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The application of aerobic yeast for treatment of high strength food processing wastewater containing furfural

by

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For the Major Program

DEDICATION

To my dad who passed away
before I came to the USA.

To my mom who has inspired me in everything, including my education.
She always looks forward to hearing of my success,
and I know she patiently waits for my homecoming to Thailand.

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ABSTRACT

Food processing industries typically produce large quantities of high strength organic wastewater. The standard approach for such wastewater would be anaerobic pretreatment. However, the wastewater from the Quaker Oats Company located in Cedar Rapids, Iowa USA contained furfural, which was found to be toxic to anaerobic microorganisms.

In this study, batch and continuous laboratory scale experiments were performed to investigate the treatment of furfural-based wastewater using aerobic yeast. Three yeasts, *Candida guillermondii*, *C. tropicalis*, and *C. utilis*, were first studied in batch tests. All yeast cultures could lower the COD in the wastewater by more than 90%.

As the work was done under non-aseptic conditions, bacterial growth also occurred. Much of the work focused on finding operational conditions to minimize bacterial growth. At a pH of 4.5, the maximum specific growth rate, μ_{max}, of *C. utilis* and bacteria were 0.13 h^{-1} and 0.03 h^{-1} and the value of half-saturation coefficient for substrate, K_s, were 160 mg COD/L and 12 mg COD/L, respectively. Accordingly, when substrate concentrations were higher than 30 mg COD/L, yeast grew faster than bacteria.

Yeast cultivation in continuously stirred aerobic reactors was studied with different yeast cultures. The effect of the age of the yeast or solids retention time (SRT) on reactor performance was investigated initially. SRT values of 1.0, 1.3, 1.5, 1.75, and 2 days were investigated and the reactors were controlled at a constant pH of 4.5 and temperature of 35°C. *C. tropicalis* and *C. utilis* and mixed cultures of these two yeast were used. The results showed that an SRT of 1.5 days was optimal to treat wastewater, maintain the yeast culture, and minimize bacterial contamination. All three yeast cultures could remove COD from the wastewater up to 90% at this SRT.

Subsequently, the effect of temperature on yeast performance was studied. *C. tropicalis* and *C. utilis* were studied in separate reactors. Two reactors were run at temperatures of 25, 30, 35, and 40°C. The SRT and pH were controlled. at 1.5 day of 4.0 respectively. The results indicated that the optimum operating temperature in terms of removal efficiency, yeast biomass yield, and prevention of bacterial contamination was 30°C. Under these conditions, both *C. tropicalis* and *C. utilis* could remove COD by 90%.

Finally, the effect of pH on yeast performance was investigated. *C. tropicalis* and *C. utilis* were studied in separate reactors. The reactors were operated at different pH values, i.e. 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. The SRT and temperature of 30°C were controlled at 1.5 days and 30°C respectively. The higher pH increased the removal efficiency. Bacterial contamination was significant at pH values of 5.0 and 5.5. Therefore, the optimum pH range in terms of treatment efficiency, yeast biomass yield, and preventing bacterial contamination was between 4.0 and 4.5. At this pH range, more than 90% COD removal was accomplished in both reactors.

CHAPTER 1 GENERAL INTRODUCTION

1.1 INTRODUCTION

Food processing industries typically produce large quantities of high strength organic wastewater. Many food processing plants discharge waste streams into municipal sewers for combined treatment with municipal wastewater. The disadvantages of discharging high strength wastewater to the municipal sewer are as follows:

- High treatment cost surcharge to be paid to wastewater treatment plant municipalities.

- High readily degradable loadings, particularly volatile fatty acids, cause septic conditions in collection systems and can promote filamentous bulking in activated sludge plants.

- Many municipal wastewater treatment plants are not designed for such high concentrations of organic content of up to 10,000 mg/L BOD or COD from food processing industries. Typically, the organic matter concentration of municipal wastewater ranges from 110 to 400 mg/L in terms of BOD and 250 to 1,000 mg/L in terms of COD (Britton, 1999). Incorporating high strength wastewater into a city's incoming wastewater can significantly increase the loading on wastewater treatment plants and even lead to over-loading.

The other option is to pretreat high strength wastewater separately. The disadvantages of this option are clear in that it requires unattractive capital investment in equipment and land and expenditure of non-productive operating costs on personnel, power, and sludge disposal. These disadvantages can be offset if the organic matter in industrial wastewater can be converted into a valuable by-product such as microbial biomass that can be used as a protein supplement. Yeast is one example of single cell protein that can be sold as animal feed. *Candida utilis* is an example of yeast that is more popular for the production of a single cell protein and contains about 50% protein on a dry basis (Forage, 1978; Prior *et al*, 1981).

Another option is to treat high strength wastewater by anaerobic pretreatment to reduce the organic content in wastewater before discharge to the municipal wastewater treatment plant. However, the wastewater from the Quaker Oats Company located in Cedar Rapids, Iowa USA contained furfural, which was found to be toxic to anaerobic microorganisms (Koh and Ellis, 2002). Furfural was a by-product of the process stream. The wastewater was discharged to the municipal wastewater facility subject to a considerable surcharge due to the organic loading. The average wastewater flow rate was approximately 565 m^3/d, and the range of COD varied from 10,000 to 20,000 mg/L including 100 to 2,000 mg/L of furfural.

Aerobic yeast cultivation with simultaneous treatment of this high strength wastewater containing furfural appeared to be a feasible option. An investigation of this possibility and optimization of the operating conditions formed the basis of this dissertation.

1.2 DISSERTATION ORGANIZATION

This dissertation consists of Chapter 1, a general introduction, Chapters 2-5, each written as a paper or publication, Chapter 6, general conclusions, and appendix that shows raw data used in this dissertation and some related research data based on this research but not presented in this dissertation. The cited references are included at the end of each separate chapter.

The title of the first paper is "Bioconversion of waste organic matter into a microbial biomass protein". It was presented at the 75th Annual Technical Exhibition & Conference, Chicago, IL USA and published in the Proceedings by the Water Environment Federation.

The second paper entitled "Treatment of food processing wastewater containing furfural using aerobic yeast" was presented at the Asian Waterqual Conference, Bangkok, Thailand, IWA–Asia Pacific Region and was published in the Proceedings.

The title of the third paper is "Production of aerobic yeast from food processing wastewater". It has been accepted by IWA–Asia Pacific Region and will be presented in July 2005 at the Waterqual Conference, Singapore and published in the Proceedings.

The fourth paper entitled "Effect of pH on the treatment of furfural-based wastewater by yeast" will be submitted to Water Science and Technology.

Each of these papers was written in a manuscript format appropriate for the target conference and journal.

1.3 LITERATURE REVIEW

1.3.1 The advantages of yeast

Yeast are more tolerant to furfural than bacteria. In addition, some yeast can reduce the toxicity of furfural by conversion to the less harmful furfuryl alcohol (Taherzadeh *et al.*, 1999).

Kazmi (2003) concluded the advantages of yeast in wastewater treatment in his report as follows:

High organic treatment and direct decomposition of oils. High organic content wastewater from food processing, marine products processing, and other industries where the BOD exceeds 1,000 mg/L is suitable for treatment by yeast. The ability of yeast to directly treat oils is a distinct advantage over other treatment methods that would require oil and grease removal pretreatment with chemical coagulants and separation by, for instance, dissolved air floatation (DAF).

High BOD and SS loading. BOD removal by yeast treatment is stable over a wide range of loading (F/M ratio). The optimum loading for yeast treatment is about 1-2 kg BOD/kg yeast

per day. This is 3-5 times higher than the F/M ratio of the conventional activated sludge process that is about 0.2-0.4 kg BOD/kg SS per day.

Reduced sludge generation. The generation of excess yeast is 0.2 kg per kg of BOD removed on a dry basis weight. It is about one third of the excess sludge produced by the activated sludge process that is about 0.4-0.8 kg per kg of BOD removed.

Good settling and dewatering properties. In a conventional activated sludge process, coagulation and flocculation induced by biopolymers may be needed. Yeast are larger than bacteria. Yeast can physically intertwine the mycelia/pseudo mycelia to form flocs. With the mentioned properties of yeast, floc forming by yeast treatment system settles easily. The SVI of yeast sludge is about 50-60 ml/g. This allows the maintenance of yeast concentrations in the reaction tanks up to 10,000 mg/L.

Low oxygen requirements. The supplied airflow can be reduced to about 60% of that required in the activated sludge process. It is because the net-structured yeast floc facilitates oxygen diffusion and eliminates the necessity of excessive dissolution of oxygen in water.

Effective use of excess yeast. Yeast contains about 50% protein as previously mentioned. Yeast also contain vitamins Therefore, the cultivation of yeast single cell protein on many kinds of wastewater should make this an attractive additive to animal feeds.

1.3.2 Some examples of the application of yeast in wastewater treatment

Arnold *et al.* (2000) used *Candida utilis* and *Galactomyces geotrichum* to reduce the polluting potential of silage effluent in their study. Silage effluent is one of the most potent agricultural wastes. It contains BOD ranging from 30,000 to 80,000 mg/L and pH about 3.0-4.5. Both yeast reduced COD of 50/50 dilution wastewater/water up to 74-79% and 25/75 dilution wastewater/water 91-95%. The highest yeast biomass was found to be 7.0 and 8.6 g dry weight/L for *C. utilis* and *G. geotrichum* respectively.

An anaerobic acidogenic reactor followed by yeast reactor (i.e.in series) was used to treat high strength wastewater from food processing industries (Elmaleh *et al.*, 1999). *C. utilis* was selected for their study. Effluent from an anaerobic acidogenic reactor was mainly volatile fatty acids such as acetic acid, propionic acid, and butyric acid and pH was very low. Yeast treatment was a compromise alternative. Volatile fatty acids in term of TOC were reduced by 97%.

The biosurfactant, BS-UC, was produced by *Candida antarctica* using *n*-undecane as the substrate (Hua *et al*, 2003). They concluded that BS-UC increased the emulsification and the biodegradation of several *n*-alkane substrates. BS-UC also changed the hydrophobicity and zeta potential of the cell surface. As a consequence, the microbial cells attached to the hydrophobic substrate easily. Cell transport experiments were carried out, and the results showed that BS-UC changed both zeta potential of the cell and that of the porous media, and thus improved the retention time of the cells in the media.

Blanch water from potato processing industries was treated and used as a substrate to produce single cell protein (SCP). A mixture of *Saccharomycopsis fibuliger* and *C. utilis* was used. *S. fibuliger* is an amylolytic yeast that can hydrolyze starch to simple sugars for growth of *C. utilis* that is an important SCP. The optimum temperature for this study was found to be 32°C, and the pH of 4.3 to 4.8 increased the amount of protein production (Lemmel *et al.*, 1979).

C. rugopelliculosa and *Brachionus plicatilis* were used as co-culture to treat fish processing wastewater (Lim *et al*, 2003). *C. rugopelliculosa* reduced the soluble chemical oxygen demand from the influent fish processing wastewater up to 70% at 6.3 h hydraulic retention time and stimulated the growth of the rotifer, *B. plicatilis*, by 18.3% over the commercial diet of *Saccharomyces cerevisiae*.

C. tropicalis can directly grow on soluble starch, corn, and cassava powders because it contains the enzyme needed to hydrolyze starch, α-amylase. Therefore, it was not required

pre-hydrolyzed. This property was used to develop a fermentation process of corn or cassava powders by *C. tropicalis* so that the resultant mixture of biomass and residual corn or cassava had an improved protein content from 10% of corn or 3% of cassava up to 20% that could be utilization as animal feed (Azoulay *et al*, 1980).

Zheng *et al*. (2001) studied the treatment of salad oil manufacturing wastewater by yeast. Five yeast including *C. tropicalis* and *C. utilis* were isolated from soil spots of a salad oil factory and utilized for continuous treatment of salad oil manufacturing wastewater. The results showed that oil removal over 97% and COD removal over 93% were achieved for the first 10 days regardless of variation of sludge load from 1.0 to 3.5 kg COD kg/MLSS/d (or volumetric load from 5 to 14 kg COD/m^3/d.

Scioli *et al*. (1997) used the yeast *Yarrowia lipolytica* ATCC 20255 to treat olive mill wastewater in their study. COD concentrations of this wastewater ranged from 100-200 g/L and were reduced up to 80% in 24 h by the treatment of *Y. lipolytica*. Biomass concentrations up to 22.45 g/L were produced.

1.3.3 Furfural

Furfural, according to **http://www.encyclopedia.com/html/f1/furfural.asp** (für´ferel) or furfuraldehyde [Lat. = bran], C_4H_3OCHO, is a viscous, colorless liquid that has a pleasant aromatic odor; upon exposure to air it turns dark brown or black. It boils at about 160°C. It is commonly used as a solvent; it is soluble in ethanol and ether and somewhat soluble in water. Furfural is the aldehyde of pyromucic acid; it has properties similar to those of benzaldehyde. A derivative of furan, it is prepared commercially by dehydration of pentose sugars obtained from cornstalks and corncobs, husks of oats and peanuts, and other waste products. It is used in the manufacture of pesticides, phenolfurfural resins, and tetrahydrofuran. Tetrahydrofuran is used as a commercial solvent and is converted in starting materials for the preparation of nylon.

Furthermore, the Occupational Safety & Health Administration U.S. Department of Labor reported that the almond-like odor of furfural is detectable when an air odor threshold concentration of 0.078 part per million (ppm) part of air is reached. (**http://www.osha.gov/SLTC/healthguidelines/furfural/recognition.html**).

According to **http://www.osha.gov/SLTC/healthguidelines/furfural/recognition.html** the toxicity of furfural to animals and humans is summarized as below:

Effects on animals: Furfural is an irritant of the skin, eyes, mucous membranes, and respiratory tract. It is a toxin to the central nervous system, liver, kidney, blood, and bone marrow. The oral LD (50) in rats is 65 mg/kg; and the inhalation LD (50) in rats is 260 ppm.

Exposure of rats to furfural by ingestion or subcutaneous injection caused unsteadiness, paralysis, seizures, coma, and changes in liver, kidneys, blood, and bone marrow. Chronic dietary exposure to furfural caused liver cirrhosis in rats.

Cats exposed to 2,800 ppm for 30 minutes developed fatal pulmonary edema.

Solutions of 10 percent and 100 percent furfural instilled in rabbits' eyes caused pain in addition to transient swelling and redness of the lids and conjunctiva. Rabbits exposed to furfural vapors for several hours daily developed liver and kidney lesions and changes in their blood profiles.

Dogs exposed at 130 ppm for 6 hours a day for 4 weeks developed liver damage, but dogs exposed at 63 ppm did not.

Furfural is mutagenic in at least one bacterial species.

Effects on Humans: Furfural is an irritant of the skin, eyes, mucous membranes, and the respiratory tract. Concentrations of 1.9 to 14 ppm produced headache, itching of the throat, and redness and tearing of the eyes in some exposed workers.

Workers exposed to furfural vapors in a plant with inadequate ventilation reported numbness of the tongue and mucous membranes of the mouth, loss of taste sensation, and difficulty in breathing.

Exposure to high concentrations has produced pulmonary edema. Damage to the eyesight of some individuals has also been reported. Chronic skin exposure may produce eczema, allergic skin sensitization, and photosensitization.

Furfural may cause a disulfiram-type reaction; that is, a worker exposed to furfural who has consumed alcohol may experience warmth and redness of the fact, a throbbing sensation and pain in the head and neck, difficulty in breathing, nausea, vomiting, sweating, thirst, chest pain, uneasiness, weakness, dizziness, blurred vision, and confusion. This effect may last from 30 minutes to several hours but does not appear to have residual side effects.

By analogy with effects seen in animals, furfural may affect the central nervous system, liver, kidneys, blood, and bone marrow of humans; however, these effects have not been reported in exposed workers.

Since the wastewater from Quaker Oats Company ranged from 100 to 2000 mg/L, it was potentially toxic to anaerobic bacteria (Koh and Ellis, 2002). Therefore, anaerobic treatment, normally suitable for the high strength wastewater from a food processing industry like the Quaker Oats Company in Cedar Rapids, Iowa was not considered possible. Aerobic treatment by yeast is an alternative for this wastewater. Kasper (2004) cited that yeast can tolerate concentrations of furfural up to 2g/L. The wastewater used in this study contained furfural at about 180 to 190 mg/L, which was not expected to have an effect on wastewater treatment by yeast.

1.3.4 Retention times in wastewater treatment

A retention time is defined as the average amount of time a constituent stays in a system (Grady *et al*, 1999). Two constituents are considered in retention time i.e. soluble and particulate fractions and their retention times are not necessary the same. Soluble constituents are intimately associated with the fluid and cannot be separated from it. Therefore, the retention time of soluble parts is equal to the mean hydraulic retention time as written in equation 1.1:

$$\tau = \frac{V}{F}$$
(1.1)

Where: τ = hydraulic retention time (HRT), d^{-1}

V = volume of liquid, m^3

F = flow rate, m^3/d

Particulates or biomass can be separated from fluid by physical means such as filtration or settling. As a result, biomass can be controlled and retained within the bioreactor. This biomass is normally called return sludge in activated sludge systems. Thus, it results in the retention time of biomass to be greater than that of the soluble part. The retention of biomass is called solids retention time or mean cell residence time and can be expressed as equation 1.2:

$$\theta c = \frac{V \cdot X}{Fw \cdot Xw}$$
(1.2)

Where: θ_C = solids retention time (SRT), d^{-1}

X = biomass concentration in reactor, mg/L

F_w = flow rate out of reactor, m^3/d

X_w = biomass concentration out of reactor, mg/L

In this study, the reactors were operated as chemostats i.e. HRT equal to SRT or biomass concentration in the reactor (X) was equal to biomass concentration out of reactor (X_w). Consequently, equation 1.2 is simplified to:

$$\theta c = \frac{V}{Fw} \qquad (1.3)$$

The effluent concentration can be predicted by using SRT as shown in equation 1.4:

$$S_e = \frac{Ks(1 + k_d\theta c)}{\theta c(\mu_{max} - k_d) - 1} \qquad (1.4)$$

Where: S_e = effluent concentration of organic matter, mgCOD/L

K_S = half-saturation coefficient, mgCOD/L

k_d = decay coefficient, d^{-1}

μ_{max} = maximum specific growth rate, d^{-1}

The SRT value below which microorganisms are washed out is called the critical SRT and can be estimated as follow:

$$\theta c, o = \frac{1}{\mu_{max} - k_d} \qquad (1.5)$$

Where: $\theta_{C,O}$ = critical SRT, d^{-1}

1.3.5 Temperature in wastewater treatment

Temperature is one of the most important environmental factors influencing the growth and survival of organisms. Madigan et al. (1997) explained this as follows:

"For every microorganism, there is a *minimum temperature* below which growth no longer occurs, an *optimum temperature* at which growth is rapid, and a *maximum temperature* above which growth is not possible. The optimum temperature is always nearer the maximum than the minimum".

These three temperatures are often called "cardinal temperatures" and generally characteristic of each microorganism but not completely fixed because they can be modified slightly by other environmental factors.

Microorganisms can be classified according to temperature range into 4 groups (Madigan *et al*, 1997).

- A psychrophile is a microorganism with a growth temperature optimum of $15^{\circ}C$ or lower and maximum growth temperature below $20^{\circ}C$.
- A mesophile is an organism that grows well between temperatures of 20 and $45^{\circ}C$.
- A thermophile is an organism with an optimum temperature between 45 and $80^{\circ}C$.
- A hyperthermophile is a microorganism that grows better at temperatures of $80^{\circ}C$ and higher.

All yeasts used in this study were mesophiles.

Temperature can affect biological reactions in two ways, i.e. it influences the rate of enzymatically catalyzed reactions and affects the rate of substrate diffusion to the cells (Grady *et al*, 1999).

The Arrhenius equation is used to explain the microbial growth rate related to temperature as shown in equation (1.4) (Grady et al, 1999; Bitton 1999):

$$k = Ae^{-u/RT} \tag{1.6}$$

Where: k = temperature independent rate coefficient, h^{-1}

A = constant

u = temperature coefficient, kJ/mole

R = gas constant, 8.3143 J/K.mole

T = absolute temperature, K

For two rate coefficients at different temperature, the Arrhenius equation can be rearranged as follows:

$$\ln (k_1/k_2) = u \cdot (T_1 - T_2)/(R \cdot T_1 \cdot T_2) \tag{1.7}$$

Since the mesophilic temperature range is small when T is expressed in K, the term $(R \cdot T_1 \cdot T_2)$ does not vary significantly and is considered as constant. Hence equation 1.5 is simplified as:

$$k_1 = k_2 \cdot e^{C \cdot (T_1 - T_2)} \tag{1.8}$$

Where: $C = u/(R \cdot T_1 \cdot T_2) \approx 0.0015u$

When equation 1.6 is used, the temperature can be written in °C because only the difference in temperature is applied in the equation. The value of C may be obtained by plotting ln (k) versus T, and the slope is equal to C.

Since $C = \ln (\theta)$, the equation 1.6 is simplified as:

$$k_1 = k_2 \cdot \theta^{(T_1 - T_2)} \tag{1.9}$$

As mentioned previously, the mesophillic temperature range is between 20 and 45°C. The increase in temperature is independent of the rate coefficient, growth rate, reaction rate, etc.

However, Ahmad *et al.* (1995) referred in his report that temperatures above 34°C affected the metabolism of the cells negatively.

1.3.6 pH in wastewater treatment

The pH plays a part in the activity of microbial enzymes. It involves the ionization of the chemicals and plays a role in the transport of nutrients and toxic substrates to the cells (Bitton, 1999).

The optimal growth pH represents the pH of the extracellular environment only. The intracellular pH must be maintained near neutral in order to prevent the destruction of acid- or alkaline-labile macromolecules in the cells (Madigan, 1997).

Biological wastewater treatment is generally optimal at neutral pH values, i.e. between 6 and 8. Bacteria typically grow better at a pH near 7, though some species are obligated to a lower pH i.e. *Thiobacillus*, and *Sulfolobus spp.* thrive at pH less than 2. Fungi (including yeast) prefer a pH of 5 or lower. The optimum pH of Cyanobacteria is higher than 7 (Bitton, 1999).

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CHEPTER 2 BIOCONVERSION OF WASTE ORGANIC MATTER INTO A MICROBIAL BIOMASS PROTEIN

A paper published in the Proceedings of 75th Annual Technical Exhibition & Conference, Chicago, IL USA Water Environment Federation

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2.1 ABSTRACT

Food processing industries produce large quantities of wastewater with a high organic content. It is expensive to treat and dispose of this wastewater, and it is a waste of organic resources. These waste streams can be converted from an economic liability to a source of revenue in the production of a high quality single cell protein. Several studies have been carried out on the production of microbial biomass protein from food processing wastewater using aerobic yeast cultures. The main challenge seems to be prevention of bacterial infection and maintaining the selected cultures in a full-scale continuous flow reactor. The hypothesis behind this research is that there is a range of operating conditions (e.g., SRT, pH, temperature) at which selected microorganism cultures have a competitive advantage and can be maintained in the system by a natural selection process. The concept was demonstrated with wastewater from the production of furfural, but the proposed methodology can be applied to any organic contaminants and microorganism cultures. The results of this study indicated that furfural production wastewater could be effectively treated by aerobic yeast cultures to produce high quality microbial biomass.

A COD removal efficiency of 92-95% and complete furfural degradation was achieved in a single stage process. A new approach for the design of biological processes aimed at the

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production of microbial biomass protein was proposed. The proposed approach is based on maintaining the selected microorganism cultures in the system using natural selection processes. A methodology to define the range of operating conditions that prevent bacterial growth was proposed and verified in continuous flow experiments. Effluent soluble COD was significantly higher than the effluent biodegradable COD predicted possibly due to formation of SMP associated with substrate biodegradation.

2.2 INTRODUCTION

Food processing industries produce large quantities of wastewater with a high organic content. Many food-processing plants discharge waste streams into municipal sewers for combined treatment with municipal wastewater. Discharge to the municipal sewer has several significant disadvantages:

- High treatment fees charged by wastewater treatment plants.
- Many municipal wastewater treatment plants were designed to treat conventional domestic wastewater with organic matter concentrations of 300-500 mg/L. Incorporating high strength wastewater (e.g., as that from food processing) into the influent can significantly increase the influent concentration of organic matter. Thus, in order to achieve the required effluent quality (i.e., to maintain the design solids retention time, SRT), the plant would have to operate at a higher suspended solids concentration. This can negatively affect operation of secondary clarifiers and overall process performance.
- Highly biodegradable substances in high concentrations lead to septic conditions in interceptors. Volatile fatty acids could then be produced and these could lead to the growth of specific filamentous bacteria such as Thiothrix species within activated sludge processes leading to sludge bulking and poor settling (Conner and van Leeuwen, 2003).

- In some cases municipal wastewater treatment plants do not have the capacity to accept additional organic loading.

The other option is to treat high strength wastewater separately. The disadvantages of this option are clear: it requires unattractive capital investment in equipment and treatment area and expenditure of non-productive operating costs. These disadvantages can be eliminated if the organic matter in industrial wastewater can be converted into a valuable by-product (e.g., microbial biomass that can be used as a protein supplement).

Several studies have been carried out on the production of microbial protein from food processing wastewaters (e.g., Zheng et al., 2001; Tauk, 1982). Of all the possible microorganisms, the most suitable seem to be fungi, molds andyeasts. (Note: yeasts are also fungi) Bacterial cultivation is easier, but it results in an inferior quality protein with a high nucleic acids content. Jin et al. (1998; 1999a; and 1999b) studied wheat starch processing wastewater treatment with filamentous microfungi. This process had a high bioconversion efficiency of starch materials and a short HRT. The fungal biomass contained 38-48% protein, and 90% organic matter removal was accomplished. Barker et al. (1982), Malnou et al. (1987), and Moriya et al. (1990) have shown that there is a potential for the production of microbial biomass protein from ethanol distillery wastewater using filamentous fungi and aerobic yeast cultures.

A variety of organic compounds have been studied as potential substrates for yeast growth. Braunegg (1975) reported results indicating that methanol could be used as a substrate for generating yeast biomass. Yeast cultures that have an ability to utilize methanol are *Candida boidinii, Hansenula hennicii*, and *Torulopsis molischiana*. Fukui and Tanaka (1981) studied biodegradation of alkanes by various yeast cultures and found that *C. tropicalis, C. oleophia*, and *Saccharomycopsis lipolytica* can be used to produce useful microbial biomass. British Petroleum has developed a process for yeast biomass production using alkanes from gas oil. *C. tropicalis* and *Saccharomycopsis lipolytica* were successfully used. The use of alkanes is limited, however, by their poor solubility and low yield coefficient values (Rehm and Reiff,

1981). Several processes have been proposed for production of yeast biomass from starch containing wastes (e.g., Potter, 1998; Chigusa and Matsamaru, 1991). The biomass produced from the above waste streams was reported to have a potential use as a protein and vitamin source in animal feed (Vazza, 1994 and Evich et al., 1981).

In spite of the significant research conducted on the production of microbial biomass protein, there is no generally accepted approach to the design of this process. The main problem seems to be prevention of bacterial contamination in continuous flow system without need to continuously reseed the reactor with the selected culture of microorganisms. The hypothesis behind this research is that there is a select range of operating conditions (e.g., SRT, pH, temperature) at which the aerobic yeast cultures have a competitive advantage and can be maintained in the system by a natural selection process. This concept was demonstrated with wastewater from the production of furfural, but the proposed methodology can be applied to a variety of organic contaminants and microorganism cultures.

2.3 OFJECTIVES

The main goal of this study was to provide the scientific background for selecting operating conditions at which yeast cultures can be reliably maintained in a continuous flow system fed with industrial wastewater. The specific objectives wereas below.

- To select yeast cultures that have an ability to degrade organic matter from the specific wastewater
- To evaluate the value of biokinetic parameters for yeast and competitive bacterial cultures
- To define the range of operating conditions at which bacterial growth can be prevented
- To evaluate the effluent quality that can be achieved

2.4 METHODOLOGY

2.4.1 Materials

Wastewater. Wastewater from the furfural production unit of Quaker Oats Co., Cedar Rapids, Iowa was used in this study. Grab samples of the wastewater were received at the Iowa State University within 6 h from sampling, acidified to pH 1.2-1.7 with sulfuric acid and stored at room temperature.

Yeast cultures Freeze dried cultures of *Candida utilis*, *C. guiliermondii*, and *C. tropicalis* were obtained from the American Type Culture Collection. After rehydration over a 24 h period, the cultures were revived using YM nutrient broth with glucose as a carbon source at 35°C. Revived yeast cultures were seeded into 250 mL flasks containing 50 mL of diluted (1:10) wastewater with pH 4.5-5 and placed on a shaker at 300 rpm and 35°C. After 1 week, the mixed liquor from the flasks was used to seed the continuous flow reactor.

Mixed bacterial culture Mixed liquor from the Cedar Rapids Water Pollution Control Facility was used to evaluate the kinetic parameters of bacterial growth on the selected wastewater. The biomass was shipped cold and tested within 24 h after sampling.

2.4.2 Experimental methods

Evaluation of kinetic parameters The Monod equation was used to describe the growth rate for both yeast and microbial cultures:

$$\mu = \mu_{max} \frac{S}{K_s + S} \tag{2.1}$$

Where: μ = specific growth rate, h^{-1}

μ_{max} = maximum specific growth rate, h^{-1}

K_S = half-saturation coefficient, mgCOD/L

S = organic matter concentration, mgCOD/L

Mixed bacterial culture Extant kinetic parameters (μ_{max}, K_S, and Y) for the mixed bacterial culture were determined using the procedure described by Ellis *et al.* (1996). Respirometers with an internal volume of 230-250 mL were used. The respirometers were water-jacketed, and the temperature during the tests was maintained by a circulating constant temperature water bath. The temperature was 25°C in all the tests except for the test in which the effect of the temperature was studied. Mixing during the tests was provided through magnetic stir bars. The concentration of DO was measured by a DO meter (YSI Model 5300 Biological Oxygen Monitor, YSI Inc., OH) that was interfaced with a personal computer. Data acquisition was automated with an analog to digital interface (Computer Boards, Middlebrow, MA) and Labtech Notebook software (Andover, MA). DO concentrations were measured at a rate of 10 Hz. Prior to the beginning of the test, the DO meter was calibrated, and the biomass was aerated for approximately 1 h to consume any residual substrate. The biomass was placed into a respirometer, the DO concentration was adjusted to 12-18 mg/L with pure oxygen, and data collection was initiated. The data on endogenous respiration was collected during the first 3 to 5 min. prior to injection of 0.2 mL of the wastewater. The DO data continued to be collected until the OUR returned to the background endogenous rate. Four to eight extant kinetic tests were performed with each biomass sample.

Yeast cultures An attempt to apply the technique described above to estimate the kinetic parameters for the yeast cultures did not result in uniquely identifiable values of μ_{max} and K_S due to a high K_S value compared with the initial substrate concentration (Ellis *et al.*, 1996). For this reason, a modification of the technique was used. After measuring the endogenous respiration rate, the wastewater was injected into the respirometer and the OUR rate was measured during 2 min after the injection. Six to eight tests were conducted with each biomass samples at initial organic matter concentrations in a range of 100 to 1000 gCOD/L. OUR at each initial concentration of organic matter is related to the specific rate of substrate utilization as follows:

$$OUR = (1-Y)qX \qquad (2.2)$$

Where: Y = yield coefficient

            q = specific rate of substrate utilization, h^{-1}

            X = biomass concentration, mgCOD/L

The value of q is a function of substrate concentration:

$$q = q_{max} \frac{S}{Ks + S} \tag{2.3}$$

Where: q_{max} = maximum specific rate of substrate utilization, h^{-1}

Based on the value of the measured OUR and Y estimated in batch tests, q was calculated from Equation 2 for each of the initial test concentrations, and the set of q and K_S values that provided the best fit of experimental data to Equation 3 was estimated.

Continuous flow system. A Bioflo 2000 fermentor (New Brunswick Scientific, New Brunswick, NJ) was operated as a chemostat at a solids retention time of 1 and 2 d, temperature 35°C, and pH 4.5. The reactor was seeded with an acclimated culture of *C. utilis* and operated over a period of 18 months at a SRT of 1 and 2 d. Influent and effluent soluble COD, VSS, organic acids, and furfural concentrations in the reactor were measured on a weekly basis, and microscopic observations of the biomass were performed periodically. A 0.5 N solution of NaOH was used to control pH. The predicted effluent concentration of organic matter was calculated as follows (Grady et al., 1999):

$$Se = \frac{Ks(1 + k_d \theta_c)}{\theta_c(\mu_{max} - k_d) - 1} \tag{2.4}$$

Where: S_e = effluent concentration of organic matter, mgCOD/L

            θ_C = solids retention time, h

            k_d = decay coefficient, d^{-1}

The value of the decay coefficient was assumed to be 0.2 d^{-1} (Grady et al., 1999).

Batch experiments. Three series of batch tests were conducted to estimate the ability of the selected yeast culture to degrade organic matter from the furfural production wastewater. In each series, 1 L of the furfural production wastewater was seeded with 3,000 mgCOD/L of one of the studied yeast cultures. After mixing, and adjusting the pH to 4.5, the mixture was distributed into 11 250-mL flasks (approximately 100 mL in each flask). The flasks were placed on a shaker at 300 rpm and 35°C. Periodically, one of the flasks was taken for analysis of soluble COD.

Batch tests were also conducted to evaluate the value of *Y* for the yeast cultures, and to compare the organic matter removal efficiency by *C. utilis*, *C. tropicalis*, and a mixture of the two cultures. 250-mL flasks containing 100 mL of wastewater were seeded with small amount of the tested yeast cultures and put on a shaker at 300 rpm and 35°C. The concentrations of organic matter, pH, and VSS were measured in the flasks at the end of the test. The following mass balance equation was used to calculate Y from the results of the batch tests:

$$\frac{dX}{dt} = Y\frac{dS}{dt} - k_d X dt \qquad (2.5)$$

Where: Y = yield coefficient

T = time, h

2.4.3 Analytical methods

High performance liquid chromatography was used to measure the concentrations of furfural. The analysis was performed using water as a mobile phase at 0.5 mL/min and ultraviolet detector at 280 nm. Data were analyzed using PeakNet 5.01 software. Concentrations of

acetic, propionic and butyric acid were measured with a gas chromatograph equipped with a flame ionization detector. VSS, COD, and total volatile fatty acids (VFA) concentrations were measured according to Standard Methods (APHA et al., 1992).

2.5 RESULTS

2.5.1 Chemical composition of the wastewater

The chemical composition of the wastewater from the furfural production unit of Quaker Oats Company. is shown in Table 2.1. The data in Table 1 indicates that more than 70% of the organic matter in the wastewater studied was identified as short chain organic acids and furfural. Nitrogen and phosphorus concentrations were low compared with the organic matter content indicating that these nutrients could limit microorganism growth. For this reason ammonium chloride and potassium phosphate were added to achieve a C:N:P ratio of 100:5:1.

2.5.2 Selection of the yeast cultures

COD removed by *C. utilis*, *C. guillermondii*, and *C. tropicalis* in batch tests are shown in Figure 2.1. A substantial lag phase was observed in all tests (12 h for *C. utilis* and about 24 h for *C. tropicalis* and *C. guillermondii*). This can be due to the fact that the cultures were revived on dextrose, whereas the major organic component of the tested wastewater was acetic acid. The degradation rate by *C. utilis* was the fastest, but all the cultures were able to remove more than 95% of soluble COD. The final pH was 6.5 to 6.8 in all the tests due to the degradation of acids indicating that pH control may be required to maintain a pH of 3.5-4 in continuous flow systems.

The observed yield coefficients were 0.34, 0.37 and 0.42 for C. *tropicalis*, C. *guillermondii*, and *C. utilis*, respectively. Based on the results in Figure 2.1, *C. utilis* and *C. tropicalis* were selected for further study.

2.5.3 Evaluation of the kinetic parameters and defining the range of operating conditions at which bacterial growth can be prevented

Once the ability of the yeast cultures to degrade organic matter in the selected wastewater was established, it was important to define the range of operating conditions at which continuous flow systems could be operated. In order to do that, kinetic parameters were estimated for *C. utilis* (see Figure 2.2) and mixed bacterial culture. At a pH of 4.5, the values of μ_{max} were 0.13 h^{-1} and 0.03 h^{-1} and the values of K_S were 160 mgCOD/L and 12 mgCOD/L for *C. utilis* and the mixed bacterial culture, respectively. The higher μ_{max} measured for *C. utilis* was expected because it is known that the low pH favors yeast cultures (e.g., Jin *et al.*, 1999b; Zheng *et al.*, 2000). However, it should be recognized that it is the value of μ rather than μ_{max} that defines which culture of microorganisms will have a competitive advantage under a given set of operating conditions.

Due to a high K_S value, even at pH 4.5, yeast will have a competitive advantage over bacteria only if the organic matter concentration in the reactor is more than 30 mgCOD/L as shown in Figure 2.3. Based on the kinetic parameter values, this organic matter concentration can be achieved at an SRT of less than 3 d.

In order to verify this finding, a continuous flow system seeded with an acclimated culture of *C. utilis* was operated over a period of one year at an SRT of 2 d. Microscopic observation indicated that after one year of operation the vast majority of the biomass was eukaryotic yeast cells, while bacterial growth was not significant (see Figure 2.4). According to Figure 2.3, at higher COD concentrations in the reactor (i.e., at a lower SRT), the competitive advantage of yeast over bacteria is expected to be even more significant. It can be concluded therefore, that the yeast population could be reliably maintained in the reactor at pH of 4.5 and a SRT of less than or equal to 2 d.

2.5.4 Evaluation of the effluent quality that can be achieved

In order to evaluate the effluent quality that can be achieved, kinetic parameters for *C. tropicalis* and the combined culture of *C. utilis* and *C. tropicalis* were estimated as shown in Figure 5 and compared with the kinetic parameters previously measured for *C. utilis*. Comparison of the data in Figure 2.5 and Figure 2.2 indicate that *C. tropicalis* exhibited slightly higher biodegradation rate than *C. utilis* due to the lower K_S value, while the values of μ_{max} were similar. A more significant difference was observed in the biodegradation kinetics exhibited by the combined culture and individual yeast cultures. The combined culture exhibited μ_{max} values approximately 30% higher and K_S values 63% and 50% lower compared with *C. utilis* and *C. tropicalis*, respectively, thus suggesting that better effluent quality can be achieved when using the combined culture of *C. utilis* and *C. tropicalis*. In order to verify this finding, batch tests were performed in which COD removal efficiency was estimated for individual and combined yeast cultures. The results in Table 2.2 indicate that better COD removal efficiency was obtained in the tests with the combined cultures, while the difference in COD removal efficiency by *C. utilis* and *C. tropicalis* was not statistically significant.

Based on the measured values of the kinetic parameters, the predicted effluent concentrations of organic matter were calculated (see Table 2.3). Data in Table 2.3 indicate that although the values of the kinetic parameters suggest that use of the combined yeast culture will result in lower effluent COD; greater than 99% biodegradable COD removal efficiency was predicted for both individual and combined cultures. The predicted removal efficiencies are significantly higher than those reported in the literature (e.g., Barker et al., 1982; Ruegger and Tauk-Tornisielo, 1996; Malnou et al., 1987).

The results of the continuous flow experiments conducted in this study also indicated that the effluent COD was significantly higher than the range of predicted based on the measured values of kinetic parameters (see Table 2.4).

It should be noted however, that in spite of higher than predicted effluent COD, more than 90% of organic matter was removed. This is higher than removal efficiencies of 55% (Malnou et al.,1987) to 75% (Gabib et al., 1983) reported in literature for distillery wastewater. Therefore, the results in Table 2.4 suggest that the furfural production wastewater can be effectively treated by yeast cultures to produce marketable microbial biomass protein.

2.6 DISCUSSION

In order to produce marketable microbial biomass from the waste organic matter, several requirements must be met:

- the selected microorganism cultures must have an ability to utilize the waste organic matter
- the chemical composition of the wastewater must be known to ensure that it contains no toxic organic compounds or all the toxic organic compounds are completely removed as a result of biodegradation or stripping
- the range of operating conditions must exist in which maximum specific growth rate of the selected cultures is higher than that of bacteria.

The results of this study show that the wastewater from furfural production can be used to produce marketable microbial biomass. Wastewater was found to contain high concentrations of short chain organic acids and furfural that could be used as a substrate for growth of aerobic yeast cultures (*C. utilis*, *C. tropicalis*, and *C. guillermondii*). Approximately 27% of the COD in the wastewater was contributed by organic compounds that were not identified. Based on our knowledge of the furfural production process, these compounds could be non-hydrolyzed cellulosic materials and hydrocarbons. High concentrations of furfural were measured in the wastewater. Furfural is a toxic compound and its sorption on the biomass can impact the biomass use as a protein supplement. The results of this study indicated that complete furfural removal occurred at an SRT of 1 d. It is unlikely that sorption of furfural

on the yeast biomass contributed to the high furfural removal efficiency due to its high water-octanol partition coefficient (Dewulf et al., 1999). It can be concluded therefore, that furfural removal observed in this study was due to its biodegradation and stripping.

Evaluation of the kinetic parameters for yeast and bacterial cultures indicated that at a pH of 4.5 and T= 35°C the maximum specific growth rate of yeast cultures was significantly higher than that of bacteria in spite of relatively low yield coefficient values measured for the yeast cultures. This made it possible to select operating conditions at which bacterial growth could be prevented, and the selected yeast cultures could be maintained in the reactor by natural selection processes. It was found that due to the high value of the half saturation coefficient measured for the yeast cultures, operating conditions must provide relatively high substrate concentrations in the reactor in order to provide yeast with a competitive advantage over bacterial cultures. This finding indicates that design approach for processes aimed at the production of microbial biomass protein should be different from the design of conventional biological wastewater treatment systems. Conventional wastewater treatment processes are designed to achieve certain effluent quality. The required effluent concentration of organic matter defines the SRT at which the system should be operated. The results of this study indicate that if the process using yeast cultures was designed to achieve the best effluent quality, bacterial growth could not be prevented and the yeast population could not be maintained. For this reason, the major design criterion for systems using yeast cultures should be the prevention of bacterial contamination.

The critical SRT value (i.e., the SRT value below which microorganisms are washed out) can be estimated as follows:

$$\theta_{C,O} = \frac{1}{\mu_{max} - k_d} \qquad (2.6)$$

Where: $\theta_{C,O}$ = critical SRT, h

Thus, the design SRT should be lower than the critical SRT for bacterial cultures and higher than the critical SRT for the selected cultures. Based on the kinetic parameters measured in this study, yeast biomass could be maintained in the reactor at a SRT range of 0.4 to 2.0 d. The results of continuous flow experiments at an SRT of 2 and 1 d support this finding. The SRT value selected to prevent bacterial growth defines the effluent quality that can be achieved. Once the range of SRT at which bacterial growth is prevented is defined, the effluent quality can be estimated.

Several important observations can be made from comparison of the measured and predicted effluent COD concentrations:

- effluent COD was significantly higher than predicted based on the measured values of kinetic parameters
- effluent COD was not affected by the SRT
- organic compounds contributing more than 70% of the influent COD were not found in the effluent

One possible explanation for these observations is that part of the organic matter present in the influent cannot be degraded by *C. utilis*. Measured values of the kinetic parameters represent the rate of utilization of the biodegradable fraction of the influent organic matter, while the concentration of nondegradable organic compounds remains unchanged resulting in higher than predicted effluent COD. This can also explain the lower K_S value measured for the combined yeast culture compared with the individual cultures (part of the organic matter that cannot be degraded by one culture is utilized by the other).

Another explanation is that the high effluent COD results from soluble microbial products (SMP). It is generally accepted that the SMP can be classified into two categories: SMP associated with biomass decay and SMP associated with substrate biodegradation (e.g., Grady et al., 1999). The first group of SMP products is not biodegradable, while the measured BOD_5 in Table 2.4 indicate that at least a part of the effluent organic matter could be degraded by activated sludge microorganisms. For this reason, data in Table 2.4 suggest

that if high effluent COD is a result of SMP formation, the SMP are likely to be associated with substrate biodegradation. In this case, the concentration of SMP can be calculated as follows (Grady et al., 1999):

$$S_{MP} = \frac{Y_{MP}}{Y} X\theta_c(\frac{1}{\theta_c} + k_d) \tag{2.7}$$

Where: S_{MP} = concentration of SMP, mgCOD/L

           Y_{MP} = SMP yield coefficient

Using the value of Y= 0.34 estimated for *C. utilis*, and the recommended value of Y_{MP} = 0.1 (Grady et al., 1999), the SMP concentration in the effluent can be estimated as 1,560 mgCOD/L and 1,680 mgCOD/L for SRTs of 2 and 1 d, respectively. These predicted values are similar to the measured effluent COD in Table 2.4. It can be seen from Equation 7 that if the system is operated without biomass recycle (i.e., biomass concentration is independent of SRT) and the SRT is low ($1/q_c \gg k_d$), the concentration of SMP is not significantly affected by the SRT; this phenomenon was also observed in this study.

2.7 CONCLUSIONS

The results of this study indicated that furfural production wastewater can be effectively treated by aerobic yeast cultures to produce high quality microbial biomass. A COD removal efficiency of 92-95% and complete furfural degradation could be achieved in a single stage process. A new approach for the design of biological processes aimed at the production of microbial biomass protein was proposed. The proposed approach is based on maintaining the selected microorganism cultures in the system using natural selection processes. The methodology of defining the range of operating conditions that prevent bacterial growth was proposed and verified in continuous flow experiments. The results of this study indicated that effluent COD could be significantly higher than predicted using the measured values of

kinetic parameters and independent of SRT in the range of 1 to 2 d possibly due to formation of SMP associated with substrate biodegradation.

2.8 ACKNOWLEDGEMENTS

Table 2.1 Chemical composition of the wastewater

Compound	Concentration, mg/L	# of samples	Analytical method
COD	15,600±1,800	6	APHA (1992)
Suspended Solids	30±14	6	APHA (1992)
Organic compounds[1]			
Acetic acid	7,100±1,200	6	GC-FID
propionic acid	2,060±1,020	5	GC-FID
Butyric acid	240±160	5	GC-FID
Furural	2,100±680	6	HPLC-UV
Inorganic compounds			
NH_4^+	5.0±2.3	4	IC
NO_3^-	21.2±6.7	4	IC
PO_4^{3-}	1.1±0.3	4	IC
SO_4^{2-}	2,200±520	4	IC

[1]Concentrations of organic compounds are shown in mgCOD/L

Table 2.2 Results of batch cultivation of the yeast cultures on the Quaker Oats Company wastewater T= 35°C, 72 h

Yeast culture	Initial COD	Initial SS	Initial pH	Final pH	Final COD
C. utilis (continuous)[1]	13,200±1,400	3,120	4.65	4.62	860±210
C. tropicalis (batch)[2]	12,800±1,100	3,060	4.81	6.55	640±140
Combined (1:1)	13,100±590	3,012	4.79	7.82	148±51

[1]Culture from the continuous flow reactor operated at an SRT of 2 d

[2]Culture from the 250 mL flasks cultivated on a shaker at 300 rpm, 35°C

Table 2.3 Predicted effluent biodegradable COD at pH 4.5 and T= 35°C

Yeast culture	Influent COD	Predicted effluent biodegradable COD, mg/L	
	mg/L	SRT = 1 d	SRT = 2 d
C. utilis	15,000	3,120	4.65
C. tropicalis	15,000	3,060	4.81
Combined (1:1)	15,000	3,012	4.79

Table 2.4 Organic matter removal by C. utilis in a continuous flow system (T= 35°C, pH 4.5)

Compound	Influent concentration	Effluent concentration, mgCOD/L		
	mgCOD/L	SRT = 2 d	SRT = 1.4 d	SRT = 1 d
COD	15,600±1,800	1,200±550	960±240	1,220±460
BOD5, 20°C			270±67	
Acetic acid	7,100±1,200	25±7	24±11	32±4
propionic acid	2,060±1,020	ND[1]	ND[1]	ND[1]
Butyric acid	240±160	ND[1]	ND[1]	ND[1]
Furural	2,100±680	ND[1]	ND[1]	ND[1]
VSS	15±3	4,461±568	3,921±268	5,328±850

[1]ND indicates that the concentration was below the detection limit (1 mgCOD/L for organic acids, and 0.03 mgCOD/L for furfural)
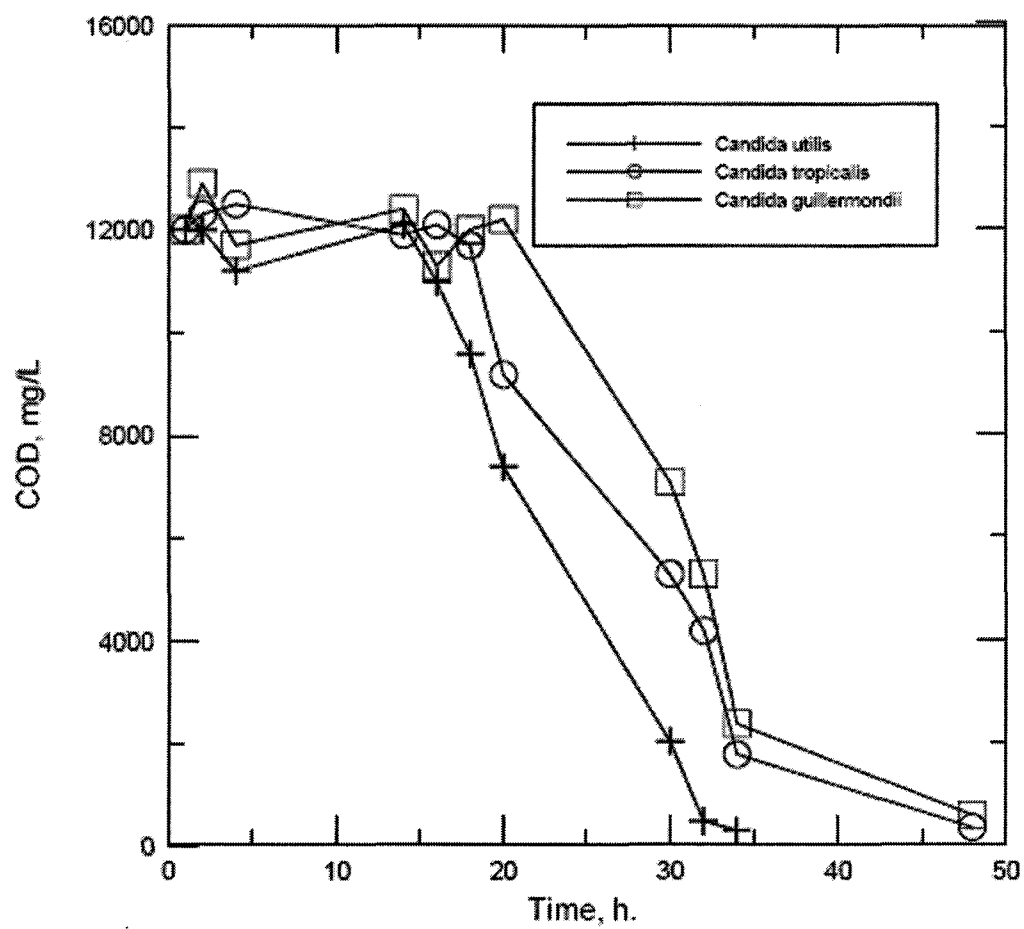
Figure 2.1 Reduction of soluble COD in batch test
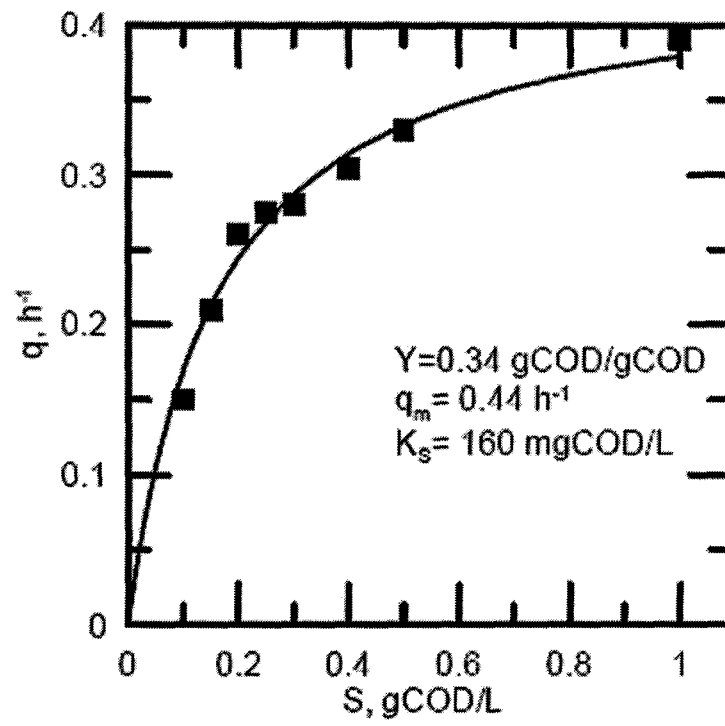
Figure 2.2 Evaluation of kinetic parameters for *C. utilis*
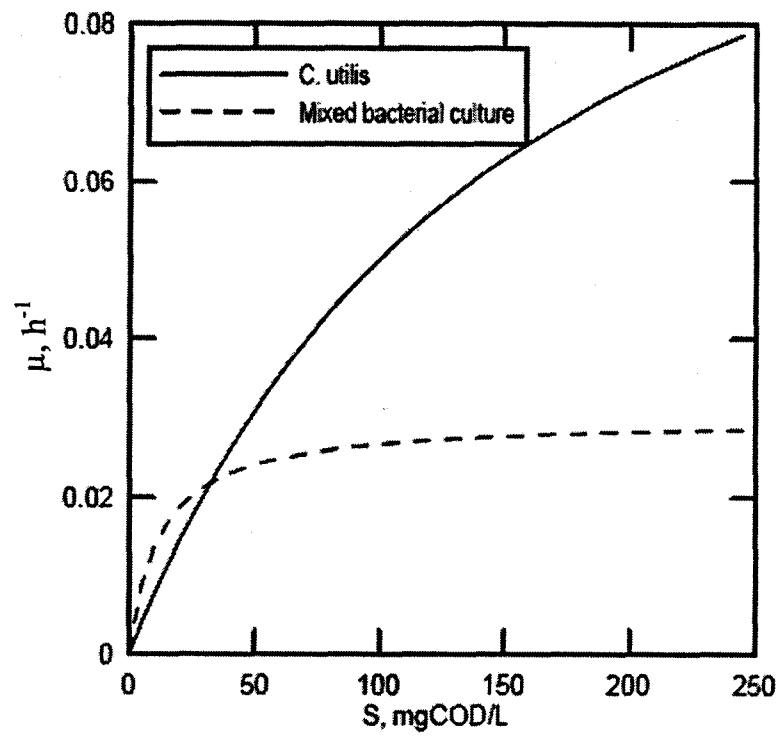
Figure 2.3 Specific growth rate of *C. utilis* and mixed bacterial culture as a function of substrate concentration at pH 4.5 and 35°C
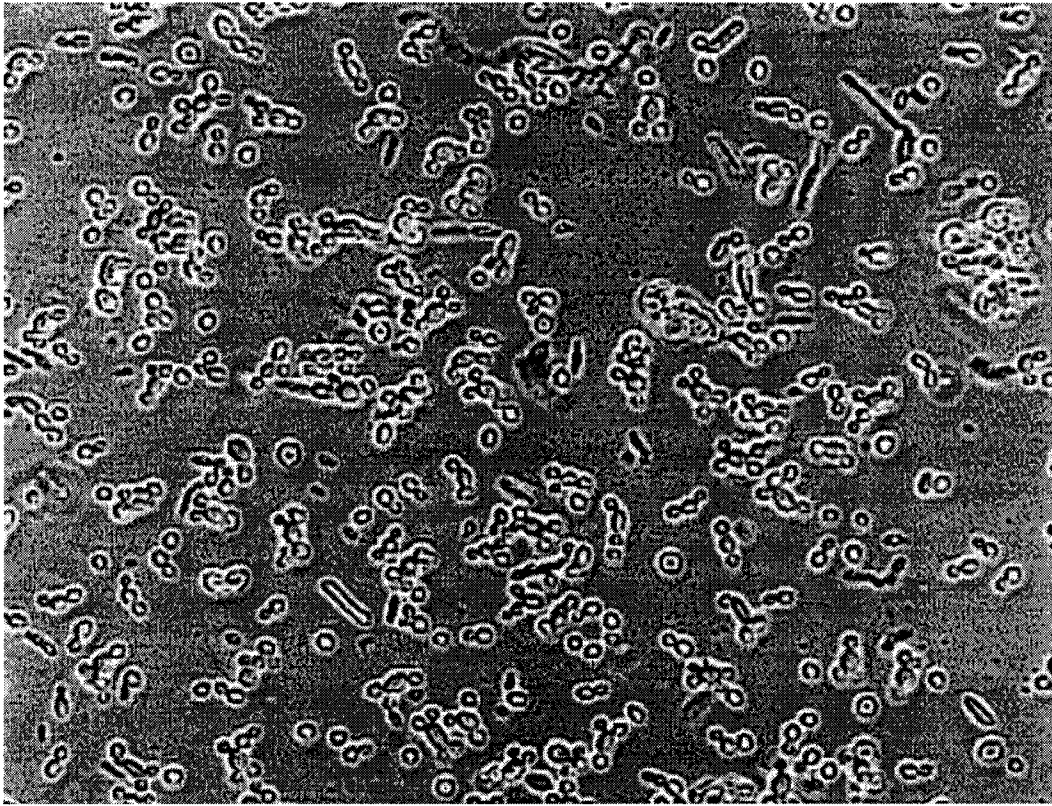
Figure 2.4 Microbial population in the continuous flow reactor after 1 year of operation at an SRT of 2 days (pH 4.5, T =35°C)
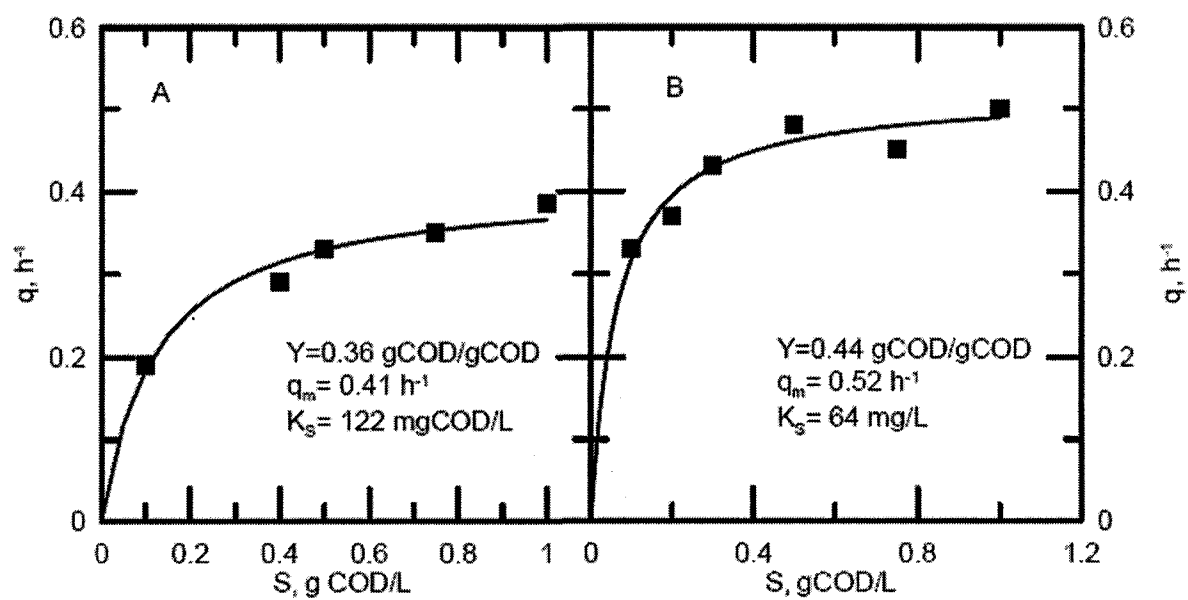
Figure 2.5 Evaluation of the kinetic parameters for *C. tropicalis* (Panel A) and combined culture of *C. utilis* and *C. tropicalis* (Panel B) at pH 4.5 and T= 35°C

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CHAPTER 3 TREATMENT OF FOOD PROCESSING WASTEWATER CONTAINING FURFURAL USING YEAST

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3.1 ABSTRACT

Food processing wastewater containing inhibitory furfural was treated with suspended aerobic yeasts in continuous lab-scale reactors. The effect of different yeast cultures and solid retention times (SRTs) on the effluent quality and the yeast biomass yield was studied. Yeast cultures of *Candida tropicalis*, *C. utilis*, and a mixture of the two species were studied. A variation of SRTs between 1 and 2 days was investigated. The results showed that there were no significant differences in effluent chemical oxygen demand (COD), biochemical oxygen demand (BOD), volatile suspended solids (VSS) and volatile fatty acids (VFA) among the three cultures. The effluent qualities were similar for SRTs of 1.5 to 2.0 days. At lower SRTs, the effluent COD, BOD, and VFA increased significantly while the effluent VSS decreased. The yield coefficient for the different cultures ranged from 0.24 to 0.30 gVSS/gCOD removed at SRT of 1.5 to 2.0 days. The yield decreased at lower SRTs for *C.*

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tropicalis and *C. utilis* while they remained constant for the mixed cultures down to SRTs of 1 day. Almost complete removal of furfural was obtained.

Keywords

Solid retention time; furfural; *Candida tropicalis*; *Candida utilis*; mixed cultures; food processing wastewater; yeast biomass yield

3.2 INTRODUCTION

Food processing industries typically produce large quantities of high strength organic wastewater, which is often subjected to anaerobic pretreatment. However, the wastewater from the Quaker Oats Company located in Cedar Rapids, Iowa USA contains furfural, which was found to be toxic to anaerobic microorganisms (Koh and Ellis, 2002). The wastewater contains furfural as a by-product of the process stream. The wastewater is discharged to the municipal wastewater facility subject to a considerable surcharge due to the organic loading. The average wastewater flow rate was 483 m³/day, and the range of COD varied from 10,000 to 20,000 mg/L including 100 to 2,000 mg/L of furfural.

Taherzadeh *et al.* (1999) concluded in their study that the toxicity of furfural could be reduced to less harmful furfuryl alcohol by yeast. Many advantages of using yeasts in wastewater treatment include their ability to cope with high strength of organic matter from food processing industries, the direct decomposition of oils, their ability to deal with high organic and suspended solids loading, sludge generation reduction, good settling and dewatering properties, low oxygen requirements, and use of yeast cells as protein and vitamin source (Kazmi, 2003). Several studies have been conducted in the treatment of high strength wastewaters using different yeast cultures (Malnou, 1987; Moriya *et al.*, 1990; Lee and Baerwald, 1991; Scioli and Vollaro, 1997; Matins, 1999). *Candida tropicalis* and *C. utilis* were popular species for studying wastewater treatment (Lemmel *et al.*, 1979; Azoulay *et al.*, 1980; Elmaleh *et al.*, 1996; Ortiz *et al.*, 1997; Ruiz-Ordaz *et al.*, 1998; Elmaleh *et al.*, 1999; Arnold *et al.*, 2000; Zheng *et al.*, 2001).

Aerobic yeast cultivation was investigated as an option for treating this high strength wastewater containing inhibitory furfural. *Candida tropicalis*, *C. utilis*, and the mixture of two cultures called "mixed cultures" were selected to reduce organic pollutants from the wastewater in parallel lab scale continuous flow reactors. The aim of this study was to determine the effect of different yeast cultures and the required solid retention time (SRT) for effective operating conditions.

3.3 METHODOLOGY

3.3.1 Materials

Influent Wastewater from the furfural production unit of Quaker Oats Company, Cedar Rapids, Iowa, USA was used in this study. Two different grab samples of the wastewater were used as influent. The characteristics of each influent are shown in Table 3.1. The original pH of each influent sample was between 2.0 to 3.5. It was acidified to a pH of below 2.0 by concentrated sulfuric acid for preservation and kept at room temperature.

Yeast Freeze dried cultures of *C. tropicalis* and *C. utilis* were obtained from the American Type Culture Collection. After rehydration over a 24-hour period, the cultures were revived using YM nutrient broth with glucose as a carbon source at 35°C. Revived cultures were transferred to numerous agar plates to keep the cultures for future use.

Nutrients Based on the initial COD, 10% nitrogen using NH_4Cl, 1% phosphorus using KH_2PO_4 were added to influent to maintain a C:N:P ratio of 100:10:1. Some macronutrients for yeasts were added to the influent, i.e. 1 g/L of calcium using $CaCl_2$, and 0.5 g/L magnesium using $MgCl_2.6H_2O$ and it was assumed that other micronutrients were available given the origin of the wastewater from processing natural organic substances.

Reactors Figure 3.1 shows the two reactors, model BIOFLO 2000 fermentors from New Brunswick Scientific, Edison, NJ, USA used in this study. The working volumes were approximately 1.8 L at 35°C. Yeast cultures were inoculated from agar plates to YM nutrient broth in 250 mL flasks and incubated in the shaker at 300 rpm and 35°C for about 2 days. Each culture was introduced into separate reactors containing wastewater diluted with water 1:10. After a few days, yeast cultures were established in the reactors. Undiluted influent was subsequently pumped into the reactors continuously and controlled through a level probe. The reactors were allowed to operate continuously for about two weeks to reach steady state. The pH of the medium was controlled at 4.0 in all experiments using 1 N NaOH. Continuous flow reactors were operated as chemostats i.e., hydraulic retention time (HRT) was equal to the SRT. SRTs of 1.0, 1.3, 1.5, 1.75, and 2.0 days were investigated.

3.3.2 Analytical methods

Samples were collected directly from the reactors at least 3 times for each SRT and culture.

COD, BOD, and VSS concentrations were analyzed according to Standard Methods (1992). The BOD tests were seeded with bacterial biomass obtained from the Boone, Iowa, USA activated sludge municipal wastewater treatment plant aerobically acclimatized to the Quaker Oats wastewater.

VFA concentrations were analyzed by the distillation method according to Standard Methods (1992). In addition, gas chromatography (GC) analysis was conducted for some samples. The distillation method was done for routine analysis. The results of the GC analysis indicated an efficiency factor in the distillation method of 0.5-0.8. Consequently, an efficiency factor of 0.8 was used in the distillation method.

Furfural concentrations were analyzed by high-pressure liquid chromatography (HPLC) using DIONEX resin and distilled water as the mobile phase at 0.5 mL/min. The HPLC comprised an AS40 automated sampler; GP40 gradient pump; and AD20 absorbance detector

that was operated at 280 nm (high intensity). The detector was connected to a personal computer for data interpretation.

Flow cytometry was performed to determine the number of yeasts compared to bacterial cells at an SRT of 1.5 days. Briefly, *Candida* cultures were diluted with a suspension of fluorescent polystyrene beads (6 μm diameter) of a predetermined concentration - usually 2 x 10^6 beads/mL - and then the cell-permeant, fluorescent nucleic acid stain SYTO 9 (Molecular Probes, Eugene, OR, USA) was added to a final concentration of 3.34 mM. SYTO 9-labeled yeast and bacteria are easily differentiated on the basis size (forward scatter) and level of nucleic acid staining (SYTO 9 fluorescence) using flow cytometry. The concentration of yeast versus bacteria in diluted *Candida* cultures was determined by comparing the number of cells in each population to the number of fluorescent polystyrene beads, which are present at a known concentration. Likewise, the average size of yeast was estimated by comparing the mean forward scatter signals of the yeast and 6 μm fluorescent beads. The level of forward scatter signal is directly proportional to cell size. An EPICS ALTRA (Beckman Coulter; Miami, FL, USA) flow cytometer was used in this study.

3.4. RESULTS AND DISCUSSION

The results of VFA by GC analysis of the two grabs of influent and two examples of effluent are shown in Table 3.2. Effluent quality, removal efficiency, yield, and standard deviation from each SRT and culture were calculated and are shown in Table 3.3 and Figures 3.2, 3.3, and 3.4. The results indicated that there were no significant differences among the three cultures used in the treatment of Quaker Oats wastewater. All three cultures could remove organic matter in terms of COD and BOD by about 90% at SRTs of 1.5, 1.75, and 2.0 days. The treatment efficiency was reduced to 78-82% when the SRT was lowered to 1.3 and 1.0 days. However, at a lower SRT, a mixed culture of the two yeasts maintained relatively higher concentrations of biomass. This implies that mixed cultures may have had a slight complementary effect.

All volatile suspended solids (VSS) were accounted for yeast cells because the bacterial contamination was below 0.5% in all cases. This will be discussed later. Yeast biomass concentration was about 3.2-4 g/L at an SRT of 1.5 day or more. With these SRTs, the yield was about 0.24-0.30 gVSS/gCOD removed. Yeast biomass concentration decreased to below 3.0 g/L at SRTs below 1.5 days. The yield coefficient values were also lower at shorter SRT except those of the mixed culture that did not change significantly. The data suggest that biomass wash out started to occur at the SRTs of less than 1.5 day. This SRT is significantly lower than that of a conventional activated sludge process where the SRT typically ranges from 4 to 15 days (Rittmann and McCarty, 2001).

Total VFA accounted for the majority of organic substrate in the wastewater influent. As tabulated in Table 2, approximately 90% or more of the VFA was acetic acid. Data in Figure 3B show that the total VFA content was reduced by all cultures to below 300 mg/L at SRTs of 1.5, 1.75, and 2.0 day – more than 97% removal efficiency. When the SRT was reduced to 1.3 and 1.0 day, the VFA concentration of effluent increased substantially and the VFA removal efficiency dropped to 73-76% and 85-89% at SRTs of 1.0 and 1.3 day, respectively.

Since the pH was controlled at 4.0 during operation, yeast cultures were favored over bacteria. Consequently, bacterial growth and contamination was minimized. All bacterial cell counts were always lower than those of yeast by an order of magnitude as illustrated in Table 4. Volumetric comparisons showed that the bacterial biomass comprised less than 0.5% of the yeast cells.

Diameters of yeast were calculated by relating to reference beads as discussed previously. The diameters of bacteria were lower than the detection limit, 1.0 μm. However to be conservative, it was assumed that all bacterial diameters were 1.0 μm. Volumes of both microorganisms were calculated as spheres from $4.\pi.r^3/3$ multiplied with cell counts. The photographs of *C. tropicalis* and *C. utilis* are shown in Figure 3.5 A and B.

The pH in reactors could be maintained within a very narrow range. This was due to yeast consuming VFA resulting in an increase in pH. However, when acidified influent was pumped into the reactors, the pH in reactors was lowered again and if it was lower than 4.0, 1 N NaOH solution was pumped into the reactors. The NaOH consumption was low: a liter of NaOH lasted for more than a month's continuous operation. Therefore, the reduction in effluent VFA was mainly due to the consumption of VFA by yeasts. This was similar to the findings of Yoon *et al.* (2002). They investigated the pH of aeration tank during wastewater treatment containing acetic acid and reported that the pH could be properly maintained when acidified wastewater was supplied.

3.5 CONCLUSIONS

According to this study, an SRT of 1.5 day is recommended as the operating condition for wastewater treatment using *Candida utilis* or *C. tropicalis*. With this SRT, COD, BOD, and VFA can be removed to 89 and 94, and 97%, respectively, with a yeast biomass yield of 0.24-0.3 g/gCOD at an SRT of 1.5 days or above. Furfural concentrations in effluent were below detection limit at an SRT of 1.5 days and above. Mixed cultures of *C. utilis* and *C. tropicalis* were equally effective in organic removal and able to produce a similar yield of biomass at SRTs down to 1 day.

3.6 ACKNOWLEDGEMENTS

Table 3.1 Characteristics of influent

Influent	Concentration (mg/L)				
taken on	COD	BOD	VSS	VFA	Furfural
[1]10/31/02	17,120 ± 393	12,660 ± 1,800	0	14,480	183
[2]04/04/03	14,550 ± 271	8,750 ± 361	0	11,535	[3]NA

[1]Mixed cultures at SRT 1.75, and 2.0 day, *C. tropicalis* 1.0, and 1.3 day, *C. utilis* 1.0, 1.3, 1.75 and 2.0 day

[2]Mixed cultures at SRT 1.0, 1.3, and 1.5 day, *C. tropicalis* 1.5, 1.75, and 2.0 day, and *C. utilis* 1.5 day

[3]Not analyzed

Table 3.2 Concentration of individual VFA by GC analysis

	Individual VFA concentration (mg/L) of samples from				
Constituents	Influent on 10/31/02	Constituents	Influent on 10/31/02	Constituents	
Acetic acid	12,920	Acetic acid	12,920	Acetic acid	
Propionic acid	180	Propionic acid	180	Propionic acid	
Isobutyric acid	160	Isobutyric acid	160	Isobutyric acid	
n-butyric acid	240	n-butyric acid	240	n-butyric acid	
2-methylbutyric acid	240	2-methylbutyric acid	240	2-methylbutyric acid	
3-methylbutyric acid	220	3-methylbutyric acid	220	3-methylbutyric acid	
n-valeric acid	520	n-valeric acid	520	n-valeric acid	
Total	14,480	Total	14,480	Total	

Table 3.3 Effluent quality from different SRTs and cultures

SRT	Mixed cultures			C. tropicalis			C. utilis		
(day)	COD (mg/L)	STDEV	Rem eff (%)	COD (mg/L)	STDEV	Rem eff (%)	COD (mg/L)	STDEV	Rem eff (%)
1	4590	260	68	5622	386	67	5625	76	67
1.3	3271	50	78	2755	383	84	3114	694	82
1.5	1549	172	89	1553	113	89	1559	184	89
1.75	1297	70	92	1322	36	91	1392	94	92
2	1284	67	93	1309	95	91	1317	40	92
SRT (day)	BOD (mg/L)	STDEV	Rem eff (%)	BOD (mg/L)	STDEV	Rem eff (%)	BOD (mg/L)	STDEV	Rem eff (%)
1	3043	104	65	3650	145	71	3942	160	69
1.3	1572	278	82	1260	124	90	1369	379	89
1.5	489	37	94	505	41	94	497	60	94
1.75	488	52	96	492	19	94	499	85	96
2	376	25	97	375	12	96	372	20	97
SRT (day)	VSS (mg/L)	STDEV	[1]Yield	VSS (mg/L)	STDEV	[1]Yield	VSS (mg/L)	STDEV	[1]Yield
1	2551	79	0.26	1473	201	0.13	1807	161	0.16
1.3	2714	65	0.24	1687	548	0.12	2888	249	0.21
1.5	3243	263	0.25	3235	81	0.25	3229	336	0.25
1.75	4129	93	0.26	3917	53	0.3	3810	81	0.24
2	4063	51	0.26	3971	343	0.3	3929	84	0.25
SRT (day)	VFA (mg/L)	STDEV	Rem eff (%)	VFA (mg/L)	STDEV	Rem eff (%)	VFA (mg/L)	STDEV	Rem eff (%)
1	2720	139	76	3857	213	73	3883	150	73
1.3	1783	417	85	1647	418	89	2067	424	86
1.5	297	40	97	269	26	98	263	20	98
1.75	217	40	99	280	65	98	217	40	99
2	229	53	98	274	17	98	217	87	99

[1]yield in g VSS/gCOD removed

Table 3.4 Cell count by flow cytometry at SRT 1.5 day

Reactor operation at SRT 1.5 day	Culture	Cell count per mL sample	Cell count (%)	Average cell diam. (μm)	Volume (μm^3)	Volume (%)
Mixed	yeast	2.57×10^8	91.33	3.23	4.54×10^9	99.72
cultures	bacteria	2.44×10^7	8.67	1	1.28×10^7	0.28
C. tropicalis	yeast	2.13×10^8	88.31	4	7.14×10^9	99.79
	bacteria	2.82×10^7	11.69	1	1.48×10^7	0.21
C. utilis	yeast	1.80×10^8	76.6	4	6.03×10^9	99.52
	bacteria	5.50×10^7	23.4	1	2.88×10^7	0.48
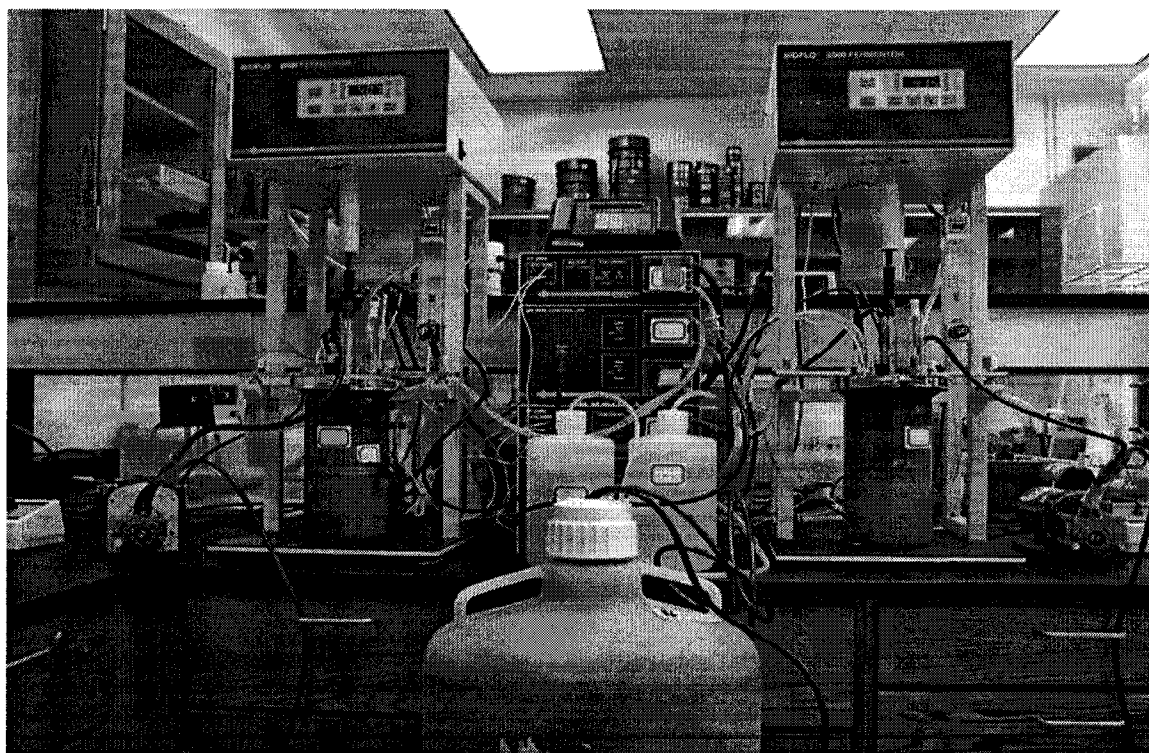
Figure 3.1 Experimental set-up of continuous flow reactors
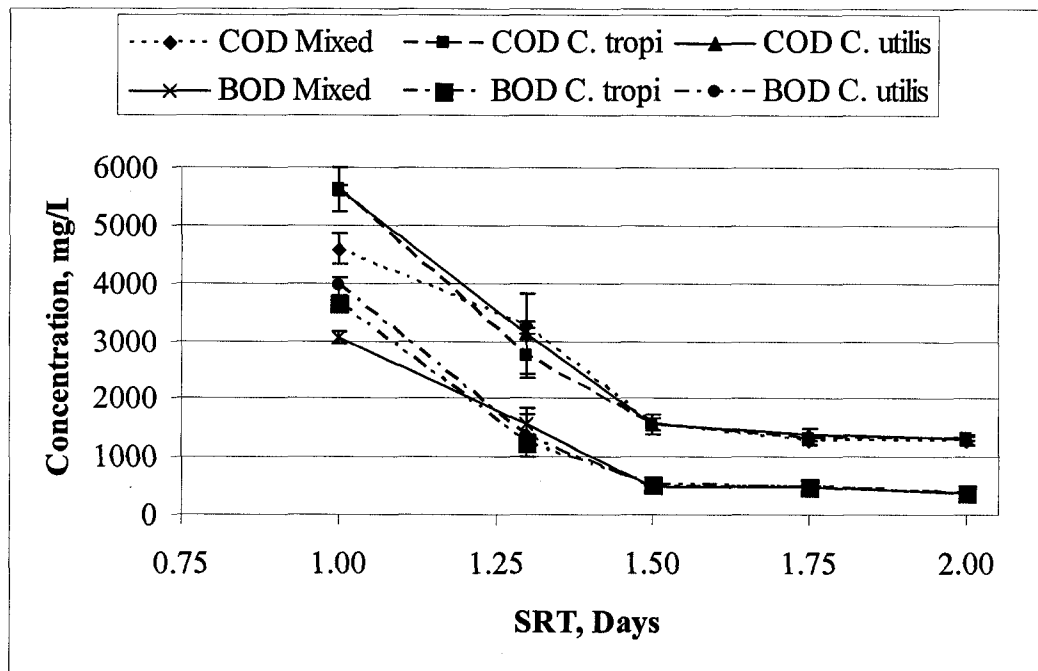
Figure 3.2 Effect of SRT on reactor performance for COD and BOD
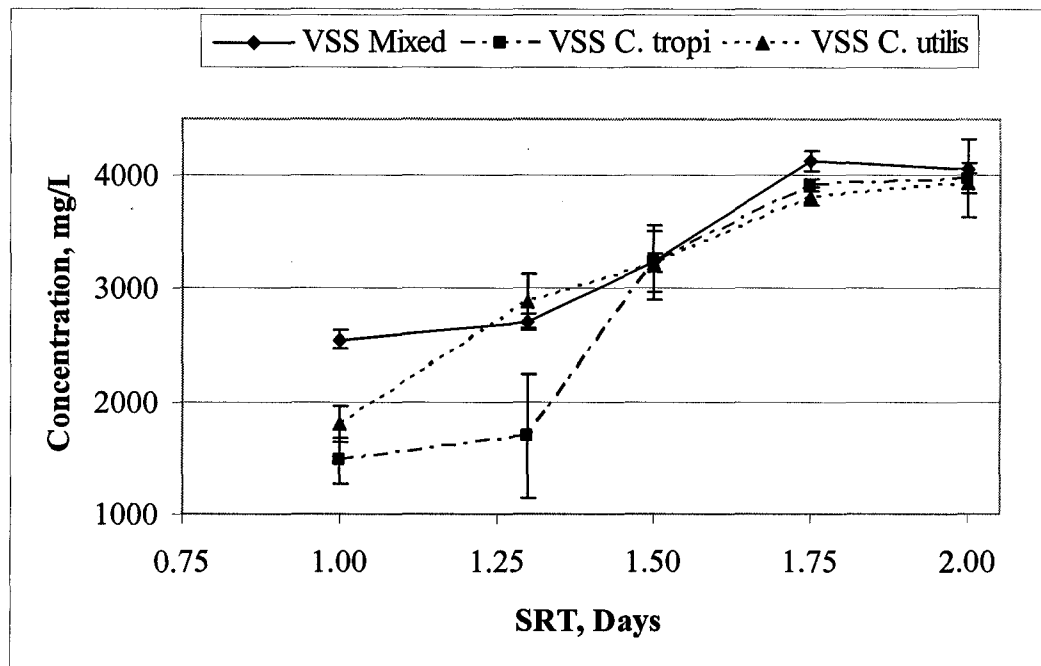
Figure 3.3 Effect of SRT on reactor performance for VSS
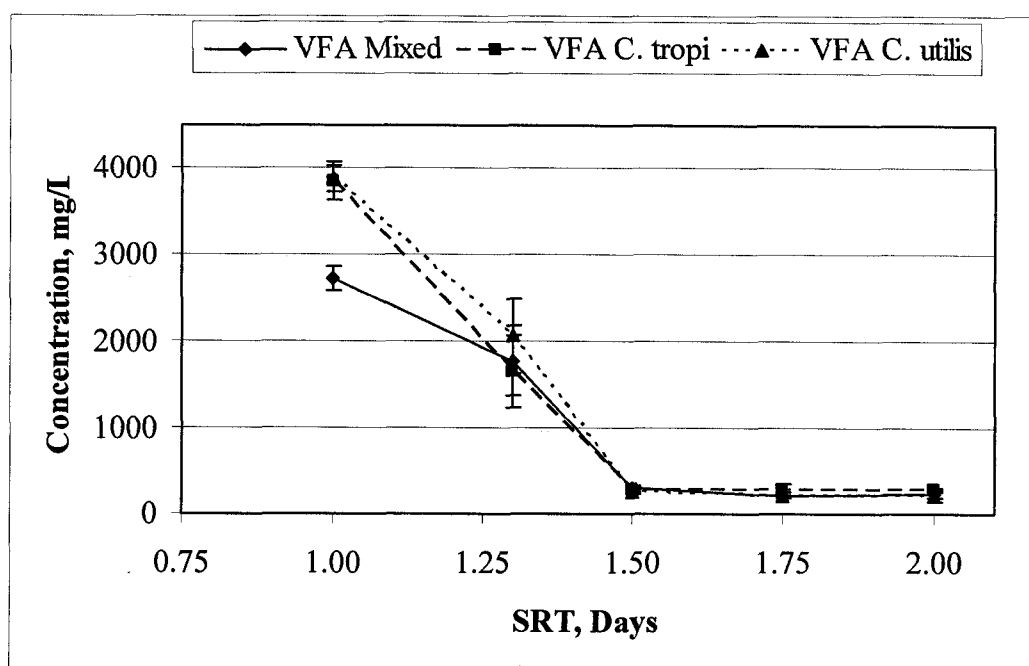
Figure 3.4 Effect of SRT on reactor performance for VFA

(A) *C. tropicalis*   (B) *C. utilis*
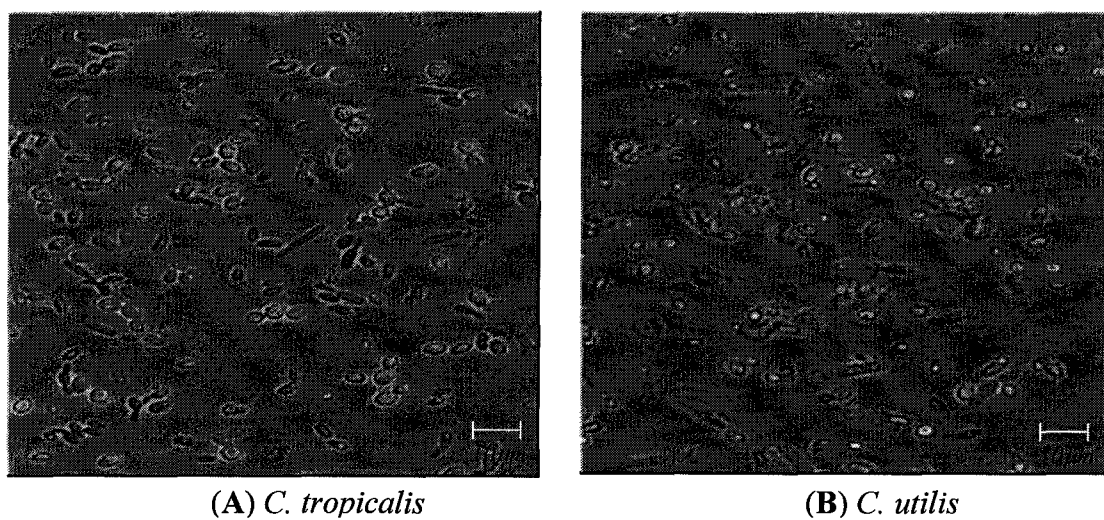
Figure 3.5 Microscopic examination of yeast cells operating at SRT of 1.5 days

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CHAPTER 4 PRODUCTION OF AEROBIC YEAST FROM FOOD PROCESSING WASTEWATER

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4.1 ABSTRACT

High organic content of food processing wastewater from the furfural production unit at the Quaker Oat Company in Cedar Rapids, Iowa USA was treated using the aerobic yeast: *Candida tropicalis* and *C. utilis*. They were cultured on the wastewater in two separate laboratory-scale continuous fermentors. The previous study found that the minimum solids retention time (SRT) of 1.5 days was optimal in terms of COD removal and prevention of bacterial contamination. In this study, the effect of temperature on COD removal and biomass yield was investigated over a range of 25 to 40°C. The sludge retention time was controlled at 1.5 days and the pH at 4 or 4.5 using 1 N NaOH. The results showed COD removal efficiencies over 90% at all temperatures. The highest biomass yield of 0.31 g biomass/g COD removed was achieved at 30°C.

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4.2 INTRODUCTION

Food processing industries typically produce large quantities of high strength organic wastewater, which is often subjected to anaerobic pretreatment. However, the wastewater from the Quaker Oats Company located in Cedar Rapids, Iowa USA contained furfural, which was found to be toxic to anaerobic microorganisms (Koh and Ellis, 2002). Furfural was a by-product of the further processing of oats hulls to make furfural. The wastewater was discharged to the central municipal wastewater facility subject to a considerable surcharge due to the organic loading. The average wastewater flow rate was about 565 m^3/day, and the range of COD varied from 10,000 to 20,000 mg/L including 100 to 2,000 mg/L of furfural.

Taherzadeh *et al.* (1999) concluded in their study that the toxicity of furfural could be reduced to less harmful furfuryl alcohol by certain yeast. Many advantages of using yeast in wastewater treatment include their ability to cope with high strength organic matter from food processing industries, the direct decomposition of oils, their ability to deal with high organic and suspended solids loading, sludge generation reduction, good settling and dewatering properties, low oxygen requirements, and production of yeast cells as protein and vitamin source (Kazmi, 2003). Eliosov *et al.* (2002) stated that yeast and other fungi are suitable microorganisms for conversion of waste organic matter into single cell protein. By selling these proteins as animal feed, it can compensate for the high cost of wastewater treatment. Fish-processing wastewater as a growth medium for *Candida rugopelliculosa* was studied by Lim *et al.*(2003). The results showed that *C. rugopelliculosa* removed 70 % of the soluble chemical oxygen demand (SCOD) from influent and stimulated the growth of the commercially produced rotifer, *Brachionus plicatilis*, by 18.3 % compared to their normal diet of *Saccharomyces cerevisiae*. Shimada *et al.* (1998) cited that *C. utilis* could be cultured on large scale as a promising source of single cell protein as well as a host for the production of many chemicals such as glutathione and RNA. *C. utilis* is also a safe and

industrially important yeast approved by the U.S. Food and Drug Administration. *C. tropicalis* has the ability to grow directly on soluble starch, corn, and cassava powders since it can produce an enzyme for hydrolyzing starch, α–amylase. Thus, addition of yeast could increase the protein in animal feed from 10% (using corn) or from 3% (using cassava) to up to 20% (Azoulay, 1980). Ruiz-Ordaz *et al.* (1998) found that *C. tropicalis* has the ability to degrade and utilize phenol up to a concentration of 2.5 g/L as a sole carbon and energy source. Hua *et al.* (2003) studied *C. antarctica* cultured on n-undecane as substrate to produce biosurfactant. The result demonstrated the significant improvement in biodegradation of hydrocarbons due to the biosurfactant produced by this yeast. Bacterial competition within the yeast bioreactor was found to be a problem during continuous yeast production. The maximum specific growth rates, μ_{max}, of *C. utilis* and bacteria were 0.13 h^{-1} and 0.03 h^{-1} and the value of half-saturation coefficient for substrate, K_s, were 160 mg COD/L and 12 mg COD/L respectively at a pH 4.5 (Eliosov *et al.*, 2002). These results also showed that when substrate concentrations were higher than 30 mg COD/L, yeast would outgrow bacteria. The optimum SRT for the continuous treatment of furfural-based wastewater using *C. tropicalis*, *C. utilis*, and the mixture of these yeast was found to be 1.5 days (Wongkarnka *et al.*, 2003). Batch test studies using Quaker Oats wastewater were performed in this paper to evaluate kinetic parameters for *Candida utilis* and mixed cultures of bacteria.

4.3 METHODOLOGY

4.3.1 Materials

Influent Wastewater obtained from the furfural production unit of Quaker Oats Company, Cedar Rapids, Iowa, USA was used in this study as influent. The characteristics of the influent are shown in Table 4.1. The original pH of the influent sample was 2.3. It was acidified to a pH of below 2.0 by concentrated sulfuric acid for preservation and kept at room temperature. It can obviously be seen that most of the organic matter from the influent is mainly volatile fatty acid, especially acetic acid.

Reactors Figure 4.1 shows the two reactors, model BIOFLO 2000 fermentors by New Brunswick Scientific, Edison NJ, USA used in this study. The working volumes were approximately 1.8 L each.

Nutrients The influent contained very low nutrients as shown in Table 4.1. Therefore, based on the initial COD, NH_4Cl and KH_2PO_4 were added to influent to maintain a C:N:P ratio of 100:10:1. Some macronutrients for yeast were added to the influent, i.e. 1 g/L of calcium using $CaCl_2$, and 0.5 g/L magnesium using $MgCl_2.6H_2O$. It was assumed that other micronutrients were available from the origin of the wastewater.

Yeast cultures Freeze-dried cultures of *C. tropicalis* and *C. utilis* were obtained from the American Type Culture Collection with the ATCC number of 750 and 9226, respectively. After rehydration over a 24-hour period, the cultures were revived using YM nutrient broth with glucose as a carbon source at 35°C. Revived cultures were transferred to numerous agar plates to keep the cultures for future use.

Start up Yeast cultures were inoculated from agar plates to YM nutrient broth in 250 mL flasks and incubated in the shaker at 300 rpm and 35°C for about 2 days. Each culture was put into separate reactors containing wastewater diluted with water 1:10. After a few days, two yeast cultures were established in each reactor. Undiluted influent was subsequently pumped continuously into the reactors controlled through a level probe. The reactors were allowed to operate continuously for about two weeks to reach steady state.

The effect of temperature on yeast and bacterial kinetics Continuous flow reactors were operated as chemostats i.e., hydraulic retention time (HRT) was equal to the SRT. The SRT was maintained at 1.5 days and the pH controlled at 4 using 1 N NaOH to study the effect of temperature on yeast production and COD removal. The temperature effects were studied at 25, 30, 35, and 40°C.

4.3.2 Analytical methods

Samples were collected directly from the reactors at least 3 times during operation at each temperature. After a temperature change, the reactors were operated for at least 5 days, i.e. more than 3 times the SRT.

COD, BOD, and VSS were analyzed according to Standard Methods (1992). The BOD tests were seeded with bacterial biomass obtained from the Boone municipal wastewater treatment plant aerobically acclimatized to the Quaker Oats wastewater.

VFA was analyzed by the distillation method according to Standard Methods (1992). In addition, gas chromatography (GC) analysis was conducted for some samples. The distillation method was done for routine analysis. The results of the GC analysis indicated an efficiency factor in the distillation method of 0.5-0.8. Consequently for the worst case, an efficiency factor of 0.8 was used in the distillation method.

Furfural was analyzed by high-pressure liquid chromatography (HPLC) using DIONEX resin. The mixture of 1/4 by volume of distilled water/methanol was used as the mobile phase at 0.5 mL/min. The HPLC comprised an AS40 automated sampler, GP40 gradient pump and AD20 absorbance detector operated at 280 nm (high intensity). The detector was connected to a personal computer for data interpretation.

Flow cytometry was performed to determine the number of yeast compared to bacterial cells in each stage. Briefly, *Candida* cultures were diluted with a suspension of fluorescent polystyrene beads (6 μm diameter) of a predetermined concentration -usually $2x10^6$ beads/mL- and then the cell-permeant, fluorescent nucleic acid stain SYTO 9 (Molecular Probes, Eugene, OR, USA) was added to a final concentration of $3.34x10^{-3}$ mol. SYTO 9-labeled yeast and bacteria are easily differentiated on the basis of size (forward scatter) and level of nucleic acid staining (SYTO 9 fluorescence) using flow cytometry. The concentration of yeast versus bacteria in diluted *Candida* cultures was determined by comparing the number of cells in each population to the number of fluorescent polystyrene

beads, which are present at a known concentration. Likewise, the average size of yeast cells was estimated by comparing the mean forward scatter signals of the yeast and 6 μm fluorescent beads. The level of forward scatter signal is directly proportional to cell size. An EPICS ALTRA (Beckman Coulter; Miami, FL, USA) flow cytometer was used in this study.

4.4 RESULTS AND DISCUSSION

The reactors were operated at an SRT of 1.5 day and the pH controlled at about 4.0 while the temperatures were varied at 25, 30, 35, and 40°C. The results are shown in Table 4.2 and 4.3. In addition, Figure 4.2, 4.3, and 4.4 illustrates the effect of temperature on reactor performance for COD and BOD, VSS, and VFA respectively.

It can be seen that COD and BOD removal efficiency of more than 90% were obtained when the reactors were operated at 25, 30 and 35°C and it dropped slightly at a temperature of 40°C. The reactors were also operated at 45°C but this resulted in the die-off of the yeast culture. It can be verified that temperatures higher than 35°C are not suitable for mesophilic yeast cultivatuin. Ahmad and Holland. (1995) explained that temperatures above 34°C had adverse effects on the metabolism of the cells. The results from continuous laboratory scale corresponded to those on batch scale where the optimum temperature for *C. tropicalis* and *C. utilis* were 35 and 33°C, respectively (Kasper, 2004). The optimum temperature for *C. utilis* and *Saccharomycopsis fibuliger* was 32°C in the study of the production of these yeast from potato processing wastewater (Lemmel *et al.*, 1979).

Arnold *et al.* (2000) studied the use of *C. utilis* and *Galactomyces geotrichum* to reduce the pollution from silage effluent with no temperature control. COD removal of 91-95% was achieved when the original influent of 16,410 mg/L COD was diluted with water 1:4. Zheng *et al.* (2001) used 5 yeast species as a mixed culture including *C. tropicalis* and *C. utilis* to treat salad oil manufacturing wastewater at a temperature of 30°C. The COD removal efficiency was up to 93%. Yeast other than *C. tropicalis* and *C. utilis* were also studied. The

yeast *Yarrowia lipolytica* was used to treat olive mill wastewaters at a temperature of 25°C resulting in COD was removalof 80% (Scioli *et al.*, 1997).

Maximum VSS or yeast biomass up to 4.1 and 4.3 g/L for *C. tropicalis* and *C. utilis* when the reactors were run at 30°C. Arnold *et al.* (2000) accomplished a yeast biomass concentration of *C. utilis* of up to 7 g/L in their experiment. It is possible that wastewater that contained no inhibitory compound like furfural was used in this study. Scioli *et al.* (1997) attained a yeast biomass of *Y. lipolytica* in their study of up to 22.45 g/L. The biomass yield was highest at an operating temperature of 30°C for both *C. tropicalis* and *C. utilis* at 0.31 and 0.32 gVSS/gCOD removed, respectively.

The effluent quality for VFA was found to be lower than 200 mg/L and a removal efficiency of 98-99% was achieved in all cases. The result is equivalent to a removal efficiency in terms of TOC at 97% was achieved by the experiment using *C. utilis* as the biomass and acetic acid or propionic acid or butyric acid or a mixture of these acids as the main carbon source (Elmaleh *et al.*, 1996; Elmaleh *et al.*, 1999). Arnold *et al.* (2000) achieved 90-100% in VFA removal.

The pH in the reactors could be maintained within a very narrow range. This was due to the yeast consuming VFA resulting in an increase in pH. However, when acidified influent was pumped into the reactors, the pH in the reactors was lowered again and if it was lower than 4.0, 1 N NaOH solution was pumped into the reactors. The NaOH consumption was low: a liter of NaOH lasted for more than a month's continuous operation. Therefore, the reduction in effluent VFA was mainly due to the consumption of VFA by yeast. This was similar to the findings of Yoon *et al.*(2002). They investigated the pH of aeration tank during wastewater treatment containing acetic acid and reported that the pH could be properly maintained when acidified wastewater was supplied.

Furfural analysis was carried out when the reactors were operated at a temperature of 30°C and pH 4.0. The effluent concentration was found to be 46 and 49 mg/L and the removal

efficiency of 76 and 75% for *C. tropicalis* and *C. utilis*, respectively. However, the almond-like smell of furfural has never been noticed in the effluent, only yeast smell, althoughthe almond-like smell was very strong in the influent. It is possible that the effluent furfural concentration might be due to the derivatives of furfural that cannot be detected by smell. From Table 4.3 and Figure 4.5, flow cytometry analysis shows that yeast cultures are the dominant population in all cases. This is because the pH was controlled at 4.0 during operation that favors yeast over bacteria. Diameters of yeast were calculated by relating to reference beads as discussed previously. The size of yeast in this study is similar to *C. antarctica* at about 5 μm (Hua *et al*, 2003). The diameters of bacteria were lower than the detection limit, 1.0 μm, however as a worst case, it was assumed that all bacterial diameters were 1.0 μm. Volumes of both microorganisms were calculated as spheres from $4/3.\pi.r^3$ multiplied with the number of cells.

Volumetric comparisons showed that the bacterial contamination was less than 0.5% in all cases when reactors were operated at 25, 30, and 35°C. The results also show that the maximum bacterial contamination at these operations was only 0.22 %. The bacterial contamination was higher, up to 4.5% at an operating temperature of 40°C.

Furthermore, the number of bacterial and yeast cell per milliliter of sample was found to be very close each other at 40°C. Bacterial cell counts were always lower than those of yeast by an order of magnitude at lower temperatures. Bacterial biomass at 2-9 mg/L contaminated the yeast biomass at an operating temperature of 30°C but this increased to 125 mg/L when the temperature was increased to 40°C.

4.5 CONCLUSIONS

Organic matter measured as COD, BOD, and VFA could be reduced by over 90% at all temperatures. The removal efficiency decreased and higher bacterial contamination was found when the reactors were operated at higher temperatures (Provide range!). However, comparing biomass generation at different temperatures, biomass yield dropped at

operational temperatures of 25 and 40°C. The highest biomass yield was achieved at a temperature of 30°C. Therefore, a temperature of 30°C and an SRT of 1.5 days will be used for further studies on the effect of pH on yeast production performance.

4.6 ACKNOWLEDGEMENTS

Table 4.1 Characteristics of influent

Constituent	Concentration, mg/L
COD	14,550 ± 271
BOD	8,750 ± 361
VSS	0
Furfural[1]	193 ± 30
Total VFA	11,535
-Acetic acid	11,500
-Propionic acid	35
-Butyric acid	‹1
TKN as N	6.6
Phosphate as P	6.7

[1]The influent was a different grab sample but very close in COD concentration of 14,510 ± 62 mg/L.

Table 4.2 Effluent quality at different temperatures

Temp	*C. tropicalis*		*C. utilis*		*C. tropicalis*		*C. utilis*	
	COD	Rem eff	COD	Rem eff	BOD	Rem eff	BOD	Rem eff
(°C)	(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)
25	972±162	93±1	878±354	94±2	337±65	96±1	244±156	97±2
30	1255±38	91±0	1125±223	92±2	413±98	95±1	268±124	97±1
35	1381±181	91±1	1363±129	91±1	471±150	95±2	412±76	95±1
40	1399±168	90±1	1462±347	90±2	581±194	93±2	635±317	93±4

Temp	*C. tropicalis*		*C. utilis*		*C. tropicalis*		*C. utilis*	
	VSS		VSS		VFA	Rem eff	VFA	Rem eff
(°C)	(mg/L)	[1]Yield	(mg/L)	[1]Yield	(mg/L)	(%)	(mg/L)	(%)
25	3806±349	0.28±0.02	3521±359	0.26±0.02	157±71	99±1	199±70	98±1
30	4148±225	0.31±0.02	4305±298	0.32±0.02	126±65	99±1	97±38	99±0
35	3874±160	0.29±0.01	4046±84	0.31±0.01	183±70	98±1	149±63	99±1
40	2788±348	0.21±0.03	2796±639	0.21±0.04	107±30	99±0	184±74	98±1

[1]Yield in gVSS/gCOD removed

Table 4.3 Cell count by flow cytometry at different temperatures

Temp (°C)	Main microorganism	Culture	Cell count per mL sample	Cell Count (%)	Average cell diameter (μm)	Volume (μm)^3	Volume (%)	VSS (mg)
	C. tropicalis	yeast	1.09×10^8	83.85	6.0	1.23×10^{10}	99.91	3803
25		bacteria	2.10×10^7	16.15	1.0	1.10×10^7	0.09	3
	C. utilis	yeast	1.25×10^8	85.62	4.5	5.97×10^9	99.82	3515
		bacteria	2.1×10^7	14.38	1.0	1.10×10^7	0.18	6
	C. tropicalis	yeast	3.40×10^8	87.40	4.6	1.73×10^{10}	99.85	4142
30		bacteria	4.90×10^7	12.60	1.0	2.57×10^7	0.15	6
	C. utilis	yeast	4.50×10^8	94.94	3.2	7.72×10^9	99.84	4298
		bacteria	2.40×10^7	5.06	1.0	1.26×10^7	0.16	7
	C. tropicalis	yeast	1.12×10^8	89.60	6.5	1.61×10^{10}	99.96	3872
35		bacteria	1.30×10^7	10.40	1.0	6.81×10^6	0.04	2
	C. utilis	yeast	3.40×10^8	87.86	4.0	1.14×10^{10}	99.78	4037
		bacteria	4.70×10^7	12.14	1.0	2.46×10^7	0.22	9
	C. tropicalis	yeast	9.28×10^8	57.68	2.5	7.60×10^9	95.51	2663
40		bacteria	6.81×10^8	42.32	1.0	3.57×10^8	4.49	125
	C. utilis	yeast	3.64×10^8	53.85	3.0	5.15×10^9	96.92	2710
		bacteria	3.12×10^8	46.15	1.0	1.63×10^8	3.08	86
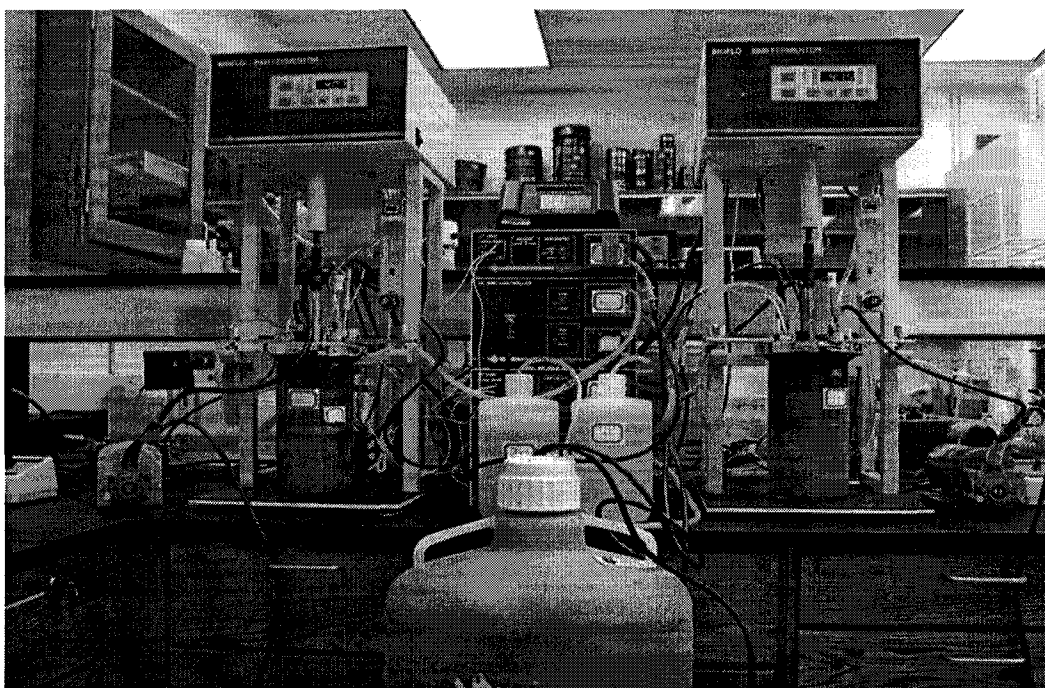
Figure 4.1 Experimental set-up of continuous flow reactors: effect of temperatures
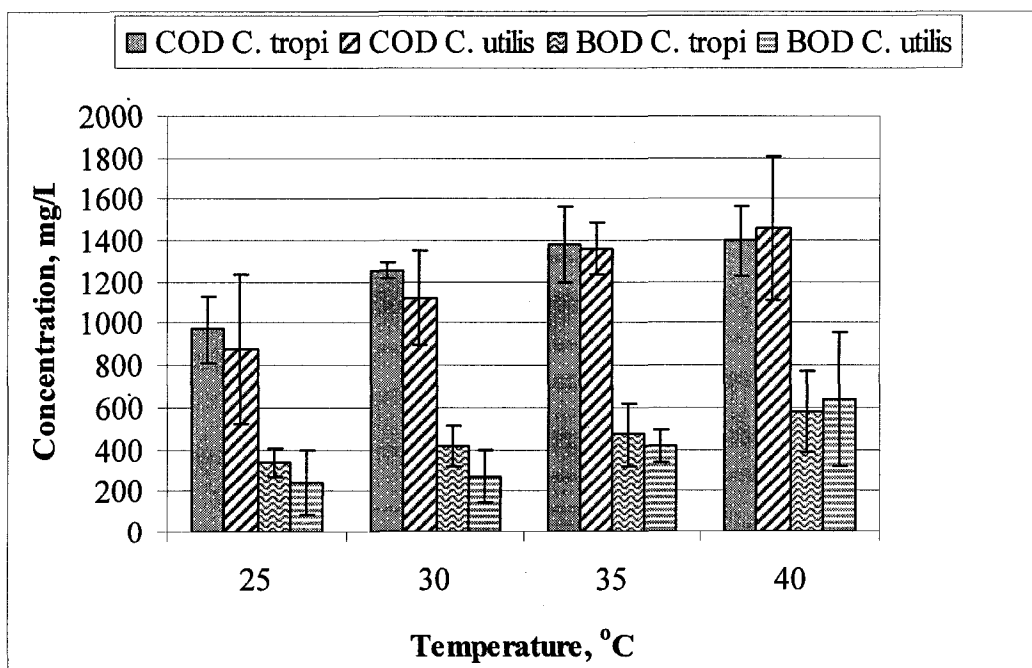
Figure 4.2 Effect of temperature on reactor performance for COD and BOD
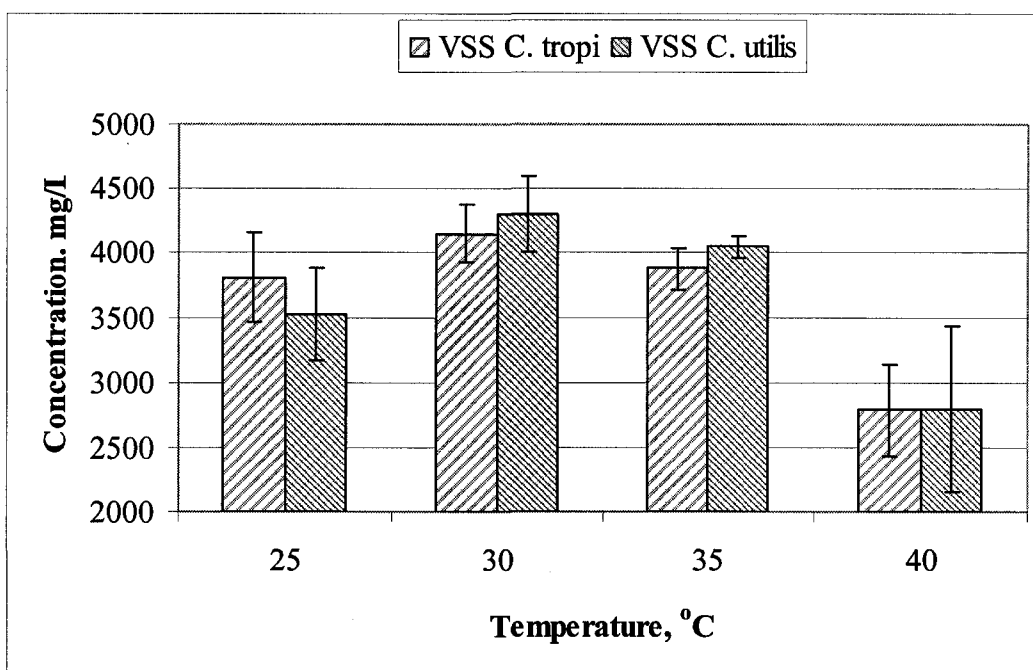
Figure 4.3 Effect of temperature on reactor performance for VSS
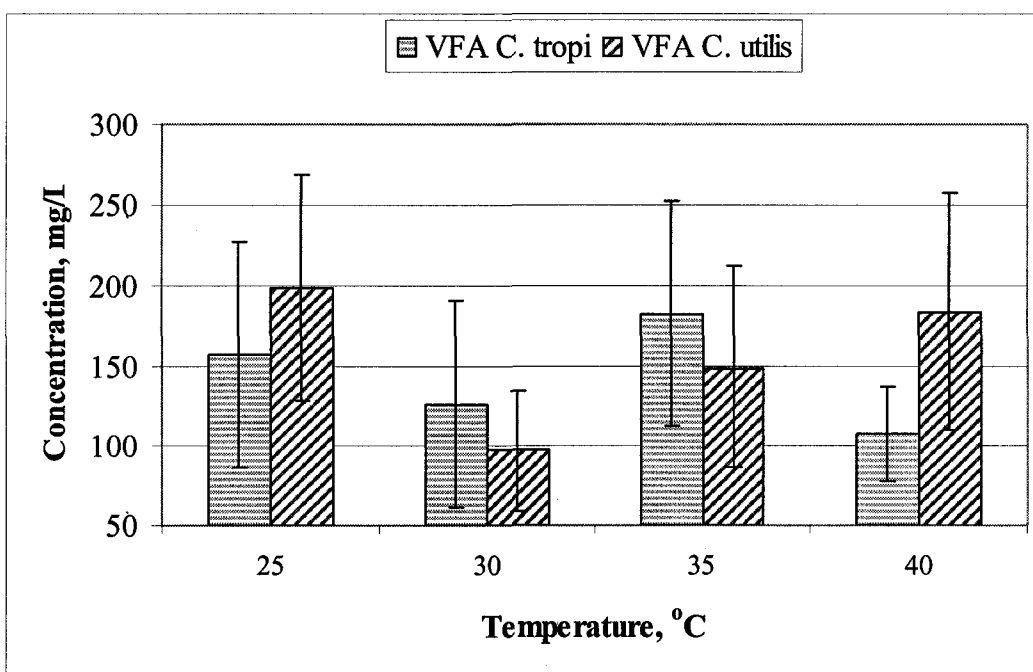
Figure 4.4 Effect of temperature on reactor performance for VFA
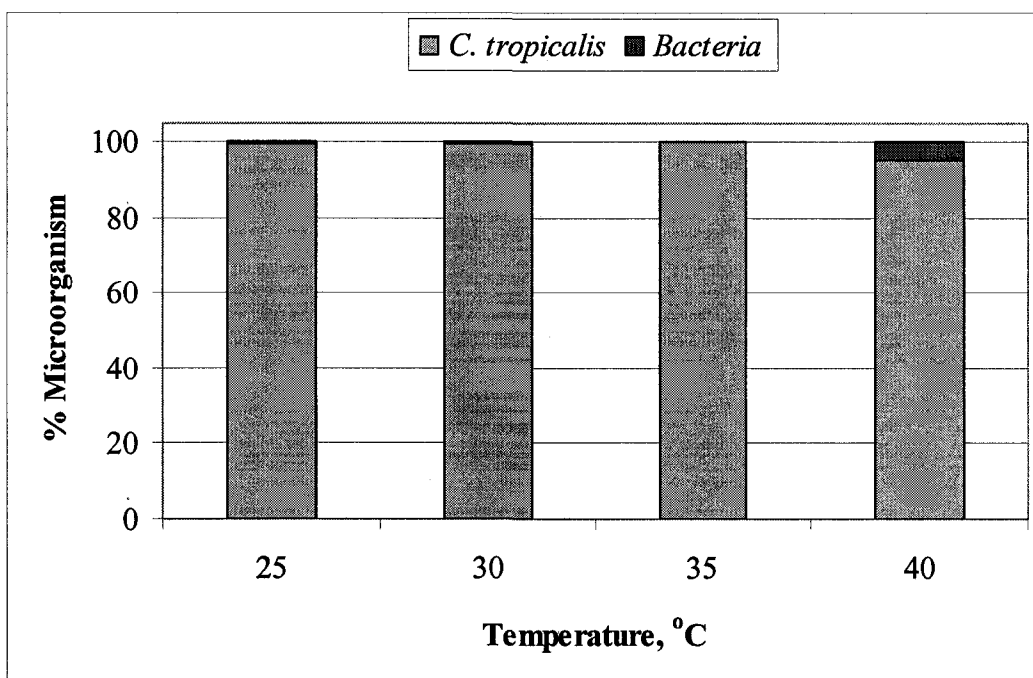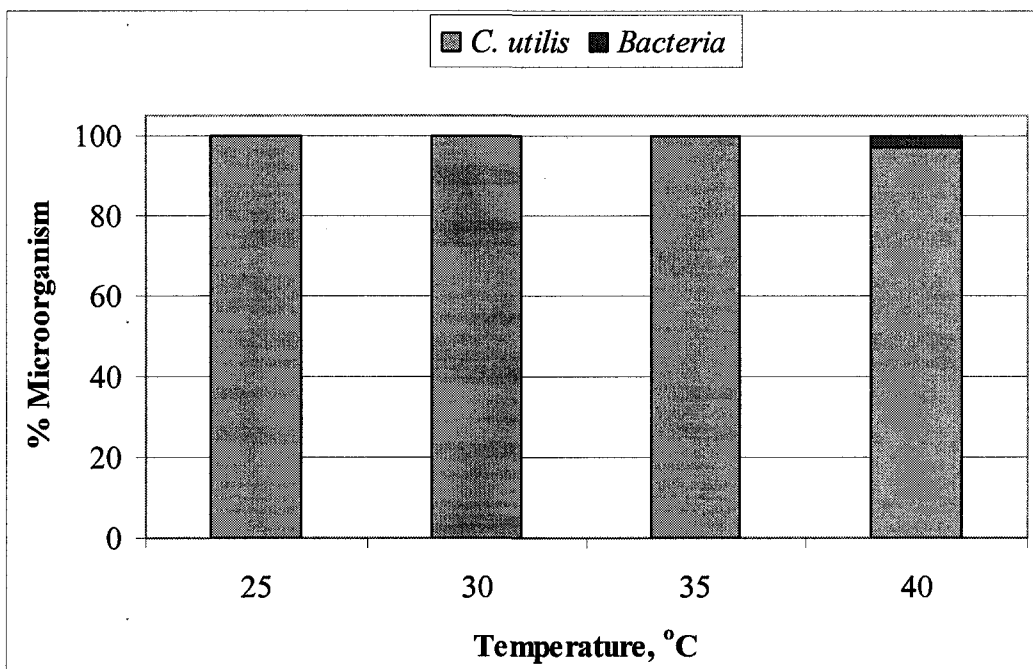
(A) *C. tropicalis*

(B) *C. utilis*

Figure 4.5 Distribution of yeast and bacteria at different temperature (A) *C. tropicalis* and (B) *C. utilis*

4.7 REFERENCES

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CHAPTER 6 GENERAL CONCLUSIONS

6.1 CONCLUSIONS

6.1.1 Preliminary study

Batch test studies indicated that furfural production wastewater can be treated effectively by aerobic yeast cultures to produce high quality microbial biomass. Up to 92-95% COD removal by all three yeast, *Candida guillermondii*, *C. tropicalis*, and *C. utilis*, was obtained and complete furfural degradation could be achieved in a single stage process. At pH 4.5, the maximum specific growth rate, μ_{max}, of *C. utilis* and bacteria were 0.13 h^{-1} and 0.03 h^{-1} and the value of half-saturation coefficients for substrate, K_s, were 160 mg COD/L and 12 mg COD/L, respectively. Furthermore, when substrate concentrations were higher than 30 mg COD/L, yeast grew faster than bacteria.

Continuous laboratory scale experiments were also done to verify all kinetic parameters from the batch tests. The predicted SRT for maintaining yeast over bacterial cultures was equal to or less than 2 days. Microscopic observation illustrated that yeast, *C. utilis*, was the main component of biomass when the reactor was run continuously over a period of 1 year at an SRT of 2 d and a pH of 4.5. Effluent quality prediction using kinetic parameters was verified in continuous tests as well. The results indicated that effluent COD was significantly higher than predicted using the measured values of kinetic parameters and was independent of the SRT in the range of 1 to 2 d. This might possibly be due to formation of soluble microbial products (SMP) associated with substrate biodegradation which would increase the effluent COD concentration.

6.1.2 Effect of SRT and yeast species on reactor performance

All three yeast cultures, *C. tropicalis*, *C. utilis*, and mixed cultures of these two yeast, had the same degree of ability to treat furfural-based wastewater at all SRTs. The results showed that SRTs of 1.5, 1.75, and 2.0 days were about the same in terms of treatment efficiency and

biomass yield. Therefore, an SRT of 1.5 days is recommended as the operating condition for wastewater treatment using *Candida utilis* or *C. tropicalis*. With this SRT, COD, BOD, and VFA can be removed to 89, 94, and 97%, respectively, with a yeast biomass yield of 0.24-0.3 g/gCOD removed at an SRT of 1.5 days or above. Furfural concentrations in the effluent were below the detection limit at an SRT of 1.5 days and above, and the volumetric bacterial contamination was below 0.5% in all three reactors.

6.1.3 Effect of temperature on yeast performance

C. tropicalis and *C. utilis* had the same ability to treat the wastewater at all operation temperatures studied. A temperature of $30^{\circ}C$ was found to be optimal in terms of removal efficiency, yeast biomass yield, and prevention of bacterial contamination. With this operational temperature, COD, BOD, and VFA could be removed to at least 91, 95, and 99%, respectively with a yeast biomass yield of 0.31-0.32 g/gCOD removed. The bacterial contamination was less than 0.2% when reactors were operated at this temperature.

6.1.4 Effect of pH on yeast performance

Both cultures, *C. tropicalis* and *C. utilis* could treat the wastewater to the same degree for all pH conditions. The higher pH operation resulted in higher removal efficiency. However, at pH 5.0 and 5.5, the contamination from bacteria was found to be up to 40%. Therefore, the optimum pH for yeast performance is recommended at 4.0-4.5. At these pH values, COD, BOD, VFA, and furfural were removed up to 93, 97, 98, and 79% respectively with a yeast biomass yield up to 0.22 g/gCOD removed. Bacterial contamination was less than 0.1% at a pH 4.0 and 1% at pH 4.5.

6.2 RECCOMMENDATIONS FOR FUTURE RESEARCH

- Other treatment systems such as airlift suspension and sequencing batch reactors (SBR) should be studied to compare the treatment efficiency, biomass yield, and bacterial contamination.

- Biomass separation should be studied further to ascertain the requirements for harvesting prior to sale as animal feed.

- Furfural concentration in dry yeast biomass should be investigated because it is toxic to both animal and human.

- Since yeast contains protein up to 50% as dry mass basis, the effect of nitrogen concentration in wastewater on single cell protein quality should be studied further.

6.3 ENGINEERING SIGNIFICANCE

An SRT of 1.5 day, temperature of 30-35°C, and pH of 4.0-4.5 were recommended for the application in wastewater treatment using aerobic yeast either *C. tropicalis* or *C. utilis* in this study. This could be applied to any wastewater with an organic content of more than 30 mg/L COD as yeast can grow faster than bacteria. Especially when anaerobic treatment cannot be used for wastewater containing inhibitory compounds. Aerobic yeast treatment is the best option for this kind of wastewater because yeast are often more tolerant to toxic compounds than bacteria.

APPENDIX

APPENDIX A: ADDITION YEAST CULTIVATION DATA

Table A-1 Raw data of the effluent quality for different SRTs (mixed cultures)

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent mixed cultures (mg/L) COD	BOD	VSS	VFA	Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent mixed cultures (mg/L) COD	BOD	VSS	VFA
04/01/03	1.75	4.0	35.0	1337	535	4050	240	04/08/03	2.0	4.0	35.0	1326	403	4116	240
04/02/03	1.75	4.0	35.0	1339	497	4232	171	04/09/03	2.0	4.0	35.0	1319	370	4060	274
04/03/03	1.75	4.0	35.0	1216	433	4104	240	04/12/03	2.0	4.0	35.0	1206	354	4014	171

Note: Wastewater from Quaker Oats received on Thursday, October 31, 2002

COD = 17,120±393 mg/L, BOD = 12,660±1,800 mg/L, total VFA = 14,480 mg/L, and furfural = 180 mg/L

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent mixed cultures (mg/L) COD	BOD	VSS	VFA	Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent mixed cultures (mg/L) COD	BOD	VSS	VFA
04/17/03	1.5	4.0	35.0	1355	447	3542	274	04/24/03	1.3	4.0	35.0	3237	1876	2664	1303
04/18/03	1.5	4.0	35.0	1682	501	3144	343	04/25/03	1.3	4.0	35.0	3247	1509	2788	2057
04/19/03	1.5	4.0	35.0	1610	519	3044	274	04/26/03	1.3	4.0	35.0	3328	1331	2690	1989
05/01/03	1.0	4.0	35.0	4334	3150	2642	2743								
05/02/03	1.0	4.0	35.0	4582	2943	2512	2571								
05/03/03	1.0	4.0	35.0	4854	3035	2498	2846								

Note: Wastewater from Quaker Oats received on Friday, April 4, 2003

COD = 14,550±271 mg/L, BOD5 = 8,750±361 mg/L, and total VFA = 11,535 mg/L

Table A-2 Raw data of the effluent quality for different SRTs (*Candida tropicalis*)

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. tropicalis* (mg/L) COD	BOD	VSS	VFA	Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. tropicalis* (mg/L) COD	BOD	VSS	VFA
01/04/03	1.3	4.0	35.0	3197	1384	1952	2116	02/02/03	1.0	4.0	35.0	6063	3794	1556	4100
01/07/03	1.3	4.0	35.0	2534	1136	2052	1514	02/03/03	1.0	4.0	35.0	5459	3650	1620	3770
01/13/03	1.3	4.0	35.0	2534	1260	1056	1312	02/04/03	1.0	4.0	35.0	5344	3505	1244	3702

Note: Wastewater from Quaker Oats received on Thursday, October 31, 2002

COD = 17,120±393 mg/L, BOD = 12,660±1,800 mg/L, total VFA = 14,480 mg/L, and furfural = 180 mg/L

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. tropicalis* (mg/L) COD	BOD	VSS	VFA	Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. tropicalis* (mg/L) COD	BOD	VSS	VFA
05/28/03	2.0	4.0	35.0	1415	389	4014	274	06/04/03	1.75	4.0	35.0	1352	514	3934	326
05/29/03	2.0	4.0	35.0	1281	370	3978	291	06/05/03	1.75	4.0	35.0	1283	485	3960	309
05/30/03	2.0	4.0	35.0	1232	366	3922	257	06/06/03	1.75	4.0	35.0	1332	478	3858	206
06/11/03	1.5	4.0	35.0	1427	488	3312	240								
06/12/03	1.5	4.0	35.0	1644	552	3150	291								
06/13/03	1.5	4.0	35.0	1587	475	3244	274								

Note: Wastewater from Quaker Oats received on Friday, April 4, 2003

COD = 14,550±271 mg/L, BOD5 = 8,750±361 mg/L, and total VFA = 11,535 mg/L

Table A-3 Raw data of the effluent quality for different SRTs (*Candida utilis*)

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. utilis* (mg/L) COD	BOD	VSS	VFA	Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. utilis* (mg/L) COD	BOD	VSS	VFA
01/04/03	1.3	4.0	35.0	3669	1388	2672	1760	03/03/03	1.0	4.0	35.0	5691	4115	1988	4046
01/07/03	1.3	4.0	35.0	2336	980	2832	1890	03/04/03	1.0	4.0	35.0	5641	3910	1680	3854
01/13/03	1.3	4.0	35.0	3336	1738	3160	2550	03/05/03	1.0	4.0	35.0	5542	3800	1754	3750
04/01/03	1.75	4.0	35.0	1500	595	3722	240	04/08/03	2.0	4.0	35.0	1537	377	3948	206
04/02/03	1.75	4.0	35.0	1328	467	3880	240	04/09/03	2.0	4.0	35.0	1317	350	3838	137
04/03/03	1.75	4.0	35.0	1348	434	3828	171	04/12/03	2.0	4.0	35.0	1277	389	4002	309

Note: Wastewater from Quaker Oats received on Thursday, October 31, 2002

COD = 17,120±393 mg/L, BOD = 12,660±1,800 mg/L, total VFA = 14,480 mg/L, and furfural = 180 mg/L

Date	SRT (Day)	Cont. pH	Temp. (°C)	Effluent *C. utilis* (mg/L) COD	BOD	VSS	VFA
04/17/03	1.5	4.0	35.0	1712	544	3616	274
04/18/03	1.5	4.0	35.0	1355	429	3042	274
04/19/03	1.5	4.0	35.0	1610	518	3028	240

Note: Wastewater from Quaker Oats received on Friday, April 4, 2003

COD = 14,550±271 mg/L, BOD5 = 8,750±361 mg/L, and total VFA = 11,535 mg/L

Table A-4 Raw data of the effluent quality for different temperatures

Date	SRT (Day)	Cont. pH	Temp. (oC)	Effluent C. tropicalis (mg/L)				Date	SRT (Day)	Cont. pH	Temp. (oC)	Effluent C. utilis (mg/L)			
				COD	BOD	VSS	VFA					COD	BOD	VSS	VFA
07/10/03	1.5	4.0	25.0	775	237	4030	274	07/10/03	1.5	4.0	25.0	403	169	3778	274
07/11/03	1.5	4.0	25.0	875	374	4126	137	07/11/03	1.5	4.0	25.0	886	252	3776	137
07/12/03	1.5	4.0	25.0	936	382	4016	137	07/12/03	1.5	4.0	25.0	855	230	3712	137
09/25/03	1.5	4.0	25.0	1148	385	3495	154	09/25/03	1.5	4.0	25.0	1402	495	2945	274
09/27/03	1.5	4.0	25.0	1128	305	3365	82	09/27/03	1.5	4.0	25.0	843	75	3395	171
07/17/03	1.5	4.0	30.0	1256	438	4074	103	07/17/03	1.5	4.0	30.0	1127	331	4052	111
07/18/03	1.5	4.0	30.0	1265	371	4100	103	07/18/03	1.5	4.0	30.0	1147	340	4156	103
09/09/03	1.5	4.0	30.0	1275	341	4374	77	09/09/03	1.5	4.0	30.0	1336	364	4674	103
09/10/03	1.5	4.0	30.0	1255	371	4260	111	09/10/03	1.5	4.0	30.0	1396	351	4364	69
09/17/03	1.5	4.0	30.0	1294	601	4320	257	09/17/03	1.5	4.0	30.0	876	138	4620	154
09/18/03	1.5	4.0	30.0	1182	355	3758	103	09/18/03	1.5	4.0	30.0	866	82	3962	43
07/23/03	1.5	4.0	35.0	1546	578	3640	163	07/23/03	1.5	4.0	35.0	1367	467	3902	137
07/24/03	1.5	4.0	35.0	1536	580	3898	189	07/24/03	1.5	4.0	35.0	1248	364	4144	154
08/27/03	1.5	4.0	35.0	1409	555	3722	274	08/27/03	1.5	4.0	35.0	1534	294	4074	103
08/28/03	1.5	4.0	35.0	1225	256	4002	69	08/28/03	1.5	4.0	35.0	1467	505	4020	69
09/03/03	1.5	4.0	35.0	1471	553	4044	231	09/03/03	1.5	4.0	35.0	1189	404	4104	180
09/04/03	1.5	4.0	35.0	1098	302	3940	171	09/04/03	1.5	4.0	35.0	1370	437	4034	249
10/01/03	1.5	4.0	40.0	1332	403	3294	137	10/01/03	1.5	4.0	40.0	1127	358	3734	214
10/03/03	1.5	4.0	40.0	1537	473	2500	129	10/03/03	1.5	4.0	40.0	1219	366	2384	137
10/08/03	1.5	4.0	40.0	1534	843	2680	86	10/08/03	1.5	4.0	40.0	1645	869	2672	111
10/10/03	1.5	4.0	40.0	1191	604	2678	77	10/10/03	1.5	4.0	40.0	1857	948	2394	274

Note: Wastewater from Quaker Oats received on Friday, April 4, 2003

COD = 14,550±271 mg/L, BOD5 = 8,750±361 mg/L, and Total VFA = 11,535 mg/L

Table A-5 Raw data of the effluent quality for different pH values

Date	SRT (Day)	Temp. (°C)	Cont. pH	Effluent C. tropicalis (mg/L)					Date	SRT (Day)	Temp. (°C)	Cont. pH	Effluent C. utilis (mg/L)				
				COD	BOD	VSS	VFA	Fur					COD	BOD	VSS	VFA	Fur
02/07/04	1.5	30.0	3.0	5006	4422	566	3943	90	02/07/04	1.5	30.0	3.0	5006	4458	1118	3857	71
02/08/04	1.5	30.0	3.0	7264	4622	582	5057	68	02/08/04	1.5	30.0	3.0	7019	4503	824	4971	76
02/09/04	1.5	30.0	3.0	7787	4670	462	5966	87	02/09/04	1.5	30.0	3.0	7798	4680	576	5966	87
05/11/04	1.5	30.0	3.5	3889	1935	1808	1560	73	02/16/04	1.5	30.0	3.5	4142	1713	1912	2829	69
05/12/04	1.5	30.0	3.5	3970	1811	1784	1629	78	02/18/04	1.5	30.0	3.5	4805	2303	1734	3446	76
05/13/04	1.5	30.0	3.5	4101	1785	1912	1406	65	02/20/04	1.5	30.0	3.5	4162	1798	1818	2863	74
04/19/04	1.5	30.0	4.0	1081	490	3112	326	46	04/19/04	1.5	30.0	4.0	1334	560	3080	343	49
04/20/04	1.5	30.0	4.0	1149	510	3020	360	46	04/20/04	1.5	30.0	4.0	1460	618	3012	394	50
04/21/04	1.5	30.0	4.0	1168	492	2276	309	46	04/21/04	1.5	30.0	4.0	1392	518	2476	257	48
04/27/04	1.5	30.0	4.5	889	345	3068	326	41	04/27/04	1.5	30.0	4.5	1247	470	2684	343	38
04/28/04	1.5	30.0	4.5	848	329	2820	309	41	04/28/04	1.5	30.0	4.5	828	300	2692	291	42
04/29/04	1.5	30.0	4.5	1319	535	3024	326	46	04/29/04	1.5	30.0	4.5	899	330	3192	291	39
05/04/04	1.5	30.0	5.0	951	351	3545	240	36	05/04/04	1.5	30.0	5.0	900	321	2660	274	33
05/05/04	1.5	30.0	5.0	1033	367	2870	257	39	05/05/04	1.5	30.0	5.0	879	292	2565	257	34
05/06/04	1.5	30.0	5.0	920	340	2665	291	37	05/06/04	1.5	30.0	5.0	1104	444	3760	291	34
04/13/04	1.5	30.0	5.5	826	307	3368	171	38	04/13/04	1.5	30.0	5.5	1148	516	2820	600	40
04/14/04	1.5	30.0	5.5	735	255	3072	240	35	04/14/04	1.5	30.0	5.5	1088	490	2948	600	30
04/15/04	1.5	30.0	5.5	977	390	3504	309	34	04/15/04	1.5	30.0	5.5	715	212	2912	291	31

Note: Wastewater from Quaker Oats received on Monday, September 15, 2003

COD = 14,510±62 mg/L, BOD5 = 12,880±911 mg/L, Total VFA = 13,340 mg/L, and Furfural = 193±30 mg/L

APPENDIX B: YEAST BIOMASS DEWATERABILITY AND SETTLING PROPERTIES

Table B-1 Raw data for filterability test (A) *C. tropicalis* and (B) *C. utilis*

(A) *C. tropicalis*

T	*C. tropicalis* at pH 4.0			*C. tropicalis* at pH 5.5			*C. tropicalis* at pH 7.0		
(s)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)
15	17.0	0.000017	882352.94	80.0	0.00008	187500	45.0	0.000045	333333.33
30	21.0	0.000021	1428571.4	100.0	0.0001	300000	50.0	0.00005	600000
45	24.5	0.0000245	1836734.7	110.0	0.00011	409090.91	55.0	0.000055	818181.82
60	27.5	0.0000275	2181818.2	115.0	0.000115	521739.13	60.0	0.00006	1000000
75	30.0	0.00003	2500000	120.0	0.00012	625000	65.0	0.000065	1153846.2

(B) *C. utilis*

| T	*C. utilis* at pH 4.0			*C. utilis* at pH 5.5			*C. utilis* at pH 7.0		
(s)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)	Tot V (mL)	Tot V (m^3)	t/v (s/m^3)
15	29.0	0.000029	517241.38	150.0	0.00015	100000	100.0	0.0001	150000
30	38.5	0.0000385	779220.78	170.0	0.00017	176470.59	110.0	0.00011	272727.27
45	44.5	0.0000445	1011236	180.0	0.00018	250000	115.0	0.000115	391304.35
60	50.0	0.00005	1200000	185.0	0.000185	324324.32	120.0	0.00012	500000
75	54.0	0.000054	1388888.9	190.0	0.00019	394736.84	125.0	0.000125	600000

Table B-2 Filtration rate through Fisher brand glass fiber filter circles G6

Time	dV (mL) of _C. tropicalis_			dV (mL) of _C. utilis_		
(Min)	pH 4.0	pH 5.5	pH 7.0	pH 4.0	pH 5.5	pH 7.0
0.25	17.00	45.0	80.0	29.00	100.0	150.0
0.50	4.00	5.0	20.0	9.50	10.0	20.0
0.75	3.50	5.0	10.0	6.00	5.0	10.0
1.00	3.00	5.0	5.0	5.50	5.0	5.0
1.25	2.50	5.0	5.0	4.00	5.0	5.0

Table B-3 Sludge volume indices (mL/g) for the effluent at different pH values

Time	_C. tropicalis_			_C. utilis_		
(hr)	pH 4.0	pH 5.5	pH 7.0	pH 4.0	pH 5.5	pH 7.0
0.5	α	265	265	α	280	125
1.0	α	250	250	α	187	93
1.5	α	250	250	α	125	62
2.0	α	250	250	α	109	50

Note: Suspended solids of the effluent from _C. tropicalis_ and _C. utilis_ are 3,574 mg/L and 3,208 mg/L, respectively.
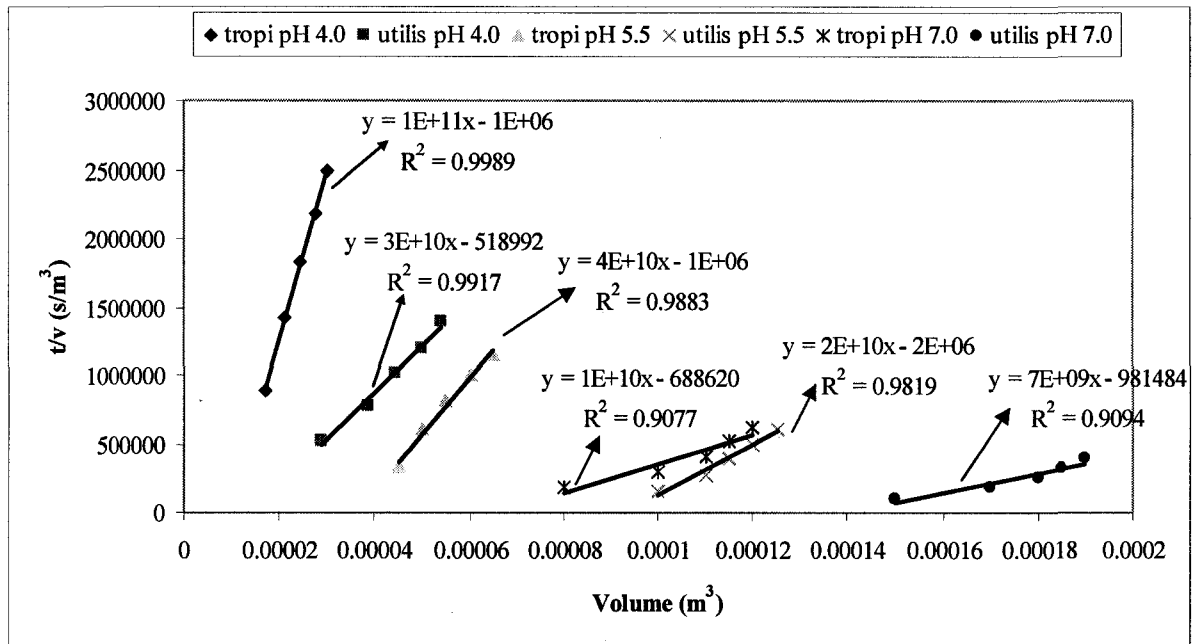
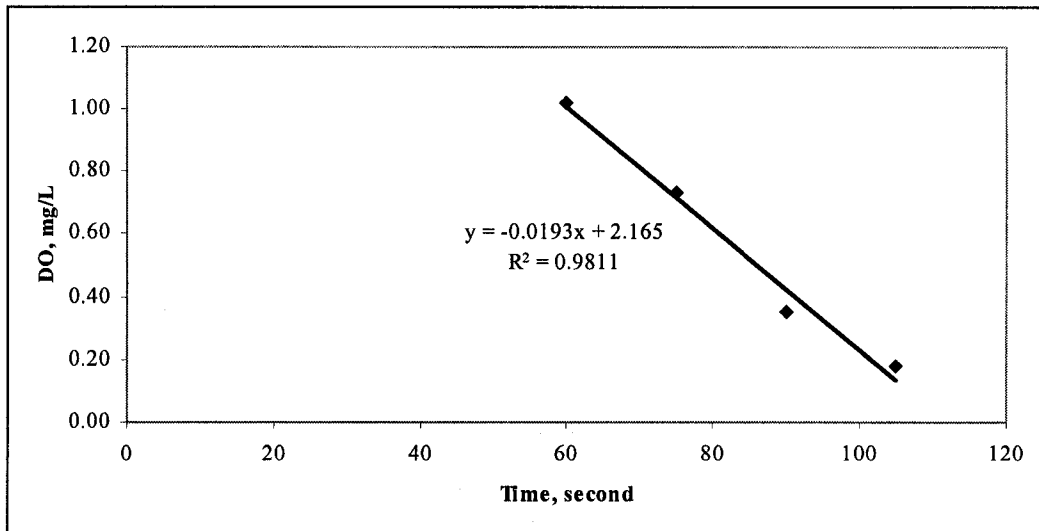**Figure B-1** Specific resistance to filtration of yeast biomass at different pH values
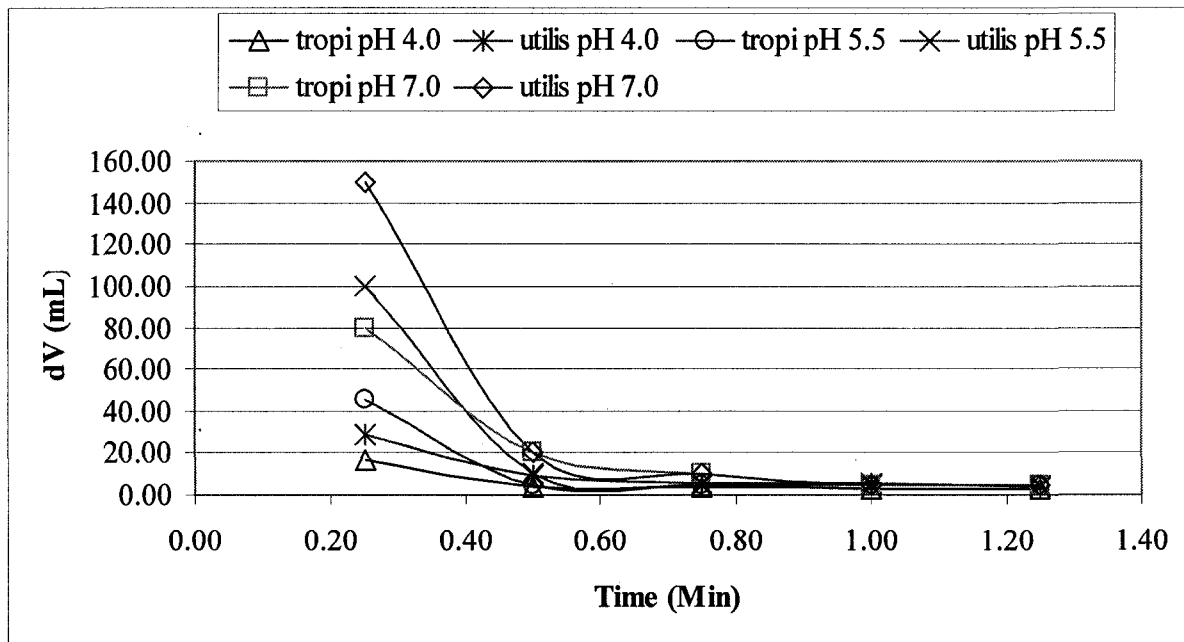
Figure B-2 Filtration rate through Fisher brand glass fiber filter circles G6
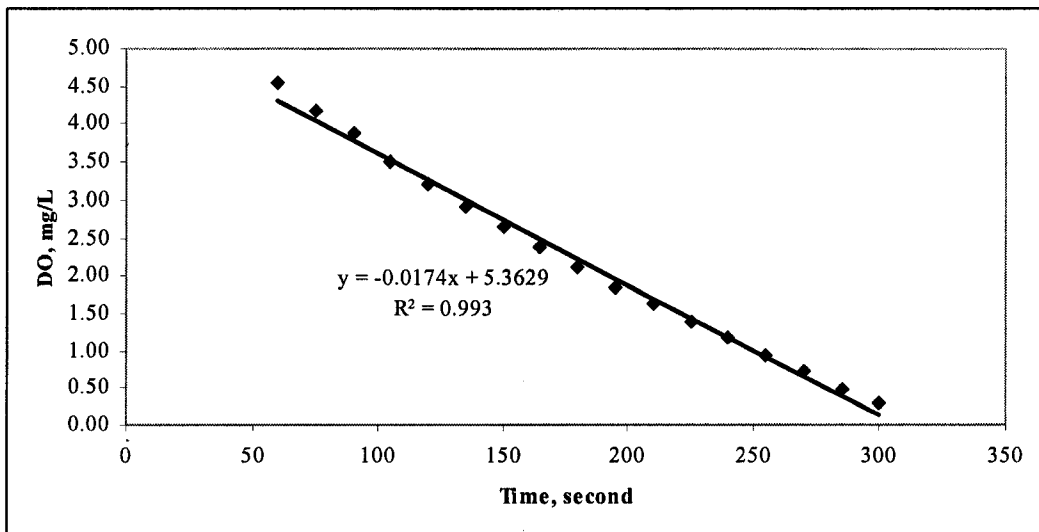
APPENDIX C: DISSOLVED OXYGEN CONCENTRATION IN YEAST REACTORS

Table C-1 Dissolved oxygen concentration in the reactor of *C. tropicalis* and *C. utilis* at 0 and 5 minutes after adding the wastewater to the reactors

Time	*C.tropicalis*		*C. utilis*	
(s)	DO, mg/L at T = 0 min	DO, mg/L at T = 5 min	DO, mg/L at T = 0 min	DO, mg/L at T = 5 min
60	1.02	4.55	1.12	4.08
75	0.73	4.17	0.80	3.65
90	0.35	3.88	0.55	3.13
105	0.18	3.50	0.30	2.83
120		3.21	0.12	2.56
135		2.91		2.26
150		2.64		2.02
165		2.37		1.78
180		2.10		1.51
195		1.84		1.28
210		1.63		1.04
225		1.40		0.83
240		1.18		0.61
255		0.94		0.40
270		0.73		0.19
285		0.49		
300		0.30		

(A) At 0 minute after adding the wastewater to the reactor, DO = 2.2 mg/L

In graph (A): $y = -0.0193x + 2.165$, $R^2 = 0.9811$

(B) At 5 minutes after adding the wastewater to the reactor, DO = 5.4 mg/L

In graph (B): $y = -0.0174x + 5.3629$, $R^2 = 0.993$
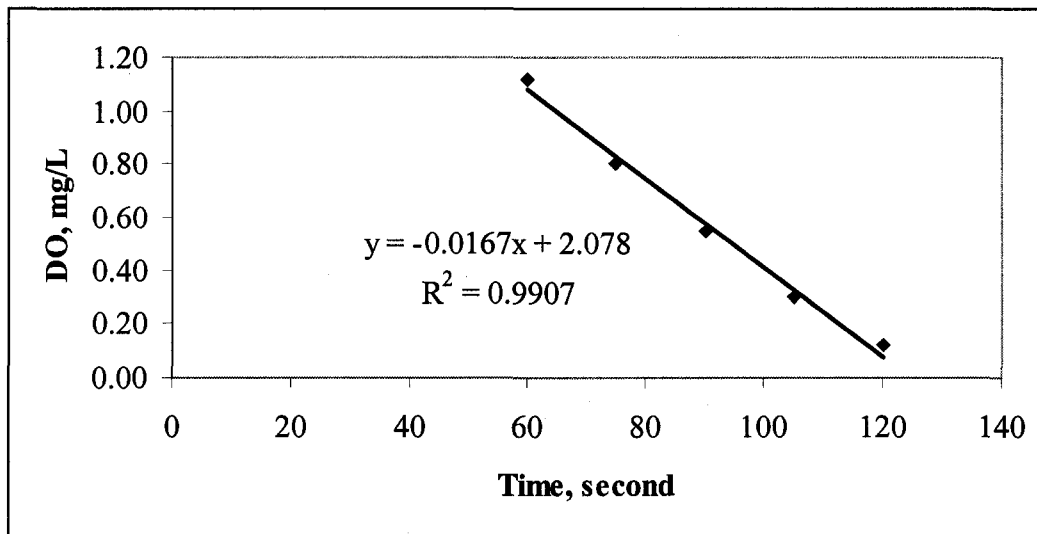
Table C-1 Dissolved oxygen concentration in the reactor of *C. tropicalis*

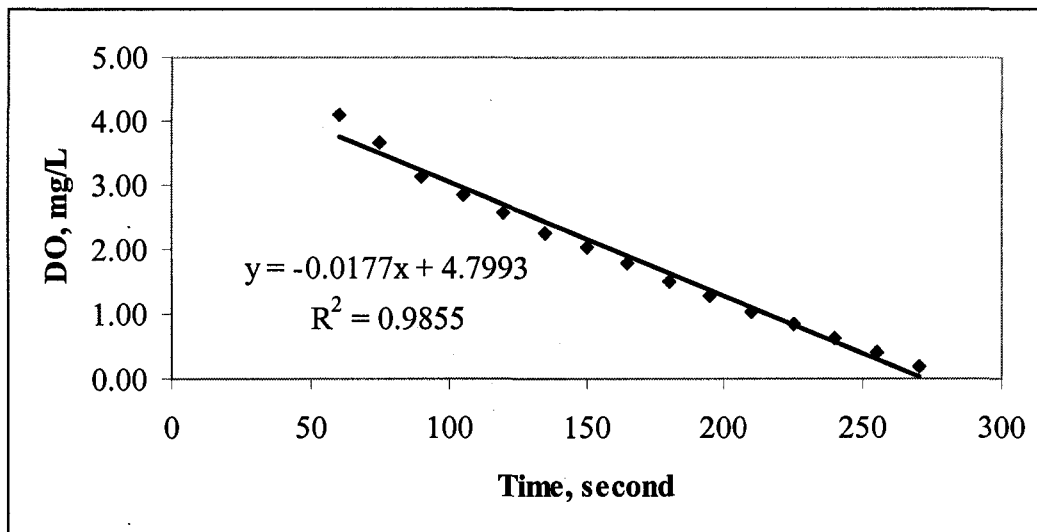(A) At 0 minute after adding the wastewater to the reactor, DO = 2.0 mg/L

(B) At 5 minutes after adding the wastewater to the reactor, DO = 4.8 mg/L

Table C-2 Dissolved oxygen concentration in the reactor of *C. utilis*