FISHERY WASTE EFFLUENTS: A SUGGESTED SYSTEM FOR DETERMINING AND CALCULATING POLLUTANT PARAMETERS

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ABSTRACT

An improved and simplified system to test for pollutants in shrimp waste effluents is presented. In addition, two methods were developed to calculate both protein and oil and grease content. The first method is based on establishing empirical regressions of protein or oil and grease on total residue. The second and preferred method, a simultaneous equation, is independent of these correlations but dependent on the total residue and chemical oxygen demand (COD) of the waste effluent obtained through routine analyses. The COD value was found to depend upon the amount of potassium dichromate remaining at the completion of the 2-h reflux period. The dichromate can vary from 0 to 6.25 meq excess and between 2 and 5 meq, the COD will vary 4.2%. A table of factors is given to correct the COD to 3.5 meq excess. Coefficients of COD were determined on a number of preparations of protein and oil and grease from shrimp waste effluent and from fish and shellfish. These coefficients (1.338 mg COD/mg protein and 2.678 mg COD/mg oil and grease) were required for the simultaneous equation. The simple analytical tests and mathematical treatment used in this system would be less expensive to the industry and would result in a more accurate and comprehensive evaluation of the waste load than currently obtainable by methods specified in the monitoring regulations.

An improved testing program for fishery waste effluents has been suggested (Collins and Tenney 1976) in which the total residue (TR) and the chemical oxygen demand of the filterable residue (COD_{FR}) were to be determined by analysis and used to calculate other parameters from equations previously established for a particular plant and process. It was also suggested that the protein and oil and grease (O&G) content could probably be calculated from COD and TR data to give more accurate values than by direct analyses.

The purpose of this study was to test the validity of such a testing-calculating system on waste effluents from a shrimp plant in Kodiak, Alaska. A further purpose was to derive equations whereby O&G and protein could be calculated from COD and TR data.

EXPERIMENTAL

Grab samples were taken at specific times during the shrimp production periods to obtain a range in values that would be useful for subsequent mathematical treatment. Waste effluents were taken from the underflow of a Bauer Hydra-

sieve[2] (1 mm, 0.04 inch) in a plant processing shrimp with combined Model A and PCA peelers. The methods of analysis and the method of calculating data are similar to those reported previously (Collins and Tenney 1976). The test for filterable residue (FR) was modified, however, to give sufficient filtrate (900 ml) for duplicate macro-Kjeldahl, COD, FR, and ash analyses. About 1,000-ml effluent, after settling 30 min, was decanted through a plug of glass wool in a powder funnel positioned over a 600-ml coarse sintered glass funnel containing GF/A glass filter paper and ¼ inch of dry base-acid-water washed ASTM standard Ottawa sand (C-190). The suction flask was evacuated briefly several times during filtration and clamped off to prevent plugging of the filter and evaporation. We have found that use of continuous evacuation causes rapid plugging of the glass filter paper and, additionally, could cause considerable errors through evaporation.

As will be discussed later, the precision of the residue and ash analyses is particularly important. Consequently, considerable attention was given these analyses to obtain good precision as well as convenience in conducting the analyses. The major steps of the procedure follow:

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[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

1. Heat 100-ml Pyrex beakers at 500°C for 1 h, air cool for 1 h, and weigh. Prior to use, new beakers should be equilibrated to ashing conditions.
2. Accurately weigh about an 80-ml sample of effluent into the dry beaker. Dry overnight at 103°C in a forced draft oven and weigh after 1 h of air cooling.
3. Calculate TR in milligrams/liter. (Note: this system, of course, gives TR in milligrams/1,000 g, but we follow the convention and express it in milligrams/liter.)
4. Heat beaker and dried sample at 500°C for 2 h, air cool 1 h, and weigh as before.
5. Calculate ash from the initial weight of sample, express as milligrams/liter as in step 3.

RESULTS

In general, these effluent samples were tested for COD, residue, ash, O&G, and protein. The data in Table 1 are averages of duplicate analyses, except O&G which is in triplicate. The data should not be considered representative of the effluent from this plant because of the specific way of taking these grab samples. Comparisons in relative data, however, can be made. For example, the COD of the filterable residue (COD_{FR}) was slightly over one-half of the total COD (COD_{TR}) and the filterable residue (FR) was 64% of the total residue (TR) on an ash-free basis. The TR contained 17% ash, but most of the ash was found in the FR fraction (92%) leaving only 8% in the nonfilterable residue (NFR) fraction.

The relationship between COD and ash-free residue is plotted in Figure 1 and that for O&G

$$COD_{TR} = 1.41\ TR_K - 28$$

$$COD_{FR} = 1.39\ FR_K - 166$$
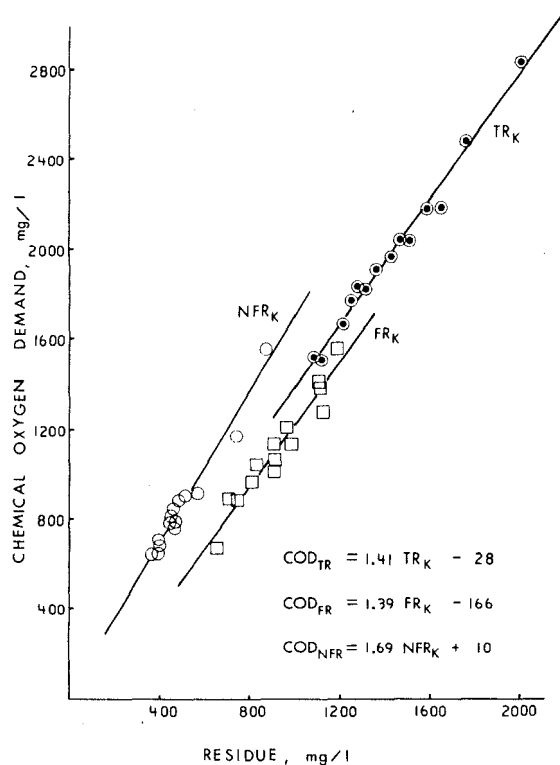
$$COD_{NFR} = 1.69\ NFR_K + 10$$

FIGURE 1.—Relationship between the COD and the concentration of the ash-free residue in waste effluents from a plant using both Model A and PCA peelers and fresh water.

and protein versus ash-free residue is given in Figure 2. The coefficients of correlation were 0.99 and 0.97 for the COD regressions on TR_K and FR_K, respectively. The F-test for linearity at the 95% level of significance was 0.015 for the TR_K line and

TABLE 1.—Analyses of screened shrimp waste effluents from a plant using both Model A and PCA mechanical peelers. [All values in milligrams/liter.]

| Sample number	Chemical oxygen demand		Residue		Ash		Protein (6.25N)		Oil and grease
	TR	FR	TR	FR	TR	FR	TR	FR	TR
1	1,517	672	1,420	946	304	291	831	522	185
2	2,839	1,280	2,328	1,441	325	310	1,319	859	486
3	2,190	1,016	1,911	1,146	264	241	1,215	785	276
4	2,182	1,413	1,897	1,400	308	288	1,281	947	258
5	1,824	1,139	1,567	1,146	261	242	1,056	790	203
6	1,917	1,210	1,602	1,182	242	220	1,075	806	230
7	2,039	1,393	1,833	1,418	324	298	1,212	944	229
8	1,771	964	1,532	1,061	280	256	1,037	744	195
9	2,481	1,565	2,137	1,522	378	332	1,425	1,072	302
10	1,969	1,066	1,750	1,197	321	284	1,175	835	204
11	1,666	883	1,460	965	247	224	1,025	703	186
12	1,829	1,046	1,573	1,093	286	263	1,116	794	175
13	2,041	1,156	1,822	1,310	352	328	1,188	863	233
14	1,522	883	1,351	946	256	228	925	644	148
Mean	1,985	1,120	1,727	1,198	296	272	1,134	808	236
SD	361	240	280	193	41	38	158	136	83
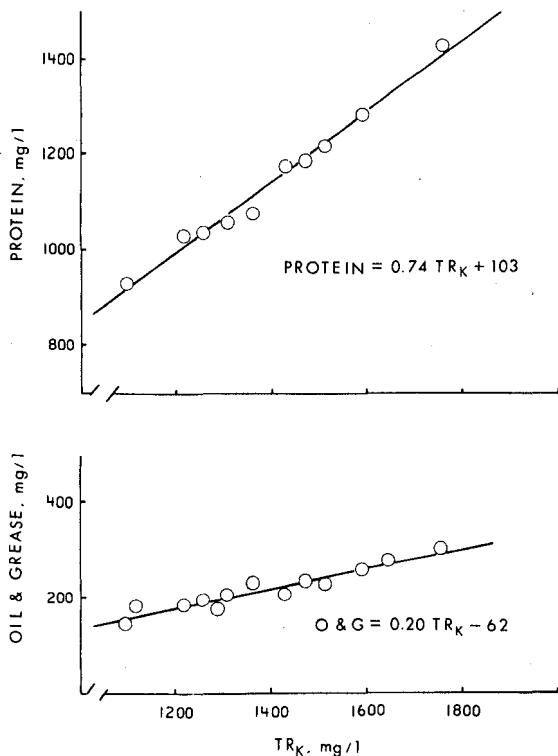
FIGURE 2.—Relationship between the concentration of protein or oil and grease and the concentration of the ash-free total residue in waste effluents from a plant using both Model A and PCA peelers and fresh water.

0.068 for FR_K. The regression lines and equations found in Figures 1 and 2 include a correction for ash content in the residue, i.e., TR − ash = TR_K. These equations, obtained by the method of least squares, are as follows:

$$COD_{TR} = 1.41\ TR_K - 28 \qquad (1)$$
$$COD_{FR} = 1.39\ FR_K - 166 \qquad (2)$$
$$COD_{NFR} = 1.69\ NFR_K + 10 \qquad (3)$$
$$Protein = 0.74\ TR_K + 103 \qquad (4)$$
$$O\&G = 0.20\ TR_K - 62 \qquad (5)$$

In our previous paper we suggested that background data for a particular plant should be determined [Equations (1), (2), and (3)] so that the other parameters could be calculated from routine tests for TR and COD_{FR}. Since usage of salt and seawater in plants tends to vary, we now also suggest that an ash analysis be done to eliminate variability in the total residue. Once background data have been established for a particular plant or product process, our testing-calculating system would proceed as follows: Determine TR and ash and substitute the difference into Equation (1) and solve for COD_{TR}. Using the mean values for TR and ash of Table 1 gives 1,431 mg/liter TR_K. Substitution into Equation (1) gives 1,990 mg COD_{TR}/liter which nearly agrees with the mean analytical COD value. Similarly, the other recommended routine test for COD of the filtrate (COD_{FR}) gives a mean value from Table 1 of 1,120 mg/liter which, when substituted into Equation (2), gives 925 mg/liter for FR_K, in agreement with the difference between FR and ash, i.e., FR − ash = 926 mg/liter. The NFR or COD_{NFR} are obtained by difference, e.g., $TR_K - FR_K = NFR_K$. In order to calculate protein and O&G, the TR_K can be substituted into Equations (4) and (5). A rough estimate of O&G content can also be obtained by dividing the COD by 9 which is the average for the ratio of COD to the weight of O&G. The ratio actually varies from about 8 to 10 and inversely with the COD. The ratio and equations only have application to this plant and processing conditions. For other processing conditions or plants, the baseline data and equations should be determined in the same manner.

CALCULATION OF O&G AND PROTEIN USING A SIMULTANEOUS EQUATION

In this section we will derive a simultaneous equation that can be used as a substitute for direct analysis so that O&G and protein can be calculated by using routine data on COD_{FR}, TR, and ash. The equation is based on the assumption that the sum of the COD of each component in the effluent equals the total COD, i.e., COD ($x_1 + x_2 \ldots x_n$) = total COD; and that the sum of the weights of each constituent having an effect on COD equals the total residue minus ash, i.e., Residue ($x_1 + x_2 \ldots x_n$) = Total residue − ash.

To develop the simultaneous equation, coefficients must first be determined that relate COD to the two major constituents of a fishery waste (protein and O&G). In addition, the residue-ash relation needs defining.

COD in Relation to Protein and O&G

To establish a relationship between COD and pollutants, we prepared samples of protein and

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O&G and determined their COD equivalent by direct analysis.

To prepare protein a sample of muscle was washed with water and centrifuged to remove the blood and other small nitrogen components, then washed with 2-propanol (IPA) to remove part of the water. The sample was blended and refluxed twice with IPA followed by filtration, washing, and refluxing with petroleum ether (PE) and overnight drying at 103°C. These oil free, white, odorless protein samples were analyzed for nitrogen by the standard macro-Kjeldahl method (Horwitz 1965:273) and for COD. The COD factor was calculated on a 100% protein basis.

To obtain O&G, the sample of fish or shellfish was briefly rinsed with water and IPA; then, using a high speed blender and anhydrous conditions (MgSO$_4$), the O&G was extracted, cold, with IPA and PE. For waste effluent, O&G was obtained by the analytical method used previously (Collins 1976). By either method, after weighing the dry O&G and diluting to volume with PE an aliquot of the final solution equivalent to 8-10 mg O&G was evaporated in the COD flask, oven-dried for 0.5 h, and used for COD determination. Since PE has a residue significantly affecting COD, freshly distilled PE was used throughout the tests.

The COD equivalent was determined on a number of different preparations of O&G and protein from fish and shellfish muscle and from shrimp waste effluent. The average values of from 5 to 30 replicate COD analyses for each material are given in Table 2.

The COD coefficients for protein are in reasonable agreement and are probably independent of

species or product form. The theoretical COD coefficient of protein was calculated using amino acid percentage composition data for snow crab reported by Krzeczkowski and Stone (1974). The theoretical figure of 1.285 mg COD/mg protein was in close agreement with our experimental figure of 1.338. The coefficients for O&G, however, are quite different and are presumably caused by errors in the COD method, differences in species, product, and perhaps slight differences in the method of extracting. There are, of course, known differences in the lipid composition of these species, especially the C-20 and C-22 polyunsaturated fatty acids. The chain length and configuration of the lipids would have a positive effect on the COD coefficient. For example, some theoretical coefficients are: acetic acid (C$_2$) 1.066, propionic (C$_3$) 1.514, myristic (C$_{14}$) 2.807, melissic (C$_{30}$) 3.115, lecithin (C$_{44}$H$_{88}$O$_9$NP) 2.458, and tristearin (C$_{57}$H$_{110}$O$_6$) 2.934. Recognizing the wide variations possible, the empirically derived coefficient of 2.678 seems reasonable.

These coefficients are used along with the concentration of protein and O&G to give the COD, i.e., (1.338 mg COD/mg protein)mg protein + (2.678 mg COD/mg O&G)mg O&G = COD$_{TR}$ and assumes that the total COD is the sum of the COD of these two major constituents. To check the validity of this equation the coefficients were multiplied by the predicted values for protein and O&G [obtained from TR$_K$ data and Equations (4) and (5)] and the resulting mean of the sums of the products (2,155 mg COD/liter) was found to be 1.083 times greater than the mean predicted value for COD$_{TR}$ (1,990 mg COD/liter) obtained from TR$_K$ data and Equation (1). Although difficult to prove or demonstrate, we believe that the lower analytical values for COD in a sample of waste effluent are caused by the unequal and competing oxidation of protein and O&G. As is well known, O&G reacts slowly and especially if the dichromate concentration has been reduced from reacting with the more easily oxidized protein. Minor constituents such as nonprotein nitrogen and carbohydrates would contribute to COD in a ratio different from the protein coefficient. Regardless, if the simultaneous equation is to be developed, the inequality must be adjusted by increasing the COD value to equal the sum of the COD of protein plus O&G, i.e.,

TABLE 2.—The COD coefficient of several preparations of oil and grease (O&G) and protein from fish and shellfish and from shrimp waste effluent.

Starting material	COD of 1.0 mg/liter of	
	O&G	Protein
Black cod, frozen		1.328
Pollock, frozen		1.328
Snow crab, frozen	2.631	
Pink salmon, fresh	2.795	1.326
	2.818	1.345
Pink shrimp, fresh	2.710	1.349
	2.505	1.270
		1.328
Pink shrimp, canned	2.757	1.414
	2.584	1.350
	2.518	
	2.736	
Shrimp waste effluent	2.788	
	2.618	
Mean	2.678	1.338
SD	0.112	0.037

$$1.338 \text{ protein} + 2.678 \text{ O\&G} = 1.083 \text{ COD}_{TR}. \quad (6)$$

COD Reaction

The oxidation reaction in the COD method follows the usual chemical reaction laws, i.e., the completeness of the reaction is dependent upon the concentration of the reactants (potassium dichromate and waste). The method uses 25 ml 0.25N or 6.25 meq $K_2Cr_2O_7$ in the reaction flask and 50 ml of effluent. If the effluent is relatively strong, most of the dichromate will be expended in the reaction which results in an incomplete reaction and a lower COD value than if the waste were weak, i.e., having a larger excess of dichromate at the completion of the reaction. Moore and Walker (1956) recommended that the size of sample should be selected so that not more than 50% of the potassium dichromate is used up during the oxidation. To illustrate the relationship between COD and amount of dichromate remaining (the excess) at the end of the 2-h reflux period, data from six protein preparations were combined and plotted in Figure 3. The equation of the regression line was then used to calculate correction factors so that if the COD were determined at an excess dichromate level above or below an arbitrary point of 3.5 meq, the value can be corrected to its value at 3.5 meq. These correction factors are listed in Table 3. To correct COD
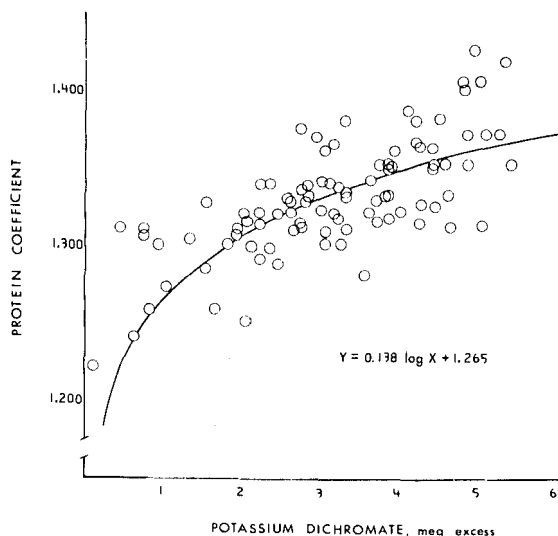
TABLE 3.—Multiplication factors to correct COD to 3.5 meq dichromate excess.

Excess dichromate (meq)	Multiplication factor	Excess dichromate (meq)	Multiplication factor
2.0	1.026	3.6	0.999
2.1	1.024	3.7	0.998
2.2	1.021	3.8	0.996
2.3	1.019	3.9	0.995
2.4	1.017	4.0	0.994
2.5	1.015	4.1	0.993
2.6	1.014	4.2	0.992
2.7	1.012	4.3	0.991
2.8	1.010	4.4	0.990
2.9	1.009	4.5	0.989
3.0	1.007	4.6	0.988
3.1	1.005	4.7	0.987
3.2	1.004	4.8	0.986
3.3	1.002	4.9	0.985
3.4	1.001	5.0	0.984
3.5	1.000		

values, determine the excess dichromate (titration value times normality) and multiply the corresponding factor from Table 2 by the COD determined in the usual way.

Since titration (Jirka and Carter 1975), sample, or reaction errors occur at either end of the curve, we suggest that COD values are valid only between 2 and 5 meq excess. All data for the protein coefficients were determined by obtaining from 10 to 30 COD values at different addition levels (5 to 30 mg protein/50 ml) and plotting the regression line. The coefficient was obtained by substituting the logarithm of 3.5 meq excess into the equation for the regression and solving for COD. In addition, all COD data in Table 1 were corrected to 3.5 meq excess dichromate.

Residue-Ash Correction

The major components of the total residue that contribute to COD are protein and O&G. In addition, various salts and dirt contribute to TR and possibly to COD. Unfortunately, there is no convenient method to measure these minor constituents so we estimate them by determining ash and then subtract to give a corrected value for TR. Since the weight of ash obtained after 500°C drying is less than its corresponding weight when dried at 103°C, the TR_K value (TR − ash) is accordingly greater than it should be. Therefore, the TR_K was reduced as follows: To eliminate variability in individual values, the O&G and protein values were predicted using Equations (4) and (5) for the regression lines in Figure 2 and TR_K data. The sum of the weight of protein plus O&G was found to be about 3% smaller than TR_K, i.e.,

FIGURE 3.—Relationship between the protein coefficient and the amount of dichromate remaining at the end of the 2-h reflux period.

protein + O&G = 0.969 TR$_K$.   (7)

This equation corrects the TR$_K$ so that it equals the sum of the protein and O&G, and is convenient to use in this form in the simultaneous equation. The constant, 0.969, is the result of increasing the analytical value for ash by 15.2% and represents, in part, the difference in weight of ash between drying at 500°C and 103°C.

Simultaneous Equation

In the preceding discussion we have shown the two parts of the simultaneous equation: the first showing the sum of the COD from protein and from O&G to be equal to an adjusted total COD, and the second showing the sum of the weights of protein and O&G to be equal to the total residue minus the ash content and corrected for the difference in weight caused by drying at 500°C or 103°C. Equations (6) and (7) are combined in the following so that a simple calculation can serve as a substitute for the difficult direct analyses for protein and O&G:

$$X + Y = 0.969 \text{ TR}_K$$
$$1.338X + 2.678Y = 1.083 \text{ COD}_{TR}$$ (8)

where: X = protein in milligrams/liter
Y = O&G in milligrams/liter.

This equation should have general application to fishery waste effluents provided: 1) TR$_K$ and COD$_{TR}$ are known or can be derived, and 2) the constant used to increase the value for COD$_{TR}$ has general application. If our assumption is correct that the COD is low because of the incomplete and competitive oxidation of protein and O&G, the constant would apply to any fishery waste having a similar relative amount of protein and O&G, i.e., about 5:1, respectively.

The mean TR and ash data from Table 1 are used to illustrate the use of this equation: From Table 1, TR − ash = 1,431 mg/liter and when substituted into Equation (1) gives a value of 1,990 mg/liter for COD$_{TR}$. These values, when substituted into the equation and solved for X and Y, give,

$$X + Y = 0.969(1,431)$$
$$1.338X + 2.678Y = 1.083(1,990)$$

where: X = 1,163 mg protein/liter
Y = 224 mg O&G/liter.

The calculated values are 29 mg higher for protein and 12 mg lower for oil than the mean analytical values of Table 1 (1,134 and 236, respectively). The differences between data obtained by the direct analysis for protein and O&G and the two methods of calculation are compared in Table 4. A negative or positive sign indicates whether the calculated value is less or more than the analytical value.

The analytical values of sample numbers 1, 2, 3, and 12 for protein and 2 for O&G are obviously in error and although these values were included in the mean values in Table 1, they were omitted from the regression lines and equations of Figure 2. The comparative data indicate that the calculated values are in reasonable agreement with analytical values. Since a regression line determined by the method of least squares is by definition the best fit of empirical data containing normal errors in precision and accuracy, and since protein and O&G are less accurate analyses than TR$_K$ or COD, it follows that a value for O&G calculated from the simultaneous equation or from the equation of the regression line should be more correct than an individually determined value. The data of Equations (4) and (5) in Table 4 are merely a measure of the fit of each value to the regression line. The data of Equation (8), however, are independent of protein and O&G but dependent upon COD and TR data.

If the simultaneous equation is used to calculate O&G, TR$_K$ and COD$_{TR}$ are required for the equation and can be obtained through analysis and calculation, respectively. Alternatively, O&G or

TABLE 4.—Comparison by difference of protein and O&G data obtained by analysis or by calculation.

Sample no.	Protein mg/liter			O&G mg/liter		
	Analysis	Eq. (4)	Eq. (8)	Analysis	Eq. (5)	Eq. (8)
1	831	+98	+104	185	−24	−39
2	1,319	+266	+265	486	−147	−129
3	1,215	+107	+206	276	−9	−101
4	1,281	−2	+33	258	−2	−32
5	1,056	+13	−1	203	−4	+8
6	1,075	+34	+9	230	−20	+4
7	1,212	+8	+63	229	+11	−42
8	1,037	−8	−44	195	−7	+25
9	1,425	−20	−24	302	−12	+2
10	1,175	−15	+1	204	+20	+5
11	1,025	−24	−22	186	−5	−13
12	1,116	−61	−102	175	+20	+59
13	1,188	+3	+9	233	−1	−6
14	925	−12	−35	148	+9	+23

protein can be calculated from the regression of O&G and protein on TR_K. For practical reasons, we prefer using the simultaneous equation because establishing the base data would be difficult at the plant level in that both protein and O&G should be determined and correlated with COD and TR_K to establish the accuracy of the analyst.

Occasionally, wild values might occur in analyses but the average of the standard deviations between duplicate analyses for TR_K, FR_K, COD_{TR}, and COD_{FR} in this paper was 6.1, 3.6, 14.4, and 10.1 mg/liter, respectively. Using the 6 mg/liter TR_K figure the predicted value for COD from $1,431 \pm 12$ mg TR_K is $1,990 \pm 17$ mg COD from Equation (1). Based on this interval of two standard deviations, protein and O&G values obtained by the simultaneous equation could vary as follows:

TR_K	COD_{TR}	Protein	O&G
1,419	1,973	1,153	222
1,431	1,990	1,163	224
1,443	2,007	1,172	226

RECOMMENDATION

We recommend that this simplified testing-calculating system be used by the fishing industry provided proper regulatory approval is obtained. The following background data will be required:

1. Determine the regression of COD_{TR} and COD_{FR} on TR_K and FR_K and calculate the equations [i.e., Equations (1), (2), (3)]. Use grab samples (about 10) to give a good spread of data.
2. For protein and O&G, either a regression or a simultaneous equation can be used.
   (A) Obtain O&G and protein data on the same samples as above and determine the equation of the regressions of protein and O&G on TR_K [i.e., Equations (4) and (5)].
   (B) Determine the ratio or weight of protein to weight of O&G on several samples and if between 4.6 and 5.9, the constant (1.083) in Equation (8) is assumed

valid. If not, the constant must be recalculated in order that the COD_{TR} equals the sum of COD from protein and O&G [see discussion for Equation (6)].
   (C) The O&G coefficient should be determined on fishery waste effluents in which the oil may give a significantly different value than 2.678.

The routine application of this system would be as follows:

1. Determine COD_{FR}, TR, and ash by direct analysis.
2. Subtract ash from TR to give TR_K.
3. Substitute into Equations (1) and (2) and solve for COD_{TR} and FR_K.
4. Obtain COD_{NFR} and NFR_K by difference or by Equation (3).
5. Obtain protein and O&G from Equations (4), (5), or (8).

Thus, three simple and accurate tests give reportable data on nine parameters which more completely describe the pollutant load released to the environment than those currently in use.

LITERATURE CITED

COLLINS, J.
    1976. Oil and grease: A proposed analytical method for fishery waste effluents. Fish. Bull., U.S. 74:681-683.
COLLINS, J., AND R. D. TENNEY.
    1976. Fishery waste effluents: A method to determine relationships between chemical oxygen demand and residue. Fish. Bull., U.S. 74:725-731.
HORWITZ, W. (editor).
    1965. Official methods of analysis of the Association of Official Agricultural Chemists. 10th ed. Assoc. Off. Agric. Chem., Wash., D.C., 957 p.
JIRKA, A. M., AND M. J. CARTER.
    1975. Micro semi-automated analysis of surface and wastewaters for chemical oxygen demand. Anal. Chem. 47: 1397-1402.
KRZECZKOWSKI, R. A., AND F. E. STONE.
    1974. Amino acid, fatty acid and proximate composition of snow crab (Chionoecetes bairdi). J. Food Sci. 39:386-388.
MOORE, W. A., AND W. W. WALKER.
    1956. Determination of low chemical oxygen demands of surface waters by dichromate oxidation. Anal. Chem. 28: 164-167.