSODA` from `scipy.integrate` to integrate and then applies `Nelder-Mead` to minimize SSE and iteratively solve for the parameters. image

Table 6.1: Fitted parameters and goodness-of-fit metrics.

Parameter	Fitted value	Units
$\mu_{\max}$	3.97	hr^{-1}
K_s	4.89	$\mathrm{g\ L}^{-1}$
K_o	0.99	$\mathrm{mg\ L}^{-1}$
K_p	1227.3×10^2	$\mathrm{g\ L}^{-1}$
$Y_{p/X}$	15.51	g product g^{-1} biomass
R^2	-6.9	
SSE	5160	

$$\mathrm{SSE} = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2, \qquad R^2 = 1 - \frac{\mathrm{SSE}}{\sum_{i=1}^{n} (y_i - \bar{y})^2}.$$

```
y = np.asarray(Cx)
yhat = np.asarray(Cx_sim)
```

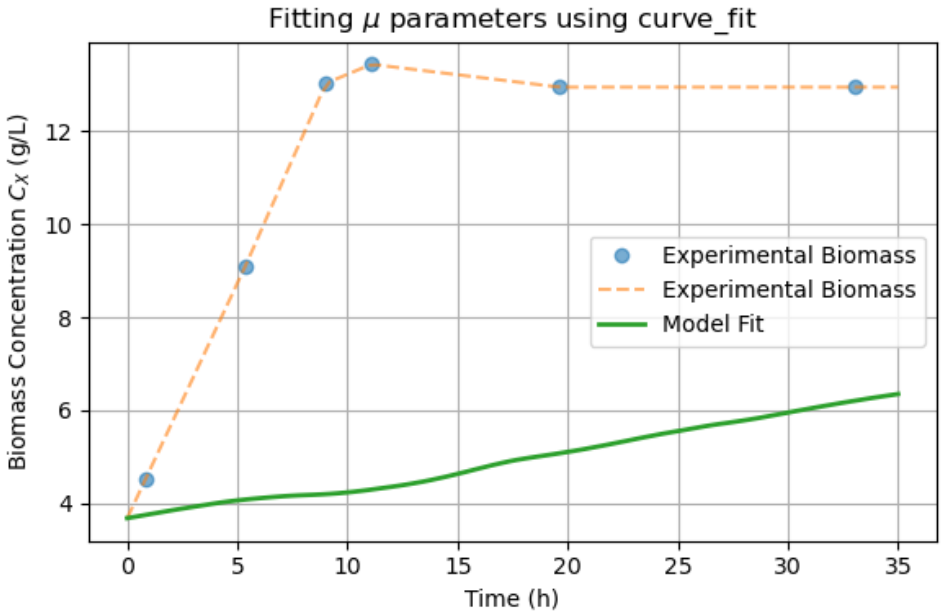

Figure 6.5: Experimental v/s fitted Biomass COncentration

```

n = len(y)
p = len(mu_params) if 'mu_params' in globals() else 1

sse = np.sum((y - yhat)**2)
mse = sse / n
rmse = np.sqrt(mse)
ss_tot = np.sum((y - np.mean(y))**2)
r2 = 1 - sse / ss_tot if ss_tot > 0 else np.nan
adj_r2 = 1 - (1 - r2) * (n - 1) / (n - p - 1) if (n - p - 1) > 0
    else np.nan
```

Listing 6.4: Simple Monod kinetics simulation in Python

Chapter 7

Downstream Processing and Separation

7.1 Overview and separation challenges

Commercial recovery of 1,3-propanediol (PDO) is challenging because fermentation broths typically contain low PDO concentrations (ca. 5-15 wt%), a complex matrix of cells, salts, organic acids and residual nutrients, and because PDO is relatively hydrophilic, high boiling and low volatility.

These properties make recovery energy-intensive and costly: a robust downstream train must

(i) remove cells and solids,

(ii) exclude inorganic and organic impurities, and

(iii) perform final concentration and polishing to a commercial product grade.

Typical unit operations used in the literature and patents include centrifugation and membrane filtration, ion exchange and electrodialysis, solvent/salting-out extraction, adsorption, pervaporation, and vacuum distillation or preparative chromatography for final polishing [1].

7.2 Primary removal of cell & Intermediate impurity removal

The first step is removal of biomass and particulate materials to avoid fouling and excessive solvent load downstream which are separated by membrane filtration, high-speed centrifugation and evaporation. Choice depends on scale, broth rheology, and downstream sensitivity to particulates. The broth still contains inorganic salts, organic acid salts, glycerol, small organic molecules and color bodies and are mostly separated by the broth still contains inorganic salts, organic acid salts, glycerol, small organic molecules and color bodies.

7.2.1 Ion exchange

Cation and anion exchange resins remove mineral ions and organic acid salts effectively. DuPont reported removal of >98% of mineral salts and organic acid salts using strong-acid cation and weak-base anion exchangers in sequence; however, resins saturate quickly and their regeneration consumes substantial NaOH and HCl[1].

7.2.2 Electrodialysis

Electrodialysis can selectively remove ionic species (organic acid salts, inorganic ions) and has been used to desalinate fermentation broths. Reports show removal of about 90% of organic acid salts, but electrodialysis suffers from significant energy consumption, capital cost and can cause PDO losses; it also generates brine waste streams that require disposal or treatment [1].

7.2.3 Liquid-liquid extraction and salting-out

Because PDO is highly hydrophilic, direct solvent extraction is often inefficient. Salting-out (two-phase extraction) uses inorganic salts (e.g. K_2CO_3, K_2HPO_4) with a water-miscible or partially miscible organic extractant (e.g. isopropanol, n-butyl acetate) to partition PDO into an organic or salted phase. Several studies report recoveries $>90\%$ and even 98.27% (e.g. K_2CO_3/isopropanol systems) depending on salt, pH and solvent choice [1]. Salting-out is attractive for high recovery but leaves large inorganic salt loads in aqueous effluent requiring treatment; solvent selection and recycle are crucial for economics and environmental performance.

7.2.4 Reactive Distillation

Reaction between PDO & aldehyde to form dioxolane which is extracted in organic solvent and hydrolsed.

Chapter 8

Market Analysis

8.1 Global Market Overview

The global market for 1,3-propanediol has expanded rapidly since its commercialization by DuPont in the early 2000s. Recent industry analyses project continued growth, driven by demand for bio-based polymers and environmentally friendly solvents [14]. At Dupont & Tate and Lyle Bioproducts' production plant which has been operating since 2006, is reportedly undergoing expansion to increase the capacity from 61–77 kt per annum.[14]

8.2 Production Capacities and Key Players

- DuPont's sugar-based fermentation process produces PDO for *Sorona*® fiber.
- Chinese producers such as Zhangjiagang Glory Biomaterial and Shenghong Group operate glycerol-based PDO plants with capacities of 20–65 kt per year [3][5][4].
- Suzhou Suzhen Biological Engineer Co., Ltd. (a subsidiary of Shenghong Group) officially opend a 20 ktons glycerol based PDO plant and would increase it to 40 kton in 2nd phase[5].
- **DSM** and **METEX NØØVISTA** (a subsidiary of METabolic EXplorer) formed an exclusive alliance to produce and market *TILAMAR® PDO with NØØVISTA*™, the first made-in-Europe, 100% bio-based, non-GMO cosmetic-grade 1,3-propanediol[15].
- DuPont Tate & Lyle Bio Products LLC's Susterra® 1,3-propanediol is a 100% bio-based glycol produced via sugar fermentation, offering a sustainable and cost-effective alternative to petrochemical routes[16].
-
- **METabolic Explorer, Genomatica, BASF** — biotechnology companies active in PDO and related diols.

8.3 Cost Factors

The main economic drivers for PDO production include:

1. **Feedstock cost:** sugars or glycerol contribute 50–70% of total production cost.
2. **Fermentation yield and titer:** higher concentrations reduce downstream energy demand.

3. **Downstream processing:** cell inactivation, ion exchange, and distillation dominate energy and capital costs.

8.4 Process Scale and Feasibility

Plants typically operate between 20 and 77 kt per year [5] capacities. [4] With further optimization of engineered *E. coli* and reduced feedstock costs, glucose-to-PDO routes are economically viable at industrial scales.

8.5 Conclusion

The global Market is projected to reach USD 690.6 million by 2025 at a cagr od 100.4% [4]. The Sale Price or Production cost of 1,3-PDO is 1.83 USD/kg[1]. The economic outlook for bio-based PDO is promising, provided feedstock prices remain low and process efficiencies improve. Both glycerol- and sugar-based routes can be commercially attractive depending on regional availability of substrates and infrastructure.

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