# PRODUCTION OF XYLANASE ENZYME FROM SPENT SULPHITE LIQUOR USING AN AIRLIFT REACTOR WITH INTERNAL LOOP

by

Sifiso Theophilus Ntuli

A dissertation presented to the University of KwaZulu-Natal in fulfilment of the requirements for the degree of MSc. Eng. (Chemical)

Under supervision of

**Mr A. F. C. Bassa**

College of Agriculture, Engineering and Science
Faculty of Engineering
School of Chemical Engineering
University of Kwazulu-Natal
Durban
South Africa

## ACKNOWLEDGEMENTS

Firstly, would like to thank my promoters Mr AFC Bassa and Prof L Christopher (University of Pretoria and Sappi Technology Centre) for their help and encouragement over the past years. To Sappi Technology Centre staff (Pretoria, South Africa) and School of Chemical Engineering (University of KwaZulu-Natal, South Africa) technical staff for their support and advice during the course of the project.

I would like to thank my late parents and the rest of the family for their consistent support and encouragement. To my baby girl Silindile, reliable friends and special people in my life, thanks for your patience and support throughout the long days and late nights.

Mostly to God almighty, for giving me the strength and the good support system to rely on when in need.


**"It's neither the environment nor the events of our lives, but the meaning we attach to the events – how we interpret them – that shapes who we are today and who we'll become tomorrow"**

*Anthony Robbins.*

## NOMENCLATURE

| | | |
|---|---|---|
| A | cross-sectional area of the column | ($m^2$) |
| $A_b$ | cross sectional area for the flow under baffle | ($m^2$) |
| $A_d$ | downcomer cross-sectional area | ($m^2$) |
| $A_r$ | riser cross-sectional area | ($m^2$) |
| a | specific interfacial area | ($m^3/m^3$) |
| $B_o$ | Bodenstein number | (-) |
| C | constant | (-) |
| $C$ | actual tracer concentration | (-) |
| $C_{ss}$ | measure of steady state tracer concentration | (mA) |
| $C^{ss}$ | steady state oxygen concentration | (ppm) |
| $C^*$ | equilibrium oxygen concentration | (ppm) |
| D | diameter | (m) |
| D | oxygen diffusivity | ($m.s^{-2}$) |
| D | axial dispersion | (-) |
| DP | degree of polymerization | (-) |
| $D_D$ | downcomer diameter | (m) |
| $D_{EX}$ | extension section diameter | (m) |
| $D_R$ | riser diameter | (m) |
| d | diameter | (m) |
| f | frictional factor | (-) |
| g | gravitational acceleration | ($m.s^{-2}$) |
| H | height | (m) |
| h | height | (m) |
| He | Henry's law constant | ($Pa.m^3.kmol^{-1}$) |
| I | mixing homogeneity | (-) |
| k | constant | (-) |
| $K$ | consistency index | ($Pa.s^n$) |
| $k_La$ | volumetric mass transfer coefficient | ($s^{-1}$) |
| L | length | (m) |
| M | molecular weight | ($g.mol^{-1}$) |
| $n$ | flow behaviour index | (-) |
| N | rotational speed | ($s^{-1}$) |
| P | total pressure | (kPa) |
| P | power | (W) |

### 2.3.1 Filamentous fungi

Fungal xylanases have been extensively studied, with *Aspergilli* (Table 2-1), and *Trichoderma* species (Table 2-2) receiving most attention. Multiplicities of xylanases are also predominant among fungal isolates, with up to five xylanases per strain being reported. The optimum pH for xylan hydrolysis is around 5 for most fungal xylanases and they are normally stable between pH 2.0 to 9.0 (Hoq et al., 1994).

**Table 2-1** Characteristics of xylanases from Aspergillus spp. (Howard et al., 2003)

| Microbial Reference origin | Molecular weight (kDa) | Optimum | | pI | Activity ($U.ml^{-1}$) | Specific activity ($U.mg^{-1}$) |
|---|---|---|---|---|---|---|
| | | pH | Temperature ($^0C$) | | | |
| *A. awamori* | NS | 4.0 | 30 | NS | 132 | NS |
| *A. fumigatus* | 9.5<br>5.7 | NS<br>NS | NS<br>NS | 6.5<br>5.0 | NS<br>NS | NS<br>NS |
| *A. kawachi* IFO 4308 | A) 35.0<br>B) 26.0<br>C) 29.0 | 5.5<br>4.5<br>2.0 | 60<br>55<br>50 | 6.7<br>4.4<br>3.5 | NS<br>NS<br>NS | NS<br>NS<br>NS |
| *A. niger* | NS | 5.0-6.0 | 30 | NS | 3.75 | NS |
| *A. niger* | 16 | NS | NS | 4.0 | NS | NS |
| *A. niger* | A) 14.0<br>B) 12.0 | 5.5<br>6.0 | 45<br>45 | 9.0<br>8.6 | NS<br>NS | NS<br>NS |
| *A. niger* | 20.8 | 4.5-5.5 | 50-60 | 6.7 | NS | NS |
| *A. niger* strain 14 | 24.0<br>41.0 | 4.0 | 50 | 3.6<br>4.2 | NS<br>NS | NS<br>NS |
| *A. ochraceus* | 48 | 6.0-7.0 | NS | NS | NS | NS |
| *A. oryzae* | 35 | 5.0 | NS | 6.9 | NS | NS |

**NS: not specified; 1 $IUml^{-1}$ = 16.67 $nkat.ml^{-1}$**

Most of the *Aspergillus* species exhibit optimum xylanase activity in the acidophilic range with the lowest being that of *Aspergillus oryzae*, in the range of 3.0 to 6.5. Optimum temperature for xylanase activity ranges between 30 $^0$C and 80 $^0$C, with least enzymes lying between 45 $^0$C and 60 $^0$C. Activity levels vary with the highest reported being that of 3000 nkat.ml$^{-1}$ by *Aspergillus oryzae* (Chipeta et al., 2005).

Of the xylanases produced by *Trichorderma* species, two main groups of enzymes can be identified (see Table 2-2 above). Both types have low molecular mass but the pI values vary. Xylanases with a high pI have been extensively studied whereas the other types of xylanase with their pI's approximated around 5.0 have been characterized for *Trichoderma* species. (John and Schmidt, 1988; Tankanen et al., 1991). More than one basic xylanase has been isolated from *Trichoderma harzian, Trichoderma koningii* and *Trichoderma longibrachiatum* (Royer and Nakas, 1989; Tan et al., 1994; Wong et al., 1988; Wood and McCrae, 1986). However, these enzymes have been reported to make major contributions to the total activity in the culture filtrate. The minor components may be products of post-transational modifications of the major enzyme. The pI 9.0 and 5.5 xylanases of *Trichoderma ressei* have been reported to be coded by different genes (Tenkanen et al., 1991).

## 2.4 Assaying of enzymes

Xylanase enzyme activity determination procedures are developed from various methods that differ considerably in the conditions of the assays and in the principle of the quantification of the enzyme activity (Haltrich et al 1996).

The most commonly assay methods are:

- Dinitrosalycylic acid method (DNS); (Miller, 1959)
- Somogyi and Nelson method (SN); (Nelson, 1944; Somogyi, 1952)
- Viscometric method (McCleary 1996).
- Chromogenic substrate method (Biely, 1985; McCleary 1996)
- Nephelometric method (Nummi et al., 1985)

One international unit (IU) of xylanase activity is defined as the amount of enzyme which catalyzes the release of 1μmol of xylose or glucose equivalent, respectively, per minute of reaction time.

### 2.5 Xylan hydrolysis by enzymes

Several enzymes are required to ensure complete breakdown of a branched acetyl xylan. Hemicellulases attack substrate by their exo- or endo-hydrolytic attack. Exo-enzymes degrade polysaccharides by the successive removal of terminal oligosaccharide units usually from the non-reducing end of the polysaccharide chain. The action of the endoenzyme occurs in a random manner, resulting in a decrease in the degree of polymerization of the substrate, and an increase in the formation of shorter fragments (mono- and disaccharides) (Biely, 1985). Until recently, the properties of accessory enzymes involved in xylan hydrolysis and the microorganisms producing these enzymes have received scant attention. The possible application of these enzymes in conjunction with xylanases has led to these enzymes being studied in greater detail.

#### 2.5.1 Xylosidases

D-Xylosidase (D-xyloside xylodrolase; E.C. 3.2.1.37) facilitates the hydrolysis of short linear xylooligosaccharides and xylosidases from the non-reducing end to liberate xylose (Wong et al., 1988). The partial liberation of xylose from the branched oligosaccharides produced by the action of xylanases is also accomplished by xylosidase (Biely, 1985). Xylosidases have been reported in bacteria, fungi and yeast, where they appear to be mainly cell associated (Reese, 1977), implying the presence of a xylobiose or xylotriose transport system (Biely, 1985). Xylosidases are generally larger enzymes than xylanases with molecular weights between 60 and 360 kDa, and they may be mono- or dimeric proteins. Extracellular xylosidase activity, however, has also been reported (Zimmermann et al., 1990). In fungi, xylosidase remains associated with mycelia during the early stages of growth. It is released, either by secretion or cell lysis into the growth medium later on in the growth phase (Wong et al., 1988).

Purified xylosidases are generally unable to attack xylan. There are, however, reports of xylosidases that are able to attack xylan slowly to produce xylose (John and Schmidt, 1988).

### 2.5.4 β-Mannanases

β-Mannanases are involved in the catalysis of the hydrolysis of β-D-1,4-monnopyranosyl linkages. These links occur within the main chain of mannans and the polysaccharides consisting mainly of mannose. β-Mannanase production by fungi is usually induced by manna-rich substrate such as locust gum, or by cellulose (Rizzatti et al., 2005). Fungal and bacterial β-mannanases molecular masses range between 34-73 kDa, respectively, and acidic isoelectric points. The pH optima ranges from 3.0 to 5.5 for fungal β-mannanase and from 5.0 to 7.0 for bacterial β-mannanases. Some fungi also secrete β-Mannanases as multiple enzyme forms e.g., *Trichoderma harzianum* and *Thielavia terrestris* (Rizzatti et al., 2005). β-Mannanase has also been purified from several bacteria (Eriksson et al., 1990).

### 2.5.5 α-Galactosidases

These enzymes have been reported to occur in microorganisms, plants and animals. Their mode of action involves the hydrolysis of terminal, non-reducing α-D-galactose residues from α-D-Galactosidase, including galactose oligosaccharides, galactomannans and galactoglucomannans (Dey and Pridham, 1972). The optimum pH and pI values are in the acidic range. Three α-*Galactosidases* have been characterized and purified. These include *Aspergillus niger* (Bahl and Agrawal, 1969), *Aspergillus tawarii* (Civas, 1984) and *Bacteriodes ovatus*. (Gherardini et al., 1996)

## 2.6 Effect of inhibitors

In almost all the cases presented in Table 2-3 below, $Hg^{2+}$ totally inhibits xylanase activity. $Hg^{2+}$ and other thiol reagents such as p-hydroxymercuribenzoate (PHMB), 3,5-dithiobis 2-nitrobenzoic acid (DTNB) and n-ethylmaleimide (NEM) inhibits *Aspergillus ochraceus* xylanase (Biswas et al., 1990). *Gloeophyllum trabeum* BAM Ebw 109 xylanase as opposed to other xylanases is only slightly inhibited by $Al^{3+}$ (Singh et al., 2000). Larger divalent metal ions are also responsible for inhibition of certain xylanases (Table 2-3).

In several other fungal organisms the addition of a complex nitrogen supplement has shown to be advantageous and produce higher xylanases than when employing inorganic nitrogen sources (Christov et al., 1999, Chipenta, 2005). Good xylanase activities had been also reported when peptones or yeast extract are used in the production medium.

Relatively cheaper complex nitrogen supplements are successfully used in certain organisms that include corn steep powder, pharmamedia (cotton derive protein), soybean meal or potato protein, (Haltrich et al., 1994; Purkarthofer et al., 1993; Gomes et al., 1993).

Suggestions of limiting the available precursor for protein synthesis under a lower nitrogen-to-carbon ratio in the medium require a more strict separate regulation of the synthesis of cellulases and xylanases occurring from *T reesei* (Biely 1985). Furthermore, the nitrogen source influences the pH of the medium during the course of fermentation. Culture pH is found to have an important effect on the production of xylanase in *T. reesei*. In addition to the medium components, different surfactants (mainly Tween 80) or fatty acids are frequently added to the medium to enhance the yield of xylanase. It is assumed that these compounds increase the secretion of certain proteins (Gamerith et al., 1992, Hrmova et al., 1986). Tween 80 surfactant commonly dosed in concentration of 0.5–3 $g.L^{-1}$ increases xylanase titers by approximately 12–60 % in certain organisms (Dubeau et al., 1987; Okeke and Obi, 1993; Singh et al., 2000). Apparently it has no effect on xylanase production in other cases (Fernandez-Espinar et al., 1992; Gomes et al., 1993).

### 2.6.2 Substrate medium selection

An appropriate choice of the substrate is vital for the successful production of xylanases. The substrate serves as carbon and energy source and also provides the necessary inducing compounds for the organism used especially for an extended time since the prolonged production phase can result in an increase on overall productivity of the fermentation process (Haltrich et al., 1996). Based on small scale experiments, xylan derived low molecular mass compounds have proven to be excellent substrate for xylanase induction (Haltrich et al., 1994, 1996). Using a number of different organisms the substrate not only results in xylanase yiled increament but, form a selective induction of xylanase with possibly no cellulose activities (Yu et al., 1987; Hrmova et al., 1986; Senoir et al., 1989; Biswas et al., 1990; Gamerith et al., 1992).

**Table 2-4** Summary of Kraft and Sulphite pulping processes (Biermann, 1993)

| Process | Chemicals | Species | Pulp properties | Uses | Yield (%) |
|---|---|---|---|---|---|
| Kraft process<br><br>pH 13-14 | NaOH+$Na_2S$ (15-25%) on wood<br><br>Unlined digester<br><br>High recovery of pulping chemicals<br><br>Sulphur odour | All woods | High strength<br><br>Brown pulps Unless bleached | Bag, wrapping, linerboard,<br><br>Bleached pulps for white papers | 64-70% for brown papers<br><br>47-50% for bleachable pulp<br><br>43-45% after bleaching |
| Sulphite (acid or bisulphite) process<br><br>pH 1.5-5 | $H_2SO_4$+$HSO_3^-$ with $Ca^{2+}$, $Mg^{2+}$, $Na^{2+}$, or $NH_4^+$ base<br><br>$Ca^{2+}$ is traditional but outdated since it is difficult to recover<br><br>Lined digesters<br><br>$Mg^{2+}$ base | Hardwoods<br><br>Birch and non-resinous softwoods<br><br>Douglas-fir is unsuitable<br><br>Almost all species pruce and true firs preferred | Light brown pulp if unbleached<br><br>Easily bleached to high brightness<br><br>Weaker than Kraft pulp but with higher yield<br><br>Same as above but lighter color and slightly stronger | Fine paper<br><br>Tissue<br><br>Glassine<br><br>Strength reinforcement in newsprint<br><br>Newsprint, fine papers etc. | 48-51% for bleachable pulp<br><br>46-48% after bleaching<br><br>50-51% for bleachable pulp<br><br>48-50% after bleaching |

### 2.7.1. Kraft pulping process

Currently, more than 70% of the world's annual pulp outputs of approximately 100 million tons are produced by the Kraft process. Despite some disadvantages, it is the most effective, versatile wood delignification method available. (Baijpai et al., 1994). The Kraft process results in the degradation and solubilization of lignin. Wood chips are cooked in a solution of $Na_2S$/NaOH at about 170 $^0$C for about 2 hours to degrade and solubilize the lignin (Rydholm, 1965; Sanyer and Chidester, 1963).

The effluents containing the chlorolignins are highly colored and can cause detrimental environmental impact because some of the compounds are in part toxic and mutagenic. In addition, high concentrations of chlorides in these effluents contribute to their corrosiveness (Rydholm, 1965). The pulp and paper mill effluents also contain high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD). Also noted are high levels of absorbable organic halogens (AOX) which makes it difficult for it to be released in receiving waters due to major environmental impacts that might be caused.

Since the present study focuses on utilizing spent sulphite liquor as a carbon feedstock to produce xylanases and the understanding of the hydrodynamic characteristics involved during the process, more attention will be on the spent sulphite liquor.

### 2.8.1 Spent sulphite liquor

Spent sulphite liquor (SSL) is the waste liquor produced during the manufacture of dissolving pulp by the acid sulphite process. This waste liquor contains a high amount of monosaccharide sugars formed when woodchips are treated under high temperature and pressure in an aqueous sulphurous solution of calcium or magnesium bisulphate (Bajpai et al., 1994, 1999).

The acidity of these materials in this process is due to the formation of highly acidic lignosulphonic acids and sugar-derived carboxyl and sulphonic acids. The hemicellulose component of the wood chips is hydrolyzed in this process, reducing the degree of polymerization. The end products of the cooking process are monosaccharides, acetic acid (from the hydrolysis of acetyl groups), furfural, levulinic acid, and formic acid among various other components (Moodely et al., 2003). The insoluble cellulose is the one required for pulp manufacture and is separated from the sulphite liquor containing dissolved material (Bajpai, 1994).

SSL composition varies with the type of wood used and the pulping process conditions. The solid component, which varies from 7-15%, consists of 65-75% lignin, 20-30% pentose and hexoses, and 6-10% mixed organic acids, resins, polysaccharides, unused bisulphate (from the pulping process) and ash (Müeller, 1976). The types of sugars and concentrations present in the SSL are shown in Table 2-5.

This is noticed as some pulp mills have gone great lengths and expenditures to reduce the chemical load on the effluent before being disposed to the environment or being recycled back to the cooking process, for example, SSL may be disposed off by evaporating the liquor and burning the solids (Muller and Walden, 1997). Though, this leads to air pollution and acetic acid accumulation in the evaporators (Forss et al., 1991). Therefore, since SSL is cheap and readily available, it is ideal for re-utilization instead of disposal.

### 2.8.3 SSL as feedstock

The carbohydrates composition of SSL varies between 12-14 %, which makes it feasible for use as a feedstock for microorganisms. SSL has been used as carbon feedstock for the production of yeast (Mueller and Walden, 1970; Chaudry et al., 1977; McKee and Quicke, 1977) and filamentous fungal biomas (Pretorius and Lempert, 1993), single cell protein (SCP) (Eriksson, 1998; Ranatunga et al., 1997) as well as as ethanol (Kosaric et al., 1981; Taherzadeh et al., 2003). The use of SSL for biomass and enzyme production has also been investigated (Eriksson, 1998; Ranatunga et al., 1997; Christov and Prior, 1996; Chipeta et al., 2005). Even though SSL can be investigated for use as carbon feedstock, a number of problems need to be overcome before SSL cab be used a fermentation substrate. This is due to SSL composition varying with chemistry and the nature of the pulping process and the type of wood used. Also of note is that SSL contains compounds which may be toxic and thus adversely affect the growth of microorganisms. These include acetic acid, sulphite, undissociated sulphurous and furfural. These, thereby, severely hamper its effective commercial exploration due to the high costs of product recovery and thus make SSL an unreliable substrate for microbial cultivation (Baja, 1999). Recent studies on xylanase production using bleach plant effluent and SSL as carbon source, however, reported activities of less than 6 $IU.ml^{-1}$ (Christov et al., 1999) and 200 $IU.ml^{-1}$ (Chipeta et al., 20005).

## 2.9 Microorganisms for xylanases production

Several criteria are essential for choosing a microorganism to produce xylanases. The selected microorganism is grown for several days in a fermentation vessel containing nutrients and oxygen under specific conditions of pH, temperature, and agitation. During this time, the organism secretes enzymes into the growth medium.

This non-metabolizable, structural analogue of xylobiose is prepared at low cost and it is successfully employed for the induction of xylanases in fungi (Biswas et al., 1988; Ghosh and Nanda, 1994; Gomes et al., 1993). Xylan degrading organisms are cellulolytic and they secret complex mixtures of xylanases and cellulases concurrently. In a number of organisms, synthesis of xylanases occurs in the presence of xylan and also in the presence of cellulose (Haltrich et al., 1996). Xylanase production tends to decrease by the strains grown on cellulose substrate with reduced xylan content. In some organisms the synthesis of xylanase and cellulase are separately regulated and in other organisms xylanase formation is closely linked to that of cellulose.
In *T. reesei*, xylan and xylobiose selectively induce the formation of xylanase, whereas sophorose induce the production of both cellulose and xylanase (Hrmova et al., 1986). Low levels of one of the specific xylanases were formed by *T. reesei* in the presence of the disaccharide endoglucanase (Hrmova et al., 1986).

In the induction of xylan-degrading enzymes by *S. commune*, cellulose or low-molecular-mass compounds derived such as cellobiose or sophorose had to be present (Haltrich et al 1996). In *Aspergillus sp.*, induction of xylan-degrading enzymes could be achieved using xylan, xylobiose or D-xylose as subtrate but in the presence of cellulose or cellobiose both xylanase and cellulase are formed (Hrmova et al., 1986). Efficient induction is observed in the presence of bacterial cellulose that contains no xylan contamination. Conversely, xylan or xylobiose are ineffective as inducers and result in constitutive levels of xylanase activity formed (Haltrich et al., 1996).

Growth of *S. cummmune* on birchwood xylan or xylose does not result in an increase in xylanase formation compared to a control that had more readily metabolized substrate such as glycerol, or no carbohydrate added to the basal medium but significant biomass formation occurred for both xylan and xylose (Halrich et al., 1996). The levels of xylanase formed by *S.cummmune* are sufficient to enable the organism to grow on xylan without specific induction of the enzyme necessary for the degradation. Growth of this fungus on a mixture of cellulose and xylan gave higher β-xylanase activities than the medium containing only cellulose. An increase in ratio of xylanase to cellulose as substrate resulted in a decrease in β-xylanase produced by *S. cummmune* (Haltrich et al., 1996).

**Table 2-6** Production of xylanases and cellulases by fungi, bacteria and yeast in submerged cultivations (Update and taken from Haltrich et al., 1996)

| Organisms | Substrate | Cultivation Conditions | Xylanase | | | Cellulose | | Protein mg/ml | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | Activity[a] IU/ml | Product[b] IU/l.h | Assay[c] | FPase[d] FPU/ml | CMCase[e] IU/ml | | |
| *Aspergillus awamori* VVT-D-75028 | wheat bran, 30 g/l | laboratory fermentation, pH 5.5, 30°C, 96 h | 12.0 | 125 | SN | < 0.1 | 3.2[g] | 0.8 | Poutanen et al., 1987 |
| *Aspergillus awamori* AANTG 43 | oat straw, 20 g/l | 4 L fermentation, pH 4.0, 30°C, 50 h | 820(97) | 16400 (1940) | DNS (SN) | N/A | N/A | 0.11 | Smith and Wood, 1992 |
| | glucose, 5 g/l + oat spelt xylan, 10 g/l | shake flask, 30°C, 7 days | 1159 (132) | 6900 (786) | DNS (SN) | N/A | N/A | N/A | Smith and Wood, 1991 |
| *Aspergillus foetidus* VVT-D-71002 | Solka floc, 30 g/l + BMX, 5 g/l | shake flask, pH 4.8, 30°C, 7 days | 276 | 1660 | DNS | 0.2 | N/A | 1.5 | Bailey and Poutanen, 1989 |
| | Solka floc, 60 g/l | 10 L fermentation, pH 4.0-5.0, 30°C, 70 h | 126 | 1800 | DNS | 0.1 | 2.49 | 1.4 | Bailey and Poutanen, 1989 |

| Organisms | Substrate | Cultivation Conditions | Xylanase | | | Cellulose | | Protein mg/ml | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | Activity[a] IU/ml | Product[b] IU/l.h | Assay[c] | FPase[d] FPU/ml | CMCase[e] IU/ml | | |
| *Aspergillus niger* KKS | rice straw, 10 g/l | 300 ml bubble column fermentation, immobilized cells, pH 7.0, 30$^0$C, 6 days | 138 | 958 | DNS | 3.9 | 1.2 | N/A | Fernandez-Espinar et al., 1992 |
| *Aspergillus niger* NRCC 401127 | wheat bran, 10 g/l | shake flask, 30$^0$C, 96 h | 27.1 | 282 | SN | N/A | N/A | 0.08 | Bailey and Viikari, 1993 |
| *Aspergillus oryzae* VVT-D-85248 | wheat bran, 30 g/l + BMX[h], 5 g/l | shake flask, pH 4.8, 30$^0$C, 7 days | 294 | 1750 | DNS | 0.3 | N/A | 1.6 | Bailey and Poutanen 1989 |
| | wheat bran, 60 g/l | 10 L fermentation, pH 4.0-5.0, 30$^0$C, 80 h | 102 | 1280 | DNS | 0.1 | 4.8[g] | 3.4 | Bailey and Poutanen 1989 |
| | steamed xylooligo-sacchrides, 10 g/l | 10 L fermentation, 29$^0$C, 42 h | 198 | 4710 | DNS | 0.2 | 4.2[g] | 1.0 | Bailey and Viikari, 1993 |

### 2.9.3 Problems with fermentation

The viscous nature of the fermentation broths of some filamentous fungi during their submerged cultivation can cause several serious problems. Generally, the more viscous a fermentation fluid is the harder and more expensive it is to achieve sufficient momentum transfer to generate a homogeneous, well mixed cell suspension. These problems can be especially serious for larger scale fermentations where mixing times in the order of several minutes have been recorded (Bylinkina et al., 1973; Vardar, 1983). However, even in laboratory-scale fermenters, true homogeneity is not assumed. If a fermentation broth is not well mixed then it is likely the system will be heterogeneous and the formation of stagnant, non-mixed zones is possible. Hence, nutrient gradients become established within the vessel, with oxygen ($O_2$) limitation particularly a problem with aerobic fermentations (Metz et al., 1979; McNeil and Harvey, 1993). It is apparent from the literature, however, that these phenomena are not always recognized.

The transfer of sufficient $O_2$ to active cells is critically important in aerobic fermentations and is a difficult task for any fermentation, because oxygen is only sparingly soluble in the fermentation fluid (Doran, 1995). Oxygen mass transfer is even more complex with viscous broths, as it must be transferred from the gas sparged into the fermentation liquid and then from the bulk liquid to the $O_2$-demanding metabolic sites within the cells. Broth rheology can influence this process in several ways. The dispersion of the sparged gas may be restricted in a viscous broth, and bubble coalescence often occurs in increasing bubble volume. Thus, efficient $O_2$ transfer may only happen in the well-mixed regions of the reactor (Loucaides and McManamey, 1973; Oosterhuis and Kossen, 1984). Therefore, rapid depletion of the dissolved $O_2$ in the broth can occur in the slower moving fluid in the outer regions of the vessel, especially if the cells are held there for any significant period of time, that is, if stagnation is established. The strong negative influence of the broth viscosity on $O_2$ transfer has been demonstrated clearly by Chisti and Moo-Young (1987).

Oxygen is not the only nutrient that may become limiting due to poor mixing, especially if the fermenter (or bioreactor) is being run continuous or fed-batch mode, but it is probably the most important physiologically, because $O_2$ limitation can result in the suppression or even total inhibition of metabolite production in aerobic fungi. It should be noted, however, that low $pO_2$ levels can also be desirable for metabolite production.

This is explained by reduced accessibility of the hemicellulosic substrate due to unfavorable pH conditions, a relatively slow release and a prolonged availability of the inducer of xylanase synthesis, thus leading to ineffective enzyme induction (Purkarthofer et al., 1993b and Haltrich et al., 1996).

Royer and Nakas, (1989) produced similar result using *T. lanuginosus* and obtain a high production of xylanase on cellulose medium with the initial medium pH at 7.0 with no substantial change during subsequent growth. The suggestion is that at this pH, which deviates significantly from the pH optima of cellulase and enzyme activities, soluble end-products of cellulose hydrolysis are slowly formed and utilized and these conditions result in limited growth and increase yields of enzymes.

Production of xylanases by *T. reesei* on both cellulose and xylan based media is favored by minimum pH control between 6.0 and 7.0 with only low levels of concomitantly formed cellulase (Bailey et al., 1996). When the minimum pH is controlled at 4.0, lower levels of xylanase are obtained in both cellulose and xylan based media whereas cellulase formation is favored in medium containing Solka floc. (Haltrich et al., 1996). It is conclusive that production of xylanases from *T. reesei* is completely dissociated from that of cellulases, not only by selecting an appropriate inducing carborn source but also the cultivation condition. The effect of an increase in xylanase production at elevated pH values is not generalized for fungal organisms. Bailey et al. (1996) found that xylanase formation by *Aspergillus fumigatus* at a pH below 3.0 during latter stage of cultivation is essential for efficient production. The reason for poor production at higher pH values is probably due to proteolytic inactivation of the xylanase. Large amounts of proteinase activity by *A. fumigatus* during growth at high pH not when cultivating at pH 3.0. Smith and Wood (1991) also pointed out the importance of the pH value for the production of xylanase by *A. awamori*. Acid pH values favours xylanase production with this organism, while pH values of 5.0 or higher causes a dramatic reduction in enzyme yield. Optimum production is obtained when pH is controlled at 3.5 to 4.0.

A low pH is required for efficient production of xylanase by *S. rolfsii* during cultivation (Haltrich et al. 1994). The pH of this organism drops from the initial pH 5.0 to values between 3.0 and 3.5 when uncontrolled. This low value is essential since the regulation of the pH to a constant value of 5.0 resulted in the excretion of large amounts of mainly oxalic acid by *S. rolfsii*, thus leading to a growth and enzyme production. (Haltrich et al. 1994).

Hoq et al (1994) concluded that using *T. lanuginosus* RT9, varying rates from 0.5 to 1.0 vvm, higher xylanase activity were formed while at the highest aeration rate of 1.5 vvm xylanase yields are significantly reduced.

2.9.4.4 Effect of agitation

Xylanase and cellulase production by *A. fumigatus* is reported to reduce at increased agitation rates in a stirred tank-type fermenter, caused by shear damage that occurred to the mycelium. By using an airlift fermenter this negative shear effect caused by the disc turbine agitator could be avoided and the xylanase activity attained could thus be increased by more than 60 %. Because of higher mass-transfer capabilities in the airlift fermenter, xylanase productivity can be doubled (Wase et al., 1985). It is of particular interest to note that xylanase productivity is particularly sensitive to changes in equipment, whereas maximum concentrations for endoglucanase are fairly similar for the airlift and the stirred-tank fermenter. The suggestion made by the authors is that the optimum conditions for the production of these enzymes in *A. fumigatus* differ, and hence by manipulation of these conditions, alterations in the ratios of the enzymes produced should be possible.

2.9.4.5 Biomass-medium separation

A number of different methods have been used to separate the media from the waste biomass. The most frequently used methods to accomplish this are by centrifuging and/or filtration. Harvesting by centrifugation usually results in the loss of some cell material, either through incomplete removal or in the case of decanting process. Filtration, on the other hand, requires careful examination of the behavior of the filter during washing and drying. Washing with an appropriate solution or solvent then removes residual traces of the medium. Non-microbial solids may sometimes be removed by special washing. The washing should be adequate to remove the medium present but not so severe as to result in leaching or lysis of the biomass. Drying conditions must similarly represent a compromise between the need to remove water and the prevention of biomass loss through votalization or oxidation. Typically 80 $^{0}$C for 24 hours or 110 $^{0}$C for 8 hours is used, although low-temperature vacuum drying or inert gas technique may be used in certain circumstances (Mallette, 1971; Pringle and Mor, 1975).

The savings in total active chlorine are found to be 20 to 35 % for hardwoods and 10 to 15 % for softwoods (Buchert et al., 1994; Sinner et al., 1991; Skerker et al., 1991; Viikari et al., 1994; Bajpai et al., 1994; Bajpai, 1999). In the elementary chlorine-free bleaching sequence, use of enzymes increases the productivity of the bleaching plant when the production capacity of $ClO_2$ is a limiting factor. This is the case when utilization of chlorine gas is abandoned. In totally chlorine-free bleaching sequences, addition of enzymes increases final brightness value, which is a key parameter in marketing of the chlorine-free bleaching pulps.

Xylanase pretreatment leads to reduction of effluent AOX and dioxin concentrations due to reduced chlorine requirement to achieve a given brightness (Senior et al., 1992). The level of AOX in effluents is lower for xylanase pre-treated pulps than for conventionally bleached control pulps (Viikari et al., 1994, Senior et al., 1992; Berry, 1989; Bajpai et al., 1994; Bajpai, 1999). The xylanase enzyme-treated pulp results in unchanged or improved strength properties (Bajpai, 1999; Senior et al., 1992; Yang, 1995). Probably this is caused by the selective removal of xylan. However, the viscosity of the pulp is adversely affected when cellulase activity is present (Bajpai et al., 1994; Puls et al., 1993). In few cases, lower mechanical strength is obtained on xylanase-treated pulp, probably due to the presence of cellulase in the enzyme preparation (Clarke et al., 2000). Therefore, the presence of cellulase activity in the enzyme preparation is not desirable. Furthermore, thermostability of xylanases for use in pulp improvement is essential since hydrolysis at elevated temperatures is key in the pulping process (Silva et al., 1994).

Thus, screening criteria for xylanase with better thermostability and possibly higher pH optima have received greater attention. To obtain the best result from the enzymes use, enzyme dosage and pulp consistancy must be optimized in each case to obtain effective dispersion of enzymes (Bajpai, 1999). In general, the optimal dose lies within the range of 2-5 $IU.g^{-1}$ dry pulp, and 5 to 10 % pulp consistency is desirable.

2.9.6.1 Effect of xylanase in pulp bleaching

Although the precise mechanism by which xylanase enhances pulp bleaching is not yet understood, it appears that a partial hydrolysis of pulp xylan improves the susceptibility of lignin removal by subsequent chlorination and alkali extraction stages (Tolan and Canovas, 1992).

Paice et al. (1988) also showed that the xylanase prebleaching effect is not simply a partial removal of xylan, but a significant decrease in the degree of polymerisation of the residual xylan. The extent of bleachability improvement with enzyme treatment seems to depend on the particular enzyme used, so that some enzymes are more effective than others. Enzyme size (smaller enzymes can penetrate further into the fibre wall than larger enzymes), the accessible area of the pulp and top chemical substrates are also important factors. This was demonstrated by Puchart et al. (1999) on treating three types of spruce wood chemical pulps with two β-mannanases.

**2.10 Airlift reactors**

Reactors (or bioreactors) of the airlift type are promising designs for aerobic fermentations. Basic knowledge required for the understanding and the prediction of performance of airlift reactors is only now beginning to emerge (Chisti and Moo-Young, 1987). The most important part of any bioprocess is the bioreactor. In the fermentation industry gas-slurry bioreactors are columns mainly used for aerobic fermentations. These vessels include bubble columns with modified internals, pulse bubble columns and airlift reactors (internal and external). Pneumatic reactors, in which all agitation is due to bubbling gas, are a relatively recent invention. Airlift reactors fall under category of pneumatic reactors.

In biological processes, the primary advantage of airlift reactors over bubble column and stirred tank reactors is related to shear rate imposed by the turbulent fluid on the cells or pellets suspended in the medium. One of the most important aspects of the flow in airlift reactors is the homogeneous field of shear, which is relatively constant throughout the reactor. Shear forces in bubble columns will be greatest adjacent to the gas sparger and will dissipate further away from the sparger. In aerated stirred tank reactors (ASTRs), a region of high shear exist near the agitator, which decreases with increasing distance from the agitator (Siegel and Robinson, 1992). The lack of uniformity in the shear field in bubble columns and ASTRs exposes the organisms to varying shear rates and environments as they pass through the reactor. This adversely affect the organisms due to the amount of shear the organisms can tolerate (Mets et al., 1979; Merchuk, 1986).

The potential applications of airlift reactors are not limited to the field of biological processes. These reactors offer possibility of very simple and highly effective fluidization which suggests a high potential for their application in three-phase processes where gas, liquid and solids must be brought into contact. Solids may be one of the reactants, catalysts or products. The airlift reactor is an attractive option for slurry reactions. Another non-conventional use of airlift reactor principle is the utilization of internal- or external-loop devices where liquid is metal (mercury) and the induced flow has the purpose of magneto-hydrodynamic power (Merchuk and Siegel, 1988).

### 2.10.2 Classification of airlift reactors

A variety of configurations of airlift reactors has been investigated and there has been occasional confusion of the terminology in the literature. The two basic classes of airlift reactors will be distinguished (Figure 2-6). These are:

(i) The internal loop reactor which is basically a simple bubble column and the separation into the riser and downcomer is achieved either with the installation of the concentric draught tube in a cylindrical column or by a vertical baffle in split cylinder geometry (see Figure 2-6 a, b).

(ii) The external loop reactor where the downcomer and riser are two separate tubes connected by horizontal sections near the top and the bottom (Figure 2-6c).

The study on airlift bioreactors has been reviewed by Blenke (1985), Onken and Weiland (1983), Merchuk (1986), Chisti and Moo-Young (1987), Chisti (1998) and Joshi et al., (1990). Some major sources are Schügerl (1990), Shah et al. (1982), Deckwer and Schumpe (1987), and Deckwer (1988, 1992).

### 2.10.3 Comparison of airlift reactors

The continuous stirred tank reactors (ASTRs), although often considered a well characterized reactor type, have several limitations (Misra and Barnett, 1987; Popovic and Robinson, 1993; Wang and McNeil, 1992). Complications and cost of construction is also raised by the need to give an effective sterile seal on a moving agitator shaft, high running costs due to continuous mechanical agitation, and ineffective mass and momentum transfer especially in highly viscous or non-Newtonian process fluid (Fumitake, 1982). Airlift reactors (ALRs) are one of the important classes of modified bubble columns (BC). They present the same advantages as conventional bubble columns over stirred tank reactors, that is, a simple construction without internals or moving parts, an excellent heat transfer capacity, a reasonable interphase mass transfer rate and good mixing properties at low energy consumption, as the gas phase serves the dual function of aeration and agitation. Particularly, additional attractive features of ALRs are good solid-liquid and gas-liquid mass transfer characteristics, a well-defined flow pattern and ease of long-term sterile operation (Chisti, 1998; Moa et al., 1992). Mostly, a hydrodynamic environment suitable for fragile biocatalysts that are susceptible to physical damage by fluid turbulence or mechanical agitation exists in ALRs. Many studies have shown that ALRs are better at suspending solids than BCs and CSTRs (Gaspillo and Goto, 1991; Goto et al., 1989; Immich and Onken, 1992).

The hydrodynamics of the aforementioned reactors are quite different from each other. In an airlift reactor the rate of liquid circulation depends on, and is determined by, the gas flow rate, whereas in the bubble column it is independent. Bioreactors require long residence time and high liquid throughput. This is possible in bubble columns if there are large recycle rates, but in airlift reactors high linear velocities result without external recycle mechanisms. The turbulence that occurs converts the incipient slugging in an airlift to higher velocities than in bubble columns (Onken and Weiland, 1981; Merchuk 1986). The operating range for gas and liquid superficial velocities is much larger for airlift reactors (see Figure 2-7) than for bubble columns (Onken and Weiland, 1981).

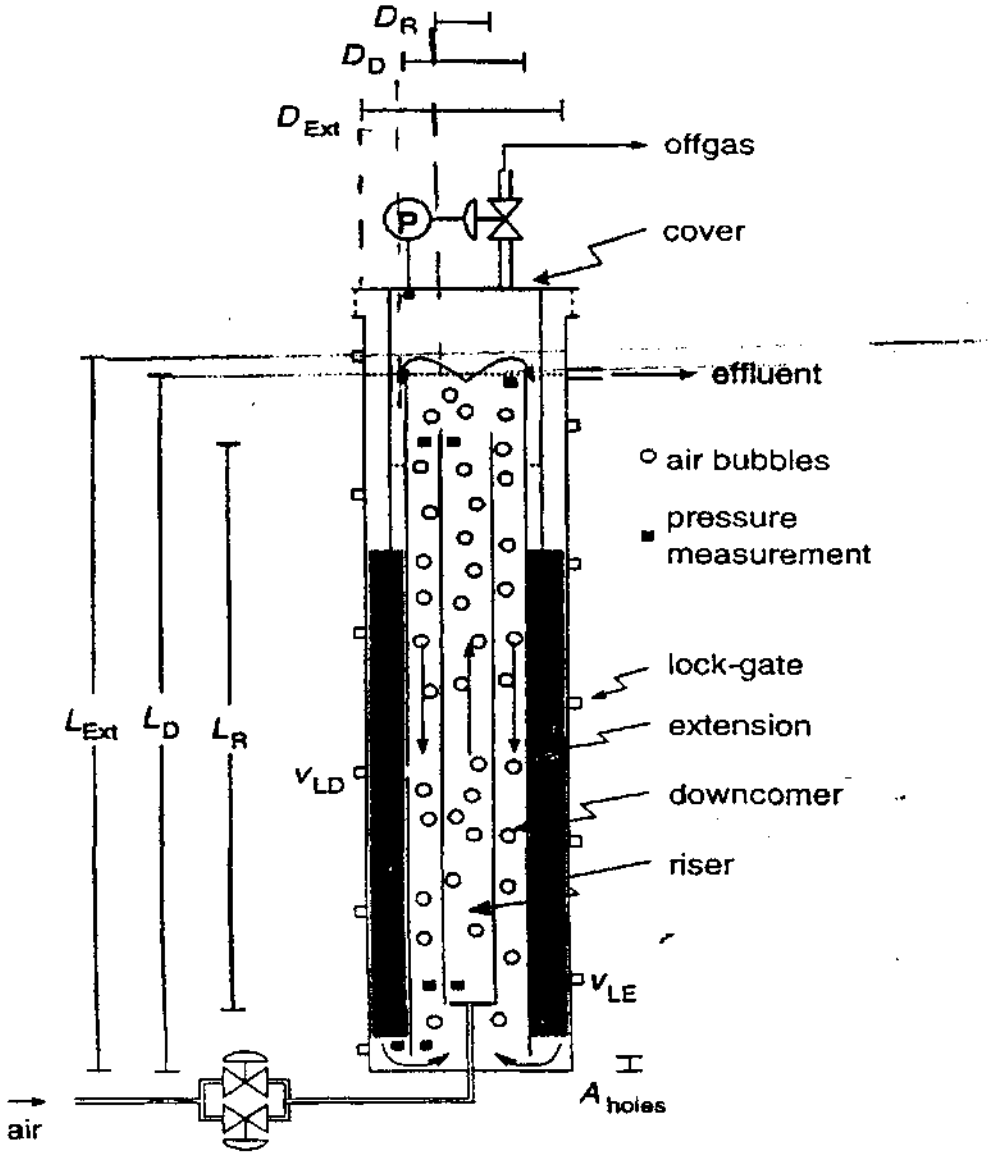


**Figure 2-8** Schematic diagram of internal-loop airlift reactor (BASE). $L_D$: length of downcomer section. $L_{Ext}$: length of the extension section. $L_R$: length of the aerated section (riser or draft-tube).

The unattractive point of BASE reactor is that of the downcomer becoming anoxic due to consumption of dissolved solid particles. This is so because a high air rate is required for either: (i) a sufficient high oxygen transfer from gas to liquid, or (ii) good suspending of the particle in the riser, or (iii) to create a well-mixed aerobic compartment. Therefore, a reactor like BASE combines good mixing and high oxygen transfer rate in the central part of the column with a controllable, small liquid flow in the downcomer.

### 2.10.5 Flow Properties

Bio-fluids and slurries such as polysaccharide fermentations and the broths of *Streptomyces, Aspergillus,* etc, fall into a category known as non-Newtonian media. In this media the viscosity of these fluids is dependent on the rate of shear (Chisti, 1988, 1998).

The power law model commonly describes the dependence as follows (Chisti et al., 1998):

$$\tau = K(\gamma'')^n \qquad (2.1)$$

where $K$ and $n$ are the consistency index and the flow behavior index, respectively, of the fluid and $\tau$ and $\gamma''$ are shear stress and shear rate, respectively.

The effective viscosity ($\mu_{eff}$) for non-Newtonian fluids that follows the power law is (Nishikawa et al., 1977; Chisti et al., 1998):

$$\mu_{eff} = K(\gamma'')^{n-1} \qquad (2.2)$$

For $n = 1$, equation (2.2) reduces to constant form and the fluid is Newtonian. For $n > 1$, the fluid becomes increasingly viscous with shear and it is termed dilatant or shear thickening. When $n < 1$, the fluid is shear thinning or pseudoplastic, i.e. apparent viscosity decreases with increasing rate. Many biological media display pseudoplastic behavior (note that all fermentation broths without non-biological suspended solids may be treated as pseudo-homogeneous liquids. A plot of shear stress versus shear rate is termed a rheogram. The shear stress and shear rate for the different fluids are shown graphically in Figure 2-10.

**Table 2-7** Mathematical Models for the viscous behavior of non-Newtonian fluids (Adapted from Whorlow, 1980).

| Mathematical Format | Name of Model | Comments |
|---|---|---|
| $\tau = k\gamma^{n-1}$ | Power Law or Otswald-de-Waele | Shows fluid behavior as a result of low solid suspension. |
| $\gamma = k_1\tau \_ k_2\tau^n$ | Ellis | Shows Newtonian behavior at low $\gamma$. |
| $\tau - \tau_y = \mu\dot{\gamma}$ | Bingham Plastic | Accommodate yield stress and shows linear shear stress-shear rate behavior above $\tau_y$. |
| $\tau - \tau_y = K\dot{\gamma}^n$ | Herschel-Buckley | Similar to power law, but includes yield stress. |
| $\tau^{0.5} = \tau_y + k\dot{\gamma}^{0.5}$ | Casson | Semi-empirical mainly for suspensions |

Some biological fluids possess viscoelastic properties which should be considered for reactor design. Properties of the broth could change over the course of operation; therefore, the reactor has to be capable of handling these variations.

Rheological parameters such as flow behavior index ($n$), the consistency index ($K$) and the yield stress depend on the concentration of solids in the broth, the morphology (length, diameter, degree of branching, shape) of the particles, the growth conditions (flexibility of the cell wall and particle), the microbial species and the osmotic pressure of the suspending liquid, among possible other factors. How these factors affect rheology remains largely unquantified.

Shi et al. (1990) adopted the analogical analysis to find a correlation for shear rate in an airlift loop reactor, demonstrated in Figure 2-11. The liquid circulation velocity in the downcomer of the airlift reactor was chosen as the measurable parameter because of its relationship to shear rate.

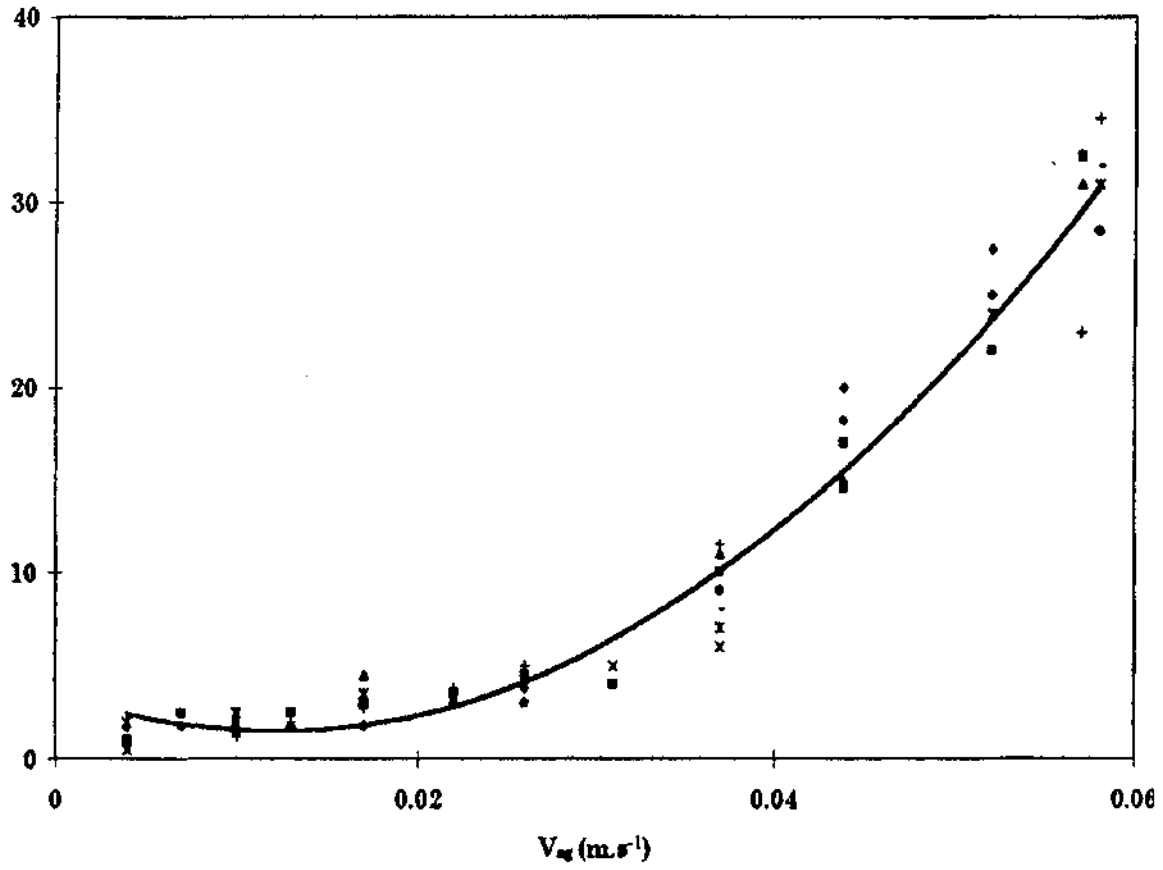


**Figure 2-11** Effective shear rate versus superficial gas velocity by Shi et al. (1990).

For the non-Newtonian system the relationship between the liquid circulation velocity and the superficial gas velocity was found for each solution. Figure 2-12 also shows that the effective viscosity of the non-Newtonian system was found by keeping the operating conditions in both systems constant. The power law indices for the non-Newtonian were obtained with a viscometer.

The experimental data were scattered along the fitted curve (Figure 2-12). For lower $U_{sg}$ the values fitted the curve closely, but not for the higher values of $U_{sg}$. There is still a need for verification in the higher range of $U_{sg}$. The parameter besides $U_{sg}$ could be influencing the effective shear rate.

Theoretical analysis by Shi et al. (1990) suggested that there is an annular channel of liquid around a bubble chain. The correlation suggested for shear rate was calculated from the velocity profile in the liquid around the bubble

$$\gamma_{eff} = \left[\left(\frac{P}{V}\right) + V_{sg}\rho_l g\right]^{0.335} U_{sg}^{\ 0.245}\mu_{eff} \qquad (2.6)$$

The following are possible criticisms of the assumptions incorporated into the above model:

i) The bubbles in the bubble chain are assumed to be spherical.

ii) The bubble rise velocity is calculated without taking into account the shearing exerted on the bubble chain by the liquid flow in the annular channel.

iii) The shear gradient in the bubble column is set equal to the maximum shear gradient with respect to mass transfer coefficient but not with respect to mixing time.

iv) The viscosity of the gas-liquid mixture is set equal to the viscosity of the liquid.

The assumptions for the model are theoretically based, therefore it does not hold under actual operation conditions.

Correlative analysis by Henzler, (1980) correlated data of $k_l a$ (for bubble column):

$$\left(\frac{k_l a}{U_{sg}}\right)\frac{\nu^2}{g^{0.33}} = B\frac{U_{sg}}{g\nu^{0.33}}\left(\frac{\nu}{D_L}\right)^{0.5} \qquad (2.7)$$

The effective shear rate was calculated from the following equation

**2.10.7 Surface active agents**

Many fermentation broths contain substances which affect interfacial behavior, frequently leading to the production of foam in all types of bioreactor configurations. Foams can be stabilized either by substances such as surfactants molecules which directly enhance foam structure or by viscous additives which increase foam drainage times (Lee et al., 1980). Antifoam compounds are therefore routinely used to suppress foam formation. The addition of antifoam is known to affect hydrodynamics, bubble behavior and interactions, and mass transfer rates in bioreactors (Dukkan, 1996, Kawase and Moo-Young, 1990). Furthermore, the nature and extent of the effect seems to depend upon the type of surfactant used.

The conditions which cause collapse of the foam structure can also favor the coalescence of bubbles in the body of the liquid. This can result in an increase in mean bubble diameter and a reduction in gas holdup (due to larger rise velocities). These effects tend to reduce the specific interfacial area available for mass transfer. Furthermore, during bubble motion, absorbed surface active materials can be swept towards the rear of the bubble thus creating a surface tension gradient at the surface. This in turn generates shear stresses which inhibit fluid motion close to the bubble and reduce interfacial circulations within the bubble. There is ample experimental evidence in the literature that a small amount of antifoam addition can reduce the volumetric mass transfer coefficient by as much as 60% (Dukkan, 1996). Kawase and Moo-Young (1990) summarized the results of relevance to bubble column and airlift designs and identified many of the discrepancies and conflicts apparent in the published data. Koide et al. (1985, 1992) investigated the effect of a selected surfactant (aqueous solution of *n*-hexanol and *n*-octanol) and the antifoaming compound (Nissan Antifoam) on the hydrodynamic characteristic of a bubble column. The gas holdup was increased by addition of alcohol surfactant while the resulting foam caused a reduction in holdup due to enhanced coalescence of bubbles.

However, size measurement suggested that, for almost the entire range of $U_{sg}$, the mean bubble size in the antifoam was less than that in the water, leading to the expectation that holdup should increase. Kawase and Moo-Young sought to solve this conflict by suggesting that the size distribution of the bubbles in the antifoam solution (which is uniform and binodal containing very small (1 mm) and relatively large bubbles ($\cong$ 5 mm) could give rise to a small holdup. They further suggested that antifoams, while enhancing coalescence, can also promote bubble break-up due to lowered surface tension. The increased coalescence was attributed to the destruction, by

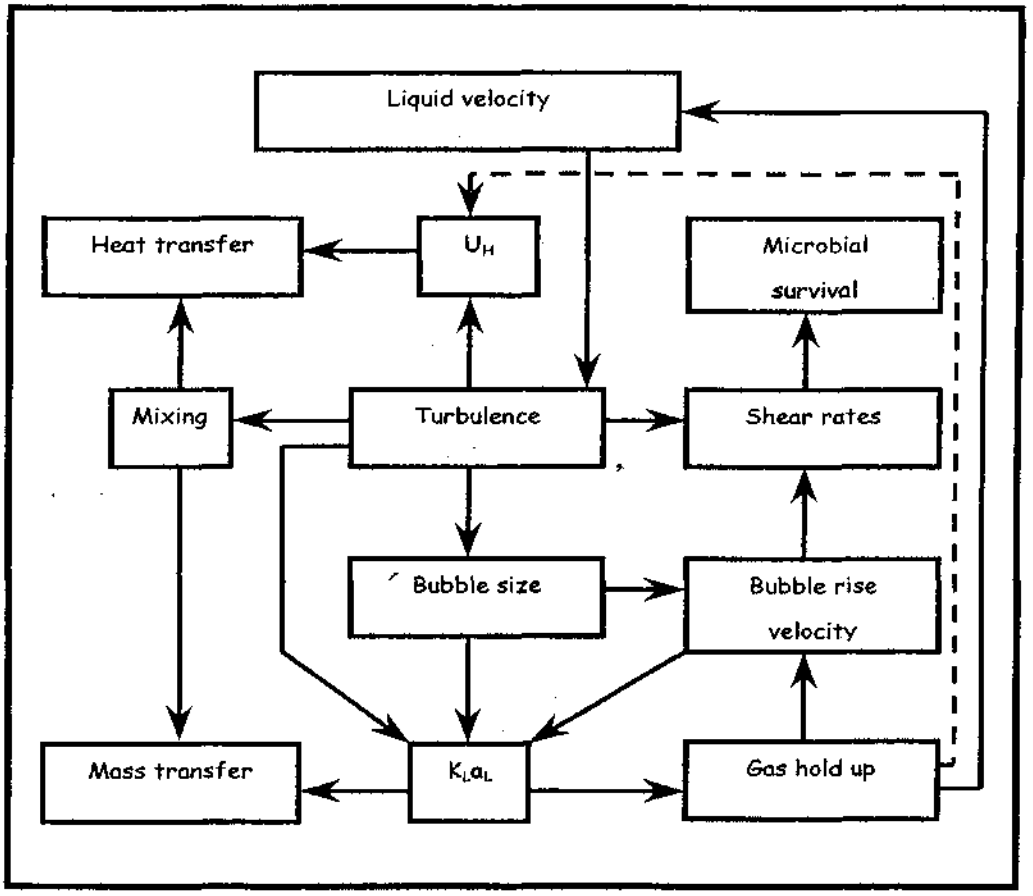


**Figure 2-13** The interrelationship between the liquid velocity and other reactor performance characteristics (Chisti, 1998)

From this Zuber and Findlay (1965) obtained:

$$U_{sg} = C(U_{sr} + U_{lr}) + (U_{bt})^{\infty} \qquad (3.1)$$

where $C$ is the distribution parameter which indicates the extent of radial non-uniformity in the gas holdup, and volumetric fluxes.

As the flow profile in the riser becomes flatter, the distribution parameter approaches unity. The intercept of equation (3.1) represents the terminal rise velocity of the bubble. Thus a linear plot of the gas velocity versus the superficial velocity of the bulk fluid in the riser will enable one to establish the flow profile in the riser. For a given reactor it is unlikely that a single equation of the form (Equation 3.1), will adequately describe all the flow regimes encountered (Al-Marsy and Abasaeed, 1998).

Figure 3-1 shows a typical plot of the drift flux versus gas holdup. When a transformation from one regime to another occurs, a clear deviation from the main curve is observed.

Freitas et al. (1999, 2001) proposed a model for estimating the gas holdup and liquid velocity in two-phase airlift reactors for both external and internal-loop airlift reactors. The riser gas holdup estimation is done using a modification of the Zuber and Findlay model:

$$\varepsilon_{gr} = \frac{U_{gr}}{C\left[U_{gr} + U_{lr}\left(1 + \frac{\varepsilon_{sr}}{(1-\varepsilon_{gr}-\varepsilon_{gd})}\right) - \varepsilon_{sr}U_{sl} + U_{bt}\right]} \qquad (3.2)$$

where $U_{bt}$ is the terminal rise velocity of a single bubble.

### 3.2 **Three-phase hydrodynamic model** (Popovic and Robinson, 1988, 1989)

Popovic and Robinson (1988, 1989) investigated the effects of liquid viscosity on loop hydrodynamics. Their study was performed on a bubble column and external loop airlift reactors using viscous non-Newtonian fluid solutions. All non-Newtonian fluids were found to obey the power law relationship between shear rate ($\gamma$) and shear stress ($\tau$), therefore, the effective viscosity ($\mu_{eff}$) can be estimated using

$$\mu_{eff} = \tau / \gamma = K \gamma^{n-1} \qquad (3.3)$$

where $K$ is the consistency index and $n$ is the flow behavior index. Some average shear rate has to be known in order to use equation (3.3). Nishikawa et al. (1977), using a bubble column presented a linear relationship between the average shear rate and the superficial gas velocity:

$$\gamma = kU_{sg} \qquad (3.4)$$

where $k$ depends on the ascending velocity of gas bubbles, or the bubble diameter and superficial gas velocity.

They found that the gas holdup increased with increasing superficial gas velocity and a relative effect on the fluid rheology data (i.e. $K$ and $n$). Both the holdup and the liquid circulation velocity were largely influenced by the reactor geometry, $A_d/A_r$. For the same operating conditions and rheological behavior ($K$ = 1.6 Pa.s), the $U_{sl}$ with $A_d/A_r$ = 0.444 was found to be four times greater than that obtained at $A_d/A_r$ = 0.111. At a given gas flow rate ($U_{sg}$) and riser cross-sectional area, increasing the downcomer area will lower the resistance to flow in the downcomer, which in turn will tend to increase the liquid circulation velocity for the same hydrodynamic driving force (Allen and Robinson, 1989).

#### 3.2.1 Gas holdup

Popovic and Robinson (1988, 1989) correlated experimental gas holdup and liquid circulation velocity data for various Newtonian and non-Newtonian solutions using Nishikawa's expression and proposed the following equations:

gas velocities. The above correlations have, in general, failed to become widely used for the description of hydrodynamics in reactors, except in cases for which they have been devised (Glennon et al., 1993).

### 3.2.3 Mixing time

It will become clear later that the degree of fluid mixing can be an important factor in the modeling and the determination of hydrodynamic characteristic effects in ALRs. Figure 3.2 is a typical response to a pulse of tracer for such a reactor. The liquid circulation time, $t_c$ is clearly identified as being that time between the passages of successive tracer concentration peak at the probe location. The mixing time, $t_m$, which is the time taken for the tracer concentration to reach a specified level of homogeneity, is frequently used to characterize the degree of mixing in the reactor.

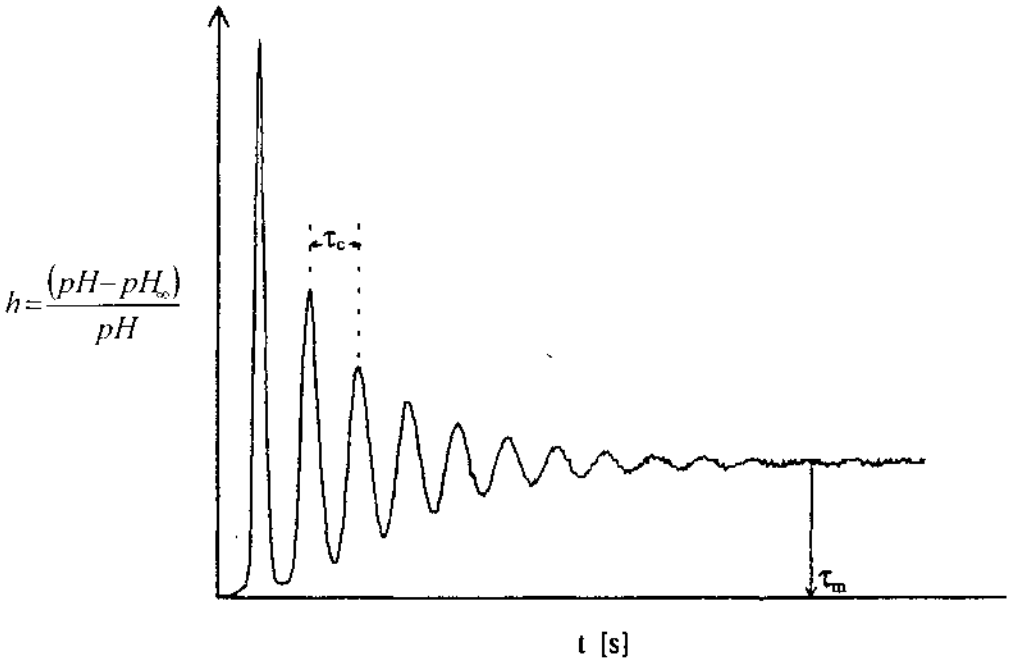


**Figure 3-2** Typical tracer response curve for airlift reactors.

### 3.3 **Filamentous fungal model** (Wang and McNeil, 1992, 1996)

#### 3.3.1 Gas hold-up

Wang et al (1992, 1996) suggested the following assumption for their models:

1. Fermentor overall mean gas holdup $\varepsilon_g$ is affected by the changes in superficial gas velocity ($U_{sg}$) and consistency index ($K$).
2. For viscous broth, $A_d\varepsilon_{gd} << A_r\varepsilon_{gr}$.

The effect of changes in the value of the consistency index ($K$) and the changes in the superficial gas velocity $U_{sg}$ upon overall mean gas hold-up can be expressed simply by the following equation (Wang and McNeil, 1992, 1996):

$$\varepsilon_g = a\,U_{sg} + b \qquad (3.11)$$

Both $a$ and $b$ in equation (3.11) are themselves the function of consistency index ($K$). By correlating $a$ and $b$ with $K$, we have:

$$a = 0.1379 + 7.8284*10^{-3} \ln K \qquad (3.12)$$

$$b = 2.4882*10^{-3} + 1.5437*10^{-3} \ln K \qquad (3.13)$$

By combining equation (3.11) and (3.12) with equation (3.13), an empirical relationship between $\varepsilon_g$ and both $U_{sg}$ and $K$ are obtained and can be expressed as:

$$\varepsilon_g = \left(0.1379 + 7.8284*10^{-3} \ln K\right)U_{sg} + 2.4882*10^{-3} + 1.5437*10^{-3} \ln K \qquad (3.14)$$

This equation indicates that $\varepsilon_g$ changes in direct proportion to $U_{sg}$.

### 3.3.3 Mixing behavior in the IL-ALR

Mixing times, $t_m$ as a function of the superficial gas velocity and viscosity of the broth are presented. Mixing times varies according to the relationship (Wang and McNeil, 1992, 1996):

$$t_m = m\,(U_{sg})^n \qquad (3.19)$$

The values of $m$ and $n$ change with the consistency index $K$, and can be expressed as:

$$m = 12.3668K^{-0.05931} \qquad (3.20)$$

$$n = -0.9664 - 0.0791 \ln K \qquad (3.21)$$

By combining equation (3.19) with equation (3.20) and (3.21), an empirical correlation for $t_m$ in terms of $U_{sg}$ and $K$ is obtained and is thus described as:

$$t_m = 12.3668K^{-0.05931}U_{sg}^{-0.9664-0.0791\ln K} \qquad (3.22)$$

The predicted values of $t_m$ are obtained by equation (3.22).

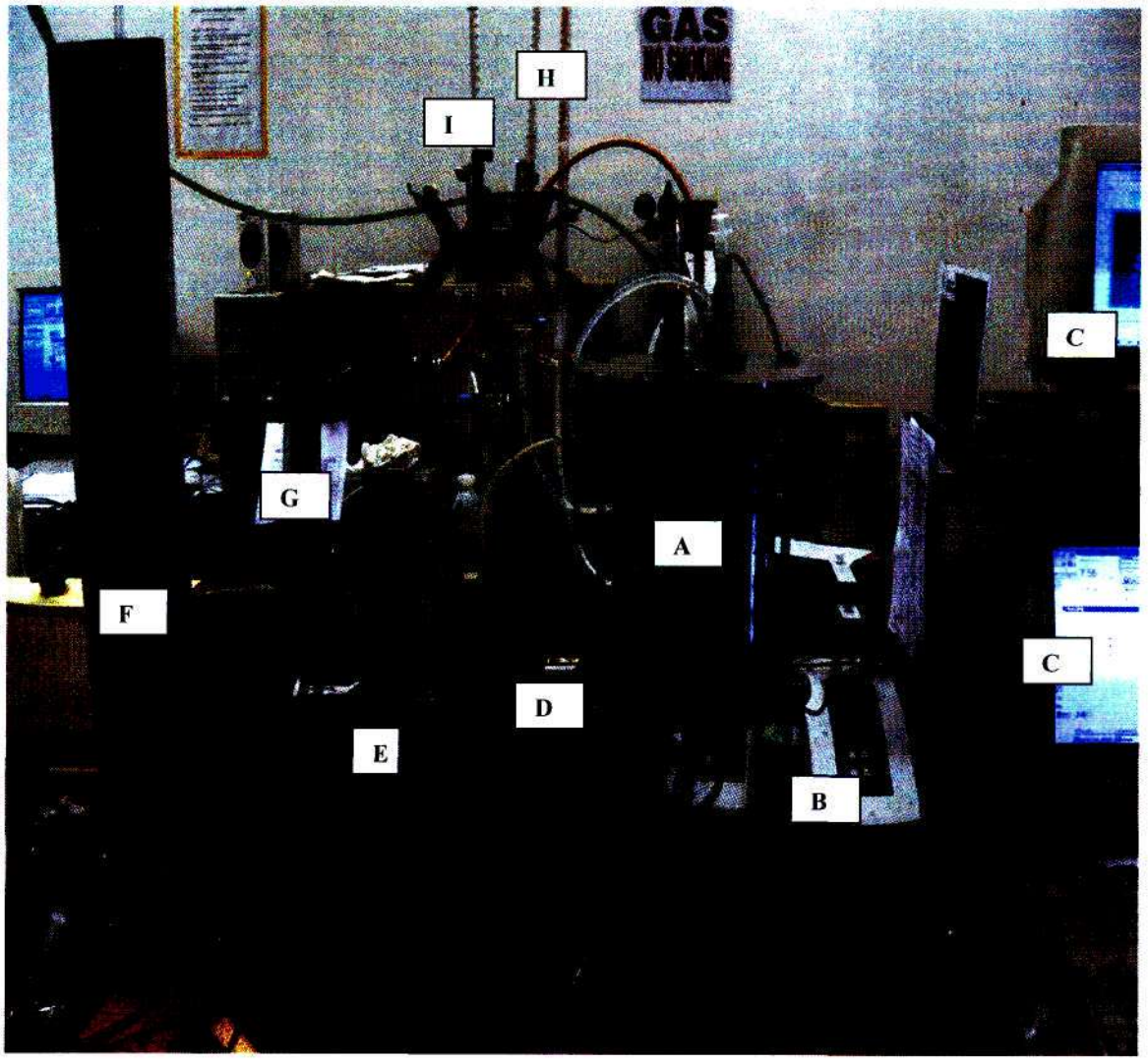


**Figure 4-1** Experimental setup. (A) Internal airlift reactor y. (B) DO/Temperature /pH and pressure gauge meter. (C) DO/Temperature/pH and gauge pressure recorder. (D) Water pump. (E) Water bath. (F) Manometer. (G) Oxygen flow meter. (H) Alkali and acid reservoirs. (I) Anti-foam reservoir.

**Table 4-1** Summary of the IL-ALR dimensions.

| **Dimension** | **IL-ALR (1 L total volume)** |
|---|---|
| Riser diameter | 0.024 m (ID) |
| Downcomer diameter | 0.048 m (ID) |
| Riser to downcomer cross-sectional area, $A_r/A_d$ | 0.25 |
| Dispersion height | 0.043 m |
| Normal riser length | 0.152 m |
| Normal downcomer length | 0.182 m |
| Reactor vessel height | 0.225 m |

## 4.1 Experimental methods

In order to determine optimum conditions for producing xylanase enzymes by cultivating *Aspergillus oryzae* strain using SSL as carbon feedstock, several batch cultivations at different superficial gas velocities, pH and temperature conditions were performed. The cultivation period per batch was 3 days. Also noted was that the bioreactor total volume restricted the range at which the superficial gas velocity can be varied due to problems that might develop beyond the aforementioned limit such as uncontrollable turbulence, which can damage microbial cells, and frequent foaming which overflows or floods the reactor. Therefore, the superficial gas velocity effect on xylanase production will be determined within the gas velocity range of 0.003 to 0.0147 $m.s^{-1}$.

The pH and temperature conditions used are based on the PhD study done by Chipeta (2005), where a pH of 7.5 and temperature of 30 $^0$C were the optimum conditions. Oat spelt xylan (Sigma) was used as a control substrate since it is a known inducer of xylanases (Siedenburg et al., 1998; Bahkali, 1996).

### 4.4 Culture medium

The spent sulphite liquor (designated SSL, $Ca(SO_3)_2$) was supplied in a concentrated form from Sappi Saiccor's acid sulphite pulp mill (in South Africa), where it had been concentrated approximately five-fold by a three-step evaporation process. This was the same liquor used for a PhD thesis by Chipeta (2005). The aforementioned carbon source was then diluted ten-fold with distilled water. The other medium constituents were (g $l^{-1}$): citric acid, 0.25; $(NH_4)_2SO_4$, 5; $K_2HPO_4$, 5; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.02 and yeast extract, 10 as well as 1 ml of a trace element solution (du Preez and van der Walt, 1983; Chipeta, 2005)..

### 4.5 Broth rheological properties

The rheological properties of the broth are influenced by the morphology and concentration of the organism, as well as the concentration of xylanases. Under the chosen operational conditions, the mould grew well and produced true mycelia filaments with few pellets. Fermentation fluids which contain filament and mycelia enzymes are generally much more viscous then those containing pellets only. Therefore, such fluids exhibits high viscosity and usually show non-Newtonian pseudoplastic behaviour (Olivier et al., 1989; Allen and Robinson, 1989). The rheological properties of the product were examined using a Brookfield cylindrical spindle digital viscometer (Model VD-II+) to measure the rheological properties of the broth at the respective temperature the media was set on. Details of the rheological techniques and an extensive comparison of various rheological methodologies have been given elsewhere (Allen and Robinson, 1989; Allen, PhD Thesis, University of Waterloo, 1987).

Their rheological behaviour has been described by the so called "power law" equation (Popovic and Robinson, 1988, 1989):

$$\tau = K(\gamma)^{n-1} \qquad (4.1)$$

where $\gamma$ is the shear rate. The shear rate was estimated from the correlation proposed by Nishikawa et al. (1977):

$$\gamma = 5000^* U_{sg} \qquad (4.2)$$

According to Nishikawa et al. (1977), correlation (4.2) holds in the slug flow regime.

The incubation time was 5 min at 30 $^0$C, and then 3 mL DNS reagent was added to stop the reaction. The color was developed by boiling the samples for 5 min and then placing them immediately on ice prior to taking an aliquot of 1 mL into a disposable plastic cuvette. The absorbance was measured at 540 nm (using Ultra Spec 1000E UV/Visible spectrophotometer, Pharmacia Biotech, Cambridge, England) against a blank prepared in the same way but with 0.2 ml of 0.05 M citrate-Na buffer pH 6.0 as a sample.

Absorbance at 540 nm due to the xylanase activity could be related to the amount of reducing sugars (xylose) present by comparison with a standard curve. Enzyme activity in a given assay took into account the volume of the assay and expressed as $IU.mL^{-1}$, where one international unit (IU) of enzyme activity was defined as the amount of enzyme that liberated 1 µmol of reducing sugar (xylose or glucose equivalents) per minute of reaction time. Results shown represent the average of two determinations and observed variations were under 5 % of mean values.

**4.7 Instrumentation and measurement**

Visual observation enabled qualitative and quantitative characterization of the hydrodynamic properties. The gas holdup was evaluated by measuring the liquid free-surface area level.

Instrumentation consisted of gauge pressure meter and a relief valve, a pH meter and a DOT meter. Relative gauge pressure meter and a relief valve controlled the pressure buildup inside the reactor.

The pH was measured by means of a probe (Applikon Winlab) connected to a pH meter (Winlab RS232). The probe was connected directly to the data acquisition unit during liquid circulation velocity measurements. Total dissolved oxygen was measured by means of a Winlab electrode based DOT probe (Applikon) connected to a transmitter (Winlab RS232).

Oxygen gas was used and passed through a flow meter (GH Automation, UK) which was used to determine the volumetric flow rate of oxygen to the sparger (entrance the reactor). The superficial gas velocity ($U_{sg}$) was determine by volumetric flow rate ($Q_g$) but corrected for pressure and temperature:

## 5. EXPERIMENTAL RESULTS

To determine the optimal cultivation conditions and the rheological and hydrodynamic characteristic effects for xylanase production by *Aspergillus oryzae* NRRL 3485 culture in an IL-ALR, several bioreactor cultivations (batch system) at different cultivation pH and temperature were performed, within a specific superficial gas velocity, using SSLc as a carbon feedstock. Oat spelts xylan (Sigma) was used as control substrate since it is a known inducer of xylanases (Siedenburg *et al.*, 1998; Bahkali, 1996). The results also summarize the investigation of the mathematical model against the experimental rheological data as a function of the hydrodynamic parameters during the fungal growth process to describe the behavior of a fungal culture.

### 5.1 Superficial gas velocity effects

Growth parameters of *A. oryzae* under various aeration rates in batch culture are shown in Table 5-1, which were obtained at pH 7.5 and temperature of 30 $^{0}$C (as optimum conditions). Superficial gas velocities of 0.0033, 0.0073 and 0.0147 $m.s^{-1}$ were selected in order to control the DOT at or above 25 % during batch cultivation and to the constraint of the reactor capacity being unable to handle rates beyond this point. It was, therefore, necessary to determine the effect of aeration rate within these limits for xylanase production.

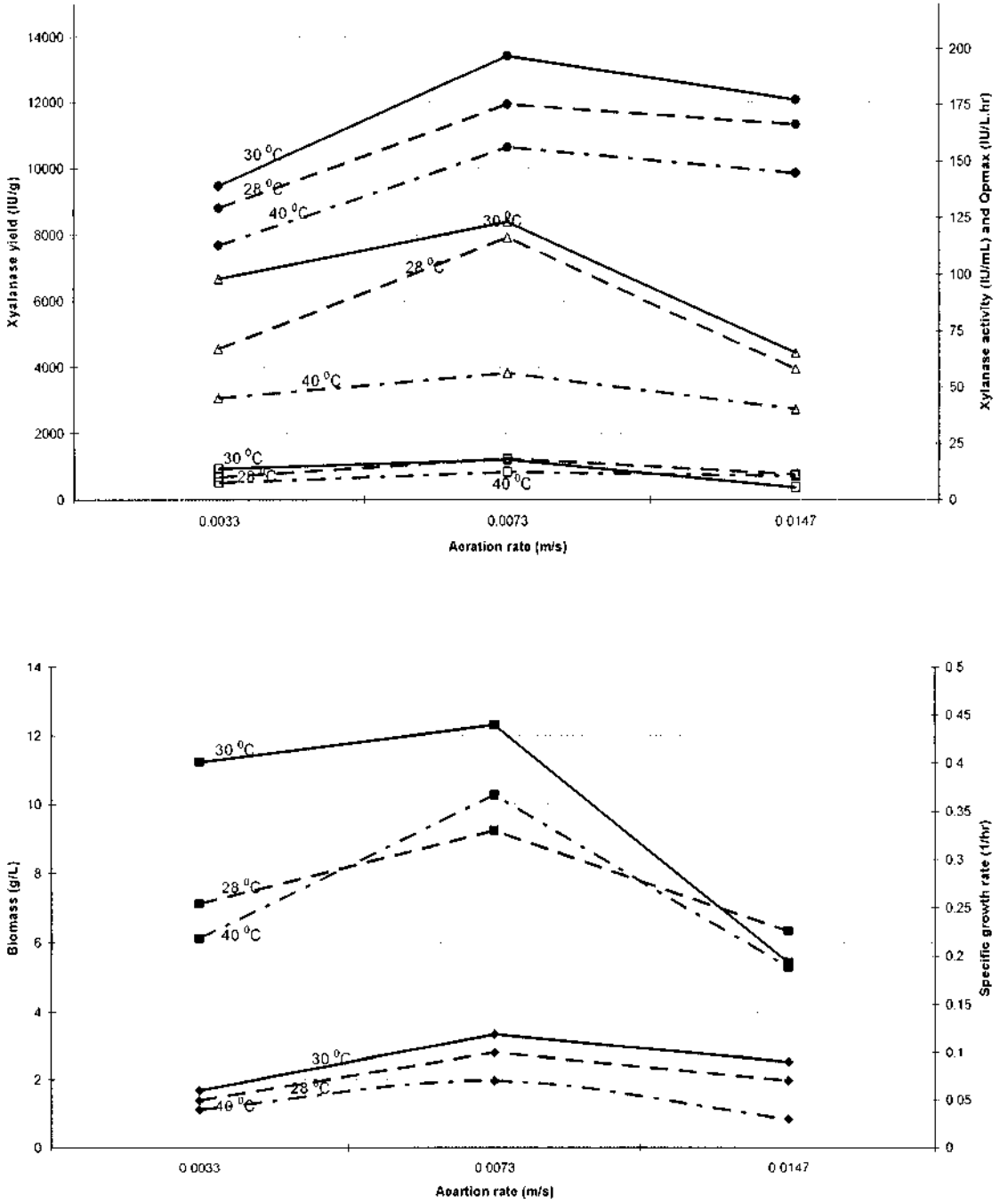


**Figure 5-1** Batch cultivation of *A. oryzae* NRRL 3485 with SSL as carbon substrate in a 1-litre IL-ALR at pH 6.0 and culture temperature set at 28, 30 and 40 $^{0}$C and superficial gas velocity of 0.0033, 0.0073 and 0.0147 m.s$^{-1}$ for a 3-day cultivation period. Symbols: Δ β-xylanase activity; ■ biomass; □ max volumetric productivity rate; • β-xylanase yield; ♦ max specific growth rate.

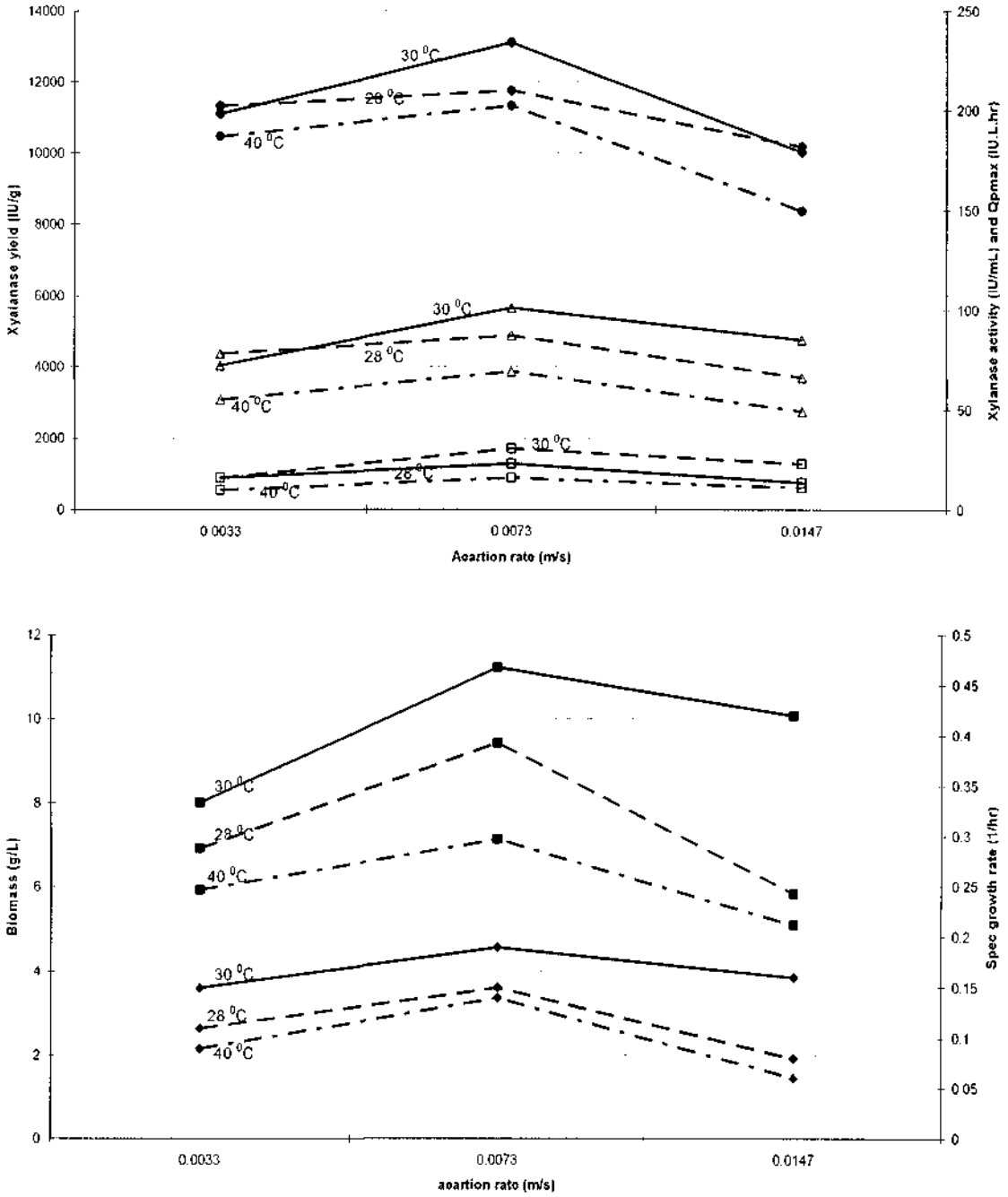


**Figure 5-3** Batch cultivation of *A. oryzae* NRRL 3485 with SSL as carbon substrate in a 1-litre IL-ALR at pH 8.5 and culture temperature set at 28, 30 and 40 $^{0}$C and superficial gas rate of 0.0033, 0.0073 and 0.0147 m.s$^{-1}$ for a 3-day cultivation period. Symbols: Δ xylanase activity; ■ biomass; □ max volumetric productivity rate; • β-xylanase yield; ♦ max specific growth rate

**Table 5-2** Growth parameters (mean values of duplicate experiments) of *A. oryzae* NRRL 3485 in batch culture at varying pH conditions (at 30 $^{0}$C cultivation temperature and superficial gas velocity of 0.0073 m.s$^{-1}$) with SSL as carbon substrate.

| **Culture parameters** | **Cultivation Temperature (30 $^{0}$C and 0.0073m.s$^{-1}$)** | | | |
|---|---|---|---|---|
| | pH 6.0 | pH 7.5 | pH 8.5 | No pH Control |
| Xylanase (IU.ml$^{-1}$) | 123 (±21.5) | 210 (±26.2) | 101 (±29.6) | 87 (±20.6) |
| Biomass (g.l$^{-1}$) | 12.31 (±2.26) | 20.03 (±2.61) | 11.22 (±1.79) | 6.25 (±2.01) |
| $\mu_{max}$ (h$^{-1}$) | 0.07 (±0.02) | 0.17 (±0.05) | 0.11(±0.08) | 0.05 (±0.03) |
| $Q_p^{max}$ (IU.ml$^{-1}$ h$^{-1}$) | 25.67 (±0.15) | 28.77 (±0.18) | 13.44 (±0.21) | 10.24 (±0.17) |
| $Y_{p/x}$ (IU.g.biomass$^{-1}$) | 13 684 (±1 323) | 15 076 (±987) | 13 116 (±1 004) | 11 832 (±897) |

### 5.3 Effect of cultivation temperature

The growth parameters for the *A. oryzae* NRRL 3485 strain with SSL as carbon substrate at the different cultivation temperatures, with pH 7.5 and 0.0073 $m.s^{-1}$, are summarized in Table 5-3. There was a noticeable deviation or influences in xylanase activity, cell biomass concentration, maximum volumetric production rate as well as xylanase yield due to temperature set point variation (see Figure 5-4).

**Table 5-3** Growth parameters (mean values of duplicate experiments) of *A. oryzae* NRRL 3485 in batch culture at different temperature conditions (at pH 7.5 and superficial gas velocity of 0.0073 $m.s^{-1}$) with SSL as carbon substrate.

| **Formation parameters** | **Cultivation Temperature (pH 7.5, 0.0073m.s$^{-1}$)** | | |
|---|---|---|---|
| | 28 $^0$C | 30 $^0$C | 40 $^0$C |
| Xylanase ($IU.ml^{-1}$) | 151 (±21.2) | 210 (±26.2) | 79 (±23.2) |
| Biomass ($g.l^{-1}$) | 12.05 (±2.26) | 20.04 (±2.02) | 10.04 (±1.89) |
| $\mu_{max}$ ($h^{-1}$) | 0.17 (±0.06) | 0.17 (±0.02) | 0.14 (±0.04) |
| $Q_p^{max}$ ($IU.ml^{-1}\ h^{-1}$) | 25.67 (±0.26) | 28.77 (±0.12) | 13.44 (±0.32) |
| $Y_{p/x}$ ($IU.g.biomass^{-1}$) | 11 474 (±1 622) | 15 076 (±1 126) | 8 165 (±826) |

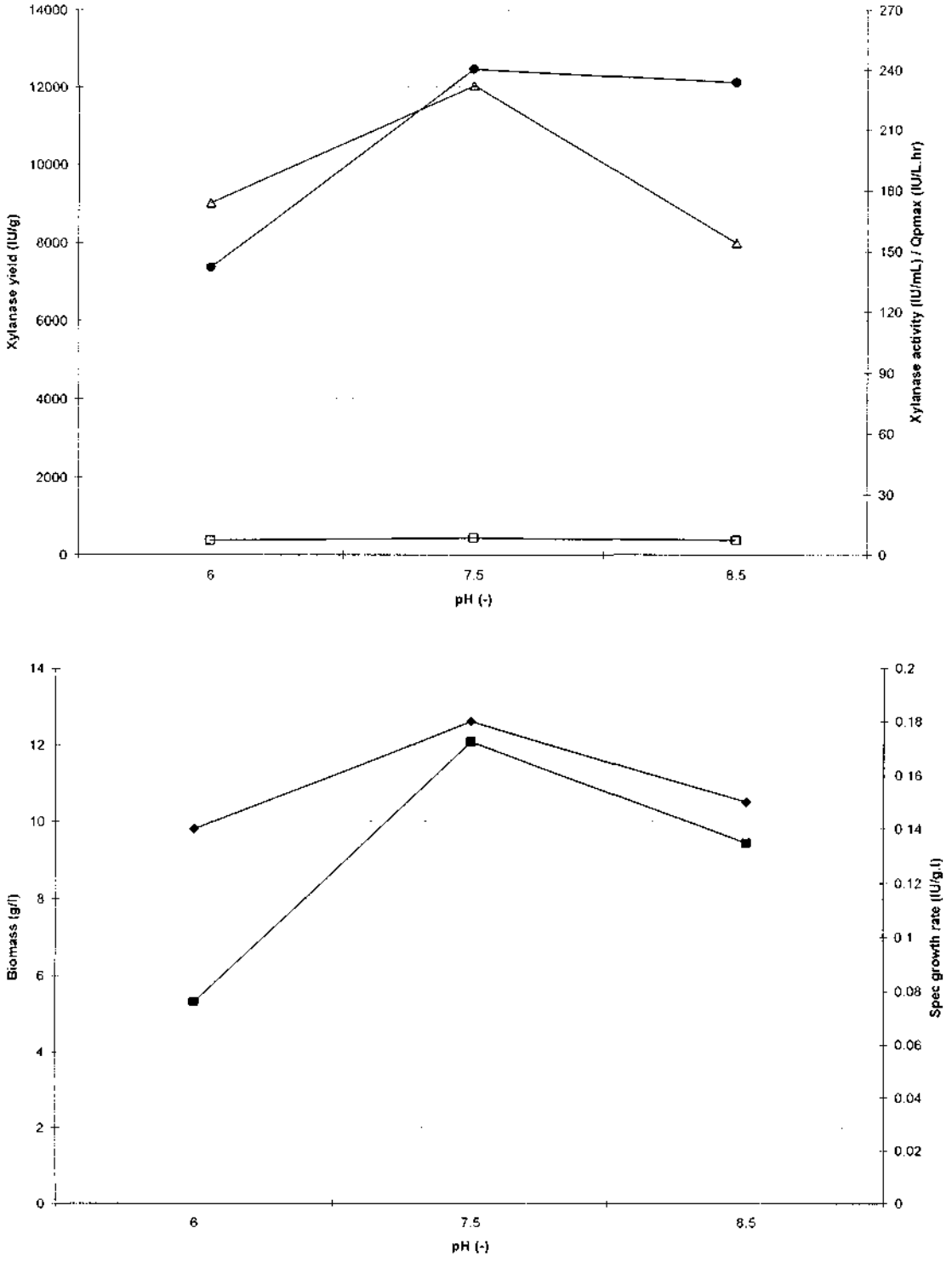


**Figure 5-5** Growth parameters of *A. oryzae* NRRL 3485 with xylan as carbon substrate in bioreactor cultures at superficial gas velocity of 0.0073 $m.s^{-1}$, cultivation pH at 6.0, 7.5 and 8.5 and culture temperature set at 30 $^0$C. Symbols: Δ xylanase activity; ■ biomass; □ max volumetric productivity rate; • xylanase yield; ♦ max specific growth rate

### 5.5 Rheology and hydrodynamic characteristics

After a series of three day cultivations of *Aspergillus oryzae* NRRL 3485 in an IL-ALR, the optimal conditions which produced maximal values were a superficial gas velocity of 0.0073 $m.s^{-1}$ and cultivation conditions of pH 7.5 and temperature 30 $^0$C. During this process, the fungus grew in a dispersed mycelial suspension form with few pellets. The rheological nature of the liquid was complex due to fungal biomass, water, nutrients and xylanases enzymes present in the highly viscous fluid. To simulate the range of the fluid viscosities observed, the final process fluid obtained at optimum conditions was diluted with varying amounts of sterile water (Table 5-5). Rheological property of the fluid was also observed during operation.

**Table 5-5** Concentration of biomass and xylanase enzymes (mean values of duplicate experiments) at various dilutions over a 3-day cultivation period at $O_2$ gas velocity of 0.0073 $m.s^{-1}$ in the IL-ALR (at optimal operating conditions of pH 7.5 and Temperature 30 $^0$C).

| **Product** | **Culture fluid (% concentration)** | | | | |
|---|---|---|---|---|---|
| | **100** | **80** | **60** | **40** | **20** |
| Biomass ($g.l^{-1}$) | 20.03 (±0.65) | 9.82 (±0.73) | 4.21 (±0.71) | 2.19 (±0.62) | 1.25 (±0.45) |
| Xylanase Enzyme ($IU.ml^{-1}$) | 210 (±26.2) | 93 (±23.2) | 46 (±21.6) | 22 (±20.3) | 7.21 (±22.7) |
| Viscosity ($Pa.s^n$) | 1.912 (±0.02) | 0.456 (±0.01) | 0.135 (±0.03) | 0.061 (±0.02) | 0.005 (±0.04) |

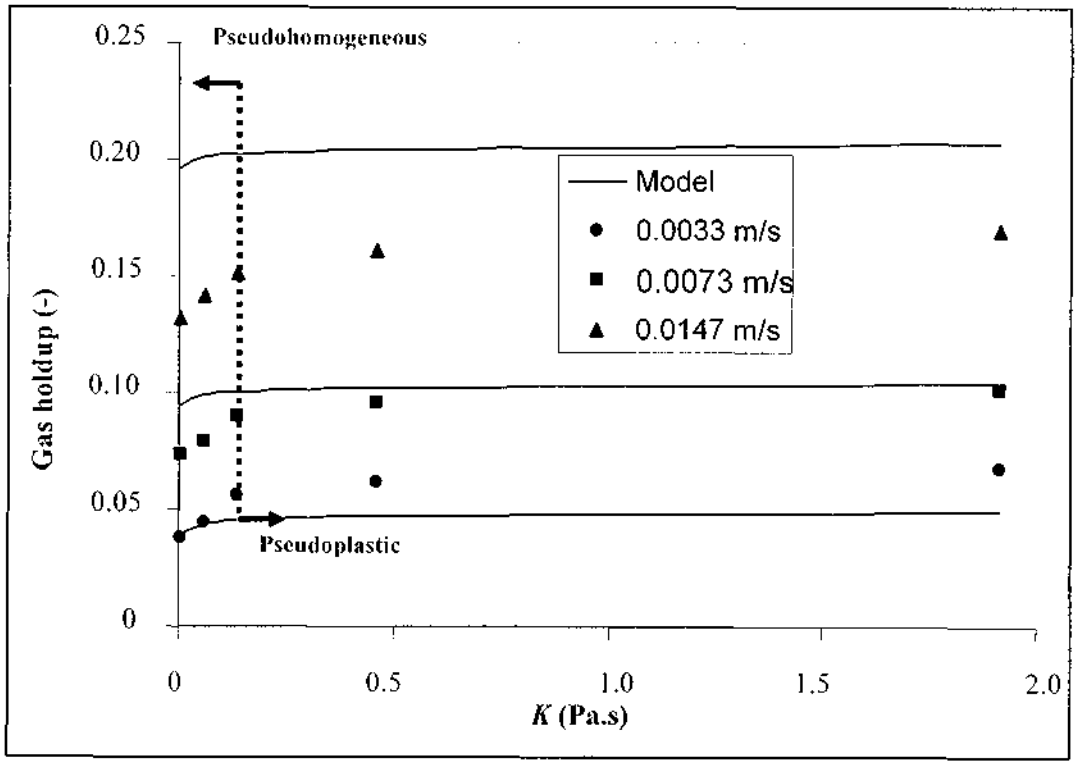


**Figure 5-7** The influence of the consistency index on mean gas hold-up.

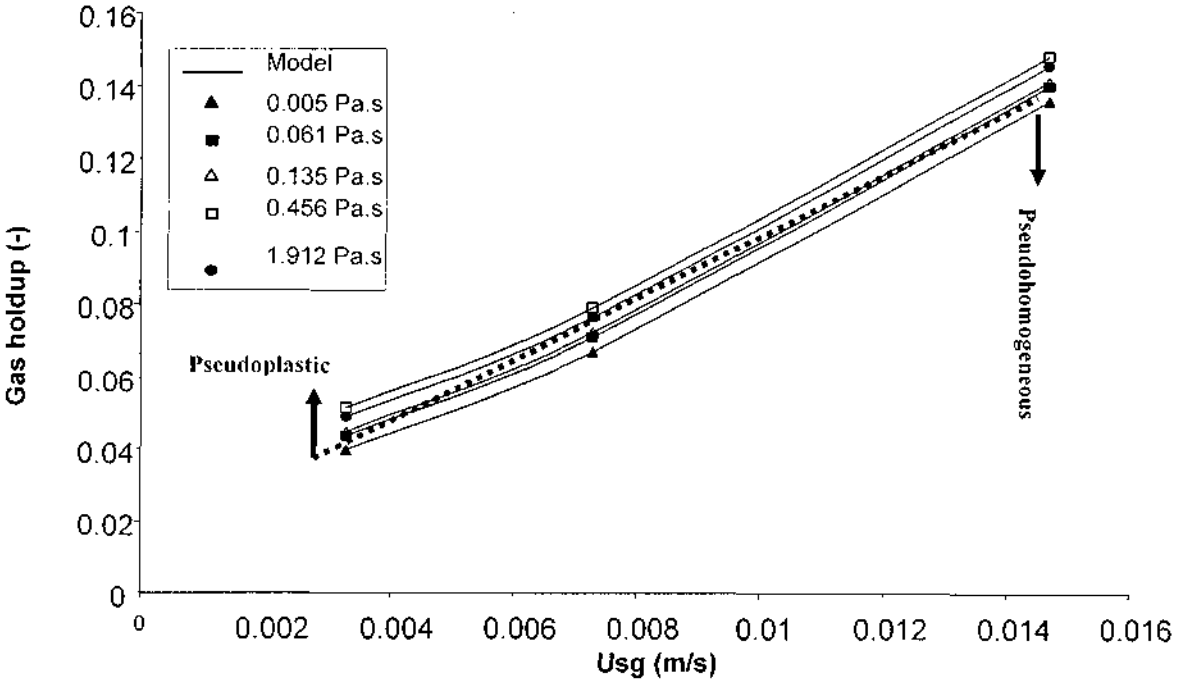


**Figure 5-8** The influence of superficial gas velocity on mean gas hold-up

### 5.5.2 Liquid circulation velocity

The liquid circulation velocity in the annular space (downcomer) of the IL-ALR is shown in Figure 5-9 as a function of the consistency index of the process fluid and in Figure 5-10 as a function of the superficial gas velocity. The expression relating $U_{ld}$ and $U_{sg}$ are described by equation (3.18) (Wang and McNeil, 1992, 1996). The mean relative standard deviation between "calculated" and measured values is ±13.5 % (Figures 5-9 and 5-10).

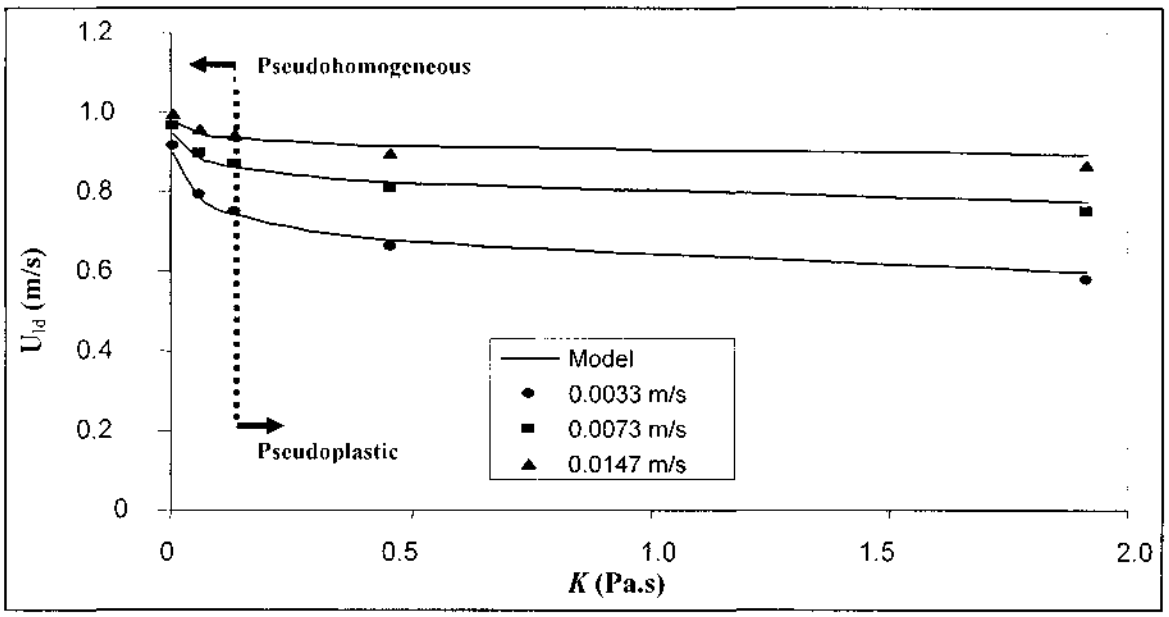


**Figure 5-9** The influence of the consistency index on the liquid recirculation velocity in the annular space.

Figure 5-10 shows the effect of superficial gas rate on the liquid circulation velocity in the annular space. The curves also shows the two different regimes divided by a K value. When $K < 0.135$, the psuedohomogeneous flow regime, liquid velocities increase with increase superficial gas flow rates. Conversely, in the psuedoplastic flow regime when $K > 0.135$, liquid circulation velocities are low and independent on $K$ (Figures 5-9 and 5-10).

### 5.5.3 Mixing time

Mixing times are plotted in Figures 5-11 and 5-12 for various oxygen flow rates and different viscosities investigated. The figures show the variation in $t_m$ as a function of the superficial gas velocity, and of the consistency index of the broth. Mixing times varies according to the relationship given by equation (3.22) (Wang and McNeil, 1992, 1996). The mean standard deviation compared to measured values is ± 15.2 %.

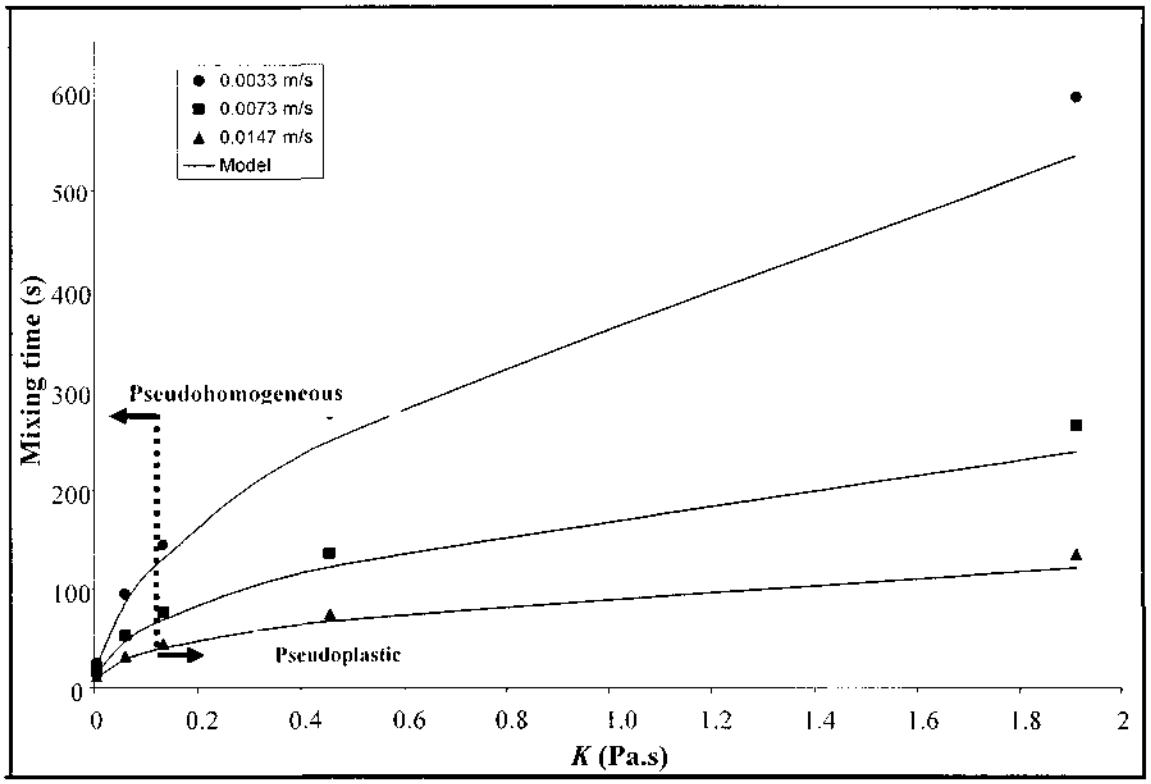


**Figure 5-11** The effect of $K$ on mixing time

## 6. DISCUSSION

This study demonstrated that spent sulphite liquor (SSLc) could serve as carbon substrate as well as inducer for the production of xylanases by *A. oryzae* NRRL 3485. Although xylan, a known powerful and expensive inducer of xylanase, yielded higher volumetric xylanase activities as well as xylanase yields (IU.g.biomass$^{-1}$) than SSLc as carbon substrate (Siedenburg *et al.*, 1998; Bahkali, 1996). Maximum xylanase production by *A. oryzae* NRRL 3485 were obtained with culture pH, temperature and superficial gas velocity set at 7.5, 30 $^{0}$C and 0.0073 m.s$^{-1}$, respectively, with SSLc as carbon sources.

Results presented in the current study clearly show that the superficial gas velocity of 0.0073 m.s$^{-1}$, favored the xylanase activity levels of the *A. oryzae* NRRL 3485 culture medium. Xylanase activity levels and biomass were reduced when gas velocity was increased to 0.0147 m.s$^{-1}$, the lowest were obtained at 0.0033 m.s$^{-1}$. The decrease in xylanase production at high superficial gas rate (0.0147 m.s$^{-1}$) has been attributed to the effects of hydrodynamic stress which may cause morphological disruption. These results are consistent with observations made by Hoq *et al.* (1994) for *T. lanuginosus* RT9. Hoq *et al.* (1994) suggested that at low gas velocity, oxygen limitation may have slowed down the growth rate but, on the other hand, the low shear stress may have aided a higher final biomass concentration, whereas at higher acration rate the conditions facilitated a higher growth rate but the increased shear stress might result in lower biomass concentrations.

Shear stress as a result of aeration intensity has also been reported to cause morphological (Purkarthofer *et al.*, 1993; Singh *et al.*, 2000) as well as physiological changes in some filamentous fungi, resulting in decreased xylanase production (Techapun *et al.*, 2003). The effect of agitation intensity on xylanolytic enzyme production has been investigated in a number of studies and this was conducted in combination with the superficial gas flowrate (Techapun *et al.*, 2003, Chisti et al., 1987, 1988), dissolved oxygen tension (Singh *et al.*, 2000) and cultivation pH (Purkarthofer *et al.*, 1993). Almost all studies use gas flowrates as the sole measure of shear stress and ignore the effects of the shear forces. This renders meaningful comparisons between studies difficult, especially when they have been conducted at different vessel scales and geometry.

The effect caused by varying the cultivation temperature is not clear. Whether it results in synthesis of thermostable enzymes or it affects the thermostability in another way is a matter for further investigation.

It can be seen from data in Table 5-5 and Figure 5-6, the fluid consistency index, $K$, increases with increasing concentration of the fungal biomass and xylanases in the fluid. When the broth percentage exceeds 60 %, $K$ increases sharply, while the flow behavior index, $n$, decreases with increasing concentration of the fungal biomass and the enzyme, xylanases. The undiluted broth shows the highest consistency index of 1.912 Pa.s$^n$ with the lowest flow behavior index of 0.74, representing markedly pseudoplastic or non-Newtonian behavior. Figure 5-6 also shows the transformation from a Newtonian fluid with low biomass and xylanolytic enzyme to a psuedoplastic (or non-Newtonian) fluid with increase in cell mass and xylanase activity levels. Figure 5-7 shows that the gas holdup increases sharply when the value of the consistency index is relatively low. When the consistency index reached a value 0.135, the gas hold up reached a constant value, with increasing consistency index. The main reason for this phenomenon is that on a more viscous broth, after the gas exits over the central tube, a fraction of the gas is broken up in the disengagement section to give large bubbles, which ascend faster than smaller ones, so residence time becomes shorter, counteracting the increase in mean gas holdup.

Figure 5-8 shows that in the range of superficial gas flow rates studied in this work, the gas holdup was significantly affected by the superficial gas velocity, which was in agreement with the result obtained from simulated fermentation systems (Lin, 1976; Robinson and Popovic, 1988; Wang and McNeil, 1996). The gas exiting from the draft or central tube was released in the disengagement section thus forming a bubble free zone in the downcomer or annulus section. Meanwhile, since the liquid circulation velocity increases with increasing gas velocity, the amount of circulating small bubbles trapped to the downcomer by the liquid increases, which leads to a longer residence time of bubbles in the liquid and an increase in the overall mean gas holdup. It was noted that as superficial gas velocity rose in an EL-ALR, the increase in turbulence increased back mixing and vortex mixing, which led to a longer residence time of gas in the liquid. Thus, even when $K$ is very high, gas holdup increases linearly with the increase of superficial gas velocity (this study).

However, when $K > 0.135$ due to frictional losses in the circuit and poor gas dispersion, as well as the rapidly escaping large bubbles, less energy is available to promote circulation between the annular space and the central tube. This may explain why $U_{sg}$ has an impact upon the liquid velocity in annular space when $K > 0.135$. When $K$ was at its highest value of 1.912 Pa.s, liquid velocity was the lowest and seemed completely unaffected by gas velocity. In such cases, the fluid in the annular space appeared to be largely stagnant. In addition, at such high viscosities, there was no visible flow in the annular space when gas velocity was low. In addition to the pronounced effect of frictional resistance in the annular space due to high viscosity of the broth, another possible explanation for the annular flow stoppage may relate to the yield stress property of mycelia process fluids. Results obtained by Wang and McNeil (1992) in a 0.12 $m^3$ EL-ALR showed increasingly pseudoplastic behavior and yield stress with increasing biomass concentration. Figure 5-10 describes the effect of the consistency index $K$ on mixing time. At gas velocity used, $t_m$ increases with increased consistency index. There may be two main contributors to the increased $t_m$ under these circumstances. Firstly, the large amount of small bubbles carried in the annular space, together with the frictional resistance, leads to a decrease in the driving force for liquid circulation, and thus a decreased liquid velocity. Meanwhile, the back mixing and turbulence in the gas bubble zone decrease correspondingly.

Figure 5-11 shows that the mixing time decreases with the increased superficial gas velocity. Two different regimes of mixing can be discerned. Once again, the critical value of $K$, which divides these two regimes, is 0.135. Below this value, mixing times are shorter and decrease smoothly with increasing $U_{sg}$, but when $K$ is above 0.135, $t_m$ values are higher and decrease significantly with increasing $U_{sg}$. At the fluid viscosities range examined, the mixing time is strongly influenced by the superficial gas velocity. On one hand, the increase in superficial gas velocity increases the density difference between annular space and the central tube, which escalates the driving force for the liquid circulation flow, and thus increases liquid circulation velocity (Fumitake, 1982 and Merchuk and Siegel, 1988). Conversely, the increase in superficial gas velocity increases radial dispersion and back mixing, and the chaos in the bubble zone, enhancing mixing and shortening the mixing time. The influence of increased superficial gas velocity is significant in the case of a relatively low consistency index (Figure 5-10 and Figurer 5-11); thus, the mixing time is short. At high $K$, however, increased gas velocity has only a weak influence on

## 7. CONCLUSIONS

Xylanase enzyme production from SSL by *A. oryzae* NRRL 3485 using an IL-ALR was studied to realize the economical feasibility to produce such enzumes. The results presented here clearly demonstrated some apparent benefits for both biomass and xylanase enzyme production process using SSL as a carbon source. This not only solve the pulp and paper mill's effluent treatment and disposal problems but, provide a potential pulp pre-bleaching agent that can reduce or eliminate the use of chlorinated chemicals during the pulp bleaching process. This minimizes the toxicity of waste generated within the pulp and paper producing processes. Although biomass and xylanase enzyme activity was favoured under defined optimum cultural operating conditions in an IL-ALR, the aforementioned cultivation parameters evaluated were markedly affected by superficial gas velocity, culture pH and temperature. A pH of 7.5, temperature of 30 $^{0}$C and superficial gas velocity of 0.0073 m.s$^{-1}$ favoured both biomass and xylanase production. Other operation conditions had adverse effects on cell mass as well as xylanase production due to shear stress as a result of agitation intensity and thus cause morphological as well as physiological changes in a microbial organ resulting in decreased xylanase enzyme production.

The concentration of free mycelia in the fermentation medium was also high during the growth phase, which indicated free mycelia, rather than pellets, were the more productive morphological forms of *A. oryzae* for optimal cell growth and xylanase enzyme production. These results also indicates that the spent sulphite liquor is a convenient substrate for the production of xylanases by *A. oryzae*, being a cheap carbon feedstock as well as serving as an inducer for xylanase synthesis. To scale-up and increase the productivity of xylanase enzymes, it is mandatory to optimize the different operation conditions.

Hydrodynamic and mixing characteristics were studied in an IL-ALR with a viscous filamentous fungal culture *A. oryzae* NRRL 3485. The decrease in flow behavior index, $n$, indicated that the pseudohomogeneous nature of the broth evolved towards a pseudoplastic behavior (high concentration of biomass and xylanases). With a fixed reactor configuration, $\varepsilon_g$, $U_{ld}$ and $t_m$ are strongly influenced by superficial gas velocity and the viscosity of the fluid. The model investigated (Wang and McNeil, 1992, 1996) were more accurate in predicting the behavior of a real fungal strain than those extrapolated from correlations based on Newtonian or simulated non-Newtonian fluids (Chisti et al., 1988, 1989).

- Make sure that the riser area, $A_r$, is greater than the downcomer area, $A_d$. Disengagement section should be enlarged to reduce excessive entrainment and consequent liquid loss, particularly when foaming control systems are being investigated.
- The bioreactor should be installed with pH and temperature sensors that will enable it to automatically control so as to reduce manual handling or adjustments of the vital parameters.

5. Another point of importance from the design perspective view is the absolute value of the reactor height, which have a direct effect on the liquid velocity, in some cases almost independent of the reactor volume. Thus, the scale-up of a process in which the hydrodynamic conditions are critical, such as sear sensitive cultures, must be done carefully.

There is a conflict between the maintenance of a riser to downcomer cross-sectional area ratio, aspect ratio and the usually accepted concept of a fixed vvm (volume of gas per volume of liquid per minute) and the maintenance of a constant range of shear rates and forces throughout the scale-up process. However, this conflict can be overcome by careful application of hydrodynamic design principles.

**Run 1A:** Superficial gas velocity of 0.0033 $m.s^{-1}$

Aeration rate = 0.13 vvm

Temperature at 28 $^0C$ and pH 6.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.31 | 0.36 | 0.54 | 1500.00 | 0.08 | 223.50 |
| 4 | 4.03 | 0.92 | 1.71 | 1858.70 | 0.25 | 276.95 |
| 8 | 2.13 | 1.95 | 2.86 | 1466.67 | 0.43 | 218.53 |
| 16 | 1.98 | 2.83 | 3.51 | 1240.28 | 0.52 | 184.80 |
| 24 | 1.62 | 3.14 | 11.76 | 3745.22 | 1.75 | 558.04 |
| 28 | 1.79 | 3.82 | 15.58 | 4078.53 | 2.32 | 607.70 |
| 32 | 1.62 | 4.18 | 23.86 | 5708.13 | 3.56 | 850.51 |
| 36 | 1.85 | 4.91 | 32.28 | 6574.34 | 4.81 | 979.58 |
| 40 | 2.02 | 5.23 | 43.66 | 8347.99 | 6.51 | 1309.87 |
| 44 | 2.12 | 5.84 | 51.34 | 8791.10 | 7.65 | 1345.42 |
| 48 | 2.16 | 6.06 | 56.82 | 9376.24 | 8.47 | 1397.06 |
| 52 | 2.22 | 6.41 | 61.76 | 9634.95 | 9.20 | 1435.61 |
| 56 | 2.19 | 6.74 | 63.23 | 9381.31 | 9.42 | 1397.81 |
| 60 | 2.26 | 6.92 | 64.48 | 9317.92 | 9.61 | 1388.37 |
| 68 | 2.38 | 7.07 | 67.01 | 9478.08 | 9.98 | 1412.23 |
| 72 | 2.5 | 7.13 | 67.28 | 9436.19 | 10.02 | 1405.99 |

**Run 3A:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 28 $^0C$ and pH 6.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 0.41 | 0.34 | 829.27 | 0.05 | 123.56 |
| 4 | 4.77 | 0.94 | 0.87 | 2366.85 | 0.13 | 352.66 |
| 8 | 3.55 | 1.15 | 1.69 | 2362.57 | 0.25 | 352.02 |
| 16 | 2.89 | 2.18 | 3.01 | 4716.93 | 0.45 | 702.82 |
| 24 | 2.56 | 2.92 | 6.64 | 6440.18 | 0.99 | 959.59 |
| 28 | 2.09 | 3.28 | 10.65 | 6997.45 | 1.59 | 1042.62 |
| 32 | 2.18 | 3.93 | 18.39 | 7968.39 | 2.74 | 1187.29 |
| 36 | 2.27 | 4.19 | 24.74 | 8268.34 | 3.69 | 1231.98 |
| 40 | 2.32 | 4.88 | 30.61 | 8723.16 | 4.56 | 1299.75 |
| 44 | 2.23 | 5.24 | 38.34 | 8663.53 | 5.71 | 1290.87 |
| 48 | 2.43 | 5.66 | 46.89 | 9068.65 | 6.99 | 1351.23 |
| 52 | 2.71 | 5.98 | 49.27 | 8847.95 | 7.34 | 1318.34 |
| 56 | 2.85 | 6.04 | 53.33 | 9392.74 | 7.95 | 1399.52 |
| 60 | 3.18 | 6.11 | 54.18 | 9629.71 | 8.07 | 1434.83 |
| 68 | 3.39 | 6.19 | 57.24 | 10478.28 | 8.53 | 1561.26 |
| 72 | 3.6 | 6.32 | 58.72 | 11326.85 | 8.75 | 1687.70 |

**Run 5A:** Varying superficial gas velocity from 0.0073m.s$^{-1}$

Aeration rate = 0.28 vvm

Temperature at 30 $^{0}$C and pH 76.0

| Time (hr) | DOT (ppm) | Biomass (g.l$^{-1}$) | Enzyme activity (IU.ml$^{-1}$) | Xylanase Yield (IU.g$^{-1}$) | Volumetric Productivity (IU.ml$^{-1}$.h$^{-1}$) | Specific Productivity (IU,g$^{-1}$,h$^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 1.95 | 6.79 | 3482.05 | 1.039 | 532.75 |
| 4 | 4.77 | 3.98 | 11.48 | 2366.85 | 1.76 | 362.13 |
| 8 | 3.55 | 5.26 | 27.15 | 2362.57 | 4.15 | 361.47 |
| 16 | 2.89 | 7.46 | 38.17 | 4716.93 | 5.84 | 721.69 |
| 24 | 2.56 | 7.73 | 52.11 | 6440.18 | 7.97 | 985.35 |
| 28 | 2.09 | 8.03 | 66.19 | 6997.45 | 10.13 | 1070.61 |
| 32 | 2.18 | 8.68 | 72.65 | 7968.39 | 11.12 | 1219.16 |
| 36 | 2.27 | 9.24 | 85.16 | 8268.34 | 13.03 | 1265.06 |
| 40 | 2.32 | 9.92 | 97.81 | 8723.16 | 14.96 | 1334.64 |
| 44 | 2.23 | 10.28 | 101.23 | 8663.53 | 15.49 | 1325.52 |
| 48 | 2.43 | 10.76 | 105.35 | 9068.65 | 16.12 | 1387.50 |
| 52 | 2.71 | 10.93 | 110.32 | 8847.95 | 16.88 | 1353.74 |
| 56 | 2.85 | 11.07 | 112.68 | 9392.74 | 17.24 | 1437.09 |
| 60 | 3.18 | 11.38 | 114.01 | 9629.71 | 17.44 | 1473.35 |
| 68 | 3.39 | 11.84 | 115.03 | 10478.28 | 17.59959 | 1603.18 |
| 72 | 3.6 | 12.3 | 116.05 | 11326.85 | 17.75565 | 1733.01 |

**Run 7A:** Superficial gas velocity of 0.0033 $m.s^{-1}$

Aeration rate = 0.12 vvm

Temperature at 40 $^0C$ and pH 6.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.31 | 0.26 | 0.44 | 1692.31 | 0.083 | 319.85 |
| 4 | 4.03 | 0.52 | 0.91 | 1750.00 | 0.17 | 330.75 |
| 8 | 2.13 | 0.63 | 1.67 | 2650.79 | 0.32 | 501.00 |
| 16 | 1.98 | 1.13 | 2.15 | 1902.65 | 0.41 | 359.60 |
| 24 | 1.62 | 1.94 | 5.61 | 2891.75 | 1.06 | 546.54 |
| 28 | 1.79 | 2.18 | 9.85 | 4518.35 | 1.86 | 853.97 |
| 32 | 1.62 | 2.85 | 13.68 | 4800.00 | 2.59 | 907.20 |
| 36 | 1.85 | 3.19 | 21.82 | 6840.13 | 4.12 | 1292.78 |
| 40 | 2.02 | 3.52 | 32.66 | 9278.41 | 6.17 | 2008.06 |
| 44 | 2.12 | 3.89 | 41.33 | 10624.68 | 7.81 | |
| 48 | 2.16 | 4.16 | 46.28 | 11125.00 | 8.75 | 2102.63 |
| 52 | 2.22 | 4.94 | 49.62 | 10044.53 | 9.39 | 1898.42 |
| 56 | 2.19 | 5.24 | 52.54 | 10026.72 | 9.93 | 1895.05 |
| 60 | 2.26 | 5.92 | 54.18 | 9152.03 | 10.24 | 1729.73 |
| 68 | 2.38 | 6.01 | 55.01 | 9153.08 | 10.39 | 1729.93 |
| 72 | 2.5 | 6.11 | 55.16 | 9027.82 | 10.43 | 1706.26 |

**Run 9A:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 40 $^{0}C$ and pH 6.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU.g^{-1}.h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 0.23 | 0.42 | 1826.09 | 0.13 | 566.09 |
| 4 | 4.77 | 0.65 | 0.76 | 2366.85 | 0.23 | 733.72 |
| 8 | 3.55 | 1.02 | 1.71 | 2362.57 | 0.53 | 732.40 |
| 16 | 2.89 | 1.95 | 1.66 | 4716.93 | 0.51 | 1462.25 |
| 24 | 2.56 | 2.16 | 2.61 | 6440.18 | 0.81 | 1996.46 |
| 28 | 2.09 | 2.89 | 4.98 | 6997.45 | 1.54 | 2169.21 |
| 32 | 2.18 | 3.03 | 6.36 | 7968.39 | 1.97 | 2470.20 |
| 36 | 2.27 | 3.15 | 8.12 | 8268.34 | 2.52 | 2563.19 |
| 40 | 2.32 | 3.62 | 16.51 | 8723.16 | 5.12 | 2704.18 |
| 44 | 2.23 | 3.94 | 22.64 | 8663.53 | 7.02 | 2685.69 |
| 48 | 2.43 | 4.13 | 31.72 | 9068.65 | 9.83 | 2811.28 |
| 52 | 2.71 | 4.45 | 36.11 | 8847.95 | 11.19 | 2742.86 |
| 56 | 2.85 | 4.67 | 38.54 | 9392.74 | 11.94 | 2911.75 |
| 60 | 3.18 | 4.91 | 38.81 | 9629.71 | 12.03 | 2985.21 |
| 68 | 3.39 | 5.04 | 39.04 | 10478.28 | 12.10 | 3248.27 |
| 72 | 3.6 | 5.28 | 40.13 | 11326.85 | 12.44 | 3511.32 |

**Run 11A:** Superficial gas velocity of 0.0073 $m.s^{-1}$

Aeration rate = 0.28 vvm

Temperature at 28 $^{0}C$ and pH 7.5

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 1.91 | 1.6 | 837.70 | 0.19 | 99.69 |
| 4 | 4.77 | 2.68 | 2.71 | 1011.19 | 0.32 | 120.33 |
| 8 | 3.55 | 3.13 | 7.22 | 2306.71 | 0.86 | 274.50 |
| 16 | 2.89 | 3.93 | 12.66 | 3221.37 | 1.51 | 383.34 |
| 24 | 2.56 | 4.11 | 28.76 | 6997.57 | 3.42 | 832.71 |
| 28 | 2.09 | 4.87 | 32.34 | 6640.66 | 3.85 | 790.24 |
| 32 | 2.18 | 5.77 | 53.53 | 9277.30 | 6.37 | 1104.00 |
| 36 | 2.27 | 7.13 | 88.22 | 12373.07 | 10.50 | 1472.40 |
| 40 | 2.32 | 7.96 | 102.45 | 12870.60 | 12.19 | 1531.60 |
| 44 | 2.23 | 8.79 | 127.2 | 14470.99 | 15.14 | 1722.05 |
| 48 | 2.43 | 9.17 | 138.25 | 15076.34 | 16.45 | 1794.08 |
| 52 | 2.71 | 10.31 | 144.33 | 13999.03 | 17.17 | 1665.88 |
| 56 | 2.85 | 10.92 | 146.58 | 13423.08 | 17.44 | 1597.35 |
| 60 | 3.18 | 11.06 | 148.13 | 13393.31 | 17.63 | 1593.80 |
| 68 | 3.39 | 11.64 | 150.46 | 12926.12 | 17.91 | 1538.21 |
| 72 | 3.6 | 12.08 | 151.44 | 12536.42 | 18.02 | 1491.83 |

**Run 13A:** Superficial gas velocity of 0.0033 $m.s^{-1}$

Aeration rate = 0.12 vvm

Temperature at 30 $^{0}C$ and pH 7.5

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.31 | 1.05 | 1.59 | 1514.29 | 0.26 | 215.03 |
| 4 | 4.03 | 2.16 | 3.71 | 1717.59 | 0.53 | 243.90 |
| 8 | 2.13 | 4.59 | 5.86 | 1276.69 | 0.83 | 181.29 |
| 16 | 1.98 | 5.38 | 12.51 | 2325.28 | 1.78 | 330.19 |
| 24 | 1.62 | 6.94 | 27.06 | 3899.14 | 3.84 | 553.68 |
| 28 | 1.79 | 8.29 | 65.58 | 7910.74 | 9.31 | 1123.32 |
| 32 | 1.62 | 10.88 | 104.68 | 9621.32 | 14.86 | 1366.23 |
| 36 | 1.85 | 11.84 | 122.18 | 10319.26 | 17.35 | 1465.33 |
| 40 | 2.02 | 13.16 | 136.73 | 10389.82 | 19.42 | 1475.35 |
| 44 | 2.12 | 13.44 | 149.33 | 11110.86 | 21.20 | 1577.74 |
| 48 | 2.16 | 13.62 | 156.28 | 11474.30 | 22.19 | 1629.35 |
| 52 | 2.22 | 14.11 | 159.76 | 11322.47 | 22.69 | 1607.79 |
| 56 | 2.19 | 14.64 | 163.32 | 11155.74 | 23.19 | 1584.11 |
| 60 | 2.26 | 15.03 | 164.48 | 10943.45 | 23.36 | 1553.97 |
| 68 | 2.38 | 15.47 | 165.17 | 10676.79 | 23.45 | 1516.10 |
| 72 | 2.5 | 15.91 | 165.86 | 10424.89 | 23.55 | 1480.33 |

**Run 15A:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 30 $^0$C and pH 7.5

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 7.91 | 1.95 | 6.79 | 3482.05 | 0.58 | 299.46 |
| 4 | 8.73 | 3.98 | 11.48 | 2884.42 | 0.98 | 248.06 |
| 8 | 6.82 | 5.26 | 27.15 | 5161.60 | 2.33 | 443.90 |
| 16 | 4.05 | 7.46 | 38.17 | 5116.62 | 3.28 | 440.03 |
| 24 | 2.74 | 7.73 | 52.11 | 6741.27 | 4.48 | 579.75 |
| 28 | 2.21 | 8.03 | 66.19 | 8242.84 | 5.69 | 708.88 |
| 32 | 1.73 | 8.68 | 72.65 | 8369.82 | 6.24 | 719.80 |
| 36 | 2.14 | 9.24 | 85.16 | 9216.45 | 7.32 | 792.61 |
| 40 | 2.03 | 9.92 | 97.81 | 9859.88 | 8.41 | 847.95 |
| 44 | 2.15 | 10.28 | 101.23 | 9847.28 | 8.71 | 846.87 |
| 48 | 2.61 | 10.76 | 105.35 | 9790.89 | 9.06 | 842.02 |
| 52 | 2.91 | 10.93 | 110.32 | 10093.32 | 9.49 | 868.03 |
| 56 | 3.08 | 11.07 | 112.68 | 10178.86 | 9.69 | 875.38 |
| 60 | 3.29 | 11.38 | 114.01 | 10018.45 | 9.80 | 861.59 |
| 68 | 4.75 | 11.84 | 115.03 | 9715.37 | 9.89 | 835.52 |
| 72 | 6.21 | 12.3 | 116.05 | 9434.96 | 9.98 | 811.41 |

**Run 17A:** Superficial gas velocity of 0.0073 $m.s^{-1}$

Aeration rate = 0.28 vvm

Temperature at 40 $^{0}C$ and pH 7.5

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 0.34 | 0.71 | 2088.24 | 0.12 | 355.00 |
| 4 | 4.77 | 0.95 | 3.71 | 3905.26 | 0.63 | 663.89 |
| 8 | 3.55 | 1.65 | 5.98 | 3624.24 | 1.017 | 616.12 |
| 16 | 2.89 | 2.08 | 8.51 | 4091.35 | 1.45 | 695.53 |
| 24 | 2.56 | 3.22 | 12.72 | 3950.31 | 2.16 | 671.55 |
| 28 | 2.09 | 4.67 | 16.58 | 3550.32 | 2.82 | 603.55 |
| 32 | 2.18 | 5.48 | 30.64 | 5591.24 | 5.21 | 950.51 |
| 36 | 2.27 | 6.37 | 47.18 | 7406.59 | 8.02 | 1259.12 |
| 40 | 2.32 | 7.19 | 58.71 | 8165.51 | 9.98 | 1388.14 |
| 44 | 2.23 | 8.01 | 63.33 | 7906.37 | 10.77 | 1344.08 |
| 48 | 2.43 | 8.73 | 67.86 | 7773.20 | 11.54 | 1321.44 |
| 52 | 2.71 | 9.68 | 71.97 | 7434.92 | 12.23 | 1263.94 |
| 56 | 2.85 | 10.11 | 74.12 | 7331.36 | 12.60 | 1246.33 |
| 60 | 3.18 | 10.89 | 76.48 | 7022.96 | 13.01 | 1193.90 |
| 68 | 3.39 | 10.28 | 78.18 | 7605.06 | 13.29 | 1292.86 |
| 72 | 3.6 | 10.04 | 79.08 | 7876.49 | 13.44 | 1339.00 |

**Run 19A:** Superficial gas velocity of 0.0033 $m.s^{-1}$

Aeration rate = 0.13 vvm

Temperature at 28 $^0C$ and pH 8.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.31 | 0.22 | 0.46 | 1514.29 | 0.09 | 378.57 |
| 4 | 4.03 | 0.87 | 1.17 | 1717.59 | 0.25 | 214.69 |
| 8 | 2.13 | 1.15 | 3.89 | 1276.69 | 0.82 | 79.79 |
| 16 | 1.98 | 1.78 | 4.15 | 2325.28 | 0.87 | 96.89 |
| 24 | 1.62 | 2.04 | 7.27 | 3899.14 | 1.53 | 139.26 |
| 28 | 1.79 | 2.88 | 13.85 | 7910.74 | 2.91 | 247.21 |
| 32 | 1.62 | 3.12 | 24.13 | 9621.32 | 5.07 | 267.26 |
| 36 | 1.85 | 3.89 | 28.81 | 10319.26 | 6.05 | 257.98 |
| 40 | 2.02 | 4.13 | 35.2 | 10389.82 | 7.39 | 236.13 |
| 44 | 2.12 | 5.21 | 46.51 | 11110.86 | 9.77 | 231.48 |
| 48 | 2.16 | 5.46 | 52.79 | 11474.3 | 11.09 | 220.66 |
| 52 | 2.22 | 6.11 | 47.29 | 11322.47 | 9.93 | 202.19 |
| 56 | 2.19 | 6.78 | 64.43 | 11155.74 | 13.53 | 185.93 |
| 60 | 2.26 | 7.03 | 66.08 | 10943.45 | 13.87 | 160.93 |
| 68 | 2.38 | 7.22 | 69.31 | 10676.79 | 14.55 | 148.29 |
| 72 | 2.5 | 8.01 | 71.86 | 10410.13 | 15.09 | 135.65 |

**Run 21A:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 28 $^{0}C$ and pH 8.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 7.91 | 0.45 | 2.18 | 3482.051 | 0.46 | 870.51 |
| 4 | 8.73 | 1.21 | 5.29 | 2884.42 | 1.11 | 360.55 |
| 8 | 6.82 | 1.77 | 8.13 | 5161.59 | 1.71 | 322.6 |
| 16 | 4.05 | 2.34 | 18.37 | 5116.62 | 3.86 | 213.19 |
| 24 | 2.74 | 3.28 | 24.1 | 6741.27 | 5.06 | 240.76 |
| 28 | 2.21 | 3.89 | 36.19 | 8242.84 | 7.59 | 257.59 |
| 32 | 1.73 | 4.32 | 43.12 | 8369.82 | 9.06 | 232.5 |
| 36 | 2.14 | 5.19 | 42.54 | 9216.45 | 8.93 | 230.41 |
| 40 | 2.03 | 6.67 | 58.43 | 9859.88 | 12.27 | 224.09 |
| 44 | 2.15 | 7.07 | 65.77 | 9847.28 | 13.81 | 205.15 |
| 48 | 2.61 | 8.28 | 72.51 | 9790.89 | 15.22 | 188.29 |
| 52 | 2.91 | 9.45 | 80.32 | 10093.32 | 16.87 | 180.24 |
| 56 | 3.08 | 10.04 | 82.14 | 10178.86 | 17.25 | 169.65 |
| 60 | 3.29 | 10.36 | 83.29 | 10018.45 | 17.49 | 147.33 |
| 68 | 4.75 | 10.94 | 84.26 | 9715.37 | 17.69 | 134.936 |
| 72 | 6.21 | 11.02 | 85.05 | 9412.29 | 17.86 | 122.542 |

**Run 23A:** Varying superficial gas velocity from 0.0073m.s$^{-1}$

Aeration rate = 0.28 vvm

Temperature at 30 $^{0}$C and pH 8.0

| Time (hr) | DOT (ppm) | Biomass (g.l$^{-1}$) | Enzyme activity (IU.ml$^{-1}$) | Xylanase Yield (IU.g$^{-1}$) | Volumetric Productivity (IU.ml$^{-1}$.h$^{-1}$) | Specific Productivity (IU,g$^{-1}$,h$^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.08 | 0.94 | 2.13 | 2265.96 | 0.13 | 135.96 |
| 4 | 4.77 | 1.02 | 4.42 | 2366.85 | 0.27 | 142.01 |
| 8 | 3.55 | 1.59 | 9.24 | 2362.57 | 0.55 | 141.75 |
| 16 | 2.89 | 2.24 | 23.63 | 4716.93 | 1.42 | 283.02 |
| 24 | 2.56 | 2.94 | 34.91 | 6440.18 | 2.09 | 386.41 |
| 28 | 2.09 | 3.63 | 49.63 | 6997.45 | 2.98 | 419.85 |
| 32 | 2.18 | 4.01 | 55.76 | 7968.39 | 3.35 | 478.10 |
| 36 | 2.27 | 4.35 | 65.16 | 8268.34 | 3.91 | 496.10 |
| 40 | 2.32 | 5.17 | 68.19 | 8723.16 | 4.09 | 523.39 |
| 44 | 2.23 | 5.97 | 71.32 | 8663.53 | 4.28 | 519.81 |
| 48 | 2.43 | 6.03 | 75.53 | 9068.65 | 4.53 | 544.12 |
| 52 | 2.71 | 6.26 | 80.65 | 8847.95 | 4.84 | 530.88 |
| 56 | 2.85 | 6.31 | 82.86 | 9392.74 | 4.97 | 563.56 |
| 60 | 3.18 | 6.58 | 85.1 | 9629.71 | 5.11 | 577.78 |
| 68 | 3.39 | 6.71 | 85.03 | 10478.28 | 5.10 | 628.70 |
| 72 | 3.6 | 6.92 | 86.89 | 11326.85 | 5.21 | 679.61 |

**Run 25A:** Superficial gas velocity of 0.0033 $m.s^{-1}$

Aeration rate = 0.12 vvm

Temperature at 40 $^0C$ and pH 8.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.31 | 0.11 | 0.34 | 1514.29 | 0.07 | 378.57 |
| 4 | 4.03 | 0.53 | 1.01 | 1717.59 | 0.21 | 214.69 |
| 8 | 2.13 | 0.95 | 2.58 | 1276.69 | 0.54 | 79.79 |
| 16 | 1.98 | 1.31 | 3.15 | 2325.28 | 0.66 | 96.89 |
| 24 | 1.62 | 1.91 | 4.27 | 3899.14 | 0.89 | 139.26 |
| 28 | 1.79 | 2.37 | 6.18 | 7910.74 | 1.30 | 247.21 |
| 32 | 1.62 | 2.42 | 8.25 | 9621.32 | 1.73 | 267.26 |
| 36 | 1.85 | 2.84 | 12.81 | 10319.26 | 2.69 | 257.98 |
| 40 | 2.02 | 3.06 | 23.71 | 10389.82 | 4.98 | 236.13 |
| 44 | 2.12 | 3.76 | 33.48 | 11110.86 | 7.03 | 231.48 |
| 48 | 2.16 | 4.08 | 42.64 | 11474.3 | 8.95 | 220.66 |
| 52 | 2.22 | 4.71 | 46.79 | 11322.47 | 9.83 | 202.19 |
| 56 | 2.19 | 5.04 | 49.21 | 11155.74 | 10.33 | 185.93 |
| 60 | 2.26 | 5.35 | 51.85 | 10943.45 | 10.89 | 160.93 |
| 68 | 2.38 | 5.79 | 51.28 | 10676.79 | 10.77 | 148.29 |
| 72 | 2.5 | 5.92 | 54.24 | 10410.13 | 11.39 | 135.65 |

**Run 27A:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 40 $^{0}C$ and pH 8.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 7.91 | 0.31 | 0.36 | 3482.051 | 0.08 | 870.51 |
| 4 | 8.73 | 0.87 | 2.11 | 2884.42 | 0.44 | 360.55 |
| 8 | 6.82 | 1.04 | 2.85 | 5161.59 | 0.59 | 322.6 |
| 16 | 4.05 | 1.21 | 3.53 | 5116.62 | 0.74 | 213.19 |
| 24 | 2.74 | 1.88 | 4.27 | 6741.27 | 0.89 | 240.76 |
| 28 | 2.21 | 2.16 | 7.81 | 8242.84 | 1.64 | 257.59 |
| 32 | 1.73 | 2.84 | 8.52 | 8369.82 | 1.79 | 232.5 |
| 36 | 2.14 | 3.13 | 11.72 | 9216.45 | 2.46 | 230.41 |
| 40 | 2.03 | 3.91 | 17.38 | 9859.88 | 3.65 | 224.09 |
| 44 | 2.15 | 4.01 | 24.41 | 9847.28 | 5.13 | 205.15 |
| 48 | 2.61 | 4.73 | 27.92 | 9790.89 | 5.86 | 188.29 |
| 52 | 2.91 | 4.98 | 31.97 | 10093.32 | 6.71 | 180.24 |
| 56 | 3.08 | 5.11 | 44.12 | 10178.86 | 9.27 | 169.65 |
| 60 | 3.29 | 5.49 | 46.56 | 10018.45 | 9.78 | 147.33 |
| 68 | 4.75 | 5.68 | 46.82 | 9715.37 | 9.83 | 134.936 |
| 72 | 6.21 | 5.83 | 49.14 | 9412.29 | 10.32 | 122.542 |

**Run 1B:** Superficial gas velocity of 0.0073 $m.s^{-1}$

Aeration rate = 0.26 vvm

Temperature at 30 $^{0}C$ and pH 6.0

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.43 | 0.31 | 1.11 | 4732.08 | 0.21 | 612.68 |
| 4 | 6.30 | 0.64 | 2.71 | 5845.00 | 0.55 | 706.60 |
| 8 | 3.31 | 0.99 | 4.98 | 5189.44 | 0.72 | 817.22 |
| 16 | 2.89 | 1.35 | 8.51 | 5557.88 | 1.65 | 736.34 |
| 24 | 2.62 | 1.91 | 14.72 | 6844.98 | 2.25 | 1021.56 |
| 28 | 1.97 | 2.17 | 25.58 | 5780.11 | 3.08 | 1125.59 |
| 32 | 1.26 | 3.25 | 48.64 | 7394.16 | 5.13 | 1165.76 |
| 36 | 1.58 | 3.48 | 62.18 | 8685.43 | 6.06 | 1273.08 |
| 40 | 2.20 | 3.89 | 83.17 | 13426.31 | 9.25 | 1344.59 |
| 44 | 2.11 | 4.06 | 108.34 | 1188.21 | 10.44 | 1166.76 |
| 48 | 2.61 | 4.33 | 115.60 | 9859.46 | 10.74 | 1729.07 |
| 52 | 2.42 | 4.71 | 126.71 | 9025.56 | 11.19 | 1830.08 |
| 56 | 2.39 | 4.84 | 132.22 | 8667.71 | 12.33 | 1977.75 |
| 60 | 2.46 | 5.13 | 151.58 | 9861.77 | 12.66 | 1939.42 |
| 68 | 2.58 | 5.22 | 169.51 | 9322.54 | 13.28 | 2015.56 |
| 72 | 2.42 | 5.30 | 173.82 | 7366.31 | 17.24 | 2111.22 |

**Run 3B:** Superficial gas velocity of 0.0147 $m.s^{-1}$

Aeration rate = 0.56 vvm

Temperature at 30 $^{0}C$ and pH 7.5

| Time (hr) | DOT (ppm) | Biomass ($g.l^{-1}$) | Enzyme activity ($IU.ml^{-1}$) | Xylanase Yield ($IU.g^{-1}$) | Volumetric Productivity ($IU.ml^{-1}.h^{-1}$) | Specific Productivity ($IU,g^{-1},h^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 8.91 | 0.11 | 1.37 | 1961.90 | 0.18 | 470.00 |
| 4 | 7.73 | 0.34 | 4.91 | 2085.19 | 0.29 | 453.89 |
| 8 | 5.22 | 0.95 | 9.84 | 3109.52 | 1.51 | 666.00 |
| 16 | 4.55 | 1.34 | 10.51 | 4645.45 | 1.86 | 699.55 |
| 24 | 3.94 | 1.90 | 22.37 | 6124.38 | 2.09 | 746.12 |
| 28 | 2.81 | 2.07 | 55.84 | 6272.37 | 2.42 | 877.20 |
| 32 | 1.93 | 2.26 | 78.64 | 7758.11 | 3.77 | 900.20 |
| 36 | 2.04 | 2.46 | 86.32 | 8899.78 | 3.83 | 1138.93 |
| 40 | 2.13 | 2.81 | 103.47 | 9718.28 | 4.27 | 1147.84 |
| 44 | 2.45 | 2.96 | 112.48 | 8708.92 | 4.89 | 1218.87 |
| 48 | 2.81 | 3.18 | 121.86 | 9904.72 | 5.15 | 1222.99 |
| 52 | 2.31 | 3.51 | 132.79 | 10556.65 | 5.79 | 2287.74 |
| 56 | 3.28 | 3.95 | 140.11 | 11213.54 | 6.07 | 1677.72 |
| 60 | 3.83 | 4.23 | 146.23 | 10345.49 | 6.38 | 1718.68 |
| 68 | 4.14 | 4.51 | 152.79 | 11987.43 | 6.34 | 1820.75 |
| 72 | 5.12 | 4.91 | 154.23 | 12119.32 | 7.15 | 1713.29 |

# APPENDIX C

## C.1 Data sheets

The following hydrodynamics data were obtained for the experiments performed for the cultivation of *Aspergillus oryzae* NRRL 3485 strain with SSL as carbon substrate and model equations for used for the hydrodynamic characteristics for the internal loop air lift reactor:

### *C1.1 Mean gas holdup*

Overall mean gas holdup, $\varepsilon_G$, as a function of superficial gas velocity , $U_G$, are expressed by the equation:

$$\varepsilon_G = aU_G + b \qquad (3.2.1)$$

where $a$ and $b$ in equation (3.2.1) are themselves the function of consistency index ($K$). By correlating $a$ and $b$ with $K$, we have:

$$a = 0.1379 + 7.8284*10^{-3} \ln K \qquad (3.2.2)$$

$$b = 2.4882*10^{-3} + 1.5437*10^{-3} \ln K \qquad (3.2.3)$$

Equation (3.2.1) and (3.2.2) in equation (3.2.3) is expressed as:

$$\varepsilon_G = \left(0.1379 + 7.8284*10^{-3} \ln K\right)U_G + 2.4882*10^{-3} + 1.5437*10^{-3} \ln K \qquad (3.2.4)$$

| $U_{sg}$ (m.s$^{-1}$) | | 0.0033 | 0.0073 | 0.0147 |
|---|---|---|---|---|
| **Model prediction** | ***K*** **(Pa.s)** | ε (-) | ε (-) | ε (-) |
| | 0.005 | 0.0397 | 0.0947 | 0.1964 |
| | 0.061 | 0.0436 | 0.0987 | 0.2006 |
| | 0.135 | 0.0449 | 0.0999 | 0.2019 |
| | 0.456 | 0.0468 | 0.1019 | 0.2039 |
| | 1.912 | 0.0490 | 0.1042 | 0.2063 |

| **Model prediction** | $U_{sg}$ (m.s$^{-1}$) | ε (-) | ε (-) | ε (-) | ε (-) | ε (-) |
|---|---|---|---|---|---|---|
| | 0.0033 | 0.0397 | 0.0436 | 0.0449 | 0.0468 | 0.0490 |
| | 0.0073 | 0.0947 | 0.0987 | 0.0999 | 0.1019 | 0.1042 |
| | 0.0147 | 0.1964 | 0.2006 | 0.2019 | 0.2039 | 0.2063 |

| **Experimental results** | $U_{sg}$ (m.s$^{-1}$) | ε (-) | ε (-) | ε (-) | ε (-) | ε (-) |
|---|---|---|---|---|---|---|
| | 0.0033 | 0.0378 | 0.0440 | 0.0559 | 0.0617 | 0.0675 |
| | 0.0073 | 0.0732 | 0.0788 | 0.0898 | 0.0952 | 0.1006 |
| | 0.0147 | 0.1314 | 0.1418 | 0.1508 | 0.1602 | 0.1694 |

**Run 2C:** Superficial gas velocity of 0.0033, 0.0073 and 0.147 $m.s^{-1}$

Temperature at 30 $^0$C and pH 7.5

| $U_{sg}$ ($m.s^{-1}$) | | **0.0033** | **0.0073** | **0.0147** |
|---|---|---|---|---|
| **Experimental results** | ***K*** **(Pa.s)** | $t_a$ **(s)** | $t_a$ **(s)** | $t_a$ **(s)** |
| *L (cm) = 5.0* | 0.005 | 1.002 | 0.98 | 0.94 |
| | 0.061 | 0.95 | 0.93 | 0.9 |
| | 0.135 | 0.91 | 0.89 | 0.88 |
| | 0.456 | 0.87 | 0.85 | 0.84 |
| | 1.912 | 0.85 | 0.84 | 0.82 |

| $U_{sg}$ ($m.s^{-1}$) | | **0.0033** | **0.0073** | **0.0147** |
|---|---|---|---|---|
| **Model prediction** | ***K*** **(Pa.s)** | $U_{ld}$ **($m.s^{-1}$)** | $U_{ld}$ **($m.s^{-1}$)** | $U_{ld}$ **($m.s^{-1}$)** |
| | 0.005 | 0.897 | 0.948 | 0.976 |
| | 0.061 | 0.784 | 0.886 | 0.947 |
| | 0.135 | 0.742 | 0.862 | 0.935 |
| | 0.456 | 0.676 | 0.823 | 0.9159 |
| | 1.912 | 0.596 | 0.774 | 0.890 |

| $U_{sg}$ ($m.s^{-1}$) | | **0.0033** | **0.0073** | **0.0147** |
|---|---|---|---|---|
| **Experimental results** | ***K*** **(Pa.s)** | $U_{ld}$ **($m.s^{-1}$)** | $U_{ld}$ **($m.s^{-1}$)** | $U_{ld}$ **($m.s^{-1}$)** |
| | 0.005 | 0.915 | 0.967 | 0.995 |
| | 0.061 | 0.791 | 0.895 | 0.956 |
| | 0.135 | 0.749 | 0.870 | 0.944 |
| | 0.456 | 0.663 | 0.807 | 0.898 |
| | 1.912 | 0.578 | 0.750 | 0.864 |

***C1.3 Mixing time***

Mixing times as a function of the superficial gas velocity, and of the viscosity if the broths are presented. Mixing times varies according to the relationship:

$$t_m = mU_{Ci}^{n} \qquad (3.2.11)$$

where $m$ and $n$ change with the consistency index $K$, and can be expressed as:

$$m = 12.3668K^{-0.05931} \qquad (3.2.12)$$

$$n = -0.9664 - 0.0791 \ln K \qquad (3.2.13)$$

Equation (3.2.11) with equation (3.2.12) and (3.2.13), yield a correlation for $t_m$ in terms of $U_{sg}$ and $K$ thus described as:

$$t_m = 12.3668K^{-0.05931}U_{sg}^{-0.9664-0.0791\ln K} \qquad (3.2.14)$$

## REFERENCES


1. **Abashar, M.E., Narsingh, U., Rouillard, A.E. and Judd, R. (1998).** Hydrodynamic modeling of an external loop airlift reactor. *Ind. Eng. Chem. Res.* **37**: 1251-1268.

2. **Allen, D.G. and Robinson, C.W. (1989).** Hydrodynamics and mass transfer in *Aspergillus niger* fermentations in bubble column and loop bioreactors. *Biotechnol. Bioeng.* **34**: 731-740.

3. **Allen, D.G. and Robinson, C.W. (1990).** Measurement of rheological properties of filamentous fermentation broths. *Chem. Eng. Sci.* **45**, 37-48.

4. **Al-Marsy, W. (1993).** An experimental investigation of mass transfer in external loop airlift reactors. *Ph.D. Thesis*, University Collage.

5. **Al-Marsy, W. and Abasaeed, A.E. (1998).** On the scale-up of external loop airlift reactors. *Chem. Eng. Sci.* **53** (24): 4085-4096.

6. **Alvarez, A. (1992).** Gas-liquid bioreactors: Maximum stable bubble size. *Chem. Eng. J.* **49**: B13-B16.

7. **Anupama, Ravindra P. (2000).** Value-added food: Single cell protein. *Biotechnol Adv.* **18**(6):459-479.

8. **Bahkali, A.H. (1996).** Influence of various carbohydrates on xylanase production in *Verticillium tricorpus. Biores Technol* **57**: 265-268.

9. **Bahl, C. and Agrawal, L. (1969).** Glycosidases of *Aspergillus niger I.* Purification and characterization of β-galactosidases and β-N-acetylglucosaminidase. *J. of Biol. Chem.* **244** (11):2970

10. **Bailey, M.J and Poutanen, K. (1989).** Production of xylanolytic enzymes by strains of *Aspergillus. Appl. Microbiol. and Biotechnol.* **30** (1): 232-245.

21. **Bello, R.A., Robinson, C.W. and Moo-Young, M. (1985).** Gas holdup and overall volumetric oxygen mass transfer coefficient in airlift contactors. *Biotechnology. Bioeng.* **27**: 369-381.

22. **Biely, P., Kratky, Z., Vrsanska, M. and Urmanicova, D. (1980).** Induction and inducers of endo-1,4-xylanase in the yeast *Cryptococcus albidus*. *Eur. J. Biochem.* **108**:323–329.

23. **Berry, S. (1989).** Contribution of autolysin to virulence of *Streptococcus pneumoniae*. *Inf. and Immun.* **57** (8):2324-2335.

24. **Biely, P. (1985).** Microbial xylanolytic systems. *Trends Biotechnol* **3**: 286-290.

25. **Biely, P., Puls, J., and Schneider, H. (1985).** Acetyl xylan esterases in fungal cellulolytic systems. *FEBS Letters* **186**:80–84.

26. **Biely, P., Markovik, O. and Mislovicova, D. (1985).** Sensitive detection of endo-1,4-β-xylanases in gels. *Anal Biochem* **144:** 147-151

27. **Biely, P. MacKenzie, C.R , Puls, J. and Schneider, H. (1986).** Cooperativity of Esterases and Xylanases in the Enzymatic Degradation of Acetyl Xylan. *Biotechnol.* **4**:731–733.

28. **Biely, Tenkanen and D. Kluepfel. (1996).** Endo-β-1,4-xylanase families: Biermann CJ (1993) Essentials of Pulping and Paper Making. Academic Press, San Diego, CA differences in catalytic properties. *Journal of Biotechnol.* **83** (3): 231-244

29. **Biermann, C.J. (1993).** Essentials of Pulping and Paper Making. Academic Press, San Diego, CA

30. **Biswas, S.R., Mishra, A.K. and Nanda, G. (1988).** Xylanase and β-xylosidase production by *Aspergillus ochraceus* during growth on lignocelluloses. *Biotechnol Bioeng.* **31**:613–616

41. **Carla, F., Fialova, M., Zahradnik, J. and Teixeira, J.A. (2000).** Hydrodynamic model for three-phase external-loop airlift reactors. *Chem. Eng. Sci.* **55**: 4961-4972.

42. **Camarasa, E., Carvalho, E., Meleiro, L.A.C., Filho, R.M., Domingues, A., Wild, G., Poncin, S., Midoux, N. and Bouillard, J. (2001).** Development of a complete model for an airlift reactor. *Chem Eng Sci.* **56**: 493-502.

43. **Chahal, D.S. (1992).** Bioconversion of polysaccharides of lignocellulose and simultaneous degradation of lignin in Kennedy et al. (eds) Lignocellulosics: Science, Technology, Development and Use. Ellis Harwood Limited, England, pp 83-93.

44. **Chahal, P.S., Chahal, D.S. and Le, G.B.B. (1996).** Production of cellulase in solid straw. *Appl. Biochem. Biotechnol.* **58:** 433-442.

45. **Chakravarty, M., Begum, S., Singh, H.D., Barwah, J.N. and Iyengar, M.S. (1973).** Gas holdup distribution in a gas-lift column. *Biotechnol. Bioeng. Symp.* **4:** 363-378.

46. **Chanda, S.K., Hirst, E.L., Jones, J.J.N. and Percival, E.G.V. (1950). The** constitution of xylan from esparto grass (*Stipa tenacissima*). *J. Chem. Soc.* **50:** 1287-1289.

47. **Chaudhary, M. Y., Shah, M. A. and Shah, F. H. (1977).** Utilization of sulfite waste liquor for production of single cell protein. *Food Technol. and Ferm.Division.* 39-43.

48. **Chaudhary, P. and Deobagkar, D. (1997).** Purification and characterization of xylanases from *Cellulomonas* sp. N.C.I.M. 2353. *Biotechnol. Appl. Biochem.* **25:** 127-133.

49. **Civas. (1984).** Glycosidases induced in *Aspergillus tamari*. *Biochem. Eng. J.* **219**:857

50. **Chipeta, Z.A. (2005).** Production of xylanase enzymes from *Aspergillus oryzae* NRRL 3485 using CSTR. PhD. Thesis, University of Orange Free State, South Africa.

51. **Chipeta, Z.A., du Preez, J.C., Szakacs, G. and Christopher, L. (2005).** Xylanase production by fungal strains on spent sulphite liquor. *Appl. Microbiol. Biotechnol.* **69**:71-78.

62. **Christov, L. P., Szakacs, G. and Balakrishnan, H. (1999)** Production, partial characterisation and use of fungal cellulase-free xylanases in pulp bleaching. *Process Biochem.* **34**: 511-517.

63. **Christov, L. P., Szakacs, G., Rele, M. V. and Balakrishnan (1999).** Screening of cellulose-free fungal xylanases and evaluation of their performance on sulfite dissolving pulp. *Biotechnol. Technique.* **13**: 313-316.

64. **Christov, L. P, Bicly, P., Kalogeris, E., Christakopoulos, P., Prior, B. A. and Bhat, M. K. (2000).** Effect of purified endo-β-1,4-xylanases of family 10 and 11 and acetyl xylan esterases on eucalypt sulfite dissolving pulp. *J. Biotechnol.* **83**: 231-244.

65. **Clarke, J.H,, Davidson, K., Rixon, J.E., Halstead, J.R., Fransen, M.P., Gilbert, H.J., Hazlewood, G.P. (2000).** A comparison of enzyme aided bleaching of softwood pulp using a combination of xylanase, mannanase and α-galactosidase. *Appl Microbiol Biotechnol.* **53**:661–667.

66. **Colella, D., Vinci, D., Bagatin, R., Masi, M. and Bakr, E. A. (1999).** A study on coalescence and breakage mechanisms in three different types of columns. *Chem. Eng. Sci.* **54**: 4767-4777.

67. **Copa-Patino, J. L., Kim, Y. G. and Broda, P. (1993).** Production and initial characterization of the xylan-degrading system of *Phanerochaete chrysosporium. Appl. Microbiol. Biotechnol.* **40**: 69-76.

68. **Coughlan, M.P. and Hazlewood, G.P. (1993).** β-1,4-D-Xylan-degrading enzyme systems: biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem.* **17**, 259-289.

69. **Coughlan, M.P., Tuohy, M.G., Filho, E.X.F., Puls, J., Claeyssens, M., Vrsanska, M. and Hughes, M.H. (1993).** Enzymological aspects of microbial hemicellulases with emphasis on functional systems. *Hemicelluloses and Hemicellulases.* G.P. Portland Press, London.pp53-84.

81. **Dukkan, A. (1996).** The effects of antifoam on the hydrodynamics and mass transfer characteristics of an EL-ALR. MSc Thesis, University of Durban-Westville, South Africa, January 1996.

82. **du Preez, J. C. and van der Walt, J. P. (1983).** Fermentation of D-xylose to ethanol by a strain of *Candida shehatae*. *Biotechnol. Lett.* **5**: 357-362.

83. **Durand, H., Soucaille, P., Tiraby G. (1984).** Substrate use and production of xylanolytic enzymes. *Enzyme Microb. Technol.* **6** (4): 175-180

84. **Duran, N., Milagress, A.M.F., Esposito, E. and Haun, M. (1995).** Xylanase delignification in traditional and chlorine-free bleaching sequence in hardwood kraft pulps. In: Enzymatic Degradation of Insoluble Carbohydrates (Saddler, J.N. and Penner M.H., eds). American Chemical Society, Washington.

85. **Eda, S., Ohnishi, A. and Kato, K. (1976).** Xylan isolated from the stalks of *Nicotiana tabacum*. *Agri. Biol. Chem.* **40:** 359-364.

86. **EL-Temtamy, S.A., Khalil, S.A., Nour-El-Din, A.A. and GAaber, A. (1984).** Oxygen mass transfer in a bubble column bioreactor containing lysed yeast suspensions. *Appl. Microbial. Biotechnol.* **19** (66):376-381.

87. **Eriksson, K. E., Blanchette, R.A. and Ander, P. (1990).** Microbial and enzymatic degradation of wood components. Springer-Verlag, Berlin.

88. **Eriksson, K. L., (1998).** Biotechnology in pulp and paper industry: An overview. *American Chem Society*: 2-14.

89. **Fernandez-Espinar, M.T., Ramon, D., Pinaga, F. and Valles, S. (1992).** Xylanase production by *Aspergillus nidulans*. FEMS *Microbiol. Lett.* **91:**91-96.

90. **Favelukis, M. and Albalak, R. J. (1996).** Bubble growth in viscous Newtonian and non-Newtonian liquids. *Chem. Eng. J.* **63**: 149-155.

101. **Gamerith, G., Groicher, R., Zeilinger, S., Herzorg, P. and Kubicek, C.P. (1992).** Cellular-poor xylanases produced by *Trichoderma reesei* RUT C-30 on hemicellulase substrates. **38** : 315-322.

102. **Gaspillo, P. and Goto, S. (1991).** Mass transfer in bubble slurry column with static mixer in a draft tube. *J. Chem. Eng. Jpn.* **24:** 1091-1105

103. **Gavrilescu, M. and Tudose, R. T. (1998).** Modelling of liquid circulation in concentric-tube airlift reactors. *Chem. Eng. J.* **69**: 85-91.

104. **Gherardini, F.C., Liu, S.Y, and Wiegel, J. (1996).** Purification and cloning of a thermostable xylose (glucose) isomerase with an acidic pH optimum from *Thermoanaerobacterium* strain JW/SL-YS 489. *J. Bacteriol.* **178**(20): 5938-5945.

105. **Gibbs, P. A., Seviour, R. J. and Schmid, F. (2000).** Growth of filamentous fungi in submerged culture: Problems and possible solutions. *Critical reviews in Biotechnol.* **20** (1): 17-48.

106. **Ghildyal, N.P., Ahmed, S.Y., Lonsane, B.K.and Ramakrishna, S.V. (1987).** Design of solid state fermentor for production of fungal metabolites on large scale. *Biotechnology Techniques.* **1** (2):97-102

107. **Ghosh, M. and Nanda, G. (1994).** Purification and some properties of xylanase from *Aspergillus sydowii* MG 49. *Appl. Environ. Microbiol.* **60**:4620–4623

108. **Glennon, B, Al-Masry, W.A., Macloughlin, P.F. and Malone, D.M. (1993).** Hydrodynamic modeling in an airlift loop reactor. *Chem. Eng. Commun.* **121**: 181.

109. **Gomes, J., Gomes, I., Kreiner, W., Esterbauer, H., Sinner, M. and Steiner, W. (1993).** Production of high level cellulase-free and thermostable xylanase by wild starin of *Thermomyces lanuginosus* using beechwood xylan. *Biotechnol. J.* **30**: 283-297.

120. **Heijenen, J.J, Hols, J., van der Lans, R.G.J.M, van Leeuwen, H.L.J.M, Mulder, A. and Weltervrede, R. (1997).** A simple hydrodynamic model for the liquid circulation velocity in a full-scale two- and three-phase internal airlift reactor operating in the gas recirculation regime. *Chem. Eng. Sci.* **52**: 2527-2540.

121. **Henzler, H.J. (1980).** Aeration of highly viscous liquids. *Chem. Eng. Tech.* **9**: 654-667

122. **Herskowitz, M. amd Merchuk, J.C. (1986).** A loop three phase fluidized bed reactor. *Canad J. of Chem. Eng.* **64**: 57

123. **Hills, J.H. (1976).** The Influence of External Airlift Loop Bioreactor Configuration on Bioreactor Hydrodynamics. *Chem. Eng. J.* **12**:89-103.

124. **Hoq, M.M., Hempel, C. and Deckwer, W. (1994)**. Cellulase-free xyalnase by *Thermomyces lanugisus* RT9: Effect of agitation, aeration, and medium components production. *Biotechnol. J.* **37**: 49-58.

125. **Howard, R.L., Abotsi, E., Jansen van Rensburg, E.L. and Howard, S. (2003).** Lignocellulose biotechnology: Issue of conversion and enzyme production. Affrican J. Biotechnol. **2**(12):602-619.

126. **Hrmova, M., Biely, P. and Vrsanska, M. (1986).** Specificity of cellulase and β-xylanase induction in *Trichoderma reesei* QM 9414. *Arch of Microbiol.* **144**: 307-311.

127. **Hrmova, M., Biely, P. and Vrsanska, M. (1984).** Induction of cellulose-and xylan-degrading enzyme complex in yeast. *Arch. Microbiol* **161**:371–376

128. **Hsu, Y.C. (1978).** Gas holdup and liquid recirculation in gas lift reactors. *D.Sc. Thesis.* Washington University, St. Louis.

129. **Hsu, Y.C. and Dudukovic, M.P. (1980).** Gas holdup and liquid circulation in gas-lift reactor. *Chem. Eng Sci.* **35**: 135-141.

140. **Joyce, T.W. abd Petke, W.H. (1983).** Effluent decolorization technologies for the pulp and paper industry. Water Resources Research Institute of the University of North

141. **Kamaranev, D.G., Chavarie, C. and Samson, R. (1996).** Hydrodynamics and mass transfer in an airlift reactor with a semi-permeable draft tube. *Chem. Eng. Sci.* **51**: 1173.

142. **Kantelinen, A., Viikari, L,, Ranua, M., Linko, M. and Sundqvist, J. (1986)** Bleaching with enzymes. In: Proceedings of the Third Conference on Biotechnology in the Pulp and Paper Industry, 16–19 June 1986, Swedish Wood Research Institute, Stockholm, Sweden, pp 67–69

143. **Kapoor, M., Beg, Q.K., Bhushan, B., Singh, K., Dadhich, K.S. and Hoondal, G.S. (2001).** Application of an alkaline and thermostable polygalacturonase from *Bacillus* sp. MG-cp-2 in degumming of ramie (*Boehmeria nivea*) and sunn hemp (*Crotalaria juncea*) bast fibers. *Proc. Biochem.*:**36**:803–807

144. **Kapoor, M., Beg, Q.K., Mahajan, L. and Hoondal, G.S. (2001).** Microbial xylanases and their industrial applications: a review. *Appl. Microbiol. Biotechnol.* **56**:326–338

145. **Kawase, Y. and Moo-Young, M. (1990).** Mathematical models for design of bioreactors: Application of Kolmogoroff's theory of isotropic turbulence. *Chem. Eng. J.* **43**: B19-B41.

146. **Kemblowski, Z., Przywarski, J. And Diab, A. (1993).** An average gas holdup and liquid circulation velocity in airlift reactors with external loop. *Chem. Eng. Sci.* **48**: 4023.

147. **Kessler, W. R., Popovic, M. K. and Robinson, C. W. (1993).** Xanthan production in an external-circulation-loop air fermenter. *Canadian Chem Eng. J.* **71**: 101-1106.

148. **Koide, K., Kurematsu, K., Iwamoto, S., Iwata, Y. and Horibe, K. (1983).** Gas holdup and volumetric liquid-phase mass transfer coefficient in a bubble column with draft tube and with gas dispersion into annulus. *J. Chem. Eng Jpn.* **16:** 413-419.

157. **Kormelink, F.J.M, De Graaff, L.H, Visser, J., van Den Brocck, H.C, Strozyk, F., Boonman, J.C.P. (1996).** Cloning and expression of acetyl xylan esterases from fungal origin. *Biotech. Adv.* **14** (3): 311-323.

158. **Korpijarvi, J., Oinas, P. and reunanen, J. (1999).** Hydrodynamics and mass transfer in airlift reactor. *Chem Eng Sci.* **54**: 2255-2262.

159. **Korte, G., Rinas, U., Kracke-Helm, H.A. and Schügerl, K. (1991).** Structured model for cell growth and enzyme production by recombinant *Escherichia coli*. *Appl. Microbiol. Biotechnol* **35** (2):185-8

160. **Kuhad, R.C. and Singh, A. (1993).** Lignocellulosic biotechnology: current and future prospects. *Crit Rev Biotechnol* **13**:151–172

161. **Kurbanoglu, E.B. and Algur, N.I. (2003).** Utilization for lactic acid production with a new acid hydrolysis of ram horn waste. *FEMS Microbiol. Lett.* **225** (*1*):29-34

162. **Kurbanoglu, E.B. and Kurbanoglu, N.I.** Ram horn peptone as a source of citric acid production by *Aspergillus niger*, with a process. *J. Ind Microbiol Biotechnol* **31**:289–294

163. **Krichnavaruk, S. and Pasavant, P. (2002).** Analysis of gas-liquid mass transfer in an airlift contactor with perforated plates. Chem Eng J. **89**: 203-211.

164. **Kvesitadze. (1999).** Cellulase end xylanase activity of fungi in a collection isolated from the southern Caucasus. *Int. Biodeterioration and Biodegradation* **43** (4):189

165. **Leathers, T.D., Detroy, R.W. and Bothast, R.J. (1986).** Induction and glucose repression of xylanase from a color variant strain of *Aureobasidium pullulans*. *Biotechnol. Lett.* **8**:867–872

166. **Lee, Y., Tsao, G.T. and Wankat, P.C. (1980).** Hydrodynamic effect of surfactants on gas-liquid oxygen transfer. *AIChE J.*: 1008-1012.

177. **McCarthy, A.J. (1987).** Lignocellulose-degrading actinimycetes. *FEMS Microbiol. Lett.* 46:145-163.

178. **McCleary. (1996).** The activation of PhoB by acetylphosphate. *Molecular Microbiology* **20** (6):1155.

179. **McNeil, B. and Harvey, L.M. (1993).** Viscous fermentation products. *Crit Rev. Biotechnol.* **13**:275-304.

180. **Merchuk, J.C., Stein, Y.and Mateles, R.I. (1980).** A distributed parameter model of an airlift fermenter. *Biotechnol. Bioeng.* **22**: 123

181. **Merchuk, J.C. and Stein, Y. (1981).** Local hold-up and liquid velocity in airlift reactors *Chem. Tech. Biotechnol.*: 105-120..

182. **Merchuk, J.C. (1986).** Hydrodynamics and holdup in airlift reactors. In encyclopedia of fluids mechanics (N.P. Cheremisinoff, Ed.) **3**: 1485, Gulf Publishing, Houston.

183. **Merchuk, J.C. and Siegel M.H. (1988).** Airlift reactor in chemical and biological technology. *J. of Chem. Technol. Biotechnol.* **41**: 105-120.

184. **Merchuk, J.C., Ladwa, N., Cameron, A., Bulmer and Pickett, A. (1994).** Concentric tube airlift reactors: Effects of geometrical design on performance. *AIChE J.* **40**, No 7: 1105.

185. **Merivouri. (1990).** Different temperature profiles of enzyme secretion by two common strains of *Trichoderma reesei*. *Biotechnol. Lett.* **12** (2):117-126.

186. **Metzner, A.B. and Otto, R.E. (1957).** The tubular loop fermentor. *AIChE J.* **3**:3-14..

187. **Metz, B., Kossen, N.W.F. and van Suijdam, J.C. (1979).** The rheology of mould suspensions. *Adv. Biochem. Eng.* **11**: 103.

198. **Nicolelella, C., van Loosdrecht, M.C.M and Heijnen, J.J. (1998).** Mass transfer and reaction in a biofilm suspension reactor. Chem. Eng. Sci. **53**: 2743-2753.

199. **Nigam, J. N. (2001).** Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. *J. Biotechnol.* **87:** 17-27.-137.

200. **Nishikawa, M., Kato, H. and Hashimoto, K. (1977).** Heat transfer in aerated tower filled with non-Newtonian liquid. *Ind. Eng. Chem. Process Des. Dev.* **16**: 190

201. **Noe, P., Chevalier, J., Mova, F. and Comtat, J. (1986).** Action of xylanases on chemical pulp fibres. Part II. Enzymatic beating. *J Wood Chem Technol* **6**:167–184

202. **Nummi, M., Perrin J.M., Niku-Paavola, M.L. and Enari, T.M. (1985)**. Measurement of xylanase activity with insoluble xylan substrate. *Biochem J.* **226**:617–620

203. **Obradovic, B., Dudukovic, A. and Vunjak-Novakovic, G. (1997).** Response data analysis of a three-phase airlift reactor. Trans IchemE. **75**, Part A: 473-478.

204. **Oey, R.S., Mudde, R.F., Portela, L.M and van den Akker, H.E.A. (2001).** Simulation of a slurry airlift using a two-fluid model. *Chem Eng Sci.* **56**: 673-681

205. **Okeke, B.C and S. Obi, S.K.C. (1993).** Production of cellulolytic and xylanolytic enzymes by an *Arthrographis* species. *World Journal of Microbiology and Biotechnology* **9**(3)

206. **O'Neil, F.H., Christov, L.R. and Prior, B.A. (1997).** Production of hemicellulotic enzymes by microorganisms. Enzyme and microbial technology. **15** (6):460-475

207. **Onken, U. and Weiland, P. (1981).** Fluidized-bed bioreactors using a floculating strain of *Zymomonas mobilis*for ethanol production *German Chem. Eng.* **4**:42-55.

208. **Onken, U. and Weiland, P. (1983).** Airlift fermentors: construction, behavior and uses. *Adv. Biotechnol. Proc.* **1.** 67-95.

209. **Olivier, A.P.C. and Oosterhuis, M.M.C. (1989).** Oxygen mass transfer in non-Newtonian fermentation systems. Proc. 8th Int. Biotechnol. Symp.398-407.

220. **Popovic M and CW Robinson. (1986).** Mass transfer and mixing characteristics of non-Newtonian systems in external-circulation loop airlift fermenters, Phase II, NRC *Biotechnology Research Institute*, DSS File no. 22ST.31949-4-002, Final Report.

221. **Popovic M and CW Robinson. (1987).** The specific interfacial area in external circulation loop airlifts and bubble column: II. Carboxymethyl cellulose/sulphite solution. *Chem Eng Sci.* **42**: (12): 2825-2832.

222. **Popovic M and CW Robinson. (1988).** External circulation loop airlift bioreactors: study of liquid circulation velocity in highly viscous non-Newtonian liquids. *Biotechnol Bioeng.* **32:** 301-312.

223. **Popovic, M. K. and Robinson, C. W. (1989).** Mass transfer studies of external-loop airlift reactors and bubble columns. *AIChE J.* **35** (3): 393-405.

224. **Popovic M and CW Robinson. (1993).** Mixing characteristics of external loop airlifts: non-Newtonian systems. *Chem Eng Sci.* 48 (8): 1405-1413.

225. **Pou-llinas, J. and Drigues, H. (1987).** D-xylose as inducer of the xylan-degrading enzyme system in the yeast *Pullularia pullulans*. *Appl. Microbiol. and Biotechnol.* **27** (22):134-138

226. **Poutanen, K., Rättö, M., Puls, J. and Viikari, L. (1987)** Evaluation of different microbial xylanolytic systems. *J Biotechnol* **6**:49–60

227. **Poutanen, K. and Puls, J. (1988).** Characteristics of *Trichoderma reesei* β-xylosidase and its use in the hydrolysis of solubilized xylans. *Appl. Microbiol. Biotechnol.* **28**:425–432

228. **Poutanen, K., Bailey, M., Sundberg, M., Rättö, M., Puls, J. (1990)** Enzymatic solubilization of xylans. In: Kirk TK, Chang H-M (eds) Proceedings of the 4th International Congress of Biotechnology in the Pulp and Paper Industry, Raleigh, USA, 16–19 May 1989. Butterworth, London, pp 559–565

238. **Ritschkoff , ?. (1995).** Effect of carbon source on the production of oxalic acid and hydrogen peroxide by brown-rot fungus Poria placenta. *J. of Biotechnol.* **40** (3):179

239. **Royer, J. C. and Nakas, J. P. (1989).** Xylanase production by *Trichoderma longibrachiatum*. *Enzyme Microbiol. Technol.* **11**: 405-410.

240. **Rizzatti, A., Polizeli, M., Monti, R., Terenzi, H., Jorge, J. and Amorim, D. (2005).** Xylanases from fungi: properties and industrial applications. *Appl. Microbiol. and Biotechnol.* **67** (5):577–591

241. **Robinson, M. (1984).** Transport of proteins into chloroplasts: partial purification of a chloroplast protease involved in the processing of imported precursor polypeptides. *European Journal of Biochemistry* **142** (2):337

242. **Royer, J.C. and J.P. Nakas. (1989).** Production of xylanase by Trichoderma longibrachiatum In: Forest and Crop Biotechnology: Progress and Prospects. F.A. Valentine (ed.), Springer-Verlag, New York. pp. 363-381.

243. **Royer, J.C. and J.P. Nakas. (1991).** Purification and characterization of two xylanases from Trichoderma longibrachiatum. *Eur. J. Biochem.* **202**:521-529.

244. **Rydholm, S.A. (1965).** Pulping Process. Wiley Interscience, New York. 503-522.

245. **Sanyer *and* Chidester, G. (1963).** In "The Chemistry of Wood", (B.L. Browning, Ed.), *J. Smelstorius*: Holzforschung, Interscience, New York.

246. **Schügerl K. (1990).** Comparison of the performance of stirred tank and airlift tower loop reactors. *J Biotechnol.* **13**: 251-256.

247. **Scragg A. H. (1991).** Bioreactors Biotechnology – A practical Approach. Ellis Horwood, New York, pp 112-125.

248. **See, K. H., Roberts, G.W. and Saez, A.E. (1999).** Effect of drag and frictional losses on hydrodynamics of gas-lift reactors. *AIChE J.* **45** (11): 2467-2471.

258. **Simpson, H.D., Haufler, U.R. and Daniel, R.M. (1991).** An extremely thermostable xylanase from the *Thermophilic eubacterium thermotoga. Biochemical Journal* **277** (2):413

259. **Singh, S., du Preez, J.C., Pillay, B. and Prior, B.A. (2000).** The production of hemicellulases by *Thermomyces lanuginosus* strain SSBP: influence of agitation and dissolved oxygen tension. *Appl. Microbiol. Biotechnol.* **54**, 698-704.

260. **Sinner, M., Purkarthofer, H. and Steiner, W. (1991).** Cellulase-free xylanase from Thermomyces lanuginosus: Optimization of production in submerged and solid-state culture. *Enzyme and Microbial Technology* **15**:88, 677-682

261. **Sjöeström, E. (1981).** Wood chemistry: Fundamentals and applications, Academic Press, New York.

262. **Skerker, P.S., Farell, R.L. and Chang, H.M. (1991).** Chlorine-free bleaching with CartazymeTM HS treatment. International Pulp Bleaching Conference.

263. **Smith, J.E., Anderson, J.G. and Senior, E.K. (1987).** Bioprocessing of lignocellulose. Phil. Trans. R. Soc. Lond. Ser. A. 321: 507-521.

264. **Smith, I.H. and Pace, G.W. (1982).** Recovery of microbial polysaccharides. *Journal of Chemical Technology and Biotechnology.*

265. **Smith, A.R. and Wood, C.K. (1991).** The Effect of Ethylene on the Growth and Development of Wild-type and Mutant *Arabidopsis thaliana* (L.) Heynh. *Annals of Botany* 68: 55-61.

266. **Smith, D.C.** and **Wood, T.M. (1991).** Xylanase production by *Aspergillus awamori.* Development of a medium and optimization. *Biotechnol. and Bioeng*

267. **Smith, D.C., Bhat, K.M. and Wood, T.M. (1991).** Xylan-hydrolysing enzymes from thermophilic and mesophilic fungi. *World J. of Microbiol. And Biotechnol.* 7 (44):475-484

277. **Tan, W.S., Dai, G.C., Ye, W. and Shen, J.P. (1994).** Local flow behavior of the liquid phase in an airlift bioreactor for potential use in animal cell suspension cultures. *Chem Eng J.* **57**: B31-B36.

278. **Techapun, C., Poosaran, N., Watanabe, M. and Sasaki, K. (2003).** Optimization of aeration and agitation rates to improve cellulase-free xylanase production. *J. Biosci. Bioeng.* **95** (3):300-314.

279. **Tenkanen, M., Puls, J., Korte, H.E and Poutanen, K. (1991).** Products of hydrolysis of beechwood acetyl-4-O-methylglucuronoxylan by a xylanase and an acetyl xylan esterase. *Enzyme and Microbial Technology* **13**(6):483-486

280. **Tenkanen, H, Puls, J. and Poutanen, K. (1992)** Two major xylanases of *Trichoderma reesei. Enzyme Microb Technol* **14**:566–574.

281. **Tenkanen, M., Viikari, L. and Buchert, J. (1997).** Use of acid tolerant xylanase for bleaching of kraft pulp. *Biotechnol Tech* **11**: 935–938.

282. **Teunissen, M.J. de Kort, G.V., Op den Camp, H.J.M. and Huis in 't Veld, J.H.J. (1992).** Production of cellulolytic and xylanolytic. *J. Gen. Microbiol.* **12**: 434-457.

283. **Tijhuis, L., Huisman, J.L. Hekkelman, H.D., van Loosdrecht, M.C.M., and Heijnen, J.J. (1995).** Formation of nitrifying biofilm on small suspended particles in airlift reactors. *Biotechnol. and Bioeng.* **47**: 585-595.

284. **Tobajas, M., Garcia-Calvo, E., Siegel, M.H. and Apitz, S.E. (1999).** Hydrodynamics and mass transfer predictions in a three-phase airlift reactor for marine sediment biotreatment. *Chem. Eng. Sci.* **54**: 5347-5354.

285. **Tolan, J.S. and Canovas, R.V. (1992).** The use of enzymes to decrease the $Cl_2$ requirements in pulp bleaching. *Pulp & paper Canada* **93** (55):39-42

296. **Verlaan, P., Tramper, J. Van't Riet, K. and Luyben, K. Ch. A. M. A. (1986).** Hydrodynamic model for an airlift bioreactor with external-loop. *Chem. Eng. J.* **33:** B43-B53.

297. **Verlaan, P., Tramper, J. Van't Riet, K. and Luyben, K. Ch. A. M. A. (1986).** Hydrodynamics and axial dispersion in an airlift bioreactor with two and three phase flow. Paper 07 presented at the *International Conference on Bioreactor Fluid Dynamics,* Cambridge, England, 15-17 April, 1986.

298. **Verlaan, P., Tramper, J. and Van't Riet, K. (1989).** Circulation liquid velocity in external-loop airlift reactors *Chem. Eng. J.* 33: 1343-1353.

299. **Verlaan, P., Van Eijs, A. M. M., Tramper, J. and Van't Riet, K. (1989).** Estimation of axial dispersion in individual sections of an airlift-loop reactor. *Chem. Eng. Sci.* **44**(5): 1139-1146.

300. **Vial, C., Poncin, S., Wild, G. and Midoux, N. (2002).** Experimental and theoretical analysis of the hydrodynamics in the riser of an external-loop airlift reactor. *Chem. Eng. Sci.* **57**: 4745-4762.

301. **Vial, C., Camarasa, E., Poncin, S., Wild, G., Mifoux, N. and Bouillard, J. (2000).** Study of hydrodynamic behavior in bubble columns and external loop airlift reactors through analysis of pressure fluctuations. *Chem. Eng. Sci.* **55**: 2957-2973.

302. **Viikari, L., Kantelinen, A., Sundquist, J. and Linko, M. (1994).** Xylanases in bleaching: From an idea to the industry. *FEMS Microbiol. Reviews.* **13**: 335-350.

303. **Vorapongsathorn, T., Wongsucholo, P. and Pasavant, P. (2001).** Performace of airlift contactors with baffles. *Chem Eng J.* **84**: 551-556.

304. **Wallis, G.B. (1969).** One dimensional two-phase flow, McGraw-Hill, New York, pp 23-35.

305. **Wallis, G.B. (1974).** Tower fermentation systems and their applications. *Chem. Eng.* **249**: 182-193.

316. **Yu, S., Wayman, M. and Parekh, S. K. (1987).** Fermentation to ethanol of pentose containing spent sulphite liquor. *Biotechnol. Bioeng.* **29**: 1144-1150.

317. **Zhao, M., Niranjan, K. and Davidson, J. F. (1994).** Mass transfer to viscous liquids in bubble columns and air-lift reactors: Influence of baffles. *Chem. Eng. Sci.* **49**: 2359-2369.

318. **Zimmermann, W. (1990).** Degradation of lignin by bacteria. *J. Biotechnol.* **13** (2-3): 119-130.

319. **Zimmerman, G. A., Venable, M. E., McIntyre, T. M. and Prescott, S. M. (1993).** By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*. *Biotech. Bioeng.* **25**: 103−121.

320. **Zuber, N. and Findlay, J. A. (1965).** Average volumetric concentration in two phase flow system. *J. Heat Transfer Trans.* **87**:453-457.