BIOLOGICAL TREATABILITY AND INHIBITORY EFFECTS OF A TEXTILE WASTE CONTAINING CAPROLACTAM

by

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I. INTRODUCTION

The activated sludge process is the most commonly used and cost effective means of treating domestic and industrial waste (56). Key criteria for the successful operation of a biological treatment facility are the biological degradability and toxicity of the influent (47).

For domestic sewage, the waste has been well characterized and shown to be easily treated by biological processes. In industry, however, wastewater is usually specific to a particular process and wide variations in content and concentration can often occur between similar industries. In addition, at any given facility, hourly fluctuations and process alterations can result in a variable effluent. Therefore, biological process application in industry must first be examined individually through the use of laboratory-scale and pilot-scale studies. From these experiments, treatment performance and biokinetic constants can be obtained so that an effective treatment system can be reliably designed.

Biological treatment owes its inception to the sewage farms of the early 1900's (56). The practice involved applying sewage to suitable farmland resulting in its partial stabilization through soil adsorption and bacterial degradation. The Allied plant in Chesterfield, Virginia presently employs a land application system without pretreatment to dispose of its wastewater. The waste applied is composed primarily of caprolactam, the cyclic monomer for nylon-6. In addition to organic

carbon, the presence of nitrogen in the heterocyclic structure of caprolactam results in a waste with a high level or organic nitrogen.

Recent investigations have shown that the irrigation sites at the Chesterfield plant are being vastly overloaded with nitrogen, producing a stressed system with a high potential for failure (9). Donley (9) has also determined caprolactam to be phytotoxic and the agent responsible for the leaf-tip burn observed in the sprayed fields. Persistent spraying at the present rate could result in ground water contamination and leaching to surface waters, promoting eutrophication.

In an attempt to avoid these problems, research was started to examine the biological treatment of the wastewater prior to irrigation. Work initiated by Anderson (2) determined the waste to be biodegradable under aerobic conditions with excellent transformation of organic nitrogen to ammonia nitrogen. In addition, he was able to obtain 40 to 50 percent reductions in total nitrogen. Difficulties encountered due to evaporation from his laboratory reactors, resulting in a concentrating of biological solids, placed certain restraints on data concerning treatability and design parameters.

Anderson's (2) observation that the organic nitrogen readily mineralized to ammonia nitrogen prompted further research into the use of biological treatment as a means of reducing nitrogen loading. However, many problems are also known to exist due to excessively high levels of ammonia. The more significant of these adverse impacts include: eutrophication, toxicity to aquatic and terrestrial organisms and depletion of dissolved oxygen in receiving waters capable of

nitrification (20). In an effort to address how these problems might impact on the system presently operating at the Chesterfield Plant, Gayle (13) conducted experiments that examined field cover-crop following irrigation with the biologically treated wastewater.

The work presented in this paper is an extension of previous studies. The objectives of the study were to evaluate the potential of using activated sludge to treat the caprolactam-containing wastewater, and to track the fate of nitrogen in the wastewater during biological treatment. A two phase approach was taken to accomplish these objectives. First, batch studies were conducted to assess the wastewater's toxic and inhibitory effects on the microbial population. The information obtained from the batch experiments was used to design the second phase studies. In the second phase, continuous-flow, activated sludge reactors operating at residence times of 5, 10 and 15 days were used to evaluate the treatability of the wastewater and for the development of biokinetic constants.

II. LITERATURE REVIEW

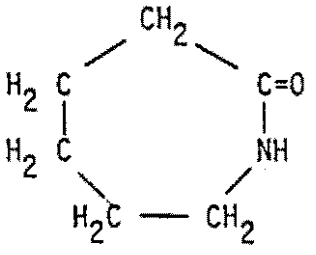
Physical and Chemical Properties of Caprolactam

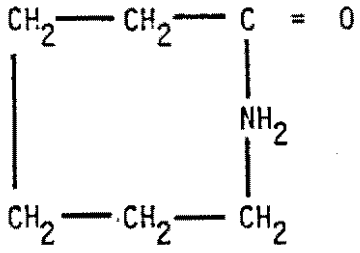
ε-Caprolactam is a major industrial chemical not naturally occurring in the environment. At present the U.S. production capacity is greater than one billion pounds per year. In the polymer industry ε-caprolactam is the most important member of the family of compounds referred to as lactams. Its uses include the production of cord, film, plastics, and resins with the bulk being used for the making of nylon-6. As a result of its large production volume and its extractability from nylon products, significant releases to the environment are likely to occur (10,33,34).

Lactams are cyclic amides with the following general formula, $\overline{R\text{-}NH\text{-}CO}$, where the R group is usually a hydrocarbon chain. In the case of ε-caprolactam the hydrocarbon chain is a five membered alkyl group yielding the molecular formula $(CH_2)_5NHCO$. Formal nomenclature of the lactams numbers the structure beginning with the ring nitrogen and continuing in the direction of the amide carbonyl. Trivial names for the lactams are derived from the corresponding open-chain amino acids (10). Table 1 illustrates the cyclic structure of ε-caprolactam and lists its physical properties.

ε-Caprolactam readily undergoes hydrolysis to yield ω-amino-caproic acid (6-amino-hexanoic acid) (Fig. 1a). This reaction occurs in the presence of mineral acids, heat, and pressure, but to the greatest extent with alkalies such as sodium or calcium hydroxides (10).

Table 1. Physical properties and structure of ε-caprolactam.

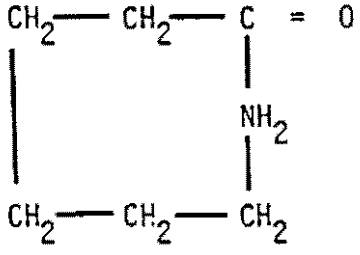
Cyclic Structure:	
Name:	formal 2-oxohexamethylenimine
	trivial ε- caprolactam
M.W.:	113.16
B.P.:	269°C
M.P.:	69°C
Form:	White crystaline solid
Solubility:	526 g per 100 g water
	very soluble in alcohol, chloroform and benzene



+ H_2O ⟶ $NH_2(CH_2)_5COOH$ [1]

ε-caprolactam ω-aminocaproic acid

Figure 1a. Hydrolysis of caprolactam.



$\xrightarrow[250°C]{H_2O}$ $[-HN(CH_2)_5CO-]_n$ [2]

ε-caprolactam polycaprolactam

Figure 1b. Polymerization of caprolactam.

Polymerization, the repetitive linking of a single compound to form a larger, more complex molecule, is one of the most important reactions of ε-caprolactam. This reaction occurs utilizing water as a catalyst at temperatures of about 250°C (Fig. 1b). In the early stages of the reaction, addition polymerization is dominant. This phase is followed by polycondensation of initial polymers, chain splitting and interchange and finally equilibrium (10,64).

Anhydrous polymerization of ε-caprolactam is also possible at elevated temperatures, catalyzed by sodium carbonate, sodium acetate, alkali and alkali earth metals, instead of water. Polymerization of ε-caprolactam is not possible without the presence of a catalyst (64).

Other significant chemical properties of ε-caprolactam are its highly hygroscopic nature and its low vapor pressure (10).

ATP and Energy Charge

In activated sludge organic matter is removed via absorption, adsorption and oxidation by biological floc. To understand and operate this type of system effectively and efficiently, a reliable means of monitoring the systems viability is essential. Currently the measurement of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) are the favored procedures because of their simplicity and lack of a reliable replacement. The validity of utilizing MLSS and MLVSS has been questioned, mainly because the measurement includes nonviable biomass, non-organic suspended solids and is slow to reflect subtle changes in biological activity. Possible

replacements for these procedures include the measurement of DNA, total protein, organic nitrogen, enzymatic activity, ATP and energy charge (EC). Of these alternatives ATP and EC seem the more promising (8,16,43,53).

The use of ATP and EC has not been extensively investigated for use with activated sludge; however, many of the test characteristics appear to make it a desirable choice. ATP is associated solely with living cells, and disappears rapidly after cell death. It has been shown to be directly proportional to both viable biomass and metabolic activity (16). ATP levels also remain relatively constant at steady state conditions, yet respond rapidly to changes in dissolved oxygen (DO), temperature, pH and substrate loading (18,43,53). In addition assay methods for ATP have become rapid, accurate and relatively convenient (16).

Energy charge (EC) is a more involved measurement, requiring not only the measurement of ATP, but also of ADP and AMP. The EC ratio has been defined as a linear measure of the amount of metabolic energy stored in the adenine nucleotide pool (19). Measurement of EC, therefore, may be useful in estimating the overall energetic state of microbial populations. Although the in situ growth rate cannot be predicted from the EC alone, it has been shown that both the rate of protein synthesis and the capacity for growth are more sensitive to the EC than to absolute levels of ATP (17,19). Energy charge is however a unitless number, which limits its usefulness in supplying information about intracellular concentrations or the rate of ATP turnover (18).

Methods for ATP extraction vary according to type of sample under analysis. Karl (18) recommends that boiling Tris buffer be used for activated sludge because it completely extracts ATP without hydrolytic loss. For any extraction procedure to be effective it must have the capacity to kill and lyse cells rapidly, release nucleotides completely, inactivate enzymes irreversibly and assure the long term stability of extracted nucleotides (18).

Several techniques for ATP measurement are in use, including chromatographic and radioisotopic procedures, but the method of choice is the firefly bioluminescence procedure due to its speed, sensitivity and reproducibility. The test uses the bioluminescent chemical, luciferin, found in the tails of fireflies and the enzyme, luciferase (18). The reactions for the emittance of light from ATP and the conversion of AMP and ADP to ATP are shown in Figure (2). The EC ratio can be calculated from the following formula:

$$EC = \frac{ATP + 1/2\ ADP}{ATP + ADP + AMP} \qquad [6]$$

In theory EC values can range from 0.0 to 1.0, corresponding to the conditions of all AMP or all ATP, respectively. In practice EC ratios of healthy, rapidly growing cells are around 0.8 to 0.9 (17). Hysert et al. (16) reported that a starved activated sludge culture had EC values ranging from 0.54 to 0.61.

Maximum levels of ATP for a bacterial culture that is 100 percent viable, has been reported to be 2 μg/mg dry weight of viable cell

1. AMP + MK + ATP ⟶ 2ADP [3]

adenosine monophosphate — myokinase — adenosine triphosphate — adenosine diphosphate

2. ADP + PK + PEP ⟶ ATP [4]

phosphokinase — phosphoenolpyrurate

3. ATP + reduced luciferin + O_2 ⟶ [5]

AMP + inorganic pyrophosphate + CO_2 + light

Figure 2. The conversion reactions for AMP and ADP to ATP and the subsequent reaction for the emittance of light.

material (16). Patterson et al. (43) found that for activated sludge fed domestic sewage, ATP concentrations were 1.8 to 2.0 and 1.4 to 1.8 µg/mg VSS in batch and continuous flow reactors, respectively.

Linearity between ATP concentration and mean cell residence time, loading rates, substrate removal and oxygen uptake was observed by Kucnerowicz and Verstraete (25). They also examined the C:ATP ratio and reported that a value of 250 was an average for microbial cells.

Interferences can occur with the bioluminescent procedure. Although luciferase is primarily specific for ATP, a number of ribose and deoxyribose nucleotides, especially guanosine triphosphate, will stimulate light emission. Also, the pyruvate kinase reaction is relatively nonspecific and will catalyze the transfer of phosphate from phosphoenolpyruvate to a number of nonadenine nucleotide diphosphates (19). In addition, inhibition of the luciferin-luciferase reaction can occur. Russel and Gauthier (53) found that phenol from a coke waste reduced light emission approximately 20 percent, while Patterson et al. (43) reported similar results with mercury.

Toxicity/Inhibition Testing Procedures

Assessing the microbial toxicity or inhibition of a waste stream is essential before undertaking more indepth and expensive biological treatability studies. This early assessment can result in the identification of toxic components in the wastewater, as well as providing information concerning treatable concentrations.

Most methods in practice involve the measurement of oxygen consumption, such as the Warburg respirometer technique. Since these procedures were originally developed for receiving waters, their direct applicability to activated sludge has been of concern (7). The majority of this concern is centered around the fact that microbial respiration can continue for some time after cell growth has stopped (1).

Broecker and Zahn (7), working with the toxicant 3,5-dichlorophenol (DCP), evaluated five toxicity tests including: oxygen consumption, oxygen consumption rate, dehydrogenase activity determined with 2,3,5-triphenyltetrazolium chloride (TTC), gas formation in fermentation tubes and inhibition of cell division in a strain of Pseudomonas.

In laboratory-scale activated sludge reactors, 5 mg/l DCP was predetermined as the toxicity limit. All tests considered obtained values close to the toxicity limit with the oxygen consumption and oxygen consumption rate predicting the exact 5 mg/l amount. Extensive replicates with the other three testing methods, revealed that they were generally not valid indicators of toxicity.

Alsop et al. (1) described a simpler technique for inhibition assessment. In this procedure turbidity measurements of those reactors being dosed with toxicant were compared against a control. The premise of this procedure is that those cultures free of toxic material will increase in turbidity, while cultures being stressed would show

reduction in turbidity. They reported a close correlation to the more involved respiration methods.

Hickman (15) utilized a series of beaker-sized batch reactors to investigate the effect of pentachlorophenol (PCP) on activated sludge acclimated to dextrose and low concentrations of PCP. Laboratory-scale reactors were established and fed one of the following three solutions: dextrose, dextrose plus 1 mg/l PCP and dextrose plus 10 mg/l PCP. In the beaker reactors, cultures were subjected to various high concentrations of PCP, and specific utilization rate was evaluated as a function of PCP dosage. He found that those cultures previously acclimated to low concentrations of PCP were more resistant to higher dosages of PCP and other closely related chlorinated organic compounds than cultures previously unexposed to the toxicant.

Inhibition/Toxicity of Caprolactam

Caprolactam, on a comparative rating scale, is considered to be a slightly toxic compound, exhibiting a low toxic potential to fish, invertebrates, plants and microbes (33). A principal reason for this is caprolactam's high solubility in water, resulting in a low octanol-water partition coefficient (K_{ow} ~ 1). The compound is therefore classified as having little ability to bioaccumulate. This was demonstrated by the lack of response shown by test organisms until LC_{50} concentrations were approached, and also by data concerning lethality response with time (33,59). Results of a static acute toxicity test are shown in Table 2.

Table 2. Results of static, acute toxicity bioassays with three species of fish and an invertebrate.[1]

	24-hour LC50 (mg/L)	48-hour LC50 (mg/L)	72-hour LC50 (mg/L)	96-hour LC50 (mg/L)
Fathead Minnow	1700	1400	1400	1400
Bluegill Sunfish	1400	1000	970	930
Channel Catfish	1600	1200	1000	1000
Daphnia magna	4100	820		

[1]LC50 data obtained from Lowengart (33).

Repetun (49), in assessing the effects of caprolactam in irrigation water, found 250 and 500 mg/l to be the maximum permissible concentrations for potatoes and cereals, respectively. He also reported that a dosage of 1000 mg/l stopped the growth of corn for three weeks. Sikka et al. (59) supported these findings by reporting no adverse effects on cucumbers, tomatoes, pinto beans, corn, ryegrass or oats at 100 mg/l. At 1000 mg/l he noted death in cucumbers with severe inhibition to the other species. Working with raw wastewater from Allied Corporation's Chesterfield, Virginia Plant which had a caprolactam concentration of approximately 10,000 mg/l, Donley (9) observed a fairly linear relationship between inhibition and increasing wastewater concentrations in corn seedling bioassay experiments. Effects on fescue and Bermudagrass under simulated irrigation conditions were also examined. He reported the evidence of leaf tip burn after 48 hours in the fescue plots at all wastewater concentrations considered, but the Burmudagrass did not appear to be effected at the levels tested.

Kundzins (26) observed swelling and increased cell membrane permeability in the algae species, Chlorella pyrenoidosa, after exposing the algae to 10,000 and 80,000 mg/l of caprolactam. Disruption of subcellular structures was also reported at the 80,000 mg/l dosage. In algal cultures grown in media containing 5,000 and 10,000 mg/l, the greatest toxic effect occurred after 96 hours. This corresponded to decreased levels of adenine containing nucleotides, uncoupling of oxidative phosphorylation and limited synthesis of organophosphorus compounds. Kundzins theorized that the

toxicity observed depended on the ratio of caprolactam molecules in the lactam-lactim tautomeric structures (27). Stupina (62) studied chlorococcal algae exposed to sewage containing 3000 mg/l caprolactam, and noted an increase in dry biomass yield, yet decreased amounts of carbohydrates and lipids. Algal cultures subjected to caprolactam concentrations greater than 8000 mg/l were inhibited.

The majority of data concerning bacterial toxicity was derived from pure cultures and naturally occurring surface waters. Fukumura (11), working with Pseudomonas aeruginosa, P. desmolytica, Achromobacter cycloclastes, Corynebacterium aurantiacum and C. roseum observed complete inhibition at concentrations in excess of 20,000 mg/l. Kinoshita (21) reported decreased degradation with increasing caprolactam concentration, with inhibition occurring at 14,000 mg/l with Achromobacter guttatus. Inhibition after exposure to 10,000 mg/l of caprolactam in Bacillus mesentericus was reported by Poi (48). He also reported that the same bacteria grown in a media containing 5000 mg/l, for five months, were capable of using caprolactam as their sole source of carbon and nitrogen.

In surface water, Sikka et al. (59) observed the highest rate of degradation at 1000 mg/l, while increasing the caprolactam to 2000 mg/l resulted in decreased microbial utilization. In a separate experiment, with glucose supplied as an additional substrate, the presence of 2000 mg/l caprolactam had no effect on the microbes ability to oxidize the glucose. Pera (44) reported no adverse impact on river microflora routinely exposed to 1000 mg/l of caprolactam.

Work by Rogovskaya (50) points out that nitrifiers appear to be one of the more sensitive types of bacteria to caprolactam. The first step in nitrification, nitrite formation, is depressed at 100 mg/l, yet a small amount of nitrite is still formed at 1000 mg/l. The second step in nitrification, nitrate formation, is reduced at 100 mg/l, with complete inhibition occurring at 500 to 1000 mg/l.

With respect to human toxicity, Gross (14) listed and described caprolactam effects on the neurological, gynecological, gastro-intestinal and cardiovascular systems. Savelova (54) reported that for normal human water consumption 30 mg/l of caprolactam would result in no health impairment.

Mechanism of Bacterial Degradation

Lowengart (33) observed in surface water, that primary degradation of caprolactam exceeded 90 percent in ten days, but only 50 percent was ultimately degraded to CO_2. He proposed the occurrence of a rapid first step, followed by slower rate-limiting reactions.

The first investigation of the bacterial utilization of lactams was carried out by Tosa and Chibata (66). They concentrated their efforts on the opening of the cyclic amides and the resulting formation of the corresponding ω-amino acids. They were able to observe this reaction using butyrolactam, valerolactam and caprolactam, referring to the enzyme responsible for the reaction as "cyclic amide hydrolase". Fukumura (12) also examined this reaction, and believed that a common enzyme was responsible for the opening of the rings, since bacteria

cultured on one lactam were capable of utilizing others. His attempts at isolating this common enzyme, however, failed. He did describe a mechanism for the reaction, which was that the ring was opened at an amide bond to give a linear oligomer, and was then split stepwise into the corresponding amino acid. Some years later, Kinoshita (22) experimented with this initial step in the bacterial degradation of lactams. He too was unsuccessful at extracting the responsible enzyme. He did theorize that since the lactams and their cyclic oligomers do not naturally occur, their breakdown is not carried out by the normally present peptidases, but is rather an acquired trait. This was demonstrated by the bacterial cultures losing their ability to utilize the compounds after being transferred to a lactam free medium, and the reappearance of the ability when reintroduced to a media containing one of the cyclic amides.

With the first step in caprolactam degradation relatively well understood, work was then focused on the subsequent reactions of bacterial oxidation. Fukumura's (11) experiments directed at examining the utilization of compounds related to caprolactam, enabled him to further speculate on the degradation process. On the basis of those studies, the pathway he proposed was as follows: caprolactam → 6-aminocaproic acid → adipicaldehydic acid → adipic acid. With some cultures he observed that transamination between 6-aminocaproic acid and 2-ketoglutaric acid could also occur. A structural representation of this degradation process can be seen in Figure 3.

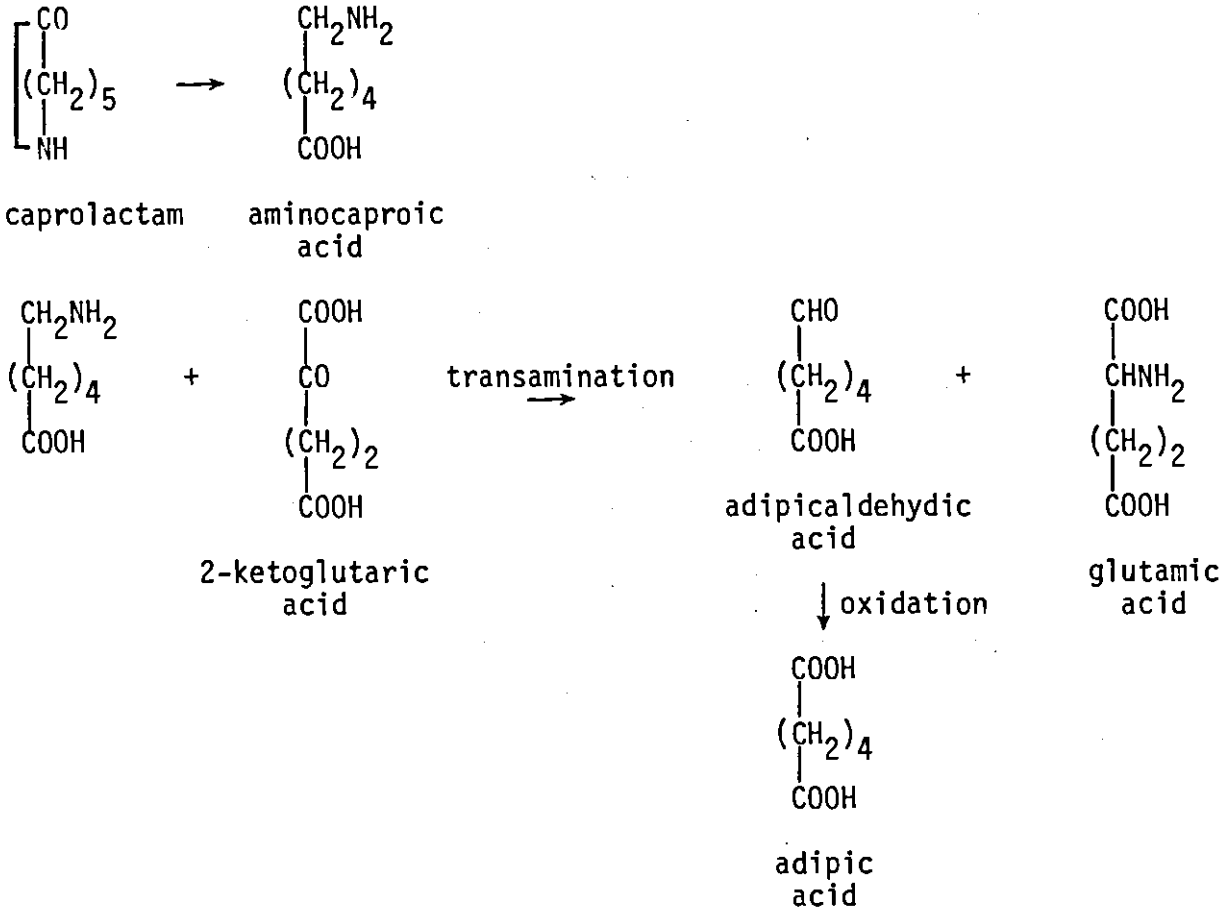


Figure 3. Proposed biodegradation pathway for caprolactam.

Naumova (39) substantiated the findings of previous researchers by developing essentially the same degradation process. He did provide an additional step, stating that the byproducts of adipic acid metabolism could be incorporated into the tricarboxylic acid (TCA) cycle. Naumova, however, offered no explanation or mechanism for this phenomenon. Anderson (2) did attempt an explanation, describing that it is possible for adipic acid to undergo β-oxidation producing the two molecules, acetyl-SCoA and succinyl-SCoA, which can directly enter the TCA cycle.

Anderson (2) also developed an important concept concerning the transamination of 6-aminocaproic and 2-ketoglutaric acid. The molecule of glutamic acid which is formed during the transamination, unless used for cellular synthesis, can undergo oxidative deamination. This reaction, therefore, is an important one regarding the transformation of organic nitrogen to ammonia. This reaction is shown in Figure 4.

Bacterial Utilization of Caprolactam

Many bacteria have been isolated that possess the ability to use caprolactam as their sole source of carbon and nitrogen. Roi et al. (51), was able to cultivate the growth of several spore forming bacteria on a solid, synthetic media containing 3000, 5000 and 11,000 mg/l concentrations of caprolactam. Kinoshita (21), working with a strain of Achromobacter guttatus observed growth at caprolactam concentrations less than 14,000 mg/l. Fukumura (11), in a very extensive study, reported optimum growth in cultures exposed to 6000 mg/l of caprolactam. The organisms he used in this study included:

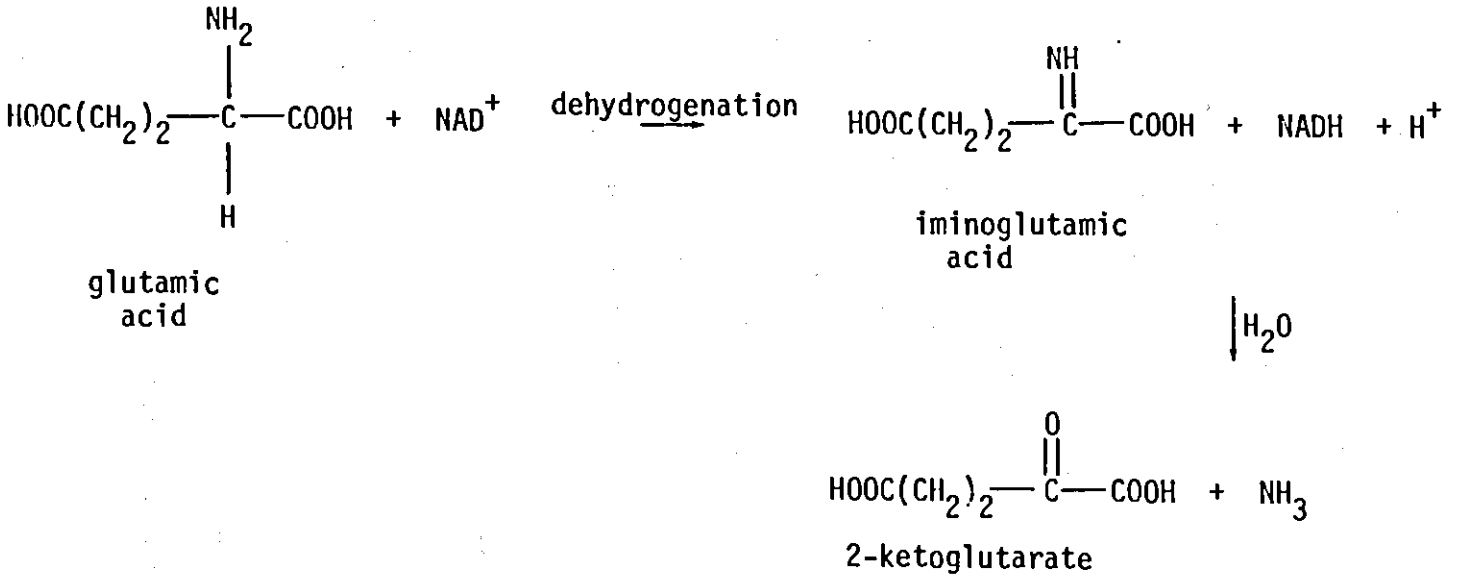


Figure 4. Formation of ammonia and 2-ketoglutarate from glutamic acid.

Pseudomonas aeruginosa, P. desmolytica, Corynebacter aurantiacum, C. roseum and Achromobacter cycloclastes.

Environmental conditions found to be the most conducive to the growth of the caprolactam degrading organisms varied among microbial species. In general, pH values ranged from 6.0 to 8.2 and temperatures were reported from 25°C to 37°C (2,4,12,21,59,66). The majority of studies specified aerobic conditions; however, Bagnyuk (4) did observe that caprolactam could be degraded anaerobically.

The effect of various utilization stimulants were examined by some investigators. Kinoshita (21), in determining if caprolactam supplied the necessary amount of nitrogen, experimented with the use of an additional inorganic nitrogen source. He observed no enhancement of growth. Additions of iron and magnesium to the basal media were reported to have stimulated growth significantly (21). Fukumura (11) noted a growth requirement for yeast extract and vitamin B1 in some bacterial cultures, concluding that the growth stimulant in yeast extract was likely vitamin B1. Rotmistrov (52) recorded increased oxidation by Bacillus subtilus in the presence of bentonite, polygoskite, montmorillonite and vermiculite.

Some studies suggest that various byproducts of the bacterial degradation of caprolactam are less readily oxidized than caprolactam itself. Fukumura (11) noted that growth on aminocaproic acid did occur, but was much poorer than on caprolactam. He believed the decrease in utilization to be due to reduced permeability of the cell membrane to the amino acid. Kinoshita (21) also observed growth on

aminocaproic acid, but was unable to establish colonies on its cyclic dimer. Levina (30), working with yeast cultures of the genus Candida, reported practically no growth in the presence of adipic or glutaric acids.

Physical and Chemical Treatment Processes for Caprolactam Removal

The majority of treatment processes directed at the removal of caprolactam are biological; however, various physical and chemical means have been tested and used. One such method has been the oxidation with air at elevated temperatures. Savelova (55), operating a reactor at 100 atmospheres, 300°C and a pH of 8.5, was able to decrease caprolactam concentration from 790 mg/l to 138 mg/l. He noted the formation of volatile acids, aldehydes and ketones following oxidation. Krysinskii (24) reported 100 percent reduction in an initial 4000 mg/l concentration of caprolactam in 30 minutes at 280 to 300°C. The oxygen requirement for complete oxidation was 9500 to 12,275 mg/l. Shurygin (57) utilized a combustion chamber which burned a mixture of natural gas and air at the chamber top, while wastewater was atomized into the bottom of the chamber. He was able to treat 150 Kg of wastewater per hour, but used 33 to 40 m^3/hr of natural gas.

Sorption experiments using bentonite (58) and activated carbon (61) have also been attempted and found to be effective for caprolactam. In static tests, the sorption capacity of activated carbon was 8 to 10 percent by weight. Sorption by activated carbon was best

at a neutral pH, with rapid decreases occurring at pH extremes in either direction.

Lastochkina (28) examined the effectiveness of a potable water treatment system on the removal of caprolactam. In a treatment facility using alum dosing followed by filtration, 9 to 20 percent of the caprolactam present was removed. The process of chlorination proved to have no effect on the caprolactam.

Wojtezak (68) discovered that films of polycaprolactam could be partially decomposed after irradiation with gamma rays or ultraviolet (UV) light. This reaction occurred in the presence of water, hydrogen peroxide and oxygen. With the aid of UV spectroscopy the breakdown products were found to be shorter caprolactam sequences with the presence of some unsaturation.

Biological Treatment of Caprolactam Containing Wastewater

Caprolactam has been shown to be relatively easy to degrade by microbial processes (42,45). As a result of those findings, research efforts and presently operating treatment facilities designed for handling waters containing caprolactam, have emphasized biological systems.

Numerous studies investigating caprolactams ability to be biodegraded have been performed. Patel and Patel (42), working with batch activated sludge reactors, addressed the question of biological treatability and removal of organics by air stripping. They obtained 88, 86 and 76 percent reductions in COD at initial COD concentrations

of 2000, 3500 and 5000 mg/l, respectively. These reductions were achieved in 24 hours at mixed liquor suspended solids (MLSS) concentrations of 3000 to 4000 mg/l. In wastewater prestripped with air prior to biological treatment, an average decrease in COD of 74 percent was noted when the initial COD concentration was approximately 2200 mg/l.

Anderson (2) examined the effectiveness of aerobic and anaerobic suspended growth treatments on a caprolactam containing wastewater. The characteristics of the wastewater were as follows: total organic carbon (TOC) 7493 mg/l, COD 34,327 mg/l, total kjeldahl nitrogen (TKN) 2040 mg/l. Results showed that anerobic treatment was ineffective, while air activated sludge demonstrated potential. In batch experiments, he evaluated the effect of pH and temperature on biodegradation, while simultaneously selecting operating conditions for continuous flow studies. For his particular culture, he determined that a pH of 8.2 and temperature of 25 ± 1°C would result in the highest effluent quality. He considered five cell residence times (θ_c), extending from 3.3 to 15.4 days, in the continuous flow studies. Reductions in TOC and COD ranged from 7.7 to 89.6 percent and 6.1 to 94.3 percent, respectively. Total nitrogen removal data showed little treatment was accomplished at θ_c's less than 8.7 days, but 47 and 57 percent decreases were observed at θ_c's of 12.8 and 15.4 days, respectively. Only trace levels of oxidized nitrogen species were detected. From these nitrogen studies, he concluded that reductions in TKN were primarily due to the volatilization of ammonia. For all parameters

measured increased treatment efficiency coincided with increasing θ_c. It was determined that a rate of evaporation of approximately 300 ml per day was occurring from the reactors. This evaporation resulted in the concentration of components in the reactors, most likely producing treatability data worse than would be expected if the rate of evaporation had not been a problem.

Tomita et al. (65), used a lab-scale activated sludge process to treat a wastewater containing 300 mg/l of caprolactam. An initial COD of 430 mg/l and BOD of 600 mg/l were reduced 45 and 97 percent, respectively.

In terms of full scale treatment systems treating caprolactam, Arnol'dov (3) reported on the performance of an activated sludge system which treated municipal sewage mixed with an industrial waste containing caprolactam. The wastewater composite had an initial caprolactam concentration of 100 mg/l, whereas effluent from the plant contained 7 mg/l caprolactam.

Shabii and Ilett (56) described a two-stage biological treatment process, specifically designed for a caprolactam polymerization plant. The primary contaminants from this facility were caprolactam and titanium dioxide. The wastewater was initially passed through a biological filter constructed of a plastic media. Wastewater flowing through the system was settled and either recycled through the filter or channeled to a contact stabilization, activated sludge system. Waste sludge was aerobically digested prior to disposal. Effluent from the treatment plant had a BOD of 10 to 54 mg/L and a COD of 20 to 236 mg/L.

Initial concentrations of BOD and COD were 700 to 3000 mg/l and 1000 to 4000 mg/l, respectively.

Several investigators suggested the need or desirability of diluting caprolactam-containing wastewaters prior to biological treatment to facilitate utilization and help prevent detrimental effects on the microbial population (31,42,46). In none of these reports is it distinctly stated what the initial caprolactam concentration was, but suggested dilution ratios ranged from 1:10 to 1:200 using water or sanitary sewage as the diluent.

Microbial Growth Kinetics

Before treatment models can be effectively used, certain parameters must be determined. For the Lawrence and McCarty model these parameters are k, K_s, Y and k_d. These coefficients can be derived from lab or pilot-scale studies.

Lawrence and McCarty (29) describe two basic equations that relate biological growth and substrate utilization and help to define each of these coefficients. The first describes the relationship between net microbial growth rate and rate of substrate utilization. This equation is written as follows:

$$\frac{dX}{dt} = Y \frac{dF}{dt} - k_d X \qquad [7]$$

where

$\frac{dX}{dt}$ = net growth rate of microorganisms per unit volume of reactor, mass/volume-time.

Y = growth yield coefficient, mass/mass

$\frac{dF}{dt}$ = rate of microbial substrate utilization per unit volume, mass/volume-time.

k_d = microorganism decay coefficient, $time^{-1}$.

X = microbial mass concentration, mass/volume.

The second equation defines the relationship between the rate of substrate utilization, the concentration of microorganisms in the reactor and the substrate concentration. This equation is written as:

$$\frac{dF}{dt} = \frac{kSX}{K_s + S} \qquad [8]$$

where

k = maximum rate of substrate utilization per unit weight of microorganisms, $time^{-1}$.

S = substrate concentration, mass/volume.

K_s = the substrate concentration at one-half the k rate, mass/volume.

Under steady state conditions equation 7 can be rewritten as follows:

$$\frac{X}{\theta_c} = Y\left(\frac{S_o - S}{\theta}\right) - k_d X \qquad [9]$$

where

θ_c = mean cell residence time, time.

θ = hydraulic detention time, time.

S_o = influent substrate concentration, mass/volume.

S = effluent substrate concentration, mass/volume.

Dividing through equation 9 by X, yields the following linear equation (36):

$$\frac{1}{\theta_c} = Y \left(\frac{S_o - S}{\theta X}\right) - k_d \qquad [10]$$

From equation 10 it can be seen that by plotting $\frac{S_o - S}{\theta X}$ versus $\frac{1}{\theta_c}$, the slope of the resulting line equals Y, while the y-axis intercept equals k_d.

Equation 8 can also be rewritten when applied to steady state conditions. The resulting equation can be written as follows:

$$\frac{S_o - S}{\theta} = \frac{kSX}{k_s + S} \qquad [11]$$

Dividing through equation 11 by X and taking its inverse yields the linearized relationship (36):

$$\frac{X\theta}{S_o - S} = \frac{k_s}{k}\frac{1}{S} + \frac{1}{k} \qquad [12]$$

From equation 12 it can be seen that a plot of $\frac{1}{S}$ versus $\frac{X\theta}{S_o - S}$, will produce a y-intercept equal to $\frac{1}{k}$ and a slope equivalent to $\frac{k_s}{k}$.

Two other relationships are important in terms of treatment system design and operation, these are specific utilization (U) and sludge age (θ_c). Specific utilization is defined as follows:

$$U = \frac{\frac{F}{t}}{X} \qquad [13]$$

where

$\frac{F}{t}$ = the amount of substrate utilized during a specified time period, mass/time.

X = the amount of microorganisms present, mass.

The θ_c can be represented by the following equation:

$$\theta_c = \frac{X}{\frac{X}{t}} \qquad [14]$$

where

$\frac{X}{t}$ = the total amount of microorganisms removed from the system, mass/time.

In a complete-mix, no-recycle system θ_c is equal to the hydraulic detention time, θ.

Deviations from the kinetic coefficients obtained from domestic waste treatment plants have been reported. Many of these changes are attributed to the treatment of known toxic and inhibitory substances. Nay et al. (40) working with a waste containing trinitrotoluene (TNT) described the inhibitory nature of the waste, evidenced by the reduced sludge growth factor (K_g) and substrate removal rate (K_r) observed with

increasing TNT concentration. Beltrame et al. (5) studied the effect of phenol on the kinetics of the activated sludge process. He reported that substrate removal rates were markedly affected by increasing influent phenol concentration. Sujarittanonta and Sherrard (63) observed variations in Y_{max} and K_d values in activated sludge cultures treating a nickel bearing waste. They were able to decrease the toxicity of the metal by increasing the COD:Ni^{2+} ratio.

III. METHODS AND MATERIALS

The experimental procedure for this study was divided into two parts. First, batch experiments were done to determine the inhibitory effect of caprolactam and thereby select an optimum concentration for biological treatment. Secondly, aerobic continuous flow, activated sludge reactors were used to evaluate the biological treatability of the wastewater and for the development of biokinetic constants.

Seed organisms were obtained from a pilot-scale, activated sludge system at the Allied Corporation Plant in Chesterfield, Virginia. This culture had been utilizing caprolactam as its sole source of carbon and nitrogen for approximately two years and, therefore, was assumed to be well acclimated. Enough sludge was removed and transferred to VPI&SU to fill three, laboratory-scale, activated sludge reactors.

The caprolactam-containing wastewater was also taken from the Allied Plant. Three, 55-gallon drums of wastewater were withdrawn at the same time from one of the plants holding ponds. Ample volume was obtained to ensure the availability of an adequate supply of wastewater to complete the study. This avoided having to sample more than once and risk the chance that the wastewater would change in composition. The wastewater was stored in lined drums at room temperature. The following characteristics were monitored frequently to ensure that the wastewater remained stable: total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), nitrate, nitrite, and ammonia.

Aerobic, Batch Experiments

Waste sludge from the three bench-scale reactors was composited to make up the biomass for the batch experiments. The composited cultures were centrifuged at 10,000 RPM for ten minutes at 20°C. The supernatant was removed and the resulting cell mass diluted to one liter. To four, 500-ml Erlenmeyer flasks, 250 ml of the biomass solution was transfered and individually aerated. To each flask, 25 ml of caprolactam standard or diluted wastewater ranging in concentration from 50 to 10,000 mg/l of caprolactam was added. In addition, a sufficient volume of a KH_2PO_4 solution was added to ensure a BOD_5:P ratio of at least 25:1. The reaction vessels were kept at room temperature (22 ± 2°C), allowed to stabilize to a pH of approximately 7.5, and aerated adequately to achieve complete mixing.

Each batch-testing period was two hours. At the beginning of each test period, samples were withdrawn for mixed-liquor suspended solids (MLSS), total adenylates, and soluble COD. At the end of the two-hour testing period, samples were again taken for total adenylates, and COD. In addition, one-hour COD samples were also taken.

Separate inhibition curves were established for both caprolactam standards and wastewater to determine if there were any other inhibitory agents present in the raw wastewater other than caprolactam. Experiments with each dilution or standard were generally duplicated, but in the more critical areas of the inhibition curves, triplicate trials were conducted.

Aerobic, Continuous-Flow Reactors

Conventional lab-scale, Plexiglas, activated sludge reactors, having volumes of 8.6 or 8.0 liters, were used for this study. The reactors were fed by (rotating and reciprocating) piston FMI pumps (Oyster Bay, New York) and (rotary) peristaltic Cole-Parmer pumps (Chicago, Illinois). Feed for each reactor was kept in individual Nalgene containers. Flow rates to each reactor were checked daily and adjusted as necessary. In addition, feed lines were periodically removed, cleaned and replaced. Air was provided by the laboratory compressed air system and was passed through glass wool and a water trap prior to entering the reactors.

The reactors were completely mixed systems operated without sludge recycle. Therefore, the hydraulic detention times and mean cell residence times were equivalent. Detention times were 5, 10 and 15 days, with residence times being varied by adjusting influent flow rate. Complete-mix conditions were attained through aeration.

The feed solution to each reactor consisted of raw wastewater diluted to provide a COD value of 2000 mg/L with distilled water. Phosphorus was added in the form of a KH_2PO_4 solution to attain a BOD_5:P ratio of approximately 25:1.

To maintain a constant temperature, the reactors were placed in a water filled aquarium and kept at 25°C by aquarium heaters. The pH in all cases stabilized at 8.0 without adjustment. Dissolved oxygen (DO) in the reactors ranged from 6.0 to 7.0 mg/L due to the high rate of aeration necessary to achieve good mixing. As a result of this high

rate of aeration, it was necessary to scrape the sides of the reactors daily to return biomass back to the tank. Plexiglas lids were placed over the reactors to help prevent the loss of reactor contents by evaporation.

Steady-state conditions were assumed to prevail when effluent COD and suspended solids (SS) concentrations remained relatively constant from seven to ten days. When steady state conditions were attained, samples were collected for a period of seven days. Analysis performed included effluent SS, soluble COD, TOC, TKN, NH_3, NO_3^-, NO_2^- and caprolactam concentration. In addition, samples for total adenylate analysis were withdrawn from the reactors.

Preliminary Aerobic, Continuous-Flow Reactor

The purpose of this experiment was to develop a system from which volatile gases could be easily recovered. The reactor was a glass cylinder in which the liquid volume was maintained at three liters. Air was provided from the laboratory compressed air system and entered at the cylinder bottom through a stainless steel sparger. The feed storage and delivery system was similar to that previously described. The temperature was maintained at 25°C by recirculating water from a heated reservoir through Tygon tubing wrapped around the cylinder.

The feed solution for this reactor consisted of undiluted wastewater, having a COD of approximately 35,000 mg/L. Phosphorus was added in the form of a KH_2PO_4 solution to attain a BOD:P ratio of about 100:2. Seed organisms were obtained from a lab-scale activated sludge

reactor that had been maintained on the wastewater for several months. The reactor was run as a completely-mixed, no-recycle system at a 20-day detention time.

Organic Carbon and Ammonia Volatilization Experiments

The nitrogen content of the raw wastewater was relatively high and was essentially all in an organic form. Because the activated sludge process is known to transform organic nitrogen to ammonia nitrogen, an ammonia volatilization experiment was attempted to determine how much nitrogen was removed by air stripping and to gain a better understanding of the nitrogen balance.

The method used was a modified version of one described by Kissel et al. (23). The apparatus consisted of a Plexiglass cylinder 20 cm tall which was clamped to a ring stand and slightly submerged into the reactor contents. Tygon tubing was attached at ports just above the water line and 7.6 cm from the cylinder top leading to an outside window and gas scrubbing bottle, respectively. In addition, an impeller was mounted on top of the cylinder to blow back aerosols created by aeration, preventing the droplets from entering the collection system. Stop-cock grease was applied to the impeller mount, and the grease, aided by the weight of the electric motor, formed an air-tight seal at the cylinder top. A vacuum pump was used to pull air through the system at a rate of 14 cc/min. A schematic diagram of the ammonia volatilization sampler can be seen in Figure 5. For each

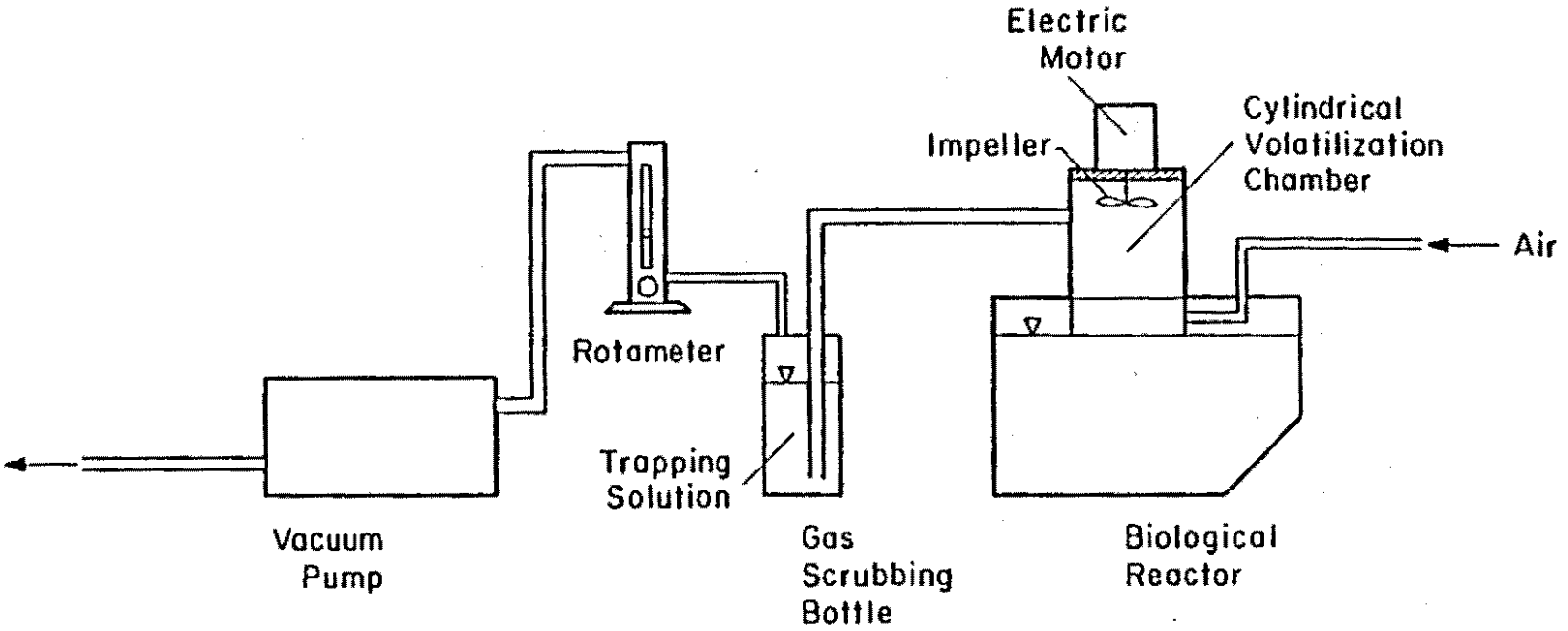


Figure 5. Schematic diagram of volatile organic carbon and ammonia recovery system.

sample, the system was run for one hour. Ammonia was trapped in a gas-scrubbing bottle containing 0.02 N H_2SO_4.

The loss of organic carbon by volatilization was also examined to see if organic loading was being reduced due to air stripping. The experimental procedure was identical to that used for ammonia with the exception of the trapping solution. Because the types of compounds that might possibly be volatilizing were unknown, four trapping solutions were evaluated, distilled water at a pH of approximately 6.0, distilled water adjusted to pH 5 and 9, and a solution composed of equal amounts of methanol and water (38). The presence of organic compounds was determined by TOC.

Analytical Procedures

Chemical oxygen demand (COD) was analyzed by the dichromate reflux method with 20 ml samples as described in Standard Methods (60). In order to obtain the soluble fraction of COD as well as the soluble portion of the other parameters measured, samples were centrifuged at 15,000 RPM for fifteen minutes followed by filtration through a 0.45 μm filter.

A Dohrmann/Envirotech Model 54 total organic carbon (TOC) analyzer was used to determine influent and soluble effluent TOC concentrations. Influent samples were diluted with distilled water to concentrations close to the carbon standard used for analyzer calibration and effluent concentrations.

Total Kjeldahl nitrogen (TKN) was measured by means of the micro-Kjeldahl method outlined in Standard Methods (60). Heat for sample digestion was provided by a gas heating mantel, and distillations were carried out on a Labcon steam distillation unit. Samples for ammonia analysis were also distilled as above, with determination of actual ammonia concentrations for both procedures performed using the titrimetric method. To obtain greater sensitivity for the lower ammonia concentrations obtained in the volatilization experiments, the phenol-hypochlorite test described by Weatherburn (67) was employed. This is a colorimetric procedure that requires two reagents: phenol plus sodium nitroprusside and sodium hydroxide plus sodium hypochlorite, to react with the ammonia, producing a pale yellow color. Absorbance was measured on a Baush and Lomb Spectronic 20 at 625 μm.

Nitrate and nitrite together comprise the nitrogen fraction referred to as "total oxidized" nitrogen and are not included as part of the TKN measurement. Both species were determined in accordance with Standard Methods (60), nitrate by the brucine method and nitrite by the azo-dye technique.

Both raw and treated wastewater were exceedingly difficult to filter, even under a high vacuum with Whatman 40, fast-filtering filter paper. The presence of caprolactam polymers and colloidal particles were believed to be responsible for plugging the filters. Because of this poor filterability, SS could not be determined directly. Instead, total solids and total dissolved solids were determined and the difference in the two measurements was assumed to be the SS concentration.

Dissolved solids samples were obtained by centrifugation and filtration as described for soluble COD samples. For the continuous-flow experiments, 20 ml of sample were used to make these two solids determinations, while 10 ml samples were used in the batch experiments. Both measurements were made in accordance with Standard Methods (60).

Caprolactam concentrations were measured by gas chromatography using a Hewlett Packard Model 5880A coupled with an integrator. Two different columns were used for the analysis. The first column was packed with one percent SP 1240 DA, a polyester packing deactivated for acidic compounds. Oven and injection port temperatures were set at 170°C and 120°C, respectively; and the carrier gas flow was 30 cc/min. This column began breaking down, producing inconsistent results and was replaced. The replacement column was packed with Alltech CS-8, a polar cyanopropyl silicone packing. Oven and injection port temperatures were set at 250°C and 220°C, respectively; and the carrier gas flow was adjusted to 40 cc/min.

Dissolved oxygen (DO) and pH were regularly checked in each of the continuous flow reactors. A Fisher Acumet, Model 230, pH/ion meter and a Yellow Springs Instrument, Model 54A, dissolved oxygen meter were used to make these measurements.

Adenosine triphosphate (ATP) and energy charge (EC) were obtained using one ml samples of MLSS and heat deactivating the samples for five minutes in 9 ml of boiling Tris buffer. This deactivation step extracts the adenine nucleotides from the cells, while simultaneously killing the cells and preventing further metabolic activity. This

solution was cooled, placed in a sterile vial and frozen until needed for analysis. The procedure used to convert ADP and AMP to ATP and the subsequent measurement of ATP was described by Karl and Holm-Hansen (19). An SAI, Model 2000, ATP Photometer, which provides a digital readout of peak height, was used in making the ATP determinations. Two-hundred microliters of sample were pipetted into the photometer sample tube, and peak height was measured within three seconds. A 0 to 3-second peak height measurement is preferred over one made after longer periods because of its reproducibility and its capacity to reduce interference from non-adenine containing nucleotide triphosphates (19).

IV. RESULTS AND DISCUSSION

As previously mentioned, the experimental procedure for this study was divided into two sections. The first consisted of batch experiments to determine the wastewater's potential for biodegradation as well as to obtain information concerning treatable wastewater concentrations. These experiments were conducted both before and after the continuous-flow studies to examine their reproducibility and the effect of prolonged exposure to the wastewater on the microbial population. In the second section, continuous-flow activated sludge reactors were utilized to evaluate the wastewaters suitability for biological treatment.

Batch Reactor Studies

Inhibition curves were developed for both the raw wastewater and caprolactam standard solutions. These were established by plotting specific utilization rate versus effluent chemical oxygen demand (COD). Specific utilization rate (q) is defined as follows:

$$q = \frac{ds/dt}{X} \qquad [15]$$

where

ds/dt = change in substrate per unit time, mg/L/time

X = MLSS, mg/L

The curve derived with caprolactam standards reproduced fairly well, although Figure 6 suggests that the standards were somewhat more

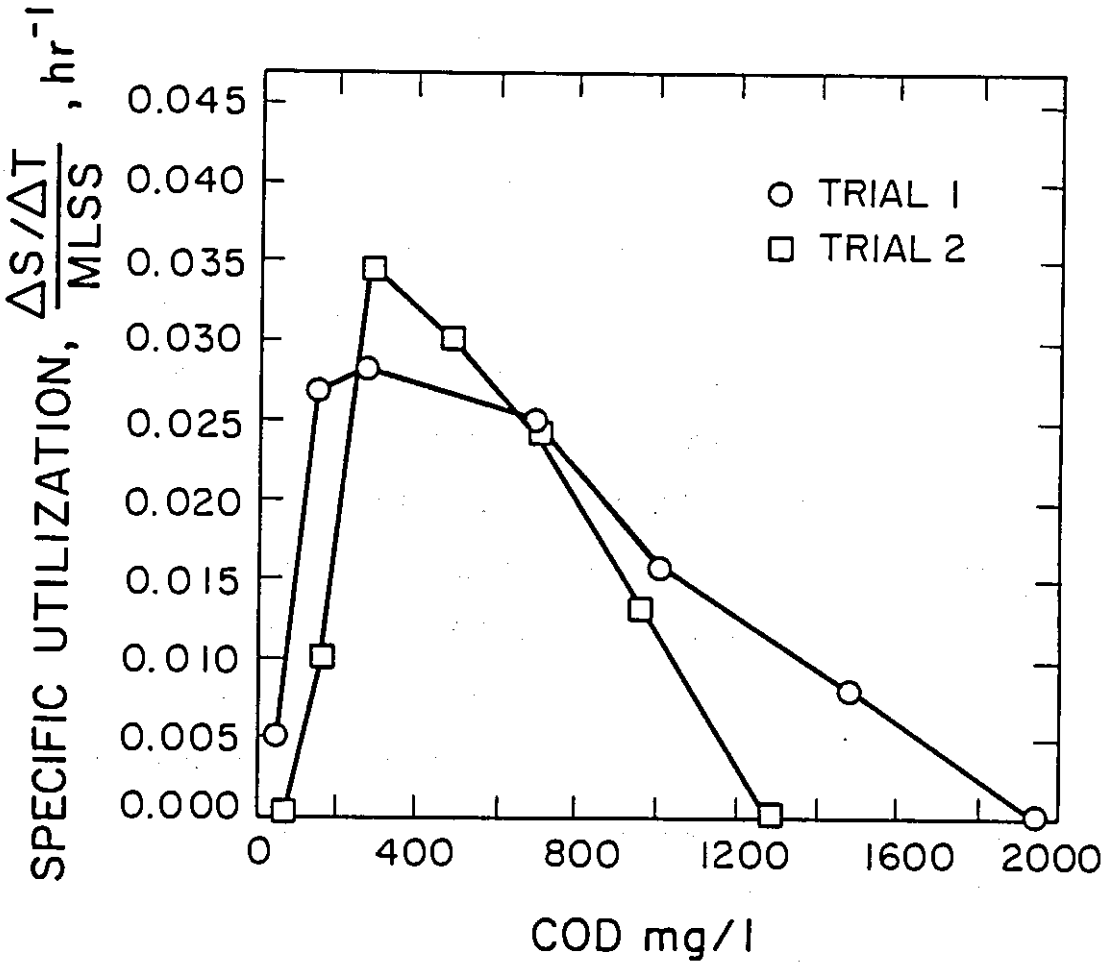


Figure 6. Relationship of initial COD to specific utilization rate of caprolactam in the two-hour batch studies.

inhibitory in trial 2 than in trial 1. The utilization rates ranged from 0 to 0.028 hr^{-1} and 0 to 0.034 hr^{-1} for the first and second trials, respectively. The maximum utilization rates were obtained at initial COD concentrations of 257 and 260 mg/L. The zero utilization rates correspond in each case to the point at which the reactors failed to produce any reduction in COD. In the first trial, this failure occurred at an initial COD of 1940 mg/L, but an initial COD of 1280 mg/L produced complete inhibition in the second trial. Initial and final COD concentrations, as well as MLSS concentrations for each reactor in trial 1 and 2, are shown in Table 3 and 4, respectively.

The curves developed for the raw wastewater showed little similarity, as can be seen in Figure 7. The utilization rates for the first trial ranged from 0.03 to 0.06 hr^{-1}, with the maximum rate occurring at an initial COD of 1240 mg/L. Note that complete inhibition was not induced by increasing wastewater levels in this trial. It can be seen, however, that a reduction in utilization rate did occur at initial COD concentrations of 1200 to 1400 mg/L; yet, a relatively high uptake rate of 0.04 hr^{-1} was still obtained at an initial 1800 mg/L COD. The utilization rates of the second trial were greatly reduced and ranged from 0 to 0.04 hr^{-1} (Figure 7). The maximum utilization rate corresponded to an initial COD of 208 mg/L. This curve also shows that utilization rates rapidly decreased with increasing wastewater concentrations (above 200 to 300 mg/L COD). Complete inhibition occurred at an initial COD of 787 mg/L. Initial

Table 3. The effect of increasing initial COD concentrations on specific utilization rates in trial 1 batch experiments using caprolactam standards.

Initial COD mg/L	$\frac{ds/dt}{X}$ hr^{-1}	Final COD mg/L	MLSS mg/L
51	0.005	40	1190
143	0.027	63	1495
257	0.028	181	1345
697	0.025	629	1385
1006	0.016	960	1350
1480	0.008	1457	1375
1940	0	1940	1350

Table 4. The effect of increasing initial COD concentrations on specific utilization rates in trial 2 batch experiments using caprolactam standards.

Initial COD mg/L	$\frac{ds/dt}{X}$ hr^{-1}	Final COD mg/L	MLSS mg/L
59	0	59	340
152	0.010	149	335
260	0.034	238	325
472	0.030	452	325
692	0.024	677	305
952	0.013	944	300
1280	0	1280	255

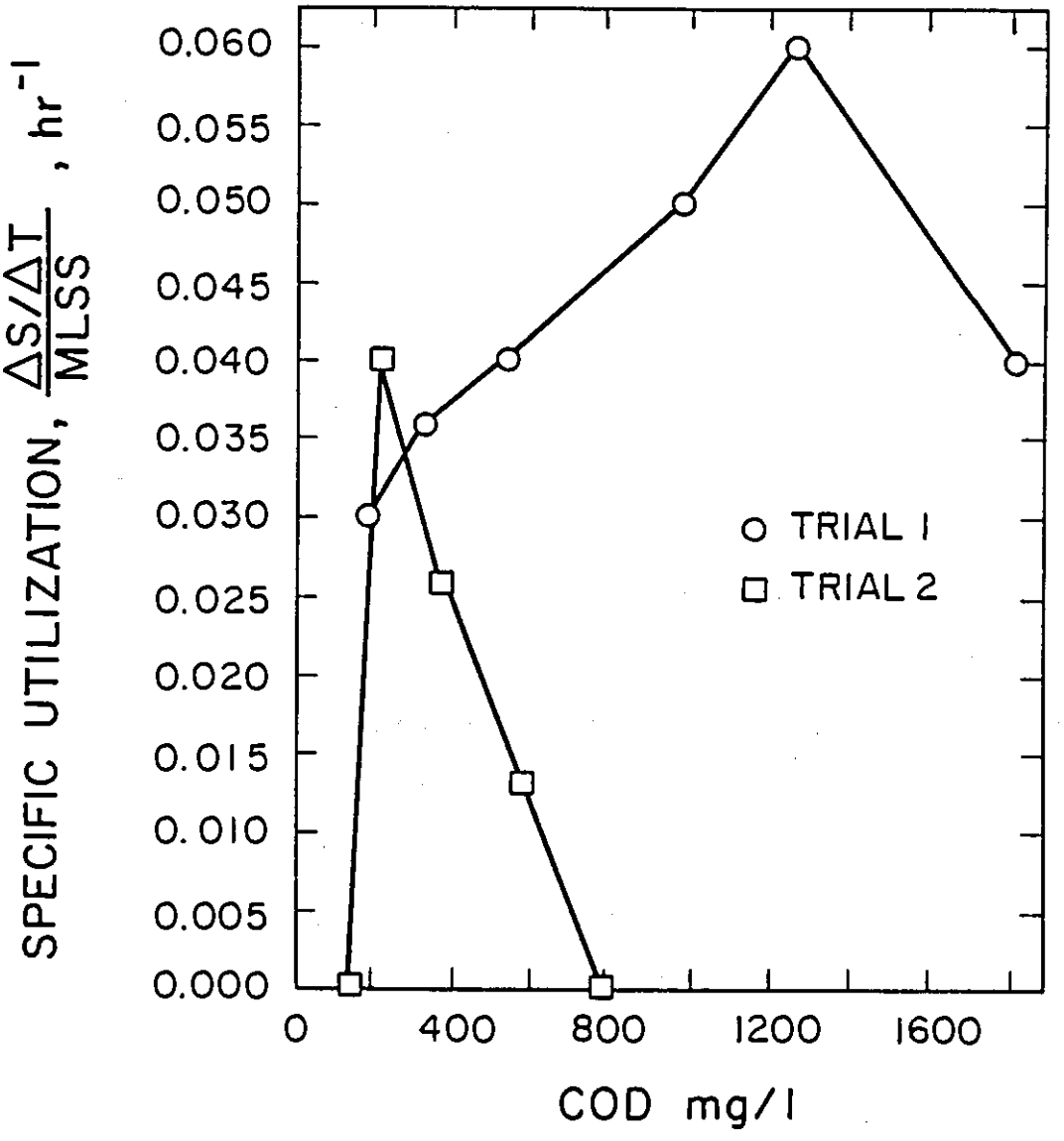


Figure 7. Relationship of initial COD to specific utilization rate of wastewater components in the two-hour batch studies.

and final COD concentrations as well as MLSS concentrations for each reactor in trial 1 and 2 are shown in Tables 5 and 6 , respectively.

The difference between trial 1 and 2 results in the batch studies was not readily apparent. More inhibition was noted in the second trials than in the first involving both the wastewater and pure caprolactam, evidenced by decreased specific utilization rates and earlier reactor failure. However, the difference between the first and second trials with the wastewater was much more pronounced than the difference between the two caprolactam trials. The differences in these curves (Figures 6 and 7) is most likely due to reductions in the MLSS concentrations that occurred between the time the first and second trials were conducted. For trials 1 and 2 of the caprolactam standards, the average MLSS concentration was 1356 mg/L and 312 mg/L, respectively. In the raw wastewater studies average MLSS concentrations were 3000 mg/L and 312 mg/L for trials 1 and 2, respectively. The reason for this decrease was that MLSS concentrations in the lab-scale reactors following the continuous-flow studies were much lower than when reactors were first established, decreasing the amount of cells available for the second set of batch experiments. However, by varying the MLSS concentration, the inhibitory effect of the caprolactam solutions and wastewater were more clearly demonstrated. By dosing with the same concentrations in both trial 1 and 2, a higher toxicant-to-cell ratio was achieved in the later trials, yielding reduced utilization rates and cultures more subject to failure. This response was similar to one obtained by Moos et al. (37) in experiments conducted with

Table 5. The effect of increasing initial COD concentrations on specific utilization rates in trial 1 batch experiments using raw wastewater.

Initial COD mg/L	$\frac{ds/dt}{X}$ hr^{-1}	Final COD mg/L	MLSS mg/L
187	0.030	20	2740
320	0.036	120	2800
613	0.040	386	2780
986	0.050	706	2720
1240	0.060	740	3975
1800	0.040	1560	3000

Table 6. The effect of increasing initial COD concentrations on specific utilization rates in trial 2 batch experiments using raw wastewater.

Initial COD mg/L	$\frac{ds/dt}{X}$ hr^{-1}	Final COD mg/L	MLSS mg/L
67	0	67	295
135	0	135	280
208	0.040	186	280
368	0.026	353	290
584	0.013	576	300
787	0	787	260

pentachlorophenol. In addition, the change in utilization rates may have been due to the physiological condition of the biomass used in the second trials. These trials were performed approximately five months after the first trials and organisms used in the later trials were taken from the continuous flow reactors of this study. The microbes in the second trials, therefore, had been exposed to the industrial waste for a prolonged period and may have become more sensitive to the wastewater and caprolactam. These two explanations, however, do not fully explain the pronounced difference observed in the wastewater trials. It may be that some undetected transformation of components occurred in the wastewater during storage and/or the microbes were sensitized to another component (or components) in the wastewater which was (or were) more inhibitory than caprolactam.

Another point of interest noted in trials with both the wastewater and caprolactam standards was the presence of what appeared to be a residual COD of 40 to 50 mg/L (Table 3-6). In other words, the fluid associated with the sludge used in the batch studies typically had an initial COD of 50 to 60 mg/L. During the course of an experiment, the COD of the fluids in control flasks which contained only sludge (i.e. no additional substrate) decreased to about 40 to 50 mg/L.

Measurement of adenylate energy charge (EC) was also attempted in the second trial of both the caprolactam standard and wastewater batch studies. Samples for analysis were taken from the control reactors at the beginning and end of each test period. These were compared to samples withdrawn at the end of the two-hour test from reactors

operating in the area of the curve representing maximum utilization and reactors at which partial inhibition had been induced. Little variation occurred in the EC at the various concentrations of wastewater and caprolactam standards. The EC ranged from 0.66 to 0.70 and 0.65 to 0.68 for the wastewater and standard solutions, respectively. Similarly, actual concentrations of ATP, ADP, and AMP remained relatively constant in all four trials. This lack of change in what has been reported as a sensitive test was probably due to the relatively short time period of the batch experiments.

Continuous Flow Experiments

Based on information derived from the initial wastewater inhibition curve, an influent COD concentration of 2000 mg/L was selected for use during the continuous flow experiments. This 760 mg/L increase in COD over the concentration (1240 mg/L COD) that yielded the maximum rate of utilization was used because it was felt that under continuous-flow conditions with a larger MLSS concentration a stronger waste could be successfully treated. In order to obtain the 2000 mg/L of COD it was necessary to make a 1:18 dilution of the raw wastewater which resulted in the following characteristics: total organic carbon (TOC) 635 mg/L, caprolactam 1102 mg/L, total Kjeldahl nitrogen (TKN) 123 mg/L, ammonia 10 mg/L, and total oxidized nitrogen 0.07 mg/L. As mentioned earlier, chemostat reactors operating at cell residence times (θ_c) of 5, 10, and 15 days were used to evaluate the biological treatability of the wastewater. Average soluble effluent concentrations of the various parameters measured for each of the θ_c values are shown in Table 7.

Table 7. Average soluble effluent concentrations obtained from the continuous flow experiments.

θ_c days	COD mg/L	TOC mg/L	Caprolactam mg/L	TKN mg/L	NH_3-N mg/L	NO_2-N mg/L	NO_3-N mg/L	MLSS mg/L
0*	2000	635	1102	123	10	0.03	0.14	0
5	1013	355	381	105	56	0.01	0.02	215
10	211	90	38	73	74	0.02	0.08	405
15	87	54	0	58	62	0.04	0.09	680

*θ_c = 0 represents influent waste characteristics.

Effluent Carbon Levels Versus θ_c. COD reductions achieved by the three reactors at θ_c values of 5, 10, and 15 days were 988, 1789, and 1913 mg/L, respectively. The corresponding reductions in TOC and caprolactam levels were 280, 545, and 581 mg/L and 721, 1064, and 1100 mg/L, respectively. The percent removals for these three systems from lowest to highest θ_c were respectively 49, 89, and 96 percent for soluble COD, 44, 86, and 91 percent for soluble TOC, and 65, 96, and 100 percent for soluble caprolactam. These removals are represented in Table 7 and Figure 8.

From the theoretical COD of caprolactam and the fact that carbon accounts for 64 percent of the compound's weight, 1 mg/L of caprolactam represents 2.12 mg/L COD and 0.64 mg/L TOC. From Figure 8, it is clear that more than caprolactam alone contributed to effluent COD and TOC levels at each θ_c. However, it appears that caprolactam readily underwent primary degradation, with intermediates of biodegradation and other carbon-containing compounds (dyes, oils, etc.) being responsible for a portion of the COD and TOC concentrations. A rapid, first step in the biodegradation of caprolactam, followed by slower rate-limiting reactions, has been documented previously (34). Table 8 shows theoretical COD and TOC concentrations calculated from observed caprolactam levels for the influent and three θ_c's. From the influent data, the accuracy of predicting COD and TOC levels from known carpolactam values is demonstrated, with actual levels for both parameters falling within 15 percent of the theoretical. That actual influent COD and TOC were 336 mg/L and 70 mg/L higher than predicted was not

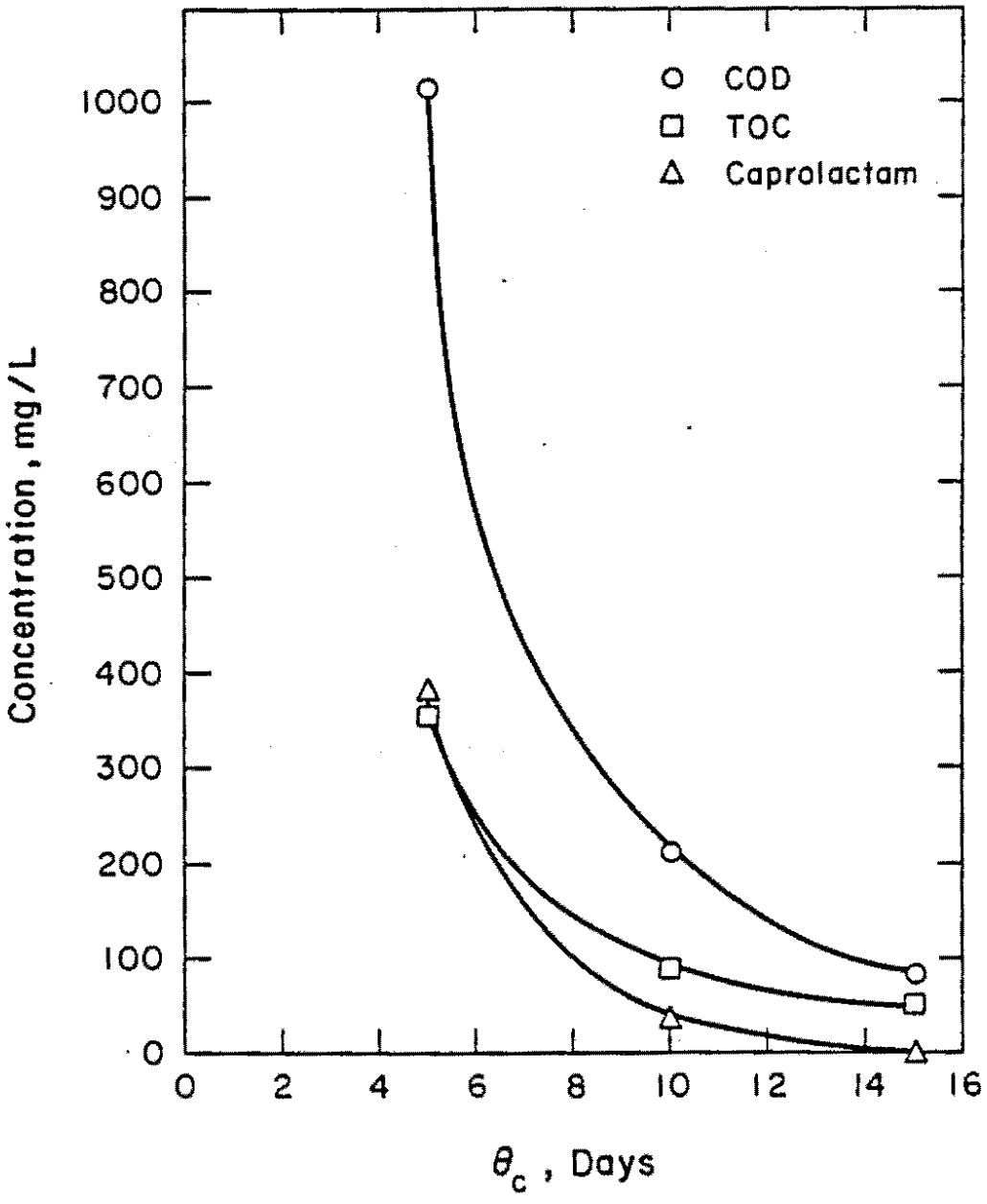


Figure 8. Soluble COD (○), TOC (□), and caprolactam (△) levels in effluents from the continuous flow reactors.

Table 8. Theoretical COD and TOC based on observed caprolactam concentrations versus actual COD and TOC measured.

θ_c days	Caprolactam mg/L	Theoretical COD mg/L	Actual COD mg/L	Theoretical TOC mg/L	Actual TOC mg/L
0*	1102	2336	2000	705	635
5	381	808	1013	244	355
10	38	80	211	24	90
15	0	0	87	0	54

* θ_c = 0 represents influent waste characteristics.

surprising. The reason for this is that other minor carbon sources do exist in the wastewater, primarily vegetable oils used during the dyeing process. Table 8 also shows the decreasing correlation between actual and theoretical values with increasing θ_c. For the 5, 10 and 15 day θ_c the actual TOC and COD concentrations were approximately 30, 70 and 100 percent greater than theoretical predictions. This trend adds support to the statement (33) that primary degradation of caprolactam readily occurs and that intermediates of biodegradation account for the observed TOC and COD concentrations.

Nitrogen Species Versus θ_c and Effluent Carbon Level. Reduction in TKN was a primary concern of this study, and the systems were able to decrease TKN levels by 18, 50, and 65 mg/L at θ_c's of 5, 10, and 15 day levels, respectively. These decreases in TKN correspond to percent removals of 15, 41, and 53 percent. A corresponding increase in NH_3 concentration occurred as the organic nitrogen was mineralized. Increases in the NH_3 concentration were 46, 64, and 52 mg/L at 5, 10, and 15 day θ_c's, respectively. Figure 9 illustrates the fate of these two nitrogen species at the various θ_c's. From the graph it can be seen that NH_3 levels at θ_c's of 10 and 15 days exceeded TKN concentrations somewhat. Since NH_3 is included in the TKN measurement, it is theoretically impossible for TKN to be less than ammonia levels and may have been due to incomplete digestion of samples in the TKN analysis or simply analytical errors. However, the important point is that at θ_c's in excess of 10 days, complete conversion of organic nitrogen to ammonia nitrogen occurred. Ammonia, of course, can be

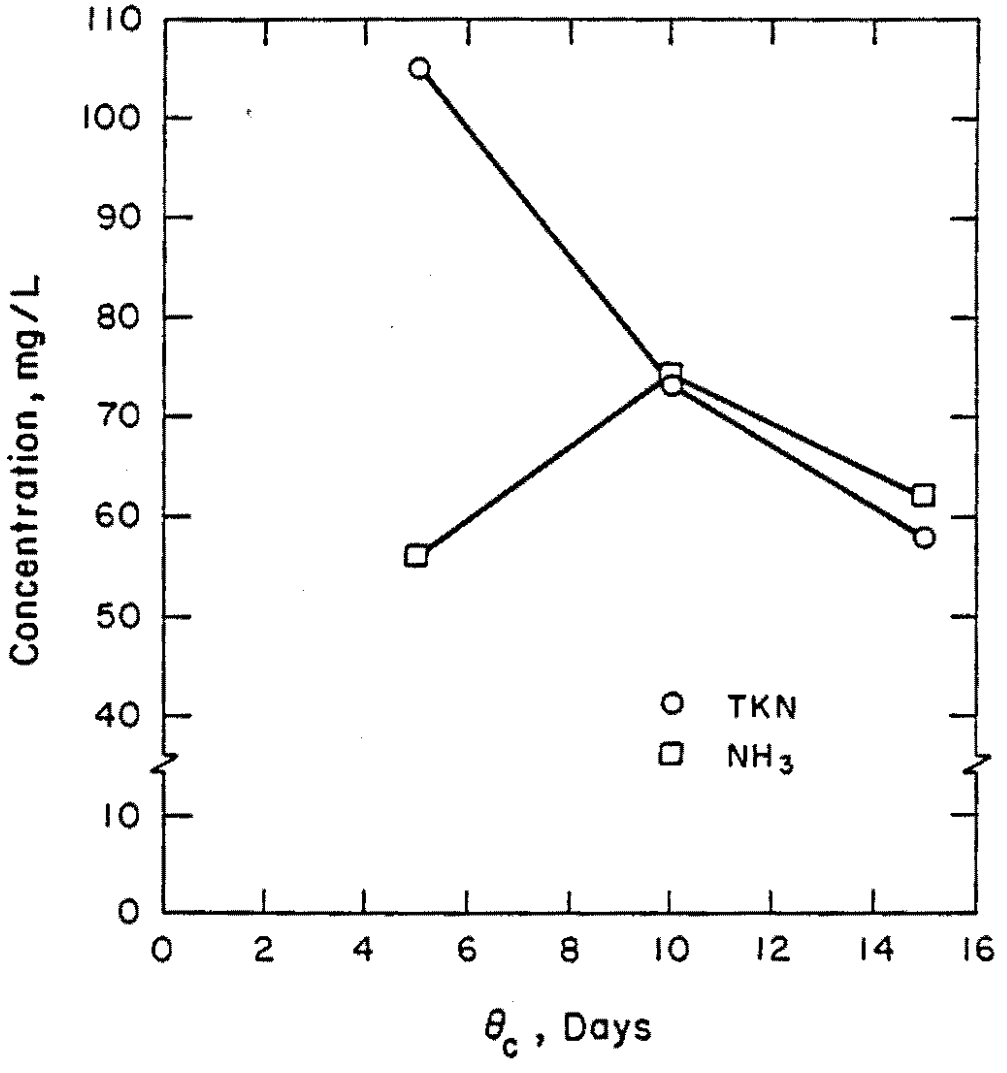


Figure 9. Fate of nitrogen in the continuous flow reactors; ○ = NH_3, □ = TKN.

efficiently removed by air stripping, selective ion adsorption, and nitrification-denitrification processes. Given the quantity of nitrogen contained in the raw wastewater, it might be feasible to biologically convert the nitrogen to ammonia and recover the ammonia for use as a fertilizer. This could be accomplished by capturing the NH_3 in acid solutions, producing ammonium salts such as ammonium sulfate or ammonium nitrate.

Only trace amounts of oxidized nitrogen were detected in the three reactors. Inhibition of nitrification was expected because nitrifying bacteria have been shown to be sensitive to caprolactam (50) and NH_3 levels in the reactors were rather high. In this particular case, the minimal amount of nitrification is actually desirable. By leaving the majority of nitrogen in the ammonia form, a greater total amount of nitrogen could be released from the wastewater, thereby reducing the amount of nitrogen applied to the irrigation sites.

Figures 10 and 11 illustrate the fate of NH_3 and TKN as functions of effluent TOC and caprolactam concentrations. Effluent TKN levels were proportional to effluent caprolactam and TOC concentrations, while ammonia levels appeared to equal TKN values and were highest at caprolactam and TOC concentrations of 38 and 90 mg/L, respectively. Thus, at concentrations below 38 mg/L caprolactam or 90 mg/L TOC, nitrogen was in a form that could readily be removed or recovered.

Nitrogen Balance. Bacteria can be represented chemically by the formula $C_5H_7O_2N$ (36). Organic material comprises 90 percent of the biomass at dry weight, with nitrogen being responsible for

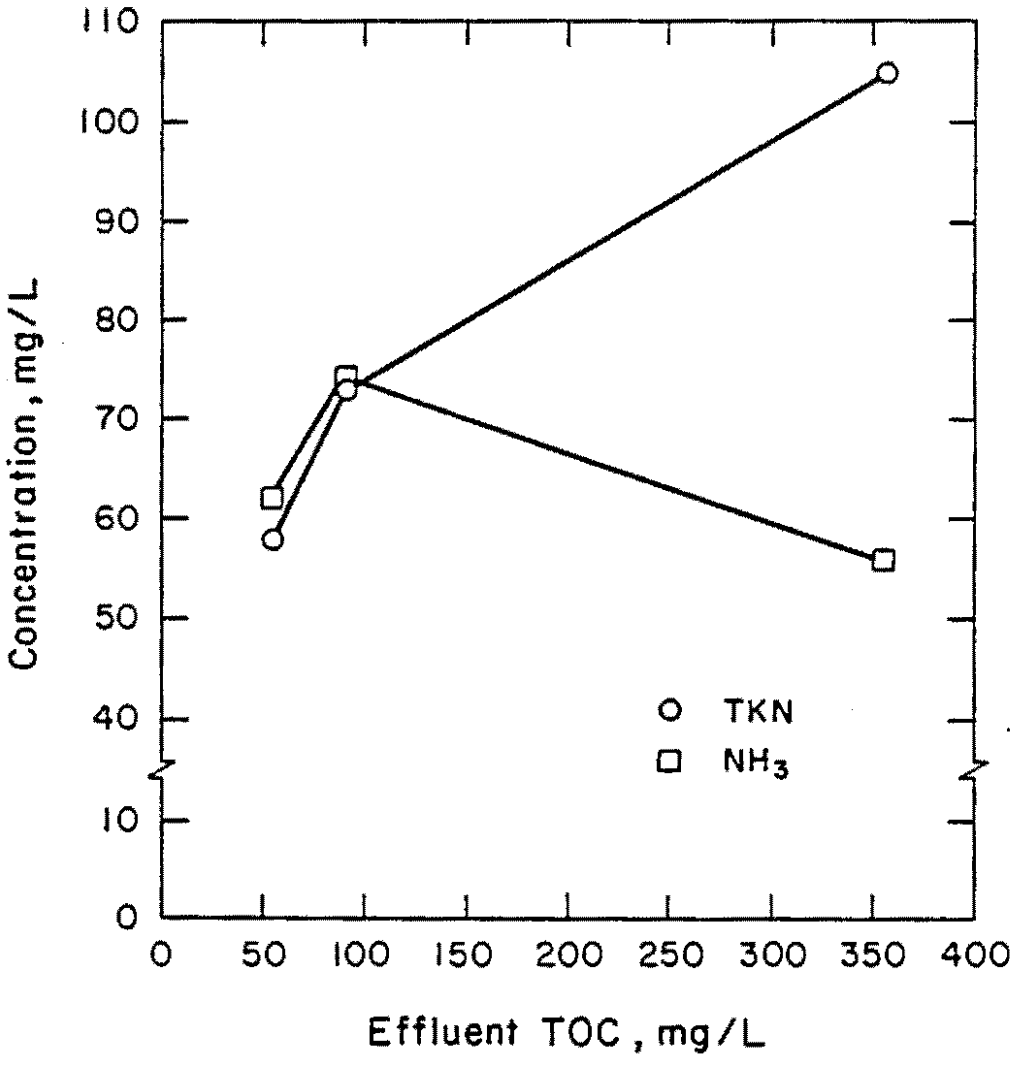


Figure 10. Relationship of nitrogen to effluent organic carbon concentration; ○ = NH_3, □ = TKN.

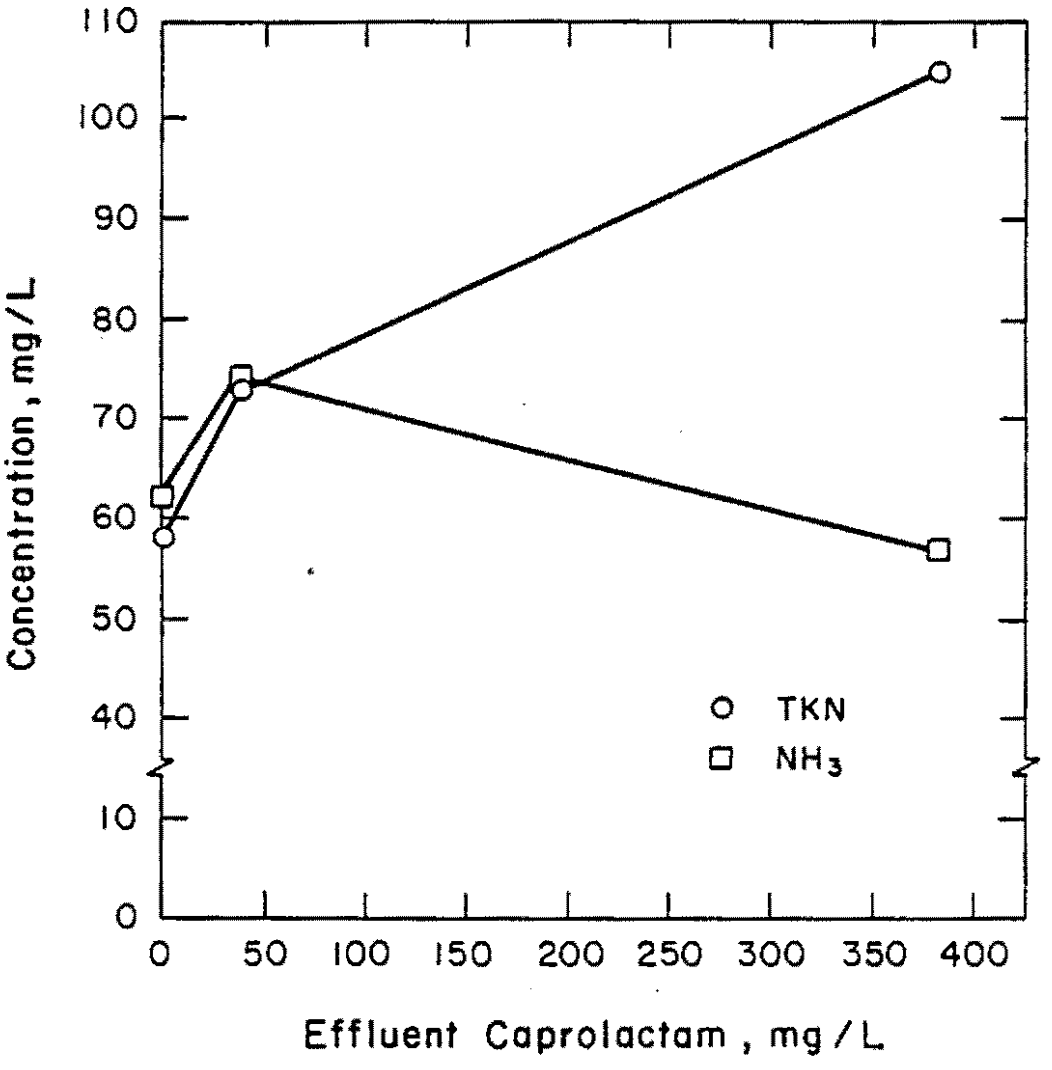


Figure 11. Relationship of nitrogen to effluent caprolactam concentration; ○= NH_3, □= TKN.

approximately 12 percent. Based on this information, nitrogen concentrations in the MLSS can be estimated, and when used in conjunction with known soluble influent and effluent nitrogen concentrations, a nitrogen balance can be obtained. At the increasing θ_c values, MLSS concentrations were, respectively, 215 mg/L, 405 mg/L and 680 mg/L, corresponding to nitrogen concentrations of about 23 mg/L, 44 mg/L and 73 mg/L, respectively. When these values are added to effluent TKN levels (see Table 7), all are within 6 percent of the 123 mg/L TKN contained in the influent.

It was, at first, somewhat surprising that the nitrogen levels balanced as well as they did, considering that the majority of nitrogen was in the ammonia form and aeration was relatively vigorous. Under these conditions air stripping of ammonia would likely occur. The explanation for the lack of volatilization observed is explained by the following:

$$NH_4^+ \rightleftarrows NH_3 + H^+ \qquad [16]$$

In aquous systems ammonia exists in both the ammonium (NH_4^+) and ammonia (NH_3) form, with the predominant species being pH-dependent (6). The equilibrium for equation 16 can be expressed as:

$$\frac{K_a}{[H^+]} = \frac{[NH_3]}{[NH_4^+]} \qquad [17]$$

where

K_a = ionization constant

$[NH_3]$ = ammonia concentration, moles/L

$[NH_4^+]$ = ammonium concentration, moles/L

$[H^+]$ = hydrogen ion concentration, moles/L

The mass balance equation for total ammonia nitrogen can be written as:

$$\text{Total ammonia nitrogen} = [NH_3] + [NH_4^+] \quad [18]$$

From the mass balance equation the percent of NH_4^+ ions present can be calculated by the following equation:

$$\% \ NH_4 = \frac{100}{1 + ([NH_3]/[NH_4^+])} \quad [19]$$

Substituting for $[NH_3]/[NH_4^+]$ from equation 17, yields the equation:

$$\% \ NH_4 = \frac{100}{1 + (K_a/[H^+])} \quad [20]$$

The continuous flow experiments were conducted at 25°C and pH values that equilibrated at 7.7, 7.8 and 8.0 for θ_c's of 5, 10 and 15 days, respectively. Based on this information, and the fact that the K_a value at 25°C is 5.6×10^{-10}, the percentage of NH_4^+ present can be determined. The 15 day θ_c represents the conditions most conducive to ammonia volatilization, operating at the highest pH and residence

time. In this reactor NH_4^+ was predominant, representing 94.6 percent of the ammonia nitrogen species, while only 5.4 percent of the nitrogen was in the volatile NH_3 form. The equilibrium conditions favoring the NH_4^+ ion, were then the principle reasons why the rather good nitrogen balances were obtained.

Volatilization Experiments. After the continuous flow studies had been completed, with the data revealing excellent transformation of organic nitrogen to ammonia nitrogen, volatilization experiments were conducted to support information concerning nitrogen loss due to air stripping and to see if longer residence times would promote ammonia removal. For this study a continuous flow reactor operating at a 20-day θ_c was used. The influent TKN loading on the reactor was 41 mg/day, while soluble TKN effluent levels stabilized at 15 mg/day. The amount of ammonia recovered from this reactor was 13 mg/day. It appears, therefore, that approximately 30 percent of the influent TKN loading was stripped from the reactor in the form of NH_3.

The results from this reactor were not included in the continuous flow data because its equilibrium conditions were in question. The MLSS concentration at the time in which these experiments were conducted was 825 mg/L (289 mg/day). Of this 289 mg/day, approximately 31 mg/day can be attributed to nitrogen. Soluble effluent and MLSS nitrogen levels combined to give 46 mg/day of nitrogen, a value already 12 percent greater than the 41 mg/day influent nitrogen loading. The amount of ammonia recovered, therefore, is a false indicator of the amount of volatile ammonia present. Explanations for the amount of

ammonia recovered include a reduction in partial pressure; which, according to Henry's law; would increase ammonia release (35), and water droplets generated by aeration could have entered the system and been captured. In addition, the phenolhypochlorite analysis for ammonia proved to be very sensitive. Replicate trials varying in recovery by only 0.1 mg/hr, translated into a 13 mg/day difference.

From the mass balance of nitrogen in the continuous flow and volatilization experiments, it would appear that to achieve a significant release of nitrogen from the biologically treated wastewater, a stripping tower or pond with adjustment in pH would be needed.

The food to microorganism ratios (F:M) for the 5, 10 and 15 day θ_c's were 2.0, 0.5 and 0.2, respectively. All the F:M ratios, except for the 5 day θ_c, were within the typical 0.2 to 0.5 range (36). Even though the F:M ratios were reasonable, the solids concentrations in all three, continuous-flow reactors were low. To evaluate the possibility of organic loading being reduced by volatilization, a set of experiments was conducted with the same 20 day θ_c reactor used in the ammonia volatilization study. The influent TOC loading was 217 mg/day, with an effluent TOC of 22 mg/day. The amount of volatile TOC recovered from the reactor was a function of the trapping solution used. For distilled water at a pH of approximately 6 and distilled water adjusted to pH 5 and 9, the volatile TOC fractions detected were 7, 5, and 15 mg/day, respectively. The considerably larger amount trapped at the higher pH can be explained by the fact that the majority of the compounds in the proposed biodegradation pathway are acidic. The

results of the trials performed with the methanol/water trapping solution were highly variable and believed to be invalid due to the loss of methanol from the gas trap during the experiments. It is likely that problems associated with the ammonia volatilization experiments were also a factor in this study. In any case, the amount of TOC recovered was small relative to the TOC loading on the reactor (less than 7 percent) and would not be expected to produce significant reductions in the MLSS concentration.

Evaluation of Biokinetic Constants. With data collected from the continuous flow studies, the values for maximum rate of utilization (k), substrate level at one-half the maximum rate of utilization (K_s), growth yield (Y) and microorganism decay (k_d) could be determined. A plot of the reciprocal of the effluent COD versus the reciprocal of the specific utilization rate was used in finding k and K_s. In this plot the reciprocal of the Y-axis intercept is equal to k and the slope is equal to K_s/k. For Y and k_d determinations a plot of the specific utilization rate versus the reciprocal of θ_c was used, where Y is the slope of the line and k_d is the Y-axis intercept. Table 9 lists the values obtained for these coefficients along with typical values reported by Metcalf and Eddy (36) for systems treating domestic wastewater. From Table 9 noticeable deviations from typical values of K_s and Y can be seen. When these derived coefficients were used in the Lawrence and McCarty model, errant predictions were obtained; particularly for effluent COD, MLSS concentration and θ_c. Based on the atypical values obtained for K_s and Y, as well as the errant

Table 9. Kinetic coefficients obtained from the continuous flow experiments with and without considering 40 mg/L residual COD.

	k d^{-1}	K_s mg/L	Y mg/mg	k_d d^{-1}
Typical Values[1]	2-10	15-70	0.25-0.40	0.040-0.075
With 40 mg/L COD	2.5	1030	0.18	0.028
Without 40 mg/L Residual COD	1.0	223	0.17	0.30

[1]Typical values obtained from Metcalf and Eddy (8).

predictability when the coefficients were used in the treatment model, further evaluation of the numbers was attempted.

In Figure 12, the curve for effluent COD versus specific utilization rate was extended to intersect the x-axis. The point of intersection is approximately at 40 mg/L of COD, which is believed to be the nonbiodegradable fraction of the waste load. Note that this is consistent with the findings of the batch studies where a 40 to 50 mg/L residual was observed. After subtracting this residual from the steady-state effluent COD levels obtained in the three continuous flow experiments, the biokinetic constants were recalculated. The revised values of k, K_s, Y and k_d are shown in Table 9. In this case, values for k and Y are lower than those normally accepted, with the K_s value remaining high, yet more in line with typical values than in the previous determination. By allowing for the 40 mg/L residual COD, this set of coefficients is believed to be the more accurate. However, when used in the Lawrence and McCarty (29) model, these coefficients also produced errant predictions. This poor fit to treatment models were also noted by Anderson (2) in his treatability study conducted with a similar wastewater. Novak et al. (41) reported that poor predictability from treatment models in general, is not an uncommon occurrence.

The reason the biokinetic coefficients were not in the range of normally accepted values is most likely due to the inhibitory nature of the wastewater. In particular, the low values obtained for k and Y are indicative of a less than ideal substrate. The relatively good

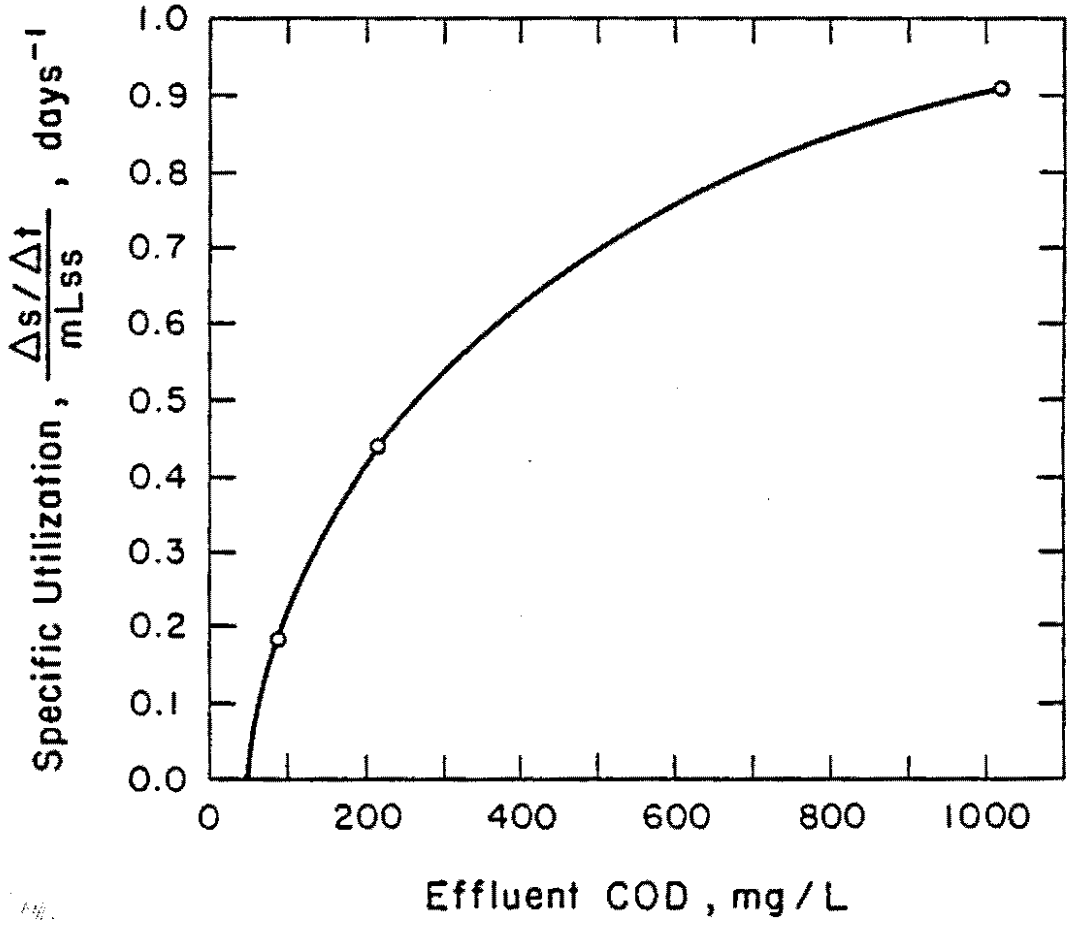


Figure 12. Specific utilization rate as a function of effluent COD.

treatment efficiencies observed for the 10 and 15 day θ_c, however, tend to complicate this issue. An explanation, which is compatible with both the low k and Y values as well as the treatment efficiencies attained, is that the caprolactam may have been readily adsorbed by the microbial floc but was either poorly transported across the cell membrane or slowly oxidized. This would then result in lower cell yields, as well as in low soluble organic carbon concentrations.

Energy Charge and Adenylate Analysis. Measurements of the individual adenylate compound (ATP, ADP and AMP) levels and the resulting energy charge (EC) of the biomass in each of the continuous flow reactors were made once equilibrium conditions were attained. Energy charge values varied only slightly among the three reactors, ranging from 0.73 to 0.79. The concentrations of the adenylate, on the other hand, tended to decrease with decreasing θ_c. The ATP, ADP and AMP levels ranged from 0.30 to 0.62, 0.21 to 0.63 and 0.00015 to 0.0005, respectively. The reduction in adenylate concentrations can most likely be attributed to the lower solids concentration associated with the shorter θ_c's. Table 10 lists the average EC ratio and adenylate concentrations obtained at each of the θ_c's.

From the results obtained, the EC ratio does not appear to be a reliable indicator of system performance. Because the relative ratios of the individual adenylates were essentially the same in each of the reactors, resulting EC values were very similar, masking the presumably stressful conditions of the systems operating at the lower residence times. Measurement of ATP alone, however, might prove to be a reliable

Table 10. Average adenylate compound (ATP, ADP, AMP) concentrations and energy charge (EC) ratios from the continuous flow studies.

θ_c days	ATP mg/L	ADP mg/L	AMP mg/L	EC
5	0.30	0.21	1.5×10^{-4}	0.79
10	0.41	0.32	7×10^{-4}	0.77
15	0.62	0.63	5×10^{-4}	0.73

parameter by which to monitor system performance. Once enough data were obtained on a particular system equating various effluent quality and MLSS concentration to ATP level, the speed and reproducibility of the ATP assay could be a useful monitoring device.

Preliminary Treatability Study. Additional data which appear to substantiate the theory that another inhibitory agent was present in the wastewater used in the batch and continuous flow experiments were derived from a preliminary study also using wastewater from Allied's Chesterfield Plant. This particular wastewater had been obtained and stored approximately two years prior to receiving the wastewater used in the experiments presented thus far. The reactor used in this particular study was operated chemostatically at a 20 day θ_c. The reactor design proved to make its operation rather difficult, in particular it was noted that solids exited the reactor at a slower and more inconsistent rate than desired. Because of the solids wasting rate the θ_c of the reactor was approximated to be 32 days. This problem also forced the abandonment of the reactor in further studies.

Table 11 lists the wastewaters characteristics and the soluble effluent concentrations of the parameters tested. From Table 11 it can be seen that relatively good treatment efficiency was achieved with 51, 83 and 83 percent reductions in TKN, COD and TOC, respectively. Although it is very difficult to make comparisons between this experiment and the continuous flow study previously presented, certain points are worth considering. First, from Table 11 and Table 7, it can be seen that the wastewater strength increased somewhat, with COD, TOC and TKN

Table 11. Average influent and effluent parameter concentrations for the preliminary treatability study conducted on the stored wastewater at a θ_c of 32 days.

Parameter	Influent	Soluble Effluent
TKN, mg/L-N	1,710	830
Ammonia, mg/L-N	75	780
Nitrate, mg/L-N	2.6	2.7
Nitrite, mg/L-N	0.0	0.0
TOC, mg/L	10,850	1,800
COD, mg/L	35,550	5,880
Caprolactam, mg/L	12,000	--*

*Analysis not performed.

concentrations increasing by approximately 500 mg/L. More striking is the fact that the reactor did not fail when subjected to the full-strength wastewater, an occurrence which happened repeatedly with the wastewater acquired later. These failures are what prompted an investigation of the inhibitory nature of the wastewater. The fact that treatment efficiencies observed in this single experiment were similar to those obtained with a 1:18 dilution described earlier tends to lead one to believe either that the more recent wastewater was contaminated with an inhibitory agent or that the wastewater has changed significantly in character.

V. CONCLUSIONS

The conclusions derived from this study are as follows:

1. Total conversion of organic nitrogen to ammonia nitrogen and good removals of organic carbon (> 85%) were achieved at cell residence times greater than or equal to 10 days.
2. Caprolactam and the textile wastewater were both inhibitory to activated sludge. This was demonstrated in the batch studies by decreased specific utilization rates at lower dilutions of the wastewater and by the low values of k and Y determined from data derived from the continuous-flow reactors.
3. Through both batch and continuous-flow studies, it appeared that there was a nonbiodegradable residual of 40 to 50 mg/L in a 1:18 dilution of the wastewater.
4. The results of the batch experiments suggested that an inhibitory agent other than caprolactam was present in the wastewater.
5. Energy charge did not appear to be a reliable indicator of suspended growth system performance and/or stress. However, ATP measurement did demonstrate promise as a useful monitoring device.

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BIOLOGICAL TREATABILITY AND INHIBITORY EFFECTS OF A TEXTILE WASTE CONTAINING CAPROLACTAM

by

Peter Christopher Brown

(ABSTRACT)

A biological treatability study was undertaken on a high-strength textile waste containing caprolactam. Caprolactam is a cyclic amide which is used as a monomeric unit in the production of nylon. The compound exhibits some unique properties and has been shown to be phytotoxic. The focus of this research was concerned with the inhibitory effect of caprolactam and the development of biokinetic growth constants.

Lab-scale, continuous-flow, conventional activated-sludge reactors operated at cell residence times (θ_c) of 5, 10 and 15 days were utilized in the kinetic studies. Short-term batch experiments, involving both caprolactam standards and wastewater, were used in the development of inhibition curves. The inhibitory effects were analyzed by comparing specific utilization rate to chemical oxygen demand (COD).

Influent wastewater characteristics, after a 1:18 dilution, were as follows: COD 2000 mg/L, caprolactam 1000 mg/L, total Kjeldahl nitrogen (TKN) 120 mg/L.

Percent reductions achieved at the 5, 10 and 15 day θ_c values were, respectively, as follows: COD, 49, 89, 96; caprolactam, 65, 96, 100; and, TKN, 15, 41, 53.

Complete inhibition, under batch conditions, was observed at initial COD concentrations in the range of 800 to 1900 mg/L.

The results suggested that primary degradation of caprolactam occurred readily at all cell residence times considered, with intermediate degradation products being responsible for the residual COD in treated effluent. Success in treating the wastewater appears to be dependent upon in-house improvements to reduce waste stream strength and/or dilution of the waste stream.