BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) IN AN UP-FLOW ANAEROBIC SLUDGE BLANKET UASB BIOREACTOR

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UNIVERSITI SAINS MALAYSIA
KAMPUS KEJURUTERAAN
2008

UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

Biological Treatment of Palm Oil Mill Effluent (POME) in an Up-Flow Anaerobic Sludge Blanket (UASB) Bioreactor

by

Dr. Mashitah Mat Don

Prof. Abdul Rahman Mohamed

PCMD

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
FINAL REPORT OF SHORT TERM RESEARCH PROJECTS

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APRIL 2007

UNIVERSITI SAINS MALAYSIA

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LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
FINAL REPORT OF SHORT TERM RESEARCH PROJECTS

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Name of Research Leader :

Ketua Penyelidik *Research Leader*	PTJ *School/Centre*
DR. MASHITAH MAT DON	**SCHOOL OF CHEMICAL ENGINEERING**

Nama Penyelidik Bersama
(Jika berkaitan) :
Name/s of Co-Researcher/s
(if applicable)

Penyelidik Bersama *Co-Researcher*	PTJ *School/Centre*
PROF. ABDUL RAHMAN MOHAMED	**SCHOOL OF CHEMICAL ENGINEERING**

2) **Tajuk Projek** :
Title of Project:

BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) IN AN UP-FLOW ANAEROBIC SLUDGE BLANKET (UASB) BIOREACTOR

3) **Abstrak untuk penyelidikan anda**

(Perlu disediakan di antara 100 – 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris**. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & luar).

Abstract of Research

(Must be prepared in 100 – 200 words in Bahasa Malaysia as well as in English. This abstract will later be included in the Annual Report of the Research and Innovation Section as a means of presenting the project findings of the researcher/s to the university and the outside community)

ABSTRACT

The development of an effective and simple method for treatment of palm oil mill effluent (POME) is a challenging task for palm oil mills. Considering the high organic character of POME, anaerobic process is the most suitable approach for its treatment. As such, up-flow anaerobic sludge fixed film (UASFF) bioreactor was designed for rapid biotransformation of organic matter to methane using granulated microbial aggregates. It is a hybrid reactor with an up-flow fixed film (UFF) part over an upflow anaerobic sludge blanket (UASB) section. It helps to shorten the start-up period at low hydraulic retention time (HRT) with increasing organic loading rate of 2.67-23.15 g COD/l.d. Granular sludge was found to develop rapidly within 20 days with an increase in size of granules from an initial pin point size to about 2 mm. Besides that, the anaerobic digestion was analyzed and modeled using response surface methodology (RSM), with HRT and influent carbon oxygen demand (COD_{in}) as the variables. Results showed that an increase in the variables resulted in a decrease in COD removal, solid retention time (SRT) and solid retention factor (SRF), respectively. However, the COD removal rate, volatile fatty acid (VFA)/alkaline used, CO_2 percentage in biogas and methane production rate tend to increase. The yield of methane was 0.287-0.348 l CH_4/g $COD_{removed}$. For biokinetic coefficients; apparent half-velocity constant *A*, half-velocity constant K_s, maximum specific microbial growth rate μ_{max}, methane yield constant Y_M and growth yield constant Y_s were found to be 0.738 g COD/ l, 0.982 g COD/ l, 0.207 d^{-1}, 0.325 l CH_4 STP/g $COD_{removed}$ and 0.174 g VSS/g $COD_{removed}$, respectively. Apparently, the reactor performance fed with pre-settled and chemically pretreated POME was also compared. An optimum condition for the digestion of POME was at feed flow rate Q_F 1.65 l/d, up-flow velocity V_{up} 0.6 m/h and Q_F 2.45 l/d and V_{up} 0.75 m/h for both cases. Nevertheless, a densely packed rod (Methanosaeta) and cocci shaped (Methanosarsina) microorganisms were found to develop in the granular sludge formed in the UASFF bioreactor at various operating conditions.

ABSTRAK

Pembangunan satu kaedah yang mudah dan efektif untuk merawat kumbahan kilang kelapa sawit (POME) merupakan satu cabaran yang besar bagi sesuatu kilang. Berdasarkan ciri bahan organiknya yang tinggi, proses anaerob merupakan cara yang sesuai untuk rawatan ini. Dengan itu, reaktor enapcemar anaerob aliran-naik saput tetap (UASFF) telah dicipta untuk biopenjelmaan cepat bahan organik kepada metana dengan bantuan daripada agregat mikrob berbutir. Ia adalah reaktor hibrid yang terdiri daripada aliran naik saput tetap (UFF) dan aliran naik enapcemar anaerob (UASB). Ia dapat membantu memendekkan tempoh permulaan pada masa penahanan hydraulik (HRT) yang rendah dengan peningkatan kadar bebanan organik 2.67-23.15 g COD/l.hari. Enapcemar berbutir didapati terbentuk dengan cepat dalam masa 20 hari dengan saiz berbutir meningkat daripada titik pin pada mulanya sehingga mencapai saiz 2 mm. Disamping itu, pencernaan anaerob telah dianalisa dan dimodel dengan menggunakan kaedah permukaan sambutan (RSM), dengan HRT dan permintaan oksigen karbon masuk (COD_{in}) sebagai pembolehubah- pembolehubah. Keputusan menunjukkan dengan peningkatan pembolehubah-pembolehubah ini menyebabkan penurunan dalam penyingkiran COD, masa penahanan pepejal (SRT) dan faktor penahanan pepejal (SRF), masing-masing. Walaubagaimnapun, kadar penyingkiran COD, asid lemak meruap (VFA)/alkali digunakan, peratus CO_2

di dalam biogas dan kadar penghasilan metana turut meningkat. Pekali biokinetik iaitu pemalar halaju separa ketara A, pemalar halaju separa K_s, kadar maksimum pertumbuhan spesifik mikrob μ_{max}, pemalar penghasilan metana Y_M dan pemalar penghasilan pertumbuhan biojisim Y_s ialah .738 g COD/ l, 0.982 g COD/ l, 0.207 hari^{-1}, 0.325 l CH_4 STP/g $COD_{disingkir}$ and 0.174 g VSS/g $COD_{disingkir}$, masing-masing. Lanjutan itu, prestasi reaktor dengan suapan POME yang melalui pra-enapan dan pra-rawatan kimia telah juga dibandingkan. Keadaan optima bagi pencernaan POME adalah pada kadar aliran suapan Q_F 1.65 l/hari, kadar aliran naik V_{up} 0.6 m/jam and Q_F 2.45 l/hari and V_{up} 0.75 m/jam bagi kedua-dua kes. Namun begitu, rod berbungkus yang padat dan mikroorganisma berupa cocci telah terbentuk di dalam enanpcemar bergranul di dalam bioreaktor UASFF pada keadaan operasi yang berbeza.

4) Sila sediakan Laporan teknikal lengkap yang menerangkan keseluruhan projek ini. [Sila gunakan kertas berasingan]
Kindly prepare a comprehensive technical report explaining the project
(Prepare report separately as attachment)

Senaraikan Kata Kunci yang boleh menggambarkan penyelidikan anda :
List a glosssary that explains or reflects your research:

Bahasa Malaysia	Bahasa Inggeris
Reaktor enapcemar anaerob aliran naik saput tetap	Up-flow anaerobic fixed film bioreactor (UASFF)
Effluen kilang kelapa sawit	Palm oil mill effluent
Pencernaan anaerob	Anaerobic digestion

5) **Output Dan Faedah Projek**
Output and Benefits of Project

(a) * Penerbitan *(termasuk laporan/kertas seminar)*
Publications (including reports/seminar papers)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).
(Kindly state each type, title, author/editor, publication year and journal/s containing publication)

Journals

1) Nagafpour, G.D., Zinatizadeh, A.A.L, Mohamed, A.R., Isa, M.H., Nasrollahzadeh, H. (2006) High rate anaerobic digestion for palm mill effluent in an up-floe slidge fixed film bioreactor. *Process Biochemistry, 41:370-379.*

2) Zinatizadeh, A.A.L., Mohamed, A.R., Nagafpour, G.D., Isa, M.H., Nasrollahzadeh, H. (2006) Kinetic evaluation of palm oil mill effluent digestion in a high rate ip-flow anaerobic sludge fixed film bioreactor. *Process Biochemistry, 41:1038-1045.*

3) Zinatizadeh, A.A.L., Mohamed, A.R., Abdullah, A.Z., Mashitah, M.D. and Isa, M.H. (2006) Process modeling and analysis of palm oil mill effluent treatment in an up-flow anaaerobic sludge fixed film bioreactor using response surface methodology (RSM). *Water research, 40:3193-3208.*

4) Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z and Nagafpour, G.D.,. (2006) Pretreated palm oil mill effluent (POME) digestion in an up-flow anaerobic sludge fixed film bioreactor: A comparative study. *Internatioanl Journal of Engineering Transaction B: Application, 19(1): 1-8.*

5) Ali Akbar Zinatizadeh lorestani, Abdul Rahman Mohamed, Mat Don Mashitah, Ahmad Zuhairi Abdullah and Mohamed Hasnain Isa (2006) Effect of organic loading rate on palm oil mill effluent treatment in an up-flow anaerobic sludge fixed film bioreactor. *Enviromental Engineering and Management Journal. September, Vol 5(3): 337-350.*

6) Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z and Isa, M.H. (2007) Characteristics of granular sludge developed in an up-flow anaerobic sludge fixed film bioreactor treating palm oil mill effluent. *Water Environmental Research. Volume 79*

7) Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z and Isa, M.H. (2007) Optimization of pretreated palm oil mull effluent digestion in an up-flow anaerobic sludge fixed film bioreactor: A comparative study. *Biochemical Engineering Journal (Accepted, inpress).*

Proceedings

1) Zinatizadeh, A.A.L., Mohamed, A.R., Abdullah, A.Z., Mashitah, M.D. and Nasrollahzadeh, H. (2005). Effects of operating variables on the performance of an up-flow anaerobic sludge fixed film reactor treating palm oil mill effluents: In *Internatioanol Conference of Chemical and Bioprocess Engineering , Universiti Malaysia Sabah, Malaysia.*

2) Zinatizadeh, A.A.L., Mohamed, A.R., Abdullah, A.Z., Mashitah, M.D. and Nagafpour, G.D. (2006) Effect of physical and chemical pretretament on palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor. In: *7th International Conference on Civil Engineering (CCE), May 2006, Tarbiat Modares University (TMU), Tehran, Iran.*

3) Zinatizadeh, A.A.L., Mohamed, A.R., Abdullah, A.Z., and Mashitah, M.D (2006) Effect of temperature on the performance of an up-flow anaerobic sludge fixed film (UASFF) bioreactor treating palm oil mill effluent, Nov. 2006, Penang, Malaysia.

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten atau impak kepada dasar dan masyakarat.**
Other benefits such as product development, product commercialisation/patent registration or impact on source and society

Hasilan daripada projek ini, sebuah bioreaktor enapcemar anaerob aliran-naik saput tetap (UASFF) telah dicipta untuk biopenjelmaan cepat bahan organik kepada metana dengan bantuan daripada agregat mikrob berbutir. Namun ia masih lagi diperingkat makmal walaupun ada prospek untuk dipertingkatkan skalanya. Bioreaktor ini pula perlulah menjalani ujian pembauran untuk mengenalpasti tahap atau kebolehannya menghasilkan gas-gas yang lain pula. Untuk itu, satu pelantar penyelidikan amatlah perlu untuk membolehkan ianya dibangunkan.

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i) Pelajar Siswazah :
Postgraduate students:
(perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Ali Akbar Zinatizadeh Lorestani (2006) Biological treatment of palm oil mill effluent (POME) using an up-flow anaerobic sludge fixed film (UASFF) bioreactor. PhD Thesis. Universiti Sains Malaysia

ii) Pelajar Prasiswazah :
Undergraduate students:
(Nyatakan bilangan)
(Provide number)

Fazira Azita Abdul Rashid (2006) Study on biological activity of granular sludge taken from an up-flow anaerobic sludge fixed film (UASFF) reactor fro palm oil mill effluent (POME) treatment in batch culture. BSc (Chemical Engineering) Thesis, Universiti Sains Malaysia

iii) Lain-Lain : Tiada
Others:

6. **Peralatan Yang Telah Dibeli :**
Equipment that has been purchased:

TIADA

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Penyelidikan telah dijalankan dengan jayanya seperti perancangan asal dalam cadangan penyelidikan

18/6

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Signature of Chairman
[Research Committee of School/Centre]

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Dekan
Pusat Pengajian Kejuruteraan Kimia
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Universiti Sains Malaysia, Seri Ampangan
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Pulau Pinang.

TARIKH
Date

BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) USING AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) BIOREACTOR

by

ALI AKBAR ZINATIZADEH LORESTANI

Thesis submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy

December 2006

RAWATAN BIOLOGI KUMBAHAN KILANG KELAPA SAWIT (POME) MENGGUNAKAN BIOREAKTOR ENAPCEMAR ANAEROB ALIRAN-NAIK SAPUT TETAP

ABSTRAK

Reaktor enapcemar anaerob aliran-naik saput tetap (UASFF) adalah satu bioreaktor cipta baru dan digunakan untuk biopenjelmaan cepat bahan organik kepada metana dengan bantuan daripada agregat mikrob berbutir. Satu bioreaktor UASFF berskala makmal dengan satu tangki pengenapan luar telah berjaya direkabentuk dan beroperasi untuk rawatan kumbahan kilang kelapa sawit (POME). Bioreaktor tersebut telah dimajukan untuk memendekkan tempoh pemulaan pada masa penahanan hydraulik (HRT) yang rendah. Bebanan organik ditingkatkan secara beransur dari 2.67 kepada 23.15 g COD/l.hari sepanjang tempoh ini. Enapcemar berbutir didapati terbentuk dengan cepat dalam masa 20 hari dengan saiz berbutir meningkat daripada titik pin pada mulanya sehingga mencapai saiz 2 mm. Pencernaan anaerob untuk POME telah dimodel dan dianalisis dengan dua pembolehubah iaitu HRT dan COD_{in} menggunakan kaedah permukaan sambutan (RSM). Kawasan eksplorasi untuk pencernaan POME telah diambil dari kawasan yang dirangkumi oleh sempadan HRT (1 hingga 6 hari) dan COD_{in} (5260 hingga 34725 mg/l). Peningkatan dalam pembolehubah tersebut mengakibatkan penurunan dalam penyingkiran COD, SRT dan SRF tetapi meningkatkan kadar penyingkiran COD, VFA/Alk, peratusan CO_2 dalam biogas dan kadar penghasilan metana. Persamaan kinetik yang dicadangkan dan satu model Monod yang dipermudahkan telah berjaya digunakan untuk menghuraikan kinetik pencernaan anaerob POME pada kadar bebanan organik antara 0.88 hingga 34.73 g COD/l.hari. Penghasilan metana adalah antara 0.287 hingga 0.348 l

CH_4/g $COD_{disingkirkan}$.hari. Pekali biokinetik iaitu pemalar halaju separa ketara (A), pemalar halaju separa (K_S), kadar maksimum pertumbuhan spesifik mikrob (μ_m), pemalar penghasilan metana (Y_M) dan pemalar penghasilan pertumbuhan biojisim (Y_x) juga telah dikira. Pemalar ketara kadar (K), dikira dengan model Monod yang dipermudah adalah dalam lingkungan 2.9 ke 7.4 l CH_4/g COD.hari. Pada kepekatan COD influen yang berbeza, nilai K menunjukkan hubungan lurus dengan perubahan kandungan VSS dalam reaktor. Dalam satu ujikaji berkelompok bagi pencernaan POME, 275 mg $CaCO_3$ kealkalian bikarbonat telah dihasilkan bagi setiap 1000 mg $COD_{disingkirkan}$. Hampir 95 % penyingkiran COD dicapai dalam masa 72 jam dengan kadar penyingkiran COD awal pada 3.5 g COD/l.hari. Model kinetik tindak balas berturutan yang telah digunakan untuk meramal data aktiviti enapcemar semasa ujikaji berkelompok memberikan padanan yang baik dengan keputusan daripada ujikaji ($R^2 > 0.93$). Langkah yang paling perlahan didapati adalah langkah pengasidan dengan pemalar kadar antara 0.015 hingga 0.083 jam^{-1} manakala pemalar kadar bagi langkah metanogen didapati antara 0.218 hingga 0.361 jam^{-1}. Prestasi jangka panjang reaktor UASFF juga telah dikaji dengan POME mentah sebagai suapan pada HRT selama 3 hari dan kepekatan COD influen sebanyak 44300 mg/l. Kaedah pra-rawatan fizik dan kimia juga telah diselidiki. Ujikaji telah dijalankan berdasarkan satu rekaan pusat rencam bermuka tengah (CCFD) dan dimodelkan mengunakan kaedah permukaan sambutan (RSM) dengan dua pembolehubah operasi iaitu kadar aliran suapan (Q_F) dan halaju aliran-naik (V_{up}). Prestasi reaktor dengan suapan POME yang melalui pra-enapan dan pra-rawatan kimia telah dibandingkan. Keadaan optima bagi pencernaan POME secara pra-enapan dan pra-rawatan kimia dengan masing-masing pada 1.65

l/hari Q_F dan 0.6 m/jam V_{up}, dan 2.45 l/hari Q_F dan 0.75 m/jam V_{up}. Dapatan ujikaji adalah berpadanan dengan jangkaan model. Pencirian enapcemar berbutir yang terhasil dalam reaktor UASFF pada pelbagai keadaan operasi menunjukkan ia terdiri terutamanya dari rod berbungkus yang padat (mikroorganisma berupa Methanosaeta) dan micoorganisma berupa cocci (Methanosarsina).

BIOLOGICAL TREATMENT OF PALM OIL MILL EFFLUENT (POME) USING AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) BIOREACTOR

ABSTRACT

Up-flow anaerobic sludge fixed film (UASFF) bioreactor is a modern bioreactor and was used for the rapid biotransformation of organic matter to methane with the help of granulated microbial aggregates. A lab scale UASFF bioreactor (3.65 lit) with an external settling tank was successfully designed and operated for palm oil mill effluent (POME) treatment. The bioreactor was developed in order to shorten the start-up period at low hydraulic retention time (HRT). The organic loading was gradually increased from 2.67 to 23.15 g COD/l.d during this period. Granular sludge was found to develop rapidly within 20 days with an increase in size of granules from an initial pinpoint size to about 2 mm. The anaerobic digestion of POME was modeled and analyzed with two variables i.e. HRT and COD_{in} using response surface methodology (RSM). The region of exploration for digestion of POME was taken as the area enclosed by HRT (1 to 6 days) and COD_{in} (5260 to 34725 mg/l) boundaries. An increase in the variables resulted in a decrease in COD removal, SRT and SRF but an increase in COD removal rate, VFA/Alk, CO_2 percentage in biogas and methane production rate. The proposed kinetic equation and a simplified Monod's model were successfully employed to describe the kinetics of POME anaerobic digestion at organic loading rates in the range of 0.88 to 34.73 g COD/l.d. The methane yields obtained were between 0.287 to 0.348 l CH_4/g $COD_{removed}$. Biokinetic coefficients i.e. apparent half-velocity constant (A), half-velocity constant (K_S), maximum specific microbial growth rate (μ_m), methane yield constant (Y_M), and biomass growth yield constant (Y_x) were also evaluated.

The apparent rate constants, K, calculated by simplified Monod model were in the range of 2.9 to 7.4 l CH_4/g COD.d. At different influent COD concentrations, K values showed a linear relationship with variations in VSS content in the reactor. In a batch POME digestion, 275 mg $CaCO_3$ bicarbonate alkalinity was produced per 1000 mg $COD_{removed}$. About 95 % COD removal was achieved within 72 h with an initial COD removal rate of 3.5 g COD/l.d. A consecutive reaction kinetic model employed to simulate the data on sludge activity in batch experiment showed good fit to the experimental results (R^2 > 0.93). The slowest step was modeled to be the acidification step with rate constants between 0.015 to 0.083 h^{-1} while those of the methanogenic step were between 0.218 to 0.361 h^{-1}. Long term performance of the UASFF reactor was investigated with raw POME as feed at a HRT of 3 days and an influent COD concentration of 44300 mg/l. Physical and chemical pretreatment methods were also conducted. Experiments on the pretreated POME digestion were conducted based on a central composite face-centered design (CCFD) and modeled using response surface methodology (RSM) with two operating variables i.e. feed flow rate (Q_F) and superficial up-flow velocity (V_{up}). The performance of the reactor fed with the pre-settled (settling for 3 h) and chemically pretreated (after flocculation) POME was compared. The optimum conditions for the digestion of the pre-settled and chemically pre-treated POME were at Q_F of 1.65 l/d, V_{up} of 0.6 m/h and Q_F of 2.45 l/d and V_{up} of 0.75 m/h, respectively. The experimental findings were in close agreement with the model prediction. The characterization on the granular sludge developed in the UASFF bioreactor at various operating conditions showed that they predominantly consisted of densely packed rod (Methanosaeta-like microganism) and cocci shaped (Methanosarsina) microorganisms.

Dr. MMD

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STUDY ON BIOLOGICAL ACTIVITY OF GRANULAR SLUDGE TAKEN FROM AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) REACTOR FOR PALM OIL MILL EFFLUENT (POME) TREATMENT IN BATCH CULTURE

FAZIRA AZITA BINTI ABDUL RASHID
Matric No.: **70484**

April 2006

ABSTRACT

Malaysia is the world largest Palm Oil Producing Country. Approximately 42.5 Mtons of Palm Oil Mill Effluent (POME) were produce in 2001. Since this huge amount of biomass waste contains organisms, we aimed to produce energy materials. Though components of POME were different in each lots and factories, analysis of POME showed that it contains useful materials for biomass to bio-energy conversion by fermentation, such as starch, glucose, glycerine, etc.

This study aimed to develop not only the conversion technology to produce bioenergy such as methane but also the waste water treating process for local and global environmental pollution issues. In this study, a 1 liter digester was used to examine the biological activity of the granules formed in an UASFF reactor used to treat palm oil mill effluent (POME). Three critical process variables, initial chemical oxygen demand (COD) concentration, initial alkalinity and biomass concentration, were varied at 3 levels, respectively (3000, 6500, 10000 mg COD/l), (200, 1100, 2200 mg $CaCO_3$/l) and (2000, 4000, 6000 mg VSS/l).The influence of the variables on the granules activity were investigated in terms of COD removal and CH_4 bicarbonate production was determined. The reactor performance was determined in terms of methane (CH_4) production, volatile fatty acids (VFA) conversion and chemical oxygen demand (COD) reduction.

Keywords: UASFF reactor, Biological activity, Granule, POME, CH_4, VFA, COD

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High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor

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Abstract

The upflow anaerobic sludge-fixed film (UASFF) reactor is one of the granular sludge bioreactors that are used for the rapid biotransformation of organic matter to methane with the help of granulated microbial aggregates. The UASFF reactor is a hybrid reactor with an upflow fixed film (UFF) part over an upflow anaerobic sludge blanket (UASB) section. The major problem associated with UASB reactors is the long start-up period (2–4 months). In this study, a UASFF bioreactor with tubular flow behavior was developed in order to shorten the start-up period at low hydraulic retention time (HRT) for palm oil mill effluent (POME) treatment. The reactor was operated at 38 °C and HRT of 1.5 and 3 days. The organic loading was gradually increased from 2.63 to 23.15 g chemical oxygen demand (COD)/l day. Granular sludge was rapidly developed within 20 days. The size of granules increased from an initial pinpoint size to reach 2 mm. High chemical oxygen demand removals of 89 and 97% at HRT of 1.5 and 3 days were achieved, respectively. Methane yield of 0.346 l CH_4/g $COD_{removed}$ at the highest organic loading rate (OLR) was obtained. The use of an internal upflow anaerobic fixed film section caused the flocculated biomass to precipitate over the sludge blanket. The precipitated biomass served as suitable and natural hydrophobic core to form a spatial arrangement of microbial species in the process of mature bio-granulation.

Keywords: UASFF; POME; Methane; VFA; COD; Biogranule; Biofilm

1. Introduction

Oil palm (*Elaeis guineensis*) is one of the most versatile crops in the tropical world. The production of palm oil, however, results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME). Typically, 1 t of crude palm oil production requires 5–7.5 t of water; over 50% of which ends up as POME. This wastewater is a viscous, brownish liquid containing about 95–96% water, 0.6–0.7% oil and 4–5% total solids (including 2–4% SS, mainly debris from the fruit). It is acidic (pH 4–5), hot (80–90 °C), nontoxic (no chemicals are added during oil extraction), has high organic content (COD 50,000 mg/l, BOD 25,000 mg/l) and contains appreciable amounts of plant nutrients [1,2].

Palm oil industries are facing tremendous challenges to meet the increasingly stringent environmental regulations. Over the past decades, several cost-effective treatment technologies comprising anaerobic, aerobic and facultative processes have been developed for the treatment of POME [2–7]. More than 85% of palm oil mills use solely ponding systems due to their low costs. It has been reported that only a few mills are equipped with biogas recovery systems [8].

One of the most notable developments in anaerobic treatment process technology is the upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor exhibits positive features, such as allows high organic loadings, short hydraulic retention time (HRT) and has a low energy demand [9]. Granular sludge formation is the main distinguishing characteristic of UASB reactors as compared

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to other anaerobic technologies. A major problem associated with the UASB reactor is that several months may be required for the development of anaerobic sludge granules [10]. It may be noted, however, that the start-up of UASB reactor and the development of granules in the reactor are two different processes with no bearing on each other. In spite of the advantages of granular sludge, effective treatment of wastewaters with flocculant sludge UASB reactors have been documented [11,12]. The high suspended solids content of POME can prevent the system from operating at high organic loading rates (OLR). Suspended and colloidal components of wastewaters in the form of fat, protein, and cellulose have adverse impact on UASB reactors' performance and can cause deterioration of microbial activities and wash out of active biomass [13].

Modification of the UASB process was required to overcome the existing deficiencies. Use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the above mentioned problem. The packing medium in the UASB reactor is intended to increase solids retention by dampening short circuiting, improving gas/liquid/solid separation, and providing surface for biomass attachment [14]. The upflow anaerobic sludge-fixed film (UASFF) reactor is an anaerobic hybrid reactor which is a combination of upflow anaerobic sludge blanket (UASB) and upflow fixed film (UFF) reactors. The lower part of the UASFF reactor is the UASB portion where flocculant and granular sludge are developed. The upper part of the UASFF reactor serves as a fixed film bioreactor. The UASFF reactor has been used successfully for the treatment of various industrial wastewaters, such as POME, slaughterhouse, swine and starchy wastewater [15–17,21].

Internal packing creates a suitable environment to accelerate biogranule formation by particles recirculation. Biogranulation is a process of cell-to-cell self-immobilization that culminates in the formation of granules. The biogranules are dense microbial consortia packed with several bacterial species and typically contain millions of organisms per gram of biomass [18]. Great attention has been paid to accelerate the start-up period and enhance granules formation in UASB reactors with the aid of chemical agents [19,20]. Borja and coworkers [2,5] have investigated the treatability of POME using ordinary and two-stage UASB reactors with HRT and start-up period of 4 and 40 days, respectively. Also, POME treatability in an anaerobic hybrid digester was studied by Borja et al. [21]. At an OLR of 16.2 g/l day and HRT of 3.5 days, COD removal efficiency and methane yield of 92.3% and 0.335 m^3/kg

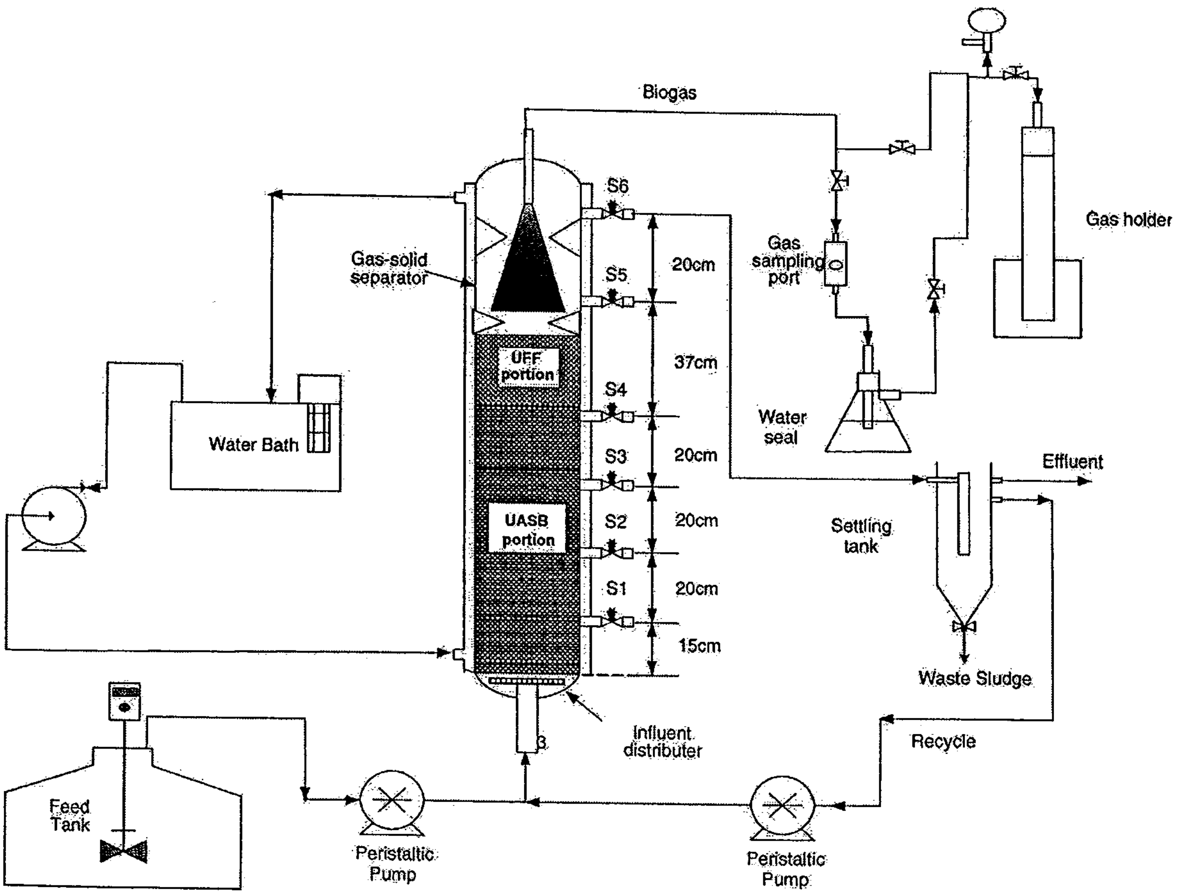

Fig. 1. Schematic diagram of the UASFF reactor.

$COD_{removed}$ were achieved, respectively. The main purposes of the present research were to explore the possibility of shortening the start-up period of the UASFF reactor, to accelerate the formation of granular sludge and to evaluate the performance stability of the UASFF reactor in the treatment of POME at high OLRs.

2. Material and methods

2.1. Experimental set up

The schematic diagram of the laboratory-scale UASFF reactor used in this study is shown in Fig. 1. The system was designed to behave as tubular flow, therefore height per diameter ratio was set at 20.3. The UASFF reactor was made of a glass column with an internal diameter of 6.5 cm and a height of 132 cm. The total volume of the reactor was 4980 ml and the working volume was 4380 ml (excluding head space). The column consisted of three compartments; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor whereas the middle part of the column with a height of 25 cm was operated as a fixed film reactor. The top part of the bioreactor served as a gas–liquid–solid separator. The middle section of the column was randomly packed with 90 pieces of pall rings with diameter and height of each equal to 18 mm. The specific surface area of the packing material was 341 m^2/m^3 with a void space of 91.25%. The purpose of the top section (i.e. the gas–liquid–solid separator) of the reactor was to allow separation of the biogas and washed out solids from the liquid phase. An inverted funnel shaped gas separator was used to conduct the biogas to the gas collector tank. A gas sampling port was provided in the connecting tubing for the determination of biogas composition. The UASFF reactor was operated under mesophilic conditions (38 °C) and the temperature was maintained by circulating hot water through the reactor jacket. The circulated flow was from a water bath maintained at the desired temperature to ensure isothermal operation. Six sampling ports (S1–S6) were placed at suitable distances along the height of the reactor (Fig. 1). POME as substrate was continuously fed to the reactor through the base using a peristaltic pump (Cole Parmer, Master flex L/s) and the effluent was collected from the top of the column. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The top of the UASFF reactor was connected to a water displacement gas meter to measure the volume of biogas produced.

2.2. Palm oil mill effluent

The UASFF reactor was fed with POME pre-settled for 1 h. The characteristics of POME are summarized in Table 1. POME samples were collected weekly from a near by palm oil mill and stored in a cold room at 4 °C before use.

Table 1
Characteristic of palm oil mill effluent (POME)

Parameter	Value
BOD (mg/l)	23,000–26,000
COD (mg/l)	42,500–55,700
Soluble COD (mg/l)	22,000–24,000
SS (mg/l)	16,500–19,500
Oil and grease (mg/l)	4900–5700
Total N (mg/l)	500–700
pH	3.8–4.4

Different dilutions of POME were prepared using tap water. The pH of the feed was adjusted to 6.8, using a 6N sodium hydroxide solution. The alkalinity was adjusted to 1500–1700 mg $CaCO_3$/l using sodium bicarbonate. Supplementary nutrients such as nitrogen (NH_4Cl) and phosphorous (KH_2PO_4) were added to yield a COD:N:P ratio of 250:5:1.

2.3. Seed sludge

The inoculum for seeding was a mixture of sludge taken from a drainage channel bed of Perai industrial zone (Butterworth, Malaysia), digested sludge from a food cannery industry and animal manure in equal proportions. The sludge was initially passed through a screen to remove debris. The total volatile solids concentration of the seed was measured as 10,300 mg/l. In order to test the microbial activity of the seed sludge, 5 ml of the sludge mixture was added to 50 ml diluted POME (as substrate with COD of 4000 mg/l) in a 150 ml serum bottle. The produced gas was analyzed after 24 h. It was found to contain CH_4 (52.7%), CO_2 (29.8%) and N_2 (18.4%). The results showed anaerobic activity of seed sludge. In the packed bed section, a 2 % agar (Merk) solution was used to make the packing surface sticky for fast development of biofilm.

2.4. Reactor operation

The UASFF reactor was inoculated with 600 ml sludge mixture. In order to acclimate the sludge with POME, the reactor was daily batch-fed with diluted POME (7000–9000 mg COD/l) for 7 days. After each feed the liquid content of the reactor was continuously recirculated for 1 day (until the next feed). The average VSS concentration of the sludge after the 7-day batch-fed period was 12,650 mg/l. A COD removal of about 65% was achieved at the end of this period.

Continuous feeding of the reactor was started with an initial organic loading rate (OLR) of 2.63 g COD/l day and an HRT of 1.5 day. The HRT was maintained constant throughout the start-up duration. The influent COD concentration was 4000 mg/l for the first 7 days, and then it was increased stepwise to 34,725 mg/l (OLR = 23.15 g-COD/l day) from 7 to 20 days (Fig. 2). In order to improve treatment performance and provide an upflow velocity of 0.44 m/h [9], fresh feed was mixed with recycled effluent in

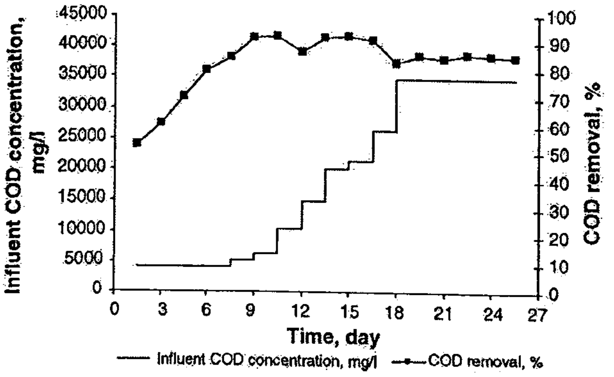

Fig. 2. COD removal efficiency during start-up period.

a ratio of 1:11.25. The purpose of the high recycle ratio was to eliminate high organic over loading and to supply alkalinity by blending the fresh feed with the low COD and high alkalinity recycled stream. Temperature was maintained at 38 °C. It was not necessary to adjust the pH of the reactor as it remained relatively constant (about 6.9–7.6) throughout the experiment. COD reduction, biomass concentration, pH, methane production and volatile fatty acids (VFA) concentration were monitored.

2.5. Analytical methods

A gas-tight syringe (Hamilton CO., Reno, Nevada, USA) was used to take gas samples from the gas sampling port. A gas chromatograph (Perkin Elmer, Auto system XL), equipped with thermal conductivity detector (TCD) and data acquisition system with computer software (Total Chrom), was used for gas composition analysis. A GC column, Carboxen 1000, with 100/120 mesh (Supelco, Park, Bellefonte, PA, USA) was used. The column temperature was initially maintained at 40 °C for 3.5 min, followed by automatic temperature increase at a rate of 20 °C/min till it reached 180 °C. The injector and detector temperatures were 150 and 200 °C, respectively. The carrier gas (He) flow rate was set at 30 ml/min.

The liquid samples for VFAs determination were analyzed by another gas chromatograph, HP 5890 Series II (Hewlett Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) and an integrator (HP 3396). A 2 m × 2 mm stainless steel, 80/120 mesh Carbopack B-DA/4% Carbowax 20 M (Supelco) column was used. The oven temperature was maintained at 175 °C. The injector and detector temperatures were 200 and 220 °C, respectively. The carrier gas (He) flow rate was set at 40 ml/min. A 2-propanol solution (1%) was used as internal standard with concentration of 20 μl/ml of sample and then the sample was acidified with 60 μl of concentrated formic acid. The injection sample volume was 0.4 μl. Samples were filtered using Whatman GF/C (934AH) to remove suspended solids before injecting into the GC column to prevent any clogging. Other parameters, viz. biochemical oxygen demand (BOD), COD, suspended solids (SS), volatile suspended solids (VSS), alkalinity and total Kjeldahl nitrogen (TKN), were analyzed using procedures outlined in Standard Methods [22].

Scanning electron microscopy (SEM) was used to examine the external structure of the granules. A specimen is bombarded with a scanning beam of electrons and then the slowly moving "secondary electrons" are collected, amplified and displayed on the cathode ray tube. The electron beam and the cathode ray tube scan synchronously so that an image of the specimen surface is formed. Specimen preparation for SEM included fixation with 5% glutaraldehyde and 1% osmium tetroxide, followed by dehydration with 50–100% ethanol before drying, finally to make the specimen conductive to electricity. The sample was examined using a Leo Supra 50 VP Field emission SEM (UK) equipped with Oxford INCA 400 energy dispersive X-ray microanalysis system [23–25].

The dry weight of the attached biofilm per unit wetted surface area of pall ring was evaluated by drying the removable section of packing before and after biofilm attachment, at a temperature of 80 °C for 24 h. The difference between the weight measurements was divided by the wetted surface area of the packing.

The sludge volume index (SVI) is the volume in millimeters occupied by 1 g of a suspension after 30 min settling. SVI was measured according to Standard Methods (2710 D) [22].

2.6. Operational and performance parameters

Operational and performance parameters included HRT, OLR, solids loading rate (SLR), food-to-microorganisms ratio (F/M) and specific methanogenic activity (SMA) and specific activity (SA) of the reactor. SLR is defined as the total suspended solids concentration introduced in a unit volume of UASFF reactor per unit time (e.g. g SS/l day). OLR takes into account the liquid flow rate and contaminant concentration and is defined as the mass of pollutant introduced in a unit volume of UASFF reactor per unit time (e.g. g COD/l day). F/M integrates contaminant concentration and microbial mass and is the mass of pollutant applied to a unit mass of microbial mass per unit time (e.g. g COD/g VSS day).

SMA can be expressed on the basis of microbial mass (liters of biogas produced per unit mass of microbial population). The specific activity of the process is described as the fraction of the organic load biodegraded in a unit mass of sludge. These parameters were determined using the following relationships:

$$\mathrm{HRT} = \frac{V}{Q} \tag{1}$$

$$\mathrm{OLR} = \frac{Q \cdot \mathrm{COD_{in}}}{V} \tag{2}$$

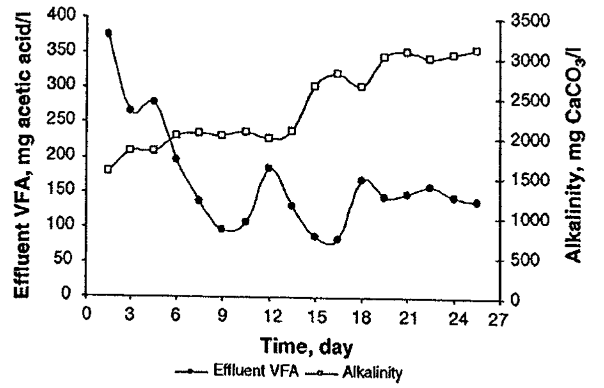

Fig. 3. Effluent VFAs concentration (mg/l).

$$\mathrm{SLR} = \frac{Q \cdot \mathrm{TSS}}{V} \quad (3)$$

$$\frac{\mathrm{F}}{\mathrm{M}} = \frac{Q \cdot \mathrm{COD_{in}}}{V \cdot \mathrm{VSS}} \quad (4)$$

$$\mathrm{SMA} = \frac{Q_{\mathrm{CH_4}}}{V \cdot \mathrm{VSS}} \quad (5)$$

$$\mathrm{SA} = \frac{Q \cdot \mathrm{COD_{in}} - \mathrm{COD_{out}}}{V \cdot \mathrm{VSS}} \quad (6)$$

where Q is the influent flow rate (l/day), V is the volume of the reactor (liters), VSS is the sludge concentration in the reactor and $Q_{\mathrm{CH_4}}$ is the methane production rate (liters CH_4/day).

3. Results and discussion

3.1. Reactor start-up

The UASFF reactor performance during the first 26 days of operation is shown in Fig. 2. In the first week the reactor was fed with an influent COD of 4000 mg/l (OLR = 2.63 g COD/l day) and the COD removal efficiency was enhanced from 53 to 85%. The influent COD concentration was then increased stepwise to 34,725 mg/l for the remaining period of the experiment. It is clear from the graph that increases in influent COD from 6260 to 10,290 mg/l and 26,210 to 34,725 mg/l caused reduction in the COD removal efficiency; implying that the system was put under stress. This is also reflected by the increase in VFAs concentration during these periods (Fig. 3). These observations are attributed to the sudden increase in COD loads which resulted in organic shocks to the microorganisms. The micro flora then took time for acclimation to the new environment. By the end of the start-up period (26 days) a removal efficiency of 85% was achieved at an OLR of 23.15 g COD/l day. This is a marked improvement over the study on POME treatment using a UASB reactor by Borja and Banks [5] wherein they achieved a COD removal efficiency of more than 90% at a much lower OLR, 1.27 g COD/l day, and after a longer period of operation, i.e. 30 days. Their reactor operation was affected by the considerable sludge loss with the effluent at the beginning of the start-up period [5]. In the present study with the UASFF reactor, the flocculated sludge wash out phenomena did not occur. Also granulation, which usually requires a longer time [26,27], was observed within the limited operational period of the UASFF reactor. The internal recirculation of dispersed bacteria probably assisted in their quicker agglomeration. In order to monitor the buffering capacity, effluent alkalinity was measured. The total alkalinity was in the range of 1570–3020 mg/l and VFA/alkalinity ratio changed from a minimum value of 0.029 to a maximum of 0.237. The results showed increase in effluent alkalinity with increase in OLR.

The UASFF reactor biomass content was monitored by determination of VSS in samples along the height of the reactor and in the effluent. Fig. 4 shows the suspended solids and residual COD distribution along the height of the reactor on the 20th day of operation. The mixed stream (feed + recycle) entering the reactor had a COD of 6160 mg/l and the HRT calculated based on the resulted flow was 3 h. The results indicated that a 27% COD removal was associated with the packed bed section. A total COD removal rate of 20.32 g COD/l day was achieved at the end of the start-up period. The average MLVSS content of the sludge blanket was 41,500 mg/l.

Fig. 5 shows the profiles of COD concentration and COD removal rate along the height of the reactor. It can be noted that different sections of the concentration profile have different gradients; thus, implying various COD removal rate along the height of the UASFF reactor. The COD removal rates were calculated based on the COD values at the bottom and top sections and the contained volumes. These rates were then plotted (Fig. 5) at the positions corresponding to the top levels of the sections. In the UASB portion of the reactor, i.e. up to 75 cm height (S4), the COD removed rate was found to decrease from 5.56 at the lowest section to 1.76 g/l day at the top. This is attributed to reduce biomass concentrations in the upper levels of the sludge blanket. The COD removal rate then increased to 6.16 g/l

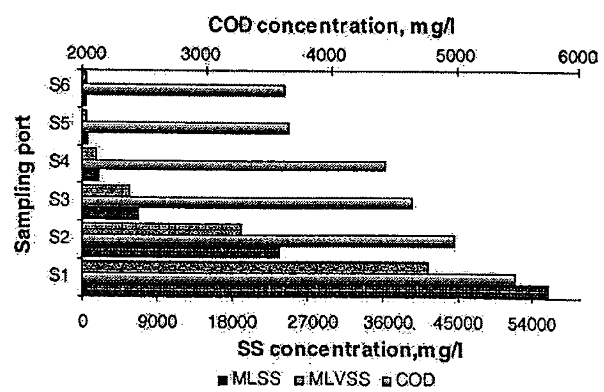

Fig. 4. VSS and COD concentration along the height of the reactor on day 20 of start-up.

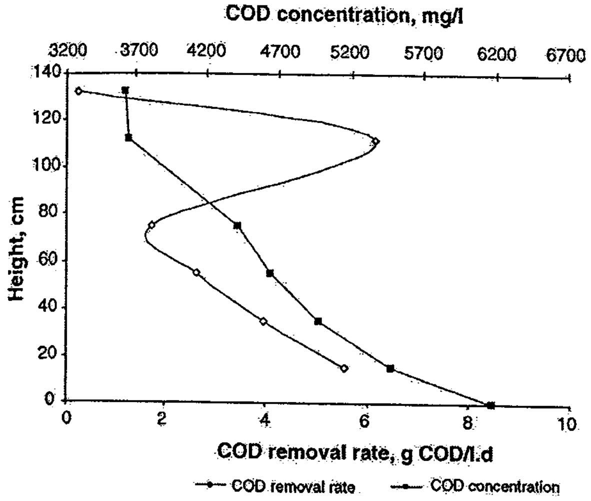

Fig. 5. Variation of COD concentration along the height of the reactor.

day at a height of 112 cm due to the presence of massive amount of anaerobic bacteria attached to the pall rings in the fixed film section of the reactor.

The biogas was found to have 62–82% methane; the balance being carbon dioxide (Fig. 6). At the end of start-up, a methane production rate of 6.23 l CH_4/l day was achieved. As the OLR was increased the CO_2 fraction in the biogas was also increased. The obtained data indicated that, even at the high gas production rate (6.23 l CH_4/l day) the CH_4 level in the biogas was 62%.

With regards to the efficiency of UASB system based on formation and retention of granules, significant growth in the height of the UASFF granule bed was observed during the start-up period (Fig. 7). An indication of sludge growth was obtained by noting the thickness of the sludge bed after stopping the feed and allowing the sludge to settle in the reactor for 1 h. With an initial bed height of 190 mm, at first the height of granulated bed, the growth rate was slow but after 8 days the rate increased substantially (Fig. 7). This is attributed to the internal recycling of sludge from the fixed film section, above the sludge blanket. While the OLR was increased from 2.63 to 23.15 g/l day, the height of sludge bed

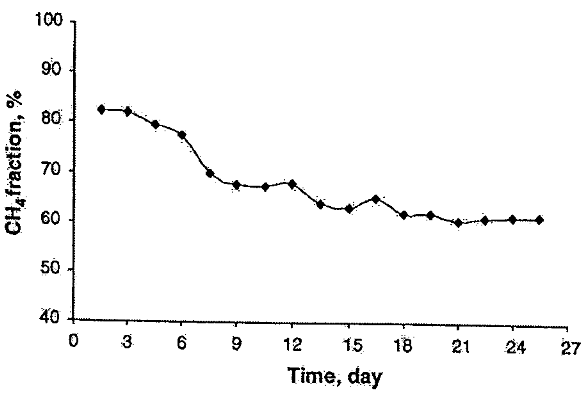

Fig. 6. Methane fraction in biogas during start-up period.

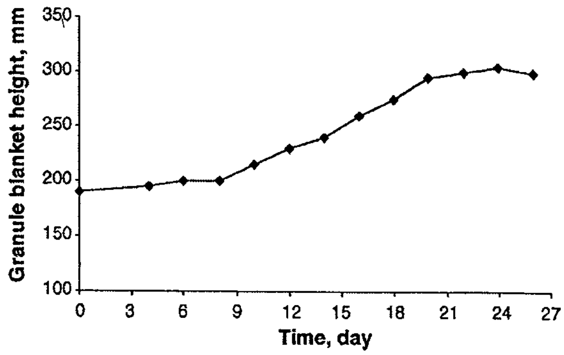

Fig. 7. Development of granule blanket height in UASFF reactor.

increased to 300 mm. The specific activity (SA) of the UASFF reactor reached 1.54 g $COD_{removed}$/g VSS day after an operational period of 20 days. The use of methanogen-enriched seed sludge for the reactor inoculation and the confinement of the dispersed and flocculated sludge in the lower part of the reactor due to the fixed bed portion were the main reasons for the accelerated start-up process.

3.2. Rapid granule formation

At the beginning of the experiment the inoculum was fine and flocculant (<0.1 mm) rather than granular. Addition of fine methanogenic sludge as seed and feeding the reactor with POME (containing high acetate, about 2300 mg/l) was effective to enhance the development of methanogenic sludge granules. The special physical features of the UASFF reactor, i.e. the presence of a UFF section that recirculates biomass to the UASB section and the resultant high interaction among bacterial consortium caused rapid biogranule development. Fig. 8 shows the sequence of bio-granule formation in the UASFF reactor during the start-up. The granules diameter increased from an initial pinpoint size to 2 mm. As the OLR was increased from 9.66 to 23.15 g/l day, the granulation rate was rapidly increased. Fig. 9a shows *methanosaeta*-like organisms packed in the core of a developed granule. Fig. 9b illustrates the porous spatial arrangement of the granule surface which confirms the gas production within the granule. Figs. 9c and d show that the structured aggregates were grown due to cellular multiplication of the entrapped bacteria, and became dense with spherical shape by the hydrodynamic shear force caused by the upflow liquid and biogas formed.

A multi layer sludge bed with granules of different sizes and densities was developed. SVI at sampling ports S1, S2 and S3 were 16.9, 37.9 and 117 ml/g, respectively. Development of microbial granules can be affected by reactor operating conditions [28]. Gas bubbles production from sludge blanket, cell precipitation from the middle fixed film part and high suspended solids content of POME also provide a high driving force to enhance rapid microbial granulation in this type of reactors.

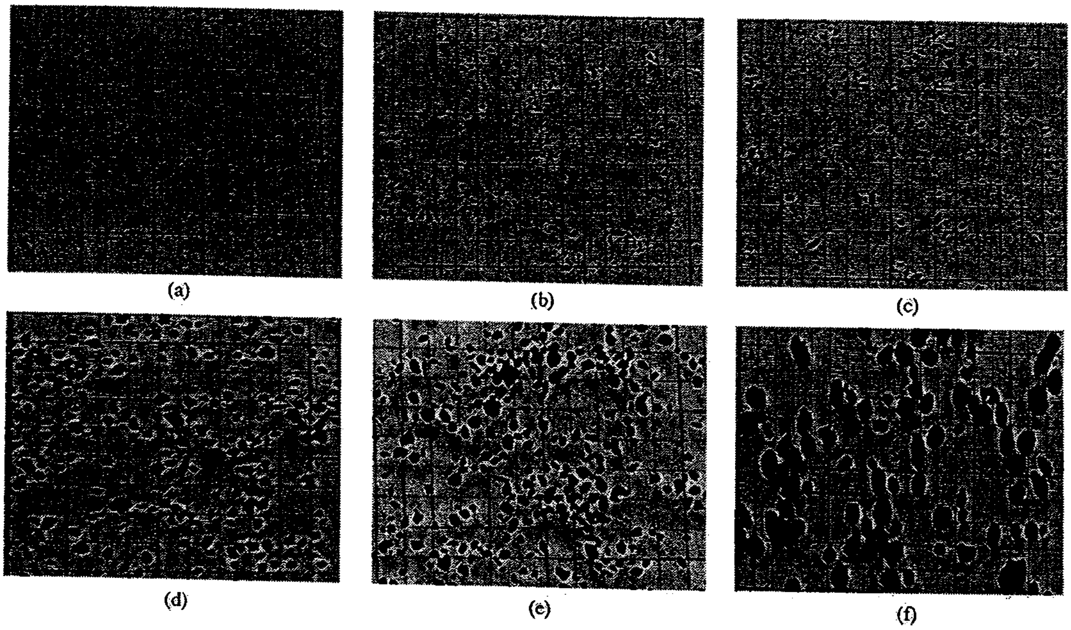

Fig. 8. Sequence of bio-granule formation in the UASFF reactor. (a) Seeding solution, (b) after 10 days, (c) after 15 days, (d) after 20 days, (e) after 25 days and (f) after 30 days.

3.3. Reactor performance

A successful operation of the UASFF reactor was achieved by rapid formation of granules with good settleability and degradation activity. After a 26-day start-up period, the reactor was operated at HRTs of 3 and 1.5 days with six different influent COD concentrations (from 5260 to 34,725 mg/l) to assess the effect of organic loadings on reactor performance. Table 2 summarizes the performance of the bioreactor under steady state conditions (effluent COD concentrations do not vary by more than 5%). In this study, the upflow velocity was maintained relatively constant at 0.44 ± 0.02 m/h while the OLR was increased from 1.75 to 23.15 g COD/l day. The fresh feed flow rate was

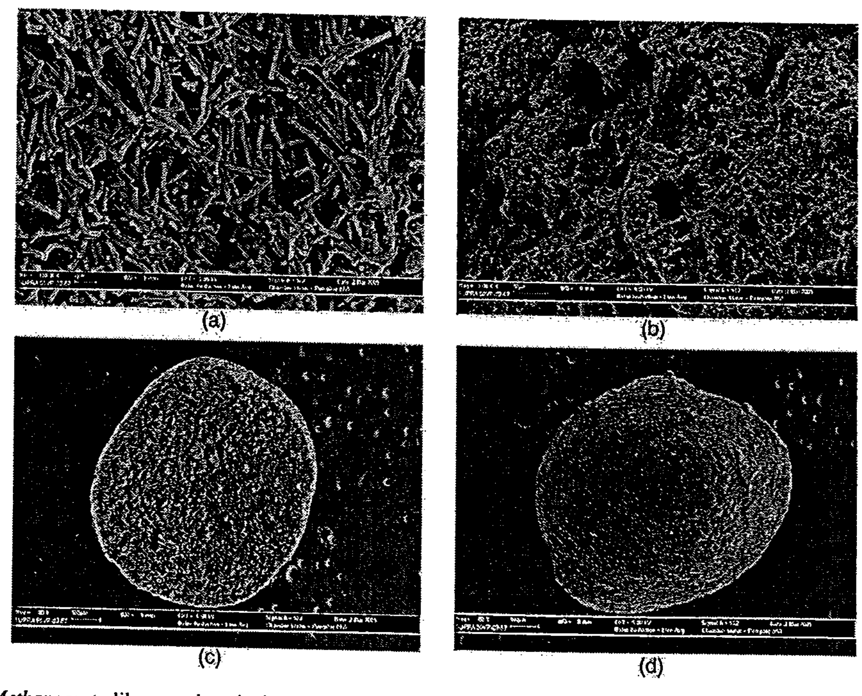

Fig. 9. (a) Aggregate of *Methanosaeta*-like organisms in the core of the granules, (b) gas cavity on the surface of the granule, (c) SEM of sectioned granule and (d) a full-grown granule.

Table 2
UASFF bioreactor performance at various HRT and OLR under steady state conditions

HRT (day)	COD_{in} (mg/l)	OLR (g/l day)	SLR (g/l day)	COD removal (%)	CH_4 fraction (%)	Methane production rate (liters CH_4/g COD day)	Methane yield (liters CH_4/g $COD_{removed}$)	Reactor sludge (g VSS)	Waste sludge (g VSS)	Specific methanogenic activity (g COD-CH_4/g VSS day)	Food/sludge (g COD/g VSS day)	Total VFA[a] (mg/l)	pH
3.0	5260	1.75	0.35	95 ± 0.5	84.0	0.29	0.310	41.7	0	0.14	0.18	20	7.05
1.5	5260	3.51	0.70	93 ± 0.5	82.3	0.32	0.338			0.29	0.37	36	7.12
3.0	10,575	3.53	0.73	96 ± 0.5	82.1	0.32	0.333			0.24	0.26	24	7.11
1.5	10,575	7.05	1.45	94.5 ± 0.5	75.9	0.32	0.333	52.9	10.05	0.47	0.51	44	7.25
3.0	14,485	4.83	1.04	97 ± 0.5	80.1	0.33	0.341			0.24	0.29	15	7.15
1.5	14,485	9.66	2.10	92 ± 0.5	72.6	0.32	0.348	74.2	7.20	0.46	0.57	60	7.42
3.0	21,310	7.10	1.53	97.5 ± 0.5	77.8	0.32	0.334			0.33	0.40	20	7.25
1.5	21,310	14.21	3.10	91 ± 0.5	69.0	0.32	0.345	77.5	41.23	0.64	0.80	80	7.60
3.0	26,210	8.74	1.81	97.5 ± 0.5	74.2	0.34	0.344			0.43	0.51	23	7.30
1.5	26,210	17.47	3.62	90 ± 0.5	64.2	0.32	0.344	75.2	52.64	0.80	1.02	115	7.83
3.0	34,725	11.58	2.34	96.5 ± 0.5	71.9	0.31	0.325			0.49	0.62	28	7.56
1.5	34,725	23.15	4.68	89.5 ± 0.5	62.0	0.28	0.346	82.4	70.36	0.97	1.23	158	7.90

[a] As acetic acid.

stepwise increased from 1.46 to 2.92 l/day for each influent COD concentration. Steady state condition was achieved at each hydraulic loading. The steady state data (Table 2) shows that the reactor was very efficient in COD removal. A minimum efficiency of 89.5% at OLR of 23.15 g/l day and HRT of 1.5 days was achieved. It is important to note that at the highest OLR (23.15 g/l day) and SLR (4.68 g/l day) while food to sludge ratio was 1.23 g COD/g VSS day, the process was stable. Instability of an ordinary UASB reactor for treatment of POME with OLR of 10.63 g COD/l day and HRT of 4 days was reported by Borja and Banks [5]. In this study, a COD removal efficiency of greater than 96% was achieved at HRT of 3 days with OLR of 11.58 g COD/l day. Two main factors controlling the stability of the UASFF reactor at high organic loading were understood to be internal packing and high ratio of effluent recycle. The internal packing was very effective in providing better solid capturing in the column and the effluent recycle created internal dilution for the elimination of high organic loading effects. As excessive built-up of sludge in the reactor can result in wash-out and turbid effluent, the MLSS content of the upper part of the sludge blanket was monitored in order to control stability of the bioreactor. The MLSS at S4 (highest level in the UASB section) was maintained in the range of 1900–2100 mg SS/l at OLR of 23.15 g/l day by removing sludge from S2.

Fig. 10 illustrates the effect of OLR on reactor performance in terms of percentage of COD removal and COD removal rate. The relationship between COD removal rate and OLR at different HRTs is linear, though with different slopes. It is an indication that the reactor has not yet reached its maximum operational limit even though at HRT of 1.5 days COD removal efficiency was slightly decreased from 93 to 89.5%. As a reactor approaches its maximum operational capacity, the COD removal rate would reach a maximum and then start to decrease. It is a sign of insufficient microbial biomass accumulation in the reactor to handle the additional organic load [29].

Fig. 11 shows that low methane production rate was obtained at OLR of 1.75 and 23.15 g/l day which

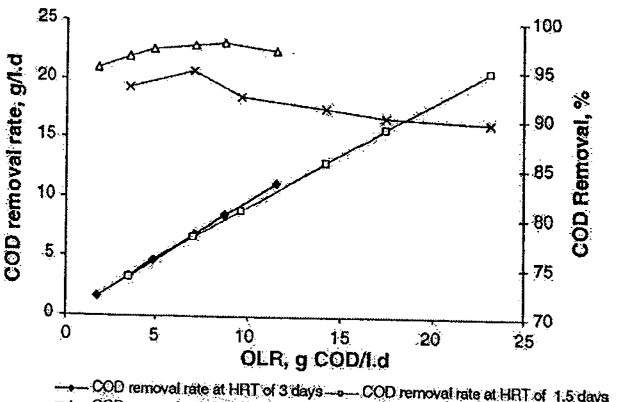

Fig. 10. COD removal rate and COD removal percent at different HRT and OLR.

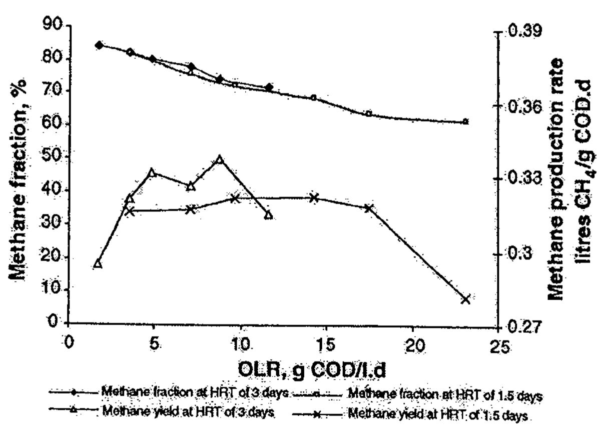

Fig. 11. Methane fraction and methane production rate as a function of OLR.

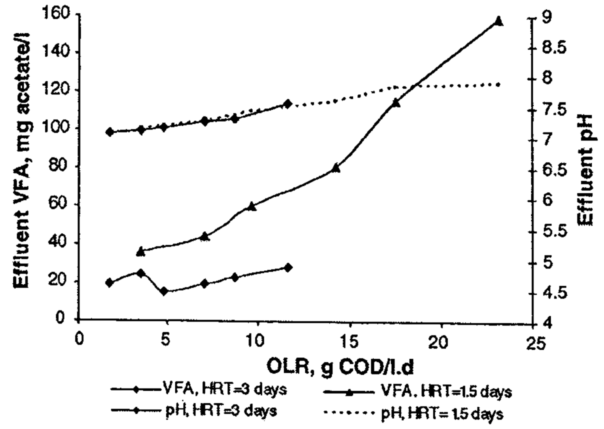

Fig. 12. Effluent VFA and pH at different HRT and OLR.

corresponds to food-to-sludge ratios of 0.18 and 1.23 g COD/g VSS day, respectively. Methane fraction of biogas gradually decreased from 84 to 62%. It may be noted that only at OLR of 23.15 g/l day was propionic acid detectable (82 mg/l) in the effluent. The increase of propionic acid in the effluent indicates overloading, implies that the acidogenic bacteria produced more VFAs and H_2 than what could be utilized by the acetogenic (propionate and butyrate consumers) and the methanogenic (acetate and H_2 consumers) bacteria. This resulted in accumulation of propionate due to hydrogen inhibition of acetogens and also caused inhibition of methanogens.

The concentration of volatile fatty acids and pH in the reactor effluent at the two HRTs and different influent COD concentrations are shown in Fig. 12. For all OLRs tested at HRT of 3 days, the total VFA concentration remained low (15–28 mg acetic acid/l) and there was no VFA built-up (Table 2). In contrast, an increase in VFA concentration was observed with increase in OLR at HRT of 1.5 days. At the highest OLR (23.15 g/l day) tested, the total VFA concentration reached 158 mg/l. It may be inferred that even though a UASFF reactor can yield good treatment efficiencies at a low HRT of 1.5 days, extra care (particularly in terms of VFA built-up) must be exercised at such low HRT levels. There was a trend for pH to increase with increasing organic load as the produced VFA was buffered by bicarbonate generated by the anaerobic bacteria and effluent alkalinity was recovered through high recycle ratio.

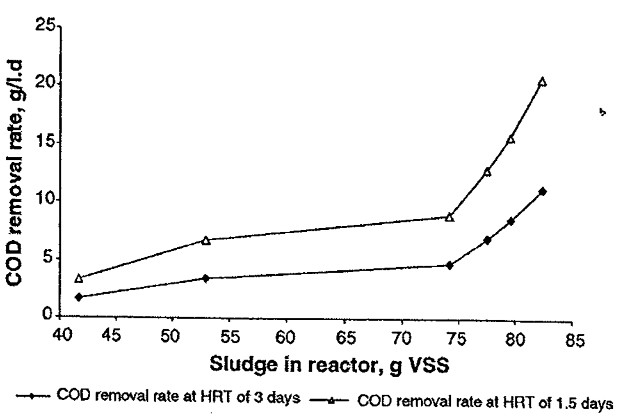

Fig. 13. Relationship between COD removal rate and VSS in the reactor as one of the biological process variables.

Fig. 13 shows the relationship between volatile solids content and COD removal rate at HRT of 3 and 1.5 days. Although it was not possible to distinguish between the nature of VSS contents (whether active biomass or organic matter from POME), this correlation confirmed that there was a logical trend between sludge bed growth and organic matter degradation rate; the specific activity of the reactor increases with the increase in sludge. The granular sludge bed was stable under all operating conditions tested, even at very high OLR of 23.15 g COD/l day (67 g COD/l of granular sludge day). The sludge was dense with low SVI (Section 3.2), there was no sludge wash-out and the reactor continued to operate stable and high COD removal efficiencies.

4. Conclusions

This study reveals that the treatability of POME using the UASFF bioreactor as a novel hybrid bioreactor with high organic load and SS concentration was successfully achieved. The use of UASFF reactor was a good strategy to accelerate anaerobic granulation and to achieve high COD removal efficiency in a short period of time. The reactor was very efficient in the treatment of diluted and high strength POME at high OLR and short HRT. High COD removals of 89 and 97% at HRT of 1.5 and 3 days were achieved, respectively. The use of packing media in the middle portion reduced channeling problem and loss of biomass due to flotation associated with poorly performing UASB reactors. Additionally, the packing material caused the flocculated biomass to precipitate over the sludge blanket to serve as suitable and natural hydrophobic core for the development of granular sludge. Biogas production was found to be close to the theoretical yield.

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Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed film bioreactor

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bstract

A kinetic study of the anaerobic digestion process of palm oil mill effluent (POME) was carried out in a laboratory-scale up-flow anaerobic sludge fixed film (UASFF) bioreactor at mesophilic temperature (38 °C). Influent COD concentrations in the range of 5260–34725 mg/l were applied at steady state conditions. Chen and Hashimoto kinetic equation and a simplified Monod's model was employed to describe kinetics of POME anaerobic digestion at organic loading rates in the range of 0.88–34.73 g COD/l day. The hydraulic retention times (HRT) ranged between 1 and 6 days. Throughout the experiment, the removal efficiency of COD was between 80.6 and 98.6%. The methane production rate was between 0.287 and 0.348 l CH_4/g $COD_{removed}$ day. Biokinetic coefficients; apparent half-velocity constant, A, half-velocity constant, K_s, maximum specific microbial growth rate, μ_m, methane yield constant, Y_M, and growth yield constant, Y_x, were evaluated. The apparent rate constants, K, calculated by simplified Monod model were in the range of 2.9–7.4 l CH_4/g COD day. At different influent COD concentrations, K values showed a linear relationship with variations of VSS content of the reactor.

Keywords: Anaerobic digestion; UASFF; POME; Methane; Monod model; Chen and Hashimoto equation

1. Introduction

In palm oil mills, liquid effluent is mainly generated from sterilization and clarification processes in which large amounts of steam and/or hot water are used. Another waste stream originate from hydro-cyclone operation where the broken shells are separated from the kernels. The mixed effluent is commonly known as palm oil mill effluent (POME). Fresh POME is a thick brownish slurry. It is hot (80–90 °C), acidic (pH 3.8–4.5) and contains very high concentration of organic matter (COD = 40,000–50,000 mg/l, BOD = 20,000–25,000 mg/l). The effluent is non-toxic as no chemical is added in the oil extraction process [1–3].

Treatability of POME has been examined by a wide range of technologies and approaches. Ngan [4], proposed evaporation technique to remove solids content of POME. Energy requirement is to be met by the burning of fiber and shell that are unwanted residues of the oil extraction process. The method, however, entails the generation of a large amount of air pollutants thereby creating another environmental problem. Chemical coagulation and flocculation using alum and flocculant agent (Profloc 4190) was optimized for POME treatment [5]. The optimum value of alum and flocculant were 15,000 and 300 mg/l at pH 6 while effluent turbidity and water recovery were 19 NTU and 76%, respectively. Membrane technology was also applied by Ahmad et al. [6]. Ultra-filtration (UF) and reverse osmosis (RO) processes were used after two pretreatment stages consisting of chemical treatment and adsorption. It is a high cost approach in terms of construction and operation. Ponding systems commonly used for POME treatment need long retention times of 45–60 days [7]. Also it is difficult to maintain the distribution of liquor to ensure smooth performance over a huge area. Aerobic degradation of POME by a tropical marine yeast (*Yarrowia lipolytica*) NCIM 3589 in a lagoon was studied [8]. Ninety-five percent of COD removal was achieved with a retention time of 2 days. Treatability of POME was also examined in an aerobic rotating biological

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contactor (RBC) [9]. Eighty-eight percentage of COD removal was obtained at an HRT of 55 h when the influent COD concentration was 16,000 mg/l.

Anaerobic treatment is the most suitable method for the treatment of effluents containing high concentration of organic carbon [10]. High-rate anaerobic reactors, which also retain biomass, have a high treatment capacity and hence low site area requirement [11]. Anaerobic treatment of POME in two stage up-flow anaerobic sludge blanket (UASB) reactors [12] and a single UASB [13] reactor was carried out. Ninety percent reduction in COD at an average OLR of 15 g COD/l day was achieved in the two-stage UASB reactors. A 96% COD removal was obtained at an organic loading rate (OLR) of 10.6 g COD/l day in the single UASB reactor at an influent COD concentration was 42,500 mg/l and HRT of 4 days. However, reactor instability was observed after 15 days of operation. Performance of an anaerobic hybrid digester was evaluated by Borja et al. [14]. At a hydraulic retention time (HRT) of 3.5 days and OLR of 16.2 g COD/l day, COD removal and methane yield obtained were 92.3% and 0.335 l CH_4/g $COD_{removed}$, respectively. A membrane anaerobic system (MAS) treating POME yielded COD removal between 91.7 and 94.2% at an average HRT of 3.03 days [15]. A clear final effluent was produced but membrane flux rate deterioration was observed due to membrane fouling. An up-flow anaerobic sludge fixed film (UASFF) reactor was examined for POME treatment [16]. High COD removal of 89 and 97% were achieved at HRT of 1.5 and 3 days. The use of packing media in the middle portion reduced channeling problem and loss of biomass due to flotation associated with poorly performing UASB reactors.

Interest in anaerobic hybrid technology (combination of different anaerobic systems into a single bioreactor) has grown as it couples the recovery of usable energy with good process efficiency and stability. The up-flow anaerobic sludge fixed film (UASFF) bioreactor as an anaerobic hybrid reactor is a combination of an up-flow anaerobic sludge blanket (UASB) reactor and an immobilized cell or fixed film (FF) reactor [17]. The FF portion positioned above the UASB section prevents sludge washout and helps in retaining a high biomass concentration in the reactor. Several researchers have successfully used the UASFF reactor to treat various kind of wastewaters such as starch, swine, slaughterhouse [18–20].

Borja and Banks [21] studied methane production kinetics for POME digestion in an immobilized cell reactor (ICR). A COD removal of 96.2% was achieved at an OLR and an HRT of 10.6 g/l day and 6.2 days, respectively. The rate of methane production followed the first order Monod's model and the product yield coefficient (Y_M) was found to be 0.325 l CH_4/g $COD_{removed}$. A modified anaerobic baffled reactor was examined for POME digestion by Faisal and Unno [22]. In this study biokinetic parameters μ_m and K_s were estimated by Monod's equation and the values were reported as 0.504/day and 0.313 g/l, respectively.

Two kinetic models were investigated to describe the anaerobic digestion of cattle waste [23]. The three major stages of anaerobic digestion, viz., hydrolysis, acidogenesis and methanogenesis, were considered in the models and the data was fitted by a non-linear multiple-response regression technique. Deveci and Ciftci [24] used a simple mathematical model to analyze kinetics of the anaerobic digestion of baker's yeast effluents. The model was based on elimination of COD during the three consecutive reactions (hydrolysis, acidogenesis and methanogenesis). The slowest step of the anaerobic treatment of this effluent was the acidification step with a rate constant of 0.004/h. Kinetic models of the anaerobic digestion of untreated beet molasses alcoholic fermentation wastewater and beet molasses previously fermented with *Penicillium decumbens*, were compared [25]. Both models followed a first order chemical kinetic rate. Average methane yield coefficients of 225 ml CH_4 STP/g $COD_{removed}$ and 305 ml CH_4 STP/g $COD_{removed}$ were obtained for untreated and pretreated molasses, respectively.

The purpose of the present work is to study the anaerobic transformation of POME to methane in a UASFF bioreactor and to determine the kinetic parameters of the process. The performance of the UASFF bioreactor under steady state conditions was investigated. The suitable rate models, Chen and Hashimoto [26] equation and the simplified Monod's model were employed to describe the anaerobic digestion of POME.

2. Materials and methods

2.1. Bioreactor and start up

A laboratory-scale, UASFF reactor (Fig. 1) was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 132 cm. Total volume of the reactor was 4980 ml and the working volume was 4380 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas–solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25% and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere [16].

2.2. Palm oil mill effluent

The bioreactor was fed with POME pre-settled for 1 h. The characteristics of raw POME are summarized in Table 1. POME samples were collected weekly from a near-by palm oil mill. The wastewater was stored in a cold room at 4 °C before use. Different dilutions of POME were prepared using tap water. The pH of the feed was adjusted to 7.0, using a 6 N NaOH solution. Supplementary nutrients, nitrogen and phosphorous, were added to adjust the COD:N:P ratio to 250:5:1. $NaHCO_3$ was added to maintain an influent alkalinity of 1500–1700 mg $CaCO_3$/l.

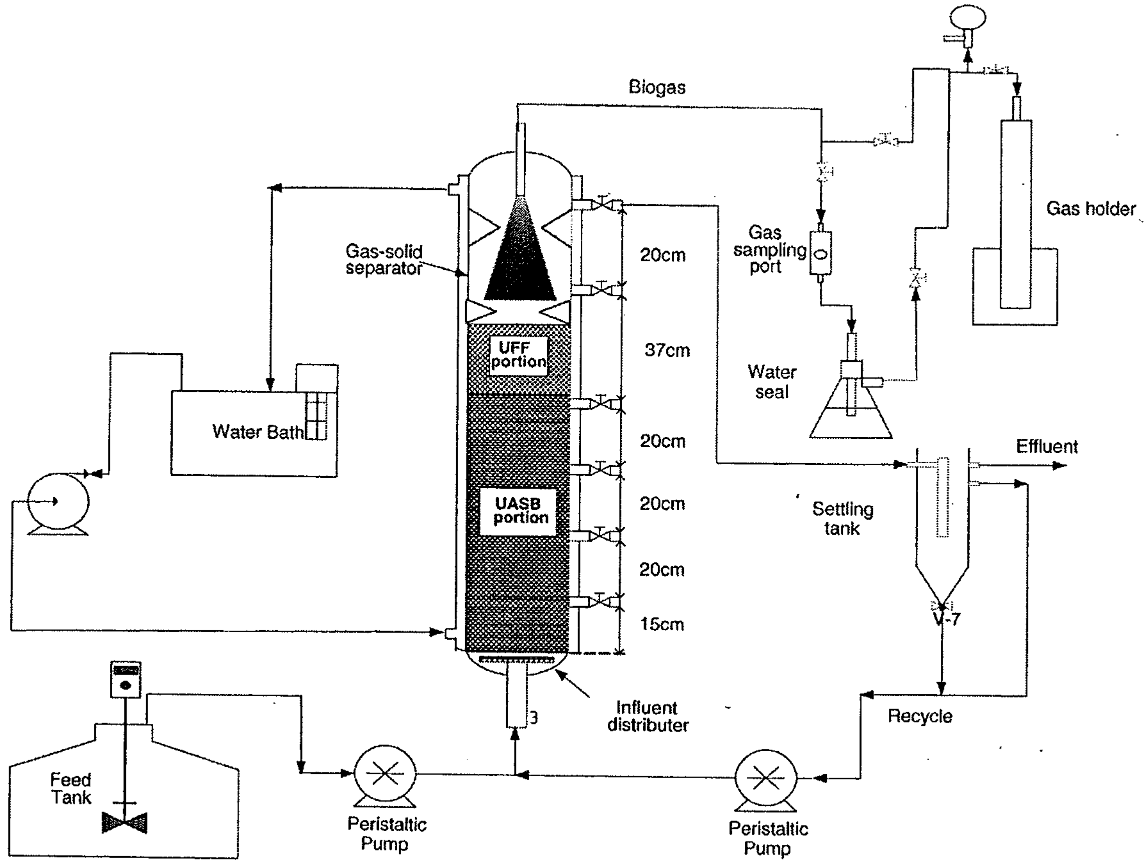

Fig. 1. Experimental set up.

2.3. Bioreactor operation

In order to improve treatment performance and provide an up-flow velocity of 0.44 m/h [17], fresh feed was initially (at HRT = 6 days) diluted with recycled effluent in the ratio of 1:47. Subsequently, this ratio was lowered as the HRT (based on influent POME) was reduced. The purpose of the high recycle ratio was to eliminate organic over loading and to supplement alkalinity by blending the fresh feed with high alkalinity and low COD recycled stream.

The steady state performance was evaluated under different influent COD concentrations (5260–34,725 mg/l) and feed flow rates (HRT of 1–6 days and OLR of 1.05–34.73 g COD/l day). Variation of ±5% in effluent COD concentration at each condition was considered as the criterion for steady state conditions. Adjustment of the reactor pH was not necessary as it remained relatively constant (6.9–7.6) throughout the experiment. COD removal, biomass concentration, pH, methane production and volatile fatty acids (VFA) concentration were monitored.

Table 1
Characteristics of palm oil mill effluent (POME)

Parameter	Amount
BOD_5 (mg/l)	23000–26000
COD (mg/l)	42500–55700
Soluble COD (mg/l)	22000–24000
TVFAs (mg acetic acid/l)	2500–2700
SS (mg/l)	16500–19500
Oil and grease (mg/l)	4900–5700
Total N (mg/l)	500–700
pH	3.8–4.4

2.4. Analytical methods

The following parameters were analyzed according to Standard Methods [26]: pH, alkalinity, TSS, VSS, BOD_5 and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co., Loveland, CO). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively [16].

The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a pall ring at 80 °C for 24 h before and after biofilm attachment. At first a pall ring was taken out from the middle part and was dried and weighed. It was then cleaned up to remove the attached biofilm, dried and weighed. This was carried out twice, before and after the experiments. No significant difference in the amount of attached biomass was noted.

3. Results and discussion

3.1. Reactor performance

After a short and successful start up period, the reactor was operated at different HRTs and influent COD concentrations. High performance of the UASFF reactor at steady state conditions with HRT of 1–6 days and influent COD concentrations of 5260–34,725 mg/l was successfully demonstrated. The performance of the UASFF reactor was evaluated in terms of effluent COD, CH_4 production, effluent VFA, effluent VSS and pH as shown in Table 2. The effluent COD and

Table 2
Performance of the UASFF reactor at different HRT and influent COD concentration under steady state conditions

Influent COD (mg/l)	HRT (day)	OLR (g COD/l day)	Effluent COD (mg/l)	Effluent VFAs (mg acetic acid/l)	Effluent pH	Effluent VSS (mg/l)	Methane production rate (mg CH_4-COD/l day)	VSS in the reactor (g/l)	Solid retention time (day)
5260	1	5.26	475	65.8	7.16	80	4277.2	9.52	119
	1.5	3.51	360	36	7.12	70	4198.9		204
	3	1.75	230	19.5	7.05	40	3924.1		714
	4	1.32	170	21.9	7.03	30	3868.8		1269
	5	1.1	160	15.1	7.02	30	3774.3		1587
	6	0.88	145	17.43	7.03	30	4143.9		1904
10725	1	10.58	1100	83.9	7.5	310	8066.6	12.1	39
	1.5	7.05	538	43.9	7.25	180	8473.2		101
	3	3.53	386	24.7	7.11	100	8578.1		363
	4	2.66	310	24.3	7.1	60	8851.6		807
	5	2.12	250	22.6	7.07	50	8363.7		1210
	6	1.76	245	19.1	7.01	50	8396		1452
14485	1	14.49	1825	93.5	7.65	640	10803.4	16.94	26.5
	1.5	9.66	1125	59.7	7.42	290	11771.3		88
	3	4.83	420	15.4	7.15	140	12141.5		363
	4	3.64	310	17.1	7.12	85	12250.9		797
	5	2.9	290	18.3	7.1	60	11854.8		1412
	6	2.41	270	18.8	7.06	60	11846.6		1694
21310	1	21.31	2830	156.1	7.6	780	14118.3	17.69	22.7
	1.5	14.21	1910	80.5	7.6	410	16932.6		65
	3	7.1	530	19.6	7.25	230	17570.7		231
	4	5.35	395	18.2	7.17	130	17415.4		544
	5	4.26	355	17.1	7.15	75	17484.5		1179
	6	3.55	340	16.3	7.11	70	17478.8		1516
26210	1	26.21	4080	365.2	7.14	960	16814.1	18.17	19
	1.5	17.47	2575	115	7.65	690	19700		39.5
	3	8.74	574	23.4	7.3	230	22340.9		237
	4	6.58	475	22.6	7.2	190	22087.5		383
	5	5.24	410	23.4	7.17	100	21683.7		909
	6	4.37	370	21.3	7.1	110	23062.2		991
34725	1	34.73	6750	843.2	6.92	1175	20352	18.81	16
	1.5	23.15	3620	158	7.63	730	23100		38.7
	3	11.58	730	28.2	7.56	260	27683.4		217
	4	8.72	560	23.1	7.34	225	29001.5		334
	5	6.95	495	21.2	7.22	140	29142.8		672
	6	5.79	480	19.8	7.15	140	31142.7		806

VFA increased with the lowering of HRT, with sharp increases at retention times of 1 and 1.5 days for concentrated POME (COD more than 14,485 mg/l). This was also reflected in the lowered CH_4 production. A comparison of the results obtained with the UASFF reactor in the present study with those obtained from other modern anaerobic techniques, i.e. a UASB reactor [13], an anaerobic hybrid reactor [14], a modified anaerobic baffled (MAB) reactor [22] and a membrane anaerobic system (MAS) [15] treating POME is presented in Table 3. About 90% COD removal was achieved with the UASFF reactor at short HRT (1.5 days) and high OLR (23.15 g COD/l day). The COD removal reached 98.6% when the HRT was increased to 6 days with an influent COD concentration of 34,725 mg/l (Table 2).

At influent COD concentration of 34,725 mg/l, the methane production decreased substantially as the HRT was decreased from 3 to 1 day, corresponding to OLR of 11.58 and 34.73 g COD/l day, respectively (Table 2) and reactor instability was observed after 4 days at OLR of 34.73 g COD/l day. It was also reflected by an increase in the effluent VFA concentration (from 158 to 843.2 mg acetic acid/l) and decrease in the effluent pH (from 7.63 to 6.92). This resulted from upsetting of the balance between acid formation and methane production in the anaerobic process due to high organic load at low HRT. The methane fraction was 55.3% in this condition. An increase in the effluent VFA (365.2 mg/l) with a concomitant decrease in the methane production was also observed at COD of 26,210 and HRT of 1 day (corresponding to OLR of 26.21 g COD/l day). Despite relatively high effluent COD and VFA concentrations, the process remained stable as enough buffering capacity was provided by the recycle ratio of 7 while this ratio was not sufficient to keep pH constant at increased COD concentration of 34,725 mg/l (OLR 34.73 g COD/l day).

The effluent pH remained within the optimal working range for anaerobic digestion (6.9–7.9) throughout the experiment. The pH stability was achieved by recovery of the effluent alkalinity through effluent recycling.

Table 3
Performance of various reactors treating POME

Type of reactor	HRT (day)	Influent COD (g/l)	OLR (g COD/l day)	COD removal (%)	Reference
UASFF	1.5	26.21	17.47	90.2	[16]
		34.73	23.15	89.5	
AHR	3.5	56.6	16.2	92.3	[14]
		65	18.6	77.9	
UASB	4	42.5	10.63	96	[13]
MAB	3	16	5.33	77.3	[22]
ICR	6.2	69	11.13	96	[21]
MAS	3.15	68.31	21.7	92.1	[15]

The amount and concentration of sludge in the reactor is related to the solids retention time (SRT) or sludge age. SRT, as a process control parameter, was also determined by measuring VSS in the reactor and in the effluent at various concentrations of influent COD. The high SRT values denote effective role of the packed bed portion on process stability due to biomass retention which allows the system to cope with changes in OLR. Minimum and maximum SRT values obtained were 16 and 1904 days at OLR of 34.73 and 0.88 g COD/l day, respectively. It was found that at the shortest HRT (1 day), the sludge age to HRT ratio was 19 which is in the range of safety factor (3–20) for the minimum SRT for successful operation of anaerobic biological reactors [27].

3.2. Kinetic evaluation of POME digestion

The anaerobic digestion of organic matter is a complex biochemical process, involving many possible intermediate compounds and reactions, each of which is catalyzed by specific enzymes or catalysts. In the first anaerobic digestion stage, liquefaction, organic substances are broken down by extra-cellular enzymes produced by hydrolytic bacteria [11]. Palm oil mill effluent contains some hydrolysable substrate, volatile acids and simple compounds that are amenable to metabolism by the acid formers. Hydrolysable substrates including suspended solids and large dissolved organic molecules constitute a considerable fraction in the total COD concentration of POME. Therefore, the hydrolysis process must be considered in the kinetic evaluation of POME digestion. Hydrolysis is generally modeled as a first order reaction with respect to substrate as the changes in $\log_e$ (particulate COD) with time is usually linear and also hydrolytic enzymes which catalyze the hydrolysis reactions, are not necessarily associated with or proportional to the active biomass, although the active biomass produces them. The fraction of substrate which is not hydrolyzed due to short HRT, will exit from the UASFF reactor with no change whereas the hydrolysable fraction is hydrolyzed and available for consumption by acidogenic and methanogenic bacteria, successively.

This mechanism is expressed by the following kinetic equations [28,29]:

Kinetics of hydrolysis reaction

$$\frac{dS_h}{dt} = K_h(S - S_h) \tag{1}$$

Kinetics of transportation of hydrolyzed substrate into the granules (it depends on amount of active biomass (X) as the biomass consumes the substrate transported into the granules)

$$\frac{-dS_h}{dt} = k(S_h - S_g)X \tag{2}$$

Kinetic of cell growth on hydrolyzed substrate, Monod's equation

$$\mu = \frac{\mu_m S_h}{K_s + S_h} \tag{3}$$

where S_h is the concentration of hydrolyzed substrate (g COD/l); S, the hydrolysable or biodegradable substrate in effluent (g COD/l); S_g, the hydrolyzed substrate concentration in the granule (g COD/l) which is assumed to be negligible due to rapid metabolism of hydrolyzed substrate in the granules; K_h, the hydrolysis rate constant (per day); k, the hydrolyzed substrate transport rate constant (per day); X, the biomass concentration (g VSS/l); K_s, the half-velocity constant (g COD/l) and μ_m is the maximum specific microbial growth rate (per day).

From Eqs. (1)–(3), we get:

$$\frac{\mu}{\mu_m} = \frac{S}{(K_s kX/K_h) + K_s + S} \tag{4}$$

Using

$$\mu = \frac{1}{\mathrm{SRT}} = \frac{Q_e X_e}{VX} \tag{5}$$

and with the assumption that the microbial growth is negligible in a short period of time

$$X = Y_x(S_0 - S) \tag{6}$$

we have:

$$\frac{S}{S_0} = \frac{A + (K_s/S_0)}{\mu_m \mathrm{SRT} + A - 1} \tag{7}$$

where S_0 is the concentration of hydrolysable substrate in influent (g COD/l) and $A = K_s k Y_x / K_h$.

As can be seen in Table 2, with increase in OLR, rate of COD removal increased. It was a good indication of high substrate mass transfer from outside to inside of the granules. Based on this, it can be assumed that there is no significant mass transfer resistance and diffusion of the hydrolyzed substrate is not rate limiting.

In order to calculate the kinetic coefficients (A, K_s, μ_m), Eq. (7) was solved by the software Sigma Plot 2000 (version 6). Fig. 2 shows the relationship between normalized effluent COD and SRT at different HRT with influent COD concentration of

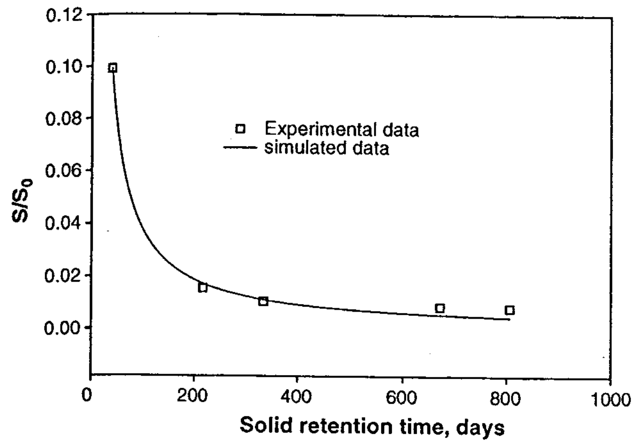

Fig. 2. Normalized COD concentration as a function of solid retention time.

34,725 mg/l. The results of analysis of variance (ANOVA) showed goodness of fit of the data with R^2 and P values of 0.99 and 0.0041, respectively. Table 4 shows kinetic coefficients evaluated for the highest influent COD concentration used in this study. The coefficient A as an apparent kinetic constant represents the apparent half-velocity constant which has reverse relationship with hydrolysis reaction rate constant (K_h). The value of A obtained in the present study is greater than that calculated by Faisal and Unno [22]. This is attributed to high concentration of initial hydrolysable substrate and low digestion time in the UASFF reactor compared to those found in literature. The maximum microbial growth rate μ_m is related to the concentration of active biomass in the reactor. The small value of μ_m (0.207/day) implies relatively high amount of biomass in the reactor. The reactor VSS determination however, includes both biomass and partially degraded influent VSS. Since the influent VSS is not acclimated anaerobic biomass, using total VSS in the reactor in kinetic expressions would bring along some error. Actual μ_m of bacterial cells is therefore, expected to be higher. High relative K_s value (0.982 g COD/l) shows that POME is not a limiting substrate at initial COD concentration of 34725 mg/l.

The rates of substrate utilization, product formation and biomass generation are proportional to one another [11].

$$\frac{-dS}{dt} \propto \frac{dX}{dt} \propto \frac{dP}{dt} \tag{8}$$

Biomass and product (methane) yields are described by Eqs. (9) and (10).

Table 4
Kinetic parameters for POME digestion in different reactors and operating conditions

Type of reactor	S_0 (g/l)	A	K_s (g/l)	μ_m (per day)	Coefficient basis	Reference
UASFF	34.75	0.738	0.982	0.207	COD	This study
MABR	16.00	0.329	0.313	0.304	COD	[22]

$$Y_x = \frac{dX/dt}{-dS/dt} \tag{9}$$

$$Y_M = \frac{dP/dt}{-dS/dt} \tag{10}$$

From Eqs. (9) and (10), we get:

$$\frac{dP}{dt} = r_M = \frac{Y_M}{Y_x}\left(\frac{dX}{dt}\right) \tag{11}$$

Noting that [30]:

$$\frac{dX}{dt} = \mu X \tag{12}$$

Thus Eq. (12) becomes:

$$r_M = \frac{Y_M}{Y_x}(\mu X) \tag{13}$$

Substituting μ from Monod's equation we get:

$$r_M = \left[\frac{Y_M \mu_m}{Y_x(K_s + S)}\right] XS \tag{14}$$

When the substrate is almost depleted i.e. $K_s \gg S$, Eq. (14) can be simplified as [21]:

$$r_M = \left(\frac{Y_M \mu_m}{Y_x K_s}\right) XS \tag{15}$$

Assuming X to be constant throughout a series of experiment, Eq. (15) becomes:

$$r_M = KS \tag{16}$$

where

$$K = \left(\frac{Y_M \mu_m}{Y_x K_s}\right) X \tag{17}$$

Methane production rate is proportional to the biodegradable fraction of organic matter. A graphical extrapolation was employed to estimate the COD concentration at infinite hydraulic retention time. This was considered to be the non-biodegradable fraction of the total COD. Fig. 3 represents effluent COD versus inverse of HRT for each data set. Due to non-linear relations of the data at retention times of 1 and 1.5 days, the data were not incorporated in the estimation of non-biodegradable COD. The data fitted well with R^2 values of 0.94 and above.

Fig. 4 presents the variation of methane production rate as a function of the effluent biodegradable COD. The plot clearly shows that the use of Eq. (16) to describe the process is valid. The rate constant (K) for each data set was

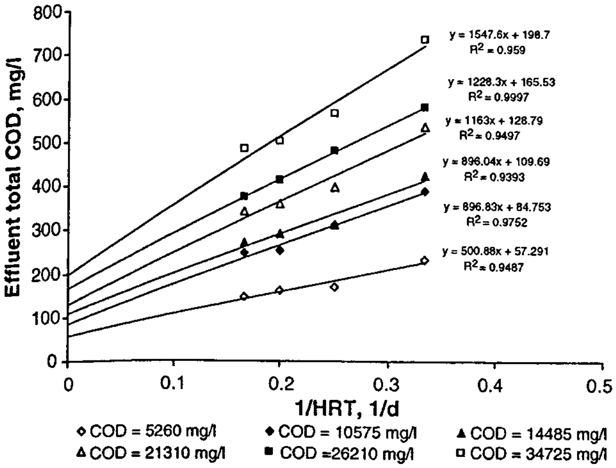

Fig. 3. Estimation of non biodegradable fraction of the total COD.

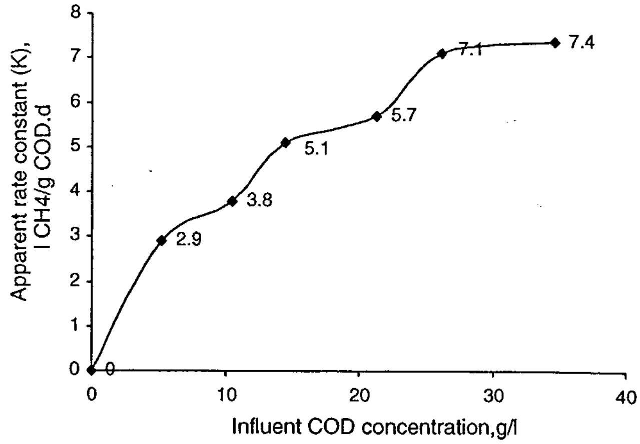

Fig. 5. Relationship between apparent rate constant (K) and substrate concentration.

calculated. Fig. 5 shows variation of the apparent rate constant as a function of the influent COD concentration. As the influent COD concentration increased, the apparent rate constant, K, also increased and became constant at about 7.4 l CH_4/g COD day.

The rate constant obtained from the biotransformation of POME in the UASFF bioreactor was five times higher than that obtained in the immobilized cell bioreactor by Borja and Banks [21] at similar influent COD concentration (34725 mg/l). This is attributed to the high specific activity of granulated sludge in the UASFF reactor compared to that of the biomass in the immobilized cell bioreactor (ICR). Besides that, the biofilm developed in the middle part of the reactor served a supplementary role for the final digestion stage to metabolize intermediates such as the remaining VFA to methane.

Fig. 6 shows the correlation between the apparent rate constant (K) and the biomass concentration (X) in the bioreactor. The data fitted well with an R^2 value of over 0.88. However, contrary to Eq. (17), a non-zero intercept is noticed. This may be attributed to the inability of the VSS determination procedure to distinguish between true biomass (living cells) and other suspended organic matter [31].

The goodness of experimental data was verified by plotting the gas production rate against the theoretical reaction rate (Fig. 7) and comparing it with the straight line obtained by plotting r_M (experimental) equal to r_M (theoretical). The theoretical rate was calculated by multiplying the apparent rate constant (K) with the effluent substrate concentration (S) (Eq. (16)). Fig. 7 shows a high correlation ($R^2 = 0.994$) between the experimental and theoretical reaction rate and that there is no significant difference between the experimental and theoretical values.

Methane yield (Y_M) is an overall kinetic coefficient of anaerobic digestion which represents reactor performance in terms of methane production. The rate of methane production (Q_M) is related to the organics removal rate [11]. The relationship is expressed as Eq. (18):

$$Q_M = Y_M Q(S_0 - S) \tag{18}$$

where S_0 is the influent COD concentration (g COD/l); S, the effluent COD concentration and Q is the volumetric feed flow rate (l/day). Using the experimental data, Eq. (18) was plotted (Fig. 8) and the Y_M value of 0.325 l CH_4 STP/g

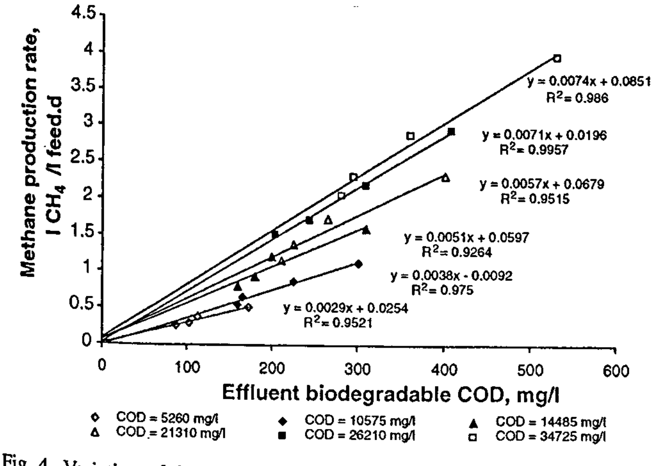

Fig. 4. Variation of the methane production rate as a function of the effluent biodegradable substrate concentration.

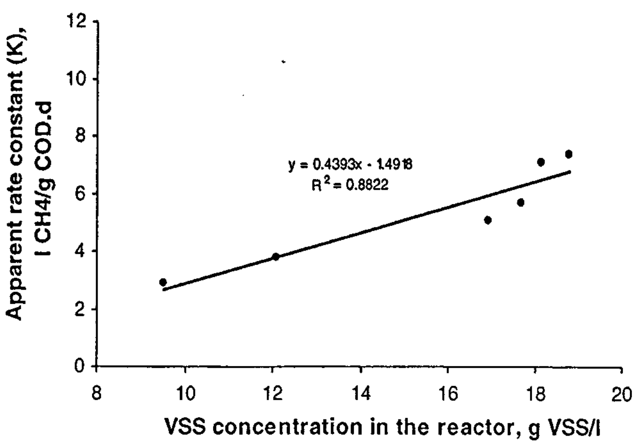

Fig. 6. Variation of the apparent rate constant, K, as a function of the biomass concentration.

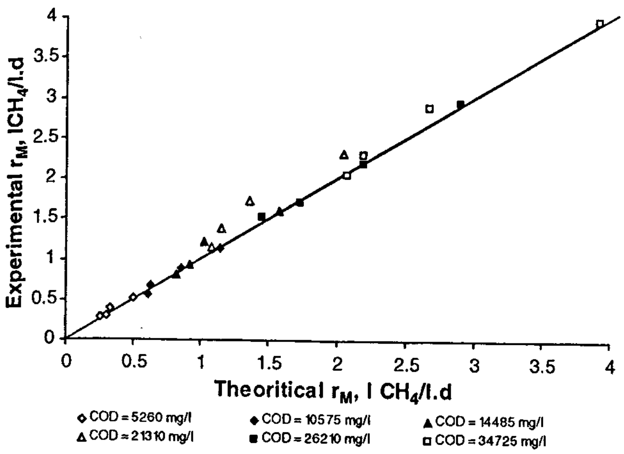

Fig. 7. Experimental r_M vs. theoretical r_M.

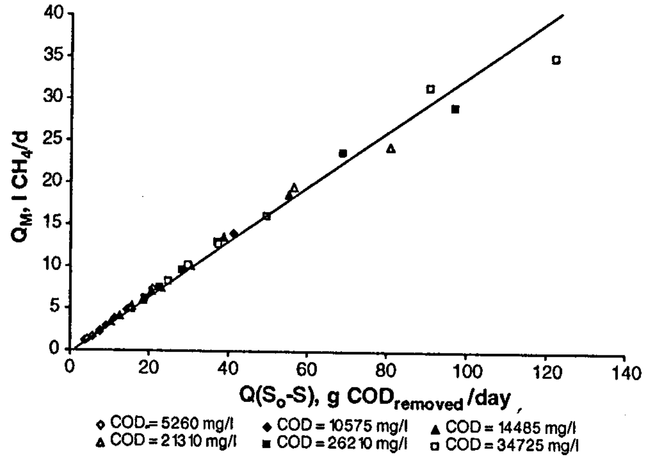

Fig. 8. Methane production rate as a function of substrate consumption rate.

$COD_{removed}$ was obtained. This is in absolute agreement with the value reported by Borja and Banks [21], despite the differences in the type of reactors and shorter HRT in the UASFF reactor.

The biomass yield coefficient (Y_x) was also estimated using the apparent rate constant (K), Y_M, K_s, μ_m, and biomass concentration (X) in the reactor at initial COD concentration of 34725 mg/l and the value of 0.174 g VSS/g $COD_{removed}$ was obtained. It was similar to the Y_x values obtained for palm oil sludge in a completely-mixed digester without solids recycle (0.14 and 0.23) [32]. It was also in agreement with typical Y_X suggested (0.18 g VSS/g COD) by Henze and Harremoes [33] for overall anaerobic process with temperature in the range of 30–40 °C.

4. Conclusions

The UASFF bioreactor was found to be a successful biological treatment system to achieve a high COD removal efficiency in a short period of time. About 90% COD removal was achieved with the UASFF reactor at short HRT (1.5 days) and high OLR (23.15 g COD/l day). The proposed kinetic equations are applicable to anaerobic treatment of palm oil mill wastewater with the UASFF reactor. The methane production rate was between 0.287 and 0.348 l/g $COD_{removed}$ day. Biokinetic coefficients A, K_s, μ_m, were found to be 0.738, 0.982 g COD/l, 0.207/day, respectively. The methane yield (Y_M) and the biomass yield (Y_x) coefficients were 0.325 l CH_4 STP/g $COD_{removed}$ and 0.174 g VSS/g $COD_{removed}$, respectively.

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Appendix A. Nomenclature

A	apparent kinetic constant in Chen and Hashimoto equation
k	transportation rate constant into the granule (per day)
K	apparent reaction rate constant (l CH_4/g COD day)
K_h	hydrolysis rate constant (per day)
K_s	half-velocity constant (g COD/l)
Q	volumetric feed flow rate (l/day)
Q_e	effluent flow rate (l/day)
Q_M	volume of gas produced per day (l CH_4/day)
r_M	methane production rate (l CH_4/l day)
S	effluent substrate concentration (g COD/l)
S_0	influent substrate concentration (g COD/l)
S_g	hydrolyzed substrate concentration in the granule (g COD/l)
S_h	hydrolyzed substrate concentration (g COD/l)
t	hydraulic retention time (day)
V	volume of the reactor (l)
X	biomass concentration (mg/l)
X_e	effluent VSS concentration (mg/l)
Y_M	methane yield constant (l CH_4/g $COD_{removed}$ day)
Y_x	growth yield constant (g VSS/g $COD_{removed}$ day)

Greek letters

μ	specific microbial growth rate (per day)
μ_m	maximum specific microbial growth rate (per day)

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Process modeling and analysis of palm oil mill effluent treatment in an up-flow anaerobic sludge fixed film bioreactor using response surface methodology (RSM)

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ABSTRACT

In this study, the interactive effects of feed flow rate (Q_F) and up-flow velocity (V_{up}) on the performance of an up-flow anaerobic sludge fixed film (UASFF) reactor treating palm oil mill effluent (POME) were investigated. Long-term performance of the UASFF reactor was first examined with raw POME at a hydraulic loading rate (HRT) of 3 d and an influent COD concentration of 44 300 mg/l. Extreme reactor instability was observed after 25 d. Raw POME was then chemically pretreated and used as feed. Anaerobic digestion of pretreated POME was modeled and analyzed with two operating variables, i.e. feed flow rate and up-flow velocity. Experiments were conducted based on a central composite face-centered design (CCFD) and analyzed using response surface methodology (RSM). The region of exploration for digestion of the pretreated POME was taken as the area enclosed by the feed flow rate (1.01, 7.63 l/d) and up-flow velocity (0.2, 3 m/h) boundaries. Twelve dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, soluble COD (SCOD) removal, effluent pH, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA), effluent total suspended solids (TSS), CH_4 percentage in biogas, methane yield (Y_M), specific methanogenic activity (SMA), food-to-sludge ratio (F/M), sludge height in the UASB portion and solid retention time (SRT). The optimum conditions for POME treatment were found to be 2.45 l/d and 0.75 m/h for Q_F and V_{up}, respectively (corresponding to HRT of 1.5 d and recycle ratio of 23.4:1). The present study provides valuable information about interrelations of quality and process parameters at different values of the operating variables.

1. Introduction

Malaysia is the world's largest producer and exporter of palm oil; contributing 49.5% of world production and 64.5% of world exports (Malaysia Palm Oil Board (MPOB), 2004). During palm oil extraction, about 1.5 tonnes of palm oil mill effluent (POME) is produced per tonne of fresh fruit bunch (FFB) processed by the mill (Ahmad et al., 2003). POME is a thick brownish liquid with average chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values of 50 000 and 25 000 mg/l, respectively. It is discharged at a temperature of 80–90 °C and has a pH typically

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between 4 and 5 (Ma and Halim, 1988; Polprasert, 1989; Singh et al., 1999).

Considering the highly organic character of POME, anaerobic process is the most suitable approach for its treatment (Perez et al., 2001). The common practice of treating POME is by using ponding and/or digestion tank systems without gas collection facilities which have particular disadvantages such as: long hydraulic retention time of 45–60 d (Chin et al., 1996), bad odor, difficulty in maintaining the liquor distribution to ensure smooth performance over huge area and difficulty in collecting biogas which could have detrimental effects on the environment (Ng et al., 1987; Yacob et al., 2005).

High-rate anaerobic reactors, which also retain biomass, have a high treatment capacity and hence low site area requirement (Droste, 1997). POME COD removal efficiencies in excess of 85% have been reported for high rate reactors such as anaerobic baffled reactor (ABR) (Setiadi et al., 1996) single up-flow anaerobic sludge blanket (UASB) reactor (Borja and Banks, 1994), two-stage UASB system (Borja et al., 1996a) and membrane anaerobic system (MAS) (Fakhrul-Razi and Noor, 1999).

Interest in anaerobic hybrid technology (combination of different anaerobic systems into a single bioreactor) has grown in recent years, as it couples the recovery of usable energy with good process efficiency and stability. The up-flow anaerobic sludge blanket fixed film (UASFF) bioreactor as an anaerobic hybrid reactor (AHR) is a combination of an UASB reactor and an immobilized cell or fixed film (FF) reactor (Metcalf and Eddy, 2003). The FF portion positioned above the UASB section prevents sludge washout and helps in retaining a high biomass concentration in the reactor. The UASFF bioreactor has been shown to be highly efficient in the treatment of POME. Borja et al. (1996b) and Najafpour et al. (2006) achieved 92% and 97% COD removals at low HRT levels of 3.5 and 3 d, respectively.

The design of UASB is often carried out based on the kinetic and mass transfer coefficients determined by steady-state models or the general practice of determining the optimal operating conditions while keeping the others at a constant level, one-variable-at-a-time technique. Anaerobic digestion process involves complex chain reactions, which generally need a number of assumptions to solve the equations derived on the basis of physical, chemical and biological concepts. Therefore, the steady-state models are basically able to predict the parameters which have been considered in mass balance relations but are unable to estimate other interrelated effluent quality parameters (responses) (Sötemann et al., 2005).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response (Box and Draper, 1987). RSM has an important application in the process design and optimization as well as the improvement of existing design. This methodology is more practical compared to the approaches mentioned above as it arises from experimental methodology which include interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process (Baş and Boyaci, 2007).

In the last few years, RSM has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes such as: optimization of single step fermentation of starch to lactic acid by *Lactobacillus amylophilus* GV6 (Altaf et al., 2005); analysis of the interactive effects of cell concentration and light intensity on hydrogen production by *Rhodopseudomonas capsulate* (Shi and Yu, 2005); application of the central composite design and RSM to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation (Ahmadi et al., 2005); selective optimization in thermophilic acidogensis of cheese–whey wastewater to acetic and butyric acids (Yang et al., 2003); response surface analysis to evaluate the influence of pH, temperature and substrate concentration on the acidogenesis of sucrose-rich wastewater (Wang et al., 2005) and so on.

In most RSM problems, the relationship between the response and the independent variables is unknown. Thus, the first step in RSM is to approximate the function (f). Usually, this process employs a low-order polynomial in some region of the independent variables. If the response is well modeled by a linear function of the independent variables, then the approximating function is a first-order model. If there is curvature in the system or in the region of the optimum, then a polynomial of higher degree must be used to approximate the response, which is analyzed to locate the optimum, i.e. the set of independent variables such that the partial derivatives of the model response with respect to the individual independent variables is equal to zero. The eventual objective of RSM is to determine the optimum operating conditions for the system, or to determine the region, which satisfies the operating specifications. Almost all RSM problems utilize one or both of these approximating polynomials (Montgomery, 1991; Mason et al., 2003; Khuri and Cornell, 1996).

This study investigates the integrated treatment of POME incorporating chemical pretreatment and anaerobic degradation in high rate digestion. This study was conducted to (a) investigate long-term performance of the UASFF reactor fed with raw POME, (b) model and analyze anaerobic treatment of chemically pretreated POME in an UASFF bioreactor using RSM with respect to the simultaneous effects of two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}), where 12 interrelated parameters were evaluated as response, and (c) develop a continuous response surface of the main parameters to provide an optimal region which satisfies the operating specifications.

2. Material and methods

2.1. *Wastewater preparation*

POME was collected from a local palm oil mill in Nibong Tebal (Penang, Malaysia). No chemical was added to POME in the first stage. In the second part of this study, raw POME was chemically pretreated to remove suspended solids and residual oil (using cationic and anionic polymers). The samples were stored in a cold room at 4 °C. This storage technique had no observable effect on its composition. The characteristics of the raw and pretreated POME are summarized in Table 1.

2.2. *Bioreactor configuration and start up*

A laboratory-scale, UASFF reactor (Fig. 1) was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml (including the section containing the gas–solid separator), and the working volume (total liquid volume excluding volume of the pall rings in fixed bed section) was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with a height of 25 cm was operated as a FF reactor and the top part of the bioreactor served as a gas–solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25% and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel-shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding

Table 1 – Characteristics of the raw and pretreated POME[a]

Parameter	Raw POME	Pretreated POME
BOD_5 (mg/l)	22 700	9750
COD (mg/l)	44 300	13 880
Soluble COD (mg/l)	17 140	13 880
TVFA (mg acetic acid/l)	2510	2760
SS (mg/l)	19 780	<20
Oil and grease (mg/l)	4850	Negligible
TKN (mg/l)	780	480
pH	4.05	4.2

[a] Values are average of three measurements. The differences between the measurements for each were less than 1%.

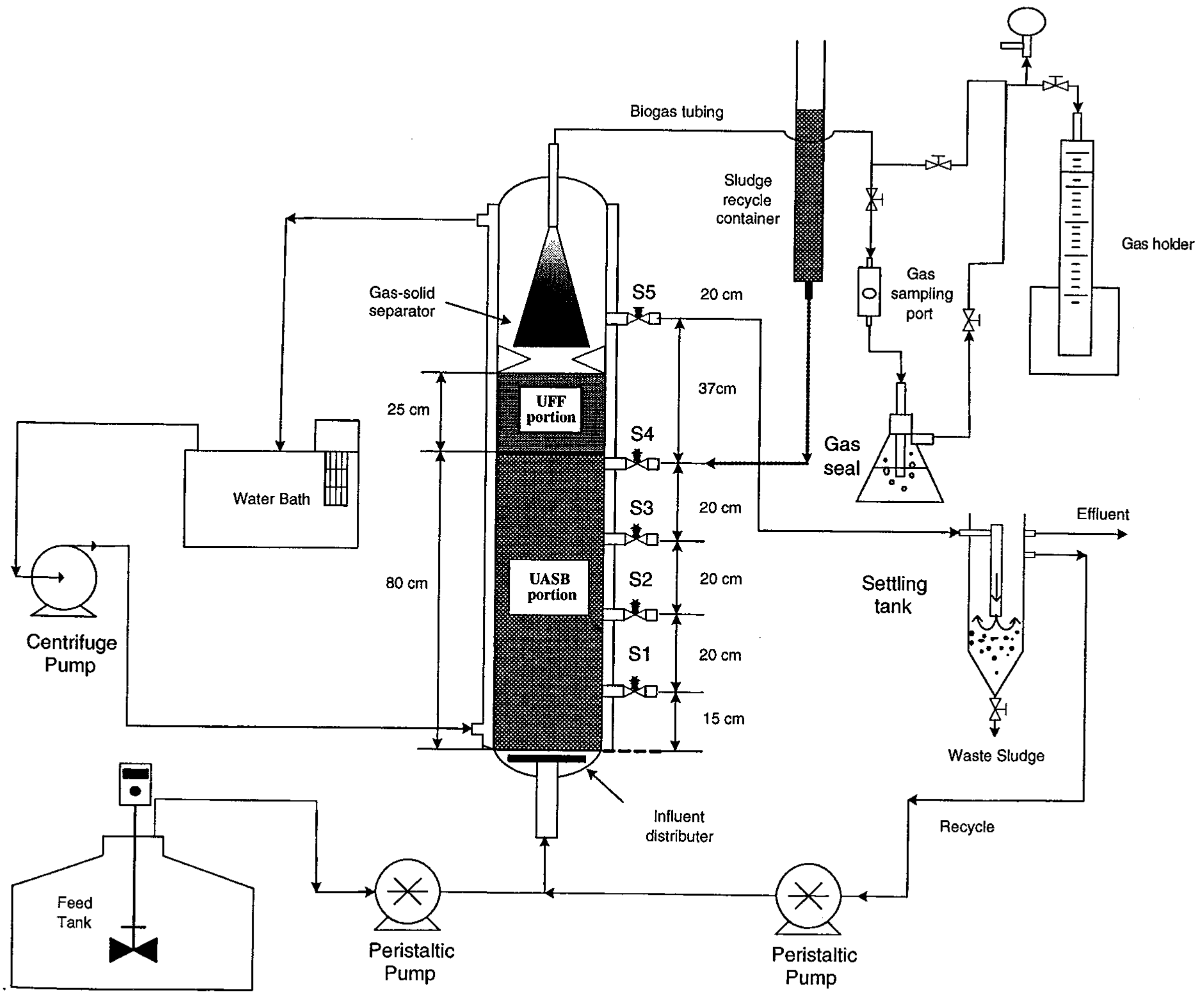

Fig. 1 – Experimental setup.

the start up procedure can be found elsewhere (Najafpour et al., 2006).

2.3. *Bioreactor operation*

In the first stage, raw POME was fed into the reactor with an HRT of 3 d and recycle ratio of 27.7:1, which provided an up-flow velocity of 0.44 m/h. In the second stage, involving modeling by RSM, the UASFF bioreactor was operated with pretreated POME and experiments were designed by Design Expert Software (Stat-Ease Inc., version 6.0.6) with two variables, feed flow rate (Q_F) and up-flow velocity (V_{up}). The Design Expert Software is a windows-compatible software which provides efficient design of experiments (DOEs) for the identification of vital factors that effect the process and uses RSM to determine optimal operational conditions. The results can be obtained as 3D presentations for visualization and also as contours to appreciate the effect of system variables on responses.

Standard deviation of operating conditions is presented in Table 2, indicative of good agreement between the designed and actual values.

2.4. *Experimental design and mathematical model*

The statistical method of factorial DOE eliminates systematic errors with an estimate of the experimental error and minimizes the number of experiments (Kuehl, 2000). Anaerobic digestion in up-flow reactors depends on a multitude of variables. Among these, five main factors which affect the process are influent COD concentration, feed flow rate, alkalinity, up-flow velocity and biomass concentration. In this study, feed flow rate and up-flow velocity were chosen as independent and most critical operating factors due to the following reasons:

1. COD concentration of the chemically pretreated POME was almost constant (13 880 mg/l) and the main aim of the present work was to investigate the reactor performance in anaerobic digestion of the pretreated POME. Due to this reason and in order to study the effect of OLR, feed flow rate (Q_F) was selected as one of the main operating variables.
2. In a batch experiment (Najafpour et al., 2005), it was found that during POME digestion, bicarbonate alkalinity (BA) is produced. Effluent recycling can therefore be considered as an important factor for providing the required alkalinity to support methanogenesis instead of external addition of alkalinity to the feed. In addition, recirculation of effluent to the reactor is widely recommended for wastewater with high COD concentration to avoid pronounced concentration gradient as a function of height (Leitão et al., 2006). Hence, V_{up} was considered as another influential operating variable which reflects the dilution coefficient of the influent feed and the rate of alkalinity recycle from effluent to influent.

Table 2 – Standard deviation of operating conditions applied in this study

Factor	Designed value in DOE	Measured value during experiment	Standard deviation
Q_F (l/d)	1.01	1.04	±0.023
	4.32	4.48	±0.113
	7.63	7.94	±0.218
V_{up} (m/h)	0.2	0.199	±0.001
	1.6	1.592	±0.006
	3.0	2.985	±0.011

It may be noted that V_{up} is related to the feed flow rate as described by following:

$$V_{up} = \frac{Q_F + Q_R}{A}, \tag{1}$$

where Q_R is recycle flow rate (l/d) and A is superficial cross-sectional area (m^2). However, as throughout the study V_{up} was adjusted by simultaneously changing Q_F and Q_R, i.e. not Q_F alone, for the purpose of modeling these two factors (V_{up} and Q_F) were thus considered as the independent variables.

Different up-flow velocities were provided by changing the recycle ratio. V_{up} also affects the physical characteristics of the granular sludge such as SVI and settling velocity (Liu and Tay, 2004). The region of exploration for POME treatment was decided as the area enclosed by the feed flow rate (1.01, 7.63 l/d) and up-flow velocity (0.2, 3 m/h) boundaries. This would cover an OLR range of 3.8–29 g COD/l.d that would include the optimum region. Selection of the range of the feed flow rate (Q_F) was based on the results obtained from an earlier study (Zinatizadeh et al., 2006). The study showed that for influent COD concentration of 14 485 mg/l at HRT of higher than 3 d; the effluent quality was almost the same in terms of COD removal and methane production. In this research, influent COD concentration of chemically pretreated POME was 13 880 mg/l. Therefore, in order to find the optimum conditions with respect to process stability and effluent quality the range of 1.01–7.36 l/d (corresponding to HRT of 3.6–0.48 d) for Q_F was chosen. A review of literature (Metcalf and Eddy, 2003; Mahmoud et al., 2003), has shown up-flow velocity typically studied in the range of 0.2–3 m/h. Influent COD concentration was maintained at 13 880 mg/l in all experiments and steady state was assumed after five turnovers.

The RSM used in the present study was a central composite face-centered design (CCFD) involving two different factors, Q_F and V_{up}. The anaerobic digestion of the pre-treated POME was assessed based on the full face-centered CCD experimental plan (Table 3). The design consisted of 2^k factorial points augmented by 2^k axial points and a center point where k is the number of variables. The two operating variables were considered at three levels namely, low (−1), central (0) and high (1). Accordingly, 13 experiments were conducted with 9 experiments organized in a factorial design (including 4 factorial points, 4 axial points and 1 center point) and the remaining 4 involving the replication of the central point to

Table 3 – Experimental conditions and results of central composite design

Run	Variables		Response											
	Factor 1 A:feed flow rate (l/d)	Factor 2 B:up-flow velocity (m/h)	Total COD removal (%)	Soluble COD removal (%)	Effluent pH	Effluent TVFA (mg acetic acid/l)	Bicarbonate alkalinity (mg $CaCO_3$/l)	Effluent TSS (mg/l)	CH_4 percentage (%)	Methane yield (l CH_4/g COD_{rem} d)	Specific methanogenic activity (g COD-CH_4/g VSS d)	Food-to-sludge ratio (g COD_{in}/g VSS d)	Sludge height in UASB portion (%)	SRT (d)
1	(−1)1.01	(−1)0.2	96.7	97.3	7.6	64.86	1394	120	62.58	0.335	0.142	0.173	48.13	1147
2	(0)4.32	(−1)0.20	84.3	86.5	7.17	146.1	1776	290	51.0	0.261	0.413	0.742	49.13	78
3	(0)4.32	(0)1.60	85.2	87.6	7.29	33.15	1896	350	61.75	0.29	0.471	0.753	60.0	66
4	(0)4.32	(0)1.60	84.5	86.5	7.26	37.49	1833	340	61.81	0.285	0.46	0.753	60.0	68
5	(−1)1.01	(0)1.60	94.3	95.4	7.51	23.1	1504	110	82.61	0.273	0.114	0.175	56.88	1132
6	(0)4.32	(0)1.60	86.0	88.2	7.33	34.7	1915	380	63.8	0.275	0.45	0.753	60.0	61
7	(1)7.63	(−1)0.2	62.2	66.4	6.64	1613.23	915	590	24.78	0.109	0.199	1.314	53.75	23
8	(−1)1.01	(1)3.00	93.24	94.3	7.22	22.1	1664	220	70.78	0.226	0.094	0.176	61.25	607
9	(1)7.63	(0)1.60	80.2	83.2	7.33	289.68	1474	430	62.2	0.244	0.665	1.341	66.88	31
10	(0)4.32	(0)1.60	87.6	89.3	7.43	33.99	1896	360	62.19	0.27	0.419	0.753	60.0	64
11	(0)4.32	(0)1.60	84.5	86.5	7.29	36.07	1854	320	62.07	0.255	0.411	0.753	60.0	66
12	(1)7.63	(1)3.00	83.8	87.0	7.19	251.02	1312	370	67.7	0.224	0.639	1.341	67.5	36
13	(0)4.32	(1)3.00	86.5	89.4	7.03	37.92	1893	370	54.98	0.228	0.378	0.758	65.63	65

get good estimate of experimental error. Repetition experiments were carried out after other experiments followed by order of runs designed by DOE as shown in Table 3. In order to carry out a comprehensive analysis of the anaerobic process, 12 dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, soluble COD (SCOD) removal, effluent pH, effluent total volatile fatty acid (TVFA), effluent BA, effluent total suspended solids (TSS), CH_4 percentage in biogas, methane yield (Y_M), specific methanogenic activity (SMA), food-to-sludge ratio (F/M), sludge height in the UASB portion and solid retention time (SRT). The last four parameters were calculated using the following:

$$Y_M = \frac{Q_{CH_4}}{Q_F(COD_{in}\text{-}COD_{ef})} \qquad (2)$$

$$SMA = \frac{Q_{CH_4}\,CF}{(V)\cdot(VSS)} \qquad (3)$$

$$\frac{F}{M} = \frac{(Q_F).(COD_{in})}{(V).(VSS)} \qquad (4)$$

$$SRT = \frac{(V)\cdot(VSS)}{(Q_F)\cdot(\text{effluentVSS})} \qquad (5)$$

where Q_{CH4} is methane production rate (l CH_4/d), VSS is the biomass concentration in the reactor (mg/l), V is the volume of the reactor (liter), CF is the conversion factor to transform volume (liter) of CH_4 at working temperature and pressure conditions to equivalent g COD at a standard temperature of 0 °C and standard pressure of 1 atm. (CF = 2.532 g COD/l CH_4 at 27 °C and 0.974 atm.).

After conducting the experiments, the coefficients of the polynomial model were calculated using the following (Khuri and Cornell, 1996):

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \ldots, \qquad (6)$$

where i and j are the linear and quadratic coefficients, respectively, and β is the regression coefficient. Model terms were selected or rejected based on the P-value with 95% confidence level. The results were completely analyzed using analysis of variance (ANOVA) by Design Expert Software. Three-dimensional plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The optimum region was also identified based on the main parameters in the overlay plot. The experimental conditions and results are shown in Table 3.

2.5. *Analytical methods*

The following parameters were analyzed according to standard methods (APHA, 1999): pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co.). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and VFA compositions, respectively (Najafpour et al., 2005). The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a Pall ring at 80 °C for 24 h before and after biofilm attachment.

3. Results and discussion

3.1. *Reactor performance with raw POME*

According to literature, high COD removal efficiency was achieved with UASFF reactors treating diluted and pre-settled POME when operated at OLRs of 16.2 and 23.15 g COD/l d with influent COD concentration of 56 600 and 34 725 mg/l, respectively (Borja et al., 1996b; Najafpour et al., 2006). However, process instability was observed when a UASB reactor (at HRT of 4 d and OLR of 10.63 g COD/l.d) and an AHR (at HRT of 3.5 d and OLR of 18.6 g COD/l.d) were fed raw POME with high influent COD concentrations of 42 500 and 65 000 mg/l, respectively (Borja and Banks, 1994; Borja et al., 1996b). In the present study, long-term performance of the UASFF reactor was thus examined with raw POME at an HRT of 3 d and influent COD concentration of 44 300 mg/l (corresponding to an OLR of 14.93 g COD/l.d). The reactor behavior over a 25-d period is shown in Fig. 2a–d.

Fig. 2a shows that COD removal efficiencies of 90% and above were achieved during the first 12 d. Thereafter, a decrease in COD removal to 82.4% was observed. This was due to some sludge washout as reflected by the increase in effluent TSS (Fig. 2d). Sludge washout suddenly occurred due to TSS accumulation in the sludge blanket. The fixed bed could not prevent the washout because the media pores were too big to retain small size flocs.

The system efficiency temporarily recovered after 15 d due to maintaining a favorable environment for anaerobic reactions, after a sludge washout. A 90% COD removal was again obtained. Extreme reactor instability was observed on day 25 when about one-third of the reactor biomass was washed out. This is also reflected in the considerable decrease in methane yield and methane fraction in biogas (Fig. 2b), increase in effluent VFA and decrease in effluent pH (Fig. 2c) and increase in effluent TSS (Fig. 2d).

Raw POME contains high concentration of suspended solids (Table 1), which require long retention time for satisfactory digestion. The non-digestable fraction of TSS gradually accumulated in the reactor by attaching on the granules. The granules activity was then decreased due to contribution of non-biomass VSS (non-biodegradable or refractory fraction) in the granules structure. Since sludge granules are formed from dense microbial aggregation, the original granules have smaller sludge volume index (SVI). The SVI was 12.5 ml/g at the beginning and it increased to 24.4 ml/g on day 25. The increase in SVI may cause process instability due to increase in the rate of sludge washout. The VSS/TSS ratio increased from 0.73 to 0.82 (the ratio for influent was 0.93). That was an indication of increasing contribution of the influent VSS on the sludge blanket. From observations, it was found that most of the granules were covered by a thin layer of fiber-like suspended solids.

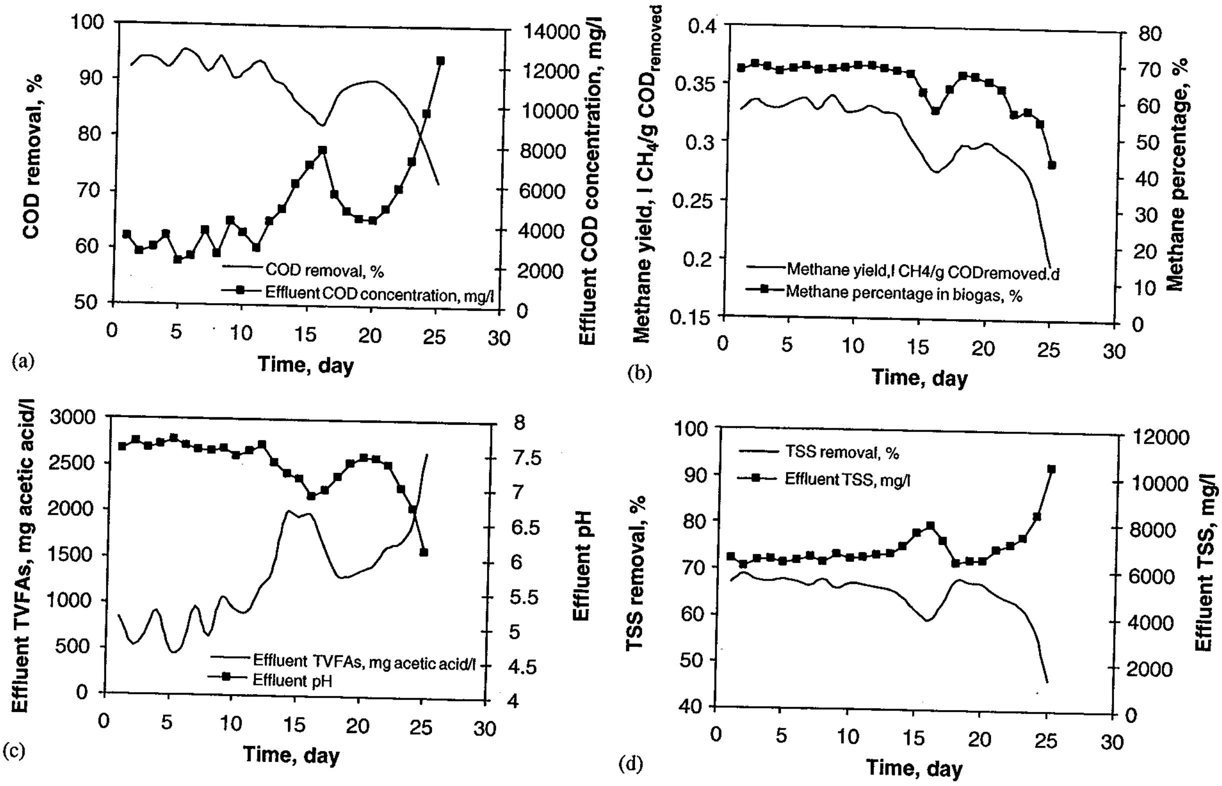

Fig. 2 – Reactor performance during 25 d: (a) COD removal efficiency and influent COD concentration; (b) methane yield and methane fraction in biogas; (c) effluent pH and VFA concentration and (d) TSS removal efficiency and effluent TSS.

3.2. *Pretreated POME digestion*

As the conclusion of the first stage of experimentation, complete digestion of raw POME without pretreatment demands high HRT, which is not easily achieved due to high volume of POME produced by most factories. Chemical pretreatment was therefore applied using two commercially available cationic and anionic polymers (coagulants). The results obtained from chemical pretreatment (Table 1) showed that about 72% and 19% total and soluble COD removal was achieved, respectively. The pretreated POME was then used as feed and its anaerobic digestion was modeled and analyzed with two operating variables, i.e. feed flow rate and up-flow velocity.

3.2.1. *Statistical analysis*

As various responses were investigated in this study, different degree polynomial models were used for data fitting (Table 4). The regression equations obtained are presented in Table 4. In order to quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations, i.e. two-factor interaction (2FI), quadratic and so on. In the Design Expert Software, the response data were analyzed by default. Some raw data might not be fitted and transformations, which apply a mathematical function to all the response data, might be needed to meet the assumptions that made the ANOVA valid. Data transformations were needed for the TVFA and SRT responses as errors (residuals) were a function of the magnitude of the response (predicted values). Therefore, $\log_{10}$ function was applied for these responses (Draper and Smith, 1998; Chapra and Canale, 2003; Ahmad et al., 2005). The ANOVA results for all responses have been summarized in Table 4. The model terms in the equations are after elimination of insignificant variables and their interactions. The interaction term, i.e. AB, was significant for all equations except those defining effluent BA, sludge height in the UASB portion and F/M ratio. Based on the statistical analysis, the models were highly significant with very low probability values (from 0.0138 to <0.0001).

It was shown that the model terms of independent variables were significant at the 99% confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). It showed high significant regression at 95% confidence level. The models adequacy was tested through lack-of-fit F-tests (Montgomery, 1991). The lack of fit F-statistic was not statistically significant as the P-values were greater than 0.05. Adequate precision is a measure of the range in predicted response relative to its associated error or, in other words, a signal-to-noise ratio. Its desired value is 4 or more (Mason et al., 2003). The value was found desirable for all models. Simultaneously, low values of the coefficient of variation (CV) (0.52–13.5%) indicated good precision and reliability of the experiments as suggested by Khuri and Cornell (1996), Kuehl (2000) and Ahmad et al. (2005). Detail analysis on the models is presented in the following sections.

3.2.2. *COD Removal*

Two-factor interaction models were selected to describe the response surface of removal of TCOD and SCOD within the

Table 4 – ANOVA results for the equations of the Design Expert 6.0.6 for studied responses

Response	Transformation	Modified equations with significant terms	Probability	R^2	Adj. R^2	Adeq. precision	SD	CV	PRESS	Probability for lack of fit
TCOD removal	—	$85.31-9.67A+3.38B+6.27AB$	<0.0001	0.9315	0.9086	22.662	2.54	2.97	229.69	0.0524
SCOD removal	—	$87.48-8.4A+3.35B+5.90AB$	<0.0001	0.9296	0.9062	22.399	2.3	2.63	188.76	0.0546
Effluent pH	—	$7.35-0.2A-0.21B^2+0.23AB$	0.0003	0.8698	0.8264	15.66	0.098	1.36	0.23	0.1437
Effluent TVFA	Base 10 log	$1.55+0.59A-0.31B+0.34A^2+0.3B^2-0.085AB$	<0.0001	0.9973	0.9954	74.417	0.037	2.01	0.078	0.0599
Effluent bicarbonate alkalinity	—	$1895.83-449.4A^2-103.9B^2+166.75A^2B-207.75AB^2$	<0.0001	0.8974	0.8632	14.309	112.61	6.86	3.17E5	0.069
Effluent TSS	—	$326.92+156.67A-80AB$	<0.0001	0.9056	0.8868	23.218	42.44	12.98	34754	0.0635
Methane yield	—	$0.27-0.043A-0.04B^2+0.056AB$	0.0004	0.8594	0.8126	15.612	0.023	9.06	0.012	0.0946
CH_4 fraction	—	$63.02-9.19A+8.15B+7.64A^2-11.78B^2+7.13AB$	0.0011	0.9172	0.858	15.083	4.43	7.26	1316.88	0.08
SMA	—	$0.45+0.28A-0.089A^2-0.083B^2+0.12AB+0.098A^2B-0.13AB^2$	<0.0001	0.9827	0.9654	22.16	0.034	9.24	0.41	0.1451
Food-to-sludge ratio	—	$0.75+0.58A$	<0.0001	0.9997	0.9997	388.014	0.0076	1.01	0.001	—
Sludge height in UASB portion	—	$60.54+3.65A+7.23B-2.98B^2$	<0.0001	0.968	0.9573	31.489	1.25	2.11	34.34	—
Solid retention time (SRT)	Base 10 log	$1.82-0.75A+0.39A^2+0.12AB$	<0.0001	0.9947	0.9930	64.992	0.048	2.39	0.061	—

A: first variable, feed flow rate (l/d), B: second variable, up-flow velocity (m/h), R^2: determination coefficient, Adj. R^2: adjusted R^2, Adeq. precision: adequate precision, SD: standard deviation, CV: coefficient of variation, PRESS: predicted residual error sum of squares

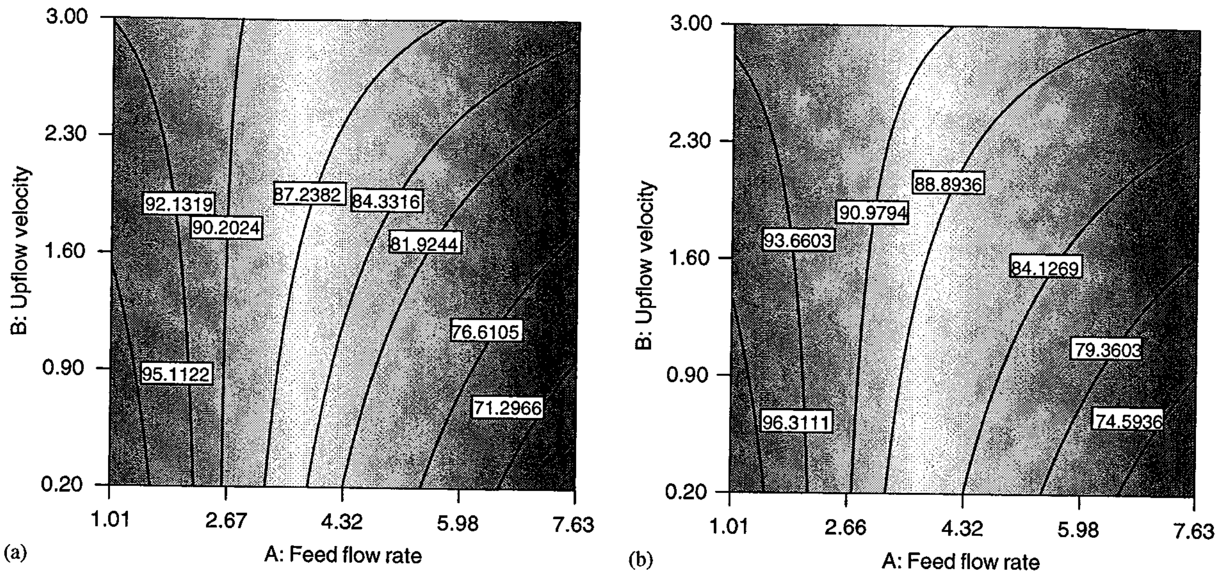

Fig. 3 – Two-dimensional contour plots of the two factor interaction models for (a) TCOD and (b) SCOD.

region (Table 4). The effect of variables on the TCOD and SCOD removal efficiencies are shown in Fig. 3 as contour plots. The influent COD concentration was constant during all the experiments. The COD removal decreased significantly with increased feed flow rate. As the up-flow velocity was increased, the effect of feed flow rate on COD removal was reduced. The change in COD removal at V_{up} of 0.2 m/h (lower limit) was 31.9% and 28.6% for total and soluble COD, respectively, while the corresponding values were 6.78% and 5% for the upper limit of V_{up} (3 m/h). The effect of V_{up} was different at constant feed flow rates. At low feed flow rates (< 2.67 l/d), the COD removal decreased with increase in V_{up}. An opposite trend was obtained at feed flow rate higher than 2.67 l/d due to high OLR at short HRT. In these conditions, V_{up} caused the anaerobic process to remain stable as the rate of effluent alkalinity recovery increased due to higher recycle ratio. OLR at the highest feed flow rate was 29.02 g COD/l d. At such high OLR, TCOD and SCOD removal reduced to 62.2% and 66.4%, respectively; under this condition, the effluent TCOD was 5250 mg/l attributed to incomplete anaerobic digestion.

Whereas the trend of COD removal at low organic loading (low Q_F) may be attributed to the reduction in the diffusion rate of substrate into granules due to higher flow velocity, (Beyenal and Lewandowski, 2000), the trend at high organic loading (high Q_F) shows that at higher organic load, the substrate concentration had a stronger effect on the diffusion rate than flow velocity. As a result, it showed that for each organic load, appropriate value of V_{up} was required for the elimination of negative effects of organic shock caused by high Q_F. It was also found that the first-order polynomial model (Table 4) could well describe the trend of TCOD and SCOD variations as function of the two variables with high coefficient of determination ($R^2 = 0.93$).

3.2.3. *Effluent pH, TVFA and bicarbonate alkalinity*

Three-dimensional plots (response surface) for pH, TVFA and BA obtained from equations presented in Table 4 are shown in Fig. 4. The curvatures of the graphs imply that there is a relatively strong interaction between the variables, which is also reflected by the corresponding low P-values (0.0003). At the highest feed flow rate (7.63 l/d, i.e. HRT = 11.5 h), BA decreased from 1890 to 915 mg $CaCO_3$/l with a TVFA to total alkalinity TA ratio of 0.78. This resulted from the accumulation of VFA that was caused by overloading (OLR = 29.02 g-COD/l d). At this condition (where reactor destabilization was observed), the effluent VFA composed of 953.5, 526.3 and 342.9 mg/l of acetic, propionic and butyric acids, respectively. After 5 d, the reactor maintained lowered but steady performance over a 5 turnover-monitoring period. The lowered reactor performance was attributed to decrease in pH (from 7.1 to 6.64) and bicarbonate alkalinity (BA) (from 1890 to 915 mg $CaCO_3$/l) as after pH adjustment, the system managed to recover.

A reverse impact of increasing V_{up} on pH was observed at different feed flow rates (Fig. 4a). At low feed flow rates; increase in V_{up} caused a decrease in pH due to higher CO_2 dissolution and low rate of alkalinity production. At high feed flow rates, however, an increase in V_{up} increased the pH. This was due to more alkalinity production resulting from increased metabolic activity at high OLR. The high effluent recycle also yielded a similar result. Lowering of the methane percentage (Fig. 7b) was a direct and combined consequence of inhibition of methanogenesis with decreased solubility of CO_2 at low pH values (Eng et al., 1986; Fongastitkul et al., 1994). The accumulation of VFA could be a typical reactor response due to overloading and/or sudden variation in hydraulic and organic loading rates (Leitão et al., 2006).

The ratio of maximum to minimum for effluent TVFA was 73. Hence, a logarithmic function with base 10 was required to fit the data. Fig. 4b shows the effect of V_{up} on effluent TVFA at different feed flow rates. The VFA 3D graph shows that as the feed flow rate increased from 2.67 l/d, TVFA increased drastically. It was observed that the effect of V_{up} values higher than 1.6 m/h on the VFA reduction was minimal. At high feed

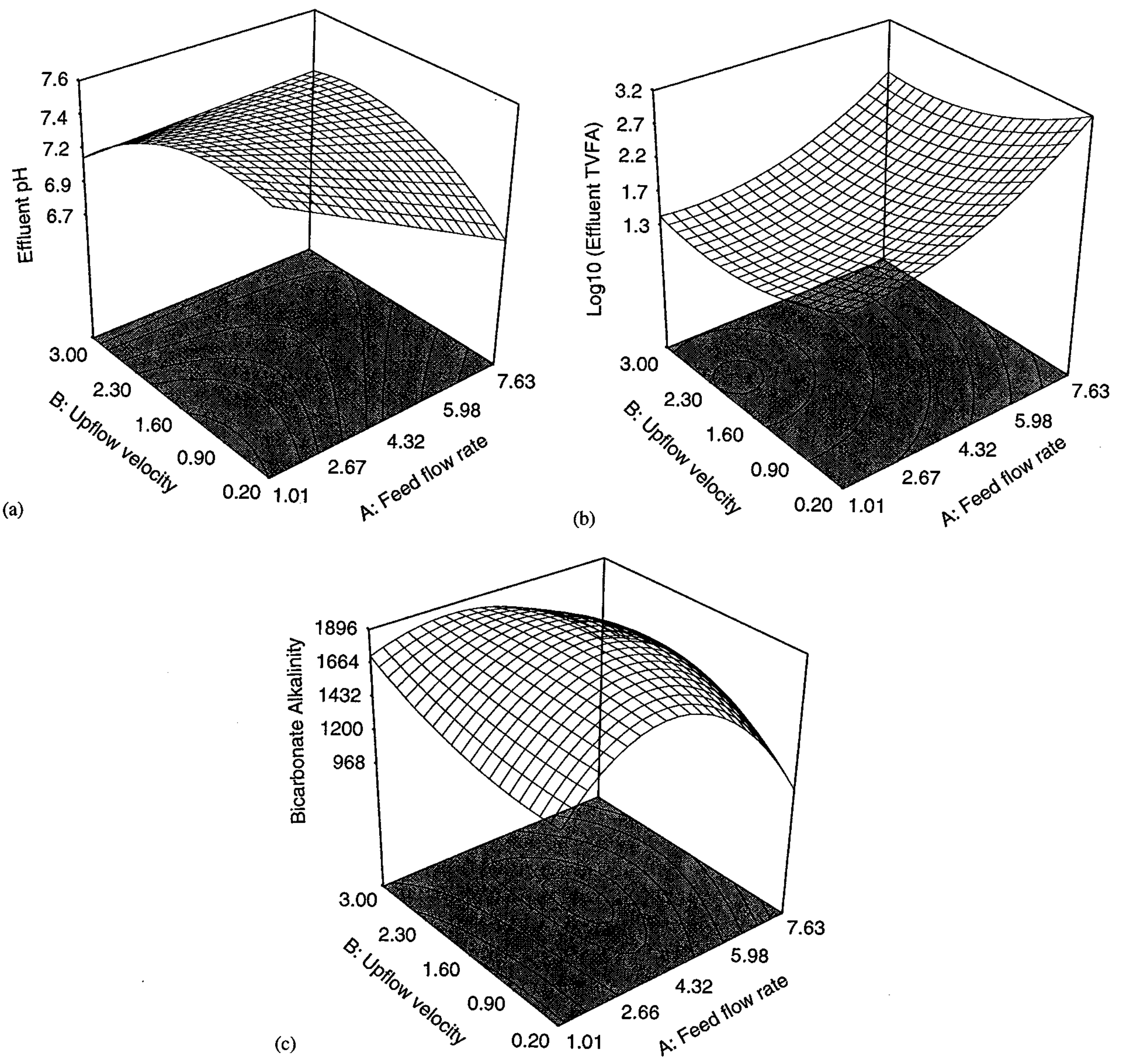

Fig. 4 – Response surface plots for (a) effluent pH; (b) effluent TVFA and (c) effluent bicarbonate alkalinity.

flow rate, when TVFA was being increased due to high OLR and biological acidification, increasing of V_{up} was not as influential as lowering of OLR as the effluent was poor in buffering capacity (915 mg $CaCO_3$/l) and in fact effluent TVFA was being recycled (effluent TVFA = 1613 mg/l).

BA is an important control parameter in anaerobic digestion. For normally operating digesters, the BA is almost the same as the total alkalinity due to the low concentration of volatile acids. However, in an unbalanced condition, an increase in VFA concentration reduces the pH and decreases the BA in accordance with the following (taking acetic acid as an example):

$$HCO_3^- + CH_3COOH \rightarrow CH_3COO^- + H_2O + CO_2. \quad (7)$$

In such situations, the total alkalinity will not change much because the VFA radicals (acetate, propionate, butyrate, etc.) will also be measured as alkalinity. But this form of alkalinity will not be available for neutralization of additional volatile acids. The following relationship was used to calculate the BA (McCarty and Brosseau, 1963).

$$BA = TA - 0.708 TVFA, \quad (8)$$

where TA is total alkalinity expressed as mg $CaCO_3$/l.

Fig. 4c shows that the variables interaction has significant effect on BA. According to the model, the maximum level for BA was predicted around the middle level of the region. It was found that BA was produced through POME digestion reactions, so that the pH remained well controlled; except under overloading conditions ($Q_F = 7.63$ l/d, $V_{up} = 0.2$ m/h) when the balance between the acidogenic and methanogenic microbial consortia was upset resulting in higher VFA accumulation (incomplete digestion). In this condition, if pH was not controlled the existing alkalinity would be neutralized. It was also found that BA decreased as Q_F deviated from the central region.

3.2.4. *Effluent TSS*

The modified two-factor interaction model shows that the main effect of feed flow rate (*A*) and two-level interaction (*AB*) effect of the variables are significant model terms. It shows that the most significant factor on the washout phenomenon was the feed flow rate, which also affected the gas production rate. The up-flow velocity (*B*) on its own did not appear to have a direct effect on effluent TSS concentration because the granules settling velocity (60 m/h) was much greater than the maximum up-flow velocity (3 m/h) adopted in this study. Since TSS content of the influent was negligible, the effluent TSS represents biomass washout rate from the reactor. The effluent VSS to TSS ratio was the same as that of the biomass in the reactor (0.72–0.78). The contour plots of the RSM were drawn as a function of the variables (Fig. 5). The combined effect of high organic load (Q_F of 7.63 l/d) and low V_{up} (0.2 m/h) was maximum effluent TSS. In this condition, an interesting observation was made. The gas produced was initially not able to escape from the sludge due to compaction of the sludge blanket. After several hours of operation, the accumulated gas suddenly buoyed the sludge and caused a relatively high washout from the reactor. It was also observed that some granulated sludge was disintegrated and then washed out due to shock load.

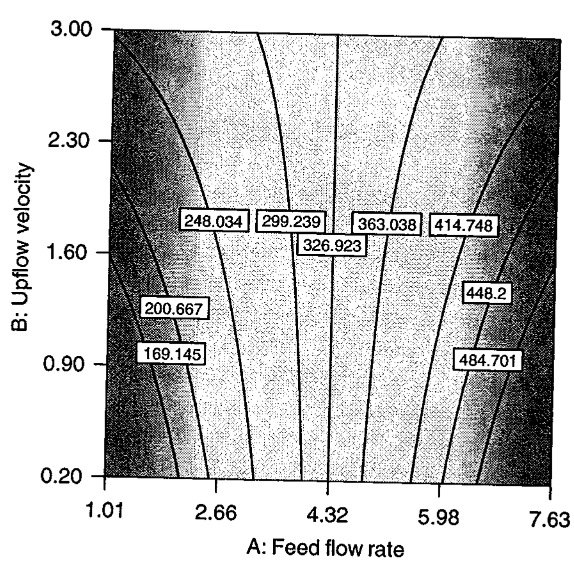

Fig. 5 – Two-dimensional contour plots of the two-factor interaction model for effluent TSS.

3.2.5. *Sludge characteristics*

In order to investigate the effect of variation in the operating parameters on the sludge blanket in the reactor, two important sludge characteristics, viz. sludge height in the UASB portion and SRT were studied. Fig. 6a presents the effects of variables on the height of sludge blanket. It was found that with a simultaneous increase in both variables, the sludge height was increased. As it can be seen in the figure, increase in sludge height caused by increase in V_{up} at constant feed flow rates was greater than increase in sludge height resulting from increase in feed flow rate at constant V_{up}.

Fig. 6b shows SRT variation as a function of the variables. The more influential variable on the SRT was found to be the feed flow rate. As the variables were increased, the sludge age was decreased due to high turbulence and hydrodynamic instability created by increase in gas bubble production and flow velocity.

The sizes of the granules developed were bigger in comparison with the granules grown on raw POME containing high TSS. Average diameter of the granules was between 2.5 and 3.5 mm while it was between 1.5 and 2.5 mm for raw POME, obtained previously. The granules also became more compacted during the experiments. For example, the SVI at sampling port S1 reached 8.13 ml/g whereas it was 11.2 ml/g at the beginning of the experiments.

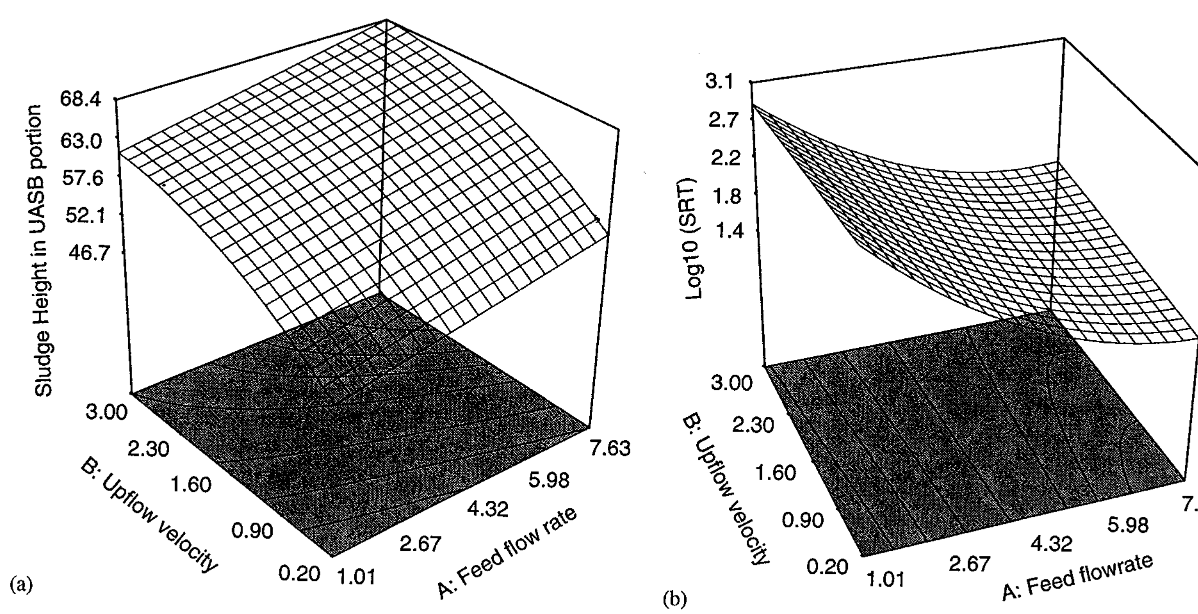

Fig. 6 – Response surface plots for (a) sludge height in the UASB portion and (b) SRT.

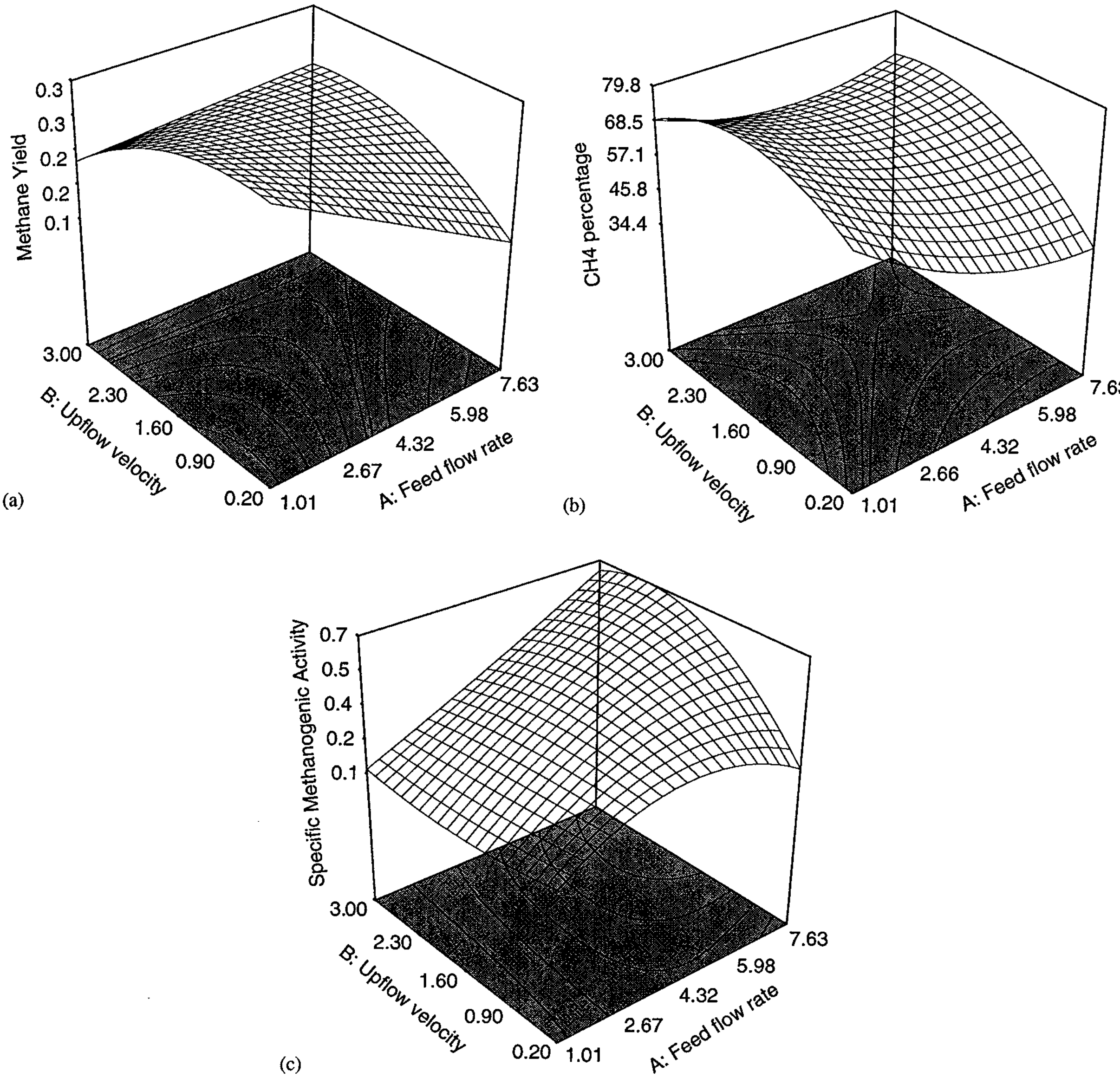

Fig. 7 – Response surface plots for (a) methane yield; (b) methane percentage and (c) SMA.

3.2.6. *Gas production rate, biogas composition and specific methanogenic activity*

The methane yield, CH_4 fraction in the biogas and SMA were also monitored to evaluate the reactor performance in terms of methanogenic activity and system stability. A reduced quadratic model describes the variation of the methane yield as a function of the variables under study (Table 4). The response surface of methane yield (Fig. 7a) showed that a decrease in the variables yielded an increase in the response. The yield reached its highest level at 0.34 when Q_F and V_{up} were 1.01 l/d and 0.2 m/h, respectively, while the value predicted by the model was 0.33 in this condition. It corresponded to HRT and recycle ratio of 3.6 d and 14.8, respectively.

Fig. 7b shows the response surface plot for CH_4 fraction in the biogas. From the figure and the model presented in Table 4, the negative effect of increase in B^2 on CH_4 fraction dominated at V_{up} values greater than 1.6 m/h while at values less than 1.6 m/h, B and AB terms dominated and demonstrated an increasing effect due to increase in CO_2 absorption caused by recycled alkalinity (increased pH). At Q_F higher than 6 l/d, as V_{up} was increased, CH_4 percentage was also found to increase. It was an indication of positive effect of V_{up} on the system recovery in terms of methane fraction in biogas. An offensive and unpleasant odor was produced when system upset occurred. The offensive odor under similar condition was also reported by Borja et al. (1996a) when POME was used in an acidogenic UASB reactor.

From the SMA 3D graph (Fig. 7c), it was found that with a gradual and simultaneous increase in Q_F and V_{up}, the SMA increased except for the region where the up-flow velocity was not high enough to neutralize the organic shock load (high Q_F). It was a good sign of initiation of an instability condition due to very high-feed flow rate relative to recycle ratio. It was also found that SMA value at the highest Q_F (where reactor destabilization occurred) was still greater than

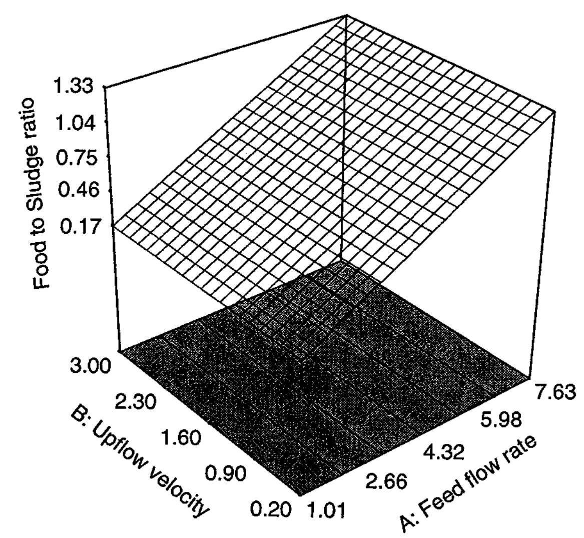

Fig. 8 – Response surface plot for food-to-sludge ratio.

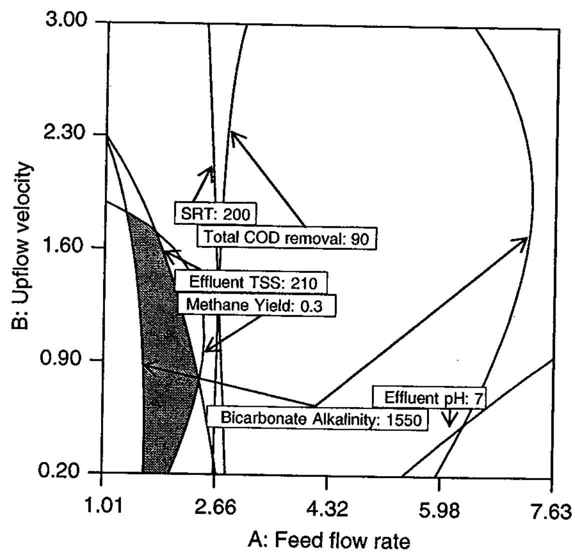

Fig. 9 – Overlay plot for optimal region.

the values at most other stable conditions due to higher F/M ratio (Table 3) as SMA is related to the amounts of substrate and biomass in the reactor. As a conclusion, the models well demonstrated the interactive effects of the variables on the responses related to methane production.

3.2.7. *Food-to-sludge ratio*

F/M ratio is an important process control parameter. Fig. 8 presents the first-order linear surface response for the ratio. It shows that the change in the ratio was independent of V_{up} while it increased with increase in feed flow rate. V_{up} does not necessarily affect the feed load per unit weight of biomass because V_{up} is not solely governed by Q_F rather it is adjusted with effluent recycle as well. Drastic increase in the F/M ratio due to increase in OLR is attributed to slow biomass growth rate in anaerobic systems. According to the model, the optimum value of 0.6 gCOD/gVSS d for biomass growth was

Table 5 – The optimization criteria for chosen responses		
Response	Limits	Unit
TCOD removal	>90	%
Effluent pH	7–8	—
Biocarbonate alkalinity	>1550	mg $CaCO_3$/l
Effluent TSS	<210	mg SS/l
Methane yield	>0.3	l CH_4/g $COD_{removed}$
SRT	>200	Day

achievable at Q_F less than the center point (4.32 l/d); this is same as the optimum value for biomass growth reported by Wu et al. (1985).

3.2.8. *Process optimization*

With multiple responses we need to find regions where requirements simultaneously meet the critical properties, the “sweet spot”. The best compromise can visually be searched by superimposing or overlaying critical response contours on a contour plot. Graphical optimization produces an overlay plot of the contour graphs to display the area of feasible response values in the factor space. Fig. 9 shows the graphical optimization, which displays the area of feasible response values (shaded portion) in the factors space. The optimum region was identified based on six critical responses (TCOD removal, pH, BA, methane yield, SRT and effluent TSS), whose criteria was adopted as shown in Table 5. These 6 parameters were chosen as they were considered most important for reliable representation and optimization of anaerobic treatment process. TCOD removal, representing the substrate metabolized in anaerobic digestion; pH, representing a very critical environmental parameter for microorganisms (methanogens in particular); BA, representing buffering capacity to support methanogenic reactions from inhibition impacts of acidogenic reactions; methane yield, representing methanogenic activity with respect to the amount of methane produced per gram COD removed; SRT, representing the amount and concentration of sludge in the reactor as a process control parameter and effluent TSS, representing the rate of biomass washout as the influent TSS is negligible in this study.

In order to check the accuracy of the models three points within the optimum region were chosen and the bioreactor was operated accordingly to compare actual responses with the predicted. Table 6 presents the results of the three experiments conducted within the optimum region (Fig. 9). The first and second experiments correspond, respectively, to the maximum feed flow rate ($Q_F = 2.45$ l/d, corresponding $V_{up} = 0.75$ m/h) and maximum up-flow velocity ($V_{up} = 1.9$ m/h, corresponding $Q_F = 1.25$ l/d), which were obtained as the intersection points from the overlay graph. The third point is a condition with a moderate Q_F and relatively low V_{up} chosen within the optimum region (not on the boundary). The accuracy of the optimum conditions from DOE experiments was checked by calculating error and standard deviation for each response. These experimental findings were in close agreement with the model prediction.

Table 6 – Verification experiments at optimum conditions

Run		Conditions	Responses									
			TCOD removal (%)	SCOD removal (%)	Eff. PH	Eff. TVFA (mg acetic acid/l)	BA (mg $CaCO_3$/l)	Eff. TSS (mg/l)	CH_4 percentage (%)	Methane yield (l CH_4/g COD_{rem}.d)	Sludge height (%)	SRT (d)
1	Experimental values	$Q_F = 2.45$ l/d	92.62	93.16	7.53	43.1	1520	200	70.83	0.313	54.4	226.3
	Model response with Cl 95%	$V_{up} = 0.75$ m/h	90.86	92.2	7.46	39.64	1725.2	211	63.8	0.299	52.49	258.3
	Error	HRT = 1.5 d	1.76	0.96	0.07	3.46	−205.2	−11	7.03	0.014	1.91	−32
	Standard deviation		±1.25	±0.68	±0.05	±2.45	±145.1	±7.78	±4.97	±0.01	±0.99	±22.63
2	Experimental values	$Q_F = 1.25$ l/d	96.1	97.21	7.53	24.41	1480	158	80.5	0.327	60.2	563
	Model response with Cl 95%	$V_{up} = 1.9$ m/h	93.75	94.81	7.47	18.33	1544.5	197.6	77.9	0.3	58.6	679.33
	Error	HRT = 2.9 d	2.35	2.4	0.06	6.08	−64.5	−39.6	2.6	0.027	1.6	−116.3
	Standard deviation		±1.66	±1.69	±0.04	±4.29	±45.6	±28	±1.84	±0.02	±1.13	±82.26
3	Experimental values	$Q_F = 2.12$ l/d	94.81	95.39	7.38	36.2	1380	150	61	0.321	51.9	343.5
	Model response with Cl 95%	$V_{up} = 0.52$ m/h	92.34	93.5	7.47	48.57	1661.1	181.9	62.86	0.304	50.77	358.7
	Error	HRT = 1.7 d	2.47	1.89	−0.09	−12.37	−281.1	−31.9	−1.86	0.017	1.13	−15.2
	Standard deviation		±1.75	±1.34	±0.06	±8.77	±198.7	±22.56	±1.32	±0.01	±0.78	±10.15

4. Conclusions

The UASFF bioreactor was a successful biological treatment process to achieve a high COD removal efficiency in a short period of time. Complete digestion of raw POME without pretreatment demands high HRT, which is not easily achieved due to high production capacity of most factories. The RSM results demonstrated the effects of the operating variables as well as their interactive effects on the responses. At low feed flow rates ($\leqslant$2.67 l/d), the COD removal decreased with increase in V_{up}. An opposite trend was obtained at feed flow rates higher than 2.67 l/d due to high OLR at short HRT. By applying RSM, the optimum region for the reactor operation was located. Experimental findings were in close agreement with the model prediction. From the present study, it is evident that the use of statistical optimization approach, response surface methodology, has helped to identify the most significant operating factors and optimum levels with minimum effort and time.

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PRETREATED PALM OIL MILL EFFLUENT (POME) DIGESTION IN AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM BIOREACTOR: A COMPARATIVE STUDY

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Abstract An up-flow anaerobic sludge fixed film (UASFF) bioreactor was used to treat the pretreated palm oil mill effluent (POME). In physical pretreatment, POME was pre-settled for 2 h and the supernatant was fed into the reactor. In chemical pretreatment, optimum dosages of cationic and anionic polymers were used. Experiments of pretreated POME digestion were conducted based on a central composite face-centered design (CCFD) with two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}). The operating variables were varied to cover a wide range of organic loading rate (OLR) from 3.8 to 29 g COD /l.d. A stable TCOD removal efficiency of 83.5 % was achieved at the highest Q_F (3.31 l/d, corresponding to OLR of 26 g COD/l.d) for pre-settled POME whereas only 62.2 % TCOD removal was achieved with chemically pretreated POME at Q_F of 7.63 l/d (corresponding to OLR of 29 g COD/l.d) and that too was coupled with process instability. At comparable OLRs i.e. 16.95 g COD/l.d (Q_F = 2.16 l/d) for pre-settled POME and 16.42 g COD/l.d (Q_F = 4.32 l/d) for chemically pretreated POME, the VFA concentrations for the two cases were also similar.

Key Words Pome, Pretreatment, UASFF Reactor, Central Composite Face-Centered Design (CCFD)

چکیده راکتور تلفیقی از بستر لجن بی‌هوازی و بستر آکنده با جریان رو به‌بالا به منظور تصفیه ثانویه فاضلاب پیش‌تصفیه‌شده کارخانه روغن نخل(POME) آزمایش شد. در پیش تصفیه فیزیکی پس از دو ساعت ته‌نشینی مایع رویی برای تصفیه بیولوژیکی به عنوان خوراک به UASFF پمپ شد. در پیش تصفیه شیمیایی، مقادیر بهینه پلیمرهای کاتیونی و آنیونی استفاده شدند. آزمایشهای هضم بی‌هوازی فاضلاب پیش‌تصفیه‌شده براساس برنامه آماری مختلط مرکزی با دو متغیر عملیاتی غیروابسته مشتمل بر شدت جریان ورودی (Q_F) و سرعت جریان رو به بالا (V_{up}) اجرا شد. تغییرات فاکتورهای عملیاتی به گونه‌ای طراحی شد که محدوده گسترده‌ای از شدت بار آلی (OLR) را از مقدار ۳/۸ تا ۲۹ گرم COD/l.d بپوشاند. مقدار ۸۳/۵٪ بازدهی حذف TCOD در بالاترین مقدار Q_F (۳/۳۱ l/d، معادل با مقدار ۲۶ گرم COD/l.d از OLR) برای فاضلاب پیش‌ته‌نشین شده بدست آمد. این در حالی است که تنها ۶۲/۲٪ حذف TCOD همراه با ناپایداری فرآیند برای فاضلاب پیش تصفیه شده شیمیائی درQ_F ۷/۶۳ l/d (معادل با مقدار ۲۹ گرمCOD/l.d از OLR) حاصل شد. در شدت های قابل مقایسه از بار آلی (به ترتیب مقادیر ۱۶/۹۵ و ۱۶/۴۲ گرم COD/l.d برای فاضلاب پیش تصفیه شده فیزیکی و شیمیایی)، غلظت های تقریباً مشابهی از VFA در خروجی راکتور برای هر دو مورد بدست آمد. در این شرایط، مقادیر Q_F و V_{up} به ترتیب ۱/۰۱ l/d و ۰/۲ m/h بودند.

1. INTRODUCTION

Palm oil is one of main agricultural products in Malaysia as it contributes 49.5 % of the total world production [1]. On an average standard palm oil mills, for each tonne of fresh fruit bunch (FFB) processed, generates about 1 tonne of liquid waste with a pollution load of biochemical oxygen demand of (BOD) 37.5 kg, a chemical oxygen demand the (COD) 75 kg, suspended solids (SS) 27 kg and oil and grease 8 kg [2]. There are currently about 265 active palm oil mills in Malaysia with a combined annual CPO production capacity of about 13 million tonnes [3]. This amounts to a population equivalent of around 80 million in terms of COD. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the economy.

Considering the highly organic character of palm oil mill effluents (POME), the anaerobic process is the most suitable approach for treatment [4]. The common practice of treating POME is by using ponding and/or open digestion tank systems which have particular disadvantages such as: long hydraulic retention times of 45-60 days [5], bad odour, difficulty in maintaining the liquor distribution to ensure smooth performance over huge areas and difficulty in collecting biogas which could have detrimental effects on the environment [6-7].

High-rate anaerobic reactors, that can retain biomass, have a high treatment capacity and hence low site area requirement [8]. POME COD removal efficiencies in excess of 85% have been reported for high rate reactors such as the anaerobic baffled reactor (ABR) [9], the single up-flow anaerobic sludge blanket (UASB) reactor [10], two stage UASB system [11] and membrane anaerobic system (MAS) [12]. In summary, the high rate anaerobic reactors mentioned above are successfully able to treat POME at short HRT.

The UASB reactor exhibits positive features, such as high organic loadings, short hydraulic retention time (HRT) and a low energy demand, especially for POME treatment [10,13]. Suspended and colloidal components of POME in the form of fat, protein, and cellulose have an adverse impact on UASB reactor performance and can cause deterioration of microbial activities and wash out of the active biomass [10,14]. The use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the mentioned problems [2-15]. Process instability was observed when a UASB reactor (at HRT of 4 d) and an anaerobic hybrid reactor (AHR) (at HRT of 3.5 d) were operated with high influent COD concentrations of 42500 and 65000 mg/l, respectively [10-15]. Consequently, complete digestion of raw POME without pretreatment demands high HRT, which is not easily achieved due to the high volume of POME produced by the mills. Various pretreatment approaches have been examined for the separation of suspended solids, oil and grease from POME. These include: Chemical coagulation and flocculation [16-18], air flotation simple and skimming [19-20], ultra-filtration [21-22], evaporation [23], centrifugation [20].

The present research is a comparative study of anaerobic digestion of POME which has been physically (primary sedimentation unit) and chemically (chemical coagulation and flocculation pretreated). Results obtained from the high rate digestion of pretreated POME were compared using the response surface methodology (RSM) with respect to the simultaneous effects of two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}).

2. MATERIAL AND METHODS

2.1. Wastewater Preparation Raw POME was collected from a local palm oil mill in Nibong Tebal, Penang, Malaysia. In the first stage, raw POME was pre-settled using an ordinary sedimentation tank. In the second part of this study, raw POME was chemically pretreated to remove suspended solids and residual oil (using a cationic and anionic polymers). The samples were then stored in a cold room at 4 °C. PMOE stored under such conditions has no observable effects on its composition. The characteristics of the raw and pretreated POME are summarized in Table 1.

2.2. Bioreactor and Start Up A laboratory-scale, up-flow anaerobic sludge fixed film (UASFF) reactor was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml, and the working volume was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height

TABLE 1. Characteristics of the Raw, Pre-Settled and Pretreated POME[a].

Parameter	Raw POME	Pre-settled POME	Chemically Pretreated POME
BOD_5 (mg/l)	22700	20100	9750
COD (mg/l)	44300	28640	13880
Soluble COD (mg/l)	17140	17140	13880
TVFA (mg acetic acid/l)	2510	2510	2760
SS (mg/l)	19780	5760	< 20
Oil and grease (mg/l)	4850	1 630	Negligible
Total N (mg/l)	780	660	480
pH	4.05	4.05	4.2

[a]Values are average of three measurements. The differences between the measurements for each were less than 1%.

of 80 cm was operated as a UASB reactor, the middle part of the column with a height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with a diameter and height equal to 16 mm. The voidage of the packed-bed reactor was 91.25 % and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1°C) and the temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere [2].

2.3. Bioreactor Operation and Experimental Design The UASFF bioreactor was separately operated with pre-settled and chemically pretreated POME and experiments were designed by Design Expert software (Stat-Ease Inc., version 6.0.6) with two variables, feed flow rate and up-flow velocity. In an earlier study [2], feed flow rate (Q_F) and up-flow velocity (V_{up}) were found to be the most critically independent operating variables which affected the performance of the reactor. The region of exploration for POME treatment was decided as the area enclosed by Q_F (1.01, 3.31 l/d) and V_{up} (0.2, 3 m/h) boundaries for pre-settled POME and Q_F (1.01, 7.63 l/d) and V_{up} (0.2, 3 m/h) for chemically pretreated POME. This would cover an OLR range of 7.9 to 26.0 and 3.8 to 29.0 g COD/l.d for pre-settled and chemically pretreated POME respectively. A steady state was assumed after five turnovers.

In order to carry out a comprehensive analysis of the anaerobic process, 4 dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA) and methane yield (Y_M).

Data analysis was carried out using the response surface methodology (RSM) under general factorial design. The results were completely analyzed using analysis of variance (ANOVA) which was automatically performed by Design Expert Software (ver. 6.0.6).Three dimensional (3D) plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The RSM used in the present study was a Central Composite Face-centered Design (CCFD) involving two different factors, Q_F and V_{up}. The experimental conditions and results are shown in Table 2.

2.4. Analytical Methods The following parameters were analyzed according to Standard Methods [24]: pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by a colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). Gas chromatographs equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively [2].

3. RESULTS AND DISCUSSION

Raw POME contains a high concentration of suspended solids (Table 1) which requires long

TABLE 2. Experimental Conditions and Results of Central Composite Design.

Run	Type of pretreatment	Variables		Response					
		Factor1 A:Feed flow rate (l/d)	Factor2 B: Up-flow velocity (m/hr)	Total COD removal (%)	Eff. TVFA (mg acetic acid/l)	BA (mg $CaCO_3$/l)	Methane Percentage in biogas %	Methane production Rate, l CH_4/d	Methane Yield (l CH_4/g COD_{rem}.d)
1	Pre-settled POME	1.01	0.2	97.3	132.4	2006	61.41	9.7	0.344
2		1.01	1.60	93.9	104.2	2026	84.4	7.8	0.298
3		1.01	3.00	93.1	80.54	2087	73.83	6.5	0.258
4		2.16	0.20	90.7	243.1	1957	60.33	15.4	0.295
5		2.16	1.60	91.3	35.17	2225	72.33	16.0	0.305
6		2.16	1.60	91.5	44.2	2208	69.98	14.8	0.303
7		2.16	1.60	91.4	36.1	2234	71.24	14.9	0.315
8		2.16	1.60	90.3	58.8	2219	71.8	15.4	0.305
9		2.16	1.60	92.7	38.3	2162	72.12	14.2	0.29
10		2.16	3.00	95.5	41.9	2210	63.33	13.0	0.25
11		3.31	0.2	82.7	573.75	1723	51.33	15.3	0.196
12		3.31	1.60	91.8	144.5	2157	74.08	25.7	0.292
13		3.31	3.00	94.2	137.8	2162	67.89	25.5	0.285
1	Chemically pretreated POME (coagulation and flocculation)	1.01	0.2	96.7	64.86	1394	62.58	4.5	0.335
2		1.01	1.60	94.3	23.1	1504	82.61	3.6	0.273
3		1.01	3.00	93.24	22.1	1664	70.78	2.9	0.226
4		4.32	0.20	84.3	146.1	1776	51	13.2	0.261
5		4.32	1.60	85.2	33.15	1896	61.75	14.8	0.29
6		4.32	1.60	84.5	37.49	1833	61.81	14.5	0.285
7		4.32	1.60	86	34.7	1915	63.8	14.2	0.275
8		4.32	1.60	87.6	33.99	1896	62.19	13.2	0.27
9		4.32	1.60	84.5	36.07	1854	62.07	12.9	0.255
10		4.32	3.00	86.5	37.92	1893	54.98	11.8	0.228
11		7.63	0.2	62.2	1613.2	915	30.96	6.3	0.109
12		7.63	1.60	80.2	289.68	1474	62.2	20.7	0.244
13		7.63	3.00	83.8	251.02	1312	67.7	19.9	0.224

retention time for satisfactory digestion. A fraction of TSS which is not digestible is gradually accumulated in the reactor by attaching to it the sludge granules in the UASFF reactor it causes reduction in process efficiency. From a practical point of view and according to various studies [2,10,11,15], the oil-bearing suspended solids need to be removed (partially or completely) before anaerobic treatment in order to have a reliable, stable and efficiently high rate anaerobic process.

3.1. POME Digestion

3.1.1. TCOD Removal The effect of the variables on TCOD removal efficiencies are shown in Figure 1a and b as contour plots for pre-settled and chemically pretreated POME, respectively. Since total suspended solids (TSS) of the pre-settled POME contained 5760 mg/l, a fraction of the OLR is in suspended solids whereas in the chemically pretreated POME, the entire OLR is solubel. In an overall comparison, the trend of changes in TCOD removal efficiency was quite similar for both conditions. The TCOD removal (%) decreased with an increase in Q_F while the rate of TCOD removal (g COD/l.d) was increased (Table 2), due to an increase in the diffusion rate of substrate at higher substrate concentration [26-27].

A stable TCOD removal efficiency of 83.5 % was achieved at the highest Q_F (3.31 l/d, corresponding to OLR of 26 g COD/l.d) for pre-settled POME whereas only 62.2 % TCOD removal was achieved with chemically pretreated POME at Q_F of 7.63 l/d (corresponding to OLR of 29 g COD/l.d) and that also was coupled with process instability. It was found that at the same OLR (center points, V_{up} from o.2 to 3 m/h), despite 33% of OLR in the pre-settled POME being suspended solids, which needs to be hydrolyzed first and greater COD removal efficiency (90-94 %) was achieved compared to chemically pretreated POME the COD removal efficiency was in the range of (82-88 %). This may be attributed to possible inhibitory effects of the polymers which were applied for chemical pretreatment.

3.1.2. Effluent TVFA The VFA concentration is a key indicator of system performance. Figure 2a and b depict the effects of the variables on the effluent VFA for the pre-settled and the chemically pretreated POME, respectively. The highest concentrations of VFA, as intermediates, was

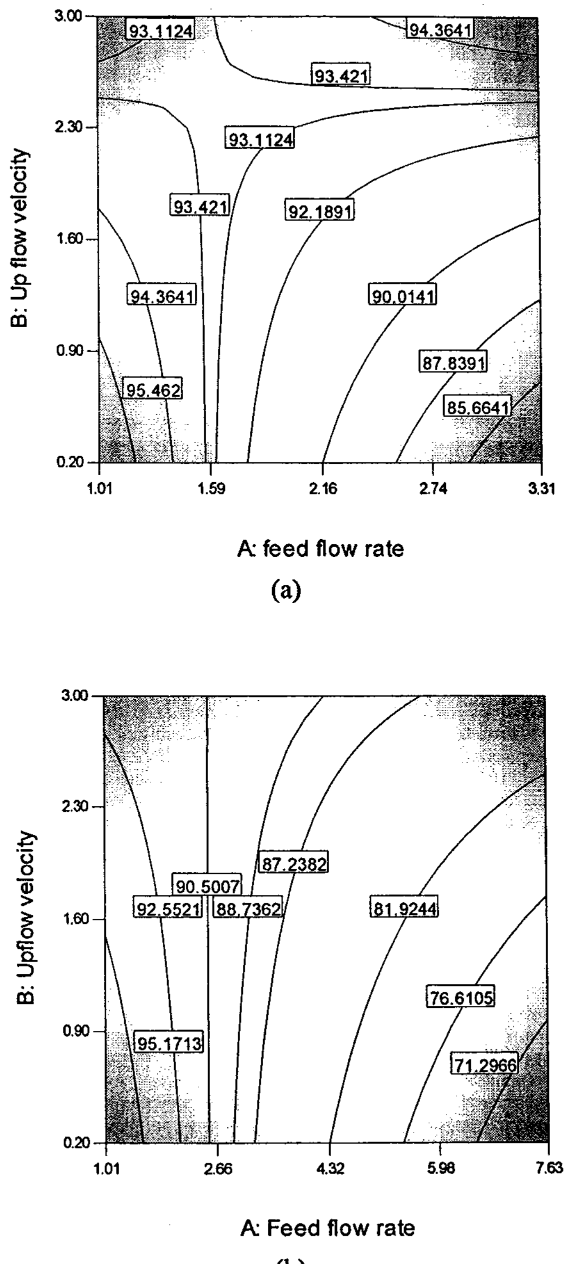
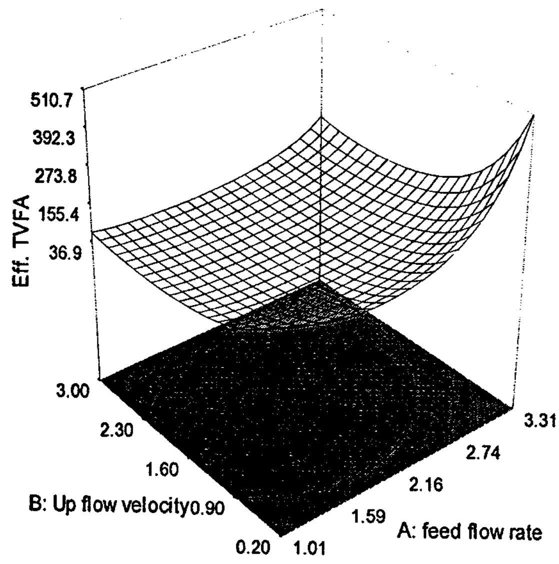
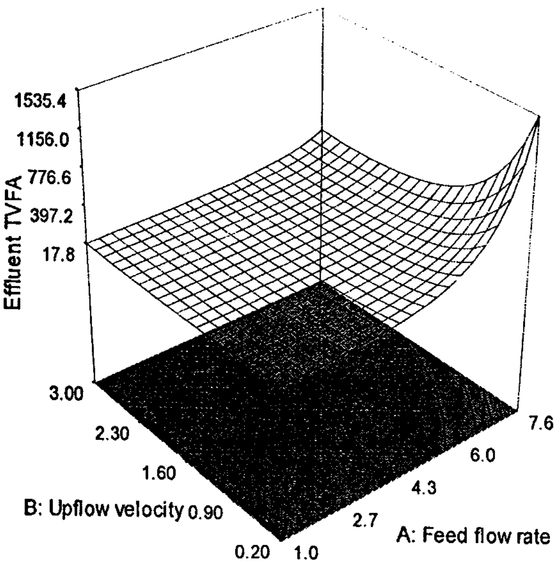


Figure 1. DESIGN-EXPERT plot. Contour plot of TCOD removal efficiency representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME.



(a)



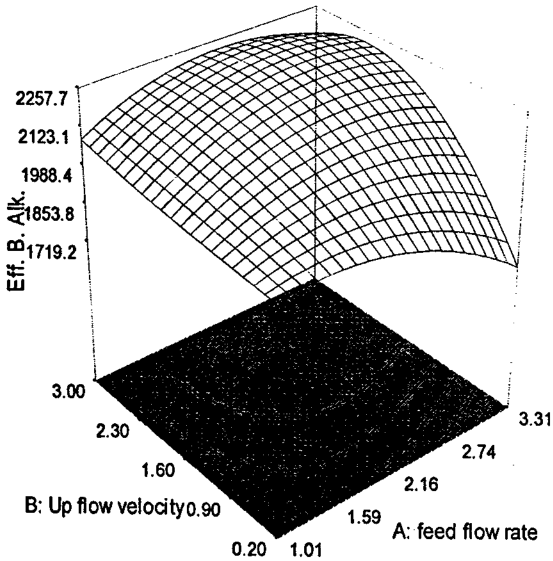
(b)

Figure 2. DESIGN - EXPERT plot. 3D graph of effluent TVFA representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME.

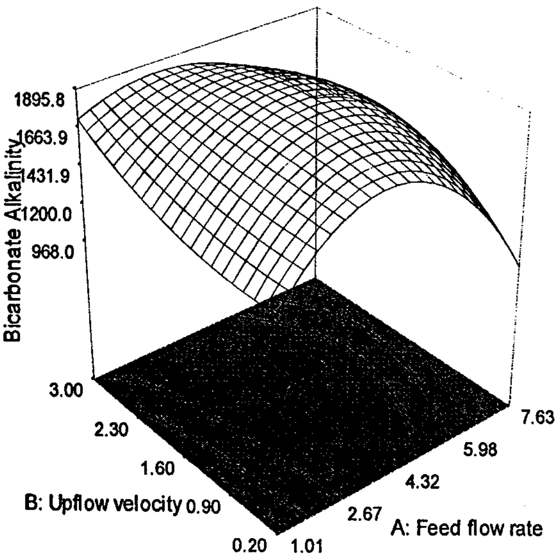
found during overloading conditions when they were 553 mg/l for the pre-settled POME at OLR of 26 g COD/l.d and 1613 mg/l for the chemically pretreated POME at OLR of 29 g COD/l.d. In both experiments, the role of V_{up} in the system recovery at a high Q_F was very significant due to its effects on recycled alkalinity and contact between substrate and biomass [28]. From the 3D graph, at comparable OLRs i.e. 16.95 g COD/l.d (Q_F = 2.16 l/d) for pre-settled POME and 16.42 g COD/l.d (Q_F = 4.32 l/d) for chemically pretreated POME, the VFA concentration for the two cases were also similar. It showed that there was a balance between acetogenesis and methanogenesis in the systems at this OLR. In the chemically pretreated POME digestion, process upset occurred as methanogenic bacteria could not metabolize the VFA as fast as they were produced; resulting in a possible reduction in pH. In the chemically pretreated POME digestion, process upset occurred when methanogenic bacteria could not metabolize the VFA as fast as they were produced; resulting in a possible reduction in pH. This condition (Q_F = 7.63 l/d, V_{up} = 0.2 m/h) had a strong influence on the biogas quality, increasing the CO_2 percentage (75.22 %). Similar behavior was observed in a secondary UASB reactor treating piggery waste at an HRT of 1 day and an influent COD of 10189 mg/l [29].

3.1.3. Effluent BA Figure 3a and b show the interactive effects of Q_F and V_{up} on bicarbonate alkalinity (BA). It was understood that the BA was produced through POME digestion reactions as no chemical was added to cneate alkalinity. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables.

From Figure 3a, the maximum level for BA was predicted to be the region where Q_F and V_F were relatively high (the values larger than center point) while it was obtained from the middle part of the design space in Figure 4b. At the highest OLR and the lowest V_{up} (corresponding to Q_F of 3.31 l/d in Figure 3a and 7.63 l/d in Figure 4b both at V_{up} 0.2 m/h), BA was obtained as 1720 and 960 mg $CaCO_3$/l for the pre-settled and the chemically pretreated POME, respectively. In this condition (V_{up} = 0.2 m/h), the BA concentration was not high enough to avoid process upset for chemically



(a)



(b)

Figure 3. DESIGN - EXPERT plot. 3D graph of effluent BA representing the effect of the feed flow rate and up-flow velocity (a) Pre - settled POME (b) Chemically pretreated POME.

pretreated POME while the process remained stable for pre-settled POME. The effect of V_{up} on system recovery was more significant at a high feed flow rate due to more alkalinity recycling.

3.1.4. Methane Yield Figure 4a and b represent the simultaneous effects of the variables on the methane yield as contour plots. It shows that a simultaneous decrease in the variables yielded an increase in the response for both pre-settled and chemically pretreated POME. It was found that the yield values for the pre-settled POME (Figure 4a) were greater than the values for the chemically pretreated one. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, in that some order where Q_F and V_{up} were 1.01 "l/d" and 0.2 m/h respectively. It was also found that a minimum retention time longer than 1.5 and 2.2 d, respectively, for the pre-settled and chemically pretreated POME digestion was needed to achieve high methane yield.

4. CONCLUSION

Response surface methodology was a potent tool to compare the results obtained from the anaerobic treatment of the two different types of pretreated POME with different characteristics. At an OLR of about 16.5 g COD/l.d (center point), despite 33% of OLR in the pre-settled POME being suspended solids, greater COD removal efficiency was achieved compared to chemically pretreated POME. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, in that some order where Q_F and V_{up} were 1.01"l/d" and 0.2 m/h respectively.

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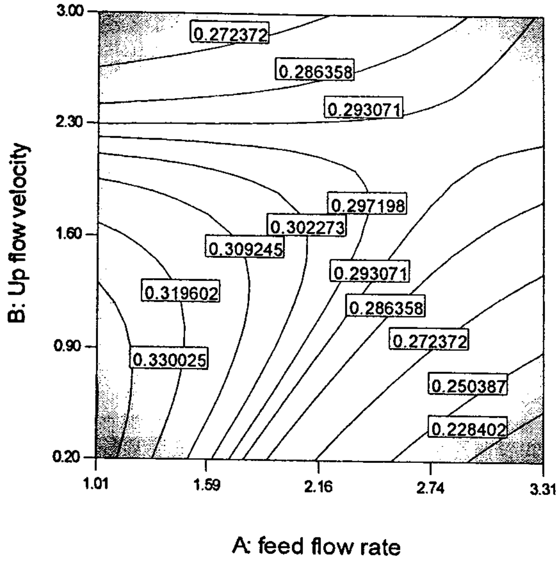
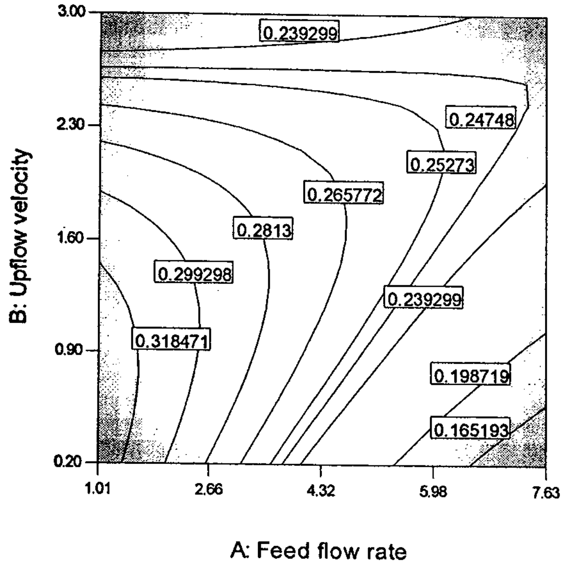


(a)



(b)

Figure 4. DESIGN-EXPERT plot. Contour plot of methane yield representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME.

the industry personnel for their full cooperation.

6. ABBREVIATION AND NOMENCLATURE

ABR	Anaerobic bafled rector
AHR	Anaerobic hybrid rector
ANOVA	Analysis of variance
BA	Bicarbonate alkalinity
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CCFD	Centerl composite face-centered design
FFB	Fresh fruit bunch
FID	Flame inoiztion detector
HRT	Hydraulic retention time
MAS	Memberane anaerobic system
OLR	Organic loading rete
POME	Palm oil mill effluent
Q_F	Feed flow rete
RSM	Response surface method
SS	Supended solids
TCD	Thermal conductivity detector
TCOD	Total chemical oxygen demand
TKN	Total kjeldahl nitrogen
TSS	Total suspended solids
TVFA	Total volatile fatty asid
UASB	Up-flow anaerobic sludge blanket
UASFF	Up-flow anaerobic sludge fixed film
VFA	Volatile fatty asid
V_{up}	Up-flow velocity
Y_M	Yield of methane production

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EFFECTS OF ORGANIC LOADING RATE ON PALM OIL MILL EFFLUENT TREATMENT IN AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM BIOREACTOR

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Abstract

The effect of OLR provided by hydraulic retention time (HRT) and influent chemical oxygen demand (COD_{in}) on the performance of an up-flow anaerobic sludge fixed film (UASFF) bioreactor treating palm oil mill effluent (POME) was studied. Anaerobic digestion of POME was modeled and analyzed with two variables i.e. HRT and COD_{in}. Experiments were conducted based on a general factorial design and analyzed using response surface methodology (RSM). The region of exploration for digestion of POME was taken as the area enclosed by HRT (1 to 6 days) and COD_{in} (5260 to 34725 mg/L) boundaries. Eight dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, TCOD removal rate, volatile fatty acids to alkalinity ratio (VFA/Alk.), CO_2 fraction in biogas, solid retention time (SRT), sludge retention factor (SRF), methane production rate per volume of the reactor and feed. A simultaneous increase of the variables determined a decrease of COD removal efficiency, SRT and SRF and an increase of COD removal rate, VFA/Alk., CO_2 fraction in biogas, methane production rate. The present study provides valuable information about interrelations of quality and process parameters at different values of the operating variables.

Keywords: anaerobic digestion, UASFF reactor, POME, HRT, COD_{in}, response surface methodology (RSM)

1. Introduction

Palm oil mill effluent (POME) is a mixture of wastewaters generated from different stages of production process, sterilizer condensates, hydro-cyclone waste, sludge separator and plant washing. It is hot (80-90 °C), acidic (pH 3.8-4.5) and contains very high concentration of organic matter (COD= 40000-

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50000 mg/L, BOD= 20000-25000 mg/L) (Najafpour et al., 2006). The palm mills in Malaysia faced the challenge of balancing environmental protection, their economic viability, and sustainable development after the department of Environment enforced the regulation for the discharge of effluent from the CPO industry, under the Environmental Quality (prescribed premises) (Crude Palm Oil) order and regulations, 1997. There is an urgent need to find a way to preserve the environment which maintaining the economy in good conditions (Department of Environment Malaysia, 1999).

Biological treatment processes, in an effort to minimize cost, utilize microbial communities of varying degrees of diversity that interact in a multitude of ways to mediate a myriad of biological reactions, (Wise, 1987). Anaerobic digestion has been widely accepted as an interesting alternative for wastewater treatment and simultaneous fuel gas production. Its successful and economic employment arises from the development of new reactor designs (Jans and Man, 1988). It presents a number of significant advantages if compared with conventional aerobic wastewater depollution systems (Droste, 1997; Metcalf & Eddy, 2003).

Due to the inherently lower efficiencies and loading rates of anaerobic filter and the occasional instability of the UASB, a hybrid of these two systems are being proposed an unpacked lower section and a packed upper section, (Speece, 1988). Employing two technological approaches in up-flow anaerobic sludge fixed film (UASFF) reactor, microbial granules development and biofilm attachment on an inert media, allow independency of sludge retention of the hydraulic retention time (HRT), (Najafpour et al., 2006).

Owing to high anaerobic digestibility of POME, a wide range of anaerobic approaches have been examined. Among them, UASFF as a hybrid reactor has been the most efficient process due to its high ability to retain biomass even under overload (Najafpour et al., 2006). Effects of organic loading rate on the performance of the different biological reactors have been investigated for POME treatment, (Ma and Ong, 1988; Borja and Banks, 1994a; Borja and Banks, 1994b; Borja et al., 1996; Fakhrul-Razi and Noor, 1999; Faisal and Unno, 2001; Norulaini et al., 2001; Najafpor et al., 2005; Najafpour et al., 2006; Zinatizadeh et al., 2006). The studies have been carried out with the aim of process investigations in different anaerobic systems for POME treatment and changes in effluent quality parameters were discussed in a certain operating condition.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response, (Box and Draper, 1987). RSM has an important application in the process design and optimization as well as the improvement of existing design. This methodology is more practical compared to the approaches mentioned above as it arises from experimental methodology which include interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process, (Baş and Boyaci, 2006).

In the present study, in addition of process analysis, a general factorial design was employed to describe and model variation trend in eight important responses (TCOD removal and removal rate, Volatile fatty acids to alkalinity ratio, CO_2 fraction in biogas, solid retention time (SRT), sludge retention factor (SRF), methane production rate per volume of the reactor and feed) as function of two independent variables, HRT and influent COD concentration.

2. Materials and methods

2.1. Bioreactor and start up

A laboratory-scale, UASFF reactor was used in this study, (Najafpour et al., 2006). The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml (including the section containing the gas-solid separator), and the working volume (total liquid volume excluding volume of the pall rings in fixed bed section) was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25 % and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere, (Najafpour et al., 2006).

2.2. Palm oil mill effluent (POME)

The bioreactor was fed with POME pre-settled for 1 hr. The characteristics of POME are summarized in Table 1. POME samples were collected weekly from a near-by palm oil mill. The wastewater was stored in a cold room at 4 °C before use. Different dilutions of POME were prepared using tap water. The pH of the feed was adjusted to 7.0, using a 6 N NaOH solution. Supplementary nutrients, nitrogen and phosphorous, were added to adjust the COD:N:P ratio to 250:5:1. $NaHCO_3$ was added to maintain an influent alkalinity of 1500-1700 mg $CaCO_3$/L.

Table 1. Characteristics of palm oil mill effluent (POME)

Parameter	*Amount*
BOD_5 (mg/L)	23000-26000
COD (mg/L)	42500-55700
Soluble COD (mg/L)	22000-24000
TVFAs (mg actic acid/L)	2500-2700
SS (mg/L)	16500-19500
Oil and grease (mg/L)	4900-5700
Total N (mg/L)	500-700
pH	3.8-4.4

2.3. Experimental design

In order to describe the interactive effects of HRT and COD_{in} on the responses, 36 continuous experiments were conducted as HRT varied from 1 to 6 days at 6 levels (1, 1.5, 3, 4, 5 and 6), and as influent COD concentration varied from 5260 to 34725 mg/L at 6 levels (5260, 10575, 14485, 21310, 26210, 34725 mg/L). HRT and influent COD concentration were chosen as two independent factors in the experiment design. TCOD removal and TCOD removal rate, Volatile fatty acids to alkalinity ratio, CO_2 fraction in biogas, solid retention time (SRT), sludge retention factor (SRF), methane production rate per volume of the reactor and feed were dependent output responses.

The experiment design is shown in Table 2. Regression analysis was performed to estimate the response function expressed by Eq. 1.

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \ldots \qquad (1)$$

where, i and j are the linear and quadratic coefficients, respectively, β is the regression coefficient. Model terms were selected or rejected based on the P value with 95% confidence level. The results were completely analyzed using analysis of variance (ANOVA) by Design Expert software. Three dimensional plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied.

2.4. Analytical methods

The following parameters were analyzed according to standard methods, (APHA, 1999): pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively, (Najafpour et al., 2006).

The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a Pall ring at 80 °C for 24 h before and after biofilm attachment.

3. Results and discussion

After a short and successful start up period, the reactor was operated at different HRTs and influent COD concentrations. In this study, the up-flow velocity was maintained relatively constant at 0.44±0.02 m/h while the influent COD concentration was increased from 5260 to 34725 mg/L (at six levels). The HRT was stepwise decreased from 6 to 1 day for each influent COD concentration, So that, the UASFF bioreactor was subjected to 36 different conditions. High performance of the UASFF reactor at steady state conditions with HRT and influent COD concentrations in the range under studied was successfully demonstrated.

In order to analyze and model the interactive effects of the two variables (COD_{in} and HRT) on the responses, Design-Expert software (Version 6) was used. In this program, general factorial design was selected. It allows the user to have factors that each has a different number of levels. The responses from the resulting 36 runs are shown in Table 2.

3.1. Statistical analysis

As various responses were investigated in this study, different degree polynomial models were used for data fitting. In order to quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations i.e. two factor interaction (2FI), quadratic and so on. In the Design Expert software, the response data were analyzed by default. Some raw data might not be fitted and transformations which apply a mathematical function to all the response data might be needed to meet the assumptions that made the ANOVA valid. Data transformations were needed for the TVFA and SRT responses as errors (residuals) were a function of the magnitude of the response (predicted values). Therefore, $\log_{10}$ function was applied for these responses, (Ahmad et al., 2005; Chapra and Canale, 2003; Draper and Smith, 1998). The interaction term, i.e. AB, was significant for all equations except the one defining TCOD removal rate. Based on the statistical analysis, the models were highly significant with very low probability values (from 0.0759 to < 0.0001).

It was shown that the model terms of independent variables were significant at the 95 % confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). It showed high significant regression at 95 % confidence level. Adequate precision is a measure of the range in predicted response relative to its associated error or, in other words, a signal to noise ratio. Its desired value is 4 or more, (Mason et al., 2003). The value was found desirable for all models. Simultaneously, low values of the coefficient of variation (CV) (1.54-9.55 %) indicated good precision and reliability of the experiments as suggested by Ahmad et al., 2005; Khuri and Cornell, 1996; Kuehl, 2000. A detailed analysis on the models is presented in the following sections.

Table 2. Experimental conditions and results of general factorial design

Run	***Variable***		***Response***							
	Factor1 A:HRT (day)	Factor 2 B:COD_{in} (mg/L)	TCOD removal (%)	TCOD removal rate (g/l.d)	VFA/Alk. (eq. acetic acid/eq. $CaCO_3$)	CO_2 fraction in biogas (%)	SRT (day)	Retention factor	Methane production rate (l CH_4/ lreactor.d)	Methane production rate (l CH_4/ lfeed.d)
1	1	5260	90.97	4.8	0.023	20.8	119	119	1.7	1.7
2	1.5	5260	93.16	3.3	0.014	17.7	204	136	1.1	1.7
3	3	5260	95.63	1.7	0.01	16.0	714	238	0.5	1.6
4	4	5260	96.77	1.3	0.011	15.4	1269	317	0.4	1.5
5	5	5260	96.96	1.0	0.008	14.9	1587	317	0.3	1.5
6	6	5260	97.24	0.9	0.008	14.7	1904	317	0.3	1.6
7	1	10575	89.60	9.5	0.025	26.5	39	39	3.2	3.2
8	1.5	10575	94.91	6.7	0.015	24.1	101	67	2.2	3.3
9	3	10575	96.35	3.4	0.01	17.9	363	121	1.1	3.4
10	4	10575	97.07	2.6	0.01	17.0	807	202	0.9	3.5
11	5	10575	97.64	2.1	0.009	16.1	1210	242	0.7	3.3
12	6	10575	97.68	1.7	0.007	15.9	1452	242	0.6	3.3
13	1	14485	87.40	12.7	0.028	30.2	26.5	27	4.3	4.3
14	1.5	14485	92.23	8.9	0.018	27.4	88	59	3.1	4.6
15	3	14485	97.10	4.7	0.005	19.9	363	121	1.6	4.8
16	4	14485	97.86	3.5	0.007	18.5	797	199	1.2	4.9
17	5	14485	98.00	2.8	0.007	15.1	1412	282	0.9	4.7
18	6	14485	98.14	2.4	0.007	17.9	1694	282	0.8	4.8
19	1	21310	86.72	18.5	0.044	35.9	22.7	23	5.6	5.6
20	1.5	21310	91.04	12.9	0.026	31.0	65	43	4.5	6.7
21	3	21310	97.51	6.9	0.006	22.2	231	77	2.3	6.9
22	4	21310	98.15	5.2	0.007	20.2	544	136	1.7	6.9
23	5	21310	98.33	4.2	0.006	19.0	1179	236	1.4	6.9
24	6	21310	98.40	3.5	0.006	17.7	1516	253	1.2	6.9
25	1	26210	84.43	22.1	0.103	42.1	19	19	6.6	6.6
26	1.5	26210	90.18	15.8	0.032	35.8	39.5	26	5.4	8.1
27	3	26210	97.81	8.5	0.007	25.8	237	79	2.9	8.8
28	4	26210	98.19	6.4	0.008	22.2	383	96	2.2	8.8
29	5	26210	98.44	5.2	0.008	20.4	909	182	1.7	8.6
30	6	26210	98.59	4.3	0.007	20.0	991	165	1.5	9.1
31	1	34725	80.56	28.0	0.288	48.7	16	16	8.0	8.0
32	1.5	34725	89.58	20.7	0.045	38.0	38.7	26	7.2	10.8
33	3	34725	97.90	11.3	0.008	28.1	217	72	4.0	11.9
34	4	34725	98.39	8.5	0.007	25.1	334	84	2.9	11.5
35	5	34725	98.57	6.8	0.007	22.8	672	134	2.3	11.5
36	6	34725	98.62	5.7	0.006	23.2	806	134	2.1	12.3

3.2. Total COD

Two reduced quadratic models were selected to describe the response surface of TCOD removal and TCOD removal rate within the range of the factors. The regression equations (built with codified factors) are as expressed by Eqs. 2, 3:

$$TCOD\ removal = 94.72+9.04A-1.61B-4.94A^2+2.57AB \quad (2)$$

$$Log_{10}\ (TCOD\ removal\ rate) = 0.92-0.45A+0.39B+0.13A^2-0.18B^2 \quad (3)$$

Fig. 1 shows simultaneous effects of the two factors on TCOD removal efficiency obtained from Eq. (2). It showed a significant decreasing trend in TCOD removal efficiency with the decrease in HRT at a constant influent COD. Whereas slight decrease in TCOD removal efficiency was observed when influent COD was increased under a constant HRT and lower than 4 days. The lowest efficiency in TCOD removal was predicted to be 84.4 % at the highest OLR (corresponds to HRT of 1 day and COD_{in} of 34,725 mg/L) while the experimental value has been 80.6 %. In this condition, the reactor instability was temporarily observed after 4 days at OLR of 34.7 g COD/L.d. But the buffer supplied by adding $NaHCO_3$ in the feed and recycling effluent alkalinity into influent prevented pH changes a lot and pH was controlled at 6.92. The main reason for relatively poor efficiency at OLR of 34.7 g COD/L.d was to accumulate suspended solids in sludge blanket due to suspended solids overload in this condition. Consequently, it caused a sludge washout followed by decreasing in COD removal from 90 to 80.4 %. It is important to be pointed out that after sludge washout the reactor efficiency remained constant for 5 turnovers.

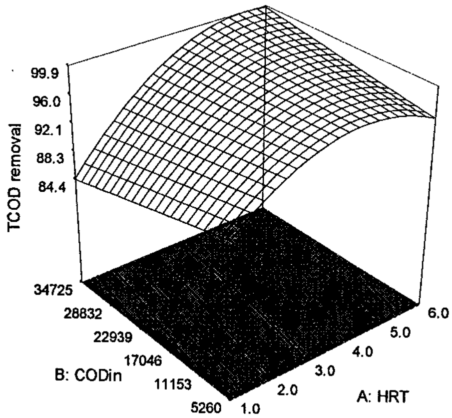

Fig. 1. Response surface plot for TCOD removal

The regression was conducted after transformation of raw data to a function of log base 10. Fig. 2 illustrates the effect of the factors on the TCOD removal rate in original scale. As can be seen, the TCOD removal rate increased with the increase in influent COD and decrease in HRT. The best COD removal rate for POME treatment in an anaerobic hybrid reactor has obtained at an OLR of 17.6 g COD/L.d (Borja, et al., 1996) while it was at 26.21 g COD/L.d (Corresponds to COD_{in} of 26210 mg COD/L and HRT of 1 day) in the present study. It might be attributed to larger amount of biomass in the form of granule due to bigger volume of UASB portion relative to total volume in a hybrid reactor.

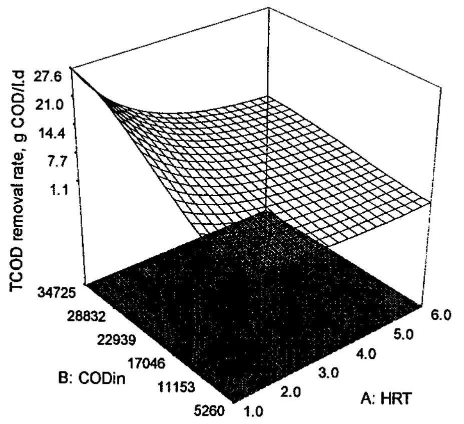

Fig. 2. Response surface plot for TCOD removal rate

3.3. VFA to Alk. ratio and CO_2 fraction

The ratio of VFA/Alk. is an important indicator of the acid-base equilibrium and process stability, (Fannin, 1987; Sanchez et al., 2005). When this ratio is less than 0.3-0.4, the process is considered to be operating favorably without the risk of acidification. The measurement of quantity and composition of the biogas produced, in terms of methane and carbon dioxide content, is of fundamental importance to evaluate the stability of the process, (Fannin 1987). When the process is stable the amount and composition of biogas are stable too. A decrease in biogas production contemporary to an increase in CO_2 content can indicate an inhibition of the methanogenesis of the system. In fact, VFA/Alk. ratio and biogas composition are strictly linked one to each other.

The following two regression equations were obtained for the variation of VFA to alkalinity ratio (p) and CO_2 fraction in biogas.

$$\log_{10}(\text{VFA/Alk.}) = -1.95-0.73A+0.077B+0.42A^2+0.11B^2-0.52AB+0.33A^2B \quad (4)$$

$$CO_2 \text{ fraction in biogas} = 24.96-12.25A+8.94B+5.54A^2-4.68AB \quad (5)$$

The ratio of maximum to minimum for p was 55. Hence a logarithmic function with base 10 was required to fit the data. Fig. 3 illustrates the effects of the variables on VFA/Alk. From the Fig.3, the maximum value of the ratio is predicted to be 0.18 at highest OLR (corresponds to HRT of 1 and COD_{in} of 34725 mg/L) whereas the actual value was 0.28 at this condition. The VFA/Alk. ratio remained lower than the suggested value throughout experiments.

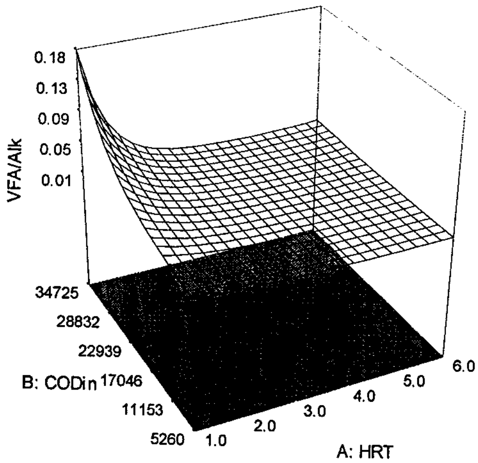

Fig. 3. Response surface plot for VFA/Alk ratio

Fig. 4 shows the variation of the CO_2 fraction in the biogas as a function of the two factors studied. The CO_2 fraction increased with simultaneous decrease and increase in HRT and COD_{in}, respectively.

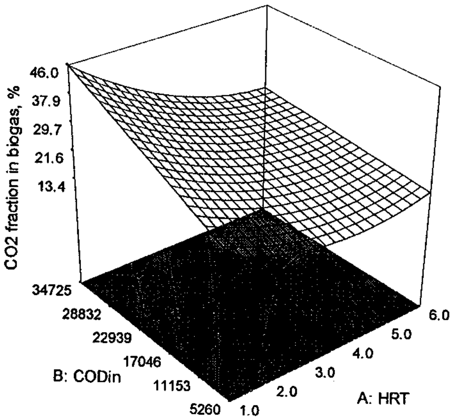

Fig. 4. Response surface plot for CO_2 fraction in biogas

The maximum CO_2 fraction in biogas was modeled to be 46.05 % whereas the actual is 48.7 %. It was also reflected by an increase in the effluent VFA concentration (from 158 to 843.2 mg acetic acid/l) and decrease in the effluent pH (from 7.63 to 6.92). This resulted from partial upsetting of the balance between acid formation and methane production in the anaerobic process due to high organic load at low HRT. The effluent pH remained within the optimal working range for anaerobic digestion (6.9 -7.9) throughout the experiment.

3.4. SRT and sludge retention factor

SRT, as a process control parameter, was also determined by measuring VSS in the reactor and in the effluent at various concentrations of influent COD. The high SRT values denote effective role of the packed bed portion on process stability due to biomass retention which allows the system to cope with changes in OLR. Minimum and maximum SRT values obtained were 16 and 1904 days at OLR of 34.73 and 0.88 g COD/l.d, respectively. It was found that at the shortest HRT (1 day), the sludge age to HRT ratio was 16 which is in the range of safety factor (3-20) for the minimum SRT for successful operation of anaerobic biological reactors, (Lawrence and McCarty, 1969).

HRT as an operating factor affecting SRT by following relationship:

$$SRT = \frac{X_r . HRT}{X_e} \tag{6}$$

where X_r, is the concentration of sludge in the reactor (g VSS/l); X_e, is the concentration of VSS in the effluent of the reactor (g VSS/l). The SRT to HRT ratio is defined the sludge retention factor (SRF). The increase in retention factor involves increasing in HRT. Large values of retention factor providing longer SRT which favoring methanogenesis and improving process performance. The best option to achieve high SRT while maintaining HRT at low levels is biomass immobilization which is applied in the UASFF reactor in the form of granular sludge and biofilm attached on the packing. In order to model interactive effects of the variables (HRT and COD_{in}) on the process control responses, SRT and SRF, two following quadratic models were obtained.

$$Log_{10}(SRT) = 2.24+1.09A-0.31B-0.35A^2+0.098B^2+0.1AB \tag{7}$$

$$Log_{10}(SRF) = 1.85+0.59A-0.31B-0.2A^2+0.098B^2+0.1AB \tag{8}$$

Since maximum to minimum ratio for SRT and SRF were 119 and 19.8, respectively, a logarithmic function with base 10 was applied to fit the data. The same variation trend for SRT and SRF was observed as shown in Fig. 5 and 6, indicating inverse proportion between OLR and the responses. In this study, the up-flow velocity was maintained constant (0.44 m/h) (controlled by recycle ratio), therefore, increase in the concentration of VSS in the effluent was attributed to an increase in gas production rate due to increase in HRT and COD_{in}.

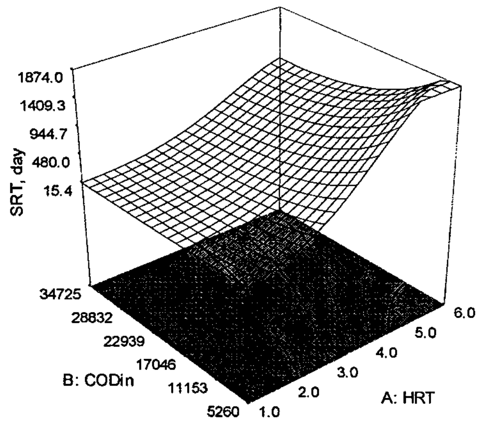

Fig. 5. Response surface plot for SRT

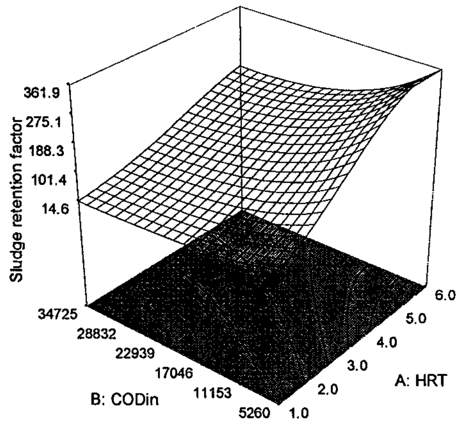

Fig. 6. Response surface plot for SRF

3.5. Methane production rate

The methane production is a function of OLR (changing HRT or/and COD_{in}). The two following regression equations were selected to describe changes in methane production rate as function of the variables.

Methane production rate per unit of reactor volume $= 2.9-2.88A+2.29B+1.19A^2-1.24AB$ (9)

Methane production rate per unit of feed $= 6.31+0.96A+4.39B-0.52A^2+0.8AB$ (10)

Fig. 7 depicts effect of the variables on the methane production rate per volume of the reactor, showing that a simultaneous increase in the variables (HRT and COD_{in}) caused an increase in methane production rate due to the increase in OLR. It was good indication of a compacted and porous sludge blanket in unit of reactor volume. A slight reduction of the methane amount produced per volume of input feed when HRT decreased at a high and fixed COD_{in}, shown in Fig. 8. It might be due to limitation of feed and product mass transport in the granules as the concentration of particulate COD is more at higher COD. Also, it may be attributed to development of non-methanogens at high OLR. However, it showed an increasing trend in the methane production when COD_{in} increased at a fixed HRT, indicating reaction rate increases with an increase in influent COD owing to high biodegradability of the substrate and sufficient microbial community.

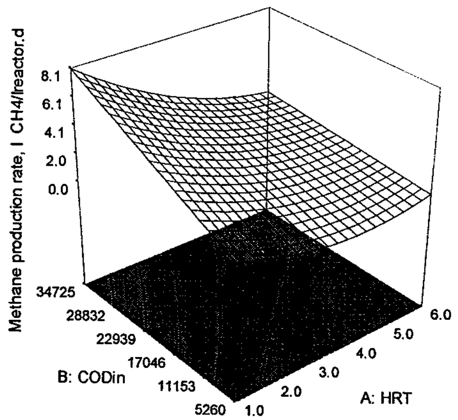

Fig. 7. Response surface plot for methane production rate per unit reactor volume

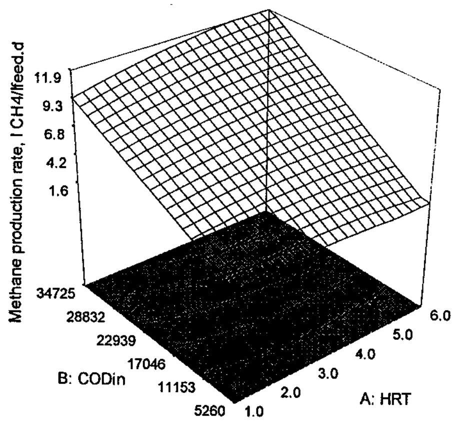

Fig. 8. Response surface plot for methane production rate per unit feed flow rate

4. Conclusions

The UASFF bioreactor was a successful biological treatment process to achieve a high COD removal efficiency in a short period of time. The response surface methodology results demonstrated the effects of the operating variables as well as their interactive effects on the responses. A simultaneous increase of the variables determined a decrease of COD removal efficiency, SRT and SRF and an increase of COD removal rate, VFA/Alk., CO_2 fraction in biogas, methane production rate. Experimental findings were in close agreement with the model prediction.

Acknowledgements

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6

Characteristics of Granular Sludge Developed in an Upflow Anaerobic Sludge Fixed-Film Bioreactor Treating Palm Oil Mill Effluent

A. A. L. Zinatizadeh[1], A. R. Mohamed[2*], M. D. Mashitah[2], A. Z. Abdullah[2], M. Hasnain Isa[3]

ABSTRACT: In the present study, characteristics of the granular sludge (including physical characteristics under stable conditions and process shocks arising from suspended solid overload, soluble organic overload, and high temperature; biological activity; and sludge kinetic evaluation in a batch experiment) developed in an upflow anaerobic sludge blanket fixed-film reactor for palm oil mill effluent (POME) treatment was investigated. The main aim of this work was to provide suitable understanding of POME anaerobic digestion using such a granular sludge reactor, particularly with respect to granule structure at various operating conditions. The morphological changes in granular sludge resulting from various operational conditions was studied using scanning electron microscopy and transmission electron microscopy images. It was shown that the developed granules consisted of densely packed rod- (Methanosaeta-like microorganism; predominant) and cocci- (Methanosarsina) shaped microorganisms. Methanosaeta aggregates functioned as nucleation centers that initiated granule development of POME-degrading granules. Under the suspended solid overload condition, most of the granules were covered with a thin layer of fiberlike suspended solids, so that the granule color changed to brown and the sludge volume index also increased to 24.5 from 12 to 15 mL/g, which caused a large amount of sludge washout. Some of the granules were disintegrated because of an acidified environment, which originated from acidogenesis of high influent organic load (29 g chemical oxygen demand [COD]/L·d). At 60°C, the rate of biomass washout increased, as a result of disintegration of the outer layer of the granules. In the biological activity test, approximately 95% COD removal was achieved within 72 hours, with an initial COD removal rate of 3.5 g COD/L·d. During POME digestion, 275 mg calcium carbonate/L bicarbonate alkalinity was produced per 1000 mg $COD_{removed}$/L. A consecutive reaction kinetic model was used to simulate the data obtained from the sludge activity in the batch experiment. The mathematical model gave a good fit with the experimental results ($R^2 > 0.93$). The slowest step was modeled to be the acidification step, with a rate constant between 0.015 and 0.083 hours^{-1}, while the rate constant for the methanogenic step was obtained to be between 0.218 and 0.361 hours^{-1}. *Water Environ. Res.*, **79** (2007).

KEYWORDS: anaerobic granular sludge, upflow anaerobic sludge fixed-film reactor, palm oil mill effluent, kinetic model.

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Introduction

Compared with conventional aerobic methods of wastewater treatment and in light of the much desirable development and implementation of sustainable methods and technologies, the anaerobic wastewater treatment concept indeed offers fundamental benefits, such as low costs, energy production, relatively small space requirement of modern anaerobic wastewater treatment systems, very low sludge production with very high dewaterability, no need to stabilize excess sludge, and high tolerance to unfed conditions (Lettinga, 1995; Metcalf & Eddy, 2003).

Previously, perceived drawbacks of anaerobic treatment systems, such as high susceptibility of microbes (in particular, methanogens) to a variety of xenobiotic compounds, low stability of the process, and long startup period, could be attributed to lack of knowledge of the basic principles of the process. The anaerobic digestion process is highly stable, provided the system is operated in the proper way. It may be necessary that optimum operational conditions be determined for the particular wastewater and, more importantly, that the process be sufficiently understood by engineers and operators.

The development of effective and simple methods for treatment of industrial wastes is a challenge to environmental engineers and scientists. In developing and tropical countries, such as Malaysia, anaerobic digestion of high-strength wastewaters, such as palm oil mill effluent (POME), becomes particularly attractive. The upflow anaerobic sludge blanket (UASB) reactor (Lettinga et al., 1980, 1984), as a high-rate anaerobic reactor, is considered desirable for high-strength organic wastewater treatment because of its high biomass retention ability and rich microbial diversity (Fang et al., 1995; Schmidt and Ahring, 1996; Wu et al., 2001).

Anaerobic treatment of POME in a single UASB and two-stage UASB reactors was investigated (Borja and Banks, 1994; Borja et al., 1996b). A 96% COD removal was reported at an organic loading rate (OLR) of 10.6 g COD/L·d in the single UASB reactor, while, at the highest influent COD concentration (42500 mg/L), reactor instability was observed, as a result of sludge washout. Meanwhile, a 90% reduction in COD at an OLR of 15 g COD/L·d was achieved in the two-stage UASB reactor.

Interest in anaerobic hybrid technology (combination of different anaerobic systems into a single bioreactor) has grown in recent years, as it couples the recovery of usable energy with good process efficiency and stability. The upflow anaerobic sludge fixed-film (UASFF) bioreactor, as an anaerobic hybrid reactor, is a combination of a UASB reactor and an immobilized cell or fixed-film reactor

Table 1—Characteristics of POME.*

Parameter	Raw POME	Presettled POME	Chemically pretreated POME
BOD_5 (mg/L)	22 700	20 100	9750
COD (mg/L)	44 300	28 640	13 880
Soluble COD (mg/L)	17 140	17 140	13 880
Total VFA (mg acetic acid/L)	2510	2510	2760
TSS (mg/L)	19 780	5760	<20
Oil and grease (mg/L)	4850	1630	Negligible
TKN (mg/L)	780	660	480
pH	4.05	4.05	4.2

* Values are average of three measurements. Differences between the measurements for each were less than 1%.

(Metcalf & Eddy, 2003). The fixed-film portion positioned above the UASB section prevents sludge washout and helps in retaining a high biomass concentration in the reactor.

Applicability of the UASFF reactor in the treatment of POME has been studied at laboratory-scale by several researches (Borja et al., 1996a; Najafpour et al., 2006; Zinatizadeh et al., 2006). The results show the ability of the reactor to treat POME at a low hydraulic retention time (HRT) and high OLR. The UASFF reactor was found to be more efficient and stable than ordinary UASB and other high-rate anaerobic systems reported in literature (Fakhrul-Razi and Noor, 1999; Setiadi et al., 1996). This prompted the need for more studies to provide suitable understanding of the POME anaerobic digestion process using such a granular sludge reactor, particularly with respect to granule characteristics under various operating conditions.

This study provides (1) illustration of structural and physical properties of the granular sludge developed in the UASFF reactor, under different operational regimes of POME treatment; (2) examination of biological activity of the granular sludge in batch experiments; and (3) kinetic evaluation of the biochemical reactions using a consecutive reaction model.

Materials and Methods

Feed for Upflow Anaerobic Sludge Fixed-Film Bioreactor. Raw POME was collected from a local palm oil mill in Nibong Tebal, Penang, Malaysia. The bioreactor was fed with POME presettled for 2 hours and POME chemically pretreated with a combination of a cationic and an anionic polymer to remove suspended solids and oil and grease. The characteristics of the presettled and chemically pretreated POME are shown in Table 1. The samples were stored in a cold room at 4°C before feeding to the reactor. This storage technique had no observable effect on their composition.

Bioreactor. A laboratory-scale UASFF bioreactor was used in this study (Najafpour et al., 2006). The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. The total volume of the reactor was 4980 mL, and the working volume was 3650 mL. The column consisted of three sections—bottom, middle, and top. The bottom part of the column, with a height of 80 cm, was operated as a UASB reactor; the middle part of the column ,with a height of 25 cm, was operated as a fixed-film reactor; and the top part of the bioreactor served as a gas–solid separator. The middle section of the column was packed with 90 pall rings, with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25%, and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel-shaped gas separator was used to conduct the biogas to a gas collection tank. Effluent was recycled to obtain various upflow velocities.

Bioreactor Operation. During operation of the UASFF bioreactor, various operational conditions were examined, including influent COD dilutions (5260 to 44 300 mg/L), HRT (1 to 6 days), upflow velocity (0.2 to 3.0 m/h), and temperature (25 to 60°C). The effect of physical and chemical pretreatment was also studied. Physical properties of the granular sludge were continuously monitored throughout the experiment. Details regarding the startup

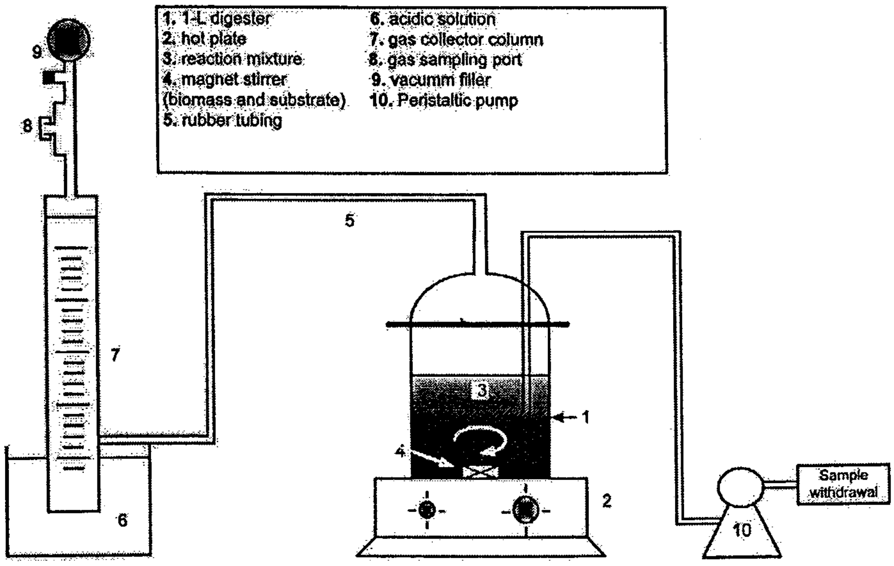
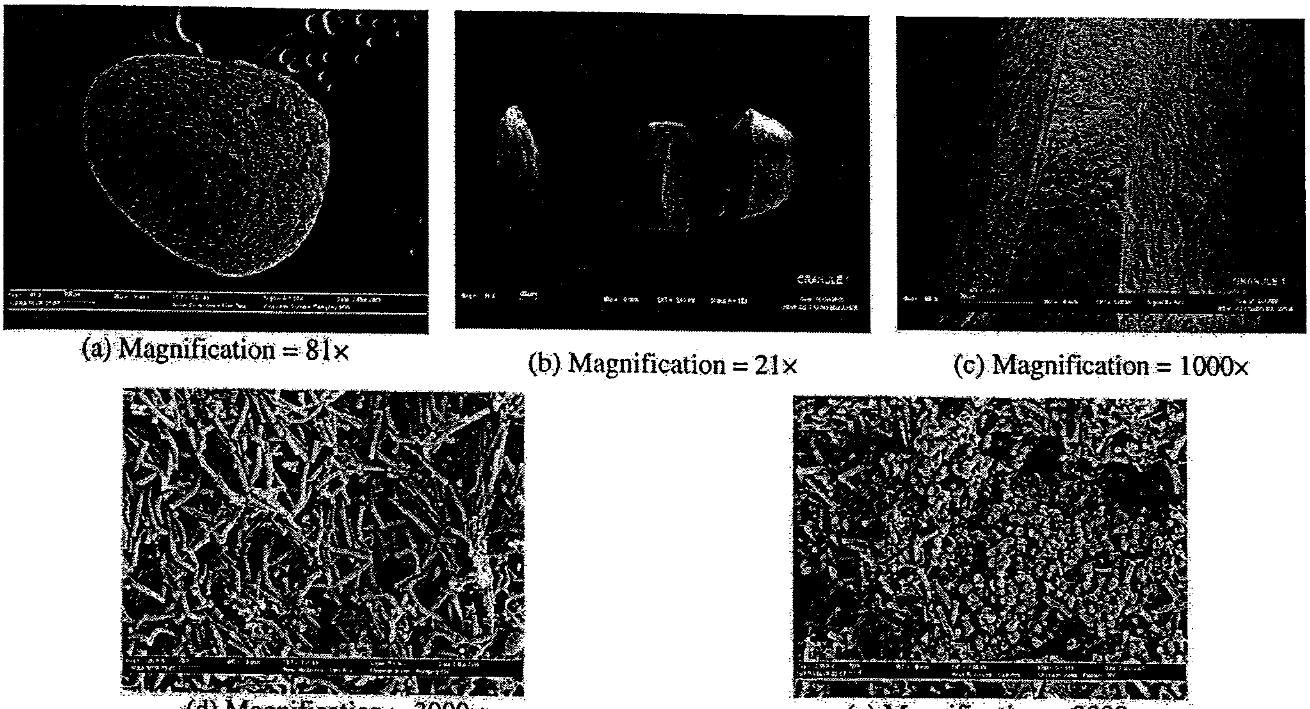


Figure 1—Batch experimental setup for methanogenic activity determination.



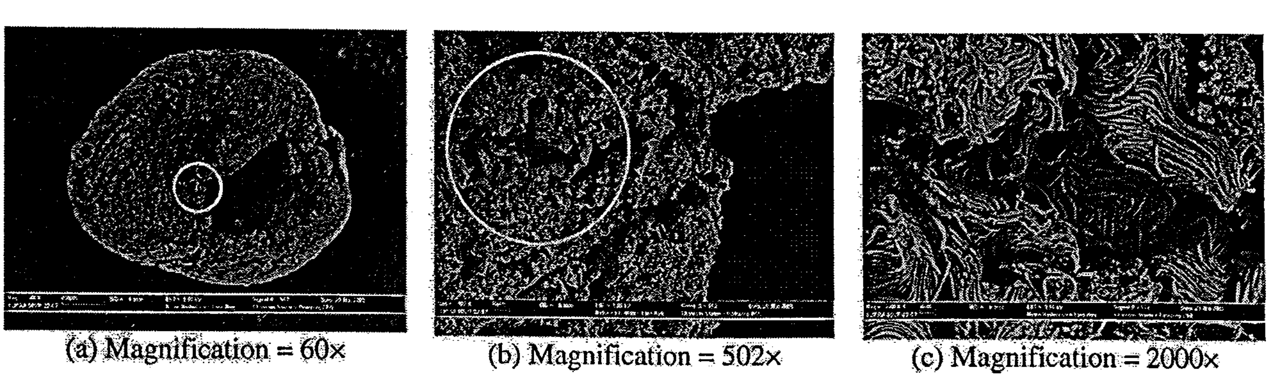
(a) Magnification = 81×
(b) Magnification = 21×
(c) Magnification = 1000×
(d) Magnification = 3000×
(e) Magnification = 3000×

Figure 2—SEM of (a, b, c) a full-grown and sectioned granule, (d) aggregate of Methanosaeta in the core of the granule, and (e) aggregate of Methanosarsina in the middle layer of the granule.

procedure and reactor performance are discussed elsewhere (Najafpour et al., 2006).

Biological Activity Test of the Granular Sludge. A simple methanogenic activity test procedure proposed by Isa et al. (1993) was adopted, with suitable modifications to suit the requirement of this study. The experimental setup is shown in Figure 1. The 1-L digester was filled with known amounts of granular sludge (volatile suspended solids [VSS] = 3240 mg/L), substrate (4160 mg/L), and bicarbonate alkalinity (2770 mg/L). Addition of nutrients was not deemed necessary, as they were available in the POME. The granular sludge samples for analysis were withdrawn from a port 15 cm above the base of the UASFF reactor, operating at an HRT of 3 days and influent COD of 26210 mg/L under a normal and stable condition. The VSS value determined at the end of the test and the slope of the linear portion of cumulative methane (CH_4) production versus time plot were used to calculate the methanogenic activity. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies were also conducted on this sludge.

Analytical Methods. The following parameters were analyzed according to *Standard Methods* (APHA et al., 1999): pH, alkalinity, total suspended solids (TSS), VSS, 5-day biochemical oxygen demand (BOD_5), and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Company, Loveland, Colorado). Gas chromatographs equipped with a thermal conductivity detector and flame ionization detector were used for the determination of biogas and volatile fatty acid (VFA) compositions, respectively (Najafpour et al., 2006). The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a pall ring at 80°C for 24 hours before and after biofilm attachment. In this procedure, a pall ring taken out from the middle part of the bioreactor was dried and weighed. It was subsequently cleaned to



(a) Magnification = 60×
(b) Magnification = 502×
(c) Magnification = 2000×

Figure 3—SEM of a sectioned granule, representing a structure of the ropelike bundle of Methanosaeta.

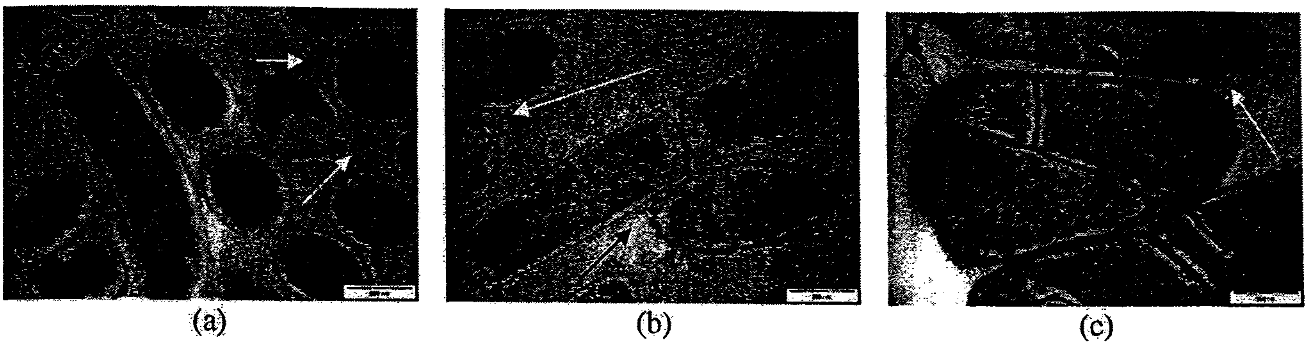


Figure 4—TEM of ultra-thin section of methanogenic cells stained with uranyle acetate and lead citrate. The cell wall is surrounded by an ECP, which appears to attach one organism to the other (scale bar = 500 nm).

remove the attached biofilm and followed by drying and weighing again. This was done twice—before and after each experiment set.

Scanning electron microscopy was used to examine the physical features of the granules. A specimen is bombarded with scanning beam of electrons; then, slowly moving "secondary electrons" are collected, amplified, and displayed on a cathode ray tube. The electron beam and the cathode ray tube scan synchronously, so that an image of the specimen surface is formed. Specimen preparation includes fixation with 5% glutaraldehyde and 1% osmium tetroxide, followed by dehydration with 50 to 100% ethanol and drying, finally making the specimen conductive to electricity. The sample was examined using a Leo Supra 50 VP field emission SEM (Leo Microscopy Ltd. Cambridge, United Kingdom) equipped with an Oxford INCA 400 energy dispersive X-ray microanalysis system (Oxford Analytical Instruments, High Wycombe, United Kingdom) (Dykstra, 1992; Glauert, 1980; Kanovsky, 1965).

A number of typical granules were fixed in a fixative consisting of 3% glutaraldehyde and 0.5% Alcian blue in 0.1 M cacodylate buffer (pH 7.2) for 2 hours at room temperature (approximately 28°C). The fixed samples were postfixed with 1% osmium tetroxide (OsO_4) and 1% lanthanum (III) mononitrate ($LaNO_3$) in 0.2 M s-collidine (pH 7.2) at room temperature and dehydrated through a graded series of ethanol solutions followed by propylene oxide. The sample was embedded in an epoxy embedding media. The sections were cut with a diamond knife mounted in a microtome and then stained with uranyle acetate and lead citrate. The TEM examination was performed using a Philips CM12/STEM electron microscope (Phillips Electronics, Hillsboro, Oregon) at an acceleration voltage of 80 KV.

To examine the stability of the granules, they were subjected to disruption by sonication. The sonication technique was based on the method reported by Forster and Quarmby (1995). The sonication energy was altered by using a constant power level of 60 W and varying the time of sonication; 10 g sample of sludge in 200 mL quarter-strength Ringers solution was used each time. A sample (1 mL) was taken at intervals (every 30 seconds), and, after diluting in quarter-strength Ringers solution, the turbidity was measured using a turbiditimeter (model PN100, Eutech Instruments Ltd., Singapore).

Approximate sizes of granules were determined by spreading them in a Petri dish placed on a graph paper. The range of diameters was then reported. The settling velocity was measured by recording the time taken for an individual granule to fall through a fixed height in a measuring cylinder (Zheng et al., 2005).

Results and Discussion

The reactor was started up with an HRT of 1.5 days using different dilutions of presettled POME. The OLR was increased stepwise, from 2.36 to 23.15 g COD/L·d, by increasing the COD concentration. At the beginning of the experiment, the inoculum was not granulated, but presented in the form of fine flocculent (<0.1 mm). The microorganisms were entrapped by the fixed bed and internally recirculated in the lower part of the reactor. This caused enhanced granulation in a stressful environment (Liu et al., 2003). By the end of the startup period (21 days), a steady COD removal efficiency of 85% was achieved, and the bottom 300 mm of the reactor had developed into a distinct granular sludge blanket having 1- to 2-mm diameter black granules. Detail information has been reported elsewhere (Najafpour et al., 2006). The reactor performance was then examined at different HRTs and initial COD concentrations.

Overall Evaluation of Microbial Aggregation in the Upflow Anaerobic Sludge Fixed-Film Reactor. *Granular Sludge Developed in the Lower Part (Upflow Anaerobic Sludge Blanket Section) of the Reactor—Scanning Electron Microscopy Examination of the Microbial Consortia.* The developed granules had a filamentous appearance, like a ball of spaghetti, formed of very long Methanosaeta-like (formerly Methanothrix) filaments (Figure 2a). The SEM of a sectioned granule (Figures 2b and 2c) showed rod- (Methanosaeta) and cocci- (Methanosarcina) shaped microorganisms densely packed together. The addition of crushed and fine methanogenic sludge (taken from the accumulated sludge in the bed of the drainage channel) as seed and feeding the reactor with POME (containing high acetate concentration, approximately 2300 mg/L) aided the development of such methanogenic sludge granules. The use of methanogen-enriched seed sludge for UASB inoculation has been emphasized to reduce the time required for startup (Liu and Tay, 2004).

Methanosaeta-like organisms were found to be the dominant species in the granules, as most of the core of the granules was observed to be Methanosaeta-like organisms. An aggregation of Methanosaeta in the core of the developed granules is shown in Figure 2d. The dense arrangement/network of these organisms indicates their importance in the formation and maintenance of the granular structure. Methanosaeta aggregates function as nucleation centers that initiate development of POME-degrading granules. More compact Methanosaeta granules (rod granules) are thought to be formed by the colonization of the central cavities of Methanosarcina clumps (Figure 2e, middle part of the granule),

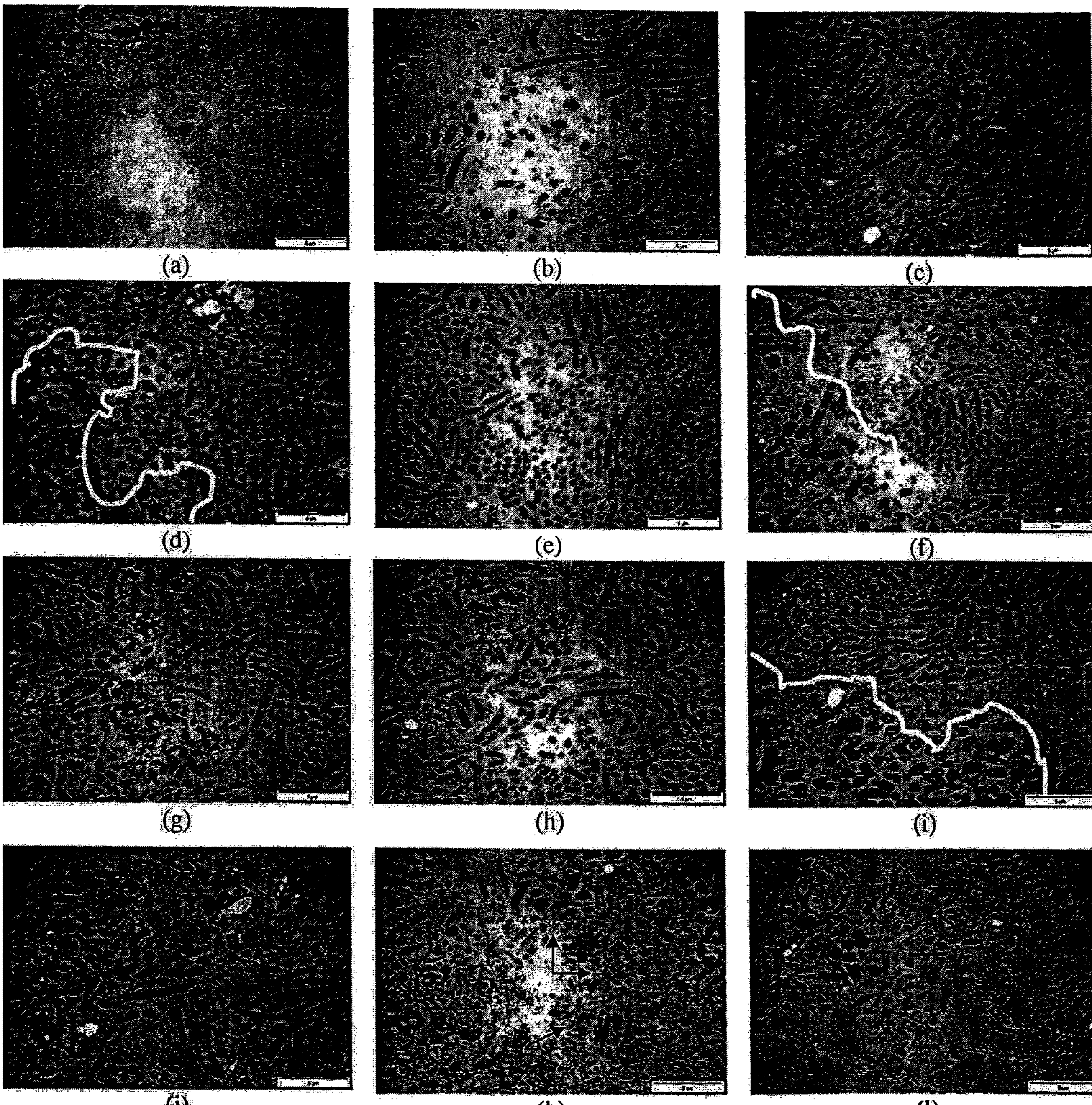
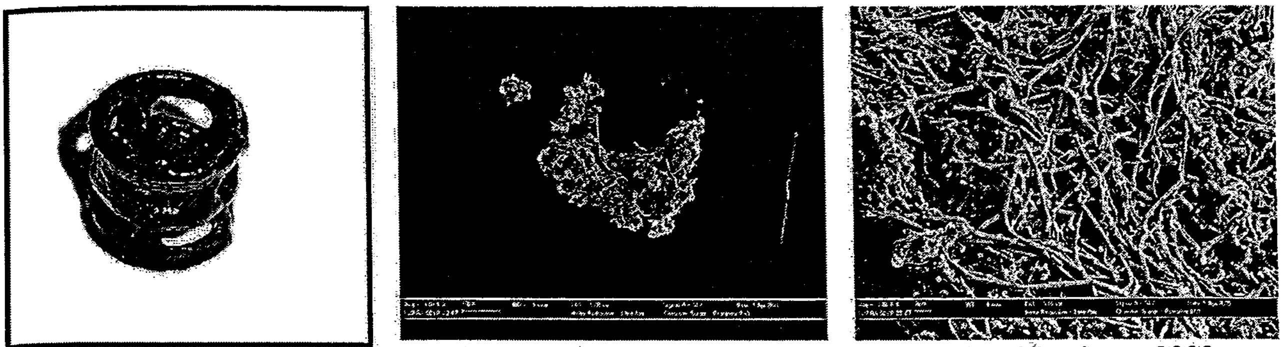


Figure 5—TEM of the ultra-thin sectioned granule (scale bar = 5 μm).

which, owing to a higher half-velocity constant (K_s) value, proliferate on the outer layer of the granules (Batstone et al., 2004). Figure 3 shows the SEM of Methanosaeta, from which, a ropelike bundle of filaments emerged. The structure of the fibers is shown in more detail in Figures 3b and 3c, which exhibit the individual filaments consisted of strings of cells.

Transmission Electron Microscopy Examination of the Microbial Consortia. According to literature (Veiga et al., 1997), extracellular polymers (ECP) produced by methanogens play an essential role in granule formation. The TEM results obtained from thin sections of granule stained with uranyle acetate and lead citrate showed significant amounts of ECP among cells to adhere the cells to one another (Figure 4), indicating that ECP forms a strong matrix for the embedding of cells in granules (Dolfing et al., 1985). This result also confirmed the production of ECP by anaerobic bacteria in the granules.

Figure 5 presents TEM of an ultra-thin section (<0.1 μm) of a 2-mm-diameter granule taken from the reactor. It shows a sequence of different structural features from one side to its diametrically opposite. From the figure, the inner layers (Figures 5c to 5k) are seen to be denser than the outer layers (Figures 5a, 5b, and 5l), indicating loose colonies in the latter. It was found that microcolonies



(a) (b) Magnification = 1010× (c) Magnification = 2000×

Figure 6—Immobilized biofilm on a pall ring taken from the fixed-film section of the reactor.

of a single microbial species formed in different parts of the granule, with overlapping heterogeneous colonies (Figures 5d, 5f, and 5i). It is essential that metabolic endproducts are able to easily escape from the aggregate. The interior of the granule is seen to be porous, which is related to the granule formation and the required diffusion of substrates and products. The size of the granule remained relatively small, so that the accessibility of organisms inside the granule to their substrate was high, as reported elsewhere (Zinatizadeh et al., 2005). The presence of particles with low electron density in some part of the granule might be attributed to accumulation of nondegradable suspended solids and/or dead cells (marked with arrows in Figure 5k, as an example) (Díaz et al., 2003).

Biofilm Attached on the Packing in the Middle Part (Fixed-Film Section) of the Reactor. The predominant microbes in the UASFF reactor were Methanosaeta, which could form very long filaments (200 to 300 μm). These microorganisms also grew on the packing in the middle part of the reactor. The developed biofilm served a supplementary role for final POME digestion to metabolize intermediates, such as the remaining VFA, to methane. Figure 6a shows a pall ring taken from the reactor with the immobilized bacteria on the surface. Figures 6b and 6c demonstrate SEM of the piece of the biofilm with different magnifications and confirm the dominance of long filamentous Methanosaeta-like bacteria, as they were the dominant genus in the granules also.

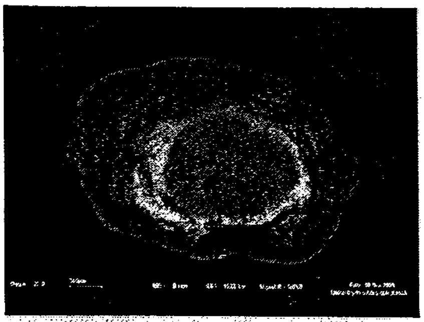
Effect of Operational Regime on Granules Structure. The UASFF reactor was subjected to various operational conditions during the study period. Process shock conditions arose from suspended solids overload (influent suspended solids of 5760 mg/L, which corresponds to suspended solids loading rate of 5.24 g SS/L·d), soluble organics overload (29 g COD/L·d), and high temperature (60°C). This upset the microbial balance and led to an abrupt massive sludge washout. The operational conditions, effluent quality, and sludge characteristics are presented in Table 2.

Effect of Suspended Solids Overload. From Table 2, the effect of the high concentration of suspended solids in raw POME and the combined effect of high organic load (26 g COD/L·d in the form of suspended and soluble solids) and low upflow velocity (V_{up}, 0.2 m/h) caused the disintegration of some granulated sludge and washout from shock load. The sludge washout was the result of a high suspended solids loading rate (5.24 g SS/L·d) and compaction of the blanket, as approximately 33% of OLR was contributed by suspended organics, which could not be hydrolyzed as fast as the soluble fraction. Before the process upset occurred under these conditions, the gas produced was not initially able to escape from the sludge, as a result of compaction of the sludge blanket. After several hours of operation, the accumulated gas suddenly buoyed the sludge and caused a relatively high washout from the reactor. The fixed-film section was not able to prevent sludge washout. The high VSS-to-TSS ratio of the sludge should not be interpreted to

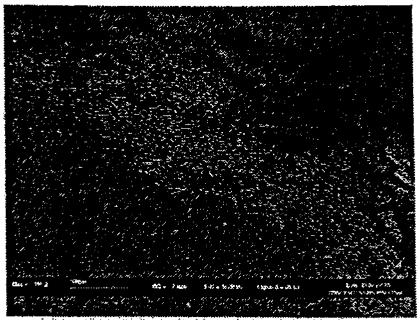
Table 2—Performance of the UASFF bioreactor at critical conditions before process upset.

	Operational conditions						Effluent characteristics					Sludge characteristics			
Type of POME	Influent COD (mg/L)	Influent suspended solids (mg/L)	HRT (days)	OLR (g COD/L·d)	Upflow velocity (V_{up}, m/h)	Temperature (°C)	COD_{ef} (mg/L)	TSS_{ef} (mg/L)	Effluent bicarbonate alkalinity (mg calcium carbonate/L)	Effluent pH	Methane yield (Y_M, L methane/g $COD_{removed}$)	VSS/TSS ratio	SVI at S2[a] (mL/g)	SRT (days)	Settling velocity (m/h)
Raw	44 300	19 780	3	14.8	0.44	38	9700	8370	—	6.73	0.243	0.82	24.4	26.9	10 to 25
Presettled	28 640	5760	1.1	26	0.2	38	4960	1250	1724	7.03	0.196	0.82	24.5	29.7	10 to 25[b]
	27 100	3910	2.2	13.6	0.6	60	8900	1280	1027	6.36	0.23	0.76	28.4	41.8	20 to 35[b]
Chemically pretreated	13 880	Not detected	0.48	29	0.2	38	5240	590	915	6.64	0.096	0.71	30.7	23	30 to 45[b]
Other than the conditions mentioned above							Favorable environment for anaerobic digestion					0.72 to 0.75	12 to 15	31 to 1146	50 to 110

[a] Sludge withdrawn from 350 mm above the base of the UASFF reactor.
[b] Values belong to washed-out granules of 0.5 to 3.5 mm in size.



(a) Magnification = 29×



(b) Magnification = 59×

Figure 7—SEM of a granule at high suspended solids loading: (a) full granule and (b) outer surface of the granule.

mean high biomass content, because it was the result of a high volatile fraction of suspended solids in the feed and not actually live biomass. Granules thus formed had a lower settling velocity (Table 2), were prone to washout, and resulted in a lower SRT. The nondigestable fraction of TSS gradually accumulated in the reactor by attaching to the granules. The granule activity then decreased, as a result of inclusion of nonbiomass VSS (nonbiodegradable or refractory fraction) in the granule structure.

Because sludge granules are formed from dense microbial aggregation, the original granules had a lower sludge volume index (SVI). A low SVI value for sludge suggests a better settling property, which is desired for more biomass accumulation to maintain a high SRT. The SVI was 12 to 15 mL/g under stable operating conditions; it increased to 24.5 mL/g when the system was upset (at an influent COD of 28640 mg/L, HRT of 1.1 days, and V_{up} of 0.2 m/h). It has been reported that, for granular sludge, SVI varies from 10 to 20 mL/g (Maat and Habets, 1987; Yan and Tay, 1997). The increase in SVI may cause process instability, as a result of the increase in the rate of sludge washout. The VSS-to-TSS ratio increased from 0.73 to 0.82 (the ratio for influent was 0.93); that was an indication of increasing contribution of the influent VSS on the sludge blanket. Observations revealed that most of the granules were covered with a thin layer of fiberlike suspended solids. Figure 7 illustrates the distinct appearance of a granule sampled from the reactor under high suspended solids loading. In this condition, the granule color changed from black to brown, showing the attachment of oil-bearing suspended solids to the outer surface of the granules. This resulted in more hydrophobic granules with poor settleability, which caused a large amount of sludge washout. The sludge floated when placed in a measuring cylinder after 5 minutes.

Effect of Soluble Organic Overload. The same observation was made as occurred at the suspended solids overload (as described in the Effect of Suspended Solids Overload section). The difference between these two was the responsible mechanism behind these observations. In the digestion of chemically pretreated POME (at an OLR of 29 g COD/L · d and V_{up} of 0.2 m/h), most of the washed-out sludge was disintegrated because of an acidified environment that resulted from the high initial hydrolyzed OLR, which was available and readily consumed by acidogenic bacteria; this was because of the higher rate of acid production than that of acid consumption. At low V_{up} (0.2 m/h), the recycle ratio (1.1) was not enough to supply the required amount of alkalinity to buffer the reactor for such overload conditions. As a result, process upset occurred. Figure 8 shows the disintegrated granular sludge under soluble organic overload.

Effect of Temperature. The reactor performance was also examined at various temperatures (24, 38, 48, and 60°C) under an OLR of 13.6 g COD/L·d and upflow velocity of 0.6 m/h. At all temperatures tested, except 60°C, no difference in granule morphology was observed. At 60°C, the rate of biomass washout increased, as a result of disintegration of the outer layer of the granules, as shown in Figure 9. Most granules exhibited surface abrasion, and the fragments were washed out. The fixed-film section could not prevent sludge washout, as the rate of granule disintegration was high, and the media pores were not small enough to retain the fragments.

Granules Strength. The sonication tests showed that granules developed under various operational conditions exhibited a very wide range of susceptibility to the effect of ultrasound (Figure 10). From the figure, the granules adapted under mesophilic condition at 38°C and fed with chemically pretreated POME (all OLRs tested

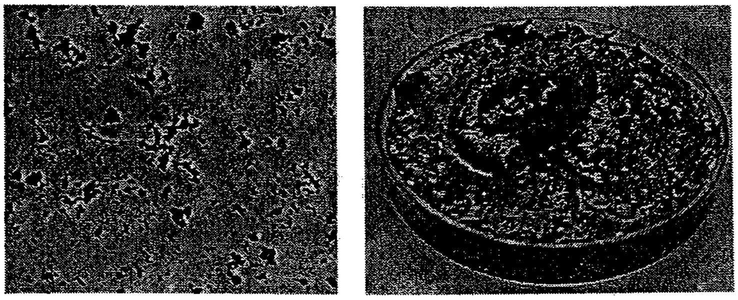
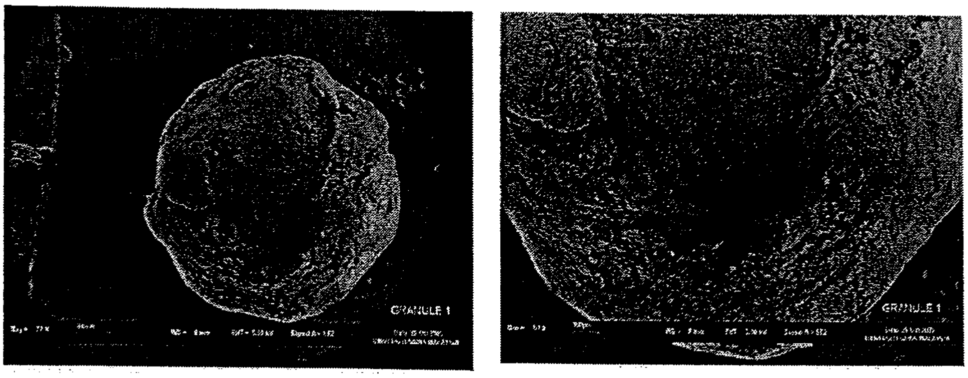


Figure 8—Disintegrated sludge that was washed-out at high soluble organic overload.



(a) Magnification = 27×   (b) Magnification = 59×

Figure 9—SEM of a granule under thermophilic condition (60°C).

except 29 g COD/L · d) appeared to be the strongest (indicated by low turbidity), whereas those that were developed under thermophilic condition at 60°C and fed with presettled POME (at OLR of 13.6 g COD/L · d and V_{up} of 0.6 m/h) and mesophilic condition fed with chemically pretreated POME at high OLR (29 g COD/L · d and V_{up} of 0.2 m/h) were found to have the weakest physical structure, as indicated by the high turbidity. Granules developed at high VFA concentration are fragile in nature and can be easily washed out of the reactor (Ghangrekar et al., 2005). It was noted that the strength of granules developed at other temperatures (24 to 50°C) under the optimum operational conditions were similar to those obtained at 38°C (results not shown). The weak granule structure was the result of partial disintegration of the outer surface caused by a high temperature and acidic environment (high soluble OLR). The relatively high stability of granules developed on chemically pretreated POME might be attributed to contribution of the cationic and anionic polymers residuals in the pretreated POME (Show et al., 2004). Granules developed on raw POME with high suspended solids (19 780 mg/L) showed medium strength, similar to mesophilic granules (fed by presettled POME, under stable condition), but had lower settling velocity, as a result of the greater VSS-to-TSS ratio consequent of high suspended solids in the feed.

Overall, the sizes of granules developed on the presettled and raw POME (with suspended solids) were smaller than those grown on chemically pretreated POME (without suspended solids). Average diameter of the granules was between 1.5 and 2.5 mm for POME with suspended solids, while it increased to 2.5 to 3.5 mm for the chemically pretreated POME. The granules also became denser during feeding with chemically pretreated POME. For example, the SVI at the bottom of the UASFF reactor reached 8.1 mL/g for chemically pretreated POME, whereas it was 11.2 mL/g for presettled POME.

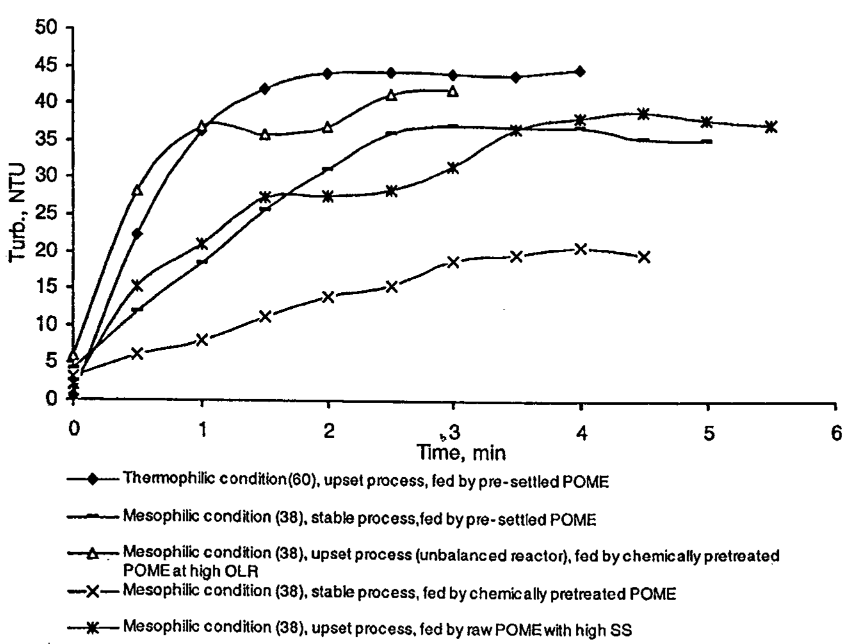


Figure 10—The effect of sonication on granules developed during POME treatment under various operational conditions, as reflected by turbidity; number in parentheses refer to temperature in degrees Celsius; SS = suspended solids.

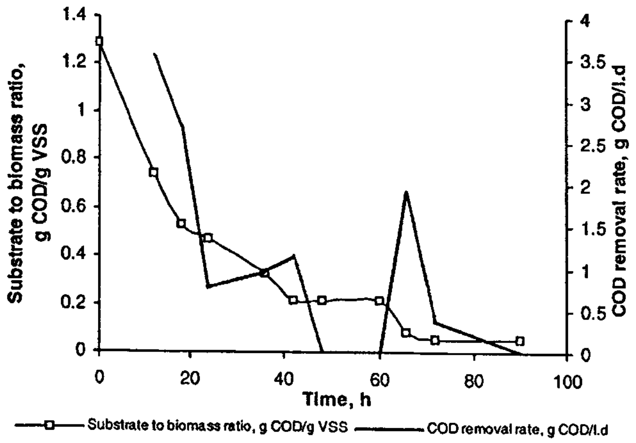


Figure 11—COD removal rate and substrate to biomass ratio versus time.

Biological Activity of the Granular Sludge. To examine the microbial activity of the granular sludge developed in the UASFF reactor, a batch experiment with initial COD concentration (from diluted presettled POME), bicarbonate alkalinity, and biomass concentration of 4160 mg COD/L, 2770 mg calcium carbonate ($CaCO_3$)/L, and 3240 mg VSS/L, respectively, was carried out. It is also noted that the TSS content of the sample tested was less than 100 mg/L, as only a small volume of supernatant was taken from the top of the settled POME for this study. Figure 11 depicts the profile of substrate-to-biomass ratio as a function of time. The maximum value of the substrate to biomass ratio was 1.28 g COD/g VSS at the beginning of the experiment (zero time). It reduced to a minimum value of 0.06 g COD/g VSS after 72 hours. The COD removal rate fluctuated over the course of the experiment. It was highest (i.e., 3.5 g COD/L · d) after 12 hours and reduced sharply to 0.77 g COD/L · d after 24 hours. The initial high COD removal rate was attributed to the presence of readily digestible substrate (such as acetic acid) in POME. The COD removal stopped after 48 hours, as a result of severe depletion in easily available substrate. After a 12-hour lag period, however, COD removal resumed, because the biologically hard organic matter underwent hydrolysis and acidogenesis to become available substrate for the methanogens. The COD removal ceased after 90 hours, at which time, 95.3% COD removal was achieved.

An interesting finding from the batch experiment was that the alkalinity increased with a decrease in total VFA (Figure 12); it resulted from the production of bicarbonate alkalinity by anaerobic bacteria. One of the possible alkalinity producing reactions is as follows:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G^\circ = -31\text{Kj/mole} \quad (1)$$

As a result of high initial VFA concentration in POME and low pH, initial alkalinity supplementation is very important, as the methanogenic bacteria are inhibited at low pH. If the system is not buffered sufficiently, VFA will accumulate and cause cessation of the anaerobic process. The pH remained within the range 7.2 to 7.4, which is optimal for anaerobic digestion.

Figure 13 shows an increase in the ratio of biocarbonate alkalinity to $COD_{removed}$, with an increase in COD removal. An average of 1.1 g alkalinity as $CaCO_3$ was produced when 95% of COD was removed, implying that approximately 275 mg bicarbonate alkalinity as $CaCO_3$ is produced for every 1000 mg COD removed. As can be observed in Figure 11, the readily degradable organic matter (COD) was metabolized up to 48 hours. The results showed that, during degradation of the biologically hard organic matters (after 60 hours, as shown in Figure 13), alkalinity remained constant, while COD reduced, resulting in a decrease in the ratio of bicarbonate alkalinity to $COD_{removed}$. Another stability parameter for anaerobic process is the ratio of VFA to alkalinity, which should be less than 0.3 to 0.4 (Bargman, 1966). In the present case, the maximum value was 0.25 initially, and it decreased to 0.01 at the end of the batch experiment.

Figure 14 represents the cumulative methane production as a function of time. From the maximum methane production rate—calculated based on the slope of the linear portion (considered up to 24 hours) of the figure—and VSS content (3575 mg/L), the methanogenic activity was obtained as 0.654 mg CH_4-COD/mg VSS · d.

Kinetic Evaluation. Consecutive reactions are complex reactions of great environmental importance; hence, equations describing the kinetics of such reactions are of real interest. In

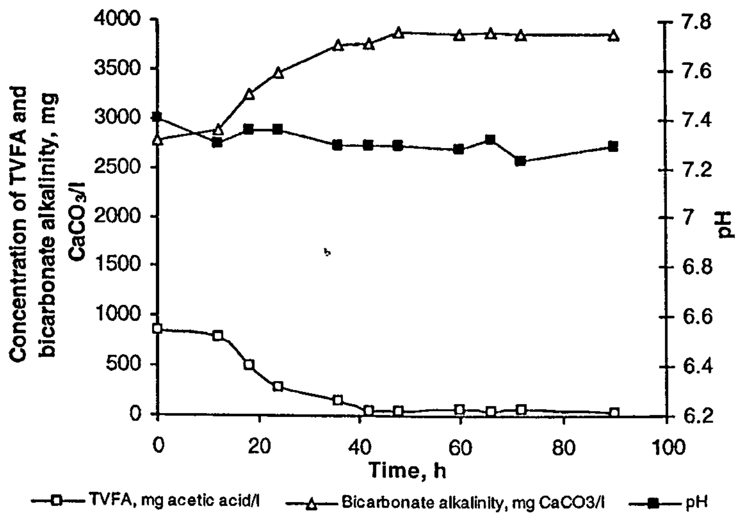


Figure 12—Total VFA (TVFA) and biocarbonate alkalinity versus time.

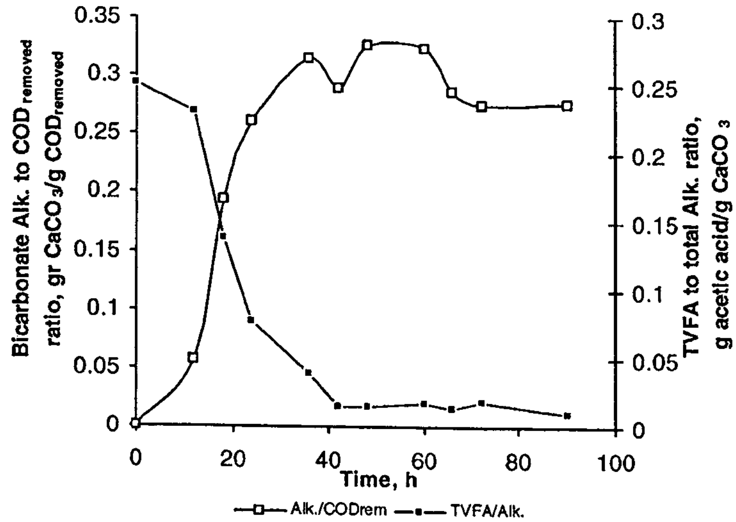


Figure 13—Alkalinity-to-COD-removed ratio versus time.

consecutive reactions, the products of one reaction become the reactants of a following reaction. A simplistic consecutive reaction was developed as a practical mathematical model for the anaerobic digestion of POME in a batch reactor. The reactions mechanism is illustrated in conversion eq 2 below, where A, B, and C represent the COD of wastewater, VFAs, and methane, respectively. The reaction rate constants are denoted by k_1 and k_2.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \tag{2}$$

The key assumptions to simplify the model are as follows:

(1) The rate of each of the consecutive reaction is first-order;
(2) The nonhydrolyzed fraction of substrate is negligible (TSS in diluted POME was only 100 mg/L compared with COD, which was 4160 mg/L);
(3) Reactions are irreversible;
(4) Microbial growth is negligible in the stoichiometry; and
(5) Methane is insoluble in water.

The differential equations that describe the rates of decomposition and formation of the reactants and products are as follows (Sawyer et al., 2003):

$$\frac{-dS_A}{dt} = k_1 S_A \tag{3}$$

$$\frac{dS_B}{dt} = k_1 S_A - k_2 S_B \tag{4}$$

$$\frac{dS_c}{dt} = k_2 S_B \tag{5}$$

At t = 0, S_{A0}= 4160 mg COD/L, S_{B0}= 905.5 mg COD/L, and S_{C0} = 0.0. Therefore, a solution for the concentration of each constituent at time, t, is as follows:

$$S_A = S_{A_o} e^{-k_1 t} \tag{6}$$

$$S_B = \frac{k_1 S_{A0}}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + S_{B0} e^{-k_2 t} \tag{7}$$

$$S_C = S_{A0}\left(1 - \frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_2 - k_1}\right) + S_{B0}(1 - e^{-k_2 t}) \tag{8}$$

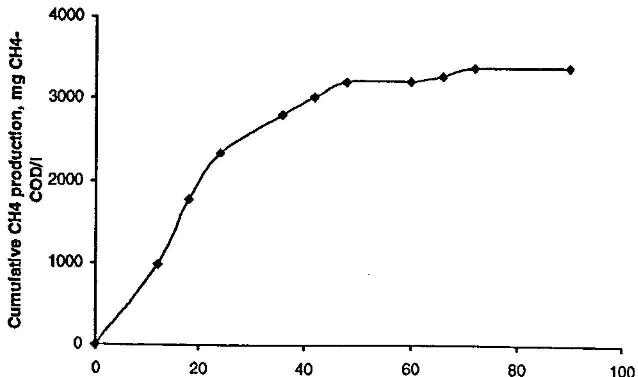


Figure 14—Cumulative methane production versus time.

Figure 15 shows the changes in each constituent, expressed as COD, which were modeled by the above equations. Table 3 shows the kinetic coefficients obtained from eqs 6, 7, and 8 (calculated by the software Sigma Plot 2000, version 6, Systat Software Inc., Richmond, California). The mathematical model gave good fit with the experimental results ($R^2 > 0.93$). Based on the statistical analysis, the models were highly significant, with very low probability values (<0.0001). The slowest step was modeled to be the acidification step, with a rate constant between 0.015 and 0.083 hours^{-1}, while the rate constant for the methanogenic step was between 0.218 and 0.361 hours^{-1}. The kinetics of anaerobic treatment of Baker's yeast effluents was studied by Deveci and Ciftci (2001) using the consecutive reactions model, including hydrolysis reaction. The slowest step was modeled to be the acidification step also, but the rate constant value (0.004 hours^{-1}) obtained was smaller than that calculated in this study for POME treatment. This might be attributed to higher biodegradability of POME, different microbial community, and not considering the hydrolysis step (as soluble COD $\geq$ 97% of TCOD) in the present work.

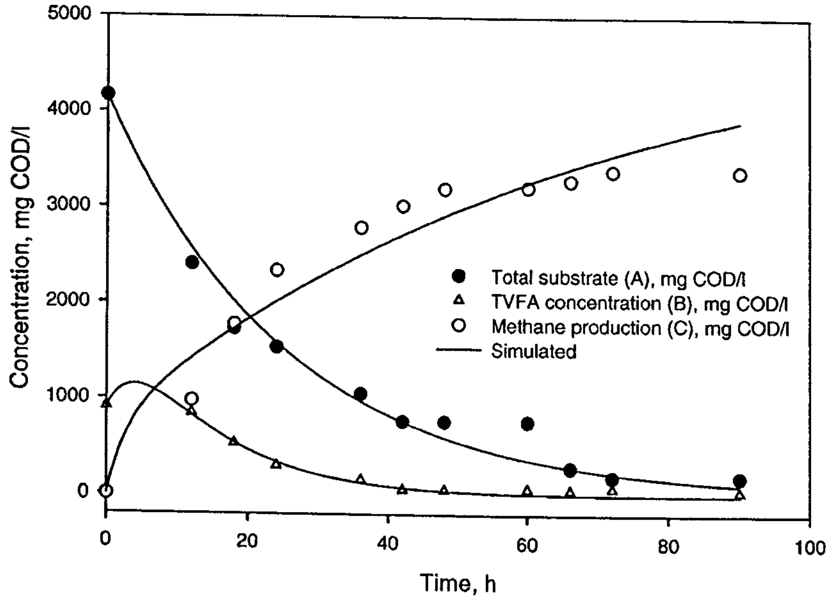


Figure 15—Simulation of batch results using a consecutive reactions model.

Conclusions

For optimum operation of the UASFF reactor for POME treatment, sludge with good characteristics should be developed in the reactor. This study included the effects of operational conditions on the physical characteristics of the granular sludge, sludge biological activity under normal conditions, and process kinetics evaluation. Methanosaeta-like microorganisms were found to be the dominant species in the granules. Operation of the UASFF reactor under different conditions showed that sludge washout occurred, as a result of suspended solids overload (influent suspended solids of 5760 mg/L, HRT of 1.1 days, and temperature of 38°C); soluble organic overload (influent COD of 13 880 mg/L, HRT of 0.48 days, and temperature of 38°C); and high temperature (60°C, HRT of 2.2 days, and influent COD of 27 100 mg/L) causing high suspended solids accumulation in the sludge blanket, unbalanced environment, and thermal shock, respectively. During the batch experiment, an average of 1.1 g alkalinity as $CaCO_3$ was produced when 95% COD was removed. Based on the sonication test, the granules adapted under mesophilic condition at 38°C and fed chemically pretreated POME (all OLRs tested except 29 g COD/L·d) appeared to be the strongest, whereas those developed under thermophilic condition at 60°C and fed presettled POME (at OLR of 13.6 g COD/L·d and V_{up} of 0.6 m/h) and mesophilic condition fed by chemically pretreated POME at high OLR (29 g COD/L·d and V_{up} of 0.2 m/h) were found to have the weakest physical structure. The methanogenic activity of the sludge (withdrawn under stable condition at HRT of 3 days and COD_{in} of 26 210 mg/L) was obtained as 0.64 mg CH_4-COD/mg VSS·d. The mathematical model derived from kinetic equations of a consecutive reaction with three constituents gave good fit with the experimental results ($R^2 > 0.93$).

Table 3—Rate constant coefficients and ANOVA report for each equation.

Equation	k_1 (hours^{-1})	k_2 (hours^{-1})	R^2	P value
6	0.040	—	0.977	<0.0001
7	0.083	0.218	0.986	<0.0001
8	0.015	0.361	0.932	<0.0001

Credits

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Optimization of pre-treated palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor: A comparative study

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Abstract

An up-flow anaerobic sludge fixed film (UASFF) bioreactor was used to treat physically and chemically pre-treated palm oil mill effluent (POME) under different operating conditions. In physical pre-treatment, POME was pre-settled for 2 h and the supernatant was fed to the reactor. In chemical pre-treatment, optimum dosages of a cationic and an anionic polymer were used. Experiments of pre-treated POME digestion were conducted based on a central composite face-centered design (CCFD) with two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}). The operating variables were varied to cover a wide range of organic loading rates (OLR) from 3.8 to 29 g COD/(l d). Six dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, effluent pH, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA), methane yield (Y_M), and solids retention time (SRT). The performance of the reactor was compared for the pre-settled and chemically pre-treated POME. The chemical pre-treatment approach was shown to be more predictable, reliable and practical as the sludge produced was very compressible and was easy to separate. At a comparable range of Q_F and V_{up}, the pre-settled POME yielded slightly better reactor performance in terms of COD removal (%), bicarbonate alkalinity and methane yield. At an OLR of about 16.5 g COD/(l d), higher COD removal efficiency (90–94%) was achieved compared to that of the chemically pre-treated POME (82–88%) despite about 33% of organics in the pre-settled POME was contributed by suspended solids. The optimum conditions for digestion of the pre-settled and chemically pre-treated POME were determined as Q_F of 1.65 l/d, V_{up} of 0.6 and Q_F of 2.45 l/d, V_{up} of 0.75, respectively. The experimental findings were in close agreement with the model prediction.

Keywords: POME; Pre-treatment; UASFF bioreactor; Central composite face-centered design (CCFD)

1. Introduction

Palm oil is one of the main agricultural products of Malaysia and constitutes 49.5% of the total world production [1]. Besides the main product, crude palm oil (CPO), the mills also generate many by-products and liquid wastes, which may have a significant impact on the environment if they are not dealt with properly. On an average a standard palm oil mill, for each tonne of fresh fruit bunch (FFB) processed, generates about 1 tonne of liquid waste with biochemical oxygen demand (BOD) 37.5 kg, chemical oxygen demand (COD) 75 kg, suspended solids (SS) 27 kg and oil and grease 8 kg [2]. There are currently about 265 active palm oil mills in Malaysia with a combined annual CPO production capacity of about 13 million tonnes [3]. This amounts to a population equivalent of around 80 millions in terms of COD. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the economy in good condition.

Biological treatment of palm oil mill effluents (POME) has been widely studied. Some aerobic treatment approaches include: degradation of POME using a tropical marine yeast (*Yarrowia lipolytica*) NCIM 3589 in a lagoon [4], trickling filter (TF) [5] and rotating biological contactors (RBC) [6]. Considering the highly organic character of POME, anaerobic process is the most suitable approach for its treatment [7]. The common practice of treating POME is by using ponding and/or open digestion tank systems which have particular disadvantages such as: long hydraulic retention time of 45–60 days [8], bad odour,

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difficulty in maintaining the liquor distribution to ensure smooth performance over huge areas and difficulty in collecting biogas which can have detrimental effects on the environment [9,10].

High-rate anaerobic reactors that can retain biomass, have a high treatment capacity and hence low site area requirement [11]. An anaerobic baffled reactor (ABR) was used to treat POME without pH adjustment at various recycle ratios [12]. A COD removal efficiency of 84.6% was achieved at an HRT of 2.5 days, an initial COD concentration of 24,850 mg/l and a recycle ratio of 25:1. Anaerobic treatment of POME in a single up-flow anaerobic sludge blanket (UASB) and two stage UASB reactors was investigated [13,14]. A 96% COD removal was reported at an organic loading rate (OLR) of 10.6 g COD/(l d) in the single UASB reactor while at the highest influent COD concentration (42,500 mg/l), reactor instability was observed. Meanwhile, a 90% reduction in COD at an OLR of 15 g COD/(l d) was achieved in the two-stage UASB reactor. A membrane anaerobic system (MAS) was also investigated to treat POME [15]. The efficiency of COD removal was between 91.7 and 94.2% at an average HRT of 3.03 days. A clear final effluent was produced but membrane flux rate deterioration due to membrane fouling was observed. POME treatability in an anaerobic hybrid digester was studied by Borja et al. [16]. A COD removal efficiency and a methane yield of 92.3% and 0.335 m^3/kg $COD_{removed}$, respectively, were achieved at an OLR of 16.2 g/(l d) and HRT of 3.5 days. A high rate up-flow anaerobic sludge fixed film (UASFF) bioreactor treating POME showed a 90% COD removal at a high OLR (23.15 g COD/(l d)) and short HRT (1.5 days) [2]. In summary, the high rate anaerobic reactors mentioned above are able to treat POME at short HRT successfully.

The UASB reactor is one of the most notable developments in anaerobic treatment technology. It exhibits positive features, such as allows high organic loadings, short hydraulic retention time (HRT) and has a low energy demand, especially for POME treatment [17]. Suspended and colloidal components of POME in the form of fat, protein and cellulose have adverse impact on UASB reactor performance and can cause deterioration of microbial activities and wash out of active biomass [13,18]. The use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the above mentioned problems [2,16]. Process instability was observed when a UASB reactor (at HRT of 4 days) and an anaerobic hybrid reactor (AHR) (at HRT of 3.5 days) were operated with high influent COD concentrations of 42,500 and 65,000 mg/l, respectively [13,16]. In a study carried out earlier [19], it was observed that the UASFF reactor became unstable under a stressful condition imposed by overloading of suspended solids at short HRT (1 day, corresponding to OLR of 34.7 g COD/(l d)). Consequently, complete digestion of raw POME without pre-treatment demands high HRT, which is not easily achieved due to the high volume of POME produced by palm oil mills. Various pre-treatment approaches have been examined for the separation of suspended solids and oil and grease from POME. These include: chemical coagulation and flocculation [9,20,21], air flotation (simple and with skimming) [22,23], centrifugation [23] and evaporation [24].

From a practical and economic point of view, optimization of the operating conditions for anaerobic digestion of POME will be beneficial. Classical and statistical methodologies are available for bioprocess optimization. Statistical methodologies involve the use of mathematical methods for designing and analyzing the results [25–27]. Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response [28]. In most RSM problems, the relationship between the dependent variable (response) and the independent variables is unknown. Thus, the first step in the application of RSM is to approximate the function (f). Usually, this process employs a low-order polynomial in some region of the independent variables. The approximating function is a first-order model if the response is well-modeled by a linear function of the independent variables. If there is curvature in the system or in the region of the optimum, a polynomial of higher degree must be used to approximate the response, which is analyzed to locate the optimum, i.e. the set of independent variables such that the partial derivatives of the model response with respect to the individual independent variables is equal to zero. The eventual objective of RSM is to determine the optimum operating conditions for the system, or to determine the region which satisfies the operating specifications. Almost all RSM problems utilize one or both of these approximating polynomials [29–31].

According to a previous study [19], pre-treatment of POME is required in order to properly operate the UASFF bioreactor for its treatment at short HRT (<3 days) with high efficiency. The present research is a comparative study of anaerobic digestion of POME pre-treated physically (sedimentation) and chemically (chemical coagulation and flocculation). Results obtained from the high rate digestion of pre-treated POME were compared using RSM with respect to the simultaneous effects of two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}). Finally, the optimum operating conditions were identified using overlay plots.

2. Material and methods

2.1. Wastewater preparation

Raw POME was collected from a local palm oil mill in Nibong Tebal, Penang, Malaysia. In the first stage, raw POME was pre-settled for 2 h using an ordinary sedimentation tank. In the second part of this study, raw POME was chemically pretreated to remove suspended solids and oil and grease (using a cationic polyacrylamide (Chemfloc 1510C) and an anionic polyacrylamide (Chemfloc 430A)). The samples were then stored in a cold room at 4 °C before feeding to the reactor. This storage technique had no observable effect on their composition. The characteristics of the raw and pre-treated POME are summarized in Table 1.

2.2. Bioreactor and start up

A laboratory-scale, up-flow anaerobic sludge fixed film (UASFF) bioreactor was used in this study. The glass bioreac-

Table 1
Characteristics of the raw, pre-settled and chemically pre-treated POME

Parameter	Raw POME	Pre-settled POME	Chemically pre-treated POME
BOD_5 (mg/l)	22,700	20,100	9750
COD (mg/l)	44,300	28,640	13,880
Soluble COD (mg/l)	17,140	17,140	13,880
TVFA (mg acetic acid/l)	2510	2510	2760
SS (mg/l)	19,780	5760	<20
Oil and grease (mg/l)	4850	1630	Negligible
Total N (mg/l)	780	660	480
pH	4.05	4.05	4.2

Values are average of three measurements. The differences between the measurements for each were less than 1%.

tor column had an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml, and the working volume was 3650 ml. The column consisted of three sections: bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with a height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas–solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25% and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank.

The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Total volatile solids concentration of the seed was initially measured as 10,300 mg/l. Details regarding the start up procedure are discussed elsewhere [2].

2.3. Bioreactor operation

The UASFF bioreactor was separately operated with pre-settled and chemically pre-treated POME and experiments were designed by Design Expert software (Stat-Ease Inc., Version 6.0.6) with two variables viz., feed flow rate and up-flow velocity. The experimental conditions are shown in Table 2.

2.4. Experimental design and mathematical model

The statistical method of factorial design of experiment (DOE) eliminates systematic errors with an estimate of the experimental error and minimizes the number of experiments [32]. In earlier studies [2,19], feed flow rate (Q_F) and up-flow velocity (V_{up}) were found to be the most critical independent operating variables which affected the performance of the reac-

Table 2
Experimental conditions and results of central composite design

Pre-settled POME

Run	Variables: Factor 1, A: feed flow rate (l/d)	Variables: Factor 2, B: up-flow velocity (m/h)	Response: Total COD removal (%)	Response: Eff. pH	Response: Eff. TVFA (mg acetic acid/l)	Response: BA (mg $CaCO_3$/l)	Response: Methane yield (l CH_4/g COD_{rem} d)	Response: SRT
1	(−1)1.01	(−1)0.2	97.3	7.76	132.4	2006	0.344	292
2	(−1)1.01	(0)1.60	93.9	7.47	104.2	2026	0.298	244
3	(−1)1.01	(1)3.00	93.1	7.46	80.54	2087	0.258	208
4	(0)2.16	(−1)0.20	90.7	7.25	243.1	1957	0.295	82.6
5	(0)2.16	(0)1.60	91.3	7.51	35.17	2225	0.305	74.7
6	(0)2.16	(0)1.60	91.5	7.55	44.2	2208	0.303	68.4
7	(0)2.16	(0)1.60	91.4	7.54	36.1	2234	0.315	72.3
8	(0)2.16	(0)1.60	90.3	7.34	58.8	2219	0.305	75.3
9	(0)2.16	(0)1.60	92.7	7.66	38.3	2162	0.29	73.5
10	(0)2.16	(1)3.00	95.5	7.16	41.9	2210	0.25	58.7
11	(1)3.31	(−1)0.2	82.7	7.03	573.75	1723	0.196	28.3
12	(1)3.31	(0)1.60	91.8	7.58	144.5	2157	0.292	50.4
13	(1)3.31	(1)3.00	94.2	7.35	137.8	2162	0.285	46.3

Chemically pre-treated POME

Run	Variables: Factor 1, A: feed flow rate (l/d)	Variables: Factor 2, B: up-flow velocity (m/h)	Response: Total COD removal (%)	Response: Eff. pH	Response: Eff. TVFA (mg acetic acid/l)	Response: BA (mg $CaCO_3$/l)	Response: Methane yield (l CH_4/g COD_{rem} d)	Response: SRT (day)
1	(−1)1.01	(−1)0.2	96.7	7.6	64.86	1394	0.335	1147
2	(−1)1.01	(0)1.60	94.3	7.51	23.1	1504	0.273	1132
3	(−1)1.01	(1)3.00	93.24	7.22	22.1	1664	0.226	607
4	(0)4.32	(−1)0.20	84.3	7.17	146.1	1776	0.261	78
5	(0)4.32	(0)1.60	85.2	7.29	33.15	1896	0.29	66
6	(0)4.32	(0)1.60	84.5	7.26	37.49	1833	0.285	68
7	(0)4.32	(0)1.60	86	7.33	34.7	1915	0.275	61
8	(0)4.32	(0)1.60	87.6	7.43	33.99	1896	0.27	64
9	(0)4.32	(0)1.60	84.5	7.29	36.07	1854	0.255	66
10	(0)4.32	(1)3.00	86.5	7.03	37.92	1893	0.228	65
11	(1)7.63	(−1)0.2	62.2	6.64	1613.2	915	0.109	23
12	(1)7.63	(0)1.60	80.2	7.33	289.68	1474	0.244	31
13	(1)7.63	(1)3.00	83.8	7.19	251.02	1312	0.224	36

tor [17,33,34]. The feed flow rate represents the organic load while the up-flow velocity reflects the dilution coefficient of the influent feed and the rate of alkalinity recycle from effluent to influent. Different up-flow velocities were provided by varying the recycle ratio. V_{up} also affects the physical characteristics of the granular sludge such as sludge volume index (SVI) and settling velocity [35]. The region of exploration for POME treatment was decided as the area enclosed by Q_F (1.01, 3.31 l/d) and V_{up} (0.2, 3 m/h) boundaries for pre-settled POME and Q_F (1.01, 7.63 l/d) and V_{up}(0.2, 3 m/h) for chemically pre-treated POME to evaluate the reactor performance at short HRTs (<3.6 days). This would cover an OLR range of 7.9–26.0 and 3.8–29.0 g COD/(l d) for pre-settled and chemically pre-treated POME, respectively, and would include the optimum regions for each treatment. Steady state was assumed after five turnovers.

The RSM used in the present study was a central composite face-centered design (CCFD) involving two different factors, Q_F and V_{up}. The anaerobic digestion of POME was assessed based on the face-centered CCD experimental plan (Table 2). Accordingly, 13 experiments were conducted with the first 9 experiments organized in a factorial design and the remaining 4 involving the replication of the central points for each stage. It was a 3^2 full factorial design with two operating variables, each considered at three levels namely, low (−1), central (0) and high (1). In order to carry out a comprehensive analysis of the anaerobic process, six dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, effluent pH, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA), methane yield (Y_M) and solids retention time (SRT). The last three parameters were calculated using the following relationships:

$$\mathrm{BA} = \text{Total Alk.} - (0.71)\mathrm{VFA} \tag{1}$$

$$Y_M = \frac{Q_{CH_4}}{Q_F(\mathrm{COD_{in}} - \mathrm{COD_{ef}})} \tag{2}$$

$$\mathrm{SRT} = \frac{V \cdot \mathrm{VSS}}{Q_F \cdot \mathrm{TSS_{ef}}} \tag{3}$$

where Q_F is the feed flow rate (l/d), Q_{CH_4} the methane production rate (l CH_4/d), VSS the biomass concentration in the reactor (mg/l), V is the volume of the reactor (l) and $\mathrm{TSS_{ef}}$ is the biomass concentration leaving the reactor with the effluent.

After conducting the experiments, the coefficients of the polynomial model were calculated using the following equation [31]:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i \cdot X_i + \sum_{i=1}^{k}\beta_{ii} \cdot X_i^2 + \sum_{i_{i \le j}}^{k}\sum_{j}^{k}\beta_{ij}X_iX_j + e \tag{4}$$

where i and j are the linear and quadratic coefficients, respectively, β the regression coefficient, k the number of factors studied and optimized in the experiment and e is the random error. Model terms were selected or rejected based on the P value with 95% confidence level. The results were completely analyzed using analysis of variance (ANOVA) by Design Expert software. Three-dimensional plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The optimum region was also identified based on the main parameters in the overlay plot. The experimental conditions and results are shown in Table 2.

2.5. Analytical methods

The following parameters were analyzed according to Standard Methods [36]: pH, alkalinity, total suspended solids (TSS), volatile suspended solids (VSS), BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, CO). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively [2]. VSS in the lower part of the reactor (UASB portion) was determined by taking samples from different sampling ports along the reactor height. The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a pall ring at 80 °C for 24 h before and after biofilm attachment. In this procedure, first, a pall ring was taken out from the middle part of the bioreactor to be dried and then weighed. It was subsequently cleaned to remove the attached biofilm followed by drying and weighing. This was carried out twice, before and after each experiment set. Finally, VSS concentration in the reactor was calculated by dividing total mass of VSS contents of the lower and middle parts by the reactor working volume.

3. Results and discussion

3.1. Overall discussion on pre-treatment methods

Raw POME contains high concentration of suspended solids as shown earlier in Table 1, which require long retention time for satisfactory digestion. A fraction of TSS, which is not digestible, is gradually accumulated in the reactor by attaching to the sludge granules in the UASFF reactor and causes reduction in process efficiency. From a practical point of view and according to various studies, in order to have a reliable, stable and efficient high rate anaerobic process, the oil-bearing suspended solids need to be removed (partially or completely) before anaerobic treatment [2,9,13,14,16].

The chemical pre-treatment approach was shown to be more predictable, reliable and practical in terms of sludge characteristics. The sludge was compressible and easy to be separated. From energy recovery point of view, the amount of methane produced per 1 l of raw POME in the UASFF reactor was relatively low as about 70% of TCOD was removed in chemical pre-treatment (including about 100% particulate COD and 20% soluble COD). Primary sedimentation was applied as another alternative to remove suspended solids and oil and grease as it is currently done in ponding system in most palm oil mills. Poor settlability of the suspended solids makes the separation difficult.

Table 3
ANOVA results for the equations of the Design Expert 6.0.6 for studied responses

Type of pre-treatment	Response	Transformation	Modified equations with significant terms	Probability	R^2	Adj. R^2	Adeq. precision	S.D.	CV	PRESS	Probability for lack of fit
Pre-settled POME (sedimentation)	TCOD removal	–	$92.03-2.6A+2.02B+3.93AB$	0.0001	0.8871	0.8495	17.588	1.34	1.45	44.27	0.1181
	Eff. pH	–	$7.52-0.12A-0.19B^2+0.16AB$	0.036	0.8955	0.8606	7.038	0.15	2.01	0.54	0.2292
	Eff. TVFA	Base 10 log	$1.65+0.17A-0.27B+0.35A^2+0.27B^2$	0.0007	0.8886	0.8330	11.245	0.15	7.8	0.68	0.0891
	Eff. BA	–	$2205.59+65.5A+128.83B-104.05A^2-112.05B^2+89.5AB-117.5AB^2$	<0.0001	0.9854	0.9707	27.287	25.13	1.19	19017.6	0.7083
	Methane yield	–	$0.3-0.021A-0.03B^2+0.044AB$	0.0006	0.8445	0.7926	14.233	0.016	5.72	5.4E−3	0.0656
	SRT	–	$72.21-103.17A-14.98B+72.62A^2+25.5AB$	<0.0001	0.9976	0.9976	82.41	5.04	4.76	1158.29	0.0590
Chemically pre-treated POME (coagulation and flocculation)	TCOD removal	–	$85.31-9.67A+3.38B+6.27AB$	<0.0001	0.9315	0.9086	22.662	2.54	2.97	229.69	0.0524
	Eff. pH	–	$7.35-0.2A-0.21B^2+0.23AB$	0.0003	0.8698	0.8264	15.66	0.098	1.36	0.23	0.1437
	Eff. TVFA	Base 10 log	$1.55+0.59A-0.31B+0.34A^2+0.3B^2-0.085AB$	<0.0001	0.9973	0.9954	74.417	0.037	2.01	0.078	0.0599
	Eff. BA	–	$1895.83-449.4A^2-103.9B^2+166.75A^2B-207.75AB^2$	<0.0001	0.8974	0.8632	14.309	112.61	6.86	3.17E5	0.069
	Methane yield	–	$0.27-0.043A-0.04B^2+0.056AB$	0.0004	0.8594	0.8126	15.612	0.023	9.06	0.012	0.0946
	SRT	Base 10 log	$1.82-0.75A+0.39A^2+0.12AB$	<0.0001	0.9947	0.9930	64.992	0.048	2.39	0.061	–

A: first variable, feed flow rate (l/d); B: second variable, up-flow velocity (m/h); R^2: determination coefficient; Adj. R^2: adjusted R^2; Adeq. precision: adequate precision; S.D.: standard deviation; CV: coefficient of variation; PRESS: predicted residual error sum of squares.

3.2. POME digestion

3.2.1. Statistical analysis

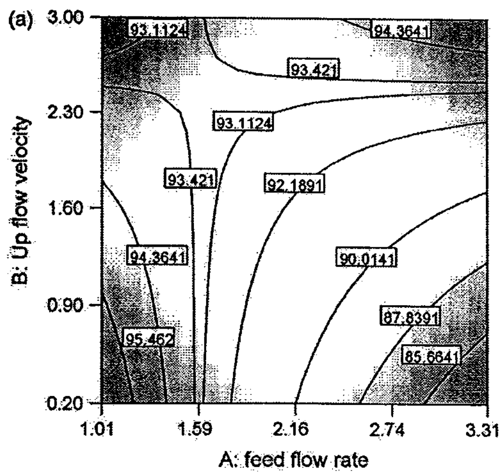
As various responses were investigated in this study, different degree polynomial models were used for data fitting (Table 3). The regression equations obtained are also presented in the table. In order to quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations, i.e. two-factor interaction (2FI), quadratic and so on. In the Design Expert software, the response data were analyzed by default. Some raw data might not be fitted and transformations which apply a mathematical function to all the response data might be needed to meet the assumptions that made the ANOVA valid. Data transformations were needed for the TVFA (both pre-settled and chemically pre-treated POME) and SRT (chemically pre-treated POME digestion) responses. Therefore, $\log_{10}$ function was applied for these responses [20,37,38]. The ANOVA results for all responses are summarized in Table 3. The model terms in the equations are after the elimination of insignificant variables and their interactions. The interaction term, i.e. *AB*, was significant for all equations except those defining effluent bicarbonate alkalinity for chemically pre-treated POME and TVFA for pre-settled POME. Based on statistical analysis, the models were highly significant with very low probability values (from 0.036 to <0.0001).

It was noted in Table 3 that the model terms of independent variables were significant at the 99 % confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). It showed high significant regression at 95% confidence level. The models adequacy was tested through lack-of-fit *F*-tests [29]. The lack of fit *F*-statistic was not statistically significant as the *P* values were greater than 0.05. Adequate precision is a measure of the range in predicted response relative to its associated error or, in simpler words, a signal to noise ratio. Its desired value is 4 or more [30]. The value was found to be desirable for all models. Simultaneously, low values of the coefficient of variation (CV) (1.19–9.06%) indicated good precision and reliability of the experiments [20,31,32]. Detail analysis of the models results and comparative studies are presented in the following sections.

3.2.2. Analysis and comparison on reactor performance

3.2.2.1. COD removal. The effect of the variables on TCOD removal efficiencies are shown as contour plots in Fig. 1a and b for pre-settled and chemically pre-treated POME, respectively. As shown in Table 4, the range of total OLR employed was comparable for the two experimental sets. Since the pre-settled POME contained 5760 mg/l of TSS, a fraction of the OLR is suspended solids whereas the entire OLR is soluble for chemically pre-treated POME. It should be noted that soluble fraction of OLR is greater for pre-settled POME at low HRT (Q_F = 1.01 l/d) due to higher initial COD concentration (Table 4). In an overall comparison, the trend of changes in TCOD removal efficiency was quite similar for both conditions as the same model terms described the variations in TCOD removal (Table 3). The TCOD removal (%) decreased with increase in Q_F while the rate of TCOD removal (g COD/(l d)) increased (Table 4), due to increase in diffusion rate of substrate at higher substrate concentration [39,40].

A stable TCOD removal efficiency of 83.5% was achieved at the highest Q_F (3.31 l/d, corresponding to OLR of 26 g COD/(l d)) and the lowest V_{up} (0.2 m/h) for pre-settled POME whereas only 62.2% TCOD removal was achieved with chemically pre-treated POME at Q_F of 7.63 l/d (corresponding to OLR of 29 g COD/(l d)) and that too was coupled with process instability. It was probably due to inhibition of the methanogenic microorganisms as a consequence of very low recycle ratio (1.1:1) [12]. As the up-flow velocity was increased, the effect of Q_F on COD removal was reduced. It was found that at the same OLR (center points, V_{up} from 0.2 to 3 m/h), greater COD removal efficiency (90–94%) was achieved compared to chemically pre-treated POME (82–88%) despite about 33% of OLR in the pre-settled POME being suspended solids, which needed to be hydrolyzed first. This might be attributed to either possible inhibitory effects of the polymers which were applied for chemical



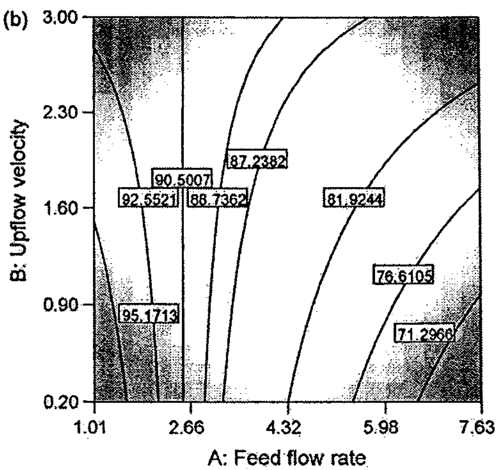


Fig. 1. Contour plot of TCOD removal efficiency representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

Table 4
OLR and COD removal rate at different operating conditions

Run	Feed flow rate (l/d)	Up-flow velocity (m/h)	Pre-settled POME			Chemically pre-treated POME	
			Total OLR (g COD/(l d))	Soluble OLR (g COD/(l d))	TCOD removal rate (g COD/(l d))	Total OLR = Soluble OLR (g COD/(l d))	TCOD removal rate (g COD/(l d))
1	Low level	0.2	7.9	5.3	28	3.8	13.5
2		1.6			27.1		13.2
3		3			26.9		13
4	Center level	0.2	16.9	11.4	56.1	16.4	50.5
5		1.6			56.5		51.1
6		1.6			56.6		50.7
7		1.6			56.6		51.6
8		1.6			56.5		52.5
9		1.6			57.3		50.7
10		3			59.1		51.9
11	High level	0.2	26	17.4	78.4	29	65.9
12		1.6			88.1		84.9
13		3			89.4		88.8

pre-treatment (inhibition effects of some cationic polymers on anaerobic bacteria in a seed sludge reported by Uyanik et al. [41]) or COD contribution of the polymers which may not have degraded under the experimental conditions.

3.2.2.2. *Effluent pH.* Fig. 2a and b represent variations of pH as a function of the operating variables for pre-settled and chemically pre-treated POME digestion, respectively. Q_F (A), the second-order of V_{up} (B^2) and the two-level interaction (AB) of the variables are the significant model terms for both conditions (Table 3). As noted in the figure, increase in V_{up} had a reverse effect on pH at different values of up-flow velocity. At low range of V_{up} (values less than 0.90 and 2.25 m/h, respectively, for Q_F of 1.01 and 3.31 l/d in Fig. 2a and less than 0.75 and 2.45 m/h, respectively, for Q_F of 1.10 and 7.63 l/d in Fig. 2b), an increase in V_{up} caused an increase in pH due to increase in recycling effluent alkalinity as no chemical was used to adjust pH or to provide buffering capacity in the feed. Whereas, at higher values, increase in V_{up} showed a slight decrease in pH. It could be attributed to insufficient contact between the sludge bed and the substrate as a consequence of mass transport at high up-flow velocity [42].

Average values of pH for pre-settled POME treatment was higher than the values for chemically pre-treated POME within the design region. It was attributed to higher organic loading at long retention times and less loading at short retention times (Table 4), which provided more favorable environment for pre-settled POME anaerobic digestion. At overload condition, pH values of 7.06 and 6.64 were obtained for pre-settled and chemically pre-treated POME, respectively. The value of 6.64 and relative cessation of methane production was evident from the higher acid production rate in digestion of the chemically pre-treated POME due to high soluble OLR [43].

3.2.2.3. *Effluent VFA.* The VFA concentration is a key indicator of system performance. The ratios of maximum to minimum for effluent VFA were 16.3 and 73.0 for the pre-settled and the chemically pre-treated POME, respectively. Hence, a logarithmic function with base 10 was required to fit the data. Fig. 3a and

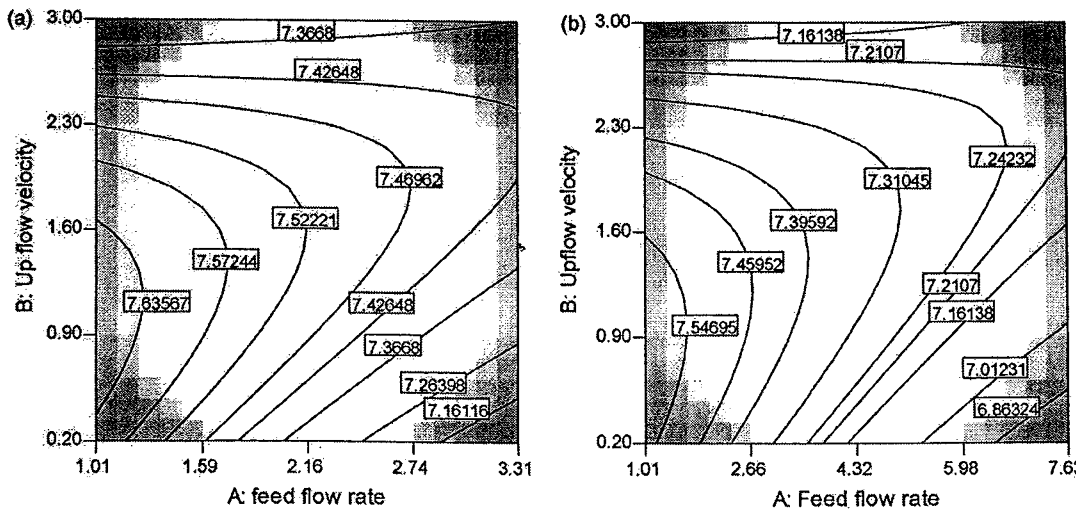


Fig. 2. Contour plot of effluent pH representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

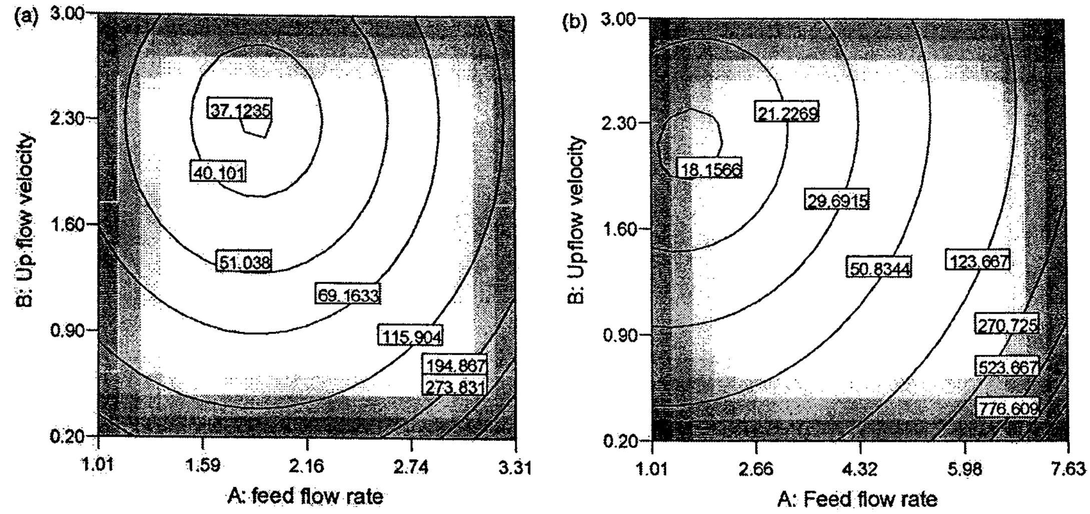


Fig. 3. Contour plot of effluent TVFA representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

b depict the effects of the variables on the effluent VFA for the pre-settled and the chemically pre-treated POME, respectively. According the quadratic models for the response (Table 3), the two-level interaction of the variables is a significant model term for the chemically pre-treated POME while it is not significant for the pre-settled POME. The highest concentrations of VFA as intermediates was found during overloading conditions (highest Q_F and lowest V_{up}) when they were 553 mg/l for the pre-settled POME at an OLR of 26 g COD/(l d) and 1613 mg/l for the chemically pre-treated POME at an OLR of 29 g COD/(l d). In both experiments, the role of V_{up} in the system recovery at high Q_F was very significant due to its effects on recycled alkalinity and contact between substrate and biomass [44]. From the contour plots, at comparable OLRs, i.e. 16.95 g COD/(l d) ($Q_F = 2.16$ l/d) for pre-settled POME and 16.42 g COD/(l d) ($Q_F = 4.32$ l/d) for chemically pre-treated POME, the VFA concentrations for the two cases were also similar. It showed that there was a balance between acetogenesis and methanogenesis in the systems at this OLR. In the chemically pre-treated POME digestion, process upset occurred as methanogenic bacteria could not metabolize the VFA as fast as they were produced, resulting in a possible reduction in pH. This condition ($Q_F = 7.63$ l/d, $V_{up} = 0.2$ m/h) had a strong influence on the biogas quality, increasing the CO_2 percentage (75.22%). Similar behavior was observed in a secondary UASB reactor treating piggery waste at an HRT of 1 day and an influent COD of 10,189 mg/l [45].

3.2.2.4. Effluent bicarbonate alkalinity (BA). Fig. 4a and b show interactive effects of Q_F and V_{up} on BA. It was understood that the BA was produced through POME digestion reactions as no chemical was added to supply alkalinity. From the quadratic models in Table 3, the main effects of the two factors (*A* and *B*) are significant model terms for the pre-settled POME while they are not significant for the chemically pre-treated one. Effluent BA values for pre-settled POME were greater than the values for chemically pre-treated POME within the tested range of the two variables. Results obtained from batch experiments showed that bicarbonate alkalinity was produced (330 mg $CaCO_3$/g $COD_{removed}$) during POME digestion [46]. According to the models, in Fig. 4a, the maximum level for BA was predicted to be the region where Q_F and V_F were 2.6 l/d

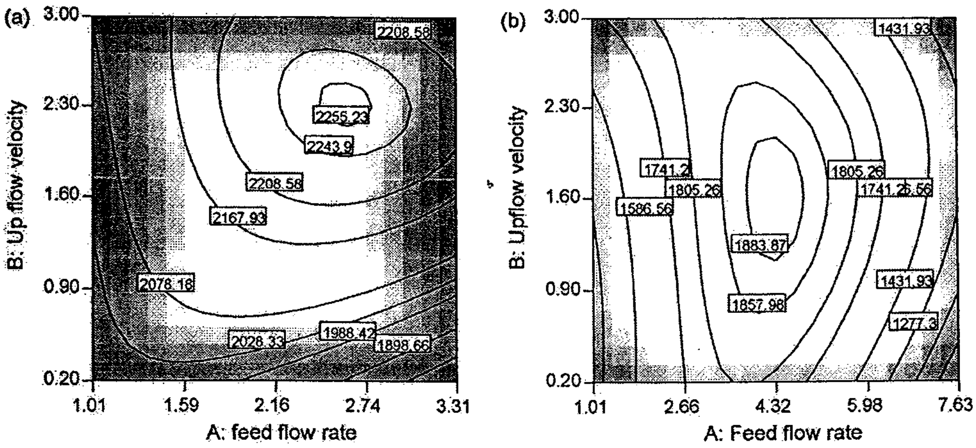


Fig. 4. Contour plot of effluent BA representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

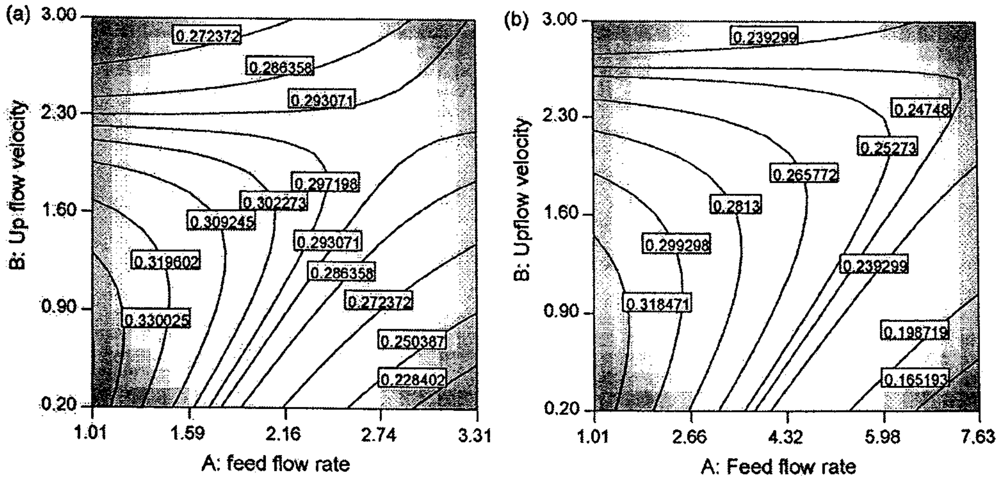


Fig. 5. Contour plot of methane yield representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

and 2.3 m/h, respectively while it was obtained as around the middle part of the design region ($Q_F = 4.32$ l/d, $V_{up} = 1.6$ l/d) in Fig. 4b. At the highest OLR and the lowest V_{up} (corresponding to Q_F of 3.31 l/d in Fig. 4a and 7.63 l/d in Fig. 4b both at V_{up} 0.2 m/h), BA was obtained as 1720 and 960 mg $CaCO_3$/l for the pre-settled and the chemically pre-treated POME, respectively. In this condition ($V_{up} = 0.2$ m/h), the BA concentration was not high enough to avoid process upset for chemically pre-treated POME while the process remained stable for pre-settled POME. The effect of V_{up} on system recovery was more significant at high feed flow rate due to higher alkalinity recycling and decreasing effective COD concentration at the bottom of the reactor by dilution.

3.2.2.5. Methane yield. Fig. 5a and b represent the simultaneous effects of the variables on the methane yield as contour plots for the two different types of feed. It showed that a simultaneous decrease in the variables yielded an increase in the response for both pre-settled and chemically pre-treated POME. The significant model terms for methane yield are A, B^2 and AB for both conditions (Table 3). It was found that the yield values for the pre-settled POME (Fig. 5a) were greater than the corresponding values for the chemically pre-treated one. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pre-treated POME, respectively. This was achieved at Q_F and V_{up} values of 1.01 and 0.2 m/h, respectively. It was also found that a minimum retention time longer than 1.5 d and 2.2 d, respectively for the pre-settled and chemically pre-treated POME digestion was needed to achieve high methane yield.

3.2.2.6. Solids retention time (SRT). Fig. 6a and b demonstrate effects of the two operating variables on SRT. The main effect of V_{up} (B) is not significant for the chemically pre-treated POME while it is significant model term for the pre-settled POME (Table 3). It should be noted that the ratio of maximum to minimum SRT was 10.32 and 49.87 for the pre-settled and chemically pre-treated POME, respectively. Therefore, a logarithmic function with base 10 was applied to model SRTs calculated for the chemically pre-treated POME while no transformation was needed for the pre-settled POME. From the contour plots, for pre-settled POME at low values of Q_F (less than 2.6 l/d), an increase in V_{up} caused a slight decrease in SRT

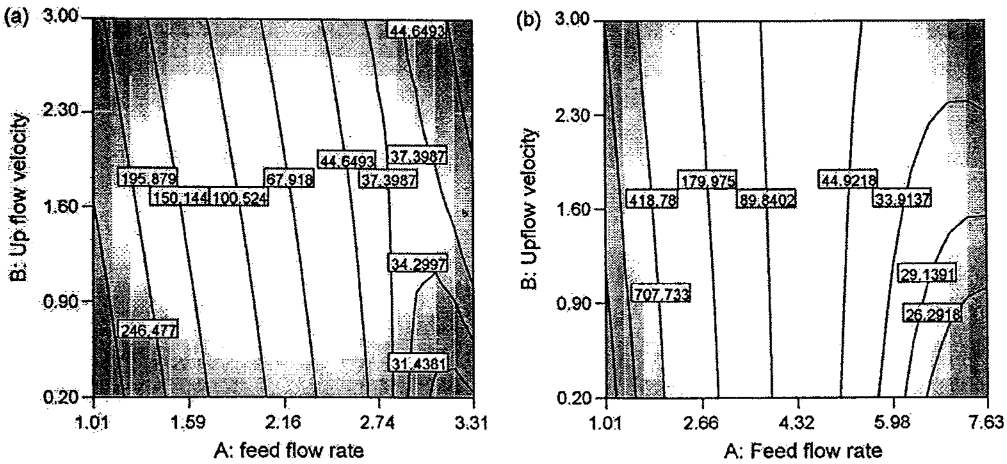


Fig. 6. Contour plot of SRT representing the effect of the feed flow rate and up-flow velocity: (a) pre-settled POME and (b) chemically pre-treated POME.

due to escape of TSS with the effluent. However, it had little effect on the response for chemically pre-treated POME. It was found that the increase in Q_F caused a decrease in SRT due to higher gas production rate, as the feed flow rate was the more influential model term to describe SRT variations as a function of the variables (Table 3). So, it can be concluded that the overall linear velocity is the effective velocity which can be calculated by addition of the liquid and gas contributions [47].

In overloading conditions (Q_F higher than 2.6 and 5 l/d, respectively, for the pre-settled and pre-treated POME), increase in V_{up} caused an increase in SRT due to improved process stability consequent of higher alkalinity recycle. It could also be due to facilitated separation of gas bubbles from the surface of the granules, which avoid gas pockets formation in the sludge bed [48]. In pre-settled POME, decrease in SRT at high range of Q_F (>4.3 l/d) and low value of V_{up} (0.2 m/h) was because of high percentage of suspended and soluble OLR (8.6 and 17.4 g COD/(l d)) and low V_{up} which made a compacted sludge blanket, so that the produced gas accumulated and suddenly buoyed the sludge. Similar condition was observed in anaerobic treatment of potato–maize wastewater in an UASB reactor [49]. The same observation was made when the reactor was operated with chemically pre-treated POME at Q_F of 7.63 and V_{up} of 0.2 m/h. The difference between these two was the responsible mechanism behind these observations. In the digestion of chemically pre-treated POME, most of the washed out sludge was disintegrated sludge due to an acidified environment that originated from high initial hydrolyzed OLR which was available and consumed by acidogenic bacteria. For this reason, the rate of acid production was larger than the rate of acid consumption. At low V_{up}, the recycle ratio (1.1) was not enough to supply the required alkalinity to buffer the reactor for such overload conditions and process upset occurred whereas with pre-settled POME sludge washout was due to high solid loading rates (SLR) and compaction of blanket as about 33% of OLR was suspended which could not be hydrolyzed as fast as the soluble fraction. Consequently, the actual OLR initially available was 17 g COD/(l d).

The sizes of the granules developed in the pre-settled POME were smaller than the granules grown on the chemically pre-treated POME. Average diameter of the granules was between 1.5 and 2.5 mm for the pre-settled POME while it increased to 2.5–3.5 mm for the chemically pre-treated POME. The granules also became denser during feeding with the chemically pre-treated POME. For example, the SVI at the bottom of UASFF reactor reached 8.1 ml/g whereas it was 11.2 ml/g at the beginning of the experiments.

3.3. Process optimization

The graphical optimization results allow visual inspection to choose the optimum operating conditions. The shaded areas on the overlay plots in Fig. 7 are the regions that meet the proposed criteria. The optimum region was identified by considering TCOD removal, pH, BA, methane yield and SRT values greater than those shown in the overlay plot. As can be seen from Fig. 7, the intersection points show the conditions where feed flow rate is maximum (1.65 l/d for pre-settled POME and 2.45 l/d for chemically pre-treated POME). Table 5 compares the responses (experimental and predicted) with respect to operating conditions (at the intersection point) for both pre-settled and chemically pre-treated POME. The results imply that pre-settled POME could be well treated with a HRT of 2.2 days and OLR of 12.9 g COD/(l d) whereas the optimum point for digestion of the chemically pre-treated POME was obtained as HRT of 1.5 days and OLR of 9.3 g COD/(l d). Based on the feed flow rate and up-flow velocity at optimum condition, recycle ratios were calculated to be 27.9:1 and 23.4:1 for the pre-settled and chemically pre-treated POME, respectively. Accordingly, the effective COD concentration at the bottom of the reactor reduced to 2.35 and 1.55 g COD/l for pre-settled and chemically pre-treated POME, respectively. This is in agreement with the value suggested by Sam-Soon et al. [50] which was in the range of 1–5 g COD/l. The accuracy of the optimum conditions from DOE experiments was checked by calculating error and standard deviation for each response. These experimental findings were in close agreement with the model prediction.

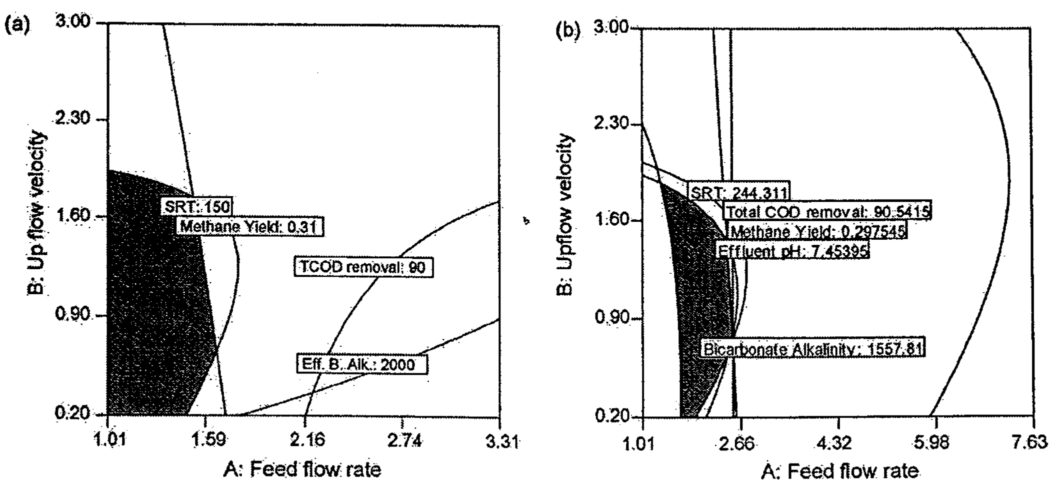


Fig. 7. Overlay plot for optimal region: (a) pre-settled POME and (b) chemically pre-treated POME.

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Table 5
Verification experiments at optimum conditions

Type of pre-treatment	Conditions	Responses					
		TCOD Removal (%)	Eff. pH	Eff. TVFA (mg acetic acid/l)	Eff. BA (mg $CaCO_3$/l)	Methane yield (l CH_4/g COD_{rem}.d)	SRT (d)
Pre-settled POME (sedimentation)							
Experimental values	Q_F = 1.65 l/d, V_{up} = 0.6 m/h	95.1	7.58	73.2	2110	0.329	147.5
Model response with CI 95%	OLR = 12.9 g/(l d), HRT = 2.2 d	93	7.53	95.8	2060	0.309	152
Error		2.1	0.05	−22.6	50	0.02	−4.5
Standard deviation		±1.48	±0.035	±15.98	±35.35	±0.014	±3.18
Chemically pre-treated POME (coagulation and flocculation)							
Experimental values	Q_F = 2.45 l/d, V_{up} = 0.75 m/h	92.62	7.53	43.1	1520	0.313	226.3
Model response with CI 95%	OLR = 9.3 g/(l d), HRT = 1.5 d	90.86	7.46	39.64	1725.2	0.299	258.3
Error		1.76	0.07	3.46	−205.2	0.014	−32
Standard deviation		±1.25	±0.049	±2.45	±145.1	±0.0099	±22.63

4. Conclusions

1. High rate anaerobic digestion of pre-settled and chemically pre-treated POME was successfully achieved at short HRTs in an UASFF bioreactor.
2. Response surface methodology was successfully applied to determine the optimum operational conditions for the anaerobic digestion of pre-treated POME. It was a potent tool to compare the results obtained from the anaerobic treatment of the two different types of pre-treated POME with different characteristics.
3. The chemical pre-treatment approach was shown to be more predictable, reliable and practical as sludge produced was very compressible and was easy to separate.
4. At a comparable range of Q_F and V_{up}, the pre-settled POME yielded slightly better reactor performance in terms of COD removal (%), bicarbonate alkalinity and methane yield. At an OLR of about 16.5 g COD/(l d) (center point), higher COD removal efficiency was achieved compared to that of the chemically pre-treated POME despite about 33% of organics in the pre-settled POME was contributed by suspended solids.
5. The optimum conditions for digestion of the pre-settled and chemically pre-treated POME were determined as Q_F of 1.65 l/d, V_{up} of 0.6 and Q_F of 2.45 l/d, V_{up} of 0.75, respectively. The experimental findings were in close agreement with the model predictions.

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EES-45

Effects of Operating Variables on the Performance of an Up-flow Anaerobic Sludge Fixed-film (UASFF) Reactor Treating Palm Oil Mill Effluent

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Abstract

Biological treatment using a combination of suspended and attached growth systems in an up-flow anaerobic sludge fixed film (UASFF) reactor was used for treatment of palm oil mill effluent (POME). Raw POME is a thick brownish colloidal slurry of water, oil and fine cellulosic fruit debris and most of its organic matter are digestable. The reactor was operated as a high rate digestion system which was the most effective, rapid and economic biological treatment method as a large amount of granular sludge could be accumulated in a small reactor. Studies of the effects of operating variables such as hydraulic retention time (HRT) and recycle ratio (R) were carried out at an average influent chemical oxygen demand (COD) concentration of 13880 mg/l and pH of 4. The HRT and recycle ratio were varied from 0.48 to 3.62 days and 1.1 to 236, respectively. The performance of the reactor was assessed based on changes in COD, bicarbonate alkalinity, pH, total and volatile suspended solids, volatile fatty acids and methane production rate. The results showed that in this system, favorable environment for POME digestion to methane could be achieved by internal alkalinity supplementation through regulation of the feed flow rate and the recycle ratio. A COD removal more than 80 percent was achieved at HRT of less than 12 h. A minimum R of 2.68 which could keep the anaerobic process stable was obtained at an OLR of 16.43 with V_{up} of 0.2 m/h while the SRT was 78 d.

Key words: UASFF reactor, anaerobic digestion, HRT, recycle ratio, POME

Introduction

Palm oil is basically a vegetable oil which is one of the most important economic sources in Malaysia. Today, Malaysia is the world's largest producer and exporter of palm oil (12.58 million tonnes production and 22.74 million tonnes world exports in 2004) [1].

During the palm oil extraction process, approximately 1.5 tonnes wastewater is produced for every one tonne of fresh fruit bunch (FFB) processed by the mill [2]. The palm oil mil effluent (POME) comprises a combination of the three main sources viz. clarification (60%), sterilizing (36%) and hydrocyclone (4%) units [3]. The raw POME is a thick brownish liquid with average values of 50000 mg/l chemical oxygen demand (COD) and 25000 mg/l biochemical oxygen demand (BOD) discharged at a temperature of between 80 °C and 90 °C. It is acidic with a pH typically between of 4-5 [4-6].

This highly polluting wastewater can therefore cause severe pollution of surface water sources due to oxygen depletion and other related effects. Considering the highly organic character of POME, anaerobic based treatment processes are the most suitable approach [7]. An anaerobic baffled reactor (ABR) was used treating POME without pH adjustment at various recycle ratios [8]. A COD removal efficiency of 84.6% was achieved at an HRT of 2.5 days, initial COD concentration of 24850 mg/l and recycle ratio of 25:1. In this study, it was concluded that a recycle of more than 15 times is needed to maintain the system pH higher than 6.8 without alkalinity addition. A membrane anaerobic system (MAS) was examined to treat POME [9]. The efficiency of COD removal was between 91.7 to 94.2% with an average HRT of 3.03 days. A clear final effluent was produced but membrane flux rate deterioration was observed due to membrane fouling.

Anaerobic treatment of POME in a two stage Upflow anaerobic sludge blanket (UASB) and a single UASB has been investigated [10, 11]. A 96 % COD removal has obtained at an OLR of 10.6 g COD /l.d in a single UASB while at highest influent COD (42500 mg/l) reactor instability was observed. A 90 % reduction in COD at an average organic loading rate (OLR) of 15 g COD/l.d was achieved in two-stage UASB. Performance of an anaerobic

hybrid digester was evaluated by Borja *et al.* [12]. At a hydraulic retention time (HRT) of 3.5 days and an OLR of 16.2 g COD /l.d, COD removal and methane yield obtained 92.3 % and 0.335 lCH_4/$COD_{removed}$, respectively. An up-flow anaerobic sludge blanket reactor was also examined treating POME [13]. High COD removals of 89 and 97% were achieved at HRT of 1.5 and 3 day, respectively. The use of packing media in the middle portion managed to reduce channeling problem and loss of biomass due to flotation associated with poorly performing UASB reactors.
The upflow anaerobic sludge blanket fixed film (UASFF) bioreactor as an anaerobic hybrid reactor is a combination of an upflow anaerobic sludge blanket (UASB) reactor and an immobilized cell or fixed film (FF) reactor [14]. The FF portion was positioned above the UASB section prevents sludge washout and helps in retaining a high biomass concentration in the reactor. Several researchers have successfully used the UASFF reactor to treat various kinds of wastewaters such as starch, swine and slaughterhouse [15-17].

Despite the advantages offered by the UASFF reactor, information on the effects of operating variables on reactor performance is still lacking. Thus, in this study the simultaneous effects of HRT and recycle ratio were investigated.

Approach and Methods

Wastewater preparation

The Palm Oil Mill Effluent (POME) was collected from a near-by palm oil mill. Raw POMÉ was chemically pretreated to remove suspended solids and residual oil using cationic and anionic polymers. The samples were stored in a cold room at 4oC. This storage technique caused no observable effect on composition. The characteristics of the raw and pretreated POME are summarized in Table 1.

Table 1 - Characteristics of the Raw and Pretreated POME[a]

Parameter	Raw POME	Pretreated POME
BOD (mg/l)	22700	9750
COD (mg/l)	44300	13880
Soluble COD (mg/l)	17140	13880
TVFA (mg actic acid/l)	2510	2760
SS (mg/l)	19780	< 20
Oil and grease (mg/l)	4850	Negligible
Total N (mg/l)	780	480
pH	4.05	4.2

[a]Values are average of three measurements. The differences between the measurements for each were less than 1%.

Bioreactor

A laboratory-scale, UASFF reactor was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 132 cm. Total volume of the reactor was 4980 ml and the working volume was 3650 ml. The column consisted of three sections; bottom, middle and top.

The bottom part of the column, with a height of 80 cm was operated as a UASB reactor and the middle part of the column with height of 25 cm was operated as a fixed film reactor. The top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with a diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25% and the specific surface area of the packing material was 341 m^2/m^3. The purpose of the top section of the bioreactor was to separate sludge and biogas from the liquid effluent. An inverted funnel shaped gas separator was used to connect and channel the biogas produced to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 °C) and the temperature was maintained by circulating hot water through the bioreactor jacket. The circulated flow in the jacket was sent to a water bath to ensure isothermal operation. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the bottom of the column. The top of the UASFF reactor was connected to a water displacement gas meter to measure the volume of biogas produced.

Start-up and Bioreactor operation

The inoculum used for seeding of the reactor was an equal proportion mixture of sludge taken from a drainage channel bed of Perai industrial zone (Butterworth, Malaysia), digested sludge from a food cannery industry and animal manure. The sludge was passed through a screen to remove debris and solid particles before seeding the reactor. The total volatile solids concentration of the seed was 10300 mg/l. Detail information has been reported elsewhere [13]. In order to investigate effects of HRT and R, the UASFF was operated at operating conditions mentioned in Table 2.

Analytical methods

The following parameters were analyzed according to standard methods (APHA, 1999) [14]: pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). A gastight syringe (Hamilton CO., Reno, Nevada, USA) was used to draw gas samples for analysis using a gas chromatograph (Perkin Elmer, Auto system XL), equipped with thermal conductivity detector (TCD) and data acquisition system with computer software (Total Chrom). The gas chromatograph had a Carboxen 1000, with 100/120 mesh (Supelco, Park, Bellefonte, PA, USA) column. The column temperature was initially maintained at 40 °C for 3.5 min, followed by an automatic increase to 180 °C at a rate of 20 °C/min. The injector and detector temperatures were 150 and 200°C, respectively. The carrier gas (He) flow rate was set at 30 ml/min.

The liquid samples for volatile fatty acids (VFAs) determination were analyzed using another gas chromatograph (GC), HP 5890 Series II (Hewlett Packard, Avondale, PA, USA) equipped with flame ionization detector (FID) and an integrator (HP 3396). A 2 m x 2 mm stainless steel, 80/120 mesh Carbopack B-DA/4% Carbowax 20M (Supelco) column was used. The oven temperature was maintained at 175 °C. The injector and detector temperatures were 200 and 220 °C, respectively. The carrier gas (He) flow rate was set at 40 ml/min. Effluent samples were filtered using Whatman GF/C before injecting into the GC to prevent clogging of the column.

Table 2 - Operating Conditions of the Experiments

Run no.	Feed flow rate (Q_F) (l/d)	HRT (d)	OLR (g COD /l.d)	Up-flow velocity (V_{up}) (m/h)	Recycle ratio (R)
1				0.2	14.79
2	1.01	3.62	3.83	1.6	125.35
3				3.0	235.9
4				0.2	2.68
5	4.32	0.85	16.43	1.6	28.48
6				3.0	54.28
7				0.2	1.09
8	7.63	0.48	29.02	1.6	15.69
9				3.0	30.29

The dry weight of the attached biofilm per unit wetted surface area of pall rings (X) was determined by drying a Pall ring at 80 °C for 24 h before and after biofilm attachment.

Results and discussion

Figure 1 shows the effects of variables on the TCOD removal efficiency. The COD removal decreased as HRT decreased (from run 1 to 9). As the recycle ratio was increased, the effect of HRT on COD removal was reduced. The effect of the R was different at constant HRT. At high HRTs (run 1-3), the COD removal decreased with an increase in the recycle ratio. An opposite trend was observed at the highest feed flow rate (7.63 l/d) due to high OLR and short HRT.

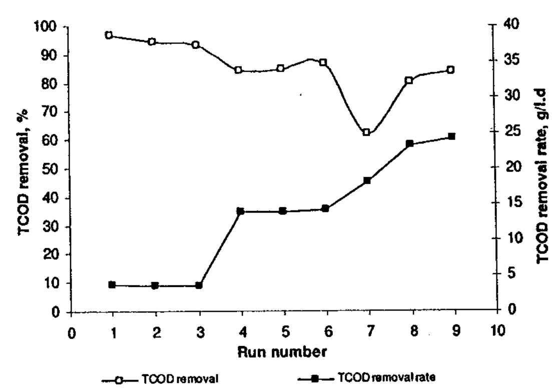

Figure 1. Profiles of TCOD Removal Efficiency and TCOD Removal Rate

In these conditions, R causes the anaerobic process to remain stable as the rate of effluent alkalinity recovery increases due to higher recycle ratio. OLR at the highest feed flow rate was 29.02 g COD/l.d. At such high OLR, TCOD removal reduced to 62.2% with an effluent TCOD of 5250 mg/l due to incomplete anaerobic digestion. Whereas the trend of COD removal at the low organic loading (high HRT) might be attributed to reduction in the diffusion rate of substrate into granules due to higher flow velocity [19], the trend at high organic loading (high Q_F) suggested that at higher organic load, the substrate concentration had a stronger effect on the diffusion rate than the flow velocity. A drastic decrease in the TCOD was observed in run 7 where the HRT and recycle ratio were 0.48 d and 1.1, respectively. It was attributed to the upset in the balance between acid production rate and acid consumption rate caused by high OLR (29. g COD/l.d) and very low recycle ratio. At this HRT, an increase in the recycle ratio produced a positive effect on process recovery through the elimination of organic shock resulted from increasing internal dilution. However, the TCOD removal efficiency and rate increased from 62.2 to 83.8% and 18 to 24.2 g COD/l.d, respectively.

Figure 2 shows the effluent TVFA and BA at different operating conditions. It was observed that the effect of R values higher than about 15 (corresponding to V_{up} higher than 1.6 m/h) on the VFA reduction was minimal. At the lowest HRT (0.48 d), when TVFA was being increased due to high OLR and acidification process, increasing of R was not as effective as the lowering of OLR as the effluent was poor in buffer capacity and in fact effluent VFA was being recycled (Effluent VFA=1613 mg/l). It was found that BA was produced through POME digestion reactions, so that the pH remained well controlled; except under overloading conditions (HRT=0.48 d, R=1.1) in which, the balance between acidogenesis and methanogenesis process was upset resulting in higher VFA production (incomplete digestion). In this condition, if the pH was not controlled the existing alkalinity would be neutralized.

At the highest feed flow rate (7.63 l/d, corresponding to an HRT of 0.48 h), BA decreased from 1890 to 915 mg $CaCO_3$/l while the TVFA to TA ratio was 0.78. This was due to the accumulation of VFA that resulted from overloading (OLR=29.02 g COD/l.d). In this condition (where reactor destabilization was observed), the effluent VFA was detected to consist of 953.46, 526.31 and 342.89 mg/l acetic acid, propionic acid and butyric acid, respectively.

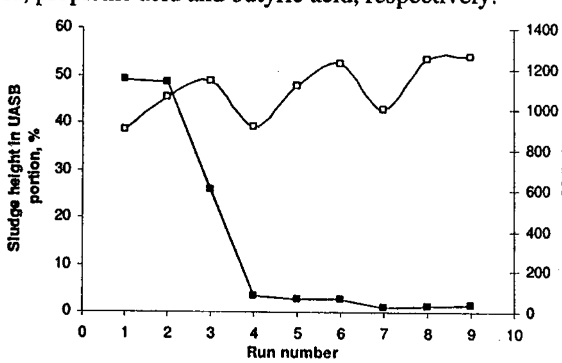

Figure 2 - Profiles of Effluent TVFA and BA

Instability of the reactor was attributed to decrease in pH (from 7.1 to 6.64) and alkalinity (from 1890 to 915 mg $CaCO_3$/l) as after pH adjustment, the system recovered. The accumulation of VFA is a typical reactor response during overloading and during sudden variation in hydraulic and organic loading rates [20].

Figure 3 depicts the effect of operating variables on the effluent TSS and VSS. It shows that the more significant factor on the washout phenomenon was the feed flow rate which also affected the gas production rate. Since TSS content of influent was negligible, the effluent TSS represent biomass wash out rate from the reactor. The VSS to TSS ratio in the effluent was same as that for the biomass in the reactor (0.72-0.78). An interesting observation was made when the reactor was operated at low HRT (0.48 d) and R of 1.1. The gas produced was initially not able to escape from the sludge due to the compaction of the sludge blanket. After several hours of operation the accumulated gas suddenly buoyed the sludge and caused a relatively high washout from the reactor. It was also observed that some of the granulated sludge disaggregated and then washed out due to shock load.

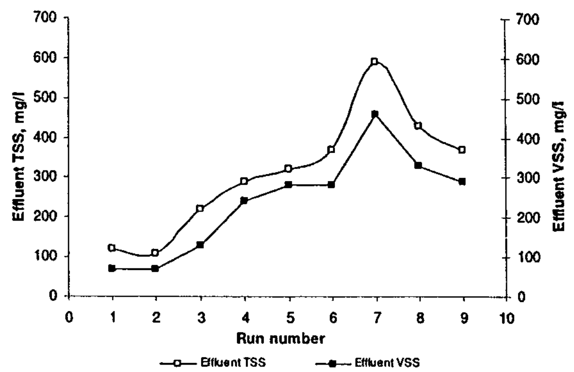

Figure 3 – Profiles of Effluent TSS and VSS

Figure 4 shows the methane production rate and methane fraction in biogas at different operating conditions. It shows that an increase in HRT along with decrease in R yielded an increase in the methane production rate except in the highest OLR (corresponding to HRT of 0.48 d) and lowest R. The maximum methane production rate was obtained with run 8 in which, the HRT and R were 0.845 d and 15.69, respectively. Reducing effect of increase in V_{up} of values higher than 1.6 m/h was observed while at lower values it had an increasing effect by the reason of adsorption capacity in recycle effluent. This effect was observed at HRT of 3.62 and 0.845 d. At lowest HRT, with increasing R, CH_4 percentage was also increased (OLR effect was dominant). It was an indication of positive effect of R in the system recovery in terms of methane fraction in the biogas. An offensive and unpleasant odor was produced when the system upset occurred (run 7). The offensive odor was also reported in literature when POME was used in an acidogenic UASB reactor [11].

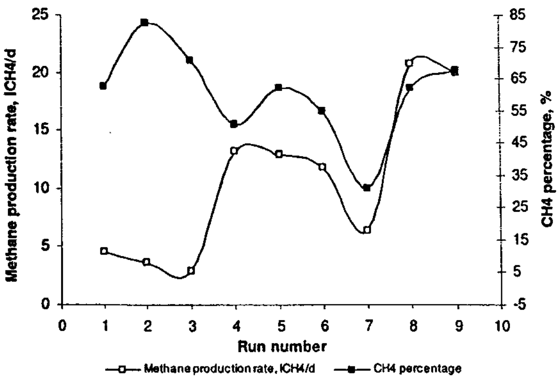

Figure 4 - Profiles of Methane Production Rate and Methane Percentage in the biogas

Figure 5 demonstrates effects of variables on the height of sludge blanket and SRT. It was found that with a simultaneous increase in both variables sludge height was also increased. The more effectual variables on the sludge height was R which is directly related to V_{up}. Figure 5 also shows the SRT variation as a function of the variables. From the Figure, as the variables increased, sludge age showed an opposite trend due to high turbulence and hydrodynamic instability created from flow velocity and gas bubble production. The size of the granules developed into bigger granules in compare with those grown in POME containing high TSS [13].

Average granules diameter of 2.5-3.5 mm was obtained as compared to 1.5-2.5 mm before [13]. The granules became denser during the experiments. However, SVI at sampling port S1 reached 8.13 ml/g while it was 11.2 ml/g at the beginning of the experiments.
Internal packing had an important role to shorten the reactor recovery time by entrapping disaggregated sludge and recirculation it to sludge blanket. As a result the system was recovered after less than 12 h at the highest OLR.

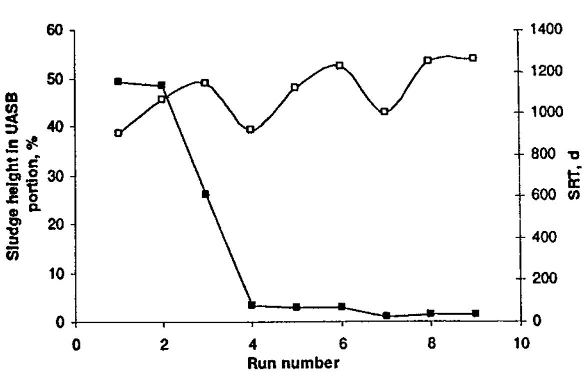

Figure 5 - Profiles of Sludge Height in UASB Portion and SRT

Conclusion

At high HRT (run1-3), the COD removal decreased with increase in recycle ratio. An opposite trend was obtained at the lowest HRT (corresponding to feed flow rate of 7.63 l/d) due to high OLR. A minimum R of 2.68 which could keep the anaerobic process stable was obtained at an OLR of 16.43 with V_{up} of 0.2 m/h while the SRT was 78 d. The internal packing had an important role to shorten reactor recovery time through entrapping the disaggregated sludge and it's recirculation it to the sludge blanket. After less than 12 h the system was recovered at the highest OLR.

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Effect of Physical and Chemical Pretreatment on Palm Oil Mill Effluent Digestion in an Up-flow Anaerobic Sludge Fixed Film Bioreactor

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Abstract

An up-flow anaerobic sludge fixed film (UASFF) bioreactor was used to treat the pretreated palm oil mill effluent (POME). In physical pretreatment, POME was pre-settled for 2 h and the supernatant was fed into the reactor. In chemical pretreatment, optimum dosages of cationic and anionic polymers were used. Experiments of pretreated POME digestion were conducted based on a central composite face-centered design (CCFD) with two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}). The operating variables were varied to cover a wide range of organic loading rate (OLR) from 3.8 to 29 g COD /l.d. Four dependent parameters were measured as response factors. These parameters were total COD (TCOD) removal, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA), and methane yield (Y_M). A stable TCOD removal efficiency of 83.5 % was achieved at the highest Q_F (3.31 l/d, corresponding to OLR of 26 g COD/l.d) for pre-settled POME whereas only 62.2 % TCOD removal was achieved with chemically pretreated POME at Q_F of 7.63 l/d (corresponding to OLR of 29 g COD/l.d) and that too was coupled with process instability. The highest level of yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, respectively, where Q_F and V_{up} were 1.01 and 0.2 m/h, respectively. The obtained results provided valuable information about interrelations of quality and process parameters at various operating conditions and also showed the effects of suspended solids on the reactor performance.

Key words: *POME, Pretreatment, UASFF reactor, Central composite face-centered design (CCFD)*

1. Introduction

Palm oil is one of main agricultural products in Malaysia as it contributes 49.5 % of the total world production [1]. On an average standard palm oil mills, for each tonne of fresh fruit bunch (FFB) processed, generates about 1 tonne of liquid waste with a pollution load of biochemical oxygen demand (BOD) 37.5 kg, chemical oxygen demand (COD) 75 kg, suspended solids (SS) 27 kg and oil and grease 8 kg [2]. There are currently about 265 active palm oil mills in Malaysia with a combined annual CPO production capacity of about 13 million tonnes [3]. This amounts to a population equivalent of around 80 million in terms of COD. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the economy in good conditions.

Considering the highly organic character of palm oil mill effluents (POME), anaerobic process is the most suitable approach for its treatment [4]. The common practice of treating POME is by using ponding and/or open digestion tank systems which have particular disadvantages such as: long hydraulic retention time of 45-60 days [5], bad odour, difficulty in maintaining the liquor distribution to ensure smooth performance over huge areas and difficulty in collecting biogas which could have detrimental effects on environment [6, 7].

High-rate anaerobic reactors that can retain biomass, have a high treatment capacity and hence low site area requirement [8]. POME COD removal efficiencies in excess of 85% have been reported for high rate reactors such as anaerobic baffled reactor (ABR) [9], single up-flow anaerobic sludge blanket (UASB) reactor [10], two stage UASB system [11] and membrane anaerobic system (MAS) [12]. In summary, the high rate anaerobic reactors mentioned above are successfully able to treat POME at short HRT.

The UASB reactor exhibits positive features, such as allows high organic loadings, short hydraulic retention time (HRT) and has a low energy demand, especially for POME treatment [10, 13]. Suspended and colloidal components of POME in the form of fat, protein, and cellulose have adverse impact on UASB reactor performance and can cause deterioration of microbial activities and wash out of active biomass [10, 14]. The use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the above mentioned problems [2, 15]. Process instability was observed when a UASB reactor (at HRT of 4 d) and an anaerobic hybrid reactor (AHR) (at HRT of 3.5 d) were operated with high influent COD concentrations of 42500 and 65000 mg/l, respectively [10, 15]. Consequently, complete digestion of raw POME without pretreatment demands high HRT, which is not easily achieved due to high volume of POME produced by the mills. Various pretreatment approaches have been examined for the separation of suspended solids and oil and grease from POME. These include: Chemical coagulation and flocculation [16-18]; air flotation simple and skimming [19, 20]; ultra-filtration [21, 22]; evaporation [23]; centrifugation [20].

The present research is a comparative studies of anaerobic digestion of POME pretreated physically (primary sedimentation unit) and chemically (chemical coagulation and flocculation). Results obtained from the high rate digestion of pretreated POME were compared using response surface methodology (RSM) with respect to the simultaneous effects of two independent operating variables, feed flow rate (Q_F) and up-flow velocity (V_{up}).

2. Material and Methods

2.1 Wastewater Preparation

Raw POME was collected from a local palm oil mill in Nibong Tebal, Penang, Malaysia. In the first stage, raw POME was pre-settled using an ordinary sedimentation tank. In the second part of this study, raw POME was chemically pretreated to remove suspended solids and residual oil (using a cationic and an anionic polymers). The samples were then stored in a cold room at 4 °C. PMOE stored under such condition had no observable effect on its composition. The characteristics of the raw and pretreated POME are summarized in Table 1.

2.2 Bioreactor and start up

A laboratory-scale, up-flow anaerobic sludge fixed film (UASFF) reactor was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml, and the working volume was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with a height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25 % and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere [2].

Table 1. *Characteristics of the raw, pre-settled and pretreated POME*[a]

Parameter	Raw POME	Pre-settled POME	Chemically Pretreated POME
BOD_5 (mg/l)	22700	20100	9750
COD (mg/l)	44300	28640	13880
Soluble COD (mg/l)	17140	17140	13880
TVFA (mg acetic acid/l)	2510	2510	2760
SS (mg/l)	19780	5760	< 20
Oil and grease (mg/l)	4850	1 630	Negligible
Total N (mg/l)	780	660	480
pH	4.05	4.05	4.2

[a]Values are average of three measurements. The differences between the measurements for each were less than 1%.

23 Bioreactor Operation and Experimental Design

The UASFF bioreactor was separately operated with pre-settled and chemically pretreated POME and experiments were designed by Design Expert software (Stat-Ease Inc., version 6.0.6) with two variables, feed flow rate and up-flow velocity. In an earlier study [2], feed flow rate (Q_F) and up-flow velocity (V_{up}) were found to be the most critical independent operating variables which affected the performance of the reactor. The region of exploration for POME treatment was decided as the area enclosed by Q_F (1.01, 3.31 l/d) and V_{up} (0.2, 3 m/h) boundaries for pre-settled POME and Q_F (1.01, 7.63 l/d) and V_{up}(0.2, 3 m/h) for chemically pretreated POME. This would cover an OLR range of 7.9 to 26.0 and 3.8 to 29.0 g COD/l.d for pre-settled and chemically pretreated POME respectively. Steady state was assumed after five turnovers.

In order to carry out a comprehensive analysis of the anaerobic process, 4 dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, effluent total volatile fatty acid (TVFA), effluent bicarbonate alkalinity (BA) and methane yield (Y_M).

Three dimensional (3D) plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The RSM used in the present study was a Central Composite Face-centered Design (CCFD) involving two different factors, Q_F and V_{up}. The experimental conditions and results are shown in Table 2.

2.4 Analytical Methods

The following parameters were analyzed according to Standard Methods [24]: pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively [2].

Table 1. *Experimental conditions and results of central composite design*

Run	Type of pretreatment	Variables		Response			
		Factor1 A:Feed flow rate (l/d)	Factor2 B:Up-flow velocity (m/hr)	Total COD removal (%)	Eff. TVFA (mg acetic acid/l)	BA (mg $CaCO_3$/l)	Methane Yield (l CH_4 /g COD_{rem}.d)
1	Pre-settled POME	1.01	0.2	97.3	132.4	2006	0.344
2		1.01	1.60	93.9	104.2	2026	0.298
3		1.01	3.00	93.1	80.54	2087	0.258
4		2.16	0.20	90.7	243.1	1957	0.295
5		2.16	1.60	91.3	35.17	2225	0.305
6		2.16	1.60	91.5	44.2	2208	0.303
7		2.16	1.60	91.4	36.1	2234	0.315
8		2.16	1.60	90.3	58.8	2219	0.305
9		2.16	1.60	92.7	38.3	2162	0.29
10		2.16	3.00	95.5	41.9	2210	0.25
11		3.31	0.2	82.7	573.75	1723	0.196
12		3.31	1.60	91.8	144.5	2157	0.292
13		3.31	3.00	94.2	137.8	2162	0.285
1	Chemically pretreated POME (coagulation and flocculation)	1.01	0.2	96.7	64.86	1394	0.335
2		1.01	1.60	94.3	23.1	1504	0.273
3		1.01	3.00	93.24	22.1	1664	0.226
4		4.32	0.20	84.3	146.1	1776	0.261
5		4.32	1.60	85.2	33.15	1896	0.29
6		4.32	1.60	84.5	37.49	1833	0.285
7		4.32	1.60	86	34.7	1915	0.275
8		4.32	1.60	87.6	33.99	1896	0.27
9		4.32	1.60	84.5	36.07	1854	0.255
10		·4.32	3.00	86.5	37.92	1893	0.228
11		7.63	0.2	62.2	1613.2	915	0.109
12		7.63	1.60	80.2	289.68	1474	0.244
13		7.63	3.00	83.8	251.02	1312	0.224

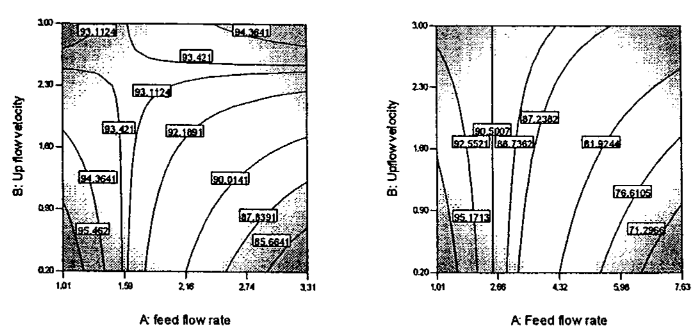
3. Results and Discussion

Raw POME contains high concentration of suspended solids (Table 1) which require long retention time for satisfactory digestion. A fraction of TSS which is not digestable is gradually accumulated in the reactor by attaching to the sludge granules in the UASFF reactor as a granular sludge reactor and it causes reduction in process efficiency. From a practical point of view and according to various studies [2, 10, 11, 15], the oil-bearing suspended solids need to be removed (partially or completely) before anaerobic treatment in order to have a reliable, stable and efficient high rate anaerobic process.

3.1 POME digestion

31.1 TCOD removal

The effect of the variables on TCOD removal efficiencies are shown in Fig 1a and b as contour plots for pre-settled and chemically pretreated POME, respectively. Since the pre-settled POME contained 5760 mg/l total suspended solids (TSS), a fraction of the OLR is suspended solids whereas the entire OLR is soluble for chemically pretreated POME. In an overall comparison, the trend of changes in TCOD removal efficiency is almost similar for both conditions. The TCOD removal (%) decreased with increased Q_F. As the up-flow velocity was increased, the effect of Q_F on COD removal was reduced. A stable TCOD removal efficiency of 83.5 % was achieved at the highest Q_F (3.31 l/d, corresponding to OLR of 26 g COD/l.d) for pre-settled POME whereas only 62.2 % TCOD removal was achieved with chemically pretreated POME at Q_F of 7.63 l/d (corresponding to OLR of 29 g COD/l.d) and that too was coupled with process instability. It was found that at the same OLR (center points, V_{up} from o.2 to 3 m/h), despite about 33% of OLR in the pre-settled POME being suspended solids, which needed to be hydrolyzed first and greater COD removal efficiency (90-94 %) was achieved compared to chemically pretreated POME (82-88 %). This may be attributed to possible inhibitory effects of the polymers which were applied for chemical pretreatment.



(a) (b)

Figure 1. *DESIGN-EXPERT plot. Contour plot of TCOD removal efficiency representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME*

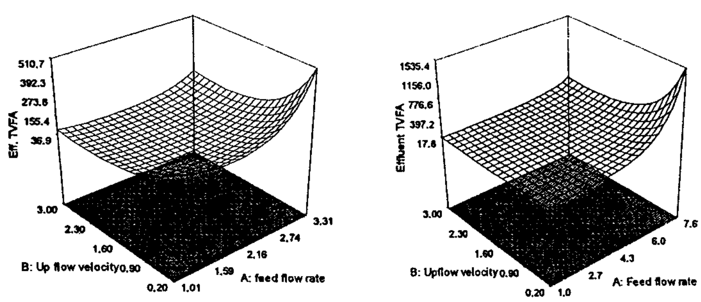
3.12 Effluent TVFA

The VFA concentration is a key indicator of system performance. Figure 2a and b depict the effects of the variables on the effluent VFA for the pre-settled and the chemically pretreated POME, respectively. The highest concentrations of VFA as intermediates was found during overloading conditions when they were 553 mg/l for the pre-settled POME at OLR of 26 g COD/l.d and 1613 mg/l for the chemically pretreated POME at OLR of 29 g COD/l.d. In both experiments, the role of V_{up} in system recovery at high Q_F was very significant due to the increase in recycled alkalinity. From the 3D graph, at comparable OLRs i.e. 16.95 g COD/l.d (Q_F=2.16 l/d) for pre-settled POME and 16.42 g COD/l.d

(Q_F=4.32 l/d) for chemically pretreated POME, the VFA concentrations for the two cases were also similar. It showed that there was a balance between acetogenesis and methanogenesis in the systems at this OLR. In the chemically pretreated POME digestion, process upset occurred as methanogenic bacteria could not metabolize the VFA as fast as they were produced; resulting in a possible reduction in pH.

3.1.3- Effluent BA

Figure 3a and b show interactive effects of Q_F and V_{up} on bicarbonate alkalinity (BA). It was understood that the BA was produced through POME digestion reactions as no chemical was added to supply alkalinity. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables.



(a) (b)

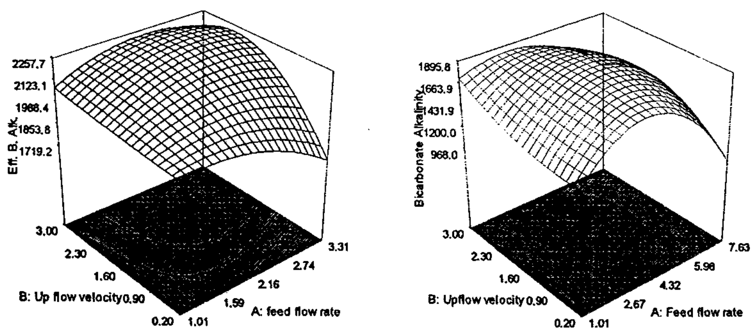
Figure 2. *DESIGN-EXPERT plot. 3D graph of effluent TVFA representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME*

From Figure 3a, the maximum level for BA was predicted to be the region where Q_F and V_F were relatively high (the values larger than center point) while it was obtained as around the middle part of the design space in Figure 4b. At the highest OLR and the lowest V_{up} (corresponding to Q_F of 3.31 l/d in Figure 3a and 7.63 l/d in Figure 4b both at V_{up} 0.2 m/h), BA was obtained as 1720 and 960 mg $CaCO_3$/l for the pre-settled and the chemically pretreated POME, respectively. In this condition (V_{up}=0.2 m/h), the BA concentration was not high enough to avoid process upset for chemically pretreated POME while the process remained stable for pre-settled POME. The effect of V_{up} on system recovery was more significant at high feed flow rate due to more alkalinity recycling.

3.1.4Methane yield

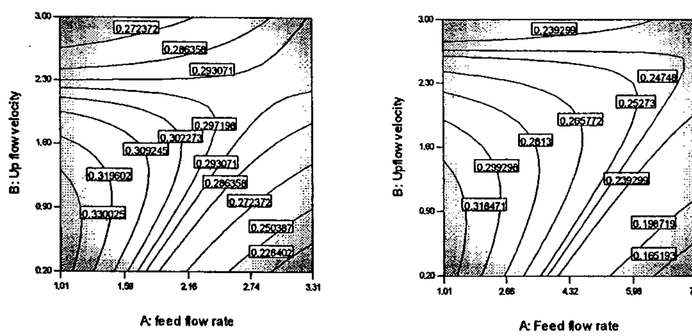
Figure 4a and b represent the simultaneous effects of the variables on the methane yield as contour plots. It showed that a simultaneous decrease in the variables yielded an increase in the response for both pre-settled and chemically pretreated POME. It was found that the yield values for the pre-settled POME (Figure 4a) were greater than the values for the chemically pretreated one. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, respectively where Q_F and V_{up} were 1.01 and 0.2 m/h respectively. It was also found that a minimum retention time longer than 1.5 and 2.2

d, respectively, for the pre-settled and chemically pretreated POME digestion was needed to achieve high methane yield.



(a) (b)

Figure 3. *DESIGN-EXPERT plot. 3D graph of effluent BA representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME*



(a) (b)

Figure 4. *DESIGN-EXPERT plot. Contour plot of SRT representing the effect of the feed flow rate and up-flow velocity (a) Pre-settled POME (b) Chemically pretreated POME*

4. Conclusion

Response surface methodology was a potent tool to compare the results obtained from the anaerobic treatment of the two different types of pretreated POME with different characteristics. At OLR of about 16.5 g COD/l.d (center point), despite about 33% of OLR in the pre-settled POME being suspended solids, greater COD removal efficiency was achieved compared to chemically pretreated POME. Effluent BA values for pre-settled POME were greater than the values for chemically pretreated POME within the tested range of the two variables. The highest level of the yield was 0.34 and 0.33 for pre-settled and chemically pretreated POME, respectively where Q_F and V_{up} were 1.01 and 0.2 m/h respectively.

5. Acknowledgements

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EFFECT OF TEMPERATURE ON THE PERFORMANCE OF AN UP-FLOW ANAEROBIC SLUDGE FIXED FILM (UASFF) BIOREACTOR TREATING PALM OIL MILL EFFLUENT (POME)

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ABSTRACT
This study investigates the performance of anaerobic digestion of palm oil mill effluent (POME) in an up-flow anaerobic sludge fixed film (UASFF) bioreactor at four different temperatures (24, 38, 50 and 60 °C) under operating conditions obtained in an earlier study (feed flow rate (Q_F) of 1.5 l/d and up-flow velocity (V_{up}) of 0.75 m/h). Influent COD concentration was 14150 mg/l. Six quality parameters viz. COD removal, methane yield, TSS removal, oil and grease removal, effluent VFA and pH were monitored as the process response. During the first few days of operation with temperature of 50 °C, levels of up to 812 mg TVFA/l occurred in the reactor (corresponding to VFA to Alk ratio of 0.37 and pH of 6.95), which was due to the shifting from mesophilic to thermophilic operation. Three days after the drop in the treatment efficiency, the reactor recovered and a stable COD removal and methane yield of 94 % and 0.33 l CH_4/g $COD_{removed}$.d, respectively were achieved. The values were greater than those obtained in mesophilic condition (38 °C) (92 % and 0.325 lCH_4/g $COD_{removed}$.d). A 94 % oil and grease removal was achieved at a temperature of 50 °C while it was 85 and 65 % at 38 and 24 °C, respectively. At temperature of 50 °C, the granules still retained their activity and structural integrity. An increase in temperature from 50 to 60 °C caused a detrimental effect on treatment efficiency as the methanogenic activity was found to almost cease within less than 6 days.

Keywords: UASFF; POME; Temperature; Anaerobic digestion

INTRODUCTION
Palm oil mill effluent (POME) is a thick brownish liquid with average chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values of 50000 mg/l and 25000 mg/l, respectively. It is discharged at a temperature of 80 °C to 90 °C and has a pH typically between 4 and 5, [8, 11, 13]. Considering the highly organic character of POME, anaerobic process is the most suitable approach for its treatment, [10]. The anaerobic digestion process is one of the technologies used to produce energy as well as to reduce the organic content of wastes. A combination of an up-flow anaerobic sludge blanket (UASB) and an up-flow fixed film (UFF) in a single reactor showed the best performance in POME anaerobic digestion. The quantity of biogas produced as a function of the quantity of

introduced feed will be variable according to several factors such as the quantity of the organic matter and the environmental parameters [13]. The intensity of the microbial activity on which the production of methane depends, is a function of the environment temperature [11] the present study, the UASFF bioreactor was operated at optimum operating conditions obtained in an earlier study (feed flow rate (Q_F) of 1.5 l/d and up-flow velocity (V_{up}) of 0.75 m/h) [17] and the effect of four different temperature (24, 38, 50 and 60 °C) on the performance of the bioreactor was investigated.

MATERIALS AND METHOD

Wastewater Preparation

POME was collected from a local palm oil mill in Nibong Tebal (Penang, Malaysia). The samples were stored in a cold room at 4 °C. This storage technique had no observable effect on its composition. The characteristics of the POME used in this study are shown in Table 1.

Table 1: Characteristics of pretreated POME

Parameter	Amount
COD (mg/l)	14150
Soluble COD (mg/l)	11400
TVFA (mg acetic acid/l)	950
SS (mg/l)	1830
Oil and grease (mg/l)	625
TKN (mg/l)	340
pH	4.0

[a]Values are averages of three measurements. The differences between the measurements for each parameter were less than 1%.

Bioreactor and start up

A laboratory-scale, UASFF reactor (Fig. 1) was used in this study. The glass bioreactor column was fabricated with an internal diameter of 6.5 cm and a liquid height of 112 cm. Total volume of the reactor was 4980 ml (including the section containing the gas-solid separator), and the working volume (total liquid volume excluding volume of the pall rings in fixed bed section) was 3650 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as a UASB reactor, the middle part of the column with height of 25 cm was operated as a fixed film reactor and the top part of the bioreactor served as a gas-solid separator. The middle section of the column was packed with 90 Pall rings with diameter and height each equal to 16 mm. The voidage of the packed-bed reactor was 91.25 % and the specific surface area of the packing material was 341 m^2/m^3. An inverted funnel shaped gas separator was used to conduct the biogas to a gas collection tank. The UASFF reactor was operated under mesophilic conditions (38 ± 1 °C) and temperature was maintained by circulating hot water through the bioreactor jacket. In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The inoculum for seeding was an equal proportion mixture of sludge taken from a drainage channel bed of Perai Industrial Zone (Butterworth, Malaysia), digested sludge from a food cannery factory and animal manure. Details regarding the start up procedure can be found elsewhere, [9].

Bioreactor Operation

In order to study effect of temperature on the reactor performance, four different temperatures (24, 38, 50 and 60 °C) were tested. The reactor was operated under optimum conditions (obtained in Zinatizadeh *et al.*[17]), HRT of 1.5 d and V_{up} of 0.75 m/h. In this study no chemical was used.

Analytical Methods

The following parameters were analyzed according to standard methods, APHA [1] pH, alkalinity, TSS, VSS, BOD and COD. Total Kjeldahl nitrogen (TKN) was determined by colorimetric method using a DR 2000 spectrophotometer (Hach Co. Loveland, Co). Gas chromatographs equipped with thermal conductivity detector (TCD) and flame ionization detector (FID) were used for the determination of biogas and volatile fatty acid compositions, respectively, Najafpour *et al.* (2005). The dry weight of the attached biofilm per unit wetted surface area of pall rings was determined by drying a Pall ring at 80 °C for 24 h before and after biofilm attachment.

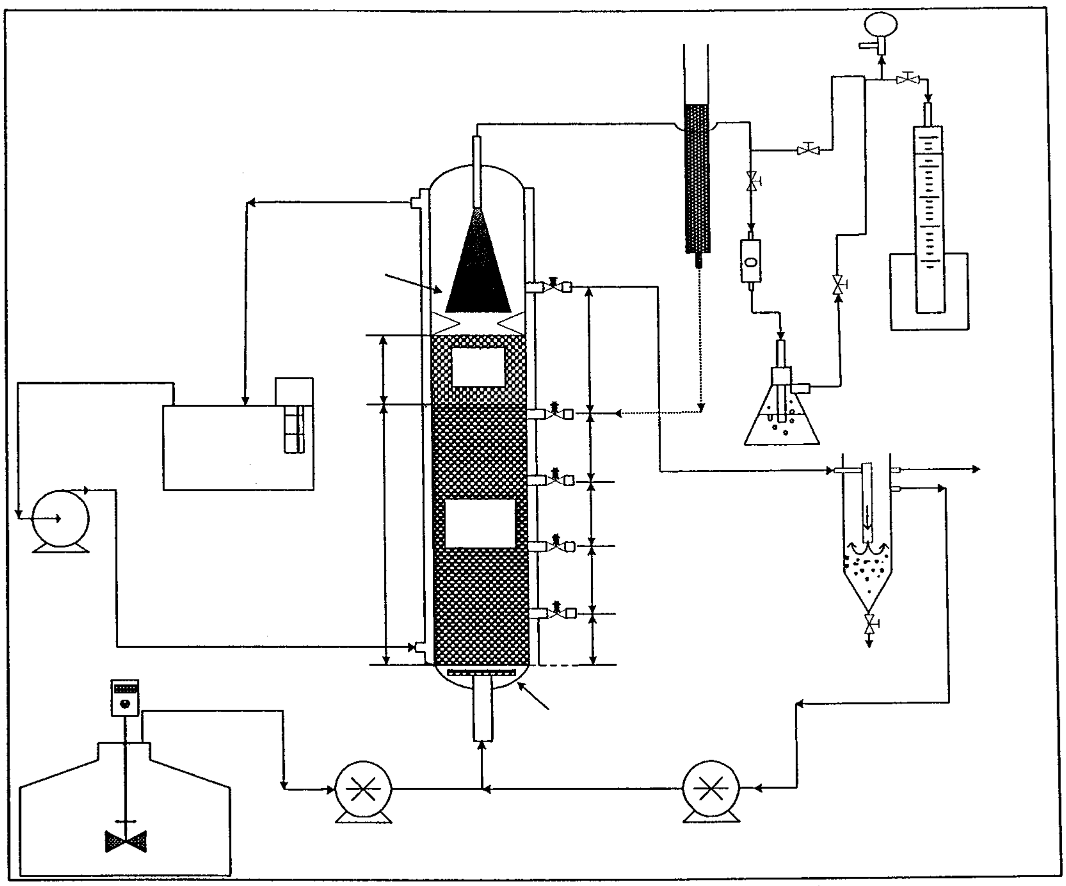

Figure 1: Schematic Diagram of the Experimental Setup.

Results and discussion

A statistic approach to compare the differences of the variation trends of each pair of the responses was carried out using the One-Way ANOVA test available in the SPSS 13.0 software. The One-Way ANOVA procedure produces a one–way analysis of variance for a quantitative dependent variable by a single factor variable. The Levene statistics obtained for each pair of the responses to test the equality of group variances are given in Table 2. The Levene statistics of the COD removal in comparison to methane yield and VFA/Alk in comparison to pH with significance value of less than 0.05 rejects the null hypothesis that the

Gas-solid separator

25 cm

UFF portion

group variances are equal [16]. The Levene statistic for comparison of the trends of TSS removal and oil and grease removal is greater than 0.05 (0.374) which indicated that the group variances of the responses were equal and temperature showed almost similar effect on the removal of TSS and oil and grease. The results are corroborated with the findings as shown in Figures 2-4.

Table 2: Levene Statistic of the Parameters Studied

	Levene Statistic	Significance
(COD removal)-(Methane yield)	29.737	<0.0001
(TSS removal)-(oil & grease removal)	0.804	0.374
(VFA/Alk)-(pH)	8.15	0.006

Figures 2-4 show effect of temperature on COD removal and methane yield (Y_M), TSS and oil and grease removal, and VFA/Alk and pH, respectively at HRT of 1.5 d. Slight changes in the responses except oil and grease removal was observed at temperatures of 24 and 38 °C. During the first few days of operation with temperature of 50 °C, levels of up to 812 mg TVFA/l occurred in the reactor (corresponding to VFA to Alk ratio of 0.37 and pH of 6.95), which was due to the shifting from mesophilic to thermophilic operation. It was also reflected in the decrease in COD removal and methane yields (Figure 2) and decrease in TSS and oil and grease removal (Figure 3). Despite the high TVFA levels, the bicarbonate alkalinity was maintained at about 1260 mg $CaCO_3$/l. Temporary drop in pH due to the increase in TVFA was reported when a mesophilic UASB was exposed to 55 °C for treatment of instant coffee production wastewater [5]. Three days after the drop in the treatment efficiency, the reactor recovered and a stable COD removal and methane yield of 94 % and 0.33 l CH_4/g $COD_{removed}$.d, respectively were achieved. The values were greater than those obtained in mesophilic condition (38 °C) (92 % and 0.325 lCH_4/g $COD_{removed}$.d). Similar results were obtained for treatment of fruit and vegetable waste in an anaerobic tubular reactor (Bouallagui *et al.*, 2004a). 90 and 65 % COD removal have been reported for POME treatment in a thermophilic completely mixed digester (HRT of 15 d) and a thermophilic anaerobic contact digester (HRT of 4.7 d), respectively [3, 4].

The effect of temperature is particularly important on the hydrolysis step (Elmashad *et al.*, 2001). The hydrolysis rate of cellulose in thermophilic conditions is about five to six times higher than that observed in mesophilic condition. A remarkable increase in oil and grease removal was observed when temperature was increased from 24 to 38 and 50 °C (Figure 3). A 94 % oil and grease removal was achieved at a temperature of 50 °C while it was 85 and 65 % at 38 and 24 °C, respectively. Oil and grease might cause inhibition to the microbial growth due to the long-chain fatty acids produced in the hydrolysis process (Rinzema *et al.*, 1994) as thermophilic organisms are more sensitive to certain inhibitory compounds than mesophilic [6, 7]. This could explain the higher TVFA levels and lower COD removal in thermophilic condition, particularly at 60 °C as suggested by Figure 4. It is noted that at a temperature of 50 °C, the granules still retained their activity and structural integrity.

An increase in temperature from 50 to 60 °C caused a detrimental effect on treatment efficiency as the methanogenic activity was found to almost cease within less than 6 days (Figure 2). It was a consequence of high concentration of VFA resulting from higher rate of hydrolysis (as shown in Figure 4) and adverse impact of the conversion process to a higher temperature on the microbial species. The detrimental effect of temperature shock from 55 to 65 °C was also reported by [15]. Negative TSS removal at 60 °C (Figure 3) indicated that in this condition, the concentration of effluent TSS was more than influent TSS due to high biomass washout. It should be highlighted that no sludge was added or recycled back into the reactor during the experiments and the amount of the sludge was maintained at about 75 g VSS/l. The solid loading rate was 0.46 g COD/g VSS.d during this study.

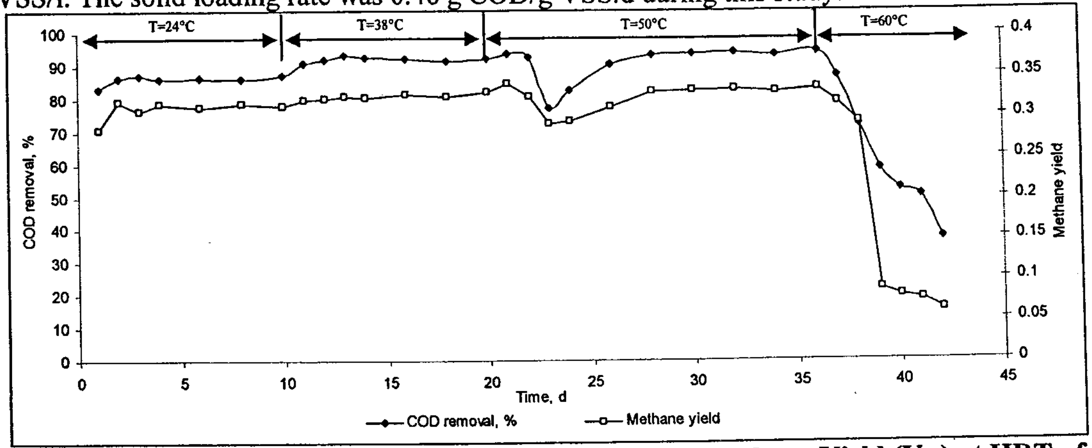

Figure 2: Effect of Temperature on COD Removal and Methane Yield (Y_M) at HRT of 1.5 d.

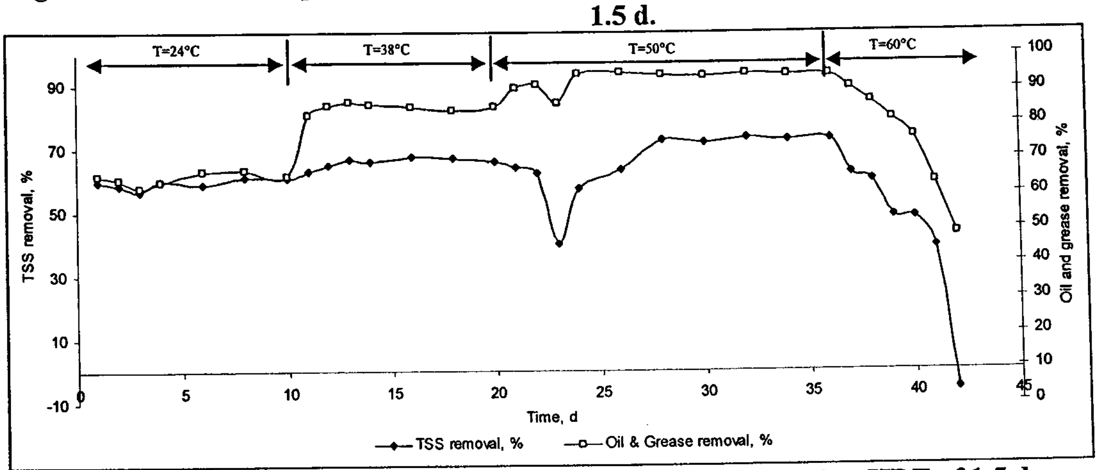

Figure 3: Effect of Temperature on TSS and O & G Removal at HRT of 1.5 d.

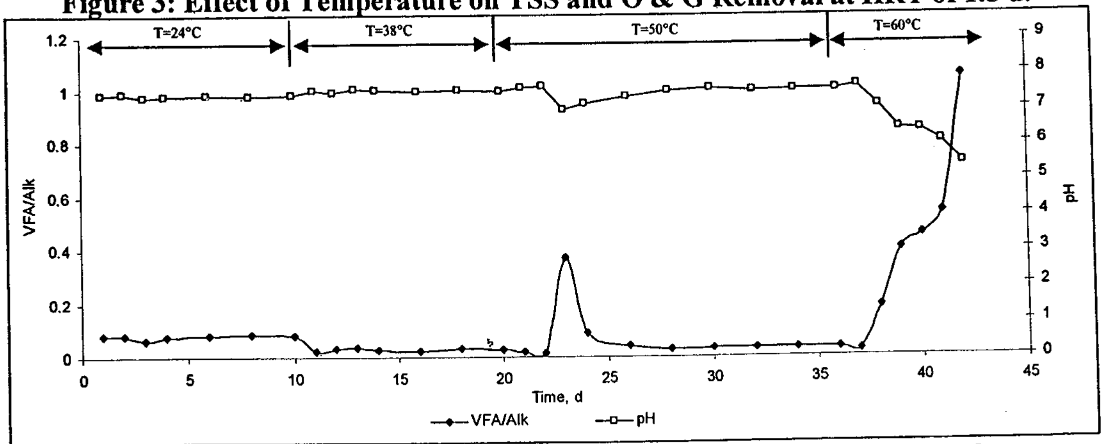

Figure 4: Effect of Temperature on VFA/Alk and pH at HRT of 1.5 d.

CONCLUSIONS

Best performance was observed in mesophilic conditions, from which it was concluded that the optimum temperature for the growth of methanogens under the conditions used for the reactors in this study appears to be 38 °C. How ever reactors could be operated even under psychrophilic and thermophilic conditions once the organisms were acclimatized to such temperatures. An increase in temperature from 50 to 60 °C caused a detrimental effect on treatment efficiency as the methanogenic activity was found to almost cease within less than 6 days.

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