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Selective disinfection for enhanced nonaseptic fungal production from food processing wastewater

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Selective disinfection for enhanced nonaseptic fungal production from food processing wastewater

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Abstract

The objective of this research was to further develop the methodology for enhancing fungal production during corn processing wastewater treatment. Food processing wastewater containing high levels of organic matter may be suitable for the growth of microfungi, and these could produce by-products of commercial interest. One challenge of fungal cultivation on wastewater is competition from bacteria for the organic substrate under nonaseptic conditions. A continuous, simple, nonaseptic, microscreen process combined with selective disinfection has been developed in this study for the treatment of corn processing wastewater (CPW) with the production of fungal biomass. Three completely mixed reactors were operated, with one of these operated as a control. Each of the reactors had a 100 μm screen fitted at the bottom. The microscreen acted as a selector to retain fungi while bacteria are washed out with the effluent due to their smaller size.

The fungus selected for investigation was *Rhizopus oligosporus* (ATCC No22959). The operating conditions selected for the study included a pH of 4, a temperature of 38°C, a 2-day solid retention time (SRT), and an 8-hour hydraulic retention time (HRT). The use of the hydrogen peroxide and also chlorine for selective disinfection in fungal cultures was studied to overcome persistent competition from bacteria. Both oxidants, when used at their respective optimal doses, successfully repressed bacterial growth and were able to reduce bacterial counts by at least 50% compared with controls. The respective optimal doses were 60mg/L of hydrogen peroxide and 10mg/L of sodium hypochlorite. Accordingly, use of either disinfectant, at the same optimal doses, resulted in better substrate utilization in terms of chemical oxygen demand (COD) removal and led to larger population growths in the

fungi. That is, a dosage of 60mg/L of hydrogen peroxide led to 85% COD removal and produced 1.8 g of dry fungal biomass from a liter of CPW; compared with the control, COD removal efficiency was increased by 10%, and fungal biomass production was increased by 45%. Similarly, a 10mg/L dosage of sodium hypochlorite resulted in 81% COD removal, 6% higher than the control; and 1720 mg/L fungal biomass, 42% higher than the control.

Higher than optimal doses of either disinfectant resulted in further reduction in bacterial counts, but COD removal efficiency and fungal biomass production decreased rapidly with increased dosages.

Chapter 1

General Introduction

Introduction

The food processing industry produces substantial amounts of wastewater containing high levels of organic materials, measured as biochemical oxygen demand (BOD) or chemical oxygen demand (COD), and large concentrations of total suspended solids and various inorganic constituents. Direct disposal of the such wastewaters to rivers and lakes creates a pollution problem and reduces water quality (Karim and Sistrunk, 1985). Traditionally, food processing wastewaters have been treated by bacteria-based aerobic or anaerobic processes. Although the bacteria efficiently degrade the organic material, aerobic bacteria convert over 50% of the organic matter into low or negative value bacterial cells, necessitating additional treatment and resulting in higher costs for treatment and disposal. Enforcement of wastewater discharge regulations has forced the food processing industries to look for more cost effective technologies to provide pretreatment or complete treatment of their wastewaters (Contreras et al., 2002). These wastewaters usually contain appreciable amounts of useful matter such as carbohydrates, protein, starch, and lipids (Barbesgaard et al., 1992). The idea of converting such organic matter into useful products for human use is gaining popularity. Fungi as a source of a variety of beneficial substances, such as protein, enzymes, chitin, and others, have recently received some interest (van Leeuwen et al., 2003). Fungal wastewater treatment for microbial biomass protein (MBP) production on wheat milling wastewaters has been investigated (Jin et al., 1999). However, without costly presterilization, bacteria already present in wastewater proliferate during treatment and

compete with fungi for the organic substrate. Removal or inhibition of bacteria is therefore important to achieve a high quality fungal product.

To overcome the bacterial challenge, different selector mechanisms for the growth of microorganisms, such as physiology, environmental factors, and physical properties, have been investigated. The growth of specific microorganisms can be favored by controlling environmental conditions, such as pH, temperature, and carbon, nitrogen, oxygen, and trace element concentrations. The concept of size-based selection of microorganisms was developed by Pretorius (1987). A microscreen system was developed in earlier work in our laboratories to separate bacteria from fungi under nonaseptic conditions (van Leeuwen et al., 2003). For this study, a continuous, simple, nonaseptic, microscreen process was developed for the treatment of corn processing wastewater (CPW) from Archer Daniels Midland Company (ADM), with the production of fungal biomass. The fungus *Rhizopus oligosporus* (ATCC No22959) was chosen for investigation, and the optimal temperature, pH, and operating conditions for fungal growth were determined by a series of experiments.

The main goal of the studies in this thesis was to develop a methodology to enhance fungal production under nonaseptic conditions by using selective chemical disinfection combined with a microscreen system.

Thesis Organization

This thesis is organized into four parts. Two papers to be submitted to journals follow the general introduction and literature review. The first paper, "Selective disinfection for enhanced nonaseptic fungal production from food processing wastewater," investigates the use of hydrogen peroxide to improve fungal production by minimizing bacteria contamination. The second paper, "The effect of chlorine on fungal production from food

processing wastewater in suspended system" reports on the ability of chlorine to inhibit bacteria and the determination of the optimal dose for fungal growth. The fourth and final chapter contains general conclusions and acknowledgements.

Literature Review

Food processing is a water-intensive operation. Water is used throughout almost all steps of the food production process in food processing plants, especially as a conveyor to transport food material throughout the process; as a result, large quantities of wastewater, generally containing high levels of organic materials, measured as biochemical oxygen demand (BOD) or chemical oxygen demand (COD), and large quantities of total suspended solids and various inorganic constituents, is produced. Direct disposal of the high organic load wastewater to rivers and lakes creates a pollution problem with respect to water quality (Karim and Sistrunk, 1985), and enforcement of wastewater discharge regulations has forced the food processing industries to look for cost-effective technologies to provide pretreatment or complete treatment of their wastewaters (Contreras et al., 2000).

Food Processing Wastewater Treatment—Current Technologies

Food production processes (e.g., fruit, corn, oils, dairy, etc.) vary widely in their approach, the technologies used, and the characteristics of the wastewater produced. The choice of cost-effective technologies for specific food processing wastewater treatment depends on the specific case. Biological treatment is the primary means of reducing BOD in food processing wastewater it costs less than chemical methods. Aerobic and anaerobic processes are the two main groups of possible biotreatment technologies.

Aerobic technologies including ponds, lagoons, activated sludge processes, sequencing batch reactors, and even controlled wetlands are the conventional techniques for

wastewater treatment, and of these, the activated sludge process is the most widely used, for food processing wastewater as well as other wastewater treatment. However, a phenomenon known as filamentous bulking, which is due to the overgrowth of filamentous microorganisms, is a serious problem in food processing wastewaters (Alejandro et al., 2003; Conner and van Leeuwen, 2004). Many studies have focused on specific solutions to the bulking problem, such as technologies of membrane bioreactors (Stephenson et al., 2000), selectors for specific methods, chlorination (Jenkins et al., 1984), and ozonation (Van Leeuwen, 1988), as well as nonspecific methods and the mesh filter activated sludge process (Fuchs et al., 2005). The disadvantage of the activated sludge process is that a large amount of excess bacterial biomass is generated. This bacterial biomass has little value or reuse potential, and 40–60% of the total operational cost of an activated sludge treatment plant will be spent on the treatment and disposal of the excess sludge (Chen et al., 2001).

Anaerobic treatment of wastewater has emerged as an economical and viable alternative for conventional aerobic treatment, particularly for concentrated wastewaters or sludge produced in many agricultural and food industries (Strous et al., 1997). Different kinds of anaerobic systems have been studied and developed. Sandberg and Ahring (1992) investigated anaerobic digestion of fish processing wastewater using upflow anaerobic sludge blanket (UASB) reactors, and to overcome the drawback of high-rate anaerobic reactors for the treatment of organic suspended solids in wastewater, two-stage systems that separate the acid-forming and the methane-forming phases of the anaerobic process also have been studied, using a continuous-flow stirred-tank system (Guerrero et al., 1999). With the increase in understanding of the anaerobic digestion process, the processes of anaerobic digestion for low strength wastewater have seen great advances, and different reactors such

as the anaerobic filter (AF), fluidized bed (FB), expanded granular sludge bed (EGSB), and anaerobic baffled reactors (ABR) (Alette et al., 2000) have been studied. Some disadvantages of the anaerobic processes must be considered; these include longer start-up time, requirements for further treatment, more sensitivity to reaction rates at lower temperatures, the possible presence of disease-causing microorganisms in the wastewater, and odors associated with treatment. In many cases, aerobic and anaerobic processes are combined in one treatment system; anaerobic treatment is used to remove organic matter from wastewater higher concentrations of organic matter, whereas, aerobic treatment is used on streams containing lower concentrations. An example is the anaerobic-aerobic treatment of potato starch wastewater (Abeilung and Seyfried, 1993)

Physical-chemical alternatives to biotreatment for BOD reduction in food processing wastewaters include settling (Wright, et al., 1979), activated carbon treatment (Panasiuk, et al., 1977), and membrane filtration systems (Goodwin and Catley, 2001). These are mainly separation and concentration technologies. Of these technologies, membrane treatment has attracted the most attention. Reverse osmosis has been installed at a dairy cheese plant to reduce BOD in the wastewater, and the filtered water can be directly discharged into a nearby creek (Anon., 1993). In many cases, membrane systems have been combined with other treatment systems such as membrane bioreactors (Stephenson et al., 2000; Scott and Smith, 1997), in which the secondary settling tank is replaced with a membrane filtration unit. A combination of high sludge concentration and excellent quality is the main advantage of this process, but the high cost of procuring and installing membrane modules and the high energy

consumption are still barriers to a more widespread application of membrane systems (Fuchs et al., 2005).

Usually, a wastewater treatment system includes different units for different functions. Examples of current wastewater treatment systems in these plants include physical screening to remove large solids, chemical flocculation to remove colloidal and suspended solids by dissolved air flotation (DAF) units, anaerobic and aerobic digestion to remove dissolved organic matter, and chlorination to kill remaining unwanted microorganisms (Chen et al., 2000).

Fungal Utilization

In recent years, a resource recovery approach, known as recycling and utilization of useful materials from food processing waste sludge, has emerged. Fungi are classified in a separate kingdom. Until now about 77,000 species of fungi have been known, and some of these are important to human welfare. One of the well-known examples is *Penicillium notatum,* used for penicillin production.

Chitin and its derivate chitosan are becoming important neutraceuticals. These are presently extracted from arthropod shells (crabs, prawns, and lobsters), but this is a seasonal and unreliable supply. An evaluation of alternative potential sources shows that cultured fungi capable of synthesizing chitin alone or in association with chitosan will play a major role in chitin and chitosan supply (Allan et al., 1978; Tan et al., 1996). Research into the production of chitosan has been conducted on different fungal species such as *Phycomyces blakesleeanus* (Knorr and Klein, 1986), *Absidiarepens* (Davoust and Hansson, 1992), and *Absidia blakesleeanus* (Rane and Hoover, 1993). The conversion of organic materials in food processing wastewater to microbial biomass protein (MBP) also has been investigated, as a source of

food for animals and humans (Stevens and Gregory, 1987). Other valuable biochemicals such as aspartic proteinase, lipase, lactoferrin, and lysozyme can be obtained from other species of fungi such as *Cephalosporium eichhorniae* 152 (Mikami et al., 1982), *Aspergillus oryzae*, *Rhizopus oligosporus,* and *R. arrhizus* (Christense et al., 1988; Huge-Jensen et al., 1989; Ward et al., 1992; Tsuchiya et al., 1992; Jin et al., 1999). Glucoamylase is one of the most important enzymes used in food processing because of its ability to hydrolyze starch almost completely into glucose (Manjunath et al., 1983), and species of *Aspergillus* and *Rhizopus* have been widely used in industry for commercial production (Imai et al., 1994). Other species also have been found to be promising species for amylase production; these include *Lentinula edodes* (El-Zalaki and Hamza, 1979) and *Neurospora crassa* (Stone et al., 1993). The bioconversion of starch processing wastewater (SPW) using yeasts has attracted attention because of their high enzymatic activity and high growth rate (Gonzatez et al., 1992), and they have been considered more suitable for MBP production than microfungi (Bergman and Hizukurii, 1988). However, the characteristics of easier separation and easier recovery of the MBP from the culture, due to the filamentous nature of the microfungi, have made microfungi more promising for bioconversion (Nigam, 1994).

Numerous trials have been conducted to enhance the fungal product or fungal by-product production process, and researchers have tried to find a way to increase such production for commercial use. Most of these processes employed monocultures under aseptic culture conditions on relatively expensive substrates such as starches or molasses (Barbesgaard et al., 1992). To keep costs down, researchers have tried to find cheaper substrates, and food processing wastewater may potentially be the desirable substrate for fungal cultivation, since large amounts of waste carbohydrates (such as starch) as well as

some protein and lipids, which are useful matter for fungal growth, are present in such wastewater. On the other hand, fungi produce enzymes such as cellulase and amylase that are more effective in hydrolyzing complex carbohydrates than bacteria are (Friedrich et al., 1986). Some trials combining wastewater treatment with production of useful biomass have been conducted (Stevens and Gregory, 1987; Jin et al., 1999b; Bergmann et al., 1988; Shah et al., 1991; van Leeuwen et al., 2003). However, without presterilization, bacteria originally contained in wastewater or from the environment proliferate and compete with fungi for the organic substrate during treatment. These bacteria also deteriorate the dewatering characteristics of the biomass and influence the quality of the dried biomass, and some bacteria also secrete toxic substances. To keep the costs of production and wastewater treatment down, nonaseptic conditions are desirable. Only a few investigations have been conducted under nonaseptic conditions (Jin et al., 1998). Finding a more effective, low cost, treatment system is the purpose of this research. Therefore, a selection mechanism that favors fungi over bacteria is required.

Selection Technologies to Enhance Fungal Production

Physiological factors, environmental factors, and operational conditions using the physical properties of microorganisms could be considered as major selection conditions in biological wastewater treatment. The physiological properties of the microorganisms form the basis for the "primary" selection stage, while their physical properties can be used as an initial basis for a "secondary" selection stage (Pretorius, 1987).

Effect of energy source

In conventional wastewater treatment, the most important factor determining primary selection of microorganisms is the energy source (the organic substrate) (Pretorius, 1987). Different types of microorganisms have diverse metabolisms, so specific microorganisms consumes specific substrates at different rates (Wainwright et al., 1992). In contrast to domestic sewage, which contains unknown and complex organic and inorganic compounds, each specific food processing process usually produces its own specific complex substrates. Therefore, a food processing wastewater of a specific, unique composition has a specific, microbial selection power as a specially designed selective culture medium for fungal growth. The metabolisms of many fungal species have been investigated in different types of food processing wastewater; some examples are introduced below.

Yeast in MBP production processes exhibit a diversity of metabolic reactions that affect fungal growth rate and yield. Jin et al. (1998) have investigated the differences in fungal growth rate and yield in relation to the type of substrate (Table 1).

Stevens and Gregory (1987) studied the performance of *Cephalosporium eichhorniae* in producing MBP from potato processing wastes and obtained 0.61 g (dry weight) of product and 0.3 g of crude protein per gram of carbohydrate supply under certain conditions (pH 3.75, ferric iron addition, and nitrogen supply). Mishra et al. (2004) evaluated two *Aspergillus* species, A. *foetidus* and *A. niger,* for their ability to grow and produce biomass and reduce the organic load of the wastewater. About 60% COD removal and 2.4 and 2.85 g/L biomass production, respectively, were obtained. The degree of bioconversion of apple distillery waste also depended on fungal species: *Aspergillus* consumed the soluble organic substances, but they could not degrade raw fibers;

Table1. Production of MBP by yeast cultivations from starch material (Jin et al., 1998).

Substrate	Species	Strain	Process	μ (L/h)	T(°C)	Yield (mg/mg)
Starch	*C. utilis*	NRRL1048	Batch	0.14	23	0.61
	L. ononenkoae	IGC4052	Batch	0.12	25	0.58
		ICG4052B	Continuous	0.04	28	0.48
	S. cerevisiae	BRG 530	Batch		25	0.50
	Sa. fibuligera	NRRL Y76	Batch	0.35	30	0.50
		ATCC2607	Batch		32	0.51
	Sc. occidentalis	IGC2829	Batch	0.21	30	0.59
		UCD54-83	Batch	0.25	30	0.62
	Sp.holsaticus	FRI Y-5	Batch	0.14	23	0.43
Potato Waste	*C.utilis*	NCYC707	Batch	0.5	30	0.55
	Sc.occidentalis	IMAT2196	Batch		28	0.54
	Sa. fibuligera	NRRL1062	Continuous	0.35	32	0.84
Cassava	*C. tropicalis*	CBS6948	Continuous	0.26	35	0.42
	Sc. occidentalis	CBS2863	Batch	0.23	28	0.44

Abbreviations for genera: *C.=Candida; L.=Lipomyces; S.=Saccharomyces; Sa.= Saccharomycopsi; Sc.=Schwanniomyces; Sp.=Sporobolomycesia*

Trichoderma reesei degraded insoluble fibers, first cellulose, and then small quantities of lignin; and *Phanerochaete chrysosporium* was the most effective in degrading lignin and cellulose, but it grew slowly and synthesized low quantities of protein (Friedrich et al., 1986). Jin et al. (1999a) investigated biomass and enzyme production with a number of strains of two different species of microorganisms, *Aspergillus oryzae* and *Rhizopus arrhizus*, using starch processing wastewater. Maximum biomass growth can be achieved in a cultivation period of 10 and 12 hours and different biomass yields of 8.45 g/m^3 and 8.06 g/m^3 were observed respectively.

Effect of pH

Microorganism growth is very sensitive to change of pH within the growth medium because the pH determines the solubility of carbon dioxide and minerals in the medium and,

directly or indirectly, influences the metabolism of the microorganism (Rafiqul et al., 2005). The effects of pH on the biomass growth activities of fungi in a dominant culture have been investigated by numerous researchers (Stevens and Gregory, 1987; Riscaldati et al., 2000; Jin et al., 2002; van Leeuwen et al., 2002; Mishra et al., 2004). The results showed that the pH for biomass production and COD removal are different; for example, the optimal pH is 6 for two *Aspergillus* species, *A. foetidus* and *A. niger* (Mishra et al., 2004) and 3.75 for *Cephalosporium eichhorniae* (Stevens and Gregory, 1987). The pH also influenced the growth rate and microbial competition in the media with mixed cultures of microorganisms. Van der Westhuizen and Pretorius (1998) reported on competition between bacteria and fungi *(Asperillus fumigatus)* at three different pHs in a mixed culture. Yield coefficients of *A. fumigatus* were 0.44, 0.39, and 0.38 g/g COD at pH 5.0, 5.5, and 6.1, respectively. As pH increased, yield coefficients increased, and the number of bacteria was 1 x 10^6, 21 x 10^6, 30 x 10^6 (CFU/mL), respectively; as pH decreased, the number of bacteria was reduced dramatically.

Jin et al. (1999) investigated the morphological characteristics and yield of three different fungal strains, *A. oryzae* DAR 1679, 3699, and 3863, using starch processing wastewater at a pH range of 3.0–6.0. The three fungi formed different types of mycelia and pellets with different pH and showed different yield coefficients according to the type of morphology (Table 2). They concluded that the pH of the growth medium affected morphological formation and biomass yield significantly.

Table 2. Morphology and biomass yield at different growth pH (Jin et al., 1999).

Growth pH	*A. oryzae* DAR 1679		*A. oryzae* DAR 3699		*A. oryzae* DAR 3863	
	Morphology	Yield	Morphology	Yield	Morphology	Yield
3.0	Dispersed mycelia	2.67	Clumpy mycelia	4.69	Dispersed mycelia	2.83
3.5	Dispersed mycelia	3.21	Compact pellets	5.03	Fluffy mycelia	3.45
4.0	Fluffy mycelia	4.32	Compact pellets	5.18	Clumpy pellets	4.26
4.5	Fluffy mycelia	5.22	Compact pellets	4.94	Compact pellets	5.1
5.0	Clumpy mycelia	5.37	Clumpy pellets	4.12	Compact pellets	5.
5.5	Clumpy mycelia	5.25	Clumpy mycelia	3.25	Compact pellets	5.42
6.0	Clumpy mycelia	4.54	Fluffy mycelia	3.05	Compact pellets	5.18

Effect of temperature

Temperature is one of the most important factors affecting microbial growth. Enzyme reactions influenced by temperature are directly related to the growth of microorganisms, and can be explained by the Arrhenius relationship (Mitchell, 1999). Generally, the rate of enzymatic reaction increases as temperature is increased within a limited range of temperatures. The reaction rate in the Arrhenius equation will double for each 10°C increase in temperature, within certain temperature limits (Jin et al., 1999). Each microorganism has a specific optimal temperature, and each temperature influences the determination of the dominant species in a mixed culture differently. The optimal temperature for *Cephalosporium eichhorniae* is 45°C (Stevens and Gregory, 1987). Maximum growth of *Sclerotium rolfsii* occurred at 25°C after 7 days of inoculation, and growth was reduced significantly below 20°C and above 35°C (Azhar Hussain, et al., 2003). Hong et al. (1997) developed a model to predict fungal spore longevity, based on temperature, and found a negative semilogarithmic relationship between longevity and temperature. The use of

temperature as a selector, to control competition between bacteria and fungi, was also investigated (Van der Westhuizen and Pretorius, 1998); the fungal culture was contaminated by bacteria at 46°C, but as the temperature increased to 50 °C, bacterial colonies decreased dramatically.

Harder and Veldkamp (1971) investigated competition between different fungal species, *Pseudomonas* sp. (optimum temperature 30°C) and *Spirillum* sp. (optimum temperature 14°C), in the range from 16°C to –4°C. These microorganisms showed different specific growth rates according to temperature. *Pseudomonas* sp. at 16°C was more competitive than *Spirillum* sp., but at –2°C, the competitiveness was reversed. At intermediate temperatures (10°C and 4°C), dominance depended on the substrate concentration of the medium; Therefore, temperature was the main selection factor at 16°C and –2°C. Production of *Rhizopus oligosporus* was studied by Jin et al (1999a) (Figure 1)

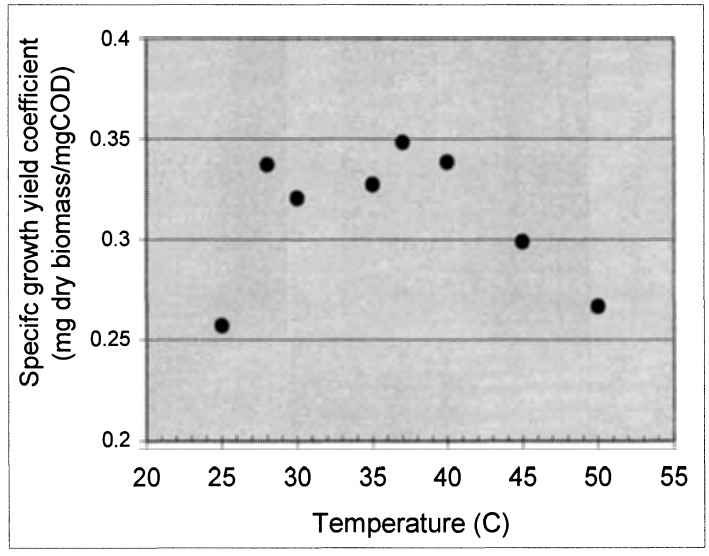

Figure 1: Specific growth yield vs. temperature for *Rhizopus oligosporus* (Jin et al., 1999a).

Effect of operating conditions based on physical properties

Physical properties of microorganisms, such as mass and size, can be also used as dynamic selection tools where two or more microorganism cultures coexist in a single reactor. An example is activated sludge systems, where filamentous bacteria, which cause a bulking phenomenon, coexist with a variety of nonfilamentous bacteria. The bulking phenomenon causes poor settling, and because settling is used to separate the biomass from the treated effluent, the bulking phenomenon can be a serious problem. Models for controlling different microorganisms in a continuous system based on the different growth rates of the groups of microorganisms coexisting in the same reactor have been developed; control over the respective populations can be exerted through operational factors such as the hydraulic retention time (HRT) and solids retention time (SRT) (Chudoba et al. 1973; Cenens et al., 1999; Contreras et al., 2002).

Size can be used as a selection principle in a microscreen system to separate bacteria from filamentous fungi (Pretorius, 1987; Lempert and Pretorius, 1997; van der Westhuizen and Pretorius, 1998; Jin, et al., 1999; van Leeuwen, 2003): bacteria are 0.5 to 2 μm in diameter, while fungi are > 5 μm in diameter with filament lengths of several hundred micrometers. Van Leeuwen et al. (2003) designed a single reactor coupled with a microscreen with an aperture of 100 μm, which is intermediate in size between the sizes of the fungi and the bacteria (Figure 2). The completely mixed culture is continuously passed through the screen so that fungi can be retained in the reactor and bacteria can be washed out. Fungal dominance can be maintained under nonaseptic conditions by the different solid retention times (SRT) for fungi and bacteria. Provided the bacteria do not attach to the fungi, the SRT of the bacteria will be equal to the hydraulic retention time (HRT) in the reactor.

R_{SF} = Biomass in the reactor/biomass harvested per hour (or day) (h or d)

$R_{SF} = X_F V/X_F q = V/q$ (1)

where

V = Working volume of the reactor (1.5 L)

q = Flow rate of harvesting stream (L/d)

X_F = Biomass concentrations in the reactor and the harvested stream (mg dry biomass/liter).

Hydraulic retention time (R_{SB}) (for nonfilamentous bacteria)

R_{SB} = Average length of time mixed liquor remains in the reactor

= V/Q (h or d) (2)

where

V = Reactor working volume (L)

Q = Influent flow rate into the reactor (L/h or L/d)

The fungal species *Rhizopus oligospous* (ATCC, No 22959) (American Type Culture Collection, Rockville, MD) characterized as fast growth has been tested using corn processing wastewater from Archer Daniels Midland (ADM) wet milling operations in Clinton, Iowa, as a substrate, and the optimal temperature, pH value, and operation parameters, namely SRT and HRT, for fungal growth in the reactor have been determined (van Leeuwen et al., 2003).

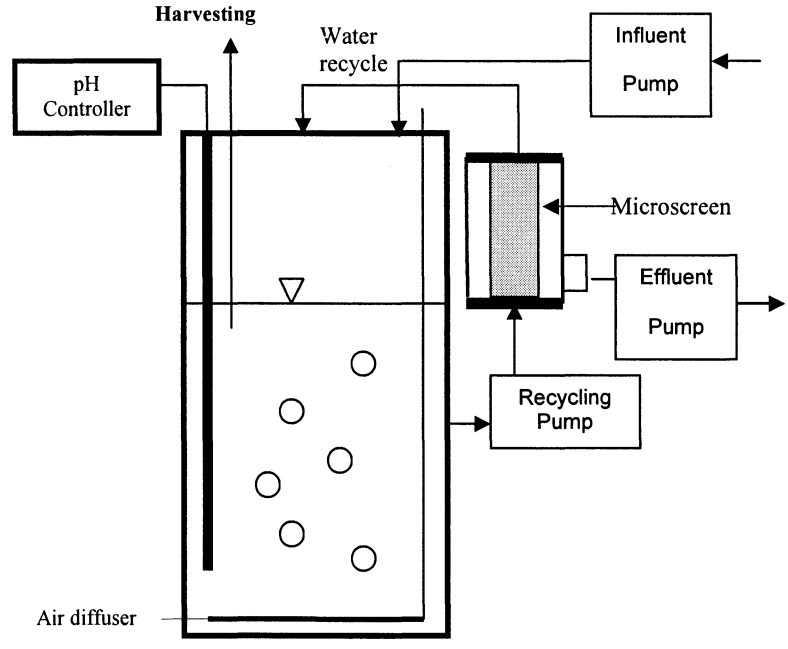

Figure 2: Schematics of continuous reactor with microscreen.

Disinfection

Disinfection usually refers to the inactivation of disease-causing organisms, and four types of disinfection (chemical and physical agents, mechanical means, and radiation), based on their different mechanisms, have commonly been described (Metcalf and Eddy, Inc., 2002). Disinfection has been used successfully to control bacteria in water and wastewater treatment. Selective disinfection with chlorine (Jenkins et al., 1982) and ozone (van Leeuwen, 1988) to discourage the growth of filamentous bacteria have been studied and found to work well. However, Van Leeuwen (1989) found that fungi in an industrial activated sludge system were much more resistant to ozone than bacteria were, and resultant fungal bulking could not be controlled with ozonation. This differential sensitivity to ozone may be the key to preventing bacterial growth within a fungal cultivation system. The aim of

this study was to find more effective disinfectants that inactivate bacteria but do not significantly inhibit fungi, and to determine the optimal dose.

Literature Cited

Abeilung, U., and Seyfried C. F. (1993) Anaerobic-aerobic treatment of potato starch wastewater. *Water Science Technol.* **28**, 165-176.

Alejandro, C., Edgardo, M. C., Leda, G., and Noemi, Z. (2003) Modeling of chlorine effect on floc forming and filamentous micro-organisms of activated sludges**.** *Water Research,* **37**, 2097-2105.

Allan, G. G., Fox, J. R., and Kong, N. (1978). A critical evaluation of the potential sources of chitin and chitosan. In R.A.A. Muzzarelli, and E.R. Pariser, *Proceedings of the first international conference on chitin/chitosan. MIT Sea Grant Report MITSG78-7, Index No. 78-307-Dmb (pp. 64-78)*. Cambridge: Massachusetts Institute of Technology.

Alette, A. M., Langenhoff, N. I., and David C. S. (2000). Treatment of dilute soluble and colloidal wastewater using an anaerobic baffled reactor: influence of hydraulic retention time**.** *Water Research,* **34**(4), 1307-1317.

Azhar, H., Muhammad, I., Najma, A., and Abdul M.H (2003). Physiological Study of *Sclerotium rolfsii* Sacc**.** Pakistan Journal of Plant Pathology **2** (2): 102-106, 2003.

Barbesgaard, P., Heldt-hansen, H. P., and Diterichsen, B. (1992). On the safty of *Aspergillus oryzae*: a review.*Applied Microbiolology Biotechnolology* **9**, 569-572.

Bergmann, F. W., Abe, J., and Hizukurii, S. (1988). Selection of microorganisms which produce raw-starch degrading amylases.*Applied Microbiology and Biotechnology,* **27**, 443-446.

Cenens, C., Smets, I. Y., and Van Impe, J. F. (1999). Modeling the competition between flocforming and filamentous bacteria in activated sludge wastewater treatment systems-II. A prototype mathematical model based on kinetic selection and filamentous backbone theory. *Water Res*earch**, 34,** 2535-2541.

Chen, G. H, Yip, W. K., Mo, H. K., and Liu, Y. (2001). Effect of sludge fasting/feasting on growth of activated sludge cultures. *Water Research*, **35**, 1029-1037.

Christensen, T., Woeldike, H., Boel, E., Mortensen, S. B., Hjortshoej, K., Thim, L., and Hansen, M. T. (1988) High level expression of recombinant genes in *Aspergillus oryzae*. *Bio-Technology* **6**:1419–22.

Chudoba, J., Grau, P., and Ottova, V. (1973). Control of activated sludge filamentous bulking—II. Selection of micro-organisms by means of a selector. *Water Research* **7**, 1389–1406.

Conner, D.B. van Leeuwen, J. Warren M. Warren T. and Morrill, M. (2003) Improving activated sludge settleability, dewatering and biomass recovery in pasta wastewater treatment. Proc. Asian Waterqual 2003, Bangkok, Thailand, October 20-22, 2003

Contreras, E. M., Glannuzzi, L., and Zaritzky, N. E. (2002). Competitive growth kinetics of S*phaerotilus natans* and *Acinetobactor anitraus*. *Wat. Sci. Tech.* **46** 45-48.

Davoust, N., and Hansson, G. (1992). Identifying the conditions for development of beneficial mycelium morphology for chitosan-producing *Absidia* spp. in submersed cultures. *Applied Microbiology and Biotechnology,* **36**, 618–620.

El-Zalaki, M. E., and Hamza, M. A. (1979). Edible mushrooms as producers of amylases. *Food Chem.* **4:**203-211.

Friedrich, J., Cimerman, A., and Perdih, A. (1986). Comparison of different cellulolytic fungi for bioconversion of apple distillery waste. *Applied Microbiology Biotechnology,* **24**, 432-434.

Fuchs, W., Resch, C., Kernstock, M., Mayer, M., Schoeberl, P. and Braun, R. (2005). Influence of operational conditions on the performance of a mesh filter activated sludge process. *Water Research,* **39**, 803-810.

Gonzatez, M. P., Murado, M. A., and Pastrana, L. (1992). Depuration and evaluation of mussel processing wastes : Characterisation of amylolytic postincubates from different species grown on an effluent. *Bioresources Technology* **42:** 1269-1273.

Goodwin, S., and Catley, G. (2001). Membranes boost water re-use. *Water Waste Treat* 13-14.

Guerrero, L., Omil, F., Méndez, R and Lema, J. M (1999) Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Research,* **33**, 3281-3290

Hong,T. D., Ellis, R. H. and Moore. D. (1997). Development of a model to predict the effect of temperature and moisture on fungal spore longevity. *Annals of botany*, **79**, 121-128.

Jenkins, D., Richard, M. G., and Daigger, G. T. (1984). Manual on the causes and control of activated sludge bulking and foaming. Water Research Commission, PO Box 824, Pretoria, South Africa.

Jin, B., van Leeuwen J. H., Patel, B., and Yu, Q. (1998). Utilisation of starch processing wastewater for production of microbial protein and fungal amylsases by *Aspergillus Oryzae. Bioresource Technology* , **66**, 201-206.

Jin, B., van Leeuwen, J. (Hans),Yu, Q., and Patel, B. (1999a). Screening and selection of microfungi for microbial biomass protein production and water reclamation from starch processing wastewater. *J Chem Technol Biotechnol* **74**:106-110.

Jin, B., van Leeuwen, J.(Hans), Patel, B. (1999b). Mycelial morphology and fungal protein production from starch processing wastewater in submerged culture of *Aspergill oryzae*. *Process Biochemistry*, **34**, 335-340.

Jin, B., Van Leeuwen, J. (Hans), Patel, B., Doelle, H. W., and Yu, Q. (1999c). Production of fungal protein and glucoamylase by *Rhizopus oligosporus* from starch processing wastewater. *Process Biochemistry,* **34**, 59-65.

Jin, B., Yan X. Q., Yu, Q., and van Leeuwen, J. (Hans). (2002). A comprehensive pilot plant system for fungal biomass protein production and wastewater reclamation. *Advances in Environmental research* **6,** 179-189.

Knorr, D., and Klein, J. (1986). Production and conversion of chitosan with cultures of *Mucor rouxii* or *Phycomyces blakesleeanus*. *Biotechnology Letters*, **8**, 691–694.

Lempert, G. G., and Pretorius, W. A. (1997). Micoprotein production on spent sulphite liquor, WRC Report No. 3/1/97, Pretoria, South Africa.

Huge-Jensen, B., Andreasen, F., Christensen, T., Christensen, M., Thim, L., and Boel, E. (1989). *Rhizomucor miehei* triglyceride lipase is processed and secreted from transformed *Aspergillus oryzae*. *Lipids,* **24**, 781–5.

Manjunath, P., Shenoy, B. C., and Rao, M. R. (1983). Review: Fungal glucoamylases. *Journa l of Applied Biochemistry*, **5**, 235-260

Mikami, Y., Gregory, K. F., Levadoux, W. L., Balagopalan, C., and Whitwill, S. T. (1982). Factors affecting yield and safety of protein production from cassava by *Cephalosporium eichhorniae*. *Appl. Environ. Microbio*l., **43**, 403-411.

Mishra, B. K., Arora Anju, and Lata (2004). Optimization of a biological process for treating potato chips industry wastewater using a mixed culture of *Aspergillus foetidus* and *Aspergillus niger*. *Bioresource Technology*, **94**, 9-12.

Mitchell, R. (1999). Wastewater Microbiology ISBN 0-471-32047-1, **57.**

Nigam, P. (1994). Process selection for protein-enrichment: Fermentation of the sugar industry by products molasses and sugar beet pulp. *Process Biochemistry,* **29,** 337-342.

Imai, Y., Suzuki, M., Masamoto, M. and Nagayasu, K. (1994). Glucoamylase production of *Aspergillus oryzae* in fed-back culture using a statistical regression model. *Journal of Fermentation and Bioengineering,* **78**(4), 310-314.

Panasiuk, O., Sapers, G. M., Ross, L. R., 1977. Recycling bissulfite brines used in sweet cherry processing. J.Food Sci. 42(4), 953-957.

Pretorius, W. A. (1987). A conceptual basis for microbial selection in biological wastewater treatment. Wat. Res. **21,** 891-894.

Rafiqul, I. M., Jalal, K. C. A. and Alam, M. Z. (2005). Environmental factors for optimization of *Spirulina* biomass in laboratory culture. *Biotechnology,* **4,** 19-22.

Rane, K. D., and Hoover, D. G. (1993). An evaluation of alkali and acid treatments for chitosan extraction from fungi. *Process Biochemistry*, **29**, 115–118.

Riscaldati, E., Moresi, M., Federici, F., and Petruccioli, M. (2000). Effect of pH and stirring rate on itaconate production by *Aspergillus terreus. Journal of Biotechnology,* **83,** 219-230.

Sandberg M., and Ahring B. K. (1992). Anaerobic treatment of fish meal process waste-water in a UASB reactor at high pH. *Appl. Microbiol. Biotechnol.,* **36**, 800-804.

Scott, J. A., and Smith, K. L (1997). A bioreactor coupled to a membrane to provide aeration and filtration in ice-cream factory wastewater remediation. *Water Research,* **31**, 69-74.

Shah, N. K., Ramamurthy, V., and Kothari, R.M. (1991). Comparative profiles of fungal amylase production by submerged and surface fermentation. *Biotechnology Letters,* **3**(5), 361-364.

Stephenson, T., Judd, S., Jefferson, B. and Brindle, K. (2000). Membrane bioreactors for wastewater treatment. IWA Publishing, London, UK.

Stevens, C. A., and Gregory, K. F. (1987). Production of Microbial Biomass Protein from Potato Processing Wastes by Cephalosporium eichhorniae, *Applied and Environmental Microbiology*, 284-291.

Stone, P. J., Makoff, A. J., Parish, J. H., and Radford, A. (1993). Cloning and sequence analysis of the glucoamylase gene of *Neurospora crassa. Curr. Genet.* **24**, 205-211.

Strous, M., Gerven, E. V., Zheng, P., Kuenen, J. G., and Jetten, M. S. M. (1997). Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Research,* **31** (8), 1955-1962.

Tan, S. C., Tan, T. K., Wong, S. M., and Khor, E. (1996). The chitosan yield of zygomycetes at their optimum harvesting time. *Carbohydrate Polymers* **30**, 239-242.

Tsuchiya, K,. Tada, S., Gomi, K., Kitamoto, K., Kumagai, C., Jigami, Y., and Tamura, G. (1992). High level expression of the synthetic human gene lysozyme in *Aspergillus oryzae*. *Applied Microbiological Biotechnology,* **38**, 109–14.

Van der Westhuizen, T.H., and Pretorius, W.A. (1998). Use of filamentous fungi for the purification of industrial effluents, WRC Report No 535/l/98, Pretoria, South Africa.

van Leeuwen, J. (1988). Domestic and industrial wastewater treatment with ozonated activated sludge. *Ozone Sci and Eng.* **10**, 3.

van Leeuwen, J. (1989). Ozonation for nonfilamentous bulking control in an activated sludge plant treating fuel synthesis waste water. *Water SA*, **15**, 2.

van Leeuwen, J. (H.), Hu, Z., Yi, T. W., and Pometto III, A. L (2002). Use of microfungi for single cell protein production during food processing wastewater treatment. Proc of the 75th Water Environmental Federation Technical Exhibition and Conference, Chicago, IL.

van Leeuwen J. (H.), Hu, Z., Yi, T. W., Pometto III, A. L., and Jin, B. (2003). Kinetic model for selective cultivationof microfungi in a microscreen process for food processing wastewater treatment and biomass production. *Acta Biotechnologica*, **23**, 289-300.

Wainwright, M. (ed.) (1992). Fungi in the food industry. In: An introduction to fungal biotechnology. *John Wiley and Sons*. USA. 126-149.

Ward, P. P., Lo, J., Duke, M., May, G. S., Headon, D. R., and Connelly, O. M. (1992). Production of biologically active recombinant human lactoferrin in *Aspergillus oryzae. Biotechnology,* **10**, 784-9.

Wright, M. E., Hoehn, R. C., Coleman, J. R. and Brzozowski, J. K (1979). A comparison use and recycled water leafy vegetable washing systems. *J.Food Sci.*, **44,** 381-389.

Chapter 2

Selective Disinfection for Enhanced Nonaseptic Fungal Production from Food Processing Wastewater Using Hydrogen Peroxide

A paper to be submitted to

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Abstract

A simple, nonaseptic, low cost single microscreen process combined with disinfection has been developed for the treatment of corn processing wastewater (CPW) with the production of fungal biomass. Three completely mixed reactors were operated, with one reactor operated as control with a 100 μm screen fitted at the bottom. Selectively, the microscreen helped to retain fungi, whereas bacteria were washed out with the effluent due to their smaller size. The system was operated under the conditions of 2-day solids retention time (SRT) and 8-hour hydraulic retention time (HRT) at 38°C and pH 4.0. The use of hydrogen peroxide led to successful repression of bacterial growth, and the results indicated

that, compared with controls, the addition of hydrogen peroxide was able to reduce bacterial counts by at least 50% at the concentration of 60 mg/L. Accordingly, at this concentration, the selected fungus, *Rhizopus oligosporus* (ATCC No22959), has the ability to covert more than 85% chemical oxygen demand (COD) in CPW to produce 1.8 g of dry fungal biomass from a liter of CPW. Compared with the control, COD removal efficiency increased 10%, and fungal biomass increased 45%. After this point, the COD removal efficiency and fungal biomass rapidly decreased as the dosing of hydrogen peroxide increased.

Keywords: hydrogen peroxide, microscreen, COD, biomass production

Introduction

Food processing industries usually discharge substantial quantities of wastewater with high concentrations of organic materials. The high organic load in the processing wastewater creates a pollution problem and reduces water quality when discharged to rivers and lakes (Karim and Sistrunk, 1985). Traditionally, bacteria-populated aerobic or anaerobic processes are used to treat food processing wastewaters. Typically, in an activated sludge system, the organic materials are converted to carbon dioxide and relatively worthless bacterial biomass, which requires additional treatment and disposal. The stricter regulation and the increasing costs of pollution abatement are forcing food processors to find alternative methods to treat these kinds of wastewaters. In recent years, a concept based on a resource recovery approach known as recycling and utilization of useful materials from food processing waste sludge has emerged.

Food processing wastewaters usually contain appreciable amounts of useful matter such as carbohydrates, protein, starch, and lipids (Barbesgaard et al., 1992), and the

conversion of such organic matter into useful matter for human use is a useful concept. Fungi as an alternative biomass for wastewater treatment that serves simultaneously as a source of a variety of beneficial substances, such as protein, enzymes, chitin, dyes, and vitamins have recently received some interest (Bergmann et al., 1988; van Leeuwen et al., 2003). Fungi produce valuable enzymes such as cellulase and amylase and are more effective than bacteria in hydrolyzing complex carbohydrates (Friedrich et al., 1986). Fungal biomass could be a good protein supplement in feed for animals and even in human diets (Stevens and Gregory, 1987). Microbial biomass protein (MBP) production for commercial use and for production of useful biochemicals has been a very desirable aim for many researchers. Using food processing wastewater as substrate for fungal treatment would be of double benefit, as it would combine wastewater treatment with the provision of a cheap substrate for fungal cultivation.

Without presterilization, bacteria originally contained in wastewater proliferate and compete with fungi for organic substrate during treatment and therefore present a problem. These bacteria also deteriorate the dewatering characteristics of the biomass and influence the quality of the dried biomass, and some bacteria secrete toxic substances as well. To keep the costs of production and wastewater treatment down, nonaseptic conditions are desirable. Most studies in this area have been conducted on autoclaved wastewater; only a few investigations have been conducted under nonaseptic conditions (Jin et al., 1998). Development of an effective, low cost system of fungal cultivation is the main purpose of the research in this paper. A selection mechanism to favor fungi over bacteria is required for this purpose.

Environmental conditions, physiological factors, and physical properties of microorganisms could be considered as major selection conditions in biological wastewater treatment (Pretorius, 1987). Environmental and physical factors such as pH, temperature, and energy source affect the growth kinetics of microorganisms. Furthermore, specific medium conditions could be beneficial to microorganisms with specific mechanisms (Jin et al., 2001). Microorganism growth is usually very sensitive to different pH value, and the effect of pH on fungal growth has been investigated by numerous researchers (Jin et al., 1999; Riscaldati et al., 2000), leading to the conclusion that the pH causes significant changes in the specific growth rate of fungi. Temperature is also an important factor in the growth rate of microorganisms. Temperature affects the metabolism regulation mechanisms of the enzymatic reactions and the cell permeability, each microorganism has a different optimal temperature (van Leeuwen et al., 2002). Physical properties of microorganisms such as mass and size can be also used as a dynamic selection tool for two or more species in a single reactor. Since the sizes of bacteria and fungi are quite different (bacteria are 0.5 to 2 μm in diameter, while fungi are > 5 μm in diameter and have filament lengths of several hundred micrometers), the idea that a microscreen, with an aperture size between the size of the fungi and the size of the bacteria, can separate fungi and bacteria was conceived, applied, and further developed by several researchers (van Leeuwen et al., 2003). In this study, a single reactor coupled with a microscreen having an aperture size of 100 μm (smaller than fungi and larger than bacteria) was designed. The completely mixed culture is continuously passed through the screen so that fungi can be retained in the reactor and bacteria can be washed out. Fungal domination can be enhanced under nonaseptic conditions by employing different solid retention times (SRT) for fungi and bacteria.

The SRT of the fungi is controlled by retaining the fungi, while the hydraulic retention time (HRT) in the reactor controls the bacterial retention time. Previous work of this research group determined optimal SRT, HRT, pH, and temperature for this wastewater and specific fungal species (van Leeuwen et al., 2002). However, none of these control parameters was able to completely eliminate bacteria, and to discourage bacterial growth, the HRT and pH values have to be maintained at values that do not coincide with optimal growth conditions for the fungi.

Some form of selective disinfection would be one way of enhancing a dominant culture under nonaseptic conditions. Disinfection refers to the partial destruction of undesirable organisms without complete sterilization. Disinfection usually refers to the inactivation of disease-causing organisms. Commonly, four types of disinfection (chemical agents, physical agents, mechanical means and radiation) have been described, based on their different mechanism (Metcalf and Eddy, Inc., 2002). Disinfection to control bacteria in water and wastewater treatment has been used successfully. Selective disinfection with chlorine (Jenkins et al., 1982) and ozone (van Leeuwen, 1988) to discourage the growth of filamentous bacteria has been studied and found to work well (Jenkins et al., 1982; van Leeuwen, 1988). Van Leeuwen (1989) found that fungi in an industrial activated sludge system were much more resistant than bacteria to ozone and resultant fungal bulking could not be controlled with ozonation. Camel and Bermond (1998) also observed that fungi are more resistant to ozone than bacteria, so this may be the key to preventing bacterial growth within a fungal cultivation system. The aim of this study was to find a more effective disinfectant to inactivate bacteria and without significantly inhibiting fungi, and to determine

an optimal dose for such a disinfectant. Given the unusual challenge of continuous ozonation at such low dosages, tests were conducted with hydrogen peroxide.

Materials and Methods

Microbial Strain

The fungal species *Rhizopus oligosporus* [American Type Culture Collection (ATCC) No. 22959, Rockville, MD] were used in this investigation. This strain was characterized as fast growing and has been well-known in the food processing industry (Manjunath et al., 1983). This culture was maintained in potato dextrose agar (PDA) slants at 4°C.

Inoculum Preparation

Potato dextrose agar (PDA) was used to grow *Rhizopus oligosporus* cultures on slant and/or petri dishes. Frozen cultures obtained from the Fermentation Facility, ISU, were thawed at room temperature and transferred to several potato dextrose slants. The slants were incubated at 28°C for 4 to 7 days for the culture to grow. Spores were harvested from the surface of each slant into 10 mL of sterile water. This suspension was used as inoculum in all studies. The suspension contained approximately 5.9×10^6 spores per milliliter, based on a hemacytometry count.

Culture Media

Corn processing wastewater from Archer Daniels Midland (ADM), Clinton, Iowa, was investigated as an organic substrate in this study. On a weekly basis, the wastewater was adjusted to pH 5.0 and stored at 4°C. The wastewater characteristics vary between batches, as shown in Table 1.

Table 1. Wastewater characteristics.

Characteristic	Wastewater	Sterilized Wastewater
TCOD (mg/L)	1870 to 3470	1650 to 3080
SCOD (mg/L)	1690 to 3190	1570 to 2820
pH	5.37 to 6.15	5.66 to 7.57
TSS (mg/L)	250 to 300	200 to 250
VSS (mg/L)	150 to 200	100 to 180
BOD_5 (mg/L)	1100 to 2200	1100 to 2200
Total N (mg/L)	TKN = 30 to 78	TKN = 34 to 80
	NH_3-N = 0.5 to 4	NH_3-N = 1.0 to 3.9
Total P (mg/L)	3 to 32	3 to 32
Protein (μg/L)	100 to 350	100 to 350
Carbohydrate (mg/L)	~400	~350
Sulfate (mg/L)	340	340
Nitrate(mg/L)	Not detectable	Not detectable
Phosphate (mg/L)	Not detectable	Not detectable
Chloride (mg/L)	2050	2050
Sodium (mg/L)	1260	1260
Potassium (mg/L)	115	115
Magnesium (mg/L)	Not detectable	Not detectable
Calcium (mg/L)	95	95

Disinfectant

The performance of different doses of the topical solution of the United States Pharmacopeia (USP) with 3% stabilized hydrogen peroxide, which is manufactured by Cumberland Swan Smyran, TN 37167, was investigated as the disinfectant in this research.

Reactor Set-up

The experiment was carried out in three parallel continuous stirred-tank reactors (CSTR) of 0.5 L working volume at as a temperature of 37 ± 0.5°C and a hydraulic retention time (HRT) of 8 hours. The reactor content was maintained in complete mixed condition by a continuous air supply at a flow rate of 1.0–1.5 L/min., and the corn processing wastewater was maintained at a temperature of 4°C to prevent biological activity during storage. The reactor was designed to house a pH electrode to provide an online pH control at 4.0. The

experimental set-up is as shown in Figure 1. The wastewater was fed at a rate of 62.5 mL/h to the CSTR, and the fungi were harvested twice per day from the effluent. Hydrogen peroxide was dosed as a 2% solution with a peristaltic pump at various dosages to two of the bioreactors.

Figure 1: Schematics of reactor set-up.

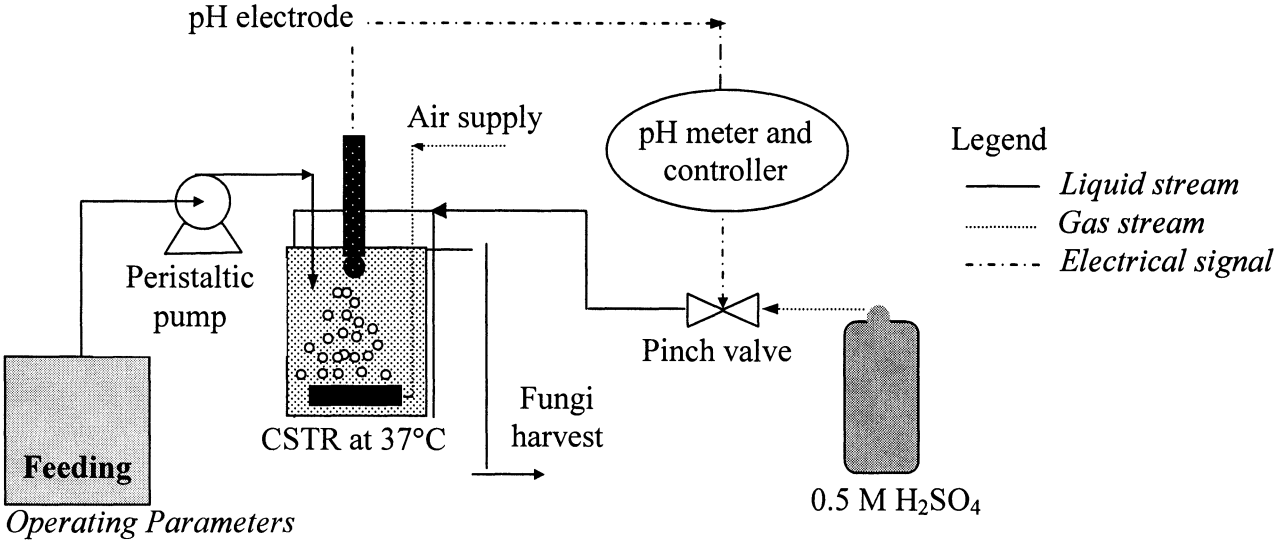


Table 2. Reactor operating conditions.

Bioreactor working volume	500 mL
Disinfectant type	Hydrogen peroxide (H_2O_2)
Disinfectant dose	Variable
Hydraulic retention time (HRT	8 hours
Solids retention time (SRT)	2 days
Influent flow rate	62.5 mL/h
Operating pH	4
Operating temperature	37±0.5°C
Air supply rate	1 to 1.5 mL/min.

Reactor Start-up

Batch cultivation tests were carried out in 250 mL Erlenmeyer flasks with 50 and 100 mL of corn processing wastewater inoculated with 3 % (v/v) spore suspension. The flasks were placed in a shaker at 150 rpm at 37°C. Each flask was inoculated with a 2 mL spore suspension (5.9×10^6 cells/mL). Significant fungal growth was noticed within a day or two. Initially, the fungi were grown in sterile wastewater and allowed to adapt to corn processing wastewater. After adaptation, the vegetative cells were transferred to reactors at equal concentration. Three CSTRs were operated in parallel, with two reactors at different disinfectant doses and one as a control reactor.

During start-up, all CSTRs were operated in batch mode. When significant fungal growth was observed in the CSTR, the system was fed continuously with raw wastewater at 1.5 L/d. The pH of 4.0 was maintained by using 0.5 N H_2SO_4. For each test run, the CSTR was allowed to operate at an SRT of 2 days. After 3 days, aiming for a 2-day SRT, harvesting of fungal biomass twice daily was started, directly from the reactor.

An SRT of 2 days was determined to be optimal, because a longer SRT (or high biomass concentration) was found to cause rapid oxygen depletion and an increase in the viscosity of the effluent. A shorter SRT may cause biomass-limiting conditions. Effluent COD was tested every 2 or 3 days, and the biomass in each CSTR was sampled regularly to examine changes in morphology with a light microscope.

Determination of Relative Fungal and Bacteria Population

To determine fungal biomass harvested from the CSTR, 100 mL of medium was filtered through a stainless steel mesh with a pore size of 100 μm, and then the biomass on the mesh was washed twice with distilled water. The supernatant was collected and filtered

again using a stainless steel mesh with a pore size of 10 μm. The collected supernatant was tested for residual bacteria population, and the bacterial biomass was measured by filtering 50 mL of filtrate (from the microscreens) through preweighed GF/C glass filters. The fungal and bacterial biomasses were dried to constant mass at 105°C for 2 hours. After weighing, the microscreen and GF/C were placed into a 550°C oven to determined the fungal and bacterial biomass expressed as volatile solids (by difference).

Specific Oxygen Update Rate (SOUR) Tests

In order to evaluate the effect of different dosages of H_2O_2 on fungal activities, a series of SOUR tests were conducted. The details of the test are outlined below:

- 5 mL of fungal biomass from the CSTR was taken to use as seed inoculum in SOUR test.
- The filtered (through 1.2 μm filter paper) corn processing wastewater was saturated with dissolved oxygen (DO) before the biomass was transferred to the BOD bottle.
- The test was conducted at 37°C, the same temperature used for the CSTR operation.
- The DO concentration was monitored at 30-second intervals until the BOD in the bottle reached 2.0 mg/L.
- A DO versus time curve was plotted for each case, and SOUR was determined using the following formula $SOUR = {}^{mg\,O_2}\!/_{g\,VSS - hr}$

Analytical Methods

The wastewater received from ADM was analyzed for chemical oxygen demand (COD), biological oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended

solids (VSS), and total phosphorus (TP) were measured according to Standard Methods for the Examination of Water and Wastewater; Total Kjeldahl Nitrogen and Ammonia.

Results and Discussion

Effect of Nutrient Supplementation

Batches of wastewater from the ADM plant varied widely in quality, especially in the organic strength (as COD) and nutrient content (nitrogen and phosphorus). Without nutrient supplementation, it took about 2–15 days to observe fungal growth in the reactor. However, with nutrient supplementation at a rate of 150:10:1 (COD:N:P), fungal growth was clearly visible in the reactor within 24 hours. Also, with nutrient addition, COD removal efficiency was 10–20% higher than that without nutrient supplementation.

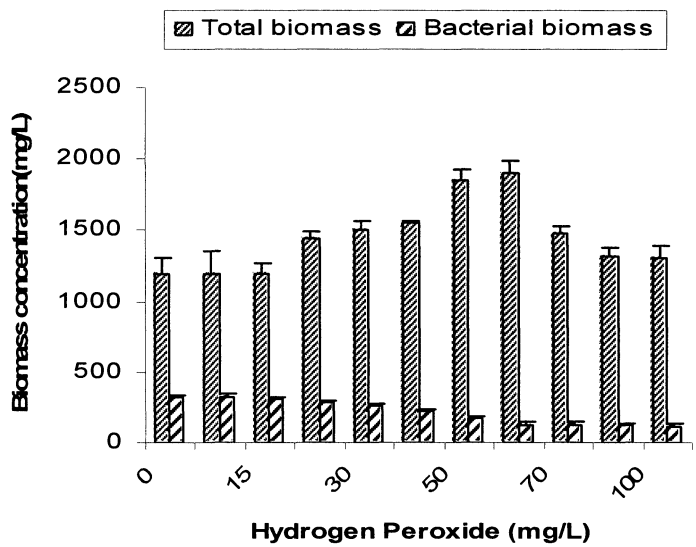


Figure 2. Total biomass and bacterial biomass at different dosages of hydrogen peroxide.

Effect of Hydrogen Peroxide on Bacterial and Fungal Growth

The results in Figure 2 indicate that the bacteria biomass concentration decreases as the dose of hydrogen peroxide increases and that, within the dose range of 0–60 mg/L, fungal biomass concentration increases as the hydrogen peroxide dose increases; if the dose is more than 60mg/L, fungal biomass concentration decreases as the hydrogen peroxide dose increases. The dose of 60 mg/L hydrogen peroxide can be regarded as the optimal dose for the fungal growth with the competition of bacteria in the culture.

COD Removal Test

Each reactor was inoculated with equal inocula of well-adapted fungal hyphae. The bioreactors were operated under the same conditions as indicated in Table 2, with a continuous dosing of H_2O_2. One of the reactors was operated as control; two other were operated at doses of 50 and 100 mg/L H_2O_2, respectively. The effluent soluble COD was measured routinely. The performance in terms of COD removal over time is shown in Figure 3.

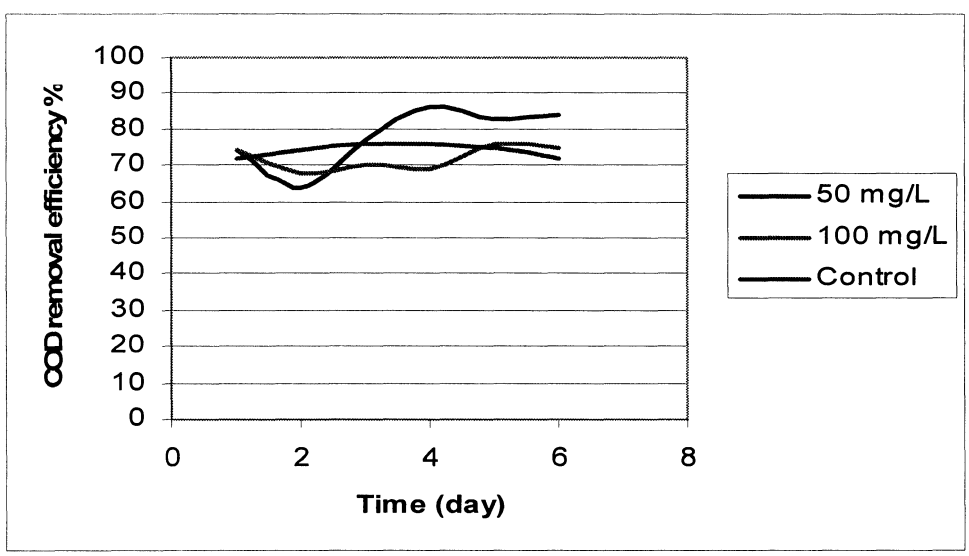


Figure 3. COD removal efficiency with time at different dosages of H_2O_2.

As seen from the figure, during the first day of H_2O_2 dosing, the COD removal efficiency declined at both 50 and 100 mg/L H_2O_2. This was likely due to inhibition not only of bacteria, but also of fungi, which needed some time to adapt to the H_2O_2. However, after 2 days of continuous operation, the COD removal efficiency improved drastically.

A more comprehensive study was conducted using 11 different dosages (10, 15, 20, 30, 40, 50, 60, 70, 75, and 100 mg/L). The soluble COD removal efficiency at different H_2O_2 dosages is shown in Figure 4. A maximum COD removal efficiency of 85% was observed at a hydrogen peroxide dosage of 60 mg/L.

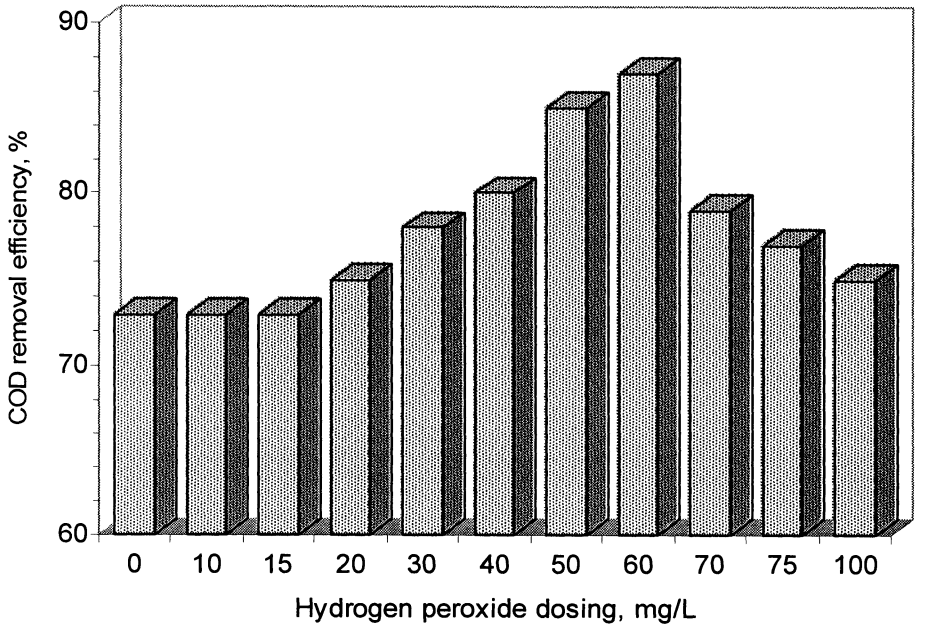


Figure 4. Combined biological COD removal at different doses of hydrogen peroxide.

H_2O_2 Contribution to COD Removal

Since H_2O_2 is a strong oxidant, part of COD removal might have been contributed by direct chemical oxidation. To estimate this contribution, a series of batch tests with different H_2O_2 dosages were conducted in 250 mL flasks at a pH of 4, temperature of 38°C, and shaker rpm of 170. Filtered sterilized effluent wastewater was used as the organic substrate. After 8 hours, the soluble COD of all samples were measured. The test results are presented in Figure 5. From the figure, it is apparent that some COD removal occurred due to H_2O_2 dosing. However, at a dosage of 60 mg/L, which was found optimal for selective disinfection of bacteria, the contribution to COD removal was only 3.6%. While this could account for one-fourth of the COD removal in the H_2O_2-dosed fungal reactor, it is quite likely that the effect during continuous processing was much lower. This statement is based on the fact that in a continuously stirred reactor, the water quality in the reactor is the same as that of the effluent.

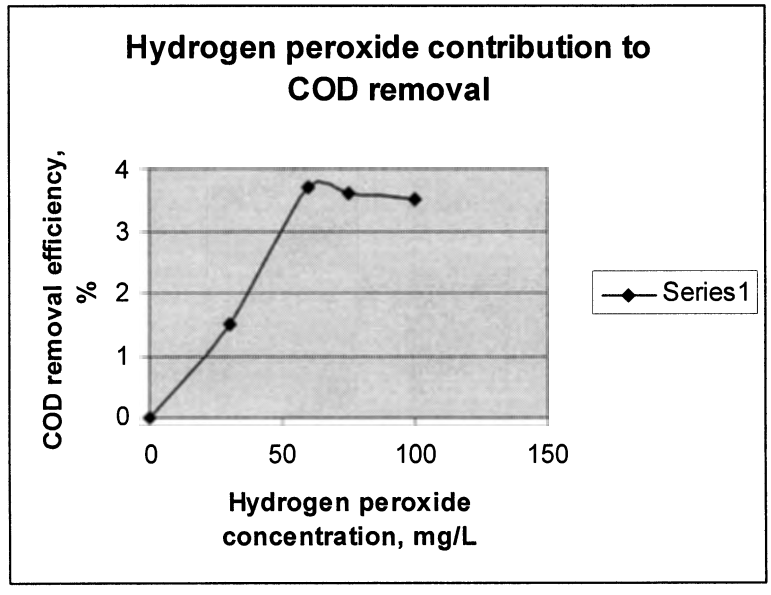
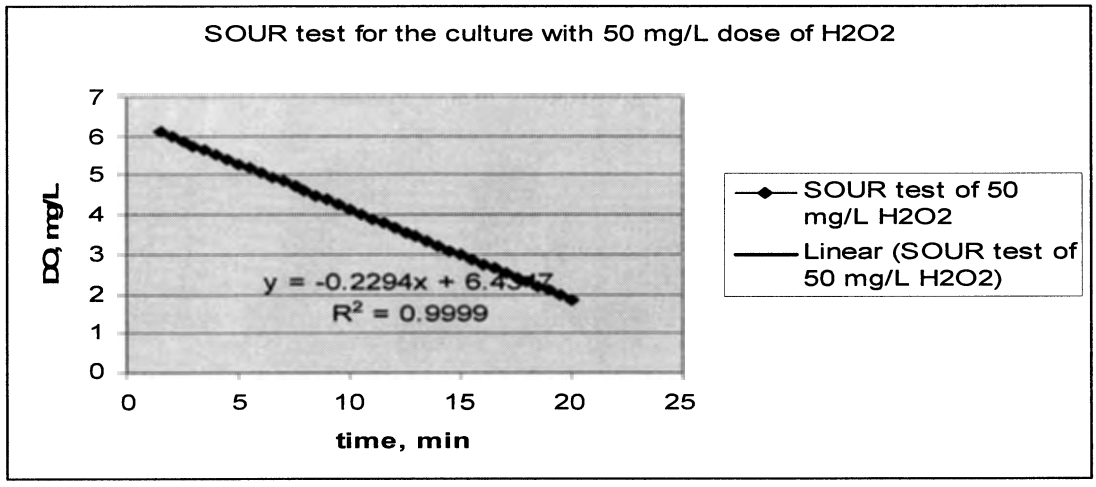


Figure 5. H_2O_2 contribution to COD removal.

SOUR Test

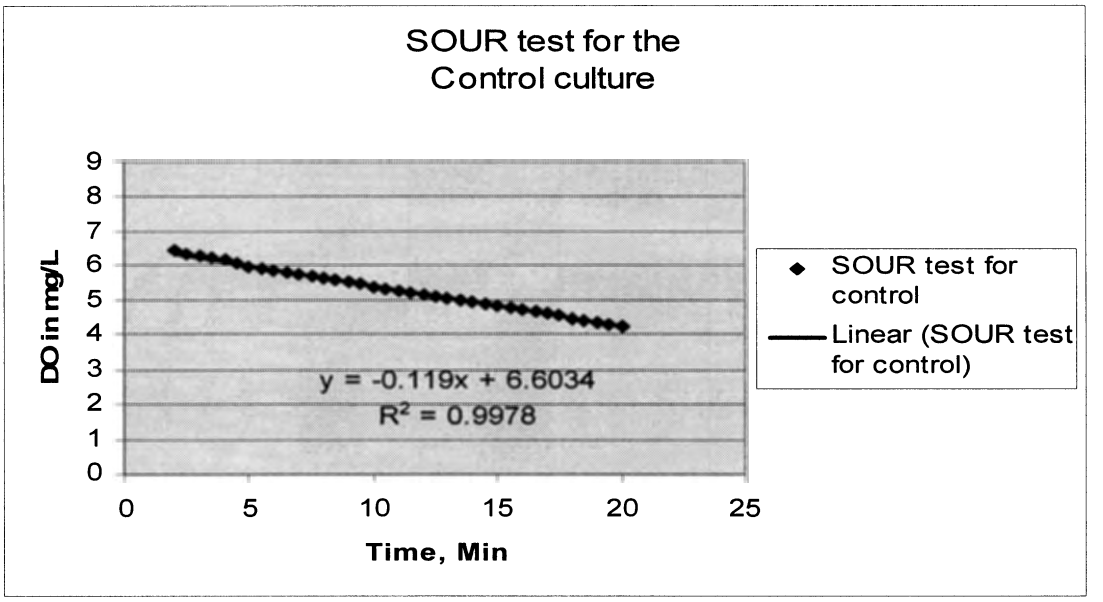
The specific oxygen uptake rate (SOUR) measures the bioactivity of fungi. The higher the SOUR value, the more active the fungi in the bioreactor. A series of SOUR tests of the fungal biomass from the bioreactor were conducted using filtered ADM wastewater. The typical oxygen uptake rates for the fungal biomass with 50 mg/L H_2O_2 and the control reactor are given at Figures 6 and 7. The results showed that the SOUR values for the fungal biomass from the reactor dosed with 50 mg/L H_2O_2 is 97 O_2/g VSS-h and 94 O_2/g for the fungal biomass from control reactor. The value is much higher than the SOUR value for bacterial biomass from an activated sludge process: the typical values for such bacterial biomass range from 30 to 60 mg O_2/g VSS-h.



*VSS = 0.142g/L,

SOUR = 0.2294*60/0.142 = 97 mg O2/gVSS-h

Figure 6. Oxygen uptake rate for fungal culture with 50 mg/L dosage of hydrogen peroxide.



* VSS = 0.076g/L, SOUR = 0.119*60/0.076 = 94 mg O2/g VSS-h

Figure 7. Oxygen uptake rate for fungal culture with 0 mg/L H_2O_2 (control).

The Effect of Wastewater Batch Variations

The characteristics of wastewater samples from ADM vary between different batches. The experiments on hydrogen peroxide selective disinfection with simultaneous enhancement of fungal production were repeated using a new batch of wastewater with a COD of 1780 mg/L. Sterile wastewater was used to start the fungal culture, with and without nutrient addition. It was found that dominant fungi grew within 20 hours in the wastewater with nutrient addition and within 30 hours in the wastewater without nutrient addition. This new batch wastewater was ideal for fungal cultivation. This was confirmed in the continuous cultivation test. The fungal biomass concentration in this wastewater (as a control) was about 1680 mg/L—higher than the fungal production in the previous work (1320mg/L). At a hydrogen peroxide concentration of 60mg/L, fungal production increased to 2160mg/L (Figure 8), also higher than in the first run.

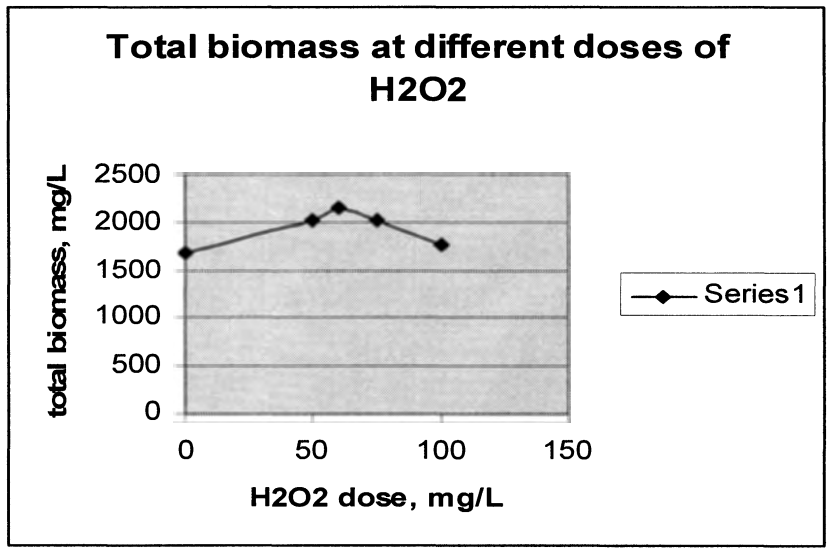


Figure 8. Total biomass concentration at different doses of H_2O_2.

The efficacy of hydrogen peroxide in bacterial control was again demonstrated, as shown in Figure 9. However, the COD removal efficiency of 81% (Figure 10) was only marginally lower than the 85% obtained in the first run at a comparable dosage of 60mg/L of hydrogen peroxide. The performance of disinfection might differ with different wastewater qualities. The disinfection model might have to be modified based on the actual situation.

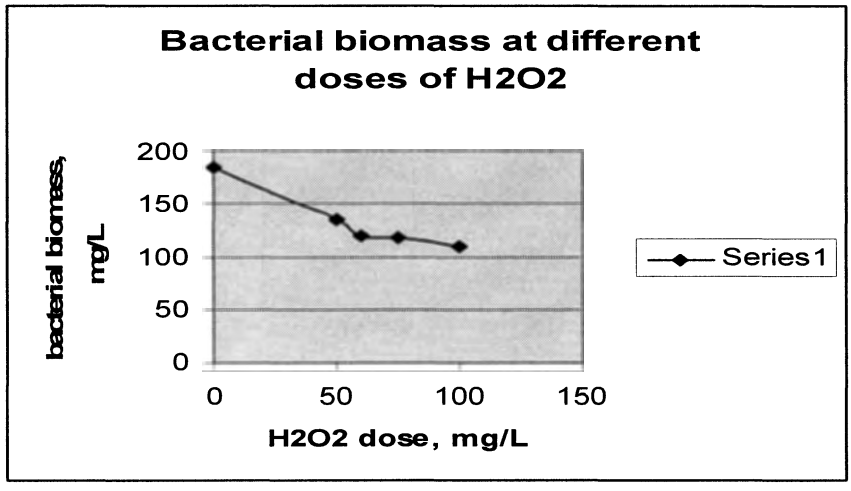


Figure 9. Bacterial biomass concentration at different doses of H_2O_2.

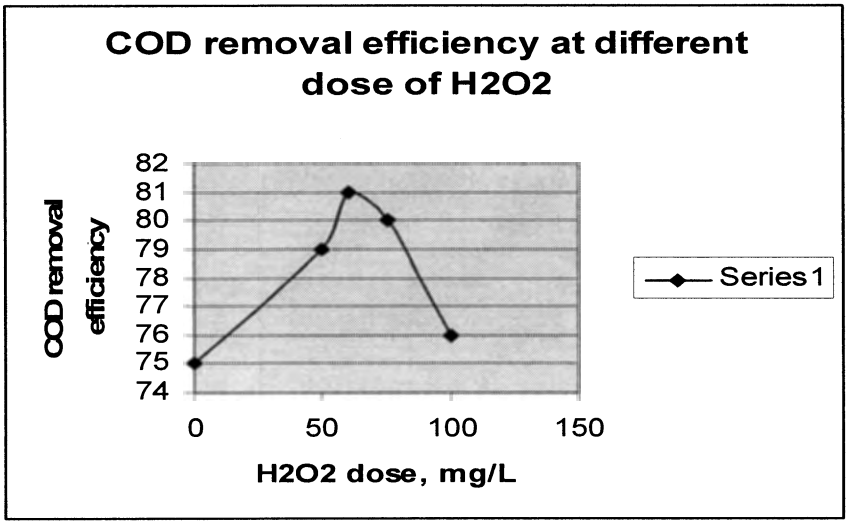


Figure 10. COD removal efficiency at different dosages of H_2O_2

Conclusions

The results indicate the selected disinfectant, hydrogen peroxide, could be used successfully for the suppression of bacteria while enhancing the production of the fungus *R. oligosporus* from CPW and cleaning up the CPW. Conclusions in respect to the single-stage process developed in this study can be drawn as follows:

1. In this system, hydrogen peroxide as a disinfectant is efficient to partially repress the growth of bacteria. With an increase of the hydrogen peroxide dosing, the counts of bacteria decrease. With a dosage of 60mg/L hydrogen peroxide, the bacteria population is decreased by 50%.
2. The optimal dose of 60mg/L hydrogen peroxide can enhance fungal production. Compared with the control, fungal biomass increased by 45%.

3. Hydrogen peroxide contributes to chemical COD removal but this is a smaller contribution that the overall COD removal in the fungal system with an optimal hydrogen peroxide dose.
4. *Rhizopus oligosporus* can adapt to corn processing wastewater with nutrient addition and also to hydrogen peroxide addition, and treat the wastewater more effectively.
5. The performance of disinfection might differ with different wastewater qualities, and the disinfection model might have to be modified based on the actual situation.
6. Substrate characteristics should be quantified and improved further to enhance fungal growth.

Acknowledgements

We gratefully acknowledge the financial support given by Archer Daniels Midland (ADM), Procter and Gamble and the United States Department Agriculture (USDA) through Iowa Biotechnology Byproducts Consortium (BBC), U.S.A.

Literature Cited

Barbesgard, P., Heldt-Hansen, H. P., and Diterichsen, B. (1992). On the safty of *Aspergillus oryzae*: A review. *Appl. Mircrobiol. Biotechnol.* **9**, 569-572.

Bergmann, F. W., Abe, J., and Hizukurii, S. (1988). Selection of microorganism which produce raw-starch degrading amylases *Appl. Mircrobiol. Biotechnol.* **23**, 443-446.

Camel, V., and Bermond, A. (1998). The use of ozone and associated oxidation process in drinking water treatment. *Wat. Res.*, **32**, 3208-3222.

Contreras, E. M., Giannuzzi, L., and Zaritzky, N. E (2000). Growth kinetics of the

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Friedrich, J., Cimerman, A. and Perdih, A. (1986) Comparison of different cellulolytic fungi for bioconversion of apple distillery waste. *Appl. Microbiol. Biotechnol.*, **24**, 432-434.

Jenkins, D., Richard, M. G., and Daigger, G. T. (1984). Manual on the causes and control of activated sludge bulking and foaming. Water Research Commission, PO Box 824, Pretoria, South Africa.

Jin, B., van Leeuwen, J. (Hans), Patel, B., and Yu, Q. (1998). Utilisation of starch processing wastewater for production of microbial protein and fungal amylsases by *Aspergillus Oryzae. Bioresource Technology* , **66**, 201-206.

Jin, B., van Leeuwen, J. (Hans), and Patel, B. (1999). Mycelial morphology and fungal protein production from starch processing wastewater in submerged culture of *Aspergillus oryzae. Process Biochemistry,* **34,** 335-340.

Jin, B., Yu, Q., and van Leeuwen, J. (Hans) (2001). A bioprocessing mode for simultaneous fungal biomass protein production and wastewater treatment using an external air-lift bioreactor. *J Chem Technol Biotechnol,* **76,** 1041-1048.

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Metcalf and Eddy, Inc. (2002) Wastewaster Engineering. McGraw-Hill, 1220.[

Pretorius, W. A. (1987). A conceptual basis for microbial selection in biological wastewater treatment. *Wat. Res.*, **21,** 891-894

Riscaldati, E., Moresi, M., Federici, F., and Petruccioli, M. (2000). Effect of pH and stirring rate on itaconate production by *Aspergillus terreus. Journal of Biotechnology* **83**, 219-230.

Stevens, C. A., and Gregory, K. F. (1987). Production of microbial biomass protein process waste by *Cephalosporim eichhorniae. Appl. Environ, Microbiol.* **53**, 824-829.

Van Leeuwen, J. (Hans), Hu, Z., Yi, T. W., and Pometto III, A. L. (2002). Use of microfungi for single cell protein production during food processing wastewater treatment. The 75th Water Environmental Federation Technical Exhibition and Conference, Chicago, IL.

Van Leeuwen, J. (1988). Domestic and industrial wastewater treatment with ozonated activated sludge. *Ozone Sci and Eng.* **10**, 3.

Van Leeuwen, J. (1989). Ozonation for nonfilamentous bulking control in an activated sludge plant treating fuel synthesis waste water. *Water SA*, **15**, 2.

Chapter 3. The Effect of Chlorination on Fungal Production from Food Processing Wastewater Treatment in a Suspended System

A paper to be submitted to

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Abstract

Rhizopus oligosporus was cultivated on corn processing wastewater in continuous reactors. Disinfection combined with a microscreen system was used to enhance the production of fungi and discourage competitive bacterial growths. The system was operated under optimal conditions for fungal growth: a pH of 4, temperature of 38°C, solids retention time of 2 days, and hydraulic retention time of 8 hours. Chlorination was tested to repress the growth of bacteria. The addition of 10 mg/L sodium hypochlorite reduced bacterial concentration by about 50% compared to control. The fungal biomass was established at 1720mg/L, and 81% COD was removed from the wastewater; these values are 39% and 6% higher, respectively, than a control without the addition of sodium hypochlorite. At dosages higher than 10 mg/L, the COD removal efficiency and fungal biomass decreased rapidly with increasing dosages of sodium hypochlorite.

Introduction

Wastewaters discharged from food processing industries are distinguished by their generally high BOD concentration. Direct disposal of such high levels of organic matter to rivers and lakes create a pollution problem (Karim and Sistrunk, 1985). Usually, aerobic or anaerobic biological treatment processes are used to remove most of the organic loading in the wastewater. Bacteria in both these systems are efficient in degrading the organic material. However, typically, in an activated sludge system, bacteria convert the organic material to carbon dioxide and relatively worthless bacterial biomass requiring additional treatment for disposal. Strict regulations as well as economic motives are driving food processors to find alternative methods to treat these kinds of wastewaters.

The high quality organic substances in food processing wastewater represent a resource that should be explored for the potential recovery of high value by-products from wastewater. Such a system would play an important role in the sustainability of the environment. With the increasing recognition of treatment technologies, fungal wastewater treatment is attracting attention since fungi are more effective than bacteria in hydrolyzing complex carbohydrates (Friedrich et al., 1986), and in addition, the fungal biomass can be a source of animal feed (Stevens and Gregory ,1987). Using food processing wastewater as substrate for fungal treatment would provide a double benefit as it would combine wastewater treatment with the provision of a cheap substrate for fungal cultivation.

Some trials combining wastewater treatment with production of useful biomass have been conducted (Bergmann et al., 1988). However, bacteria originally contained in wastewater proliferate and compete with fungi for organic substrate during treatment without presterilization. To keep the costs of production and wastewater treatment down, nonaseptic

conditions need to be acceptable. Most studies in this area have been conducted on autoclaved wastewater—only a few investigations have been conducted under nonaseptic conditions (Jin et al., 1998). An effective, low cost system of fungal cultivation is the main purpose of the research in this paper. A selection mechanism to favor fungi over bacteria is required for this purpose.

Environmental conditions, physiological factors, and physical properties of microorganisms could be considered as major selection conditions in biological wastewater treatment (Pretorius, 1987). A specific medium condition could be beneficial to a microorganism with a specific mechanism (Jin et al., 2001). For example, numerous researchers have drawn the conclusion that the pH causes significant changes in the specific growth rate of fungi (Jin et al., 1999; Riscaldati et al., 2000). Furthermore, temperature affects the metabolism regulation mechanisms of the enzymatic reactions and the cell permeability, and each microorganism has a different optimal temperature (van Leeuwen et al., 2002). In addition, physical properties of microorganisms such as their mass and size can be used as a dynamic selection tool for two or more species in a single reactor. Since the sizes of bacteria and fungi are quite different (bacteria are 0.5 to 2 μm in diameter and fungi are > 5 μm diameter with filament lengths of several hundred micrometers), the idea that a microscreen with an aperture size intermediate between the sizes of of fungi and bacteria could separate fungi and bacteria was conceived, applied, and further developed by several researchers (Pretorius, 1987; Jin et al. 1999, 2001; van Leeuwen et al., 2003).

The fungus *Rhizopus oligosporus* (ATCC No. 22959) was selected for this study, and a single reactor coupled with a microscreen with aperture size 100 μm was designed. The completely mixed culture is continuously passed through the screen so that fungi can be

retained in the reactor and bacteria can be washed out. Fungal domination can be enhanced under nonaseptic conditions with such a system by different solid retention times (SRTs) for fungi and bacteria. The SRT of the fungi can be controlled by retaining the fungi, and the hydraulic retention time (HRT) in the reactor controls the bacterial retention time. Previous work of this research group determined optimal the SRT, HRT, pH, and temperature for this wastewater and specific fungal species (van Leeuwen et al., 2002). However, none of these control parameters was able to completely eliminate bacteria, and the HRT and pH values that are necessary to discourage bacterial growth do not coincide with the optimal growth conditions for fungi.

Some form of selective disinfection would be one way of enhancing a dominant culture under nonaseptic conditions. Disinfection usually refers to the inactivation of disease-causing organisms. Commonly, four types of disinfections (chemical agents, physical agents, mechanical means, and radiation) have been described, based on their different mechanisms (Metcalf and Eddy, Inc., 2002). Disinfection to control bacteria in water and wastewater treatment has been used successfully. Selective disinfection with chlorine (Jenkins et al., 1982) and ozone (van Leeuwen, 1988) to discourage the growth of filamentous bacteria has been studied and found to work well (Jenkins et al., 1982; van Leeuwen, 1988). Van Leeuwen (1989) found that fungi in an industrial activated sludge system were much more resistant to ozone than bacteria, and the resultant bulking due to fungi could not be controlled with ozonation.. This differential sensitivity to the disinfection agent may be the key to preventing bacterial growth within a *fungal* cultivation system. The aim of this study was to find a more effective disinfectant to inactivate bacteria while not significantly inhibiting

fungi and to determine an optimal dose for such a disinfectant. Given the unusual challenge of continuous ozonation at such low dosages, tests were conducted with chlorine.

Materials and Methods

Microbial Strain

Fungi of the species *Rhizopus oligosporus* [American Type Culture Collection (ATCC); No. 22959, Rockville, MD, U.S.A] were used in this investigation and maintained in potato dextrose agar (PDA) slants at 4°C. This strain was characterized as fast growing and is well-known in the food processing industry (Manjunath et al., 1983).

Preparation of Inoculum

Phialospore suspensions were prepared from potato dextrose agar (PDA) slants on Petri dishes. The slants were incubated at 28°C for 4 to 7 days for the culture to grow. Spores were harvested from the surface of each slant into 10 mL of sterile water. This suspension was used as the inoculum in all studies. The suspension contained approximately 5.9 x 10^6 spores per milliliter as determined by hemacytometer count.

Culture Media

Wastewater samples for this study were obtained from the cornstarch industry, Archer Daniels Midland (ADM), Clinton, Iowa. The characteristics of wastewater samples are variable between each run, so that the COD of this wastewater varied from 1690 mg/L to 3190 mg/L, and the pH varied from 5.37 to 6.15. On a weekly basis, the wastewater was adjusted to pH 5.0 and stored at 4°C. The wastewater characteristics are given in Table 1.

Table 1. Wastewater characteristics.

Characteristic	Wastewater	Sterilized Wastewater
TCOD (mg/L)	1870 to 3470	1650 to 3080
SCOD (mg/L)	1690 to 3190	1570 to 2820
pH	5.37 to 6.15	5.66 to 7.57
TSS (mg/L)	250 to 300	200 to 250
VSS (mg/L)	150 to 200	100 to 180
BOD_5 (mg/L)	1100 to 2200	1100 to 2200
Total N (mg/L)	TKN = 30 to 78	TKN = 34 to 80
	NH_3-N = 0.5 to 4	NH_3-N = 1.0 to 3.9
Total P (mg/L)	3 to 32	3 to 32
Protein (μg/L)	100 to 350	100 to 350
Carbohydrate (mg/L)	~400	~350
Sulfate (mg/L)	340	340
Nitrate(mg/L)	Not detectable	Not detectable
Phosphate (mg/L)	Not detectable	Not detectable
Chloride (mg/L)	2050	2050
Sodium (mg/L)	1260	1260
Potassium (mg/L)	115	115
Magnesium (mg/L)	Not detectable	Not detectable
Calcium (mg/L)	95	95

Disinfectant

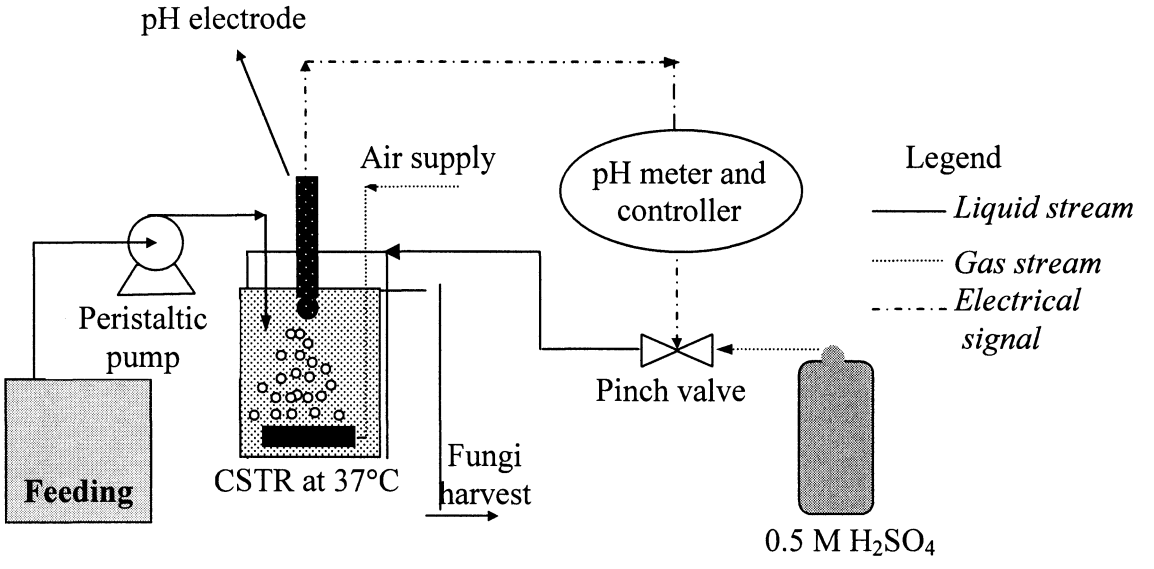
The performance of different doses of a solution of sodium hypochlorite, which is manufactured by Cumberland Swan Smyran, TN 37167, was investigated as the disinfectant in this research.

Reactor Set-up

The experiment was carried out in continuous stirred-tank reactors (CSTR) of 0.5 L working volume at a temperature of 37 ± 0.5°C and a hydraulic retention time (HRT) of 8 hours. The reactor content was maintained in completely mixed condition by a continuous air flow at a rate of 1.0–1.5 L/min., and the corn processing wastewater was maintained at a temperature of 4°C to prevent biological activity during storage. The reactor was designed to house a pH electrode to provide online pH control at 4.0. The wastewater was fed at a rate of

62.5 mL/h to the CSTR, and the fungi were perpetually harvested from the effluent. The experimental set-up is shown in Figure 1.

Figure 1. Schematics of reactor set-up.



Operating conditions

The important operating parameters of the CSTR are shown in Table 2.

Table 2. Reactor operating conditions.

Bioreactor working volume	500 mL
Disinfectant type	Sodium hypochlorite
Disinfectant dose	Variable
Hydraulic retention time (HRT)	8 hours
Solids retention time (SRT)	2 days
Influent flow rate	62.5 mL/h
Operating pH	4
Operating temperature	37 ± 0.5°C
Air supply rate	1 to 1.5 mL/min.

Batch Cultivation Optimization

Batch cultivation tests were carried out in 250 ml Erlenmeyer flasks with 100 ml corn processing wastewater, inoculated with 3% (v/v) spore suspension, with an initial pH of 4 adjusted by 0.5 N H_2SO_4. The flasks were placed in a shaker at 150 rpm at 38°C. Each flask was inoculated with a 2 ml spore suspension (5.9×10^6 cells/ml). Significant fungal growth was noticed within a day or two.

Reactor Start-up

Initially, the fungi were grown in sterile wastewater to allow them to adapt to the wastewater. After adaptation, the vegetative cells were transferred to reactors at equal concentration. Three CSTRs were operated in parallel, with two reactors at different disinfectant dose rates and one as a control reactor.

During start-up, all CSTRs were operated in batch mode. When significant fungal growth was observed in the CSTR, the system was fed continuously with raw wastewater at 1.5 L/d. The pH of 4.0 was maintained with 0.5 N H_2SO_4. For each test run, the CSTR was allowed to operate at an SRT of 2 days. After 3 days, based on a 2-day SRT, harvesting of fungal biomass directly from the reactor was begun.

An SRT of 2 days was determined to be optimal (van Leeuwen, 2003). Two of the three CSTRs were dosed with different doses of disinfectant, whereas the third was operated as a control. At intervals of 2 or 3 days, effluent COD was tested. Also occasionally, samples from the CSTR were observed under a microscope to examine the change in their morphology.

Determination of the Relative Fungal and Bacterial Concentration

After harvesting from the CSTR, fungal biomass was determined by first filtering 100 mL of medium through a stainless steel mesh with pore size of 100 μm, and then washing the biomass on the mesh twice with distilled water. The supernatant was collected and filtered again through a stainless steel mesh with pore size of 10 μm. The collected supernatant was then tested for residual bacterial population. The bacterial biomass was measured by filtration of 50 mL of filtrate (from the microscreen) through preweighed GF/C glass filters. The bacterial and fungal biomasses were both dried to constant mass at 105°C for 24 hours. After weighing, the microscreen and GF/C were placed into a 550°C oven to determine the fungal and bacterial biomass expressed as volatile solids (by difference).

Analytical Methods

Chemical oxygen demand (COD), biological oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), Total Kjeldahl Nitrogen and Ammonia were determined according to Standard Methods for the Examination of Water and Wastewater;

Results and Discussion

Effect of Chlorination on Bacteria Growth

Since the main purpose of this study is to find out disinfectants that can inhibit bacteria growth while enhancing fungal production, the first concern would be whether chlorine would be effective against bacteria. Figure 1 shows the effect of chlorination on bacteria. Chlorine, dosed as sodium hypochlorite, was seen to be effective in repressing bacterial growth as the addition of sodium hypochlorite resulted in a substantial decrease in bacterial population.

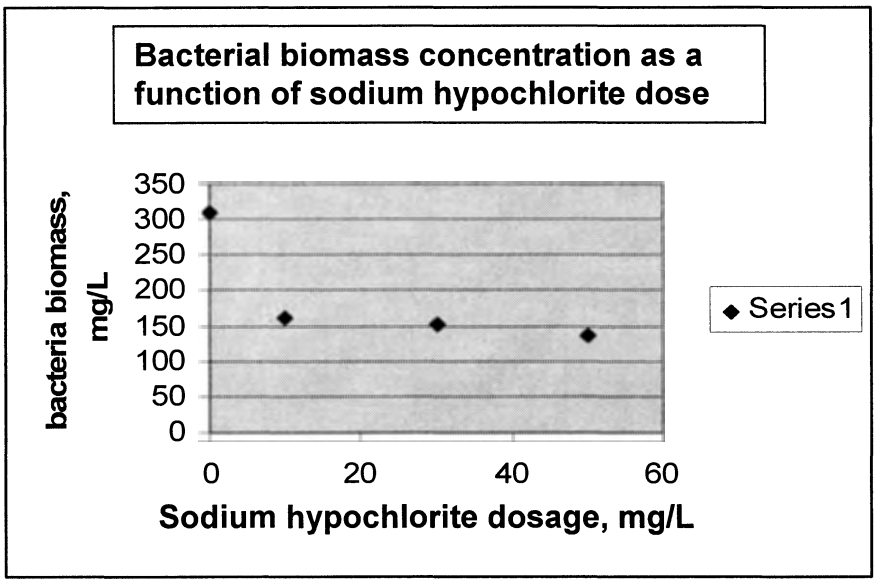


Figure 1. Bacterial biomass at different sodium hypochlorite doses

Effect of Sodium Hypochlorite on Fungi

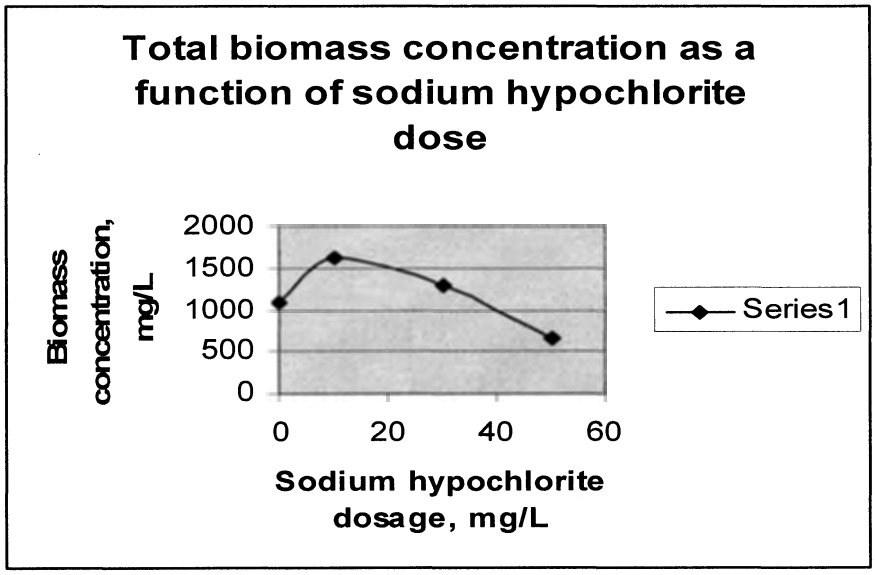


Figure 2. The effect of chlorination on fungi growth

The effect of chlorine on fungal growth, shown in Figure 2, is also important. Of the four points of sodium hypochlorite doses tested, 10mg/L yield the highest total biomass.

Beyond 10 mg/L, total biomass decreased rapidly as the dosage increased. While the total biomass level in the bioreactor was the highest (Figure 2) at a dosage of 10 mg/L, the bacterial concentration was the lowest (Figure 1) at that dosage.

Since the selected range of sodium hypochlorite was too broad, the 10mg/L sodium hypochlorite is only considered a near-optimal dose for controlling bacterial contamination during nonaseptic treatment.

Effect of Chlorine on COD Removal

The effect of chlorine on COD removal efficiency was studied, and it was found that biological treatment during chlorination at 10 mg/L effected was an increased COD (see Figure 3). Above 10 mg/L, as sodium hypochlorite dose increased, the COD removal efficiency decreased.

Since sodium hypochlorite is a strong oxidant, part of the COD removal may have been due to direct chemical oxidation. To estimate this contribution, a series of batch tests were conducted in 250 mL flasks at a pH of 4.0, a temperature of 38°C, and a shaker rpm of 170 at different sodium hypochlorite doses (from 10mg/L to 300 mg/L). After 8 hours, the soluble COD of all samples was measured. From Figure 4, it is apparent that the COD removal was less effective at sodium hypochlorite dosages above 10 mg/L. At an SCOD concentration of 2780 mg/L, only about 50 mg/L, or 1.8% of COD removal, was contributed by chemical oxidation.

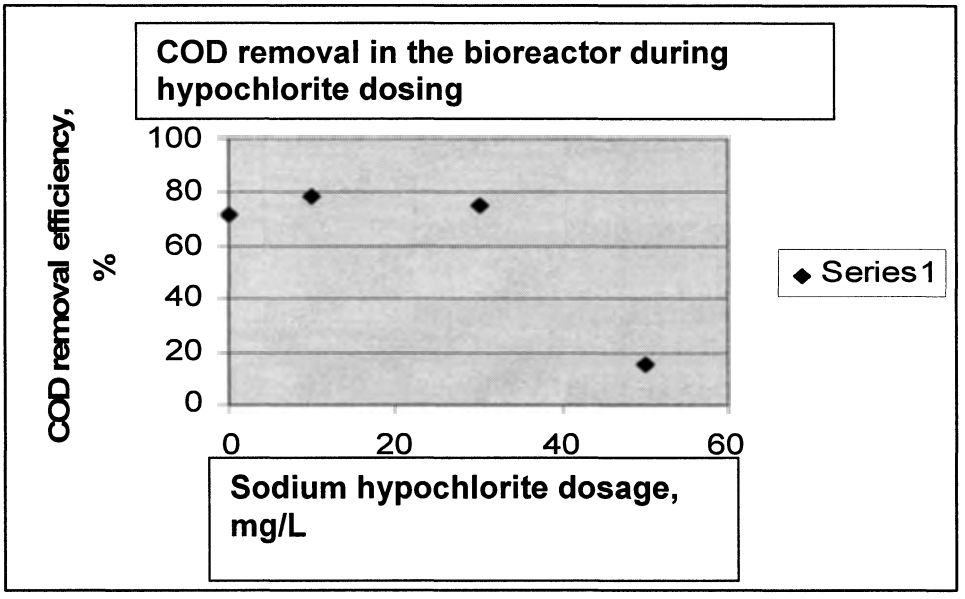


Figure 3. COD removal efficiency at different doses of sodium hypochlorite.

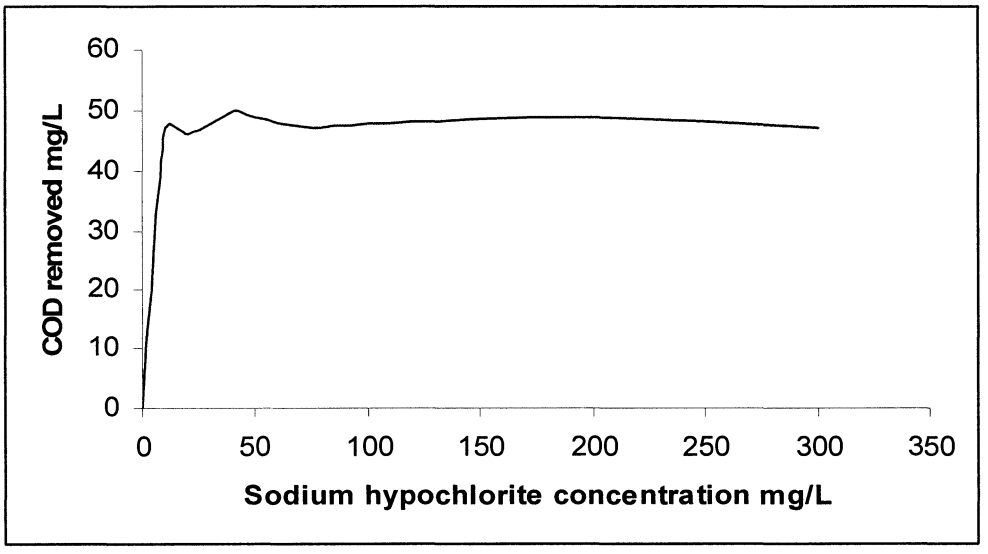


Figure 4. Sodium hypochlorite contribution to COD removal by direct oxidation.

Conclusions

The feasibility of using chlorine as a selective disinfectant, combined with a microscreen separation system, to enhance fungal production using wastewater as a substrate was demonstrated by growing *R. oligosporus* in nonaseptic culture. Chlorination effectively repressed bacteria growth and enhanced fungal production. General conclusions based on this research are as follows:

1. Chlorination effectively repressed bacterial growth. With the increase of the sodium hypochlorite dosing, the population density of bacteria decreased. At a dosage of 10mg/L sodium hypochlorite, the bacterial population is reduced by more than 50%.
2. A dosage of 10 mg/L of sodium hypochlorite enhanced fungal production. Compared with the control, fungal biomass increased by 45% at this dosage. Chlorine dosages close to around 10 mg/L have not been studied so this value is not optimized.
3. The sodium hypochlorite contribution to COD removal by direct oxidation is much smaller than the increase in COD removal by the combined effect of chlorination and fungal treatment when compared to a control of fungal treatment without chlorination.

Acknowledgements

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Literature Cited

Bergmann, F. W., Abe, J., and Hizukurii, S. (1988). Selection of microorganism which produce raw-starch degrading amylases *Appl. Microbiol. Biotechnol.*, **23**, 443-446.

Contreras, E. M., Giannuzzi, L., and Zaritzky, N. E (2000). Growth kinetics of the filamentous microorganism *Sphaerotilus natans* in a model system of a food industry wastewater. *Wat. Res.*, **34**, 4455-4463.

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Jin, B., van Leeuwen, J. (Hans), Patel, B., and Yu, Q. (1998). Utilisation of starch processing wastewater for production of microbial protein and fungal amylsases by *Aspergillus Oryzae. Bioresource Technology*, **66**, 201-206.

Jin, B., van Leeuwen, J. (Hans), Patel, B. (1999). Mycelial morphology and fungal protein production from starch processing wastewater in submerged culture of *Aspergillus oryzae. Process Biochemistry,* **34,** 335-340.

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Pretorius, W.A. (1987) A conceptual basis for microbial selection in biological wastewater treatment. *Wat. Res.*, **21,** 891-894.

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Van Leeuwen, J. (H.), Hu, Z., Yi, T. W., Pometto III, A. L, Jin, B. (2003). Kinetic model for selective cultivation of microfungi in a microscreen process for food processing wastewater treatment and biomass production. *Acta Biotechnologica*, **23**, 289-300.

Chapter 4. General Conclusions

The overall objective of this research was to further develop previously researched methodology to enhance fungal production combined with corn processing wastewater treatment.

The first paper evaluated the effect of hydrogen peroxide for the above purpose. The feasibility of producing fungal biomass using corn processing wastewater as substrate was demonstrated by growing *R. oligosporus* in a nonaseptic culture. Microscreen separation systems effectively retained fungal biomass and washed out bacterial biomass from the reactor. Hydrogen peroxide was shown to effectively inhibit bacterial growth, but did not inhibit fungal growth.

An optimized dose of 60mg/L hydrogen peroxide resulted in bacterial biomass decrease of 50% compared with the control. Nutrient supplementation at a ratio of 150:10:1 (COD:N:P) was necessary because of the low nutrient content in ADM wastewater. Average COD removal efficiency was around 75% for the control in a run period of 10 days and fungal biomass production in terms of VSS was around 1300 mg/L
under operating conditions of HRT 8 hours, pH 4, temperature 38°C, SRT 2 days. At a 60 mg/L dose of hydrogen peroxide, the COD removal efficiency increased to around 85%, and VSS (fungal biomass) was 1820 mg/L. At doses above 60 mg/L, COD removal and fungal biomass decreased as the dosing of hydrogen peroxide increased. Therefore, the 60mg/L hydrogen peroxide dosage was determined to be optimal for fungal production in this run. The wastewater quality from the ADM plant varied widely from batch to batch, especially in the organic strength (as COD) and nutrient content (nitrogen and phosphorus). Comparison of hydrogen peroxide performance in wastewater samples from two different batches showed

hydrogen peroxide was effective in inhibiting bacterial growth while enhancing fungal production in both samples, but performance expressed as VSS and COD removal varied. The disinfection model may be modified based on the actual situation for actual application.

The impact of chlorine on fungal production in corn processing wastewater treatment was evaluated in the third chapter. Sodium hypochlorite was used to produce chlorine as the disinfectant in the system. Chlorination inhibited the bacterial growth. At a dosage of 10 mg/L sodium hypochlorite, bacterial population was reduced by about 55% and COD removal efficiency and fungal biomass were increased by 6% and 39%, respectively, compared to the control. Since sodium hypochlorite is a strong oxidant, part of COD removal may be due to direct chemical oxidation. A series of batch tests showed that sodium hypochlorite only contributed to 1.8% COD removal of wastewater. Of the dosages tested, 10 mg/L of sodium hypochlorite yielded the highest COD removal and fungal biomass. At doses above 10 mg/L, COD removal and fungal biomass decreased as dosing increased. Since the selected range of sodium hypochlorite was large in this study, the 10mg/L sodium hypochlorite is only considered a near-optimal dose for controlling bacterial contamination during nonaseptic treatment.

Overall, selectors for fungal growth generally include substrate, environment, and physical properties. This research further studied selective disinfection to enhance fungal production in a suspended growth system with a microscreen and showed the effectiveness of the disinfection agents.

The mechanisms of disinfection for fungal growth and their effects on fungal production and quality sustainability should be studied further.

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