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FINAL REPORT to
Grape and Wine Research and Development Corporation
Project CSS 98/1

Dr G.J. Churchman

Grape and Wine Research and Development Corporation

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NON-TECHNICAL SUMMARY

Much of the solid residue from wineries and distilleries is sent to landfill for disposal. Availability of space for landfill will inevitably decrease so that simple disposal of these wastes will become impossible or prohibitively expensive. Apparently the absence of space for more landfill precludes further winery development in at least one important wine-producing area overseas. Therefore it is desirable to recycle useful components of the wastes such as bentonite and also diatomaceous earth. This laboratory feasibility study was aimed at devising and comparing procedures for the recycling of bentonites used for the stabilisation of heat-sensitive proteins in wines.

A hypothesised procedure involving displacement of the protein in bentonite-protein complexes using alkalis and salts - thereby reversing the process for protein uptake by bentonites – proved to be ineffective for substantial removal of the protein. It became clear that protein needed to be destroyed before the bentonite could be recycled.

Protein in wine lees could be destroyed using hot concentrated acids but these also degraded, and eventually destroyed, the bentonites. Among strong oxidising agents, sodium hypochlorite, which is used in bleaching agents, destroyed the protein quite effectively. However, it also destroyed the bentonite.

Hydrogen peroxide was quite effective in destroying protein, especially when followed by a treatment with sodium carbonate, which appeared to displace the products of protein breakdown from their association with bentonite. The product of this treatment was more effective for removing heat-sensitive protein from an unstable wine than the original bentonite that had given rise to the lees. Nonetheless, not all of the organic matter was removed from the bentonite by this procedure, which contributed considerable amounts of polluting nutrients to waste water.

A photo-oxidation treatment with ultraviolet radiation was most effective in removing organic carbon from lees and was also quite effective in removing its organic nitrogen, which particularly indicates residual protein. The bentonite remaining after this treatment also appeared to be essentially unaffected by its application. This treatment, which does not require the addition of chemicals, is likely to contribute

fewer nutrients to pollute wastewater than the peroxidation-based procedure. Nonetheless, it is untested in this regard.

A simple method was demonstrated to separate bentonitic materials, particularly bentonite-protein complexes, from other solids in winery and distillery wastes. The method involves the addition of salts which are later removed by washing.

The possible use of lees as a medium for plant growth was also examined. It was found that additions of lees to soils had toxic effects on plants. Composting of lees with soil produced considerable quantities of nitrogen, particularly in its ammonium form. This could be useful as a source of nitrogen for plants. However, prior composting of lees did not overcome the toxicity of lees towards plants. Furthermore, composting alone does not enable recycling of bentonite for its further use in wine processing.

A procedure involving hydrogen peroxide and sodium carbonate could enable recycling of bentonite from lees with further refinement to complete removal of organic products of the peroxidation. However, photo-oxidation using ultraviolet radiation may be the most viable method for recycling bentonites from solid winery and distillery wastes. This procedure requires further research to determine the effectiveness of its products for the removal of proteins from heat-unstable wine.

BACKGROUND AND RESEARCH NEED

Soluble proteins are released from grapes on pressing. These proteins are unstable and with time and excessive temperatures can lead to unsightly hazes and sediments which particularly affect the value of white wines. Naturally-occurring bentonite clay has been found to be effective for removing the problem proteins from wine. Since it was first introduced into winemaking in 1934, it has come to be used universally for fining and stabilisation in winemaking (Rankine, 1995).

After its use in the fining and stabilisation of wine, used bentonite, together with other components of the lees, including pulp, tartrates and yeasts, and also diatomaceous earth, is generally sent to distilleries, along with plant material in the marc. Following distillation, the solid residue, which includes bentonite, goes to landfill for disposal, although some may be used for animal feed. Probably the main concern for the industry into the future arises from the limited amount of land close to wineries and distilleries which will continue to be available for use as landfill for solid waste from the processing of wines and fortified wines. In California, the lack of availability of land for waste disposal has placed a major constraint to the further expansion of the wine industry (Peter Hayes, GWRDC, pers. comm., 1998).

Approximately 410 tonnes of bentonite is used in the Australian wine industry each year (figure calculated from information supplied by a major wine company and for whole industry by Australian Bureau of Statistics, 1997). Substantial solid waste is generated from the combination of proteins with the bentonite. From an environmental viewpoint it would clearly be desirable to re-use, rather than dispose of, such a large tonnage of material. Furthermore, re-use in place of disposal would be consistent with the main principles of management of winery and distillery effluent, as set out in ARMCANZ/ANZECC (1995). These include both “waste minimisation” and also “effective and feasible recycling and reuse”.

PROJECT OBJECTIVE

To establish the best procedures and optimum conditions for the removal of proteins from bentonite in distillery wastes.

The project consitutes a laboratory-scale feasibility study.

TECHNICAL PROBLEMS TO BE SOLVED, AND SOLUTIONS PROPOSED AT THE START OF THE PROJECT

(a) The displacement of proteins from complexes with bentonites

Bentonites attract proteins because, as negatively charged and finely-divided materials, they have a high capacity for the uptake of grape proteins, which carry a positive charge. Grape proteins carry a net positive charge in the low pH (ie. acidic) solutions typical of wines. Hence the proteins are attracted to the bentonite by electrostatic forces. They are held within the crystalline structures, in the interlayer region, of the smectite clay minerals comprising bentonite (which are most commonly "montmorillonite), as illustrated in Fig. 1.

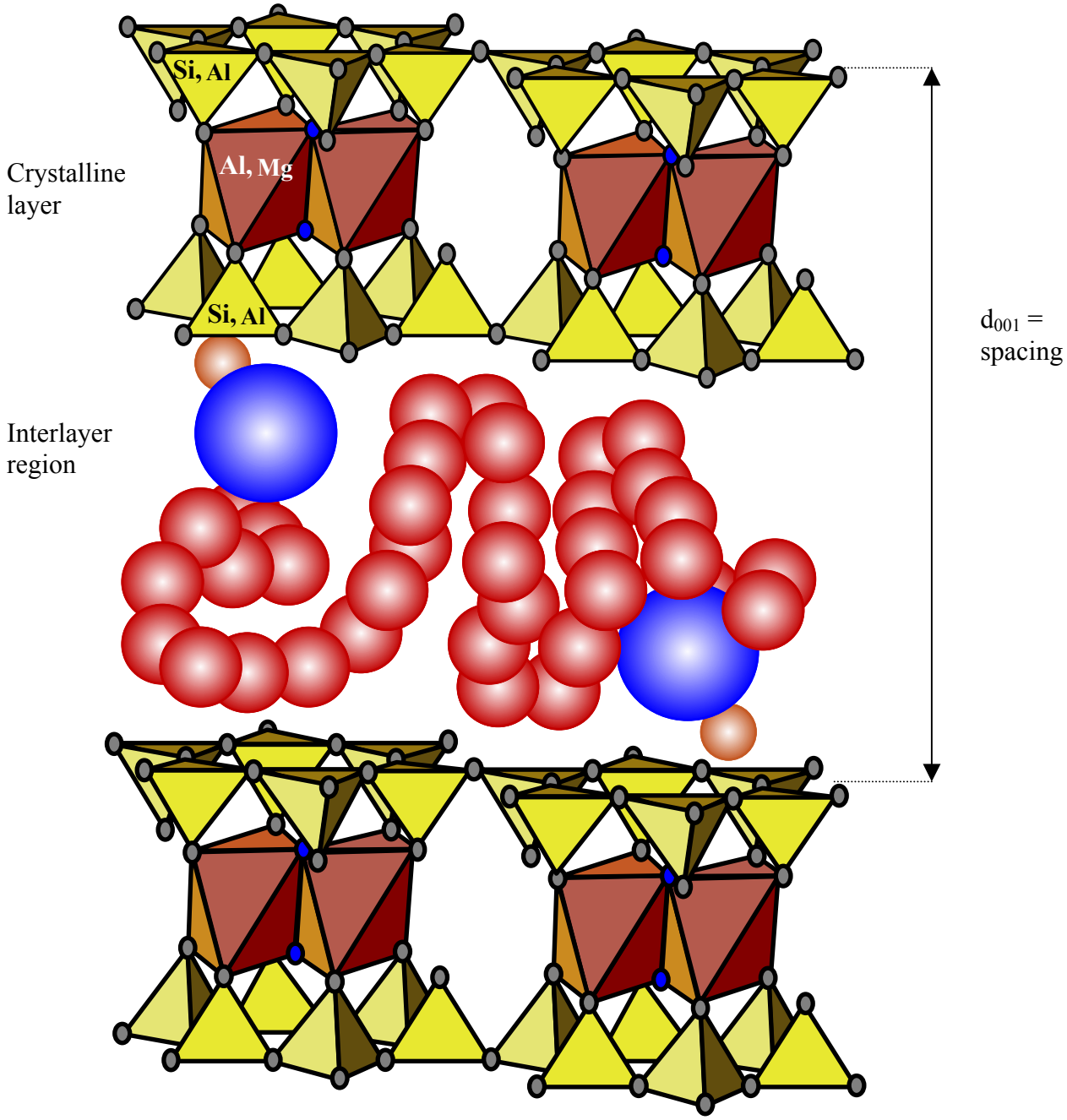


Fig. 1. Diagrammatic representation of the incorporation of protein molecules between the layers (i.e. in the interlayer region) of the smectite clay minerals comprising bentonites. The crystal structure is much more extensive laterally than is shown in the illustration.

While the charge on bentonites is permanent, it is significant that the charge on the proteins varies with pH and can be manipulated by altering the pH of the solution. The net charge on proteins is positive at low pH and negative at high pH. For each protein, there is an isoelectric point (pI), which is the pH value where the contributions of positive and negative charges are equal to each other and the molecule thus has no net charge. The majority of grape proteins have a pI between 3.5 and 6.0 (Hsu and Heatherbell, 1987; Waters and Williams, 1996).

Beginning in 1939, there have been many studies of the uptake (i.e. "adsorption") of proteins by bentonite (note: bentonite is a geological term for deposits which comprise mainly "montmorillonite" from the smectite group of clay minerals). A book by Theng (1979) summarises the earlier work. The adsorption of proteins by bentonite remains an active topic for research (e.g. Quiquampoix *et al.,* 1989, 1992, 1995; Violante *et al.,* 1995). Much of the work has been motivated by the importance of enzymes, which are proteins, in both agriculture and medicine.

On the other hand, there is little written on the removal of proteins (i.e. "desorption") from their associations with bentonites. We know of just one such study (Armstrong and Chesters, 1964). Armstrong and Chesters (1964) were able to remove 63% of pepsin (pI = 2.8) which had been adsorbed on a bentonite at pH 3.0 by simply raising the pH to 5.2. While they did not obtain complete removal of the protein, this result was encouraging, mainly because the pH was adjusted by addition of NaOH. As a strong base, only a low concentration of NaOH would be needed. Hence the concentration of cation (Na^+) to assist in the displacement of protein would also be low.

Armstrong and Chesters' (1964) study was limited in scope. However, principles that are likely to indicate the most favourable conditions for desorption of proteins from bentonites may be gleaned from the optimal conditions that have been established for their adsorption in the many studies on this topic.

The reaction for the release is represented simply (without balancing, and assuming only singly-charged species) in Equation (1).

$$\underset{\text{soluble}}{\text{Protein}^{+}} + \underset{\text{solid}}{\text{M}^{+}\text{-bentonite}} \rightarrow \underset{\text{solid}}{\text{Protein}^{+}\text{-bentonite}} + \underset{\text{solution}}{\text{M}^{+}} \qquad (1)$$

The principal hypothesis for this project was that protein would be released from its complex with bentonite if pH was raised to a sufficiently high value in relation to the pI value of the protein.

In accordance with equation (1), other factors likely to aid the release of protein from the complex would include a high concentration of cations in solution. This could be achieved by the use of a weak base, eg. sodium carbonate rather than a strong base such as sodium hydroxide.

However, there is likely to be some hysteresis in the removal of protein from bentonites. Hysteresis denotes a tendency for adsorbed soluble species to remain attached to the solid phase under conditions (pH in this case) in which they would not have been adsorbed in the first place. It is extremely common in surface reactions. It generally arises because there are other types of bonds besides electrostatic attractions, particularly van der Waals bonds from atomic interactions, and other mechanisms e.g. physical entrapment, which hold proteins on bentonite surfaces. The strength of van der Waals forces is related to the number of atoms in close contact.

Hence there may be an energy barrier to overcome in order to remove adsorbed proteins completely from bentonite surfaces.

It was proposed that some heating and/or agitation might be needed to assist in overcoming the energy barrier to the displacement of proteins.

Furthermore, the valency of the displacing cation affects its ability to displace proteins from surfaces.

It was further proposed that a divalent cation like Ca^{2+} would be much more effective than a monovalent cation like Na^{+} for displacement of protein.

(b) The recovery of used bentonite for recycling

Prior to displacement of proteins from complexes with bentonite, used bentonite, with associated proteins, needs to be separated from the solid residue where it is mixed with other material, such as pulp and plant debris, yeasts, tartrate crystals and diatomaceous earth, as well as dissolved material. Separation of bentonites from the mixture would be based on the following principles:

1. Bentonite particles are very small. Most are smaller than 0.2 μm or two ten-thousandths of a millimetre. Therefore they tend to stay in suspension in water, at least for a long time.
2. Bentonite particles carry a charge, which is high when they are uncomplexed, although it may be reduced in complexes, but the other waste material in the mixture is uncharged or has little charge.

Flocculation, whereby the fine particles are brought together into voluminous flocs, which settle out of suspension, can be achieved with bentonite particles because of their charge. Because bentonite particles have a negative charge, they repel one another and therefore tend to remain dispersed. However, when their charges are neutralised, there is no longer any barrier to their association, so they come together in loose, but large, flocs, which settle out of suspension. The addition of an excess of ions to the suspension (as salts) is a very effective way of neutralising the bentonites and causing flocculation. Other, uncharged, fine-grained material remains in suspension even after the addition of salts.

It was hypothesised that bentonite would be separated from the other fine-grained waste materials after large solid particles had precipitated out. The separation would be achieved by flocculation of the bentonite using salts.

RESEARCH METHODOLOGY

Lees containing bentonite-protein complexes was supplied by a number of South Australian wineries (BRL Hardy, Reynella; Yalumba, Angaston and D'Arenberg,

McLaren Vale). Moist solid waste that included bentonite-protein complexes was supplied by Tarac Distilleries, Nuriootpa.

A. Treatments carried out to remove protein from bentonite

Lees was either treated as received, following removal of supernatant wine separated by centrifugation, or following washing with centrifugation. Lees was kept moist and refrigerated whether or not separated from wine and/o washed.

Test of displacement of protein by alkalis and high cation concentrations. 24 distinct treatments were given. Solid: solution ratio, strength of sodium carbonate, additions of other chemicals, times of shaking, pH, and also other means of agitation (ultrasonics and also Spex milling) were varied in many of these treatments, which involved sodium carbonate for the displacement of protein from complexes with bentonite in winery lees. Other alkalies, and other cations were also examined as alternatives to sodium carbonate for this purpose.

Treatments varied in:
Solid:solution ratio. From 2:1000 to 1:10
Concentrations of Na_2CO_3. From 2-6%
Additional compounds: KCl, NaCl
Alternative compounds: KCl, $CaCl_2$, NaOH, Deconate
Time of shaking: 1 hour-5 days
Other means of agitation: ultrasound, Spex mill (both 10 mins)

Treatments with strong acids. 3 treatments were carried out with hot concentrated hydrochloric acid. 200 ml of 6M HCl was added to a moist centrifuged sample of lees of 2.5g oven-dried (o.d.) equivalent weight in a flask. The mixture was reacted at 70^{o}C for either 6 hr or 24 hr. In a further treatment, the lees was washed several times, to remove other organic constituents besides the bentonite-protein complex, before reaction with the acid for 24 hr.

15 treatments were carried out with hot concentrated sulphuric acid. 6M H_2SO_4 was reacted with moist centrifuged lees in a flask at 70^{o}C. Treatments varied in weight of solid used in relation to the same volume of acid (either 0.25, 1.25, or 2.5g o.d. equivalent), duration (between 1 and 40hr), the extent of pre-washing of the lees, and in whether the reagents were stirred during treatment.

Treatments with strong oxidising agents. Treatments were carried out with sodium hypochlorite NaOCl, following the procedure of Mikhail and Briner (1978). For this purpose, 100ml of 12.5% NaOCl which had been adjusted to pH 8 with 6M HCl was added to 2g. o.d. equivalent of moist centrifuged lees and the reagents left to stand for 16 hr at room temperature. Following centrifuging, the product was washed with 0.2M HCl and dried at 70^{o}C. Both washed lees and also lees that had been pre-treated with hot 6M H_2SO_4 for 24 hr were treated with NaOCl as described.

In another treatment, 10ml of 100 vol. hydrogen peroxide H_2O_2 was added to up to 2.5g o.d. equivalent of lees in a beaker with a watchglass cover and left overnight to react. On the following day, the mixture was heated in a waterbath at nearly 100^{o}C until frothing ceased. The method is based on that given by Jackson (1956) for the destruction of organic matter in soils. The treatment with hydrogen peroxide H_2O_2, was given following1hr and 24hr sulphuric acid treatments, and also alone.

Hydrogen peroxide treatments were also given to clay (<2μm) fractions of the lees which had been separated from the whole material by centrifugation after the sample was dispersed by adjusting the pH to 9 through addition of sufficient M NaOH.

A photo-oxidation treatment with ultra-violet radiation was also given to lees L3 (Table 1), after supernatant wine had been removed by centrifugation, following the

procedure of Skjemstad *et al.* (1996). This treatment was carried out on the clay (<2μm) fractions of the lees after its separation as in the immediately preceding paragraph.

Alternative uses for bentonite-protein complexes

B1 Use as a plant growth medium Three trials have been carried out in pots. The main growth medium for each of the pot trials was a sandy soil from Cooke Plains, South Australia. This soil has a pH of 6.35, carbon content of 0.7%, and contains 6% clay, 2% silt and 92% sand.

In order to prepare lees to add to mix with the soil for the trials, lees (L2 – see Table 1 later) was centrifuged, the supernatant wine poured off, and the lees then washed two more times with deionised water. It was finally washed with ethanol and then air-dried and gently ground. Dry soil and lees were mixed intimately in the appropriate proportions for each pot.

The basis for the trial and the different media for plant growth in each of the trials were as follows:

(a) The soil was amended with different levels of washed lees and also with bentonite alone.

Pots contained:

(i) Soil only

(ii) Soil + 5% fresh bentonite, from the same source used by the vineyard to obtain lees L2 (Table 1)

(iii) Soil + 2% lees

(iv) Soil + 5% lees

(v) Soil + 8% lees

Each was given the same fertiliser dressings and also the same amount of water. 75g of water was added to 400g of soil, and was added to maintain this weight at each watering. The nutrient solution comprised $CaCl_2.2H_2O$ (6.84g/100g soil), KH_2PO_4 (3.64g/100g soil), NH_4NO_3 (3.72g/100g soil), K_2SO_4 (6.96g/100g soil), $MgSO_4.7H_2O$

(0.984g/100g soil), $ZnSO_4.7H_2O$ (0.4g/100g soil), $CoSO_4$(0.02g/100g soil), $MnSO_4.H_2O$ (0.34g/100g soil), H_3BO_3 (0.032g/100g soil), $CuSO_4.5H_2O$ (0.052g/100g soil), $(NH_4)_6Mo_70_{24}$ $4H_2O$ (0.001176g/100g), $FeSO_4.7H_2O$ (0.4g/100g).

pHs and electrical conductivities (ECs) of the growth media (both measured in suspensions of 1:5, soil: water) and also the lengths of the roots and oven-dried weights of the plants were measured after plants were harvested.

(b) Prior composting was employed to raise starting pH (see Treatment B2). In this case plants growing in 5% lees in the soil was compared with those in the soil alone and also those in the bentonite in the soil when each were depleted in N fertiliser, to test the lees as a source of N.

Pots contained:

(i) Soil only

(ii) Soil + 5% fresh bentonite

(iii) Soil + 5% lees

Pots were watered as in (a) above. Each was given the same fertiliser dressings as in (a), except that no NH_4NO_3 was added, so as to starve the pots of the major source of added nitrogen used in (a).

(c) The lees. and also the bentonite, were tested as a source of moisture by supplying each pot with half as much water as before.

Pots contained the same set of media as in (b). In this case, only 37.5g of water was added to 400g of dry soil and the weight was maintained at the lower level of 437.5g at each watering. Nutrients were added as in (b), i.e. as in (a), but without any NH_4NO_3.

In each case, wheat (Frame variety) seeds were first germinated on filter paper, an appropriate nutrient solution was washed into the pots and then 4 seedlings planted into each pot. Following a watering every 2 hours over the first day, pots were maintained at constant weight through the addition of water by spraying with reverse osmosis water every day.

When plant growth was completed, photographs of a set of the pots were taken. The lengths of roots was also recorded for some trials.

B2 Composting Lees L2 was used either with or without washing and also without any prior treatment (i.e. including wine). The unwashed lees was separated from supernatant wine after centrifuging. The washed lees was prepared as for the plant growth trials (B1, above). Lees of each type was stored in a refrigerator without drying. The moisture content of the unwashed lees was 87.7% and that of the washed lees was 86.3%.

Each type of lees was mixed with samples of the same sandy soil from Cooke Plains that was use for the plant growth trials and a sample this soil was also composted alone and with untreated bentonite. The proportions used in each composting trial, carried out in plastic bags, were:

(i) Soil only
(ii) Soil + 5% bentonite
(iii) Soil + 2% unwashed lees
(iv) Soil + 5% unwashed lees
(v) Soil + 8% unwashed lees
(vi) Soil + 5% washed lees
(vii) Soil + 1.8% raw lees

Each trial included between 460 and 550g soil, and, after a weight of lees or bentonite chosen to give the appropriate proportion, water was added if necessary to give a moisture content of at least 20%. Trials were sampled immediately, and also after 1,2,4, and 8 weeks for NH_4-N, NO_3-N, moisture, pH and electrical conductivity. Ammonia nitrogen was determined colorimetrically following gas diffusion by segmented flow analysis. Nitrate nitrogen was determined colorimetrically following cadmium reduction of nitrate to nitrite by segmented flow analysis. pH and electrical conductivity were determined with electrodes using the appropriate meters and moisture was determined gravimetrically.

C. Analyses carried out following treatments of trials

The common analytical methods for detecting the effectiveness of displacement of proteins and of the extent of recovery of the original bentonite were:

X-ray diffraction (XRD)

Use of this technique provided an indication of the presence of protein in the interlayer space (Fig. 1). Two types of patterns were obtained, as follows:

Powder (i.e. randomly oriented) patterns. Bentonite which is not complexed with large molecules such as those of proteins gives a main peak (d_{001}) with a spacing of ~ 14 Å when air-dry and between 10 and 14 Å after oven drying, depending on ease of rehydration. Therefore d_{001} peaks with spacings >14 Å represent an expansion brought about by intercalated protein molecules. A spacing >14 Å in oven-dried samples was usually sufficient to indicate residual protein and there was no need for further analyses to confirm this point.

Sedimented (i.e. oriented) patterns. When a sedimented sample is solvated with glycerol, the crystal expands to give a relatively sharp d_{001} peak at 17-18 Å, unless entry of glycerol into the smectite interlayers in the bentonite is precluded by protein molecules already occupying interlayers. If this occurs, expansion is inhibited.

X-ray diffraction, both of oriented and unoriented samples could also show changes to the underlying bentonites as a result of treatments.

Analysis of residual carbon and nitrogen content of the solids

Residual C and N were measured using a Leco combustion furnace. Complete removal of proteins would result in virtually zero residual carbon and nitrogen in the otherwise inorganic bentonites.

Analysis of protein released into solution

Protein in solution was measured using the standard Bradford assay (Bradford, 1976). This analysis gave a measure of the relative efficiencies only of different treatments, as the reagent could not be used to measure the amount of protein originally in the solid bentonite-protein complex.

Analysis of the chemical composition of the solid, and particularly its Cation Exchange Capacity (CEC), was carried out using X-ray Fluorescence (XRF), following the procedure described by Churchman *et al.* (1994).

These analyses revealed any changes brought about by the treatments to the bentonite.

Infrared spectroscopy

Infrared spectra, using the Diffuse Reflectance Fourier Transform (DRIFT) technique enabled the analysis of the chemical nature of residual organic material.
They were obtained from 5mg of finely-ground samples resulting from some of the treatnments described which were each mixed with 45 mg dry KCl.

Trial of re-cycled bentonite for wine stabilisation

The results of the two most promising treatments were tested for optimal rate of addition for the stabilisation of heat-sensitive protein in a wine, in comparison with a bentonite supplied by the winery that supplied the particular batch of lees used to obtain the recycled bentonite. The Australian Wine Research Institute carried out the trials following AWRI method GM25 (Issued 24 April 1998).

D. Analyses of waste waters from treatments

Solutions from a number of the treatments were analysed for elemental composition using Inductively Coupled Plasma (ICP) spectroscopy

E. Optimisation of procedure to separate bentonite complexes from other wastes

Bentonite complexes were separated from both lees from a winery and also distillation residue from a distillery. Residue from the distillery was supplied in either liquid or solid form. The liquid residues were left to flocculate. Water was added to the solid residues for the same purpose. In some cases flocculation occurred spontaneously. Otherwise, a few scoops of sodium chloride crystals were added on a spatula to bring it about. The flocculated material was then separated by centrifugation, and washed 4 times with water, using ultrasonification to re-disperse the precipitate. After the final washing and dispersion, M NaOH was added slowly until the pH of the suspension was 9. The <2 μm fraction was removed as the supernatant by centrifuging using appropriate centrifuge settings (speed and time), following Stokes' Law for settling (Jackson, 1956).

F. Discussion of procedures developed with industry

The procedures developed were presented to a committee comprising representatives of various wineries (the Interwinery Environment Committee of the SA Wine and Brandy Association) by way of a half-hour presentation, with discussion to them on 9 November 1999. They have also been outlined to the technical manager of a distillery and an international consultant to the wine industry.

G. Determination of economic feasibility of favoured procedure

When the focus of the project changed from an emphasis on displacement of protein using sodium carbonate, shortage of time precluded the further development of prescribed processes for the alternative procedures beyond laboratory testing. Instead, some prices of components involved in these procedures, and also useful contacts, were obtained. These should assist in further exploration of the economics of these procedures.

DETAILED RESULTS

Characterisation of lees

Different samples of lees varied widely in their composition. 3 different samples were used in the course of this work.. Their C and N contents are given in Table 1.

Table 1. Carbon and Nitrogen Contents of samples of Lees used in this study

Lees No.	Date of collection	Origin	%C	%N
L1	11/8/98	BRL Hardy	23.8	5.35
L2	25/8/98	BRL Hardy	19.7	4.75
L3	24/2/99	D'Arenberg	25.1	2.32

Results of treatments to remove protein from bentonite

Displacement by alkalis and high concentrations of cations

Table 2 (below) shows:

- More protein was removed when lees was shaken with sodium carbonate at a low solid: solution ratio and when agitation was maximised, whether through a long time of shaking or by ultrasonication. Protein removal was also maximised at a very high pH (13), where bentonite is likely to become strongly degraded.
- Considerable N (and also C) was retained by the lees even under conditions that were most conducive to the removal of protein with sodium carbonate.

Table 2. Protein displaced and C and N contents of products of treatment of Lees (L1) with alkalis and salts

Treatment No.	Alkali/salt	Concentration	Solid:solution ratio	Agitation	Time	Protein released mg g^{-1}	C retained %	N retained %
T1	Na_2CO_3	2%	1:10	Shaking	1 hour	3.07	22.2	4.1
T2	"	4%	"	"	"	3.2	-	-
T3	"	6%	"	"	"	3.52	-	-
T4	"	2%	1:40	"	16 hours	11.34	-	-
T5	"	"	1:50	"	"	14.18	21.2	4.2
T6	Na_2CO_3 + NaCl	2%+10% (NaCl)	"	"	"	5.19	-	-
T7	Na_2CO_3 + KCl	2%+ 10% (KCl)	"	"	"	6.10	-	-
T8	KCl	10%	"	"	"	2.68	-	-
T9	Na_2CO_3	2%	1:100	"	96 hours	28.24	-	-
T10	Na_2CO_3 + KCl	2%+ 2% (KCl)	"	"	"	20.53	-	-
T11	Na_2CO_3	2%	1:200	"	16 hours	44.9	19.0	3.8
T12	"	4%	"	"	"	51.4	-	-
T13	"	6%	"	"	"	48.3	-	-
T14	"	2%	"	"	120 hours	38.6	-	-
T15	"	4%	"	"	"	38.8	-	-
T16	"	6%	"	"	"	37.5	-	-
T17	"	2%	1:500	"	96 hours	63.2	19.0	3.6
T18	"	2%	"	Ultrasound	10 min.	66.45	-	-
T19	$CaCl_2$ (pH 10.5 with $Ca(OH)_2$)	2M	"	Shaking	16 hours	0	-	-
T20	$CaCl_2$ (pH 10.5 with NaOH)	2M	"	"	"	0	-	-
T21	Deconate	3%	"	"	"	58.6	-	-
T22	NaOH	0.01M (pH 12.0)	"	"	"	52.54	-	-
T23	"	0.1M (pH 13.0)	"	"	"	65.67	-	-

- not determined

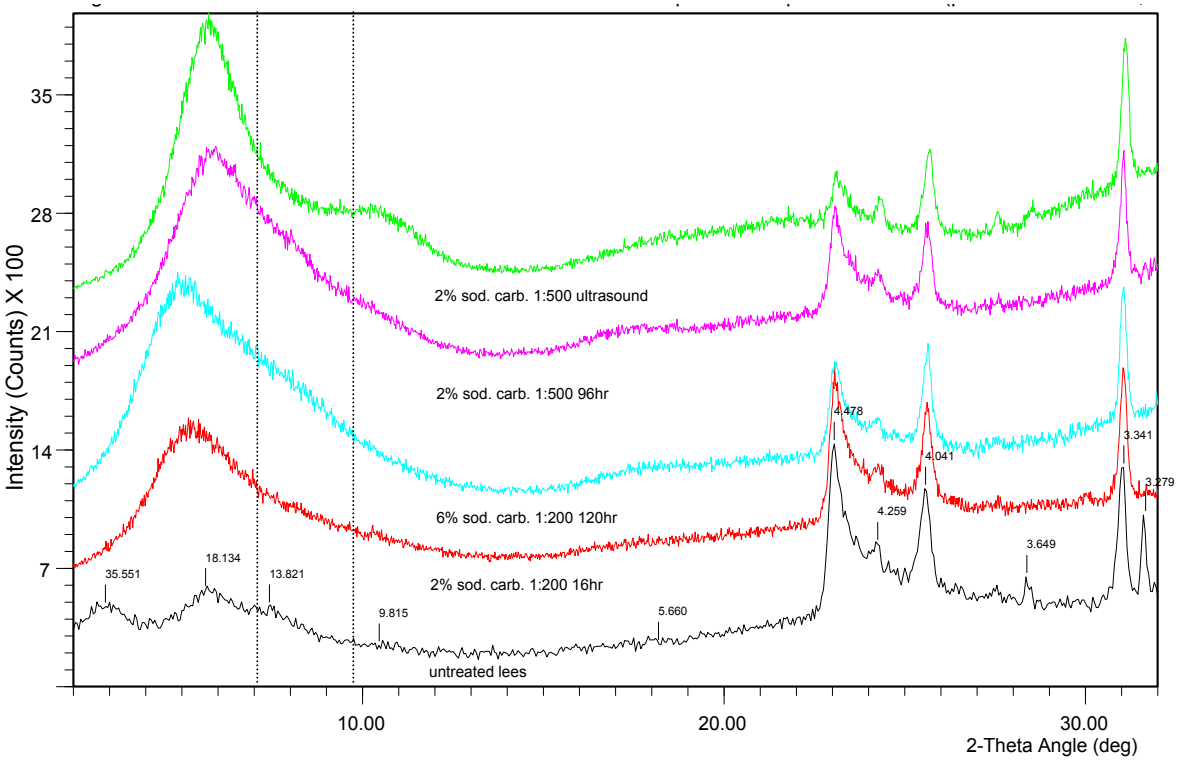


Fig. 2. Effect of treatments with sodium carbonate on X-ray diffraction patterns of bentonite-protein complexes in lees L1. Products oven-dried and examined as unoriented powders.

Fig.2 (above) shows the XRD effects of some of the treatments with sodium carbonate of the lees L1 and compares these with the XRD pattern of original untreated lees. In particular, the dashed vertical lines represent the range of angles over which an uncomplexed bentonite would show its highest spacing after drying and exposure to ambient water vapour. Instead, all samples have shown peaks at considerably enhanced spacings. This shows that all the sodium carbonate treatments examined resulted in expanded bentonites even after oven-drying. The treatments represented in Fig.2 produced the most protein of all those covered in Table 2, except, marginally, for NaOH treatment at pH 13. This indicates that treatments with salts and alkalis are incapable of removing all of the protein from within complexes with bentonite in lees.

Since it was not possible to determine the protein contents of the original lees, these results could not be expressed in terms of the proportion of protein removed. Protein determinations were not carried out following further treatments.

Effects of treatments with strong acids

Table 3 shows that all treatments with concentrated acids, both hydrochloric and sulphuric, reduced C and N contents below those attained with salt and alkali treatments (Table 2). The most effective treatments for this purpose were those with sulphuric acid, with continuous stirring, and for a long time (at least 24hr). However, such treatments also removed considerable Mg and Al from the bentonite, indicating degradation of the mineral framework. Analyses with X-ray fluoresence showed that the bentonite used to produce Lees L2 contained 10.6% Al (106 mg g^{-1}) and 1.44% Mg (14.4 mg g^{-1}). The lees contains other (mainly organic) solid material than the bentonite. Thus the untreated lees contained <106 mg g^{-1} Al and <14.4 mg g^{-1} Mg. Hence the acid treatments that were most effective in removing N, therefore protein, viz. Nos. A15-A18 inclusive, also removed >50% of Al and ~50% or more of Mg from the aluminosilicate framework of the bentonite.

Fig. 3 shows the XRD effects of the acid treatments that were most effective in removing protein and compares them with the XRD pattern of the original lees L2. It confirms the indication from Table 3 that destruction of the protein by acid is accompanied by destruction of the aluminosilicate layers in the bentonite. Prior to layer destruction occurring, peaks persist at ~ 13 Å (indicating. a repeat distance for the layers of 1.3 nm). This spacing falls within the range for smectite incorporating ambient water vapour, as shown by the dashed lines, so could be attributable merely to interlayer water. However, since it is greater than the 10 Å spacing for completely collapsed interlayers, its appearance does not preclude the intercalation of material propping open the interlayers at this enhanced spacing. This material could include persistent protein, although this appears to give either even higher spacings (Fig. 2 and also lowest pattern in Fig. 3), but also could include hydroxy- aluminium polymeric ions resulting from aluminium leached from the clay structure and strongly attracted to clay layer surfaces. Hydroxy-aluminium “pillars” are formed in both acid soils (e.g. Barnhisel and Bertsch, 1989) and as a result of acid treatments in the

laboratory (Churchman and Jackson, 1972). The 13 Å peak persists for 16hr treatments, both with and without stirring, but there are no peaks at the spacings expected (i.e. at spacings at or above those of the collapsed layers) for longer treatments. The periodicity of the layer arrangement has been destroyed by treatments for 24 or 40hr. Hence, both chemical data (Al and Mg removed) in Table 3, and also structural data, from Fig. 3, indicate that removal of protein by strong acid is accompanied by destruction of the bentonite.

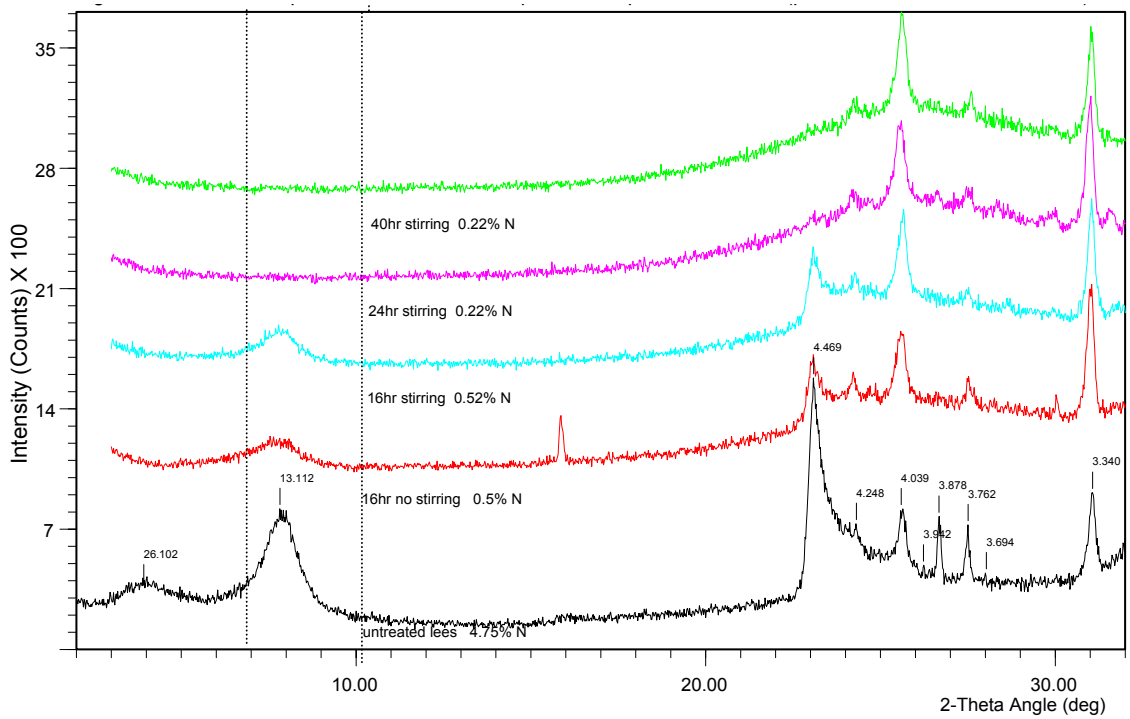


Fig. 3. Effects of hot 6M sulphuric acid on XRD patterns for bentonite-protein complexes in lees L2. Products oven-dried and examined as unoriented powders.

Elemental analyses with X-ray fluorescence XRF of the solid resulting from 24hr treatment with sulphuric acid (Table 6) showed the resulting materials to be 85% silica SiO_2 . From their black appearance, it is likely that much of the remainder was black carbon.

Table 3. Effect of treatments of lees with strong acids on contents of C and N remaining and on release of ions to solution

No.	Lees No.*	Acid	Conc.	Solid: Solution	Pretreatment of lees	Time	Temp. °C	Agitation	C retained in lees %	N retained in lees %	Al released mg g^{-1}	Mg released mg g^{-1}
A1	L1	HCl	6M	1:40	none	6 hr.	70	Occasional	12.5	2.68	3.67	3.86
A2	"	"	"	"	"	24 hr.	."	"	10.7	1.87	8.53	4.7
A3	"	"	"	"	washed	"	"	"	9.9	1.94	13.46	3.04
A4	L2	H_2SO_4	"	1:400	"	2 hr	"	"	9.1	1.75	6.8	1.0
A5	"	"	"	"	"	8 hr	"	"	9.0	1.35	48.3	6.1
A6	"	"	"	"	"	24 hr	"	"	8.1	0.5	64.2	7.9
A7	"	"	"	"	none	4 hr	"	"	9.0	1.35	3.7	2.3
A8	"	"	"	"	"	16 hr	"	"	10.1	0.5	19.8	4.4
A9	"	"	"	"	washed and dried	4 hr	"	"	8.3	1.35	21.3	3.0
A10	"	"	"	"	"	8 hr	"	"	8.3	1.40	18.1	2.6
A11	"	"	"	"	wine removed	4 hr	"	"	9.1	1.65	9.8	2.2
A12	"	"	"	"	"	8 hr	"	"	10.4	1.05	30.0	4.4
A13	"	"	"	"	"	16 hr	"	"	9.3	1.1	24.4	4.2
A14	"	"	"	"	"	24 hr	"	"	9.65	0.45	50.2	6.7
A15	"	"	"	1:40	"	24 hr	"	Stirring†	9.9	0.22	56.6	7.6
A16	"	"	"	1:80	"	"	"	"	10.1	0.25	54.7	6.8
A17	"	"	"	1:40	"	40 hr	"	"	10.3	0.25	57.8	7.6
A18	"	"	"	1:80	"	"	"	"	8.9	0.22	56.2	6.8
A19	"	"	"	1:40	"	1 hr	"	"	8.6	1.63	4.8	1.2
A19	"	"	"	"	"	2 hr	"	"	8.5	1.36	9.6	2.0
A20	"	"	"	"	"	4 hr	"	"	8.3	1.12	20.4	3.2
A21	"	"	"	"	"	8 hr	"	"	9.4	1.01	30.0	5.2
A21	"	"	"	"	"	16 hr	"	"	9.5	0.52	45.6	6.4

†continuous

Effects of treatments with strong oxidising agents

Table 4. Effect of treatments of lees with oxidising agents on C and N remaining

No.	Lees No.*	Oxidising agent	Pretreatment	Post-treatment	C	N
					% retained	
O1	L2	NaOCl	washed	none	5.4	0.14
O2	"	"	" +24hr 6M H_2SO_4	"	6.6	0.67
O3	"	"	" 1hr "	"	5.4	0.5
O4	"	H_2O_2	"	"	6.2	1.23
O5	"	"	" + 1hr 6M H_2SO_4	"	2.75	1.4
	(L3	*n.a.*	*Bentonite used for lees L3*	*n.a.*	*0.3*	*0.01)*
O6	L3	H_2O_2	none	Na_2CO_3*	4.26	0.36
07	"	"	" , <2µm separated	"	5.63	0.63
08	"	UV/photox.†			2.0	0.26

* 2%, continuous shaking 16hr, solid:solution ratio = 1:500

† photox = photo-oxidation

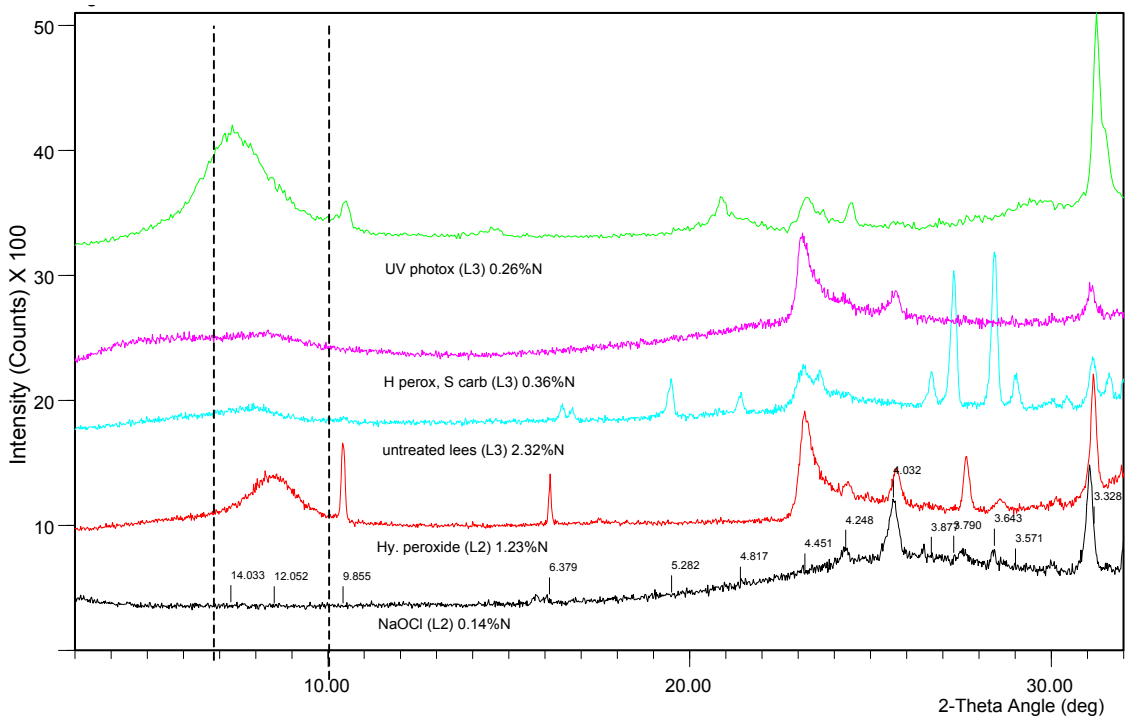


Fig. 4. Effect of oxidising agents on XRD patterns of bentonite-protein complexes in lees L2 and L3. Products oven-dried ands examined as unoriented powders.

Fig. 4 shows that treatment with sodium hypochlorite led to no peaks for aluminosilicate layers (i.e. peaks at, or greater than, spacings for the collapsed

bentonite as indicated by the dashed vertical line). This indicates destruction of layers and hence loss of layer periodicity as a result of this treatment, which removed more nitrogen than any other (Table 4, cf also Tables 2 and 3). The XRF analysis of the product of sodium hypochlorite treatment (Table 6) indicated that the clay mineral in the bentonite (viz. montmorillonite) had been largely reduced to silica, but a Cl-rich product had also been formed. Clearly, NaOCl was effective in largely, if not completely, destroying protein. However, it led to an unknown product, which while highly siliceous, also contained a chloride compound.

On the other hand, treatment with hydrogen peroxide led to a strong, although broad, peak for a spacing near 12 Å. Although this spacing is still larger than that of a fully collapsed layer (10 Å), it represents a lower spacing than attained with any other treatments (Figs 2 and 3). It is a realistic spacing for an oven-dried bentonite which has not been pretreated with potassium to aid layer collapse and which therefore has been able to adsorb some water to partially expand the interlayer spacing. It suggests that protein has been lost from the interlayers. The peroxide-treated sample also shows sharp peaks (at 9.89 and 6.38 Å) These probably represent primary minerals, respectively mica and a feldspar, which were components of the original bentonite, but which had their peaks suppressed in the pattern for the untreated lees (Fig. 3).

Considerable N remained after hydrogen peroxide treatment alone (Table 4). However, when the treated material was treated again with sodium carbonate, in order to displace oxidised products from the interlayer, nitrogen content was greatly reduced. Comparison of the patterns in Fig. 4 for the untreated lees L3 and this material treated this way ("H perox, S carb") shows that a very broad low peak for spacings extending between ~17 and ~13 Å for the lees was replaced by a strong peak centred near 12 Å. As in the case of peroxide-treated L2, this indicates loss of interlayer protein. There is a considerable concomitant loss of N (Table 4).

The content of N remaining after ultraviolet/photo-oxidation (Table 4) was lower than that remaining following all treatments except that with NaOCl, which is unsuitable because it destroys the bentonite. The residual content of C after UV/photo-oxidation was lower than after any other treatment (Tables 2-4). It may be possible to reduce the contents of both C and N even further by a longer photo-oxidation treatment.

Experience with soils (J.O. Skjemstad, CSIRO Land and Water, pers. comm.) has shown that reduction in C and N continues as photo-oxidation is prolonged.

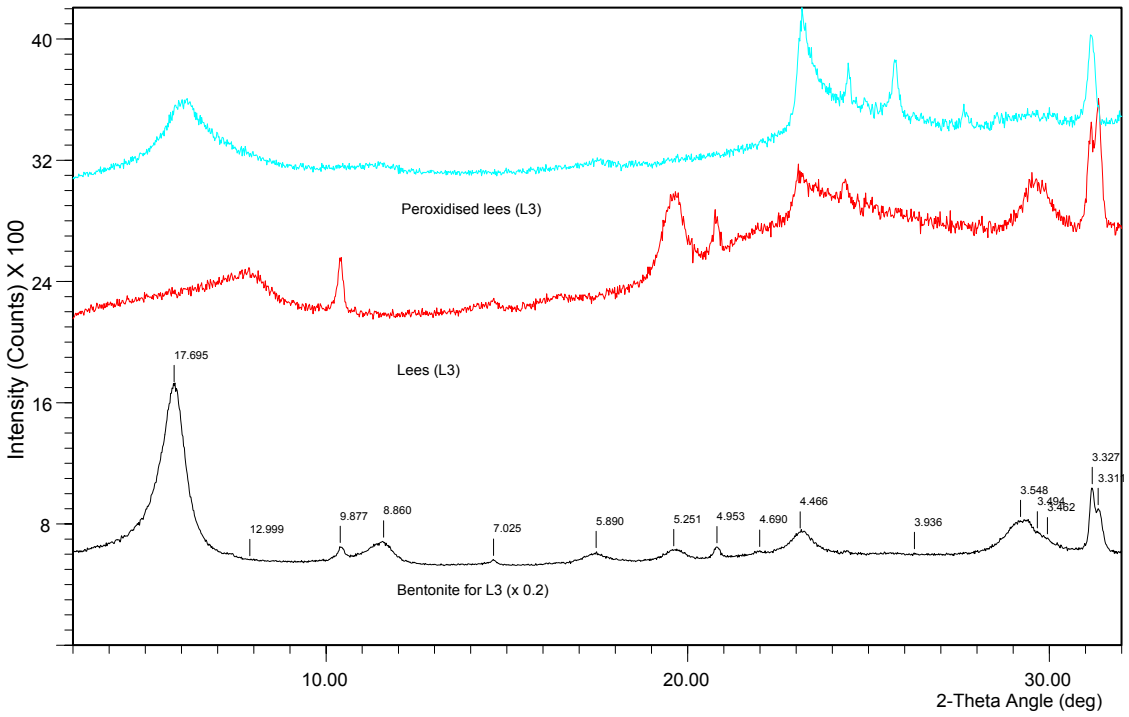


Fig. 5. XRD patterns of an oriented, glycerol-solvated sample of lees L3 after peroxidation (top pattern) compared with those of the lees (middle pattern) and the original bentonite (lower pattern, for which the intensity has been reduced by 5 times).

The XRD patterns of the peroxide-treated lees as an oriented, glycerol-solvated sample is compared with those of the original bentonite and also the untreated lees, also as oriented, glycerol-solvated samples in Fig. 5. Fig. 5 shows that the lees treated with H_2O_2 is able to accommodate glycerol in the interlayers of the clay mineral and thereby expand, like the original bentonite, whereas the untreated lees is unable to do so because of the prior occupation of protein in the interlayers. This indicates that peroxide treatment, with sodium carbonate, has re removed sufficient protein, at least, from the interlayers to open them to the entry of other molecules, such as the diagnostic glycerol.

Use of sulphuric acid in association with oxidising agents did not improve loss of N, although sulphuric acid helped reduce C when used with H_2O_2 treatment (Table 4).

Treatment with ultra-violet/photo-oxidation also resulted in sharp peaks for repeat layer spacings (Fig. 5), although this was at a higher spacing (near 14 Å) than those resulting from peroxide treatments (Fig. 4). However, the sharpness of the peak, especially relative to that for the untreated lees, suggests that it represented the constituent smectite (montmorillonite) clay mineral with intercalated water, even following oven drying, rather than with intercalated protein. This is confirmed by the close comparison between the pattern for the treated material and the original bentonite, which is shown in Fig. 6. The major difference between the two patterns, which are not offset from one another, is that the treated material gives stronger peaks. This results from its greater degree of orientation which came about because the sample was very small and had to be held in a shallow holder, in which true random orientation was difficult, if not impossible, to achieve. However, it also

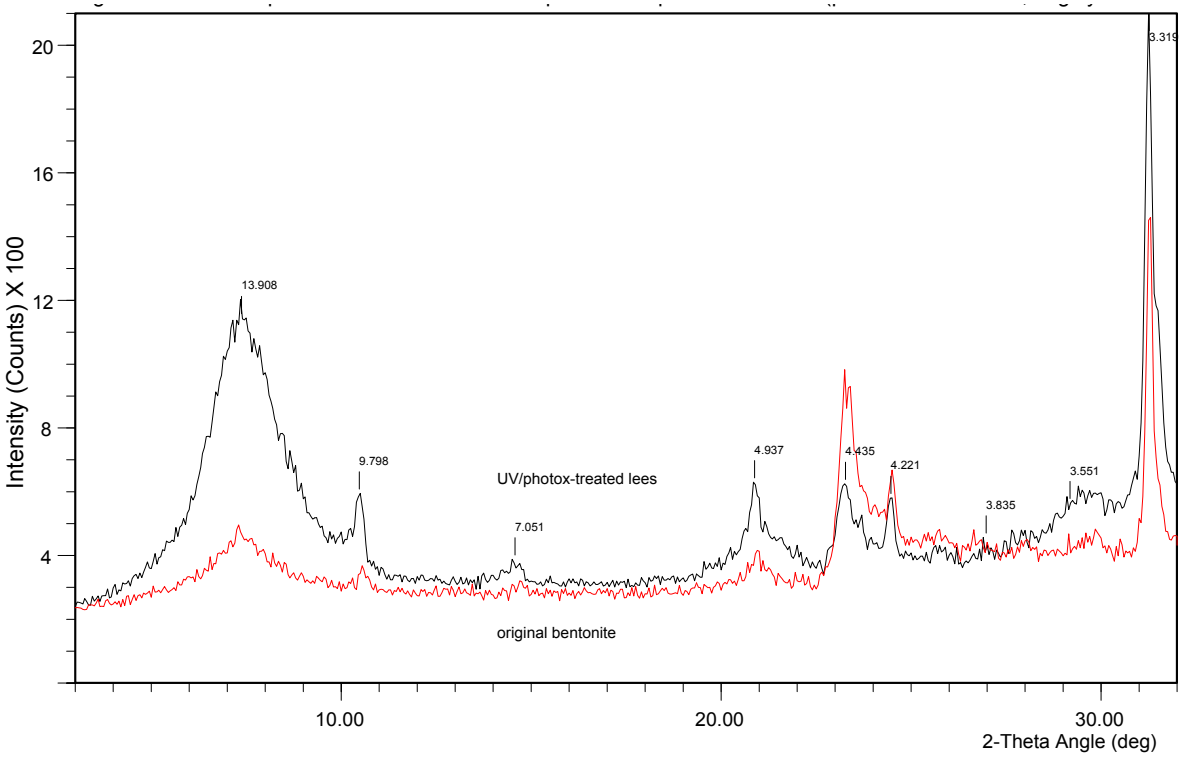


Fig. 6. XRD patterns of lees L3 after photo-oxidation treatment with ultraviolet radiation and of the original bentonite for lees L3. Oven-dried products examined as largely unoriented powders. No off-set between patterns.

indicates that the clay mineral was essentially unaffected by the UV/photo-oxidation treatment.

Analysis of the composition of the bentonite remaining after treatments (Table 5) shows that, of all treatments included in the analyses, it was only after those with hydrogen peroxide that the products were left with a comparable CEC to those of the original bentonites The product of photo-oxidation treatment with ultraviolet radiation was not analysed this way as this treatment was included late in the project.

Table 5 shows that, treatments of lees L3 with hydrogen peroxide and sodium carbonate increased the CEC over that recorded for the untreated bentonite. The high CECs were confirmed by analyses of ignited calcium-saturated samples of these products. It is possible that organic products of the treatments contributed to the high CECs, which are consistent with the apparent enhancement in the ability of the treated product to remove heat-unstable protein from wine (Table 6). It is also possible that some structural changes have been brought about to the aluminosilicate layers by the peroxide treatment that have resulted in a decrease in the residual negative charge on the layers. This result could be investigated further.

Table 5. Effect of treatments on composition of bentonites

Sample	Fe_2O_3	MnO	TiO_2	CaO	K_2O	P_2O_5	SiO_2	Al_2O_3	MgO	CEC	Cl
	%									Cmol charge kg^{-1}	
Bentonite for L2	3	0.01	0.12	0.15	0.3	0	65	21	2.5	95	0
Lees L2	3	0	0.07	0.02	0.4	0.7	66	23	2.1	25	0
24hr Sulphuric Acid	0	0	0	0.03	0.1	0	97	2.3	0.1	6	0
NaOCl	0	0	0	0.03	0	0	97	1.6	0	0	43
NaOCl + 24hr SA	0	0	0	0.04	0.2	0	96	2.2	0.1	0	35
NaOCl + 1hr SA	2	0	0.02	0.05	0.2	0	68	20	2.0	0	57
Hydrogen peroxide	4	0	0.09	.05	0	0	65	24	2.8	74	0
Hy. Perox. + 1hr SA	3	0	0.09	0.05	0.1	0	72	20	2.2	28	0
Bentonite for L3	5	0.04	0.27	0.12	1	0.1	63	21	3.6	84	8
Lees L3	4	0.02	0.33	0	1.8	2.2	63	22	3.1	39	4
Hy. Perox.+ sod. carb.	6	0.03	0.37	0.06	1.6	0.3	59	21	3.2	134	0
Hy. Perox. + s.c., clay frn.	5	0.03	0.32	0.06	1.4	0.1	56	20	3.2	189	0

Infrared spectral analysis of residual material

Infrared spectra of products of the most successful treatments for both removal of nitrogen and restoration of the properties of the bentonites (i.e. Hydrogen peroxide with sodium carbonate and ultraviolet/photo-oxidation) are shown in Fig. 7.

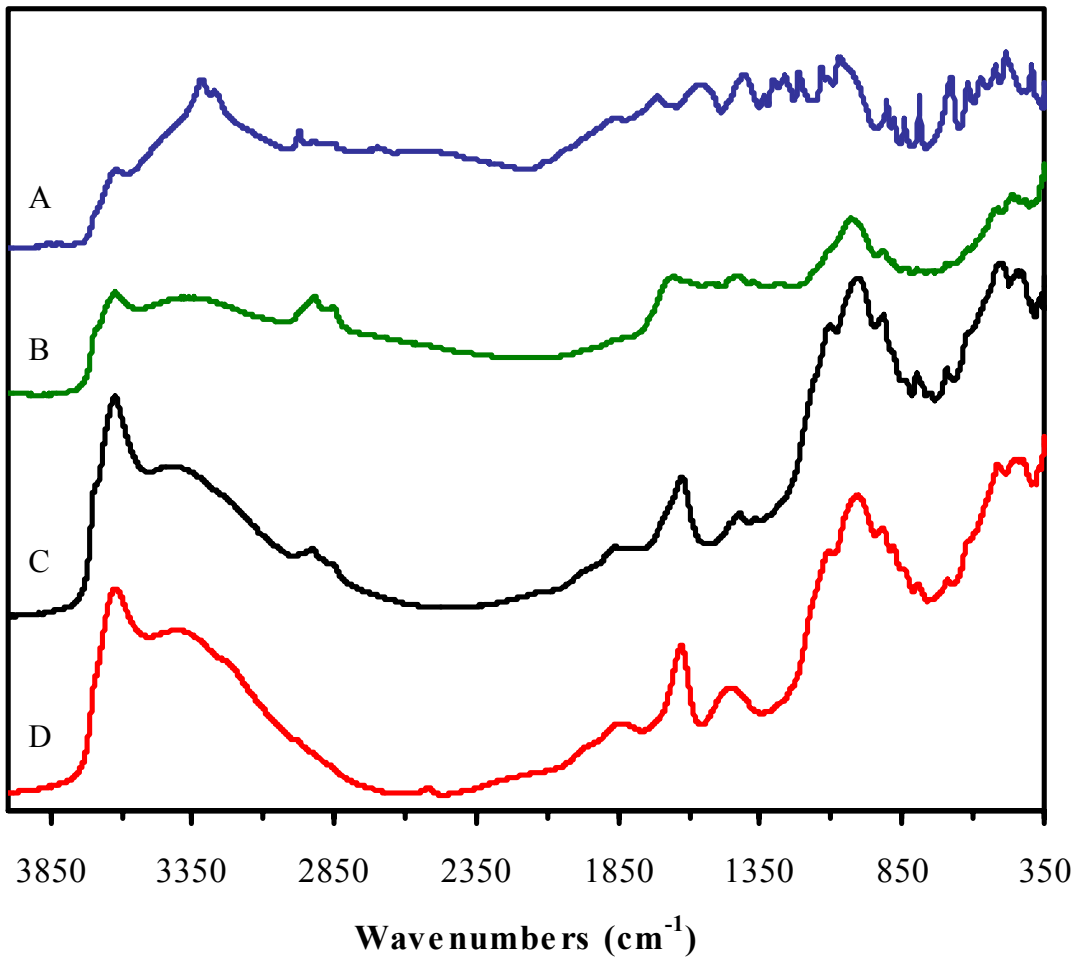


Fig. 7. Infrared spectra of a. L3 lees; b. product of treatment of L3 with hydrogen peroxide, followed by sodium carbonate; c. product of UV/photo-oxidation treatment; d. bentonite used to produce L3 lees.

Fig. 7 shows that most of the many peaks at wavenumbers below ~ 1850 cm^{-1} in spectrum a from the lees are absent from spectra c and d for the treated products. These peaks are largely attributable to proteins. Spectrum c for the product of uv/photox. treatment is virtually identical to that of the treated bentonite, although it shows small (C-H stretching) peaks near 3000 cm^{-1} for residual organic matter. These

peaks are more prominent in spectrum b for the product of peroxide/carbonate treatment, consistent with the higher contents of C and N in the products of these treatments than in those from photo-oxidation with ultraviolet radiation.

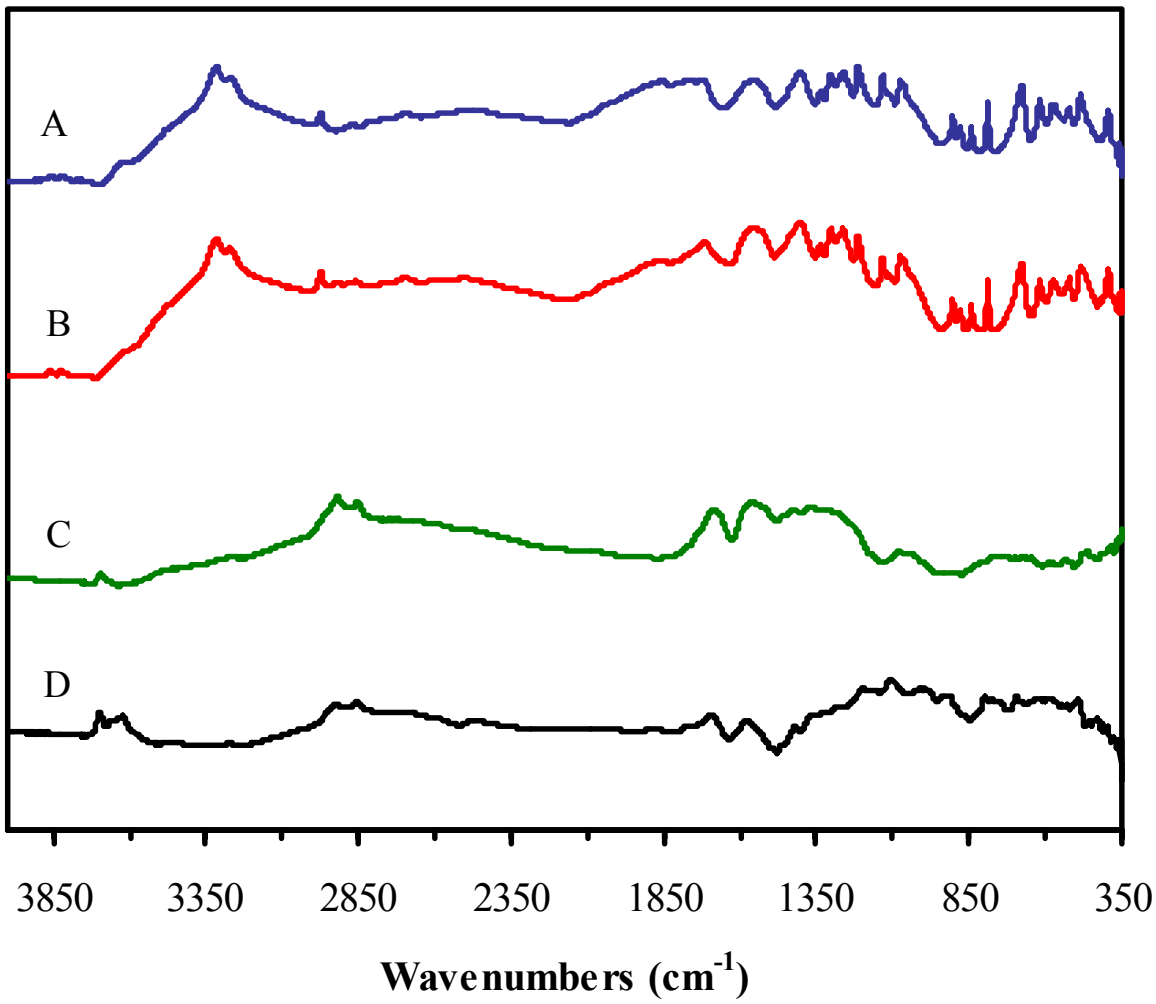


Fig. 8. Difference infrared spectra, showing the residuals of subtractions of spectra for a. peroxide/carbonate treatment of L3 from L3; b. Uv/photox. treatment for L3 from L3; c. bentonite for L3 from its peroxide/carbonate treatment; d. bentonite for L3 from its UV/photox treatment.

In Fig. 8, the multitude of protein-related peaks below ~ 1850 cm^{-1} are clear in the differences between untreated and treated lees (a and b). The differences between the two treatments and the original bentonite are greater in c, for the peroxide/carbonate treatment than in d, for the UV/perox. treatment. They are largely shown as peaks

near 3000 cm^{-1}. The residual 3000 cm^{-1} peaks for the peroxide/ carbonate treatment are larger than those for the UV/photox treatment, reflecting the relatively higher contents of both C and N resulting from the former treatment (Table 4).

Test for stabilising wines

The results of tests for the removal of heat-unstable proteins from a very unstable 1999 Chardonnay wine are given in Table 6 for products of the two peroxide/carbonate treatments of lees L3 (treated whole lees and clay fraction of treated lees), as well as for the bentonite used to produce L3. The products of the later ultraviolet/photo-oxidation treatment of the lees were not tested this way.

Table 6. The minimum concentrations of bentonite required to remove protein from an unstable 1999 Chardonnay wine.

Sample	**Min. bentonite requirement g L^{-1}**	**Observations**
Perox./Carb.-treated	3.5	Settled quickly, but frothy, with some smell
P/C treated, <2μm frn.	>4.0	Settled quickly, but frothy
Original bentonite	>4.6	Slow settling- bentonite in granular form

The data in Table 6 show that the bentonite resulting from treatment of lees with hydrogen peroxide followed by sodium carbonate is capable of removing heat-sensitive protein from wine. It was more effective than the original bentonite in this regard. Clay fractionation subsequent to the chemical treatment did not improve its effectiveness. The increased effectiveness of the treated lees is consistent with the enhanced CEC that was measured for this material (Table 5). However, the froth, and, more particularly, smell, associated with the material during the wine stabilising process detract from its immediate usefulness. They very likely reflect the residual organic material associated with the bentonite (Table 4 and Figs. 7 and 8). More treatment would be needed to clean the product of peroxide/carbonate treatment before it was useful for wine stabilisation.

Test for use of lees in plant growth media

Trial B1a, in which plants were grown in soil with different proportions of lees and also with bentonite showed:

- Plants in soil amended with 5 or 8% lees showed poor growth and symptoms of distress from early stages. The plants in these media were always the smallest in the trial and also showed fewer leaves than plants in other pots and these also showed yellow tips within 7 days of the trial. They continued to deteriorate throughout the trial.
- Plants in soil amended with 2% lees also grew less well than those in soil alone or in soil with bentonite addition. In this case signs of distress, including wilting and dying tips on leaves look ~ 19 days to appear, but became more marked beyond that stage.

The pHs and electrical conductivities (ECs) of the growth media and also the lengths of the roots and oven-dried weights measured after plants were harvested are given in Table 7. Results of the composting trial (below) showed that soils with added lees had a pH of ~3.8. The EC of the medium with soil containing 5% lees was 1-1.5 mS cm^{-1}.

Table 7. Properties of the growth media and the plants following harvesting after pot trial B1a (33 days duration). All data are means of 3 replicates.

No.	**Medium**	**Soil properties**		**Plant properties**	
		pH	EC dS cm^{-1}	Root length mm	Dry weight g
(i)	Soil only	5.09	0.04	235	1.91
(ii)	Soil + 5% bentonite*	6.10	0.06	293	1.92
(iii)	Soil + 2% lees L2	7.28	0.41	40	0.30
(iv)	Soil + 5% lees L2	8.86	0.69	25	0.10
(v)	Soil + 8% lees L2	8.84	1.15	31	0.12

* for lees L2



Fig. 9. Effects of 33 days of growth of wheat seedlings in (l. to r.): (i) soil, (ii) soil + 5% bentonite, (iii) soil + 2% lees L2, (iv) soil +5% lees L2, and (v) soil + 8% lees L2. The medium in each pot was given the same nutrient solution and had its moisture content maintained at the same level throughout the trial.

The soil data in Table 7 explain the plant data there and also the visual effects of the treatments including lees on the plants (Fig. 9) by means of the very high EC's which would constitute unsatisfactory saline conditions for plant growth (Shaw, 1999). Furthermore, pHs are excessively high for the two higher loadings of lees (Nos. (iv) and (v), so that nutrient deficiencies could occur (Slattery *et al.,* 1999). In addition, it is noted that the low pH early in the treatments containing lees may have caused damage to roots mainly through the availability of toxic aluminium (Slattery *et al.,* 1999).

Trial B1b, in which the possibility that protein complexed with bentonite in lees could act as a slow-release nitrogen fertiliser, showed again that incorporation of lees in the growth medium had a toxic effect on plants. In this case, the pH had been raised at the start of the trial to prevent toxic Al affecting roots. The effects of the treatment for 21 days is shown in Fig.10.

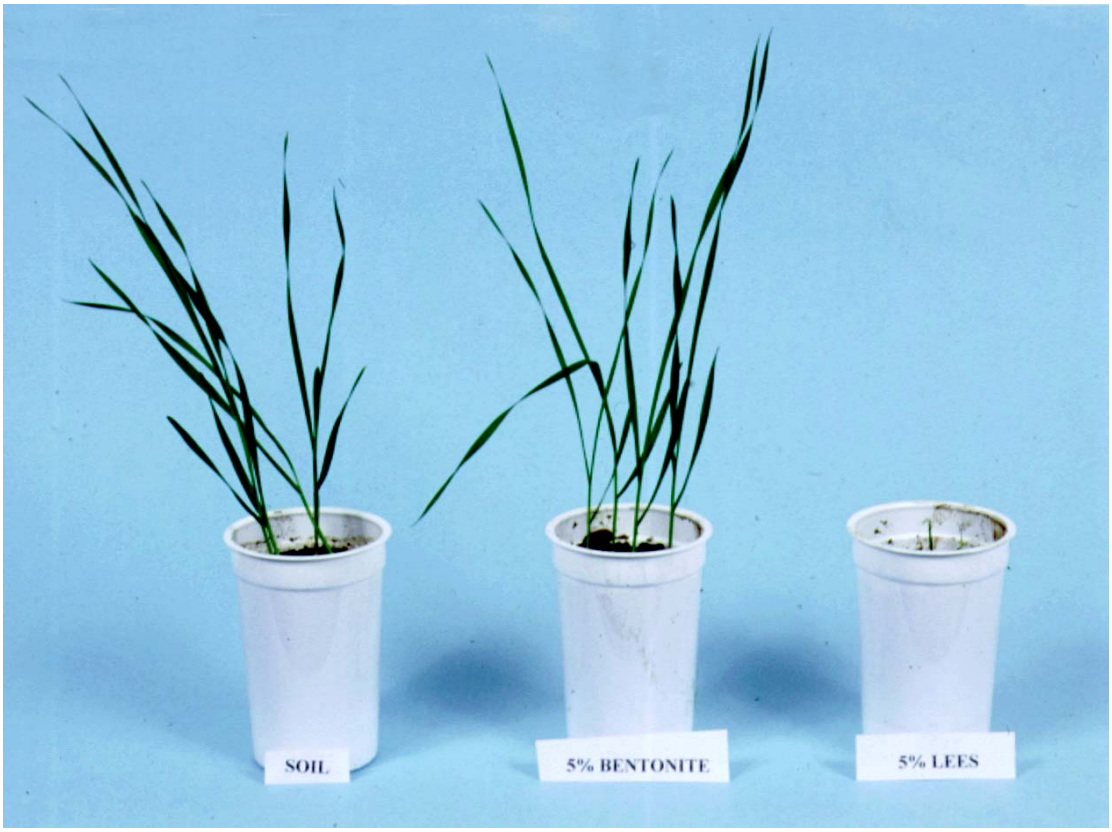


Fig. 10. Effects of 21 days growth of wheat seedlings in (l. to r.): (i) soil, (ii) soil + 5% bentonite, and (iii) soil + 5% lees L2 when the medium in each pot was given adequate amounts of all required nutrients except nitrogen. Moisture contents were maintained at the same levels as for the trial shown in Fig. 9.

Lees had the same effect on plants in Trial B1c, in which the possibility that lees, and also bentonite could supply water to plants in a dry soil was investigated. Fig. 11 shows that plants died in lees-amended soil regardless of the increased water-holding capacity of the medium. Even the addition of bentonite itself led to decreased plant growth under conditions of water-starvation (Fig.11).

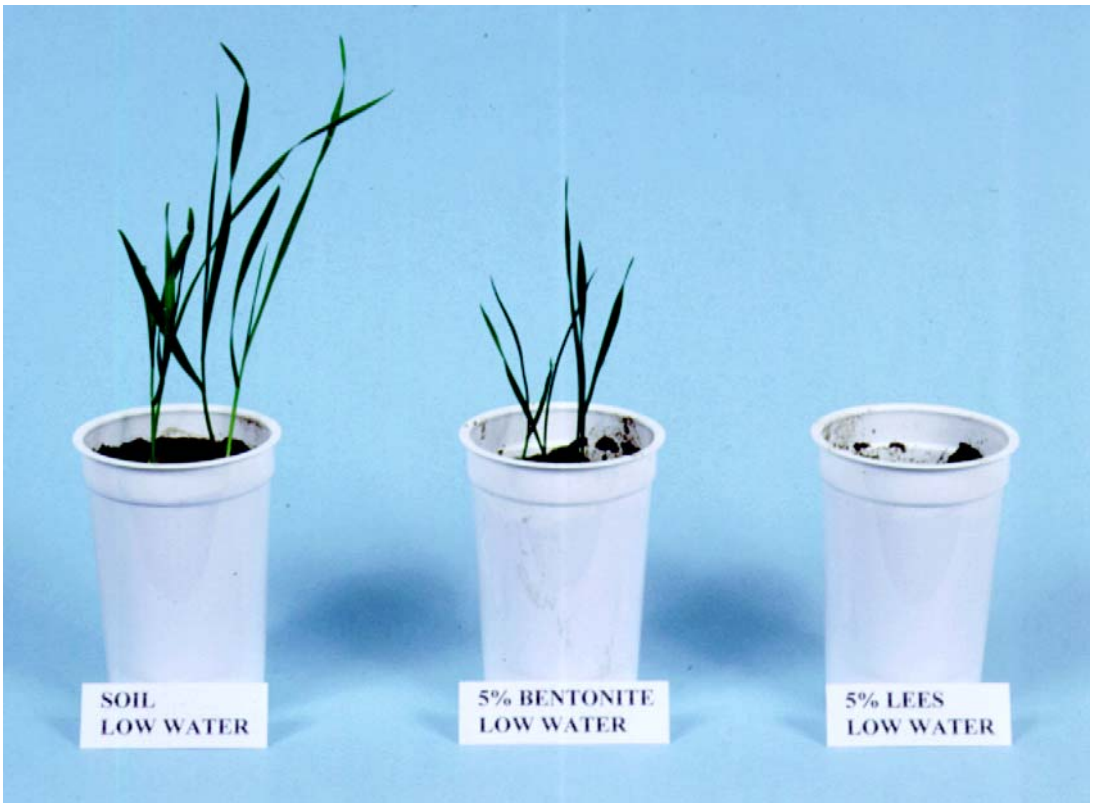


Fig. 11. Effects of 21 days growth of wheat seedlings in (i) soil, (ii) soil + 5% bentonite, and (iii) soil + 5% lees L2 when the medium in each pot had the same complete suite of nutrients added but was maintained at only one-half of the moisture content as the trials shown in Figs. 9 and 10.

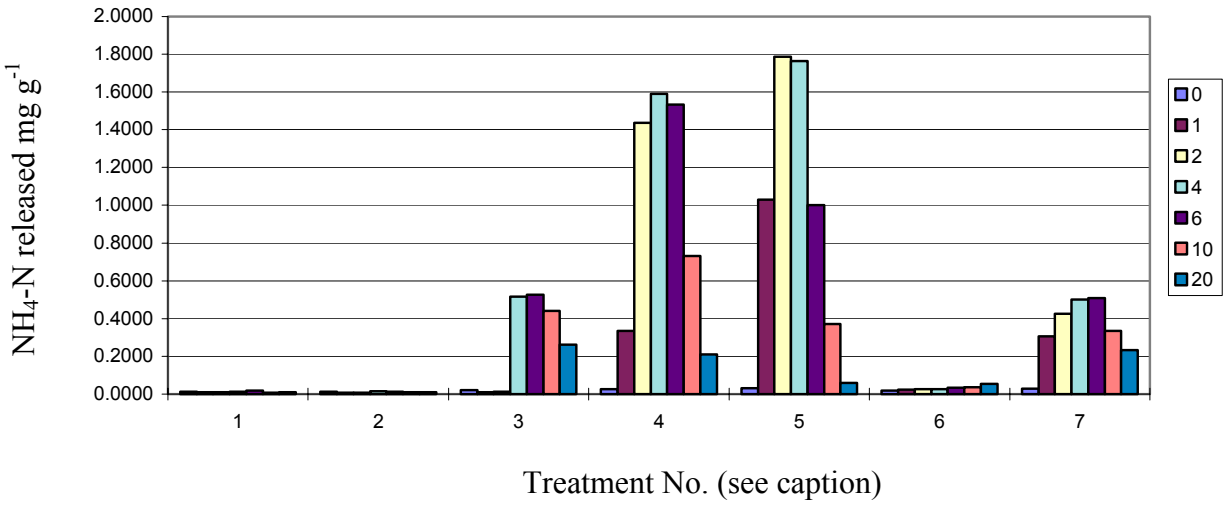
In general, the plant trials showed that lees is not a suitable material for plant growth, at least without pretreatments.

Composting of lees

Composting led to considerable production of N as ammonia from mixtures with soil involving lees that was untreated except for removal of supernatant wine (Fig. 12a). Nitrate N was also released from the mixture with the highest loading of lees from which wine only had been removed (Fig. 12b). Mixtures involving lees which had been washed released little of either form of N while a high loading (1.8%) of completely untreated lees contributed some NH_4-N, but only as much as a much lower loading (5%) of a mixture containing wine-depleted lees. pHs (values not given) ranged between ~4 for the mixture with washed lees (No. 6) to between 8 and 10 for other mixtures involving lees. The pH of the soil was maintained at ~6, while that of the mixture with bentonite (No. 2) was between 7.4 and 8. ECs (values not given) were all < 0.4 dS/m. They were highest for the mixture containing 8% lees (No. 5).

Composting lees may be a useful way of producing N, especially in the form of the ammonium ion, to enhance plant growth. However, as shown in the previous section, use of composted lees as a medium for plant growth does not diminish the toxicity of lees towards plants to any noticeable extent. In any case, composting does not enable recycling of bentonite for its further use in wine processing.

(a)



(b)

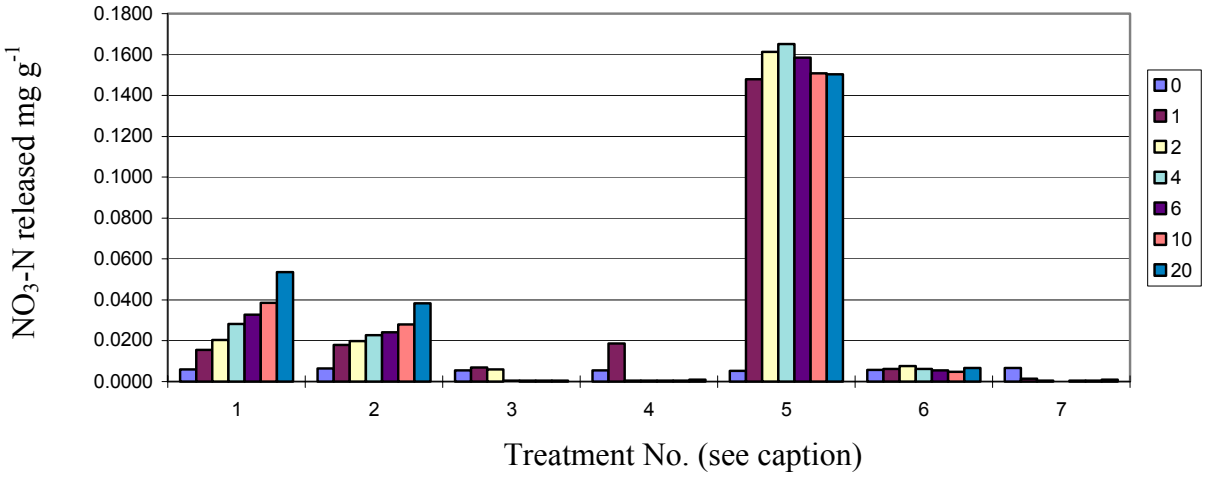


Fig 12. Release of N as (a) NH_4 and (b) NO_3 from each of the following composting treatments (as numbered along x-axis):
1. Soil only; 2. Soil + bentonite for lees L2; 3. Soil + 2% lees L2 with wine removed; 4. Soil + 5% lees L2 with wine removed; 5. Soil + 8% leesL2 with wine removed; 6. Soil + 5% lees L2 after washing; 7. Soil + 1.8% lees L2 untreated. The times of composting (in weeks) are indicated by the key to the right of each graph.

Analyses of waste waters from treatments

Table 8 shows that all treatments produce a considerable amount of nutrients in waste-waters. The chemical treatments with hydrogen peroxide, sodium hydroxide and sodium carbonate yield particularly high amounts of nitrogen, calcium, potassium and sodium, with peroxide releasing most N, the alkalis (NaOH and Na_2CO_3), the most Na.

Waste waters from the ultraviolet photo-oxidation treatment were not examined, but there would be no chamical additions from reagents in this treatment, as in the case of treatments involving NaOH and Na_2CO_3, which add sodium to waste waters.

Table 8. Analysis of waste waters from various stages during treatments of lees

Sample	**NH4-N mg/L**	**NO3-N**	**TKN**	**Al**	**B**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**S**	**Zn**
Wine supernatant	59.9	0.7	1650	<0.2	4.2	34.5	0.4	7.8	788	87.3	2.0	25.3	476	315	0.9
1st water wash	7.2	0.1	280	0.9	0.8	46.5	1.0	1.2	759	9.8	0.2	2.0	33.4	24.4	<0.1
2nd water wash	10.7	0.0	270	5.5	0.7	29.8	0.6	6.2	1986	0.7	<0.1	1221	40.9	20.3	<0.1
3rd water wash	30.8	0.3	130	1.4	0.5	10.3	0.3	2.8	938	<0.1	<0.1	558	20.4	10.2	<0.1
4th water wash	13.0	0.0	110	2.8	0.5	41.9	0.4	8.0	639	2.3	<0.1	342	13.9	5.8	<0.1
1st H_2O_2 treatment	157	0.9	4700	9.8	8.2	123	7.3	15.7	1175	140	3.4	40.7	782	512	1.8
2nd H_2O_2 treatment	145	0.9	4400	11.2	7.6	166	0.4	20.9	1269	123	3.0	36.5	659	449	1.6
3rd H_2O_2 treatment	136	0.7	3100	15.0	5.5	155	0.1	46.6	1583	90.0	2.2	23.5	429	300	1.1
4th H_2O_2 treatment	87.2	0.5	1860	17.0	3.5	133	0.3	26.3	1264	58.6	1.4	13.6	257	181	0.6
From NaOH to pH 9	16.2	0.1	550	14.0	1.6	61.4	1.4	23.2	4761	2.7	<0.1	3035	95.2	48.5	<0.1
From 2% Na_2CO_3	1.0	0.1	36	33.4	0.1	0.3	0.5	38.6	202	0.8	<0.1	7240	8.2	5.6	<0.1

Separation of bentonite from other wastes

Fig 13 shows that the fine (<2µm) fraction of both the lees (L3, Table 1) in (a), and also a residue from distillation in (b) show broad peaks at higher spacings (~14 and ~18 Å respectively) for bentonite-protein complexes. In contrast, the coarse fractions in each case show a dominance of sharp peaks, mainly for primary minerals such as quartz (at ~3.3 and ~4.2 Å), and mica (at ~9.9 and ~5 Å) in the lees (Fig. 13a), and for salts, possibly tartrates in the distillery residue (Fig. 13b).

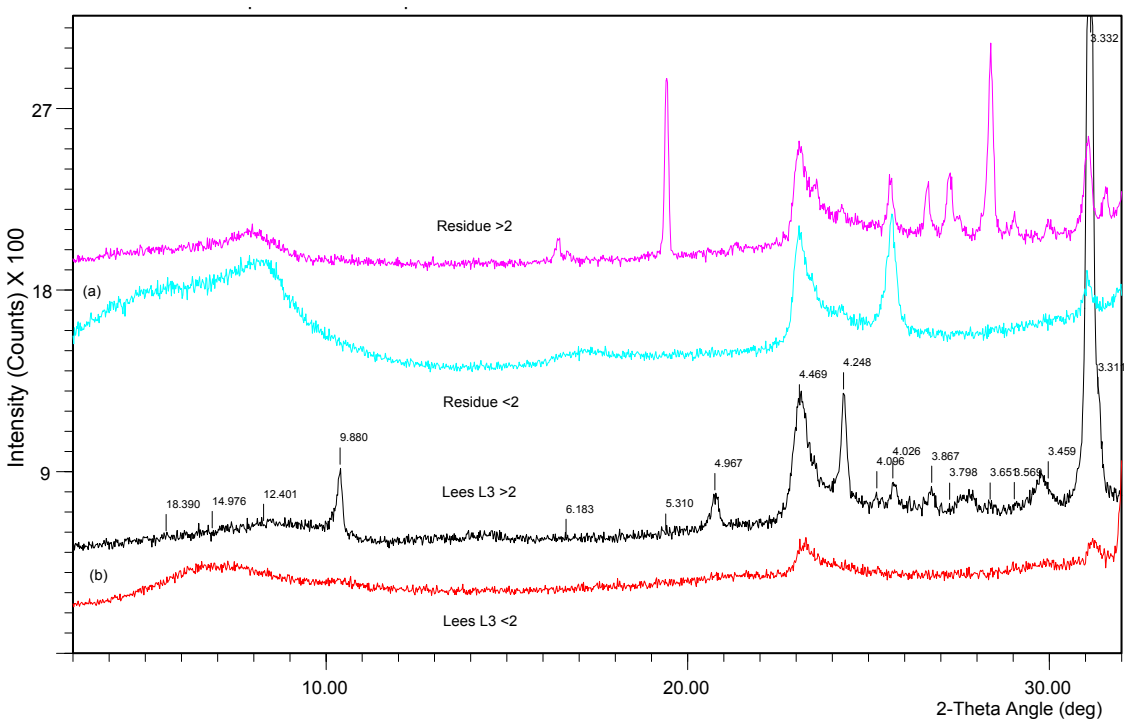


Fig. 13. XRD patterns of unoriented, oven-dried samples of the >2 µ and < 2 µm fractions of (a) Lees L3 and (b) a distillation residue. The pattern from the first sample removed by dispersion is shown for the fine fraction in each case while the coarse fractions are represented by the material remaining following exhaustive removal of fine material.

Discussion of procedures developed with industry

Following the presentation of the results to the Interwinery Environment Committee of the SA Wine and Brandy Association, most interest was shown in the feasibility of the procedure using UV/photo-oxidation , or something similar. There was interest in the way that the waste would be exposed to the UV radiation. This could be done by shining the lamp through quartz glass sleeving to isolate the waste from the lamp. However, quartz glass is fragile and expensive and alternatives exist. These include

AFP tubing, a trade-marked product of Ultraviolet Technologies of Australasia (see "Determination of economic feasibility of favoured procedures" below). In either case, the waste would be pumped past the radiation beam top as to obtain an even exposure of its components.

There was also interest in the possibility of using ozone for oxidation. Its usefulness would depend, in the first place, on its economic feasibility.

Another possibility raised at the meeting was the use of chlorine gas. The same economic considerations would apply, but there may also be a possibility in this case that Cl would degrade the bentonite in a similar way to NaOCl.

A distillery view of the procedures considered was that they would be useful if they also dealt with diatomaceous earths, clearly a major component of distillery wastes and hence a major problem for its disposal. Since diatomaceous earth, as an uncharged siliceous material would not bond chemically with proteins, it would require less severe treatments than bentonite to remove associated protein. Hence the procedures proposed should be even more effective for cleaning up wastes containing diatomaceous earth than those containing bentonite.

The procedures were thought to be useful for distilleries insofar as they decreased the BOD (biological oxygen demand) of wastes.

Discussions with an international consultant remain to be held when he next visits Adelaide (within November 1999).. However, he had already expressed the view that there was a pressing need for procedures to minimise the solid waste produced by the wine industry world-wide.

Determination of economic feasibility of favoured procedures

Although it is impossible to give a meaningful costing for as yet undeveloped processes, background information was obtained as follows:

1. For the hydrogen peroxide-based procedure, hydrogen peroxide costs $89.25 for 25 kg (~22 litres) of 30% hydrogen peroxide (~ "100 volume", as used in experiments herein) (quotation from Chem Supplies Ltd). Clearly there would also be capital costs associated with reaction vessels etc.
2. For the UV/photo-oxidation-based procedure, a standard ultraviolet lamp for the purpose would cost between $91 and $134 and would be expected to last for one year. This quotation came from Ultraviolet Technology of Australasia Pty Ltd, Glynde, South Australia, who are specialists in the provision of this technique for industrial use. More information can be obtained from their web page (www.uvta.com.au). More information would need to be sought regarding pumps, sleeving materials and reaction vessels, at least.

DISCUSSION OF RESULTS IN RELATION TO OBJECTIVE

The results of this study are summarised in Table 9

Table 9.a. Results of treatments of bentonite-protein complexes

Treatment	Effect on protein	Effect on bentonite
2. Displacement by alkalis and salts	Only partial displacement	Undetermined
2. Hydrochloric acid	Some destruction	Undetermined
3. Sulphuric acid	Eventual destruction	Decreased charge, eventual destruction
4. Sodium hypochlorite	Destruction	Destruction
5.Hydrogen peroxide	Largely destroyed	None detected
6. Hydrogen peroxide, then sodium carbonate	Largely destroyed, and removed	Apparently positive – bentonite reacts with protein
7. Ultraviolet/photo-oxidation	Largely destroyed	None apparent

b. Results of trials of other methods of use of lees

Use	Effectiveness
1 In plant growth media	Toxic to the plants; ineffective
2. For Composting	Nitrogen mineralised, so potentially useful

The protein in the complex was never released to any substantial extent using Na_2CO_3. No more protein was released by any of the other alkalis of salts used than was released by Na_2CO_3 treatments. In general, it can be concluded that protein can not be displaced without the prior alteration of either the protein or the bentonite.

Hot concentrated HCl effected considerable breakdown of the bentonite but did not appear to remove a great deal of the complexed protein. Hot concentrated H_2SO_4 was more promising insofar as it was more destructive of protein and other organic material in the complexes. At the same time, it removed constituent inorganic elements from the bentonite at a slower rate than HCl. However, the product retained considerable organic material.

Strong oxidising agent of the type commonly used to destroy organic matter in soils in laboratories were tested in order to remove the organic matter remaining after acid treatment. NaOCl, which is available commercially in bleaching agents, was effective in largely, if not completely, destroying protein. However, it led to an unknown product, which while highly siliceous, also contained a chloride compound.

Hydrogen peroxide H_2O_2, also used for destroying organic matter in soils in laboratories, was also tested. It brought about considerable reductions in the contents of both Na and C in lees, although some of each of C and N remained after treatment. However, characterisation of the product of the peroxide treatment by infrared spectroscopy indicated that there was little or no protein remaining following peroxidation and XRD suggested that the residual material was not held strongly within clay mineral structures. XRD, and also a high CEC, also indicated that interlayers were also free of intrercalated hydroxides. Further treatment with sodium carbonate reduced N content in particular.

Although not completely identical, the products from peroxidation treatments were more similar in composition to the original bentonite than those from acid treatments**.** They appeared to have an even higher CEC than the original bentonite**.** The trial for protein stabilisation of wine showed that it was more efficient for this purpose than the original bentonite. Nonetheless, further refinement of the treatment needs to be carried out to remove the undesirable traits of colour and smell, which is undoubtedly associated with oxidation products of the protein and organic matter.

A photo-oxidation treatment with ultraviolet radiation was shown to produce a product with the lowest C content and also with an N content that was lower than that obtained with peroxide treatments while retaining the XRD characteristics of the montmorillonite clay in bentonite. This treatment involves no additions of chemivcals so is likely to lead to a lower contribution of polluting nutrients to wastewater than any of the other treatments. It offers the best prospects for recycling bentonite but requires further investigation.

Since it are likely to form only a physical bond with organic wastes, removal and recycling of diatomaceous earth from winery and distillery wastes using the same procedures should produce a clean product more easily than with bentonite.

Biological solutions were also trialled. Lees proved to be an unsuitable medium for plant growth. Composting may be a suitable method of disposing of lees, and may produce useable nutrients for plant growth, but it does not bring about recycling of the bentonite.

IMPLICATIONS AND RECOMMENDATIONS

Recycling bentonite in winery and distillery wastes requires destruction of the protein. The process is shown in Fig. 14.

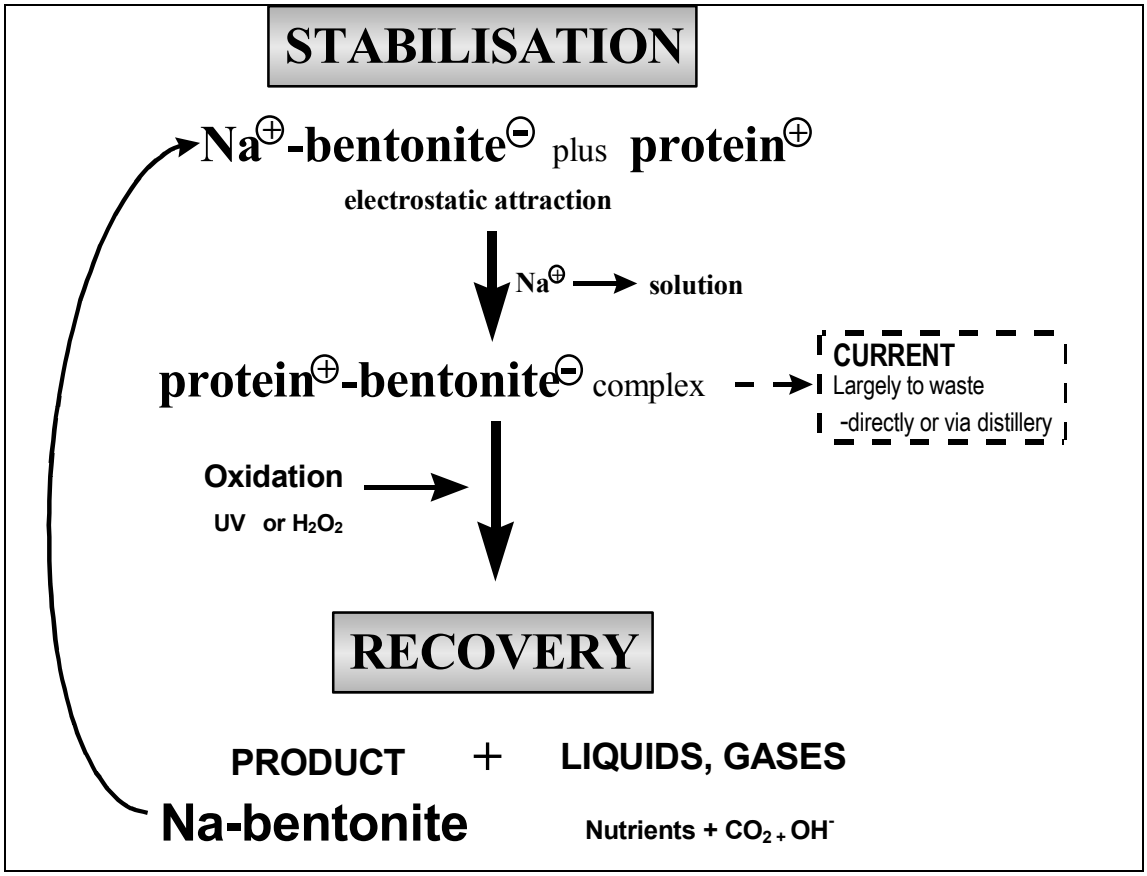


Fig. 14. Proposed scheme for recycling bentonite from winery and distillery residues and preventing solid waste

However, this study was a feasibility study at laboratory scale only. A study would be required at pilot scale in order to determine the economic and practical feasibility of

the procedures which were found to be most viable. Photo-oxidation using ultraviolet radiation showed most promise. This requires even more laboratory work as it was a late choice for study in this project. Peroxidation may also merit further study, especially at pilot scale.

Description of Intellectual Property arising from the research

As a laboratory feasibility study, this project has not developed any intellectual property..

Technical summary of information developed as a part of the research

- Prior destruction of the associated protein is essential if bentonite is to be recycled.
- Treatment with hydrogen peroxide, followed by displacement of the products of the treatment with sodium carbonate was effective in producing a recycled bentonite that was as effective as the original bentonite for removing proteins from wine.
- Photo-oxidation with ultraviolet radiation was most effective for removing carbon and nitrogen from lees that contained bentonite without apparently degrading the bentonite

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