

IMPLEMENTING ALTERNATIVE CONTROL STRATEGIES FOR BIOREACTOR APPLICATIONS

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IMPLEMENTING ALTERNATIVE CONTROL STRATEGIES FOR BIOREACTOR APPLICATIONS

by

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.....Dedicated
to my
Parents and sister

CERTIFICATE

This is to certify that the thesis entitled “**IMPLEMENTING ALTERNATIVE CONTROL STRATEGIES FOR BIOREACTOR CONTROL APPLICATIONS**” being submitted by **Mr. VIKI RAJENDRA CHOPDA** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him. VIKI RAJENDRA CHOPDA has worked under our guidance and supervision and has fulfilled the requirements for the submission of the thesis.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

We certify that he has pursued the prescribed course of research.

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ABSTRACT

This thesis documents a study of implementing advanced control schemes towards bioreactor control applications. Bioreactor operation is a primary operation in biotherapeutics manufacturing which significantly impacts both the amount and quality of the product being manufactured. Complexity of the control strategy increases with reactor size, which may vary from thousands to tens of thousands of liters in commercial manufacturing. The regulatory initiative of Process Analytical Technology (PAT) has highlighted the need for having robust monitoring tools and effective control schemes that are capable of taking real time information about the critical quality attributes (CQA) and the critical process parameters (CPP) and executing immediate response as soon as a deviation occurs. However, the limited flexibility that present commercial software packages offer may create a hurdle. We have developed an integrated program using a visual programming environment for carrying out bioreactor operations through which various peripheral devices are interfaced. The proposed program facilitates real-time access to data and allows execution of control actions to follow the desired trajectory.

Apparently, regulation at the cellular level is a complex phenomenon and as a result highly non-linear state-time profiles are used to characterize bioprocesses. In such circumstances, special attention must be devoted to design and to implement advanced controllers. Baker's yeast fermentation process possesses an appropriate degree of complexity so that successful demonstration of implementing an advanced non-linear controller for this process presents a challenge. Consequently, by solving a control problem for this process, in turn, becomes equivalent to providing a solution for a range of bioprocesses.

A model for baker's yeast fed-batch fermentation was selected from the literature. This model was further examined from a system perspective to ensure that it possessed correct structure in

terms of controllability and observability. The model was validated by experiments. Using this model, a decoupled input-output linearizing controller (DIOLC) was developed. This controller possesses two separate control loops – substrate controller by substrate feeding rate and dissolved oxygen (DO) regulated by air flow rate. The performance of DIOLC was benchmarked against the performance of a proportional–integral–derivative (PID) controller under different set of conditions. A case study also demonstrated comparing performances of DIOLC and PID controller for biomass maximization in the baker’s yeast process. Superiority of DIOLC was demonstrated over PID controller due to the ability of DIOLC to negate the interactions between the inputs and outputs.

A different control strategy that did not have a strong dependence on the quality of the model was used to control fed-batch fermentation of *Pichia pastoris* for the production of recombinant human serum albumin (rHSA). Suitability of adaptive control was examined. Using principles of decoupling (as used in the formulation of DIOLC), a decoupled adaptive controller (DAC) was implemented for this process. Results of the performances of DAC (in spite of some disturbances in DO) were found to be at par compared to that with PID controller. DAC was better at controlling substrate than DO as measurements of DO showed disturbances in the transition phase (from glycerol batch to methanol induction phase). As a result, augmenting the strengths of both DAC and DIOLC was utilized to develop a third hybrid control scheme.

Another important aspect that has been examined is the proteolytic degradation of recombinant products by host-specific proteases that hinder effective production and purification of heterologous proteins at high cell density fermentation from yeasts such as *S. cerevisiae* and *P. pastoris*. We demonstrated that use of conductivity sensor as a potential PAT tool for monitoring real-time measurements of medium conductivity can lead to higher product titer with minimal protease formation.

सार

यह शोध बायोरिएक्टर नियंत्रण अनुप्रयोगों की ओर उन्नत नियंत्रण योजनाओं को लागू करने के एक अध्ययन का दस्तावेजीकरण करता है। बायोरिएक्टर ऑपरेशन बायोथेरेप्यूटिक्स निर्माण में एक प्राथमिक ऑपरेशन है जो उत्पाद की मात्रा और गुणवत्ता दोनों पर काफी प्रभाव डालता है। रिएक्टर आकार के साथ नियंत्रण रणनीति की जटिलता बढ़ जाती है, जो हजारों से वाणिज्यिक उत्पादन में हजारों लीटर तक भिन्न हो सकती है। प्रोसेस एनालिटिकल टेक्नोलॉजी (पीएटी) की विनियामक पहल में मजबूत निगरानी उपकरण और प्रभावी नियंत्रण योजनाएं होने की ज़रूरत पर प्रकाश डाला गया है जो महत्वपूर्ण गुणवत्ता विशेषताओं (सीक्यूए) और महत्वपूर्ण प्रक्रिया मापदंडों (सीपीपी) के बारे में वास्तविक समय की जानकारी लेने और तत्काल निष्पादित करने में सक्षम हैं। जैसे ही विचलन होता है, उतनी ही प्रतिक्रिया। हालांकि, सीमित लचीलापन जो वर्तमान वाणिज्यिक सॉफ्टवेयर संकुल प्रदान करता है, वह बाधा पैदा कर सकता है। हमने बायोरिएक्टर ऑपरेशन के लिए एक विज़ुअल प्रोग्रामिंग परिवेश का उपयोग करके एक एकीकृत प्रोग्राम विकसित किया है जिसके माध्यम से विभिन्न परिधीय डिवाइस इंटरफ़ेस कर रहे हैं। प्रस्तावित कार्यक्रम डेटा पर वास्तविक समय तक पहुंच की सुविधा देता है और वांछित प्रक्षेपवक्र का पालन करने के लिए नियंत्रण कार्यों के निष्पादन की अनुमति देता है।

जाहिर है, सेलुलर स्तर पर विनियमन एक जटिल घटना है और इसके परिणामस्वरूप अत्यधिक नॉन-लीनियर स्टेट टाइम प्रोफाइल का उपयोग बायोप्रोसेसों को चिह्नित करने के लिए किया जाता है। ऐसी परिस्थितियों में, उन्नत नियंत्रकों को डिजाइन करने और लागू करने के लिए विशेष ध्यान समर्पित होना चाहिए। बेकर की किण्वन प्रक्रिया में जटिलता की एक उपयुक्त डिग्री है, ताकि इस प्रक्रिया के लिए एक उन्नत गैर-रेखीय नियंत्रक को लागू करने का सफल प्रदर्शन एक चुनौती प्रस्तुत करता है। नतीजतन, इस प्रक्रिया के लिए एक नियंत्रण समस्या को सुलझाने के द्वारा, कई तरह के बायोप्रोसेसों के लिए एक समाधान प्रदान करने के बराबर बन जाता है।

बेकर'स यीस्ट के फेरमेंटेशन प्रोसेस के लिए एक मॉडल को साहित्य से चुना गया था। इस मॉडल को एक सिस्टम परिप्रेक्ष्य से आगे की जांच करने के लिए यह सुनिश्चित किया गया था कि यह नियंत्रण योग्यता और निगरानी के मामले में सही ढांचा रखता

है। मॉडल प्रयोगों द्वारा मान्य किया गया था। इस मॉडल का इस्तेमाल करते हुए, एक डिकप्लेड इनपुट-आउटपुट रेखीयरिंग नियंत्रक (डायओलसी) विकसित किया गया था। इस नियंत्रक में दो अलग-अलग नियंत्रण लूप हैं - सबस्ट्रेट फीडिंग रेट द्वारा सबस्ट्रेट कंट्रोलर और वायु प्रवाह दर द्वारा नियंत्रित ऑक्सीजन (डीओ) भंगा। डायओलसी का प्रदर्शन विभिन्न परिस्थितियों के तहत आनुपातिक-अभिन्न-व्युत्पन्न (पीआईडी) नियंत्रक के प्रदर्शन के खिलाफ बेंचमार्क था। एक मामले का अध्ययन भी बेकर'स यीस्ट के फेरमेंटेशन प्रोसेस में बायोमास के अधिकतमकरण के लिए डायओलसी और पीआईडी नियंत्रक के प्रदर्शन की तुलना करता है। निविष्टियों और आउटपुट के बीच बातचीत को नकारने के लिए डायओलसी की क्षमता के कारण डायओलसी का श्रेष्ठता पीआईडी नियंत्रक पर प्रदर्शित किया गया था।

एक अलग नियंत्रण रणनीति जिसकी मॉडल की गुणवत्ता पर एक मजबूत निर्भरता नहीं थी, को पुनः संयोजक मानव सीरम एल्बुमिन (आरएचएसए) के उत्पादन के लिए पिचिया पस्टोरिस के खिलाए गए बैच किण्वन को नियंत्रित करने के लिए उपयोग किया गया था। अनुकूली नियंत्रण की उपयुक्तता की जांच की गई। डिकॉप्लिंग के सिद्धांतों का उपयोग (जैसा कि डायओलसी के निर्माण में प्रयुक्त होता है), इस प्रक्रिया के लिए एक डिकप्लेड अनुकूली नियंत्रक (डीएसी) लागू किया गया था। डीएसी (डीओ में कुछ गड़बड़ी के बावजूद) के प्रदर्शन का परिणाम पीआईडी नियंत्रक के साथ तुलना में बराबर पाया गया। डैक की तुलना में डीओ की तुलना में डीएसी की तुलना में सबस्ट्रेट को नियंत्रित करने में डीएसी बेहतर था। संक्रमण चरण में ग्लिसरॉल बैच से मेथनॉल आयन चरण तक की गड़बड़ी दिखायी गयी। परिणामस्वरूप, डीएसी और डायल दोनों की शक्तियों को बढ़ाने के लिए तीसरी संकर नियंत्रण योजना विकसित करने के लिए उपयोग किया गया था।

एक अन्य महत्वपूर्ण पहलू की जांच की गई है जो मेजबान-विशिष्ट प्रोटेक्शन द्वारा पुनः संयोजक उत्पादों के प्रोटीलायटिक डिग्रेडेशन है, जो कि एस. स. सर्विसिए और पिचिया पस्टोरिस जैसे खमीर से उच्च कोशिका घनत्व किण्वन पर प्रोटीनों के प्रभावी उत्पादन और शुद्धि को रोक देता है। हमने दिखाया है कि चालकता संवेदक का उपयोग मध्यम चालकता के वास्तविक समय माप की निगरानी के लिए एक संभावित पीएटी उपकरण के रूप में कम प्रोटीज गठन के साथ उच्च उत्पाद हो सकता है।

किसी भी बायोप्रोसेस के नियंत्रण के लिए उत्पाद टिटार का तीव्र मात्रा का एक महत्वपूर्ण इनपुट है। एक एकीकृत पद्धति विकसित की गई है जिसमें लक्ष्य प्रोटीन और कुल प्रोटीन एकाग्रता दोनों का अनुमान क्रमशः आंतरिक रिबोफ्लविन प्रतिदीप्ति और बाहरी फ्लोरोसेंस के उपयोग से किया गया है। प्रस्तावित दृष्टिकोण दो अलग-अलग बायोथेरेप्यूटिक्स उत्पादों, मानव सीरम एल्ब्यूमिन और ग्रैन्यूलोसाइट कॉलोनी उत्तेजक कारक के लिए मान्य किया गया है, क्रमशः पिचिया पस्टोरिस के म्यूटी और मट्स के उपभेदों के माध्यम से व्यक्त किया गया। प्रस्तावित दृष्टिकोण तेजी से और पीकी पिचिया पस्टोरिस किण्वन में पीएटी कार्यान्वयन के लिए एक महत्वपूर्ण संबल होने का प्रदर्शन किया गया है।

इस थीसिस का उद्देश्य विभिन्न चुनौतियों का सामना करना है जो विभिन्न हार्डवेयर सेंसरों के एकीकरण, पर्यवेक्षी कार्यक्रम के विकास, मॉडल विकास और विभिन्न नियंत्रण योजनाओं को डिजाइन करने और कार्यान्वित करने, पीएटी आधारित प्रक्रिया विकास और उत्पाद आकलन उपकरणों की जांच सहित विभिन्न चुनौतियों का सामना करना है। हम आशा करते हैं कि इन प्रस्तावित रणनीतियों और उपकरणों का उपयोग अत्यधिक कुशल और उत्पादक बायोफार्मासिटिकल प्रक्रियाओं के निर्माण के लिए किया जा सकता है।

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