Recovery of Residual Oil from the Centrifuge Sludge of a Palm Oil Mill: Effect of Enzyme Digestion and Surfactant Treatment[1]

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Residual oil recovery from the centrifuge sludge of a palm oil mill was investigated by treating with enzyme (Celluclast) followed by washing the digested substrate with surfactant. The optimal conditions for enzyme digestion with respect to pH, temperature, reaction time, concentrations of enzyme and surfactant were evaluated. The possible role of the surfactant in the oil recovery process is discussed. The chemical composition and physical properties of the sludge before and after treatment were determined and its significance in the subsequent effluent treatment/utilization of the sludge is discussed.

KEY WORDS: Enzyme digestion, palm oil mill centrifuge sludge, residual oil recovery, surfactant treatment.

In the production of palm oil from fresh fruit bunches, a screw press is usually used to squeeze out the oil from the fruit after steam sterilization. The oil is later separated from the sludge by centrifugation. Under the best operating conditions, the centrifuge sludge still contains about 1% (w/v) oil. This residual oil, which is strongly associated with the sludge, is discharged together with the sludge. In view of the enormous volume of sludge produced in the milling process, this represents a considerable oil loss. Residual oil also increases the pollution load of the sludge. Thus it is important that as much oil as possible is recovered from the sludge before it is discharged.

Palm oil is found in the oil-bearing cells of the mesocarp of the palm fruit. Rupturing by the screw press releases the oil from the oil cells. However, some oil cells in the mesocarp do escape being ruptured during the process. Thus, intact and ruptured oil cells, cell debris and oil droplets are the constituents of the sludge (1). Only 30% of the residual oil associated with the sludge can be removed from the cell debris by centrifugation at high speed. The rest of the oil is either trapped within the cells or remains tenaciously bound to the cell debris and protoplasmic materials.

A preliminary study (2) has indicated that treatment of the sludge with enzyme is able to degrade the remaining unruptured oil cell, thereby releasing the entrapped oil. Subsequently, a surfactant can be used to dislodge the released oil from the matrix of cell materials. This paper presents details on the enzymic treatment and the effect of sodium dodecyl sulfate (SDS) on the detachment of oil by centrifugation from the enzyme-treated sludge.

EXPERIMENTAL PROCEDURES

Sludge sample. Centrifuge sludge was collected from a 60-ton/hr mill that employed nozzle Stork centrifuges. Samples were analyzed on the same day, where possible. In instances when this was not possible they were preserved in a cold room at 4°C for no more than one week.

Chemicals. The main enzyme used, Celluclast, was a cellulase preparation of *Trichoderma reesei* provided by Novo Industri A/S (Bagsvaard, Denmark). The enzyme activities of this preparation have been determined previously (3) and are shown in Table 1. The other enzymes that have been screened for this work are Alcalase (an alkaline proteinase), Termanyl (a heat-stable α-amylase), Neutrase (a neutral proteinase) and Pectinex 3XL (a pectinase preparation), all from Novo Industries. SDS, especially pure grade, was from BDH (Poole, U.K.) and was used as received. The other chemicals were all AR-grade materials.

Enzyme incubation procedure. Equal volumes of sludge, containing the same percentage of solids and oil but different pH values, were reacted with 0.5% (w/v) of Celluclast and incubated in a shaker water-bath maintained at 50 ± 1°C for 5 hr. pH was adjusted by addition of hydrochloric acid or sodium hydroxide. At the end of the incubation period, 100 g of the treated sample was taken and centrifuged at 10,000 rpm for 20 min at 30°C. The clear supernatant was withdrawn and analyzed for glucose content by the dinitro salicylate method of Miller (5). The sediment was redispersed in 100 mL of 0.01 mol dm^{-3} SDS solution for 1 min with a Polyton Kinematic mixer, and then incubated at a temperature of 60°C for 2 hr before recentrifugation, as described above, to remove more oil. The sediment thus obtained after centrifugation was dried at 105°C overnight to determine its solids content, and then Soxhlet-extracted with hexane to determine its oil content.

The effect of incubation temperature on enzyme digestion was investigated by varying the incubation temperature from 30 to 60°C while keeping initial pH constant

TABLE 1

Cellulase and Pectinase Activities in Celluclast Preparation

Activity	Celluclast
Cellulase	
FPase[a]	2.0×10^2
CWase[b]	7.9×10^3
CMCase[c]	6.3×10^3
Pectinase[d]	2.1×10^2

[a]Filter paper activity in filter paper units per mL (ref. 4).

[b]Cottonwool activity in μmol reducing sugar released per mL per hr at 50°C, pH 4.8.

[c]Carboxymethyl-cellulose activity in μmol reducing sugar released per mL per min at 50°C, pH 4.8.

[d]Pectinase (polygalacturonase) activity in μmol glucose equivalent released per mL per min at 50°C, pH 5.5.

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at 4.6 and using the same dosage of enzyme (0.5%, w/v). Incubation time was 5 hr.

To study the effect of enzyme concentration on digestion, a series of incubations were carried out in which a fixed volume of sludge was treated with various concentrations of Celluclast and incubated at 30°C and pH 4.2 for 5 hr, and the products of the digestion were analyzed as described above. The effect of reaction time was monitored by incubating samples of the sludge with 0.5% (w/v) of Celluclast at 30°C and pH 4.6 for varying durations up to 16 hr. The possibility of further treating the Celluclast-treated sludge with various other types of enzymes for further enhancement in oil removal was also evaluated by using Alcalase, Teramnyl, Neutrase and Pectinex. A sludge sample was first treated with 1.0% (w/v) Celluclast and incubated at 55°C overnight. The sludge was then divided into portions of 100 mL each and the pH of these treated samples was then adjusted to the optimum pH for the enzyme to be used. One gram of each enzyme was then introduced and left to incubate for 2 hr before being removed and centrifuged, and the oil content of the sedimented solids was determined. Another set of sludge samples was allowed to continue incubation overnight (16 hr) before analysis of products of digestion the following day. A control with Celluclast was carried out concurrently. The efficiency of SDS on oil removal from the enzyme-treated sludge was studied by evaluating several 100-mL SDS solutions containing up to 40 millimol dm^{-3} of SDS for redispersing the enzyme-treated sediment at 60°C for 2 hr before recentrifuging as described above.

Characterization of sludge. Physical and chemical characterization of the sludge before and after enzyme treatment, such as suspended solids content, total and ammoniacal nitrogen, were determined according to the American Public Health Authority Methods (6). Viscosity of the sludge was determined at 55°C in a Haake rotary viscometer (Haake Mess Technik Co., Karlsruhe, Germany). The settling rate of the sludge at 55°C was determined in a 1-L measuring cylinder; the height of the interface between the supernatant and the solid zone was recorded as a function of time. Total oxygen demand (TOD), total carbon (TC) and total organic carbon (TOC) were determined with an Ionics/270M TOD/TOC/TC microcomputer-controlled analyzer.

RESULTS

Preliminary observation showed that the Celluclast-treated sludge did not yield significantly more oil upon centrifugation, even when the enzyme concentration was increased up to 1.00% (w/v) (see Table 2, measured in terms of oil retained in sludge). It was thought that perhaps after the cell walls were degraded, the oil droplets were still enmeshed by the protoplasmic materials and that they could be released by further treating the Celluclast-treated sludge with other types of enzymes. Table 3 shows that none of the enzymes used were able to release more oil from the Celluclast-treated sludge, even after overnight incubation. Only when the Celluclast-treated sludge was centrifuged and the sediment (solids) redispersed and washed with SDS solution could the enmashed oil droplets be released or separated by further centrifugation. Thus, this was the procedure adopted for the results obtained below.

TABLE 2

Effect of Celluclast Concentration on Oil Retained in Sludge Solids After Digestion

Conc. of Celluclast (% w/v)	Weight (g) of oil retained in solids per 100 g sludge
0	0.61
0.05	0.63
0.25	0.37
0.50	0.56
1.00	0.55

TABLE 3

Effect of Other Enzymes on Sludge Already Treated with Celluclast

Enzyme	Weight (g) of oil retained in solid per 100 g sludge[a]
Celluclast only (control)	0.59
Alcalase	0.60
Termanyl	0.58
Neutrase	0.59
Pectinex	0.63
Solids from control after washing with 100 mL 0.01 mol dm^{-3} SDS	0.13

[a]Incubation time, 16 hr.

Enzymic activity was optimized to: i) increase oil removal from sludge; ii) reduce suspended solids; and iii) increase glucose content in supernatant. Celluclast was active up to 60°C, but most active at 30°C (Fig. 1) with respect to glucose production. However, the variation in the level of glucose concentration was small over this temperature range. A significant reduction of oil (in the suspended solids) was noted when the reaction temperature was lowered to 30°C. This was accompanied by only a marginal reduction in suspended solids. The optimum pH for suspended solids reduction and minimal oil retention in the solids was 6, but at this pH the glucose concentration was much reduced (Fig. 2). The effect of incubation time on glucose yield was marginal, but a significant increase was noted in the reduction of both suspended solids and oil retention with increasing reaction time (Fig. 3).

In the presence of 1% (w/v) Celluclast, a contact time of 30 min was sufficient to disintegrate the cell walls. No intact cell walls could be observed under the microscope after 5 hr of reaction time at this Celluclast concentration. When all the cell walls were broken down, reduction in suspended solids also ceased. Glucose production also tapered off after 5 hr of reaction time. Hence, the substrate is the limiting factor in the enzymic digestion of the sludge. A minimum enzyme concentration of 0.5% (w/v) was deduced to be effective for maximum removal of oil from the solids and also for achieving a reasonably high glucose yield for a reaction time of 5 hr at 30°C (Fig. 4). These reaction conditions are relatively mild compared with those, for example, in the enzymatic saccharification of steam-pretreated willow with the same enzyme preparation (7).

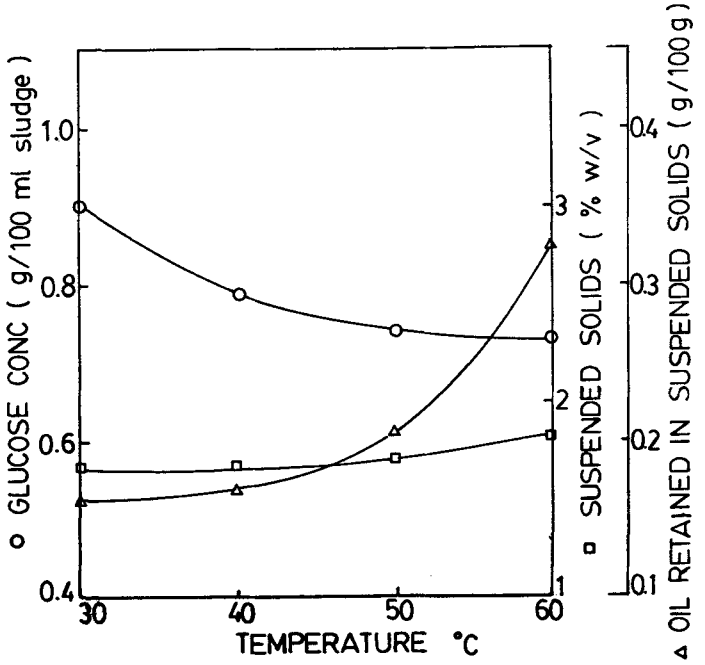

FIG. 1. Effect of reaction temperature on the digestion of sludge with respect to glucose production, suspended solids and oil retained by suspended solids (per 100 g of sludge) after digestion. Reaction conditions: pH, 4.6; reaction time, 5 hr; enzyme (Celluclast) concentration, 0.5% (w/v).

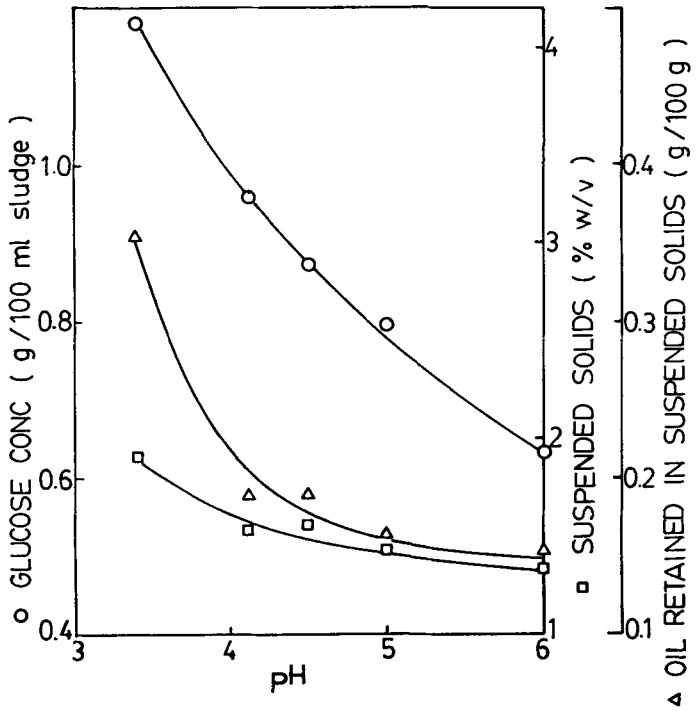

FIG. 2. Effect of pH on the digestion of sludge with respect to glucose production, suspended solids and oil retained by suspended solids (per 100 g of sludge) after digestion. Reaction conditions: reaction temperature, 50°C; reaction time, 5 hr; enzyme (Celluclast) concentration, 0.5% (w/v).

Washing the enzyme-digested solids with SDS brings about the release and separation of oil from the solids by centrifugation. A sharp reduction in oil retained by the solids was observed when SDS concentration was increased from 0.01 mol dm^{-3} to 0.02 mol dm^{-3} (Fig. 5). An increase beyond this value did not further improve oil removal. Thus, for maximum oil removal from the solids, an SDS concentration of 0.03 mol dm^{-3} is best. At this concentration, which is well above the critical micellar concentration of SDS, there are enough SDS molecules not only to detach the oil droplets from the matrix of cellular debris, but also to prevent their redeposition.

Some chemical and physical properties of the sludge before and after enzymic treatment are given in Table 4. The high TOD and TOC values after enzyme treatment were direct consequences of digestion of the organics in the sludge. The enriched soluble organics would be available, for example, for consumption by microorganisms during anaerobic digestion in subsequent treatment of the sludge. The increase in nitrogen is an added advantage for its possible use as fertilizer.

It was noted that there was a one-third reduction in suspended solids after treatment. The settling behavior of the suspended solids was also modified by the enzymic treatment. The particles of the treated sludge settled rapidly and gave a clear supernatant within one hour, unlike the untreated sludge, which hardly settled at all, even after prolonged standing (Fig. 6). After an hour the sedimentation volume of the solids was a mere 50% of the original sludge volume. The treated sludge was found to be Newtonian in flow behavior, with a viscosity of less

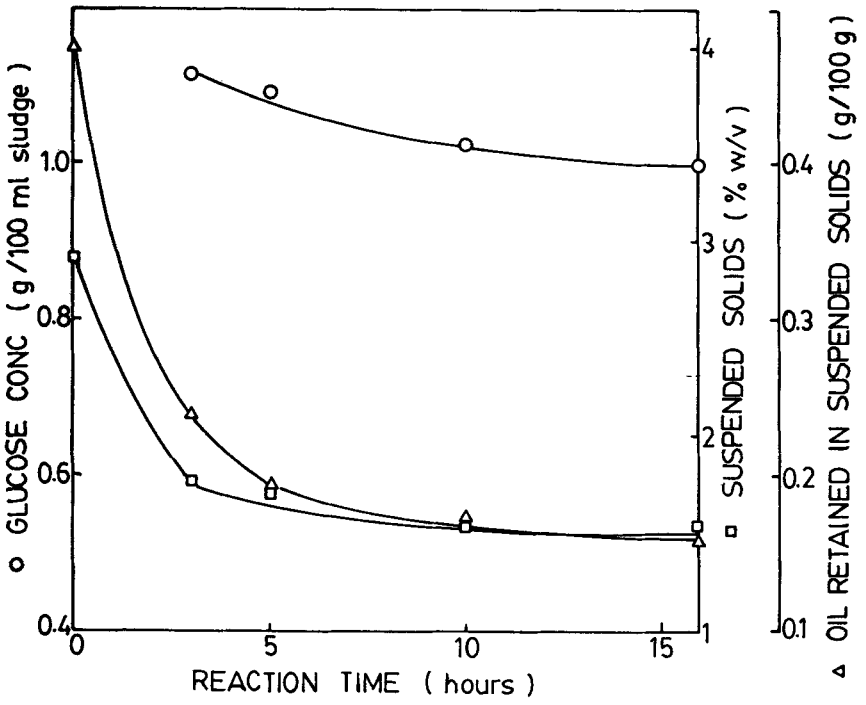

FIG. 3. Effect of reaction time on the digestion of sludge with respect to glucose production, suspended solids and oil retained by suspended solids (per 100 g of sludge) after digestion. Reaction conditions: pH, 4.6; reaction temperature, 30°C; enzyme (Celluclast) concentration, 0.5% (w/v).

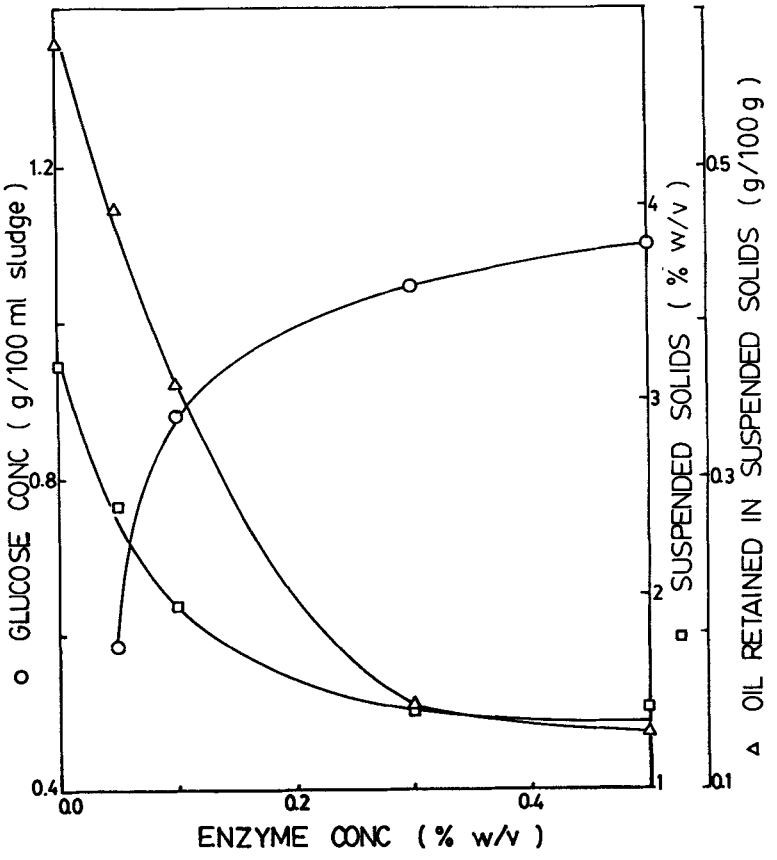

FIG. 4. Effect of enzyme concentration on the digestion of sludge with respect to glucose production, suspended solids and oil retained by suspended solids (per 100 g of sludge) after digestion. Reaction conditions: pH, 4.2; reaction temperature, 30°C; reaction time, 5 hr.

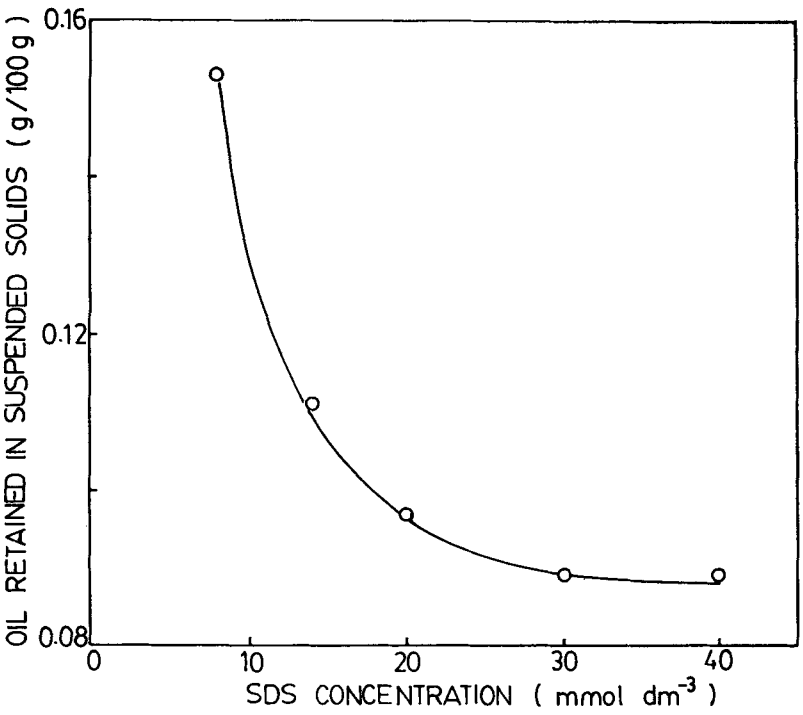

FIG. 5. Effect of SDS concentration on residual oil retained by suspended solids after Celluclast treatment.

TABLE 4

Average Characteristics of Sludge Before and After Celluclast Digestion

Parameter	Sludge	
	Before	After[a]
Suspended solid[b]	3.18	2.08
Soluble solids[b]	2.92	4.05
Oil retained in solids[b]	0.59	0.59
	—	0.13[e]
Total oil content[b]	0.78	—
Glucose content[b]	0.31	0.92
Total nitrogen[c]	888	1110
Ammoniacal nitrogen[c]	33	37
Total oxygen demand[c]	22030[d]	50340[d]
Total carbon[c]	8430[d]	12400[d]
Total organic carbon[c]	7910[d]	12040[d]

[a]Excluding the SDS washing step, except as stated otherwise. Enzyme digestion carried out under optimum conditions as indicated in text.

[b]In units of g/100 mL sludge.

[c]In units of ppm.

[d]Values are for the supernatants only, after removal of solids by centrifugation.

[e]After washing with 100 mL 0.01 mol dm^{-3} SDS.

less 3 mPa.s (Fig. 7) at 55°C, whereas the original untreated sludge was pseudoplastic in nature, with a viscosity of 850 mPa.s at the highest shear rate used, *i.e.*, 2620 s^{-1}. The markedly reduced viscosity of the digested sludge therefore would facilitate separation of the residual suspended solids from the liquid phase.

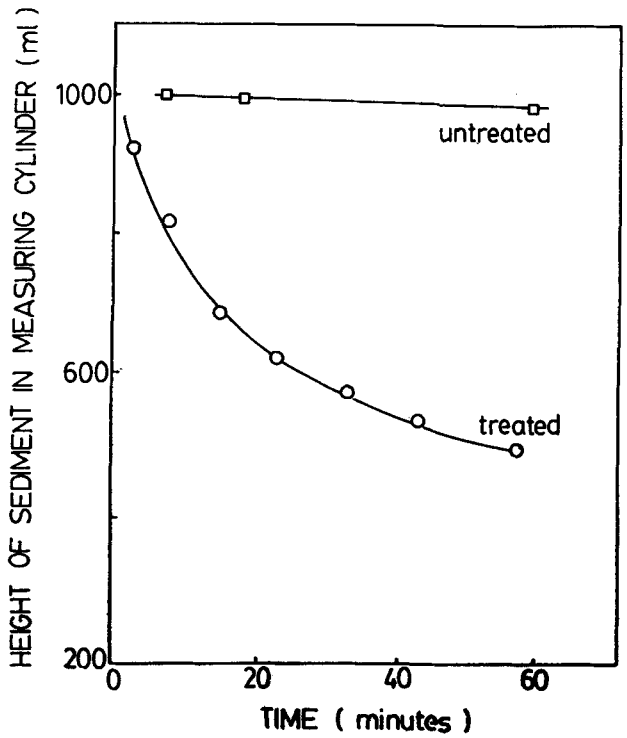

FIG. 6. Sedimentation volume of suspended solids (recorded as the height of sediment layer in a 1-L measuring cylinder) at 55°C as a function of time for sludge before and after enzyme digestion.

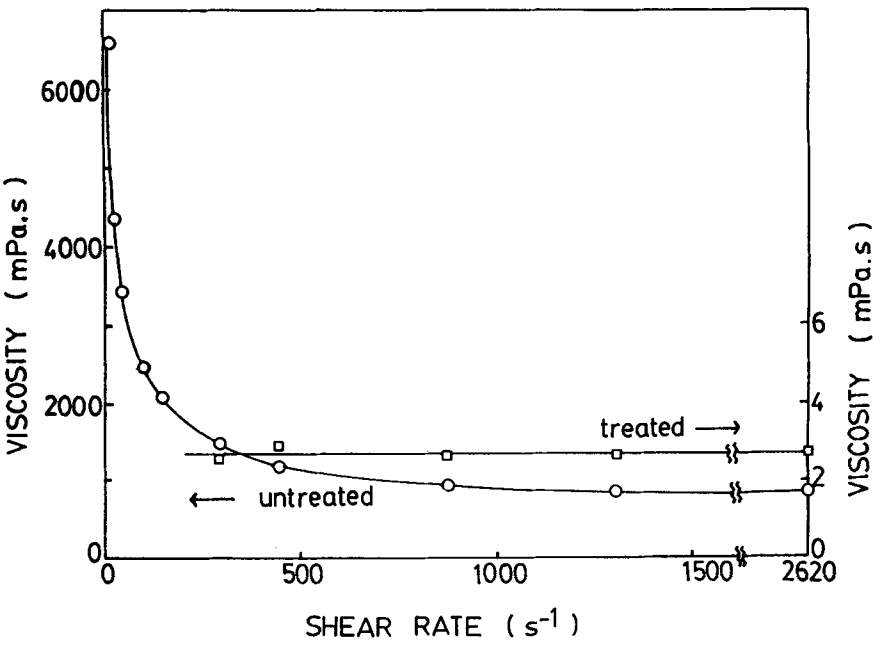

FIG. 7. Variation of viscosity of sludge as a function of shear rate measured at 55°C.

DISCUSSION

The use of enzymes in the direct extraction of oil from oil-bearing seeds and fruits has been reported previously (3,8-11). However, the application of enzyme digestion in the recovery of residual oil from cell debris or sludge after mechanical oil extraction appears to be new. Enzymic hydrolysis is a complex reaction involving product inhibition and substrate-specific adsorption of enzymes. Each substrate must be investigated to obtain its optimal hydrolysis conditions. The emphasis of this study was on the optimization of enzymic treatment conditions for maximum oil recovery from sludge. It should be noted that in this system the enzyme is acting on a substrate that has already been steam-sterilized and mechanically macerated.

The oil in the sludge can be divided into three types (2)—the tiny free oil droplets (type I) originally present in the sludge, which are very stable and separable only by centrifugation at >7600 *G*; oil entrapped by unruptured cells and adhered strongly to the cell debris (type II), separable only after enzyme treatment and detachment by SDS; and Soxhlet-extractable oil with hexane from the above enzyme-treated solids after drying (type III). The wet solid residue obtained after enzyme treatment and SDS washing followed by centrifugation was devoid of free oil droplets. Thus, it appears that type III oil is imbibed in the plant cell materials and removable only by solvent extraction.

The present study demonstrates conclusively that enzyme treatment alone of the sludge is not sufficient to ensure the release of oil from the digested debris. Because the enzymes investigated included most of those that would act upon typical cell wall constituents, it is clear that the problem is not so much the oil droplets held back in the unruptured cells, but the strong interaction between the oil droplets and the digestion products and protoplasmic materials. It appears that these macromolecules are adsorbed on the surface of the oil droplets, which thus become enmeshed in a matrix of macromolecules from which the oil drops are difficult to separate. Initial centrifugation of the enzyme-treated sludge to remove the supernatant simultaneously removes a large portion of these soluble constituents. Redispersion of the sediments with SDS solution then further displaces those macromolecules that were adsorbed at the oil drop surface, thereby decreasing its density and rendering them separable upon recentrifugation. It is important that the enzyme-treated sludge be centrifuged first before redispersion in SDS to ensure that all the SDS is utilized in displacing the adsorbed macromolecules and not in interacting with the free macromolecules that were originally present in the supernatant. The amount of SDS used should be sufficient to effect maximum displacement of the adsorbed macromolecules and to prevent their redeposition. It is known that SDS forms association complexes with carbohydrates and cellulosic materials (12-14) and also causes lysis of protein-lipid complexes (15).

To adopt the above enzyme and surfactant technique for commercial operation in a mill for residual oil recovery would require prior cooling of the sludge from about 90°C to ambient temperature. This means retention of sludge in holding tanks for a day or two with subsequent pH adjustment with lime for optimum digestion conditions. An additional stirred tank is required in which the enzymic reaction can be carried out. After digestion, the solids would have to be separated by centrifugation, and further warm SDS washing needs to be carried out in a large tank with stirrer before recentrifugation. A cost evaluation (16) shows that the process is not economically viable just for residual oil recovery alone.

On the other hand, from a technological viewpoint, enzyme degradation can be expected to break up some of

the macromolecules, which explains the much reduced viscosity of the enzyme-treated sludge. Lower suspended solids after digestion would have the same effect. This enables the enzyme-treated sludge to sediment, giving a clear supernatant within a short time as compared to the stable untreated sludge, which does not normally settle under gravity. Both these factors are added advantages in the subsequent biological treatment of the sludge. Currently raw sludge is treated by the ponding system, in which both anaerobic and aerobic reactions are employed for its degradation. Thus, retention time for the biological treatment and the frequency of de-sludging of oxidation ponds later in the effluent treatment process of the sludge could be reduced. The presence of a small amount of SDS is not expected to materially affect the performance of the treatment process of the effluent.

The enzyme preparation used in this work is capable of effectively degrading the cell materials found in the sludge. The optimal conditions for residual oil recovery from the sludge were evaluated. Digestion at a pH of 4.6 and 30°C for 5 hr at an enzyme concentration of 0.5% (w/v), followed by washing with 0.03 mol dm^{-3} SDS solution gave the best results. The improved chemical and physical properties of the digested sludge offer several advantages over the original untreated sludge. Its reduced viscosity and suspended solids would make effluent treatment much easier and faster. Its enriched chemical composition, especially with respect to glucose and nitrogen contents, open up the possibility of its use in fermentation or as fertilizer. The operation conditions are easily attainable in the mill. Thus, the potential of the process, taken on a wider perspective than just for oil recovery, merits some serious consideration by the industry.

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REFERENCES

1. Ho, C.C., and Y.K. Tan, *Water Research 17*:613 (1983).
2. Chow, M.C., C.C. Ho, J. Sutanto and S.H. Ong, *J. Am. Oil Chem. Soc. 64*:393 (1987).
3. Cheah, S.C., M.A. Augustin and L.C. Ooi, paper presented at the *National Symposium on Oil Palm/Palm Oil, Recent Development*, October 11-15, 1988, Kuala Lumpur.
4. Mandels, M., and D. Sternberg, *J. Fermentation Technol. 54*:267 (1976).
5. Miller, G.L., *Anal. Chem. 31*:426 (1959).
6. Standard Methods for the Examination of Water & Wastewater, American Public Health Authority Methods, 16th edn., 1985.
7. Eklund, R., M. Galbe and G. Zacchi, *Enzyme Microb. Technol. 12*:225 (1990).
8. Sosulski, K., F.W. Sosulski and E. Coxworth, *J. Am. Oil Chem. Soc. 65*:357)1988).
9. Buenrostro, M., and C.A. Lopez-Munguia, *Biotechnology Letters 8*:505 (1986).
10. Fullbrook, P.D., *J. Am. Oil Chem. Soc. 60*:428A (1983).
11. Cintra, M.O., C.A. Lopez-Munguia and C.J. Vernon, *J. Food Sci. 51*:695 (1986).
12. Takagi, T., and T. Isemura, *Bull. Chem. Soc. Japan 33*:437 (1960).
13. Breuer, M.M., and I.D. Robb, *Chem. & Ind.*, 530 (1972).
14. Winnik, F.M., and M.A. Winnik, *Polymer Journal 22*:482 (1990).
15. Attwood, D., and A.T. Florence, *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman & Hall, London, 1983, Chapter 10.
16. Chow, M.C., M.Phil. Thesis, University of Malaya, 1987.

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