EFFECT OF ACCLIMATION TO LIME-SULFIDE EFFLUENT FROM UNHAIRING CATTLEHIDES ON THE COMPOSITION OF ACTIVATED SLUDGE*

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ABSTRACT

The composition of activated sludge from sanitary wastes was modified by acclimation to full strength, lime-sulfide unhairing effluent during bio-oxidation. The protein content of the acclimated-activated sludge was 30 to 36 percent compared to 28 to 32 percent for activated sludge. The ash (especially high in calcium), the inorganic sulfur compounds, and the protein sulfur were increased.

The amino acid content of the protein in the lime-sulfide unhairing effluent was close to that of native hair and included lanthionine and lysinoalanine, which are formed during unhairing. The lime-sulfide effluent also contained free amino acids. Activated sludge before acclimation contained all the common amino acids but was low in cystine. It contained no free amino acids. The acclimated-activated sludge contained lanthionine as well as all the common amino acids, including some free amino acids, and was a composite of degraded hair protein and biomass. Lanthionine can possibly be used as an indicator for the presence of alkali-degraded hair protein.

The acclimated-activated sludge was free of toxic heavy metal contaminants and could possibly be used as a protein supplement in poultry layer, swine, or catfish feed, or as a high-lime fertilizer.

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INTRODUCTION

The removal of hair from cattlehides by the high-sulfide hair-pulping process produces effluents with high loads of biochemical oxygen demand (BOD) resulting from the solubilized, degraded proteins from the pulped hair and the

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noncollagenous protein in the hides (1). The need for the development of feasible processes for the reduction of the waste load of unhairing effluents is urgent because of increasingly stringent legislation and Environmental Protection Agency Guidelines concerning water pollution (2).

We have previously reported three different approaches to the lime-sulfide effluent treatment problem: removal and recovery of the protein with suggested recycling of the sulfide (3), reduction of the protein and sulfide contents by bio-oxidation with acclimated-activated sludge (4), and removal of the organic and inorganic suspended solids by flocculation and sedimentation (5). A preliminary cost comparison of the following three most promising alternatives for treating unhairing effluents was prepared by the Commercial Feasibility Group, Engineering and Development Laboratory, at our Center: Czechoslovakian Process, which includes recovery of a crude protein product precipitated by acidification, and sulfide recycling (6); Air Oxidation Process, which involves Mn^{++}-catalyzed, air oxidation of the effluent to remove sulfide ions (7, 8), followed by acidification to recover a semipure protein (3); and Conventional Waste Treatment, which involves chemical-biological waste treatment but no recovery of byproducts or recycling of unhairing chemicals (9). Based on the cost analysis and the present market value of hair and hide protein, bio-oxidation appears to be the most economical method of treatment.

Hoshino *et al.* (10) in Japan have shown that the type and composition of the feed (substrate) have considerable effect on the composition of the activated sludge. They found that the activated sludges produced from bio-oxidation of the effluents from seven different food and other industries varied in ash and crude protein content and in amino acid composition. The content of sulfur-containing amino acids was generally lower than that of the reference protein, and some of the samples were lower in lysine and tryptophan.

The present work was done to determine whether or not the composition of the activated sludge changed on acclimation to the lime-sulfide effluent, and to provide an indication of the possible feed and fertilizer value of the acclimated-activated sludge product produced by the continued growth of the acclimated microorganisms, using lime-sulfide effluent as the substrate. The acclimated-activated sludge examined in this study was prepared as described in our earlier investigation (4). Acclimation, as defined in this report, is the adaptation of the activated sludge microorganisms to their new environment, and their continued growth.

EXPERIMENTAL

Lime-sulfide effluent was obtained from a side leather tanner who unhairs fresh cattlehides in paddle vats by the high-sulfide, hair-pulping process. The full strength effluent was obtained directly from the unhairing operation and did not include any wash water. The effluent was put through a 50 x 50 mesh polypropylene screen to remove the larger solids before being placed in the bioreactor.

Return activated sanitary sludge was obtained from the Abington, Pa., municipal sewage treatment plant and kept under aeration for use in the bio-oxidation units. The effluent from primary sedimentation tanks was also obtained from the same treatment plant for use as a feed for the activated sludge.

Acclimated-Activated Sludge

The activated sanitary sludge was acclimated to lime-sulfide effluent, and acclimated-activated sludge was produced in a Horizon Ecology Bench Scale BioOxidation System‡ by the procedure reported by Cooper *et al.* (4).

The mixed liquor suspended solids (MLSS) concentration of the Abington activated sludge, about 800 mg./l., was built up to about 1700 mg./l. by adding sanitary waste for about five days, to provide sufficient microorganisms for acclimation. Acclimated-activated sludge was then prepared by adding lime-sulfide effluent, diluted 1:20 with tap water, to the activated sludge in Unit I, at a rate of 0.5 liter per hour, until a steady state concentration of 1700 mg./l. of MLSS was maintained (4). The dilution was required by the immediate dissolved oxygen demand (IDOD) and the chemical oxygen demand (COD) of the unhairing effluent after screening. After five days, the concentration of the lime-sulfide influent to the reactor was increased to 1:10 dilution with tap water and added at the same rate of 0.5 liter per hour. The MLSS in the acclimated-activated sludge unit had to be maintained at a level (2500 to 3000 mg./l.) sufficient to treat the increased COD loading and to allow for harvesting of at least 10 percent of the MLSS each time. The effluent from the acclimated sludge (Unit I) was run through the activated sludge (Unit II) for further reduction of the pollutants; then the final effluent from Unit II was used as a diluent of the lime-sulfide waste.

The total solids, settleable solids, suspended solids, mixed liquor dissolved oxygen (DO), and pH were determined daily prior to, during, and after addition of the lime-sulfide effluent to the activated sludge. The mixed liquor DO was maintained at a minimum of 1 mg./l. by controlling the aeration rate.

Harvesting of Acclimated-Activated Sludge

After sufficient growth of the microorganisms, indicated by an increase in MLSS to 2500–3000 mg./l., small portions of acclimated-activated sludge (10 to 20 percent of the volume of the bioreactors) were harvested at intervals during a four-week period to obtain two lots of product. Each lot was washed three times by alternate resuspension in distilled water, centrifugation in an International centrifuge at 2100 r.p.m. (988 gravity) for 10 min., and decantation.

‡Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

The sludges then were freeze-dried. Lots #1 and #2 contained 79 gm. and 37 gm., respectively, on a dry weight basis. The supernatant solutions or centrifugates were decanted and analyzed for free amino acids. A third lot (43 gm.) was harvested by centrifugation at 2100 r.p.m. as above, and then freeze-dried without washing.

Activated Sludge

For comparative analysis only, additional samples of return activated sanitary sludge were obtained from the northeast Philadelphia, Pa., and Abington, Pa., municipal sewage treatment plants and centrifuged, washed, and freeze-dried immediately by the procedure described above, except that the Abington sludge was centrifuged in a Beckman Ultra-centrifuge at 19,000 r.p.m. The first centrifugates were analyzed for free amino acids.

Free Amino Acids in Lime-Sulfide Effluent

A fresh sample of lime-sulfide effluent was screened as described above, then centrifuged at 2100 r.p.m. for 10 min. in the International centrifuge, and clarified by filtration through filter paper. The filtrate was analyzed for free amino acids.

ANALYTICAL METHODS

The official methods of analysis of the American Public Health Association, American Water Works Association, and the Water Pollution Control Federation were used for determinations of pH, BOD, COD, total organic carbon (TOC), total alkalinity, total ash, fat, total solids, total volatile solids, suspended solids, and volatile suspended solids (11). The dissolved oxygen concentration was measured with a Weston and Stack Model 3 galvanic probe and a Tech-Line Instruments Model A-30 BOD Analyzer with a digital readout (4).

Nitrogen was determined by the semimicro Kjeldahl method (12). Total organic and inorganic sulfur were determined by the AOAC Carius microchemical combustion methods (13). Sodium sulfide was determined by the Official Method of Analysis of the Society of Leather Trades' Chemists (14). A semiquantitative scan for all metals was made by a commercial testing laboratory, using a 1.5 meter Applied Research Laboratories Emission Spectrograph. Samples of the activated and acclimated-activated sludges were prepared for the quantitative determination of metals by ashing, dissolving in 1:1 HCl-H_2O, evaporating to dryness to precipitate the SiO_2, dissolving in distilled water, filtering, and then diluting to volume. Aliquot samples were used to determine calcium, magnesium, iron, potassium, and sodium with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer equipped with a triple-slot burner. Chemical interference in the calcium determination was suppressed with an acidified lanthanum chloride solution. Aluminum, silicon, sulfate, and total P_2O_5 were determined by standard gravimetric methods. The Official Methods of Analysis of the AOAC was used to determine the available P_2O_5 (13).

Weighed, previously dried samples of recovered activated and acclimated-activated sludges and aliquots of the centrifugates of the sludges and of the lime-sulfide effluent were prepared for amino acid analysis by hydrolyzing in 6 N hydrochloric acid solution for 24 hours under an atmosphere of nitrogen. Excess hydrogen chloride was removed by repeated evaporations under reduced pressure with intermittent additions of distilled water. The final residues then were diluted to a known volume with 0.1 N hydrochloric acid. The amino acid analyses were run in duplicate on a Phoenix amino acid analyzer with a Piez-Morris-type ion-exchanger column, using a continuous, gradient-elution buffer (15). Tryptophan was determined on separate samples of the sludges by the standard chromatographic technique after hydrolysis for 22 hours with 4 N methanesulfonic acid, in the presence of 3(2-aminoethyl)indole, under an atmosphere of nitrogen (16). Unhydrolyzed portions of the centrifugates of the two sludges and of the lime-sulfide effluent were used to determine the free amino acids.

RESULTS AND DISCUSSION

Composition of Lime-Sulfide Effluent

The composition of the full strength lime-sulfide effluent as received from the tannery is given in Table I. The data show that the effluent is an alkaline, highly polluting material with a high oxygen demand, containing substantial quantities of soluble and insoluble solids and residual sulfide. On the basis of 20,000 gallons of such an unhairing waste stream, the total solid discharge to waste amounts

TABLE I

COMPOSITION OF LIME-SULFIDE UNHAIRING EFFLUENT*

	p.p.m.		
BOD†, 15 min.	280	pH	12.5
BOD, 30 min.	480	Na_2S, %	0.35
BOD, 5 days 20°C.	21,600	Total N, %	0.49
COD‡	72,800	Organic N, %	0.45
TOC**	20,700	Settleable Solids††, ml./l.	200
Total Solids	55,900	Total Alkalinity ($CaCO_3$), mg./l.	11,386
Total Volatile Solids	29,068		
Suspended Solids	33,680		
Volatile Suspended Solids	10,105		

*Fresh hides, paddle vat, no wash water.
†BOD — Biochemical oxygen demand.
‡COD — Chemical oxygen demand.
**TOC — Total organic carbon.
††Thirty minutes at 25°C.

to almost 9500 lbs. per day. The total daily BOD discharge has a population equivalent of a municipality of about 30,276 in terms of domestic sewage.

Analyses of Recovered Activated and Acclimated-Activated Sludges

The analyses of the freeze-dried activated and acclimated-activated sludges are shown in Table II. Included for comparison is the analysis for recovered, degraded hair protein reported previously (3). The data are on a moisture-free basis. The fat contents of the activated sludges, 4.3 to 10.4 percent, were greater

TABLE II

ANALYSES OF RECOVERED ACTIVATED AND ACCLIMATED-ACTIVATED SLUDGES*

	Activated Sludge		Acclimated-Activated Sludge†			Recovered, Degraded Hair Protein**
	Phila-delphia	Abington	Lot 1	Lot 2	Lot 3‡	
Fat, %	10.4	4.3	3.6	2.0	0.7	3.0
Total S, %	1.0	0.2	1.1	2.2	0.9	3.8
Protein S, %††	0.2	0.2	0.5	1.1	0.9	3.2
Total N, %	6.4	7.1	6.3	7.4	7.6	15.5
Protein, %††	27.5	32.0	29.5	34.4	35.7	89.7

*Moisture-free basis, average of duplicate analyses.
†All three lots were derived from Abington activated sludge.
‡Dried without washing.
**Reported previously (3).
††Calculated from the amino acid analyses.

than those of the acclimated-activated sludges, 0.7 to 3.6 percent, and all varied considerably from each other. The total sulfur values of the acclimated-activated sludges, except for Lot 3, were 0.1 to 2.0 percent higher than those of the activated sludges, indicating the presence of additional inorganic and organic sulfur compounds contributed by the lime-sulfide effluent. The protein sulfur values of the acclimated-activated sludges, calculated from the amino acid analyses, were 0.3 to 0.9 percent higher than those of the activated sludges and accounted for a large portion of the total sulfur. This was due to the inclusion of sulfur-containing amino acids, especially cystine and lanthionine, which are present in relatively high concentrations in the unhairing effluent, as well as methionine. We have found 7.5 percent cystine and 3.9 percent lanthionine in protein recovered from lime-sulfide effluent from fresh hides unhaired in a paddle vat (3). Bio-oxidation of the unhairing effluent with activated sludge containing 0.2 percent cystine and no lanthionine produced acclimated-activated sludges containing 0.8 to 2.2 percent cystine and 1.0 to 1.8 percent lanthionine, on a weight percent, moisture-free product basis. However, all five sludges had considerably less protein sulfur than the recovered hair protein had. The total Kjeldahl nitrogen contents of the five sludges were not appreciably different from each other. The average protein contents of the activated and acclimated-activated sludges, calculated from the amino acid analyses, were 30 percent and 33 percent, respectively, about one third that of recovered hair protein. Judged by the protein or nitrogen content, these sludges would appear to be less attractive than degraded hair protein as a source of protein in animal feed.

Spectrographic Analyses of Acclimated-Activated Sludge

An emission spectrographic analysis of the acclimated-activated sludge (Lot 3, unwashed) was made by a commercial metallurgical laboratory to test for the presence of heavy metals. Table III shows the results of a semiquantitative scan for all metals. The data are on a moisture-free product basis. Sixteen elements

TABLE III

EMISSION SPECTROGRAPHIC ANALYSIS OF ACCLIMATED-ACTIVATED SLUDGE. SEMI-QUANTITATIVE SCAN OF LOT 3, UNWASHED.

Element	Range (%*)	Element	Range (%*)
Ag	.0001–.0009	Mg	.1 – .9
Al	.1 –.9	Mn	1.0 –5.0
B	.0001–.0009	Na	.1 – .9
Ca	>5.0	Ni	.01 – .09
Cr	.001 –.009	P	>5.0
Cu	.01 –.09	Si	1.0 –5.0
Fe	.1 –.9	Sr	.01 – .09
K†	.4	Ti	.001– .009

*Moisture-free product basis.

†Determined by atomic absorption at Eastern Regional Research Center.

were present in the quantities indicated. Potassium was determined by atomic absorption, since the concentration (0.4 percent) was less than the 0.5 percent limit of the spectrograph as set up. The sludge was free (within the limits of detection of the spectrograph) of toxic heavy metal contaminants such as Cd, Hg, Pb, Sn, or Zn, which might limit its use in feed.

Inorganic Composition of Recovered Activated and Acclimated-Activated Sludges

The inorganic composition of the activated and acclimated-activated sludges is given in Table IV. The data are on a moisture-free basis. The activated sludges had 25 percent ash. Acclimation increased the ash content to 30 to 41 percent. This was obviously due mainly to the lime from the unhairing effluent, as shown by the considerable increase in CaO content from five or six percent to 23 to 29

TABLE IV

INORGANIC COMPOSITION OF RECOVERED ACTIVATED AND ACCLIMATED-ACTIVATED SLUDGES*

	Activated Sludge		Acclimated-Activated Sludge†		
	Philadelphia	Abington	Lot 1	Lot 2	Lot 3‡
Ash, %	24.5	24.6	35.3	29.9	41.0
CaO, %	6.2	5.2	28.9	23.3	26.6
MgO, %	1.1	0.7	0.1	0.4	0.2
Fe_2O_3, %	1.2	1.2	0.6	0.4	0.9
Al_2O_3, %	1.3	2.1	2.1	2.0	1.6
SiO_2, %	8.7	7.7	0.9	2.0	0.9
K_2O, %	0.1	0.4	0.3	0.2	0.5
Na_2O, %	1.0	1.1	0.6	0.9	1.6
SO_4, %	0.6	0.6	2.9	1.9	2.7
Total P_2O_5, %	0.6	2.4	2.0	2.2	3.2
Available P_2O_5, %	0.3	1.9	2.0	2.0	0.7

*Moisture-free basis, average of duplicate analyses.
†All three lots were derived from Abington activated sludge.
‡Dried without washing.

percent. The calcium was probably present as carbonate in the acclimated-activated sludge because of the reaction with carbon dioxide formed during bio-oxidation. Acclimation produced a considerable decrease in magnesium (from 0.9 to 0.2 percent MgO), iron (from 1.2 to 0.6 percent Fe_2O_3), and silica (from 8.2 to 1.3 percent SiO_2) contents and a significant increase in sulfur compounds (from 0.6 to 2.5 percent SO_4). There were no significant changes in the aluminum, potassium, sodium, and phosphorus contents. The unwashed acclimated-activated sludge (Lot 3) contained approximately 8.4 percent more ash, 0.4 percent more Fe_2O_3, 0.3 percent more K_2O, 1.7 percent more Na_2O, and 1.1 percent more total P_2O_5, but 1.3 percent less available P_2O_5 than the washed Lots 1 and 2.

Free Amino Acids in Unhydrolyzed Centrifugates of Lime-Sulfide Effluent and Acclimated-Activated Sludge

Table V shows the presence of free amino acids in the unhydrolyzed centrifugates of the lime-sulfide effluent and the acclimated-activated sludge. The data are given in p.p.m. The activated sludge centrifugate contained no free amino acids. The lime-sulfide effluent had measurable amounts of fourteen amino acids, seven of which are essential nutritionally, resulting from the alkaline degradation of the keratin and possibly proteins from the hide. The other amino acids were probably still combined, possibly as polypeptides, in the degradation products. Glutamic acid was most abundant, approximately 2.4 to 22 times more

TABLE V

FREE AMINO ACIDS IN UNHYDROLYZED CENTRIFUGATES OF LIME-SULFIDE EFFLUENT AND ACTIVATED AND ACCLIMATED-ACTIVATED SLUDGES*

	Unhydrolyzed Centrifugate				Unhydrolyzed Centrifugate		
Free Amino Acid	Lime-Sulfide Effluent	Activated Sludge (Abington)	Acclimated Sludge (Lot 2)	Free Amino Acid	Lime-Sulfide Effluent	Activated Sludge (Abington)	Acclimated Sludge (Lot 2)
Ala	16	0	7	Lys†	14	0	0
Arg†	trace	0	0	Met†	8	0	2
Asp	6	0	6	Phe†	7	0	3
Cys	trace	0	0	Ser	19	0	0
Glu	66	0	4	Thr†	5	0	0
Gly	27	0	4	Tyr	5	0	3
Ile†	3	0	0	Val†	7	0	5
Leu†	7	0	8	Unknown	4	0	0

*The data are given in p.p.m.
†Essential amino acid.

abundant than the other amino acids. The concentrations of alanine, glycine, lysine, and serine were also higher than those of the nine remaining amino acids.

The acclimated-activated sludge centrifugate had small but measurable amounts of nine amino acids, of which leucine, methionine, phenylalanine, and valine are essential. The greatest decreases in concentration, compared to the lime-sulfide effluent, were in alanine (16 to seven p.p.m.), glutamic acid (66 to four p.p.m.), glycine (27 to four p.p.m.), lysine (14 to zero p.p.m.), and serine (19 to zero p.p.m.). The data show that at least ten of the free amino acids present in the lime-sulfide effluent had been utilized by the microorganisms.

Amino Acids in Hydrolyzed Centrifugates of Lime-Sulfide Effluent and Activated and Acclimated-Activated Sludges

Other portions of the centrifugates were hydrolyzed and analyzed. The hydrolyzed activated sludge centrifugate had only traces of alanine, aspartic acid, glutamic acid, glycine, serine, leucine, threonine, and valine, the latter three of which are essential amino acids. The hydrolyzed lime-sulfide and acclimated-activated sludge centrifugates had all twenty of the amino acids typical of recovered hair protein (3).

Amino Acid Composition of Recovered Activated and Acclimated-Activated Sludges

The amino acid compositions of the recovered activated and acclimated-activated sludges are given in Table VI. The data, in percent by weight, were calculated on a moisture-free *product basis,* so that the amino acid contents would

TABLE VI

AMINO ACID COMPOSITION OF RECOVERED ACTIVATED AND ACCLIMATED-ACTIVATED SLUDGES*

Amino Acid	Activated Sludge		Acclimated-Activated Sludge		
	Philadelphia	Abington	Lot 1	Lot 2	Lot 3†
Essential					
Arg	1.79 ± .01	1.85 ± .02	2.43 ± .04	3.16 ± .02	3.20 ± .02
His	0.63 ± .01	0.70 ± .01	0.56 ± .07	0.45 ± .01	0.52 ± .02
Ile	1.31 ± .05	1.61 ± .01	1.33 ± .01	1.25 ± .02	1.48 ± .01
Leu	2.29 ± .06	2.53 ± .01	2.13 ± .03	1.82 ± .02	2.33 ± .01
Lys	1.92 ± .08	1.84 ± .01	1.45 ± .07	1.02 ± .03	1.28 ± .01
Met	0.71 ± .01	0.74 ± .01	0.46 ± .03	0.33 ± .01	0.42 ±0
Cys‡	0.20 ± .01	0.20 ± .01	0.79 ± .01	2.16 ± .01	1.52 ± .03
Phe	1.30 ± .02	1.71 ± .08	1.23 ± .02	1.15 ± .03	1.50 ±0
Tyr**	1.06 ± .03	1.25 ± .01	1.21 ± .02	1.24 ± .02	1.45 ±0
Thr	1.43 ± .03	1.81 ± .06	1.73 ± .04	2.29 ± .05	2.39 ± .02
Trp††	0.12	slight trace	slight trace	0.13	slight-trace
Val	1.89 ± .03	2.08 ± .01	1.79 ± .04	1.87 ± .01	2.20 ± .01
Nonessential					
Ala	2.22 ± .07	2.40 ± .05	1.99 ± .03	1.51 ± .01	2.03 ± .02
Asp	3.10 ± .03	3.50 ± .06	2.75 ± .04	2.35 ± .05	3.43 ± .03
Glu	3.16 ± .05	3.88 ± .02	3.62 ± .05	3.51 ± .07	4.04 ± .02
Gly	1.55 ± .05	1.84 ± .02	1.41 ± .02	1.52 ± .03	1.69 ± .02
Pro	1.02 ± .02	1.28 ±0	1.59 ± .05	2.55 ± .02	2.35 ± .05
Ser	1.17 ± .01	1.42 ± .01	1.52 ± .06	2.21 ± .05	1.92 ± .01
Uncommon					
Lan‡‡	—	—	0.98 ± .01	1.77 ± .02	1.56 ± .01
Lya***	—	—	—	—	—
Unknown†††	0.13 ± .01	0.25 ±0	0.15 ± .01	0.24 ± .02	0.10 ± .01

*Concentrations are weight percent, moisture-free basis; actual experimental range found in two separate runs.
†Dried without washing.
‡Sparing amino acid for methionine.
**Sparing amino acid for phenylalanine.
††Run only once on a separate sample.
‡‡Lanthionine; may have sparing effect on methionine.
***Lysinoalanine.
†††Unknown, elutes between methionine and isoleucine. Calculated using the leucine color constant.

be in a form suitable for determining potential uses for the acclimated-activated sludge. An application of the data is discussed later. A detailed comparison of the amino acid composition of the sludges on an *equal protein basis* is given in Table VII.

TABLE VII

EFFECT OF ACCLIMATION TO LIME-SULFIDE EFFLUENT ON THE AMINO ACID COMPOSITION OF ACTIVATED SLUDGE

Amino Acid	Recovered Degraded Hair Protein*	Activated Sludge		Acclimated-Activated Sludge		
		Philadelphia†	Abington†	Lot 1†	Lot 2†	Lot 3†‡
Essential						
Arg	8.8	6.3	5.8	7.6	9.2	8.5
His	1.0	2.0	2.2	2.0	1.4	1.4
Ile	3.6	4.5	4.7	4.1	3.7	4.0
Leu	7.2	7.9	7.5	6.6	5.1	6.2
Lys	3.5	6.5	5.4	4.9	2.9	3.4
Met	0.6	2.5	2.2	1.6	0.8	1.1
Cys**	7.7	0.7	0.6	2.5	6.3	4.0
Phe	2.5	4.5	5.2	3.9	3.3	4.0
Tyr††	4.0	3.8	3.9	3.9	3.3	4.0
Thr	5.6	5.0	5.4	5.5	6.7	6.3
Trp‡‡	0.4	0.4	slight trace	slight trace	0.4	slight trace
Val	4.6	6.5	6.5	5.7	5.5	5.8
Nonessential						
Ala	3.2	7.7	7.3	6.4	4.3	5.2
Asp	7.1	10.8	10.5	9.0	6.9	8.9
Glu	14.7	11.1	11.8	11.5	10.0	10.6
Gly	2.9	5.6	5.4	4.5	4.3	4.5
Pro	5.5	3.4	3.9	5.1	7.5	6.3
Ser	6.0	4.1	4.3	4.9	6.3	5.1
Uncommon						
Lan***	4.0	0	0	3.1	5.1	4.2
Lya†††	0.2	0	0	—	—	—
Unknown‡‡‡	0.3	0.5	0.9	0.6	0.6	0.3

*Concentrations are weight percent, moisture-, ash-, fat-free basis, reported previously (3).
†Concentrations are weight percent, moisture-, ash-, fat-free basis, converted to the 93.4 percent protein content of the recovered hair protein.
‡Dried without washing.
**Sparing amino acid for methionine.
††Sparing amino acid for phenylalanine.
‡‡Run only once on a separate sample.
***Lanthionine; may have sparing effect on methionine.
†††Lysinoalanine.
‡‡‡Unknown, elutes between methionine and isoleucine. Calculated using the leucine color constant.

Effect of Acclimation to Lime-Sulfide Effluent on the Amino Acid Composition of Activated Sludge

To compare the amino acid compositions of the sludges with each other and with degraded hair protein on an equal protein basis, it was necessary to calculate the amino acid concentrations shown in Table VI on comparable ash-free and fat-free, as well as moisture-free, bases. This was done by calculating the protein levels of the sludges to the 93.4 percent protein content of the recovered degraded hair protein (Table VII). Concentrations are given as percent by weight. In general, the amino acid composition of the acclimated-activated sludge indicated a composite of degraded hair protein and activated sludge protein. Lots 2 and 3 appeared to incorporate more amino acids of hair protein than Lot 1 did, since the arginine, cystine, threonine, proline, serine, and lanthionine contents were greater. All eight amino acids essential for human nutrition, as well as histidine and arginine, were present. Rats require histidine in addition to the eight essential amino acids, and chicks require both histidine and arginine. Cystine and tyrosine are listed with the essential amino acids because nutritionally, according to Rose and Wixom (17, 18) and others, they can substitute for some of the methionine and phenylalanine, respectively. The acclimated-activated sludges grown on unhairing effluent had approximately a 2.3 percent increase in arginine, a 3.6 percent increase in cystine, no significant changes in histidine, isoleucine, threonine, and tyrosine, and the following decreases: leucine 1.7 percent, lysine 2.3 percent, methionine 1.2 percent, phenylalanine 1.2 percent, valine 0.8 percent, compared to these amino acids in activated sludges.

The increase in protein sulfur could be beneficial for poultry feed. A typical average summer ration from Maddy Associates (19) egg production data bank, layer phase II, Diet No. 11, requires a minimum of about 0.61 percent methionine + cystine. The average total concentrations of methionine + cystine given in Table VI were 0.93 percent for activated sludge (average of Philadelphia and Abington) and 1.89 percent for acclimated-activated sludge (average of Lots 1, 2, and 3). Therefore, if used as 10 percent of the ration, the acclimated-activated sludge would supply about one third of the minimum required methionine + cystine, whereas the activated sludge would supply only about one sixth of the minimum. While the acclimated sludge had lower levels of lysine and methionine, the levels of those and the other amino acids were still high enough to have a good overall value as a protein supplement in poultry feed. Poultry rations usually contain proteins from several sources for nutritional balance. To evaluate the nutritional quality of the acclimated-activated sludge, bioassays and animal feeding studies will be required to determine its digestibility and biological availability.

The nonessential amino acids, alanine, aspartic acid, glutamic acid, glycine, proline, serine, and several unknown amino acids (in addition to cystine and tyrosine) were present in the sludges. The unknown amino acid listed in Table VII, eluting between methionine and isoleucine, was the same as that in the recovered hair protein, as reported by Feairheller *et al.* (20). The recovered hair protein also contained 4.0 percent lanthionine, formed during unhairing, and 0.2 percent lysinoalanine, which Feairheller *et al.* (21) found was also formed during unhairing. Lanthionine and lysinoalanine were not found in either of the activated sludges. However, 3.1 to 5.1 percent lanthionine was present in the acclimated-activated sludges, a fact which indicated that degraded hair protein had been either admixed with or incorporated into the activated sludges. Lysinoalanine was probably present in small quantities in the acclimated-activated sludges, but could not be determined quantitatively because of an interfering peak in the activated sludges, which was carried over into the acclimated-activated sludges. Acclimation produced in the acclimated-activated sludges a 2.2 percent decrease in alanine, a 2.4 percent decrease in aspartic acid, and a 1.1 percent decrease in glycine, no significant change in glutamic acid, a 2.6 percent increase in proline, and a 1.2 percent increase in serine compared to these amino acids in activated sludges. The amino acid composition of the unwashed acclimated-activated sludge, Lot 3, was approximately the same as those of the washed Lots 1 and 2.

Further investigation will be needed to determine whether or not the indicated changes in amino acid composition are due to inclusion, occlusion, absorption, or adsorption of the degraded hair protein by the activated sludge, or to changes in composition of the microorganisms.

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DISCUSSION

MR. RUSSELL LAKOSKI (Ocean Leather Corporation): Tanners certainly are not happy with the fact that the cost of treating tannery effluent does not add one cent of value to the tanners' leather, but they all realize that something must be done about treating tannery effluent. The point that ought to be made is that it should be done as economically as possible. Therefore, work such as we have just heard may be the stepping stone for a better procedure to solve some of our effluent problems.

Tanneries can be divided into three groups with regard to treating their effluent: those who have spent large sums of money to treat their effluent; those who pay a surcharge to a municipal system to handle their effluent; and those who don't know how lucky they are who presently don't pay anything at all. However, I believe that all three groups are in for future surprises.

Bill, what type of tannery do you believe will benefit most from the work presented in this paper?

MR. W. F. HAPPICH: We think the acclimated-activated sludge process could be used in several different situations. First, in those situations where the municipal treatment plant is already overloaded with high BOD waste water effluents entering the treatment plant, the tannery could possibly set up its own small treatment plant just to handle its beamhouse effluent which has a very high BOD. Also, where a tannery is located in a small community whose treatment system does not have the capacity to handle the tannery wastewater load or where a tannery might be situated in the country and no municipal treatment system is available, this process could be an advantage. Furthermore, since up to 65 percent of the total BOD of the tannery could be contained in the unhairing effluent, there would be a distinct advantage for the tanner to treat perhaps only 20,000 gallons of effluent per day instead of 500,000 to 1,000,000 gallons per day for the total effluent.

Mr. S. M. De (Chestnut Operating Company): What is the retention that you maintain?

Mr. Happich: In our unit it was approximately five hours.

Mr. De: Using that time, you could reduce the sulfide from 3,500 to around 15 parts per million?

Mr. Happich: That is correct.

Mr. De: Have you tried this system on a pilot plant scale?

Mr. Happich: We have not yet operated on a pilot plant scale, but we intend to do so to get data to be used for full production estimation.

Mr. De: You are using diffused air for aeration. Since beamhouse sludges are very thick and heavy and thus could clog the submerged diffusers, do you think that floating aerators could do the job?

Mr. Happich: We have not tested floating aerators.

Mr. De: We have found that, in sulfide oxidation by aeration with surface aerators in a 55,000 gallon tank, the bottom sludge, about a foot or so, stays essentially unchanged in character from that with which we start.

Mr. Happich: I think that in activated sludge processes, it is very important to have the air come up from the bottom to give the proper oxygen contact and also circulation of the activated sludge.

Mr. De: Do you see any problem with clogging of the diffusers? Also what about the economics?

Mr. Happich: In the British work where manganese catalyst is being used for sulfide oxidation with air, they have developed large diffusers for the bottom of their tower. They do mention the need for cleaning these diffusers.

Dr. Edward M. Filachione (Eastern Regional Research Center): We might point out that we hope to tie this work into our work on flocculation studies. Thus a preliminary treatment with a flocculating agent to remove most of the suspended solids may be highly desirable.

Mr. Happich: In this present work, we used a 50x50 mesh polypropylene screen to remove large solids. However, in the work that Dr. Filachione just mentioned we are getting rid of a lot of the suspended solids by flocculation and sedimentation. So, we could use a combination of these two studies and avoid a lot of the clogging mentioned by Mr. De.

MR. HERBERT ELLISON (Armira Corporation): I noticed in one of your charts that you had a reduction of 63–65 percent in organic nitrogen. What temperature did you maintain in your activated sludge system?

MR. HAPPICH: This work was carried on at laboratory room temperatures (25–28°C.).

MR. ELLISON: What were the levels in milligrams per liter?

MR. HAPPICH: I recall that we started at 0.40 percent total nitrogen or 4,000 mg. per liter.

MR. LAKOSKI: I certainly want to thank Bill Happich and the USDA for their continuing work trying to assist us in handling our waste waters efficiently.