



**UNIVERSITI PUTRA MALAYSIA**

**PERFORMANCE ANALYSIS OF HELICAL RIBBON IMPELLER IN  
PRODUCTION OF RED PIGMENT BY *MONASCUS PURPUREUS* FTC  
5391**

**MOHD SHAMZI MOHAMED**

**FBSB 2009 14**



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**By**

**MOHD SHAMZI MOHAMED**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the  
Degree of Master of Science**

**April 2009**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**April 2009**

**Chairman : Professor Arbakariya Ariff, PhD**

**Faculty : Faculty of Biotechnology and Biomolecular Sciences**

Agitation in stirred tank bioreactors require a great deal of attention as damage to microorganisms limit the extent of impeller speed or power input, which in effect will disturb the actual mass transfer capability and productivity of a bioreactor. A new variant of low shear impeller under helical ribbon class (Paravisc) was investigated for its potential to replace the Rushton turbine which was found to exert excessive shearing in mixing processes and proved less efficient in handling non-Newtonian fluids. Performance analysis on Paravisc impeller covered the aspect of physical mixing as well as the integration model fungal system. Under non-biological environment, the impeller torque measurement gave Newtonian power-flow relationship with a power constant ( $K_p$ ) of 424.7 for mixing under laminar condition. Reynolds number ( $Re$ ) at 60 marked the critical point,  $Re_{crit}$ , where flow shifted to transitional regime. In case of non-Newtonian fluids, impeller power draw decreases as shear thinning behavior increases. The vessel shear rate was calculated from the superposition of both Newtonian and non-Newtonian power data. The vessel shear rate constant ( $K_s$ ) was then derived as a function of  $K_p(n)$  of the given

power law fluids. The mixing time analysis produced a mixing time constant ( $K_m$ ) of 53.8 throughout laminar regime but decreases when approaching  $Re_{crit}$  before stabilized again at 11.8 in turbulent flow regime. The volumetric oxygen transfer coefficient ( $k_La$ ) was more affected by the agitation of Paravisc than it had with superficial gas velocity for non-viscous fluids when mixing was above a certain critical impeller speed ( $N_C$ ). However, gassing rate became more influential on oxygen transfer for tests using 0.2 % to 0.8 % w/v carboxymethylcellulose sodium (CMCNa) solutions. Predictive  $k_La$  correlations in the form power law equations derived via multiple linear regressions resulted in separate correlations for water, electrolytes and viscous simulant fluids. Application of Paravisc in red pigment production by *Monascus purpureus* FTC5391 was based on the theoretical  $k_La$  attainable in media using calculations from the developed model. The highest yield and productivity by Paravisc mixing was achieved at 250 rpm and 1.5 VVM with  $Y_{P/S}$  and  $P$  equaled to 0.47 UA<sub>500</sub>/g.glucose and 0.15 UA<sub>500</sub>/h, respectively. These were 58% and 14% more than what was obtained by Rushton impeller. Operation-wise, mixing with a single Paravisc was more efficient since it require 42% less energy than a typical double Rushtons operation. Consequently, this led to two-fold red pigment yield per energy consumed ( $Y_{P/E}$ ) for helical ribbon impeller, of which 152.1 UA<sub>500</sub>/kW.h was measured from stirred tank bioreactor retrofitted with the novel agitator compared to 69.2 UA<sub>500</sub>/kW.h produced by Rushtons at 600 rpm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**ANALISA PRESTASI PENGADUK REBEN BERLINGKAR DALAM  
PENGHASILAN PIGMEN MERAH OLEH  
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Keadaan percampuran di dalam tangki pengaduk memerlukan perhatian yang teliti oleh kerana kerosakan pada mikroorganisma menghadkan kelajuan pengaduk atau pembekalan kuasanya, akibatnya ini boleh menjejaskan keupayaan sebenar pemindahan jisim dan produktiviti sesebuah bioreaktor. Sejenis pengaduk variasi baru di dalam kelas reben berlingkar (Paravisc) dikaji potensinya untuk menggantikan pengaduk turbin Rushton yang didapati mengenakan ricihan berlebihan dalam proses pencampuran dan kurang efisien dalam mengendalikan bendalir non-Newtonian. Analisa prestasi ke atas Paravisc mengambilkira aspek percampuran fizikal serta penglibatan sistem model fungus. Untuk analisa tanpa kehadiran unsur biologi, ukuran tork pengaduk memberikan hubungan kuasa-aliran dengan pemalar kuasa ( $K_p$ ) bersamaan 424.7 untuk percampuran keadaan laminar. Nombor Reynolds pada 60 menandakan titik kritikal,  $Re_{crit}$  di mana aliran berubah kepada rejim transisi. Di dalam kes bendalir bukan Newtonian, penggunaan kuasa oleh pengaduk berkurangan apabila sifat penjarangan ricih bendalir meningkat. Kadar geseran tangki dikira dari pertindihan antara data kuasa pengaduk bendalir

Newtonian dan bukan Newtonian. Pemalar kadar geseran, ( $K_s$ ) pula diterbit sebagai fungsi kepada  $K_p(n)$  sesebuah bendalir hukum kuasa. Analisa masa percampuran menghasilkan pemalar masa percampuran ( $K_m$ ) pada lingkungan 53.8 di sepanjang rejim laminar, tetapi ianya menurun apabila mendekati  $Re_{crit}$  sebelum kembali stabil pada 11.8 dalam aliran turbulen. Koefisien volumetri pemindahan oksigen ( $k_La$ ) pula lebih dipengaruhi oleh kadar putaran Paravisc berbanding halaju gas superfisial untuk bendalir berkelikatan rendah apabila percampuran melangkaui satu kelajuan kritikal pengaduk ( $N_c$ ). Namun, keadaan kadar penggasan lebih memberi kesan terhadap pemindahan oksigen untuk larutan carboxymethylcellulose bergaram natrium (CMCNa) yang berkepekatan 0.2% hingga 0.8% w/v. Korelasi ramalan  $k_La$  yang menyerupai persamaan hukum kuasa diterbit melalui regresi linear berganda menghasilkan korelasi berlainan untuk air, elektrolit dan bendalir simulasi likat. Aplikasi Paravisc ke atas penghasilan pigmen merah oleh *Monascus purpureus* FTC 5391 dibuat berdasarkan nilai  $k_La$  yang dicapai secara teori berpandukan pengiraan menggunakan model yang dibangunkan. Hasil dan produktiviti tertinggi dari percampuran dengan Paravisc dicapai pada 250 rpm dan 1.5 VVM dengan  $Y_{p/s}$  dan  $P$  bersamaan 0.47 UA<sub>500</sub>/g.glukos dan 0.15 UA<sub>500</sub>/h. Nilai-nilai ini 58% dan 14% lebih tinggi dari yang dicapai oleh Rushton. Dari segi operasi, percampuran dengan Paravisc lebih efisien kerana ia menggunakan 42% kurang tenaga berbanding operasi bioreaktor dengan dua Rushton. Ini menyebabkan hasil pigmen per tenaga digunakan,  $Y_{P/E}$  adalah dua kali ganda untuk pengaduk reben berlingkar, di mana 152.1 UA<sub>500</sub>/kW.h diukur dari bioreaktor yang diubahsuai dengan sistem terbaru ini berbanding 69.2 UA<sub>500</sub>/kW.h yang dijana oleh Rushton pada 600 rpm.

## ACKNOWLEDGEMENTS

First and foremost, all gratitude and utmost praise to Allah, the eternally Most Gracious and Most Merciful Creator, for He has gracefully sustained me throughout every endeavors and challenges in this lifetime.

The successful completion of this thesis would not had see the light if it wasn't supported by the continuous motivation, constructive guidance and a little bit of coaxing by the ever patient Professor Dr Arbakariya Ariff throughout the course of this study. My appreciations and gratitude also goes to the members of my supervisory committee, Assoc. Prof. Dr Rosfarizan Mohamad for her valuable comments on the direction of this research and Mr. Musaalbakri Abdul Manan of Biotechnology Research Center, MARDI who has greatly assisted me in kick starting this project. Special thanks are accorded to Mr Rosli Aslim for his invaluable and practical handyman tips and approaches during the design of experiments.

Heartfelt appreciation is also due to all faculty members of FBSB, laboratory staffs and fellow graduate students of Fermentation Technology Unit, Institute of Bioscience for their kind co-operation and assistance during the period of this study. The author wishes to recognize the contribution of two Nashruls, one who had enabled me to be part of UPM family and another who has generously helped me in the course of my research. Not to be forgotten are the rest of compadres; Mr Saiful Bahri, Zukhrufuzzaman, Masanto, Rizal Ibrahim, Dena, Faidz, Ida Farida, Rizal Kapri, Sobri, Azilah, Liyana, Zuha, Farliahati, Shikin, Mariam Aisha, Azlina Mansor, the too many



Fazli in existence (Fazly Wong, Mohd Fadly, Fazli Asras and Fadzli Mat Aris), Azman, Azlan, Radin, Helmi, Alawi, Azhari, Rafein, Zulkhairi, Yamin, Hj Fadhil and the rest of the tutors as well as countless undergraduates.

Special appreciation and deepest gratitude is extended to my family and Cheeta for their love and support. Finally, I would like to acknowledge the generous financial support of Universiti Putra Malaysia on sponsoring this Masters degree.

*“Mixing one's wines may be a mistake,  
but old and new wisdom mix admirably”*

**- Bertolt Brecht -**

I certify that a Thesis Examination Committee has met on 27<sup>th</sup> April 2009 to conduct the final examination of Mohd Shamzi bin Mohamed on his thesis entitled “Performance Analysis of Helical Ribbon Impeller in Red Pigment Production by *Monascus purpureus* FTC 5391” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institution.

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**MOHD SHAMZI MOHAMED**

Date: 22 June 2009

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## LIST OF ABBREVIATIONS

<b>Symbols</b>		<b>Units</b>
$A$	Gas-liquid interfacial area per unit volume	$\text{m}^2 / \text{m}^3$
ANOVA	Analysis of variance	-
$C$	Clearance between impeller blade and wall	m
$C_2$	Distance between two turbines	m
$C_b$	Off-bottom clearance	m
$C_L$	Actual dissolved oxygen concentration in liquid	mg/L
$C_L^*$	Saturated dissolved oxygen concentration in liquid	mg/L
$C_p$	Oxygen concentration registered by electrode in media	mg/L
$C_s$	Actual quasi steady state dissolved oxygen concentration during fermentation	mg/L
$C_{ps}$	Measured quasi steady state dissolved oxygen concentration by probe during fermentation	mg/L
CCD	Charged coupled display	-
CMCNa	Carboxymethylcellulose sodium salt	-
CFD	Computational Fluid Dynamics	-
DOT	Dissolved oxygen tension	%
DO	Dissolved oxygen	mg/L
$D_i$	Diameter of the impeller	m
$D_T$	Internal diameter of mixing vessel	m
$d_{32}$	Sauter mean bubble diameter	mm
$d_i$	Diameter of an equivalent sphere	mm
$d_1, d_2$	Diameter of major and minor axis of an ellipsoid	mm
$E$	Energy supplied to impeller	kW.h
EMPS	Extracellular microbial polysaccharide	-
$F_{d\theta}$	Drag force in $\theta$ direction	N
$Fr$	Froude number	-
$G$	Gravitational acceleration	$\text{m/s}^2$
$H_i$	Height of helical ribbon impeller	m
$H_L$	Height of liquid	m

$H_T$	Height of tank	m
$H_l$	Liquid height at gassed condition	m
$H_0$	Liquid height at ungassed condition	m
HRI	Helical ribbon impeller	-
HRS	Helical screw impeller	-
HR-RT	Coaxial combination of Helical ribbon-Rushton turbine	-
$H_x$	Liquid height in inclined tube manometer	m
$K$	Fluid Consistency index	Pa.s <sup>n</sup>
$k_e$	Oxygen electrode sensitivity	s <sup>-1</sup>
$k_{La}$	Volumetric oxygen transfer coefficient	s <sup>-1</sup>
$k_L$	Liquid side oxygen transfer coefficient	m/s
$K_m$	Dimensionless mixing time or number of stirrer revolutions required to achieve a degree or a level of homogeneity	-
$K_p$	Impeller power constant	-
$K_s$	Metzner and Otto shear rate constant	-
LOF	Lack of Fit	-
$M$	Torque generated by mixing system	Nm
$M_0$	Torque at zero load	Nm
$m$	Growth associated rate constant of product formation	g.product /g.cell
$n$	Non-growth associated rate constant of product formation	g product/g cell.h
$n$	Flow behavior index	-
$N$	Impeller rotational speed	rev/min or rev/s
$N_C$	Critical impeller speed	rev/min or rev/s
$N_{CD}$	Speed of complete dispersion	rev/min or rev/s
$N_r$	Number of ribbon	-
OD	Optical density	-
OTR	Oxygen transfer rate	mg.O <sub>2</sub> /L.h
OUR	Oxygen uptake rate	mg.O <sub>2</sub> /g.cell.h

$P$	Productivity	g.cell/L.h or UA <sub>500</sub> /h
$P$	Ungassed power input to the impeller	W
$P_g$	Gassed power input to the impeller	W
$P_o$	Power number	-
PDA	Potato dextrose agar	-
$Q$	Volumetric air flow rate	m <sup>3</sup> / s
$QO_2$	Specific microbial oxygen uptake rate	mg.O <sub>2</sub> /g. cell/ h
$Re$	Reynolds number	-
$Re_{eff}$	Effective or Generalized Reynolds number	-
$Re_{pl}$	Power law Reynolds number	-
rpm	Revolution per minute	s <sup>-1</sup>
RSM	Response surface methodology	-
RT	Rushton turbine	-
$s$	Ribbon pitch	m
$Sc$	Schmidt number	-
$Sh$	Sherwood number	-
$t$	Time	s
$t_m$	Mixing time	s
$T_i$	Local temperature detected by thermistor	°C
$T_\infty$	Temperature at equilibrium mixing	°C
$U_g$	Superficial gas velocity	m /s
$X$	Cell concentration	g/L
$V$	Volume of working liquid	m <sup>3</sup>
$VVM$	Gas volume per liquid volume per minute	min <sup>-1</sup>
$W$	Width of ribbon blade	m
$W_b$	Width of baffle	m
$Y_{x/s}$	Yield of cell per substrate consumed	g.cell/g.glucose
$Y_{p/s}$	Yield of pigment per substrate consumed	UA <sub>500</sub> /g glucose

## Greek Symbols

$\alpha$	Growth associated rate constant for substrate consumption	g glucose/ g cell
$\beta$	Non-growth associated rate constant for substrate consumption	g glucose/g cell.h
$\mu_{max}$	Maximum specific growth rate	$h^{-1}$
$\eta_a$	Apparent viscosity	Pa.s
$\eta_{eff}$	Effective viscosity	Pa.s
$\rho$	Density of liquid	$kg/m^3$
$\sigma^2$	Mixing time variance	-
$\tau$	Shear stress	Pa
$\tau_p$	Electrode time constant	s
$\mu$	Dynamic viscosity	Pa.s
$\dot{\gamma}$	Shear rate determined by rheometer measurement	$s^{-1}$
$\dot{\gamma}_{eff}$	Effective vessel shear rate	$s^{-1}$
$\varepsilon_g$	Gas hold-up	-
$\phi$	Angle of inclined tube manometer	$^{\circ}$
$\Gamma$	Normalized response to exponential change in oxygen concentration in medium	-
$\Gamma^1$	Normalized response to stepwise change in oxygen concentration in medium	-

## CHAPTER 1

### INTRODUCTION

Ever since the advent of “Schüttelkultur” or shake flask technique heralding the birth of modern fermentation (Finn, 1954), mixing for microbial proliferation has been the subject of extensive researches. Scientific knowledge gained has led to better understanding on the transport phenomena involved, by which a major consequence being the improvement of processes and equipment efficacy. For industrial-scale mixing, the works of Rushton had been credited for many of the early development in agitation devices for stirred vessel system.

In general, the centrally submerged impeller-driven agitation system is the heart of any stirred tank operation. Specifically for aerobic fermentation, the use of disk-style flat blades turbine had been explicitly recommended for gas-liquid dispersion operation (Rushton, 1945). Together with propeller and paddle, this remote clearance impeller had a long standing reputation in industrial practices since 1940's. This is in a way reinforced by a prevailing notion perpetuated by most engineering training and textbooks, which held the presumption that open type impellers are able to solve 95% of all multiphase mixing problems (McCabe *et al.*, 2001). The rest of 5% accounts for mixing particulate solids requiring specialized agitators. As a result, their widespread applications had unnecessarily curbed research effort on newer design, for industrial establishments would naturally assumed the geometries to be the standard in any types of operations. Thereby owing to such perception, these impellers are almost always selected without due consideration for other better alternatives (Tatterson, 1991).



Evidence pertaining to imperfect mixing of such preset geometries had already been highlighted through revelation of segregation phenomenologies in a seemingly single phase system (Makino *et al.*, 2001; Alvarez *et al.*, 2005). These stratifications are physical barriers for effective mass transfer process and is expected to be severely amplified in viscous, non-Newtonian broths (Amanullah *et al.*, 2004). The complexity of these fluids have proven to be the major challenges in designing agitator for fermentation processes. So much so, given that pseudoplastic fluids in industries are more of a rule rather than exception.

The gravity of deficient mixing is more serious when concerning high density cells cultivation. As Lara *et al.* (2006) pointed out, heterogeneity inevitably creates spatial gradient in culture parameters, i.e., dissolved gases, pH and substrates concentrations within the microenvironment of bioreactor. Continuous exposure to fluctuating conditions as cells travel through various active and quiescent zones will adversely affect their metabolism, yield and ultimately, the quality of the product of interest. In a more extreme circumstances, rapid pressure fluctuations and shear stresses at the turbine region may also lead to excessive cell damage or death (Papagianni, 2004).

However, optimal mixer geometry for rheologically evolving fluids is still debatable. Any good design should represent a compromise in mixing efficiency over the course of reaction and liquid phase modification (Espinosa-Solares, 1997). Several workers (Jolicoeur *et al.*, 1992; Côté *et al.*, 1998; Tatterson, 2002) had suggested a different class of agitator known as helical ribbon impeller for mixing of viscous non-Newtonian fluids. The main feature of this agitator lies in its ability to generate important three dimensional flow patterns, resulting in intimate interaction of all