

# Improvement in Treatment Efficiency of a Soybean Fermentation Wastewater Treatment Plant by the Addition of an Enzyme-Surfactant Mixture

Christopher A. McSorley, Donald R. Wagnitz, Shuichi Sasaki and Betty Olson, Ph.D.\*

## ABSTRACT

The process wastewater from a soy sauce fermentation plant was studied. Plant production has increased for the last 20 years, especially from 1989 to the present, with a resultant increase in average wastewater discharge of 284 m3 (75,000 gallons) per day. Soybean fermentation wastewater is characterized by high concentrations of protein and starch. The increase in effluent releases resulted in increased surcharges in two waste discharge categories: Biochemical oxygen demand (BOD) and total suspended solids (TSS). The high concentrations of protein in the waste water also contributed to strong odors in the treatment works. The plant instituted the use of a novel product, EcoSystem Plus (ESP), a proprietary enzyme surfactant mixture. After treatment, effluent BOD levels decreased an average of 48% and TSS levels decreased an average of 51% from the period before treatment. Aeration tank dissolved oxygen (DO) increased by almost five fold from the period before treatment. Data are presented that indicate EcoSystem Plus can be used to effectively reduce BOD and TSS concentration, eliminating the need to add on-site plant treatment capacity.

Key Words: enzyme, surfactants, aerobic, brewery wastewater, dissolved oxygen.

#### \*Corresponding Author

Since the passage of the Clean Water Act, many industries have had to institute treatment programs for their wastewater to protect receiving waters from pollution. These programs often include an on-site wastewater treatment process, discharged to a publicly owned treatment works, or both. Industries often pay surcharges based upon the pollution load of the discharged wastewater (Besselievre and Schwartz, 1976). The Kikkoman Foods, Inc. soy sauce brewery in Walworth, Wisconsin is one such industrial discharger. Kikkoman Foods, Inc. discharges its pre-treated wastewater to the region's publicly owned treatment works (POTW), Fontana-Walworth Water Pollution Control Commission's facility. This plant treats approximately 3,785 m3 (one million gallons) per day (MGD) of which the Kikkoman Foods, Inc. discharge accounts for one-fifth of the volume. The company contributed funding for the construction of the facilities at the Fontana-Walworth plant and also pays surcharges to the Commission based on pounds of biochemical oxygen demand (BOD) and total suspended solids (TSS) discharged daily. The on-site pretreatment system is a biological process contained in two continuously stirred tank reactors (CSTRs) that are mixed by continuous aeration. Kikkoman Foods, Inc. is permitted to discharge a maximum of 530 kg (1170 lbs) and 396 (875 lbs) of BOD and TSS, per day, respectively in its retreated wastewater. The company is subject to fines if these pollution levels are exceeded. Surcharges, permit maxima, and the responsibility to fund further improvements in capacity at the Fontana-Walworth P01W are encouragements to Kikkoman Foods, Inc. to control BOD and TSS in its effluent.

Wastewater from a soy sauce brewery falls into two types: One is process waste such as heat treated wheat and soy meal which contains high concentrations of protein. The second is fermentation residues including soy cake and soy sauce which contains amino acids, sugar, lactic acid, fiber and some vegetable oil. The wastewater from soybean fermentation plants varies widely in volume and BOD depending upon which portion of the soy sauce manufacturing process generates the wastewater discharged. For example, wastewater produced in the boil process accounts for a very small proportion of the total wastewater flow volume but has a very high BOD. This segment of the process is the largest source of BOD loading. In contrast, bottle washing generates the largest volume of wastewater, but the BOD loading from this source is moderate (Okada et al., 1990).



Soy sauce production at the plant has increased each year since it went on line in 1973 and production is expected to continue to increase into the future. The volume of wastewater produced is expected to increase in step with soy sauce production as it has in the past. Because BOD and TSS concentrations in the effluent have not decreased, the total loading of these pollutants has also increased. The total BOD and TSS discharges have risen to levels approaching the maximum permitted discharge limits and have caused a significant increase in the amount of surcharges assessed to Kikkoman Foods, Inc. The high protein wastewater also created odor problems at the on-site treatment plant that affected the surrounding area. In order to accommodate further increases in soy sauce production, Kikkoman Foods, Inc. had to consider strategies for decreasing the amount of BOD and TSS in its effluent.

BOD and TSS are removed from the wastewater by bacteria present in the pretreatment tanks. Bacterial degradation of compounds that cause BOD is enhanced by the continuous mixing of the tanks and also by maintaining an excess of dissolved oxygen in the system. Bacteria breakdown these compounds through metabolic processes through which they derive energy and carbon for cellular growth. Bacteria initialize the metabolism of these compounds by the excretion of enzymes and by the transport of the compound across the cell membrane into the cytoplasm (Dills et al., 1980). This process may be further enhanced by increasing the rate at which the bacteria absorb and metabolize the pollutants. The addition of surfactants has been shown (Zhang and Miller, 1995) to increase the rate of hydrocarbon degradation by bacteria.

This paper describes an enzyme-surfactant treatment, EcoSystem Plus, that increased dissolved oxygen in the aeration tank and reduced TSS and BOD in the effluent. These decreases avoided the need for expensive expansion of treatment facilities at the brewery and at the POTW. Also, this treatment regime reduced surcharges paid by the company. The odor that was once persistent at the on-site treatment plant is now gone.

## MATERIALS AND METHODS

**Brewery Wastewater Treatment Plant.** A schematic diagram of the treatment plant is shown in Figure 1. Wasted process waters from the brewery were collected and passed through a 200 mesh screen to remove larger sized solids. The wastewater then entered a round 757 m<sup>3</sup> (200,000 gallon) aeration tank that is 16 m (52 ft) in diameter and 4 m (13 ft) in depth. The wastewater in the tank was continuously aerated at a rate of 29.5 Lm<sup>-1</sup> (835 cfm) using coarse bubble diffusers. Float activated pumps transferred the wastewater to the second stage of treatment, a square 2,270 m<sup>3</sup> (600,000 gallon) aeration tank 23 m x 23 m (75 ft x 75 ft) and 4 m (13 ft) in depth. The square aeration tank was equipped with flexible membrane disc air diffusers which aerated the tank of 44.7 Lm<sup>-1</sup> (1266 cfm). The retention time was approximately 24-hours in each tank under continual aeration. The average wastewater discharge from the brewery was approximately 700 m<sup>3</sup> per day (185,000 gallons). The plant's treated effluent was measured daily for levels of TSS, BOD and total wastewater flow by the Fontana-Walworth Pollution Control Commission to calculate surcharges.

**Treatment Scheme.** EcoSystem Plus enzyme-surfactant solution was manually added directly to both tanks beginning on April 21, 1994. EcoSystem Plus is a proprietary solution of buffered enzymes and surfactants (Neozyme International, Inc. - Newport Beach, California). In August 1994, a pump system was installed which mixed the product with tap water and injected the mixture directly into the process wastewater stream approximately 200 meters up stream of the first aeration tank. An average of 17L (4.5 gallons) of the enzyme surfactant solution was added per day during this study. The actual concentration of enzyme-surfactant in the aeration tanks varied as the daily wastewater flow varied. Enzyme-surfactant solution by the effluent flow for that day. This estimation method gave an average ESP concentration of29.5 ppm (v/v) with a minimum of 6.69 ppm (v/v) and a maximum of 857.14 ppm (v/v).

**Analytical Tests**. The effluent was monitored for biochemical oxygen demand and total suspended solids (5210 Band 2540 D, respectively) of *Standard Methods for the Examination of Water and Wastewater* 





(APHA, 1994). Dissolved oxygen concentration was measured by brewery personnel in the second of the two aeration tanks at the brewery. Dissolved oxygen measurements were made weekly until August when measurements were made each weekday. Measurements were made using a DO probe (YSI 5739) and a DO meter (YSI model 57).

## **RESULTS AND DISCUSSION**

The results of this fifty-two week study (Figure 2) showed decreases in BOD and TSS in the final waste water discharge and increases in dissolved oxygen levels in the second aeration tank when EcoSystem Plus was added as a treatment. The data shown in Figure 2 indicated that TSS increased significantly between the ninth and eleventh week of monitoring from 750 to 1250 mg/L and then began to decrease. This spike in TSS concentration is attributed to the first aeration tank being taken off-line and the water level in the square tank being maintained at a higher than normal level. Before the addition of ESP, BOD and TSS levels fluctuated widely over mean values of 456 mg/L and 768 mg/L, respectively. Mean aeration tank DO was 1.3 mg/L for the same period. Over the 33 week period during which the wastewater was treated with ESP, the average concentrations of TSS and BOD in the effluent were 395 mg/L and 219 mg/L, respectively. Treatment with ESP resulted in an average decrease in effluent TSS concentration of 51% and an average decrease in effluent BOD concentration of 48%. Average DO concentration in the second aeration tank was 6.4 mg/L during the 33 weeks of ESP treatment representing almost a five-fold increase in DO. Within a few days of initiating the ESP treatment, odor coming from the aeration tanks had subsided and did not return for the duration of the study.

Because the volume of ESP being added to the treatment system varied somewhat over the study period and the volume of wastewater being treated varied from day to day, the actual concentration of ESP in the system varied considerably. Concentrations of TSS, BOD and DO were plotted as histograms according to the concentrations of ESP present in the system (Figures 3a, b, c). The aim was to determine if the concentrations of TSS or BOD in the effluent or of DO in the aeration tank changed significantly in relation to the ESP concentrations in the system. The mean TSS, BOD or DO concentration of each ESP concentration class was compared to that of each other class by the Student Newman-Kuhls (SNK) test (release 6.08 SAS Institute, Inc.). TSS, BOD and DO concentrations for ESP concentrations because of the number of data points occurring in each class of ESP concentrations was too small to be statistically significant.

The concentration of effluent TSS (Figure 3a) as it relates to ESP dosage was examined. SNK analysis indicted a significant (p < 0.01) decrease in TSS between samples taken during treatment with ESP and samples before treatment. The mean TSS concentrations for all concentrations of ESP was not significantly different from one another. No additional advantage appears to be derived from increasing the dosage> 15 ppm (v/v) of ESP.

The mean concentration of BOD (Figure 3b) exhibits similar behavior over different ESP concentration ranges as TSS. The mean BOD concentration for samples taken after the initiation of treatment with ESP was 221.6 mg/L, significantly lower than 429.7 mg/L, the mean BOD before treatment. The mean BOD for all concentrations of ESP during treatment was not significantly different from one another.

Aeration tank DO (Figure 3c) shows a different response to different concentrations of ESP than BOD and TSS. DO when ESP concentration was > 0 and ~ 10ppm (v/v) was 3 mg/L, not significantly different from 1.4 mg/L, the mean DO before treatment (p (0.01). DO for ESP concentrations) 10 and 5: 35 ppm (v/v) averaged 6.5 mg/L. This was approximately 4.5 times greater than the mean DO concentration before treatment. When ESP was > 35 and ~ 50 ppm (v/v), mean DO was 9.44, significantly higher than the mean DO for ESP> 10 and ~ 35 and 6.7 times higher than the DO before treatment with ESP.

In Figure 4, BOD is shown to be greater than 300 mg/L when DO is less than 1.5 mg/L. When DO is greater than approximately 3.5 mg/L, BOD fluctuates between 100 and 375 ppm regardless of the DO concentration. This behavior is similar to that observed in Figure 3b when BOD concentration and the





concentration of EcoSystem Plus are compared. These findings suggest that further decreases in BOD are not achieved as dissolved oxygen increases suggesting that some factor other than DO is limiting microbial utilization of the carbon. This could result from lack of required growth factors or trace elements and/or the presence recalcitrant carbon compounds in the wastewater. The fact that ESP does not further decrease BOD suggests that ESP is not the rate limiting factor in the breakdown of the carbon compounds. BOD data presented in Figure 4 suggests that Michaelis-Mentenkinetics are displayed in the reaction and that a plateau is quickly reached which is not affected by either increased ESP concentration or the resultant increase in dissolved oxygen concentration (Alben A. Lenninger, 1975).

Another benefit of increased DO concentrations due to the addition of the enzyme-surfactant solution in the square aeration tank was the elimination of odor. The reduction in odor may have been due to a combination of factors including elimination of anaerobic conditions within the tank because of increased DO (Tchobanoglous and Bunon, 1991), increased oxidation of odor compounds from increased DO concentrations (Bowker et al., 1989), and/or suppression of volatilization of odor producing compounds from the aqueous phase by surfactants in ESP.

## CONCLUSIONS

Although the exact mechanism of action is not delineated, it is likely that the breakdown of the Carbonaceous waste is facilitated by the addition of ESP. This enhancement could come about from the surfactant in the product increasing the solution of the carbon material or by disassociating bacterial aggregations, thus increasing the surface area of bacteria in the aeration tanks. Tests on municipal sewage (data not presented) showed that surfactants, in the absence of enzymes, were not sufficient to enhance bacterial degradation of BOD. Laboratory tests on pure compounds (data not presented) suggest that direct enzyme reaction is not the dominant mode of degradation, although it may accelerate bacterial breakdown of carbonaceous compounds.

Treatment of the wastewater from the Kikkoman Foods, Inc. soy sauce brewery with ESP has had several beneficial effects: Dissolved oxygen in the aeration tank increased and remained consistently high. The strong odors that used to come from the aeration tanks are gone. The significant decreases in both TSS and BOD have caused an immediate savings due to decreased surcharges and additional savings from deferment of building costly additional treatment plant capacity. Adding ESP has made Kikkoman wastewater treatment plant operate more efficiently and has increased the amount of TSS and BOD loading that can be treated.

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**Authors.** Christopher McSorley is a laboratory assistant in the Environmental Microbiology and Genetics Laboratory at the University of California, Irvine. Betty H. Olson, Ph. D. is a professor in the Department of Environmental Analysis and Design at the University of California, Irvine. Donald R. Wagnitz is an environmental consultant with extensive experience working with EcoSystem Plus. Shuichi Sasaki is the Assistant Manager of Quality Assurance at the Kikkoman Foods, Inc. soy sauce brewery in Walworth, Wisconsin.





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#### Figure 1.

Schematic drawing of the wastewater treatment facilities at Kikkoman Foods, Inc. Soy sauce brewery Walworth, Wisconsin. Wastewater is screened before undergoing continuous aeration in two tanks for 48 hours. The treatment system consists of two CSTR's in series.

#### Figure 2.

TSS, BOD and DO levels during the study. Weekly averages of TSS, BOD and DO data are shown. Average daily amount of ESP added for each week is shown in gallons. TSS and BOD levels are consistently lower during treatment with the product EcoSystem Plus than prior to treatment. TSS and BOD levels exhibit much less fluctuation during treatment than before treatment with ESP. DO concentrations in the second aeration tank during treatment increased more than four times the concentration of DO before treatment.

#### Figure 3a.

Effluent BOD at different concentrations of EcoSystem Plus. Effluent BOD decreased significantly during treatment with ESP was used from before treatment (concentration of ESP = 0). However, the effluent BOD does not differ significantly from one ESP concentration to another. The BOD when ESP concentration is) 0 and  $\sim$  10 is not significantly different from any other concentration range.

#### Figure 3b.

Effluent TSS at different concentrations of EcoSystem Plus. As in the case of BOD, increasing ESP concentration does not result in a decrease in TSS. However, TSS is significantly lower with ESP than without.



## Figure 3c.

Dissolved oxygen concentration in the second aeration tank at different concentrations of EcoSystem Plus. The concentration of DO when the ESP concentration is> 0 and  $\leq$  10 ppm is not significantly greater than when there was no ESP in the system. The DO is significantly greater when the concentration of ESP is> 10 and  $\leq$  35. When the ESP concentration is > 35 and  $\leq$  50 ppm, the DO concentration is significantly greater than all other ESP concentrations.

## Figure .4.

Effluent BOD plotted in relationship to the DO concentration in the second aeration tank. There appears to be an inverse correlation between DO and effluent BOD from 0 mg/L to approximately 2 mg/L DO. Beyond that concentration, there appears to be no relation between aeration tank DO and effluent BOD. Further increases in DO do not appear to result in lower BOD concentrations.

## Figure 5.

Forecasts of average daily wastewater flow, TSS and BOD loading are plotted to the year 2010. Continuing increases in plant production will result in an increase in total wastewater flow as shown in figure 5a. Figure 5b shows effluent BOD in pounds discharged per day the maximum permittable discharge is represented by the horizontal line at 1175 lbs/day. The decrease in BOD discharged between 1993 and 1995 is due to the use of ESP. Daily average BOD levels are not expected to reach 1993 levels until after 2010. IA similar decrease is seen in Figure 5c in the amount of daily TSS discharged. Daily TSS discharge is currently not expected to exceed permitted levels until approximately 2003.





FIGURE 1

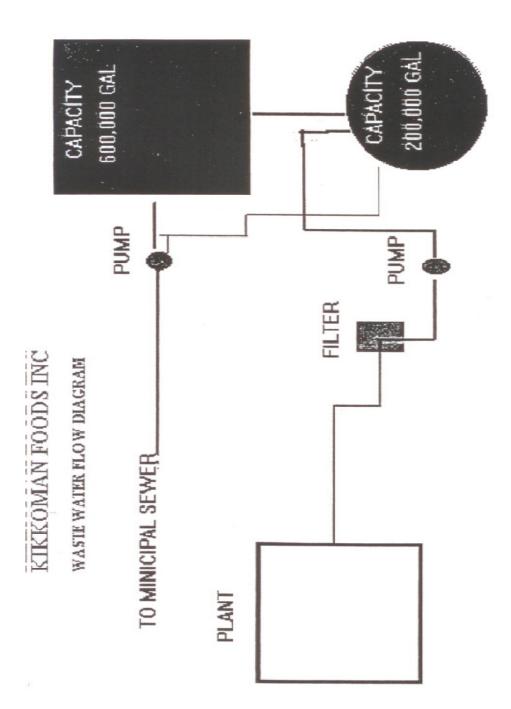
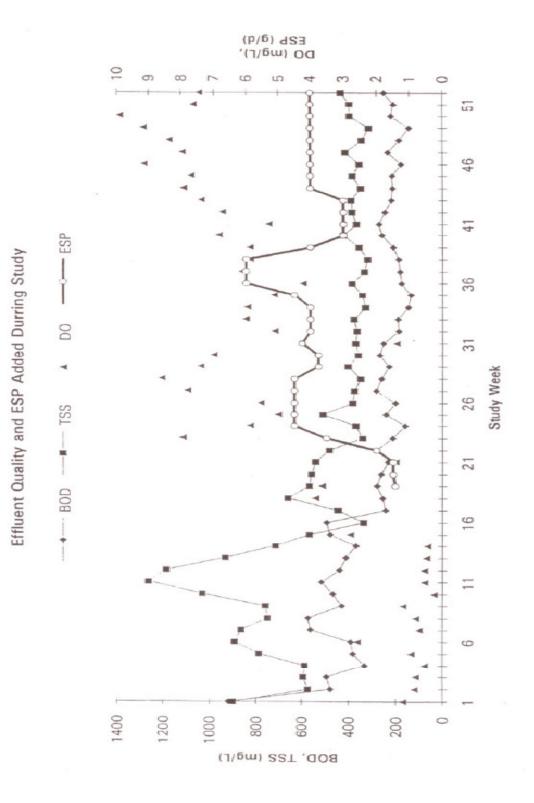






FIGURE 2

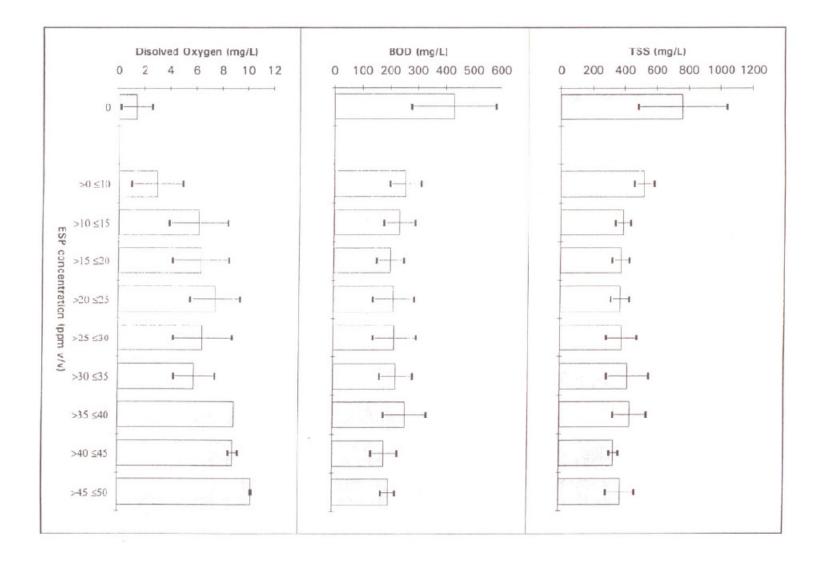








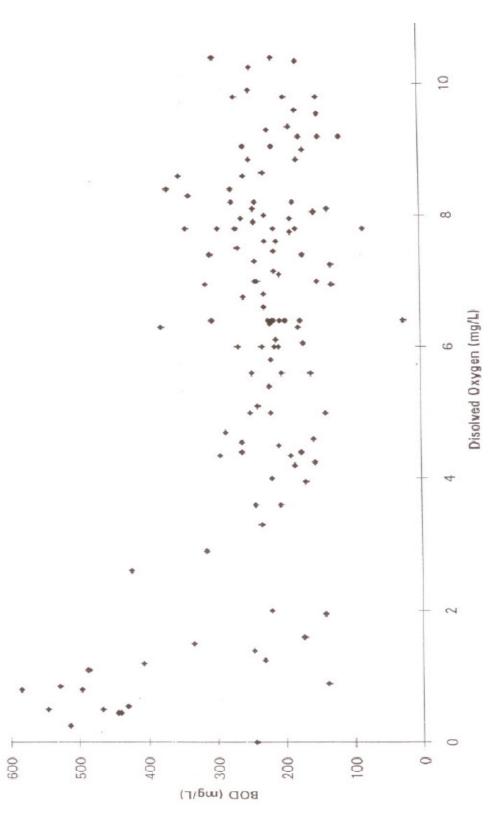
## FIGURE 3















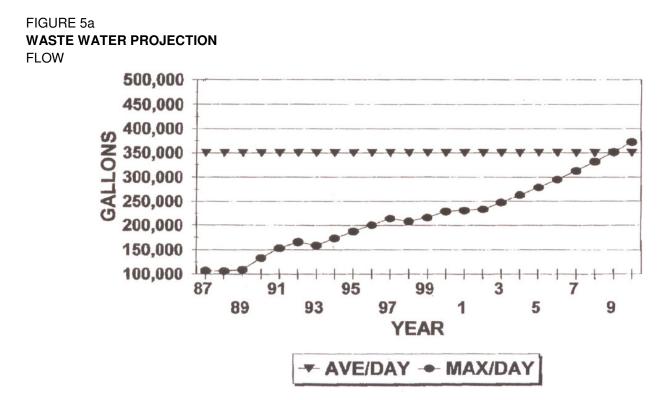
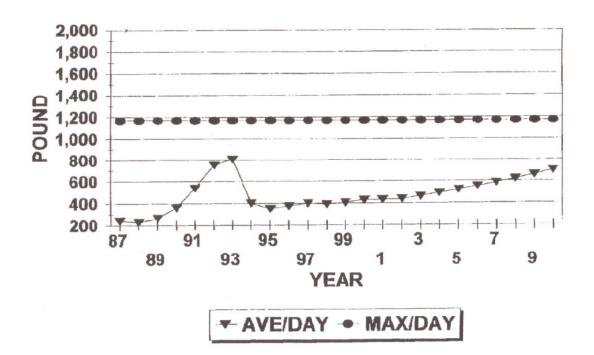


FIGURE 5b WASTE WATER PROJECTION BOD







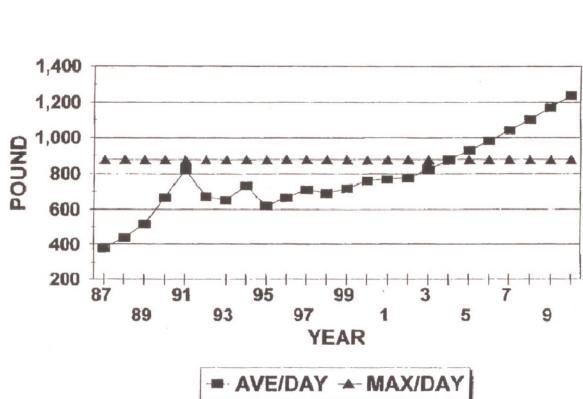


FIGURE 5C WASTE WATER PROJECTION TSS

