FUNGAL TREATMENT OF PETROCHEMICAL EFFLUENT BY AN *ASPERGILLUS PENICILLIOIDES* (*SPEGAZZINI*) SPECIES USING THE CROSS-FLOW MICRO-SCREEN AND pH-AUXOSTAT CONCEPT

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ABSTRACT

An Aspergillus penicillioides fungal species was used to treat a petrochemical effluent with the following short chain fatty acids (SCFA), acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and n-valeric-acid. Limited information is, available on the application of Aspergillus penicillioides to industrial organic acid effluents and synthetic substrates. The general and specific growth characteristics of the species are also inadequately studied and limited. Aspergillus penicillioides (Spegazzini) was cultivated in a continuous flow reactor to treat a petrochemical effluent using a cross-flow micro-screen and pH-auxostat (pH-controller). An organic loading rate of 0.439 kg COD/m^3.d and 0.348 kg SCFA/m^3.d was applied to the reactor and more than 75 % (COD) and 80 % (SCFA) was removed. The size selective ability of the cross-flow micro-screen and the pH-metabolic rate regulating ability of the pH-auxostat (pH-controller) enhanced effective species cultivation (selection) and further treatment of the petrochemical effluent. A true protein content of 39 % and crude protein content of 55 % was determined, which compares favourably with that of other species e.g. Aspergillus fumigates (>37 % and 49 %) and Geotrichum candidum (46 % and 55 %) respectively. The growth kinetic parameters obtained for Aspergillus penicillioides on the petrochemical effluent were as follows: μ_{max} = 0.344 hr^{-1}; K_s = 103 mg COD/L; k_b = 0.0034 hr^{-1} and Y_o = 1.45 mg cells COD/mg COD removed; and on the synthetic media utilizing the same species were: μ = 0.305 hr^{-1}; k_b = 0.0022 hr^{-1} and Y_o = 0.700 mg cells COD/mg COD removed.

ABBREVIATIONS

μ	:	Specific growth rate, hr^{-1}
μ_{max}	:	Maximum specific growth rate, hr^{-1}
K_s	:	Half-saturation constant, COD/L
k_b	:	Decay constant, hr^{-1}
Y_o	:	mg cells COD/mg COD removed

INTRODUCTION

The petrochemical industry is one of the generators of organic acid effluents associated with fuels and chemicals extracted from coal. The total concentration of SCFA in these industrial effluents has varied somewhat over the decades, but has remained in the order of 1.1 to 1.3 % (m/v) (1); (10); (4). The dominant organic acids encountered display the following approximate composition (m/v) with acetic acid (HAc) the predominant acid (70 %), propionic acid (15 %), iso-butyric acid (2 %), n-butyric acid (8 %), iso-valeric acid (1 %), and n-valeric-acid (3 %) (1), (17), and (10).

According to (3), a cheaper approach to the disposal of such organic by-products has been, bioconversion to single-cell-protein (SCP) production, especially when the organic acids are readily biodegradable SCFA, e.g. acetic acid, propionic acid and butyric acid. In this regard, a simple bioreactor system can be employed to treat the effluent.

A dynamic cross-flow micro-screen technique was developed at the University of Pretoria for the selection and cultivation of microbial monocultures (8). The technique proved successful in the selection and maintenance (continuous cultivation) of fungal monocultures and the subsequent treatment of industrial organic effluents (e.g. spent sulphite liquor and petrochemical effluent).

Two different fungi, Aspergillus fumigates (11) and Geotrichum candidum (10), were selected and maintained, and showed to be effective in the treatment of the latter two effluents and the subsequent production of SCP.

Author in (12) Selected and maintained a stable microbial monoculture of Aspergillus penicillioides using the cross-flow micro-screen on petrochemical effluent. Subsequently the fungus was identified as Aspergillus penicillioides (Spegazzini) according to the keys and descriptions of (9), (20) and (16).

Furthermore, Aspergillus penicillioides is a widely distributed xerophilic fungal species (2), and is found to be a common contaminant of stored grains, nuts, spices and cereal products (9), (Pitt and Hocking, 1997 quoted by (6). SCFA oxidation studies by this species are non-existent, with insignificant publications on its application to industrial effluent treatment.

The first focus of the study combined the cross-flow micro-screen technique and the pH-auxostat (pH-controller) in an aerated continuous flow reactor. The reactor was inoculated with Aspergillus penicillioides to select and maintain the species; and subsequently treat the petrochemical effluent. The organic COD removed and cell mass concentration (total suspended solids - TSS) produced, served the following purpose: 1. To assess the treatability of the petrochemical effluent, 2. Evaluate the protein quality of the single cells produced (by comparing the amino acid profile of Aspergillus penicillioides with that of other protein sources (e.g. Aspergillus fumigates, Geotrichum candidum species, and soybean meal), and 3. to determine the growth kinetic parameters of Aspergillus penicillioides.

The second focus of the study employed an aerated batch reactor with a synthetic media (using acetic acid as carbon source). The batch data was used to assess the treatability of the synthetic media and determine comparable kinetic parameters to the continuous study. Both substrates were nutrient supplemented. After the treatability studies, Oxygen utilisation rate (OUR) data were generated for both studies, to assist with kinetic parameter determination.

MATERIALS AND METHODS - EXPERIMENTAL SET-UP

Continuous Cross-Flow Micro-Screen and Ph-Auxostat (Ph-Controller)

The cross-flow micro-screen and pH-auxostat (pH-controller) were combined into the continuous flow reactor (Figure 1) under aerated conditions. The reactor had a working capacity of 15-L during the cultivation process. A stainless steel tube reactor was used with diffused aeration for mixing. The cross-flow micro-screen was made of tubular stainless steel wire and a screening sieve. The micro-screen was positioned inside a tube internal to the main reactor.

The compressed air (having an airlift principle) maintained homogenous cross-flow conditions of the reactor content and across the internal micro-screen. The overflow level on the internal micro-screen assembly controlled the liquid level in the reactor. The temperature was controlled with a thermostat heater and the pH with the pH-controller. The reactor was fed at a constant rate (using a peristaltic pump) while simultaneously harvesting cell mass directly from the reactor (Figure 1).

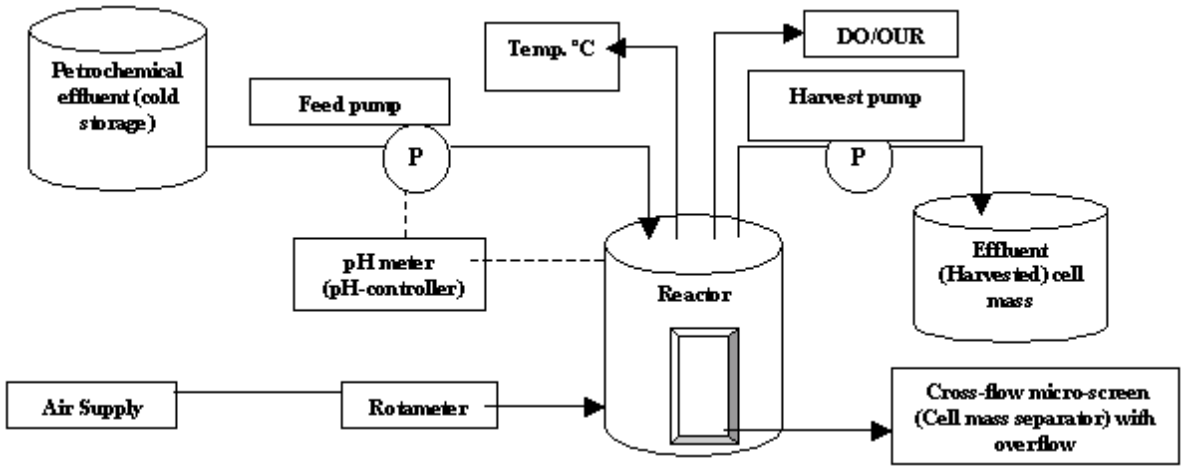

Figure 1. Combined cross-flow micro-screen and pH-auxostat (pH-controller) in the continuous flow reactor.

Aerobic Batch Reactor

An aerobic batch reactor of 5-l working volume was operated utilizing a synthetic substrate, with similar process stability variables (pH, temperature and dissolved oxygen (DO)) to the continuous flow reactor. The batch reactor served two purposes: 1. To treat the synthetic substrate, and 2. To act as an assessment measure for the kinetic parameters determined during continuous cultivation.

The OUR data were generated by the automatic aeration mechanism of the DO/OUR controller and electronic data capturing (15). The OUR data was used to determine the cell decay constant (k_b), and subsequently calculate the cell yield constant (Y). COD and SCFA data coupled with the cell yield and cell decay constants determined the specific (μ) and maximum specific growth (μ_{max}) rates as well as the half-saturation constant (K_s) of the substrates.

Petrochemical Effluent Feed and Synthetic Media (Substrate) Composition

Petrochemical Effluent Feed

A concentrated petrochemical effluent was delivered to the Water Utilization Division, South Campus. The effluent had an average organic concentration of 3191 mg COD/L; and 2784 mg SCFA/L. For experimental purposes, the effluent was diluted with tap water to a feed organic COD concentration of 1595 mg COD/L and corresponding SCFA concentration of 1392 mg SCFA/L. The diluted effluent was then supplemented with nutrients (Table 1) as described by (19) and (4), and stored at 4 ^{O}C. The cold environment provided the inhibition of bacteria and other organisms (e.g. yeast cells) competing for the same substrate.

Fresh diluted petrochemical effluent was prepared daily to feed the reactor. The nutrient supplemented petrochemical effluent had a COD: N: P ratio of 30: 2: 1 (m/m). This composition was approximate to the stoichiometric requirement of COD: N: P ratio of 33: 3: 1 (m/m) for the conversion of organic acids (4); (19), and (7), and sufficient for microbial growth.

Synthetic Media (Substrate)

The synthetic media utilized during the batch studies had the following chemical compositions in terms of macro and micronutrients, Table 1.

Macro-nutrient(s) added as a combination from (19) and (4). After nutrient addition, the pH was automatically maintained with a pH – controller (a Hanna pH502523 with PID control and analog output and a Hanna HI2911 B/5 pH probe) by using a 1N HCl solution (Figure 1). According to (6), and (11), a pH range of 4.5 - 5.5 is regarded as sufficient, at which Aspergillus species can survive and grow optimally.

Table 1. Nutrient supplemented synthetic media (substrate) and petrochemical effluent.

	Synthetic media (substrate)		Petrochemical effluent
Chemical	**Macronutrient (mg/L)**	**Micronutrient (mg/L)**	**Macronutrient (mg/L)**
SCFA	**2180 (COD) and 1744 (acetic acid - HAc)**	**-**	**1595 (COD) 1392 (HAc)**
NaOH	0.45 as Na	-	0.25 as Na
$MgSO_4.7H_2O$	0.21 as Mg	-	0.003 as Mg
$(NH_4)_2SO_4$	1.29 as S	-	104 as N
NH_4Cl	7.60 as N	-	NA
H_3PO_4 and KH_2PO_4	NA	-	53 as P
H_3PO_4	3.03 as P	-	NA
KCl	0.30 as K	-	NA
$CaCl_2.2H_2O$	0.09 as Ca	-	NA
$FeSO_4.7H_2O$	-	0.15 as Fe	NA
H_3BO_3	-	0.004 as B	NA
$MnSO_4.5H_2O$	-	0.072 as Mn	NA
$ZnCl_2.2H_2O$	-	0.060 as Zn	NA
$CuCl_2.2H_2O$	-	0.114 as Cu	NA
$CoCl_2.6H_2O$	-	0.027 as Co	NA
$NiCl_2.6H_2O$	-	0.002 as Ni	NA
$Na_2MoO_4.2H_2O$	-	0.020 as Mo	NA

Not added (NA)
* Total of H_3PO_4 and KH_2PO_4

Inoculation, Enrichment and Reactor Start-Up

Continuous Flow Reactor

The continuous flow reactor was filled with petrochemical feed to a level of 5-L and inoculated with a 3 g screen-dewatered and dried monoculture of Aspergillus penicillioides. The monoculture was obtained from a cross-flow micro-screen reactor previously operated by (12) under optimised operating conditions. The fungus was acclimatized overnight, and the petrochemical feed consisted of 1577 mg COD/L. After a 24-hour acclimatization period, the reactor volume was increased to its working capacity of 15-L, using a peristaltic pump (Watson-Marlow 313U pump with an analog input, linked to the pH-controller) at constant feed rate.

During continuous cultivation, the HRT was maintained at 3.8 ±0,2 hours and the SRT, stepwise increased from 7.0 to 17 hours. The reactor temperature was thermostatically maintained at 45 ± 2^{O}C, and reactor pH maintained at 5.5 ± 0.2 (using 1N HCl solution with the pH-controller). Air supply was maintained with a Fischer-Porter Rotameter to ensure a DO concentration of more than 2 mg O_2/l (measured with a DO meter YSI 85/10FT). To prevent wall growth and micro-screen clogging the reactor was cleaned on a daily basis with a small brush. Harvesting (waste effluent) was with a Gilson Minipuls 3 peristaltic pump.

Aerobic Batch Reactor

For the batch cultivation utilizing the synthetic substrate, acclimatized cell mass was removed from the continuous flow reactor and inoculated under aerated conditions in several batch experiments. The batch reactor (5-L working capacity) employed similar process stability variables (pH, temperature and DO) as the continuous flow reactor. OUR data was generated for both the continuous flow and batch reactor, with an automatic aeration mechanism of the DO/OUR controller and electronic data capturing (15).

Sampling and Analysis

The diluted petrochemical feed and reactor effluent was analysed for COD, TSS, NH_3-N and PO_4-P (18). The SCFA in the feed substrate and reactor effluent was determined by a SCFA titration method (13). Total Nitrogen (N) of the recovered cell mass was determined by the Kjeldahl technique (18) and the crude protein content expressed as Kjeldahl x 6.25.

Zeiss Axioskop optical microscope was used to monitor microbial growth (phase contrast, x150 – x400 magnification) and to assess any bacterial and yeast contamination in the continuous flow reactor. Similar observations were conducted for the batch reactor using the synthetic media. Analysis for the batch studies constituted COD and cell mass concentrations (TSS), and monitoring of process stability variables (pH, temperature, and DO).

Dissolved oxygen (DO) concentrations were regularly recorded with a DO meter (YSI 85/10FT). An amino acid analysis for the continuous culture was determined in duplication on two dried samples. The amino acid concentrations were reported as g / 100 g freeze dried sample and true protein expressed as the sum of individual amino acid concentrations.

RESULTS AND DISCUSSION

Continuous Culture Enrichment and Development (Selection)

One week after inoculation, Aspergillus penicillioides filamentous growth was dominant with very few bacterial and yeast cells present. Under microscopic observations fungal hyphae were observed (Figure 2). The cross-flow micro-screen and pHauxostat combination enabled the germination of all fungal hyphae into a stable filamentous monoculture / cell mass as shown in Figure 2.

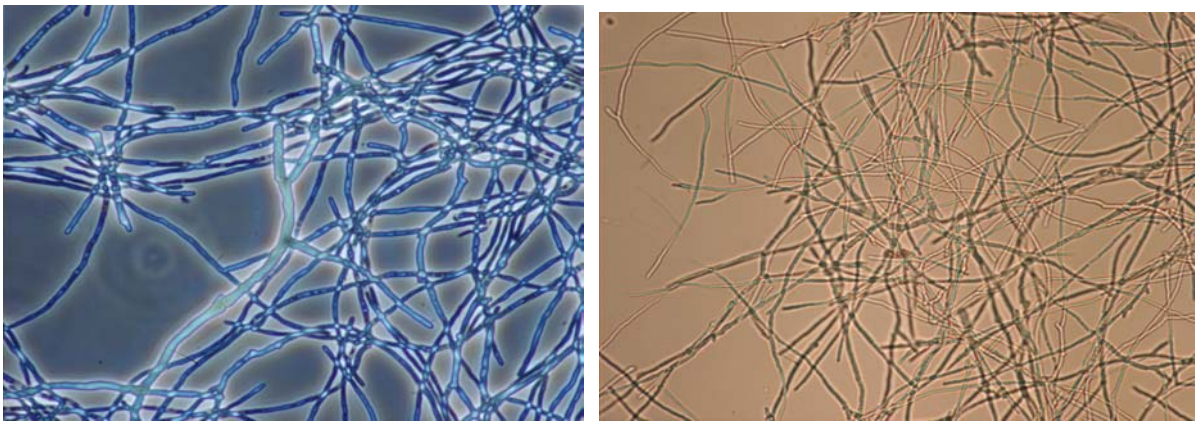

Figure 2. Aspergillus penicillioides monoculture at 200x magnification phase contrast (left) and 400x magnification bright contrast (right) (Filamentous growth form pH 5.6 and SRT of 8 hours).

To prevent micro-screen clogging and wall growth in the continuous flow reactor, the micro-screen and reactor wall had to be brushed on a regular basis. Temporary foaming problems were experienced, but no major instability occurred during continuous cultivation. Various petrochemical effluent components and fungal secretions coupled by aeration may have been the cause of foaming.

Isolation and species identification showed that the microbial species cultivated under these conditions (pH of 5.55 $\pm$ 0.2 and temperature of 45 $\pm$ 0.2 °C) consisted only of a monoculture fungus (Figure 2). Experimental work by (12) classified the fungus under the Aspergillus species, and subsequently identified as Aspergillus penicillioides (Spegazzini) according to the keys and descriptions of (9), (20) and (16).

Microscopic observations showed no spore formation in the continuous flow and batch reactors. This showed that the fungus was successfully cultivated and maintained as a filamentous monoculture. The process and concept also indicates that the petrochemical effluent is conductive for fungal cultivation. Although continuous cultivation studies at long solids retention times and low

hydraulic retention times (11) are prone to bacterial contamination, this reactor remained stable for the duration of the experiment.

Reactor Performance

Continuous Flow Reactor

During continuous cultivation of Aspergillus penicillioides, organic COD, SCFA (feed and effluent), total suspended solids (TSS) and DO were measured (Table 2). Cell mass concentrations (TSS) were measured directly in the reactor, with no waste cell mass concentrations measured. According to (7), when harvesting directly from a continuous flow reactor, the total cell mass COD in the harvest solids is equal to the total cell mass COD in the reactor (namely the Garrett flow scheme).

Table 2. Performance and process stability variables.

τ	Θc	COD Feed	COD Effluent	COD Removed	SCFA Feed	SCFA Effluent	SCFA Removed	DO	X
hr		mg COD/L		%	mg SCFA/L		%	mg/L	mg TSS/L
3.8	7.0	1651	345	79	1458	225	85	0.070	1031
3.6	8.0	1565	350	78	1320	215	84	0.040	1191
3.7	11.5	1542	350	77	1350	235	83	0.080	1431
3.8	15.0	1548	329	79	1354	254	81	0.063	1536
4.0	17.1	1671	353	79	1479	240	84	0.051	1884

τ Hydraulic retention time; Θc Solids retention time; DODissolved oxygen; XCell mass concentration.

Table 2 and Figure 3 shows that when the solids retention time (Θc) is stepwise increased 7.0 to 17 hours, the cell mass concentration (TSS) in the reactor increased from 1031 to 1884 mg TSS/L at an average feeding concentration of 1595 mg COD/L and 1392 mg SCFA/L. This shows that the process was able to maintain a stable monoculture at each solids retention time (Θc) operated. The feed organics (as COD and SCFA) were removed at a constant rate, with little variation between higher and lower solids retention times (Θc).

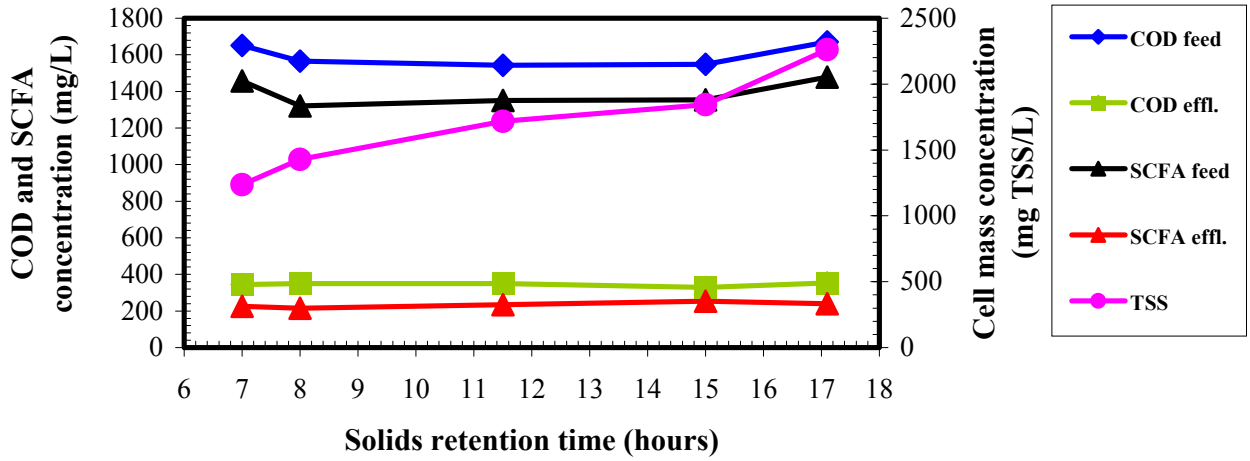

Figure 3. COD, SCFA (feed and effluent) and cell mass concentrations against solids retention time (Θc) in the continuous flow reactor with Aspergillus penicillioides grown on petrochemical effluent. A data point represents an average of 3 measurements.

The COD and SCFA % removal in the continuous flow reactor was more than 75 and 80 % respectively (Table 2 and Figure 4). The organic COD removal in the reactor remained constant (steady) at all solids retention time (Θc) and showed effective in the petrochemical effluent treatment (purification). The petrochemical effluent was previously proven to be more than 95 % biodegradable and had an approximate COD of 1115 mg/L and biodegradable COD of 1073 mg/L (12).

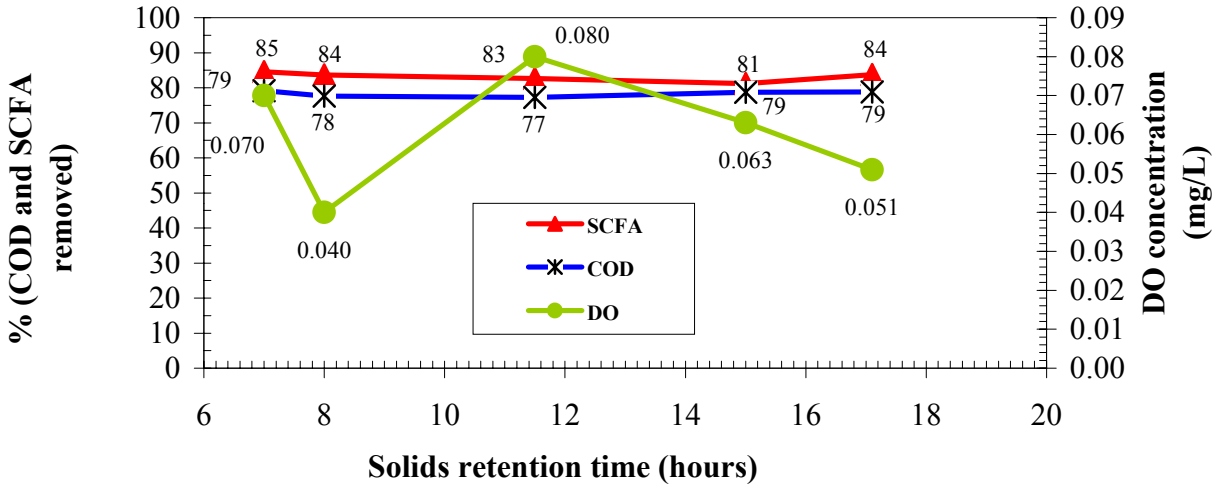

Figure 4. % (COD and SCFA removed) and DO concentration against SRT (□) in the continuous flow reactor with Aspergillus penicillioides grown on petrochemical effluent. A data point represents an average of 3 measurements.

The DO concentration decreased from a set point of more than 2.0 mg O_2/L to less than 0.10 mg O_2/L; and remained variable throughout the cultivation process at all solids retention times (Θc) (Figure 4). The maintained organic feed concentration (as SCFA and COD) of less than 2000 mg/L resulted in a constant cell mass increase, with no significant dissolved oxygen (DO) increase, even at lower solids retention times (Θc). These conditions remained throughout the operation of the reactor operation (Figure 4).

Authors in (19) stated that certain fungi (including Aspergillus species) have the ability to develop and grow under limited oxygen supply as well as anaerobic or micro-aerophilic conditions, which enables them to metabolize some of the organic components of sewage and industrial wastes. This fungus grew steadily under the observed limited dissolved oxygen (Figure 4) and thermophilic conditions (of 45 $\pm$ 0.2 ^{O}C). Although authors in (11) suggested that at lower organic COD of less than 2000 mg COD/L, the dissolved oxygen (DO) concentration of continuous flow reactors tends to increase.

To ensure steady state conditions, the hydraulic retention time (τ) and cell mass concentration (TSS) was measured until recorded values differed by less than 10 %. Ammonia (NH_3-N) and ortho-phosphate (PO_4-P) concentrations were maintained in excess and the process stability variables (temperature, pH and hydraulic retention time (τ)) also kept constant.

Aerobic Batch Reactor

For batch reactor cultivations, organic COD (feed and effluent), cell mass concentrations (TSS) and DO were measured to assess performance, coupled with process variables of time, pH and temperature. The batch experiment was operated for more than 35 hours and a steady increase of TSS was observed during the first 12 hours (Figure 5), with maximum COD removal proceeding after 20 hours as the reactor COD decreased over time.

Maximum COD removal of more than 90 % was achieved for batch the experiment within 12 hours (Figure 6), probably due to a higher dissociation of HAc and presence of readily biodegradable acetate ions (HA^-).

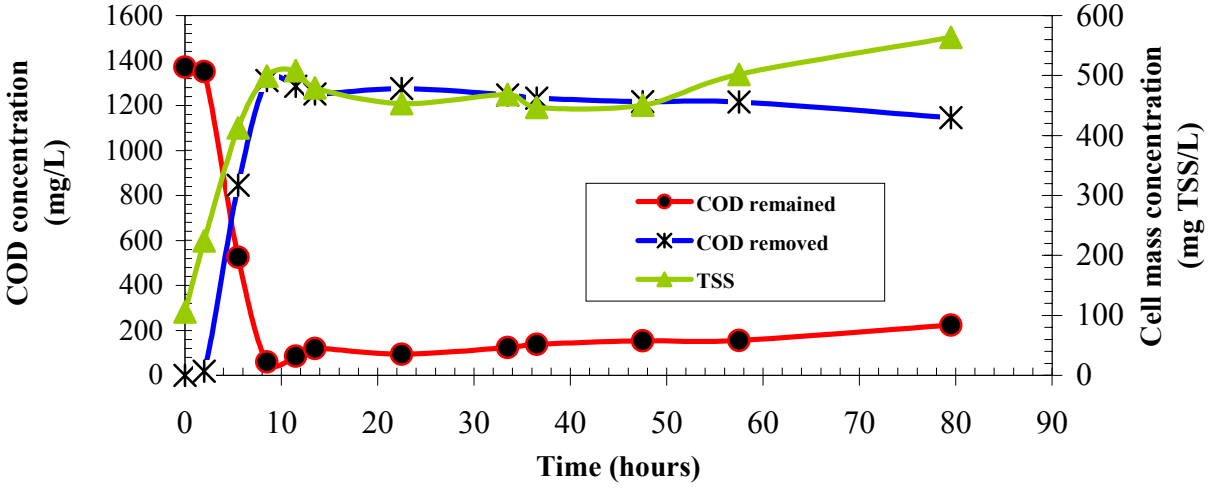

Figure 5. Batch experiment: COD (removed and remained) and cell mass concentrations against time for Aspergillus penicillioides grown on synthetic media (HAc).

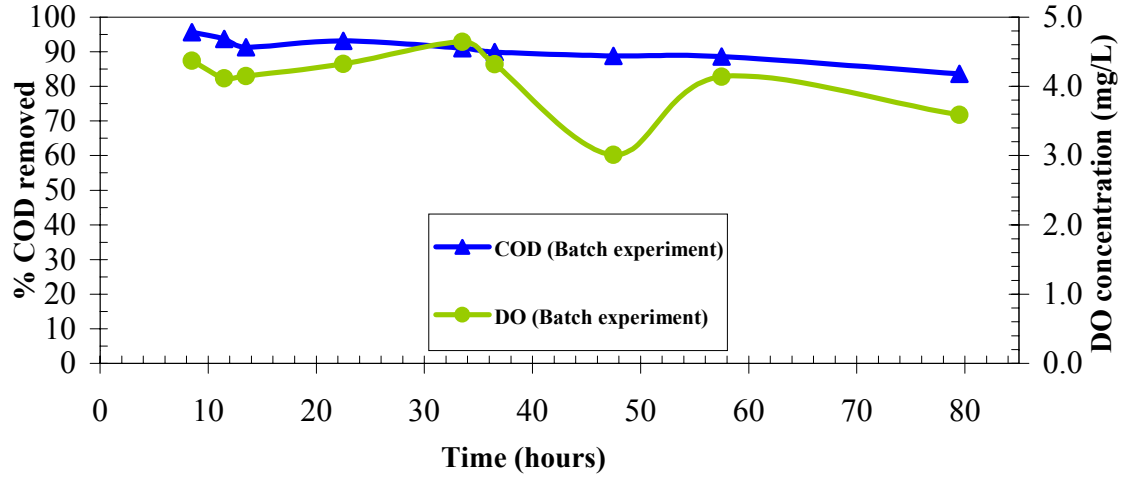

Figure 6. % COD (removed) and DO concentration against time for the batch experiment with Aspergillus penicillioides grown on synthetic media (HAc).

There were no significant correlations observed between the COD removed, growth behaviour of the species compared to the DO concentration profiles (Figure 6). The batch experiment had a consistent DO concentration of 4 mg O_2/l with slight decreases to 2.5 mg O_2/l at 2 hours and 3 mg O_2/l after 36 hours.

Growth Kinetic Parameters

Batch and Continuous Kinetics for the Aspergillus Penicillioides Species

Table 3. Batch and continuous kinetics for Aspergillus penicillioides (Spegazzini) species.

Parameter(s)	Batch kinetics	Continuous flow kinetics	Units
Specific growth rate $(\mu)^{*}$ and $(\mu_{max})^{**}$	**0.305** *	**0.344** **	hr^{-1}
Cell yield constant (Y_o)	**0.700**	**1.45**	mg cells COD/mg COD removed
Cell decay constant (k_b)	**0.0022** ***	**0.0034**	hr^{-1}
Half-saturation constant for substrate (K_s)	**Not determined**	**103**	mg COD/l

*** Average value of slope 1 and 2.

Kinetic parameters determined for batch and continuously cultivated Aspergillus penicillioides species (Table 3). The parameters were determined by methods described adopted from, (7); (5); (4); and (3). The overall parameters (especially decay constants) determined, compare favourably, but are unlikely to be exactly the same due to the significant differences in macro and micronutrients available in feed media.

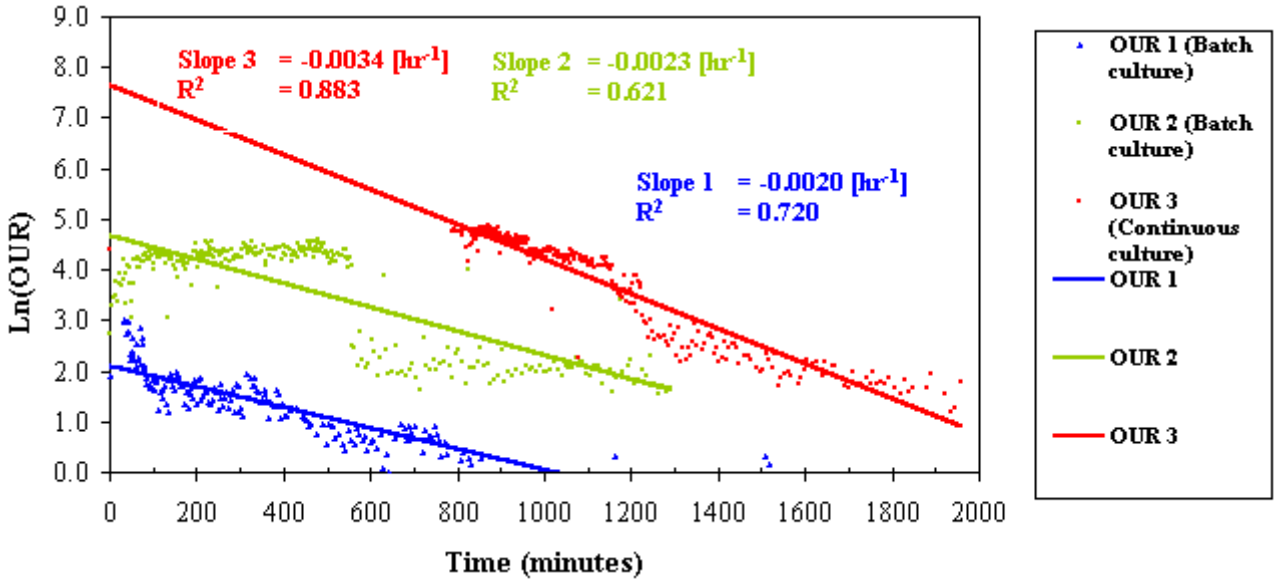

Figure 7. Determination of decay constant (k_b) for Aspergillus penicillioides, with fitted trend line and slope values indicated.

Protein Content and Amino Acid Profile Determined for Aspergillus Penicillioides (Spegazzini)

The protein contents and amino acid profiles were only determined on the continuously cultivated culture. The monoculture of Aspergillus penicillioides had an average crude protein content of 54.5 %, and compared well with that of other fungal species (Table 4). The true protein content of 38.8 % was significantly lower than that of soybean meal (66 %), but correlated well to the fungal species. The amino acid profile was more consistent and favourable in composition to the protein sources in Table 4, which indicates suitability as a feed supplement.

CONCLUSION

Synthetic Media (Substrate) – Aerobic Batch Reactor

Aspergillus penicillioides was effective in treating a nutrient supplemented synthetic media (using HAc as carbon and energy source) in an aerated batch reactor. COD of 95 % was achieved after 12 hours. There were no significant correlations observed between the COD removed, species growth behaviour observed and DO concentration profiles. A low pH of 5.5 was maintained to suppress any bacterial contamination, with a thermostatic temperature of 45 $\pm$0.2^{O}C. The kinetic parameters were determined and were found to be: (1) μ = 0.305 hr^{-1}; (2) k_b = 0.0022 hr^{-1} and (3) Y_o = 0.700 mg cells COD/mg COD removed.

Table 4. Comparison of amino acid profiles and % protein content(s) of various protein source(s) and fungal species.

Amino acid	[1] *Aspergillus fumigates* – g/100 g dried sample	[2] *Geotrichum candidum* – g/100 g dried sample	[3] Soybean Meal	[4] *Aspergillus penicillioides* (Spegazzini) – g / 100 g dried sample
Alanine	2.8	3.4	2.1	**2.5**
Argenine	2.8	3.2	3.5	**2.4**
Aspartic acid	3.6	4.8	5.4	**3.8**
Cystine	ND	1.8	0.6	ND
Glutamic acid	5.4	6.0	8.7	**5.6**
Glycine	2.1	2.2	2.1	**2.1**
Histidine	0.9	1.0	1.5	**1.0**
Isoleucine	1.9	2.1	2.1	**1.8**
Leucine	3.1	4.2	3.6	**3.2**
Lysine	2.6	3.4	4.9	**2.8**
Methionine	0.7	1.0	0.6	**0.8**
Phenylalanine	1.7	1.8	2.4	**1.8**
Proline	2.1	1.6	2.2	**2.0**
Serine	1.9	2.1	2.6	**3.1**
Threonine	1.6	2.4	2.5	**2.3**
Tyrosine	1.2	1.5	1.9	**1.3**
Valine	2.2	2.8	2.1	**2.3**
% N	7.9	8.8	ND	ND
*% Crude Protein	**49.4**	**55.3**	ND	**54.5**
** % True Protein	**> 36.5**	**43.5**	**66**	**38.8**

ND – not determined
* Kjeldahl x 6.25
** Sum of amino acids analysed.
[1]Aspergillus fumigates: (adopted from (21) and (11))
[2] Geotrichum candidum: (adopted from (10))
[3]Soybean (adopted from (10) quoted from Lategan et al., 1980)
[4]Aspergillus penicillioides (Spegazzini): * Crude protein percentage is represented by an average of two samples, and the ** true protein by the sum of amino acids analysed.

Petrochemical Effluent (Substrate) - Continuous Flow Reactor

The fungal treated petrochemical effluent, supplemented with nutrients contained mainly SCFA. Constant temperature and pH variables used as selection mechanisms (14) enabled continuous cultivation of the Aspergillus penicillioides fungus. The selection process was also promoted by the size selective cross-flow micro-screen (for specific culture containment) and pH-auxostat (pH-controller) (with a self-regulating ability). A stable fungal monoculture with a filamentous growth form was achieved for all the SRT, under minimal non-aseptic conditions. The fungus has shown capability to grow consistently under the concept utilized.

The simulated process stability variables were kept constant during the cultivation process with the hydraulic retention time (τ) set at 3.8 $\pm$0.2. The COD and SCFA reduction achieved in the continuous flow reactor was greater than 75 and 80% respectively, with the cell mass concentration increasing as the solids retention time (Θc) increased.

The DO concentration remained less than 0.1 mg O_2/l throughout the continuous study. Although the petrochemical effluent was diluted to limit the cell mass concentration to levels, where oxygen transfer could be in excess. The fungal species adapted well to the prevailing limited dissolved oxygen (DO) conditions (of less than 0.1 mg O_2/l), and constant pH of 5.55 $\pm$ 0.2 and thermophilic temperature of 45 $\pm$ 0.2^{O}C.

The kinetic parameters determined for the continuous flow reactor were found to be:

- μ_{max} = 0.344 hr^{-1}
- K_s = 103 mg COD/L
- k_b = 0.0034 hr^{-1} and
- Y_o = 1.45 mg cells COD/mg COD removed

Products

The amino acid profile of Aspergillus penicillioides was successfully determined and the true and crude protein content was found to be 39 and 55%, respectively. On the basis of nutritional value the amino acid composition and crude protein content compared favourably with that of other protein sources e.g. Geotrichum candidum, 55%; and Aspergillus fumigates, 50%. The Aspergillus penicillioides true protein content of 39 % was significantly lower than that of soybean meal (66 %), but compared well to the other fungal species, Geotrichum candidum, 44%; and Aspergillus fumigates, 37%.

Furthermore, additional research is required to optimise cell mass concentration and nutritional (including toxicological and pathogenic properties) value assessment. Further kinetic studies of the Aspergillus penicillioides species will enable performance studies of the species under different reactor configurations and substrate conditions. Therefore, optimisation of operational conditions (process stability variables e.g. pH, temperature, HRT, SRT and DO) of similar industrial organic effluent treatment will be necessary. The industrial application of this concept (cross-flow micro-screen and pH-auxostat (pH-controller)) may also prove invaluable in SCP production.

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