
Process monitoring and control using innovative optical sensors for fermentative lactic acid production in membrane bioreactor system

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千里之行，始於足下
《道德经》-老子
A journey of a thousand miles begins with a single step
Tao Te Ching - Laozi

Declaration/Confidentiality Clause

This thesis presents the results of work done in the group of membrane technology and the group of bioprocess engineering, Institute of bioprocess engineering and biopharmaceutical technology, the University of Applied Sciences Mittelhessen, under the supervision of Professor Peter Czermak.

I declare that I have finished the enclosed dissertation entitled "Process monitoring and control using innovative optical sensors for fermentative lactic acid production in membrane bioreactor system" entirely by myself and have not used sources or means without declaration in the text. All thoughts or quotations which were inferred from these sources are clearly marked.

Rong Fan

Giessen Germany

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Summary

Lactic acid (LA) is an important organic acid, traditionally used in food, pharmaceutical, leather and textile industries as an acidulant, a flavouring or a preservative. Since the beginning of the 21st century, the expanding demand of LA is mainly caused by the application to synthesize polylactic acid (PLA), which is a biodegradable, compostable and biocompatible plastic. However, the current price of LA has strongly limited the competitiveness of PLA compared with petroleum-derived plastics. Therefore, reducing the production cost by increasing the volumetric productivity has become a critical goal, and various methods focused on increasing the titre, production rate and final yield of LA have been employed in both manufacturing and downstream separation processes.

In industry, the microbial conversion of sugars (in fermenters) is preferable to the chemical synthesis of LA, because fermentation can achieve stereospecific LA production by using different strains of microorganisms, whereas chemical synthesis provides only racemic mixture of D,L-LA. However, the productivity of LA in conventional batch or fed-batch fermentations is often limited by the product inhibition. Commonly, the inhibitory effect occurs when the LA concentration in the fermentation broth reaches a critical level. In this study, the investigations of batch fermentation indicate that the growth of *Bacillus coagulans* PS5 is obviously inhibited at the concentration of LA > 40 g·L⁻¹.

A membrane unit integrated reactor system, i.e. membrane bioreactor (MBR) system is a promising candidate to surmount this limit by continuous removal of LA and supplement of substrates. The alleviation of product inhibition allows the cells to keep growing during the entire fermentation process, achieving a high level of cell density and LA productivity. The LA productivity in MBR systems, like traditional continuous fermentation, depends on the dilution rate as well as the product concentration. Because the dilution rate in MBR systems is limited by the filtration flow, a high and stable permeate flow is desired for the long-term continuous fermentation. The flux control, achieved by optimizing the operational mode of membrane filtration processes, can enhance the membrane efficiency and in the meantime reduce the capital and operational costs. Obviously, the optimization of the operational mode has to base on the knowledge of decline in filtration flux, which can be obtained by the fouling analysis using the resistance-in-series model. The analysis suggests that the major resistance (up to ~80% of total resistance) in the filtration of LA fermentation broth is attributed to the cake layer formed by the deposition of cells. Due to the high compressibility of the cake layer (compressibility index $n = 2.09$), the decline in flux during the filtration process could be conveniently controlled by increasing crossflow velocity rather than transmembrane pressure.

Considering the importance of cell density both in fermentation and filtration unit, determining the profile of cell density during the fermentation (growth curve), is integral to the process control. Conventional offline biomass measurement cannot provide a real-time cell growth profile, thus optical sensors allowing online monitoring in real-time are promising candidates to monitor the biomass concentration online during fermentation. Optical sensors based on diverse measuring

Summary

principles usually have different responses to the measured objects. In order to verify the feasibility, two types of optical sensors were tested in the fermentation broth. The appropriate optical sensor was selected and then, incorporated into the bioreactor for the online monitoring of cell growth during both batch and continuous fermentation processes. The corresponding system outputs in the membrane bioreactor were regulated with the cell growth profile to maintain a stable productivity with desired dilution rate and product concentration. In order to achieve the most cost-efficient operation, the influences of process parameters such as feed concentration and dilution rate on the LA productivity were investigated in the MBR system. During these processes, the optical sensor was used to: (i) online monitor the cell growth, (ii) analyse and identify the reasons of different process errors, (iii) enhance the process safety, efficiency and stability. Under the online monitoring with the optical sensor, a stable continuous production of LA in the MBR system was achieved with the highest productivity of $8.6 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and the highest yield of $0.87 \text{ g}\cdot\text{g}^{-1}$. This productivity is 2.5 times as high as the best overall productivity obtained in batch fermentations.

Keywords:

Lactic acid, fermentation kinetics, membrane filtration, membrane bioreactor, optical sensor, online process monitoring and control

Zusammenfassung

Milchsäure ist eine wichtige organische Säure, die traditionell in der Lebensmittel-, Pharma-, Leder- und Textilindustrie als Säuerungsmittel, Aroma oder Konservierungsmittel verwendet wird. Seit Anfang des 21. Jahrhundert wird die steigende Nachfrage nach Milchsäure hauptsächlich von der Anwendung für die Synthese der Polymilchsäuren (PLA) verursacht, welche ein biologisch abbaubarer, kompostierbarer und biokompatibler Kunststoff ist. Allerdings wird die Wettbewerbsfähigkeit der PLA im Vergleich mit aus Erdöl gewonnenen Kunststoffen stark vom aktuellen Preis der Milchsäure begrenzt. Daher müssen die Herstellungskosten der Milchsäure reduziert werden. Dies ließe sich durch die Erhöhung der Raum-Zeit-Ausbeute im Prozess realisieren. Verschiedene Verfahren zur Erhöhung der Titre, der Produktionsrate und der Ausbeute an Milchsäure kommen sowohl in der Herstellung als auch in der nachgeschalteten Aufarbeitung zum Einsatz.

Zur industriellen Milchsäure-Herstellung wird die mikrobielle Umwandlung von Zuckern (in Fermentern) der chemischen Synthese vorgezogen. Der Grund hierfür ist die stereospezifische Milchsäure-Produktion, die durch den gezielten Einsatz verschiedener mikrobieller Stämme erreicht wird, während die chemische Synthese in ein racemisches Gemisch aus D- und L-Milchsäure resultiert. Die Milchsäure-Produktivität konventioneller Batch- oder Fed-Batch-Fermentationen wird allerdings oft durch Produktinhibition limitiert, sobald die Milchsäure-Konzentration ein kritisches Niveau erreicht. In der vorliegenden Arbeit zeigten Batch-Untersuchungen mit *Bacillus coagulans* PS5 einen wachstumsinhibierenden Effekt bei einer Milchsäure-Konzentration von $> 40 \text{ g L}^{-1}$.

Ein Reaktorsystem mit integrierter Membraneinheit, bzw. Membranbioreaktor-System (MBR-System) ist ein attraktiver Lösungsansatz derartige Wachstumsinhibitionen durch kontinuierliche Produktentfernung und Substratzugabe zu minimieren. Eine Verringerung der Produkthemmung ermöglicht ein verlängertes Zellwachstum während des gesamten Fermentationsprozesses und somit eine gesteigerte Zelldichte und Milchsäure-Bildung. Die Milchsäureproduktion in MBR-Systemen, wie auch andere traditionelle kontinuierliche Fermentation, hängt dabei von der Verdünnungsrate und Produktkonzentration ab. Da in MBR-Systemen die Verdünnungsrate durch den Filtrationsfluss limitiert wird, ist ein hoher und stabiler Filtrationsfluss für kontinuierliche Fermentationen notwendig. Die Kontrolle des Filtrationsflusses, ermöglicht durch optimierte Filtrationsbedingungen, kann die Membranleistung erhöhen und gleichzeitig die Investitions- und Betriebskosten reduzieren. Grundlage für die Optimierung der Filtrationsbedingungen ist ein ausgeprägtes Verständnis für das Filtrationsverhalten, welches über Fouling-Analysen unter Verwendung des Widerstand-in-Serien Modells erlangt werden kann. Die Fouling-Analysen zeigten, dass der Hauptfiltrationswiderstand (bis zu ~80% des Gesamtwiderstandes) auf den Kuchenwiderstand zurückzuführen ist. Aufgrund der hohen Kompressibilität der Kuchenschicht (Kompressibilitätsindex $n = 2,09$) konnte eine Abnahme der Filtrationsflusses primär über die Erhöhung der Überströmungsgeschwindigkeit anstatt des Transmembrandruckes kontrolliert werden.

Zusammenfassung

In Anbetracht der Wichtigkeit der Zelldichte für die Fermentation und Filtrationseinheit, ist die Ermittlung des Zelldichteprofiles (Zellwachstumskurve) während der Fermentation ein integraler Bestandteil der Prozesssteuerung. Konventionelle Offline-Messungen der Biomasse können hierfür keine Zellwachstumsprofile in Echtzeit liefern. Somit rücken optische Sensoren als vielversprechende Kandidaten für eine geeignete Online-Überwachung der Fermentation in den Fokus. Je nach Messprinzip reagieren diese aber unterschiedlich auf das zu vermessende Objekt. Zur Auswahl eines geeigneten optischen Sensors wurden somit in der vorliegenden Arbeit zwei Sensor-Typen auf ihre Eignung hin untersucht (im Batch- sowie kontinuierlichen Betrieb). Der am besten geeignete optische Sensor ist schließlich zur Online-Überwachung des Zellwachstums im MBR-System integriert worden. Dabei wird die Leistung des MBRs über die Zellwachstumskurve reguliert, zur Aufrechterhaltung einer stabilen Produktivität bei einer definierten Verdünnungsrate und Produktkonzentration. Für eine kostengünstige Betriebsweise wurden hierzu die Einflüsse von Prozessparametern, wie Zulaufkonzentration und Verdünnungsrate, auf die Milchsäure-Produktivität des MBRs untersucht. Während dieser Untersuchungen ist der optische Sensor dazu genutzt worden: (i) das Zellwachstum online zu verfolgen, (ii) Prozessfehler zu analysieren und zu identifizieren, (iii) die Prozesssicherheit, Effizienz und Stabilität zu erhöhen. Unter sensor kontrollierten Bedingungen konnte im MBR eine stabile, kontinuierliche Milchsäure-Produktion erreicht werden mit einer Produktivität von bis zu $8,6 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ und einer Ausbeute von $0,87 \text{ g} \cdot \text{g}^{-1}$. Die erzielte Produktivität ist um das 2,5-fache höher als in den Batch-Fermentationen.

Schlüsselwörter:

Milchsäure, Fermentationskinetik, Membranfiltration, Membranbioreaktor, optischer Sensor, online Prozessüberwachung und -kontrolle

Abbreviations

Nomenclature

A	Membrane area	m^2
a	Growth associated constant	$g \cdot g^{-1}$
b	Non-growth associated constant	h^{-1}
c	Concentration	$g \cdot L^{-1}$
D	Dilution rate	h^{-1}
D	Diffusion coefficient	$m \cdot s^{-1}$
d	Diameter	m, cm, mm
F	Volumetric flow	$L \cdot h^{-1}$
J	Flux	$L \cdot m^{-2} \cdot h^{-1}$
k	Cake resistance coefficient-related constant, defined in Eq.4.3-1	$bar^{2.09} \cdot m^{-1}$
k	Mass transfer coefficient	$m^3 m^{-2} \cdot s^{-1}$
K	Constant defined in the individual sections	
l	Length	m, cm
M/m	Mass	kg
$\dot{m}$	Mass flow	$kg \cdot h^{-1}$
N	Number	
n	Compressibility index	
P	Pressure	bar
Q	Rate of production	$g \cdot L^{-1} \cdot h^{-1}$
r	Specific resistance	m^{-2}
R	Resistance	m^{-1}
S	Cross-sectional area	m^2
Sh	Sherwood number	
t	Time	s, min, h
V	Volume	L
v	Velocity	$m \cdot s^{-1}$
Y	Yield	$g \cdot g^{-1}$
AFG	Afguard	
ANOVA	Analysis of variance	
CDW	Cell dry weight	$g \cdot L^{-1}$
CFV	Crossflow velocity	$m \cdot s^{-1}$
DO	Dissolved oxygen	$mg \cdot L^{-1}$
EMP	Embden-Meyer-Parmas	
EPS	Extracellular polymer substance	
LA	Lactic acid	
LAB	Lactic acid bacteria	
NIR	Near infrared	
PLA	Polylactic acid	
OD	Optical density	
Re	Reynolds number	
RO	Reverse osmosis	
TMP	Transmembrane pressure	bar
MBR	Membrane bioreactor	
MRS	De Man, Rogosa and Sharpe	
MWCO	Molecular weight cut off	kDa
MF	Microfiltration	
NF	Nanofiltration	

Abbreviations

UF	Ultrafiltration	
<u>Greek letters</u>		
α	Cake resistance coefficient	$\text{m}\cdot\text{kg}^{-1}\cdot\text{bar}^{-2.09}$
α_0	Specific cake resistance coefficient	$\text{m}\cdot\text{kg}^{-1}$
β	Concentration related specific cake resistance coefficient	$\text{m}^2\cdot\text{g}^{-1}$
ϵ	Porosity	%
σ	Standard deviation	
μ	Growth rate	h^{-1}
δ	Cake layer thickness	m
κ	diffusion coefficient	$\text{m}^2\cdot\text{s}^{-1}$
η	Viscosity	$\text{mPa}\cdot\text{s}$
π	Osmosis pressure	Pa
ρ	Density	$\text{kg}\cdot\text{m}^{-3}$
τ	Shear stress	Pa
$\dot{\gamma}$	Shear rate	s^{-1}
<u>Subscripts</u>		
0	Initial value	
a	Adsorption	
b	Boundary layer	
b	Bulk of solution	
bio	Biomass	
c	Cake layer	
cp	Concentration polarization	
d	Death	
f/ feed	Feed	
g	Gel layer	
glu	Glucose	
i	Inhibition	
i	Individual component	
in	Input	
l	Laminar	
LA/lac	Lactate	
m	Membrane	
m/max	Maximal	
out	Output	
p	Particle	
p	Product	
p	Production	
p	Permeate	
p	Pore blockage	
R	Reactor	
r	Reaction	
s	Substrate	
ss/ ∞	Steady state	
t	Turbulent	
tot	Total	
w	Water	
1	Pressure at inlet	
2	Pressure at outlet	
600	At 600 nm	

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