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CONTINUOUS COUNTERCURRENT ION EXCHANGE RECOVERY OF CATALASE FROM BEEF LIVER EXTRACT

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INTRODUCTION

When the ion exchange cellulose "Protion"[1] was used on a pilot plant scale to produce a re-usable water from a proteinaceous treated meatworks effluent[2], it withstood such industrial handling without excessive degradation. A desire to exploit this characteristic of "Protion", coupled with a wish to overcome problems like the blockage of downflow resin beds by fine suspended solids encountered in the meatworks effluent project, suggested using "Protion" in fluidised beds. However, since the introduction of batch fluidised bed processes seemed reasonably straightforward, it was decided instead to develop a truly continuous process in which "Protion" might have an even greater advantage over more fragile sorbents. Since continuous systems require a greater level of instrumentation and engineering skill than batch processes, it was later decided to apply the technique, not for effluent treatment but for the extraction of enzymes from protein

broths. It was thought that the greater product value in this type of system would better offset the higher instrumentation costs.

If a proteinaceous broth containing a required enzyme or protein is passed batchwise through a sorbent bed, a pH and ionic strength may usually be chosen so that the enzyme is adsorbed on the bed while some of the accompanying protein and non-protein materials are not. When the resin becomes exhausted it may be eluted at a different pH or ionic strength so that the required enzyme is desorbed. The specific activity of the enzyme in the eluant with respect to unwanted protein and non-protein impurities will be higher than it was in the original broth. After desorption of the enzyme the resin bed may be regenerated and washed so that it may be used again.

This paper describes a bench-scale apparatus developed to carry out these essential operations of sorption, desorption, regeneration and wash in a truly continuous, fluidised bed system. The efficiency of the equipment was evaluated for the extraction of catalase from a beef liver extract. A major problem was the achievement of reliable resin circulation under conditions in which the ionic strength in each operational stage could be monitored and adjusted for maximum efficiency. Although several design types were investigated only one gave promising results with a catalase system. This was a single multi-purpose column with one fluidised bed per stage, and is the only column described in this paper. Large specific activity increases cannot be expected since chromatographic separation does not occur - fluidised beds are one theoretical separation plate systems. Any application will be for an initial extraction not a final purification, unless an affinity resin is used. Potential advantages include:

- reduced problems with suspended solids in the broth
- continuous processing
- shorter process times which may give higher yields of easily denatured enzymes

- possible by-passing of the usual preliminary organic solvent or ammonium sulphate precipitations which often require a large capacity for high speed centrifuging.

The apparatus is referred to as CCCIX (a continuous counter-current ion exchange) equipment.

EXPERIMENTAL

Equipment

Column Design:

The column had a rectangular cross-section and consisted of six identical units of the type shown in Fig. 1. The assembled column, made from 6 and 12 mm perspex sheet was 600 mm high with internal cross-section 150 x 21 mm. Each tray unit was 65 mm high and contained a stainless steel gauze tray with slope 1° leading to a stainless steel downcomer plate 5 mm from the end wall with its bottom 5 mm above the gauze below. A stainless steel plate 12 mm underneath and parallel to the gauze formed a sealed compartment into which fitted a 6 mm dia. stainless steel inlet tube with approximately 20 spaced 0.5 mm dia. outlet holes facing away from the gauze. Underneath the plate was a second tube, the outlet for the unit below. This had a continuous slot the full length of the tube rather than small holes which would be more easily blocked by small resin particles.

For ease of assembly and disassembly the column sections were stuck together using Dow-Corning "Selastic" 732 RTV silicone rubber adhesive-sealant.

The resin, after descending the column, was air lifted and returned to the top unit. The control lift-water line added fluid to prevent resin clogging the base of the column. It was found to give control over the liquid flow through the downcomers as is discussed later.

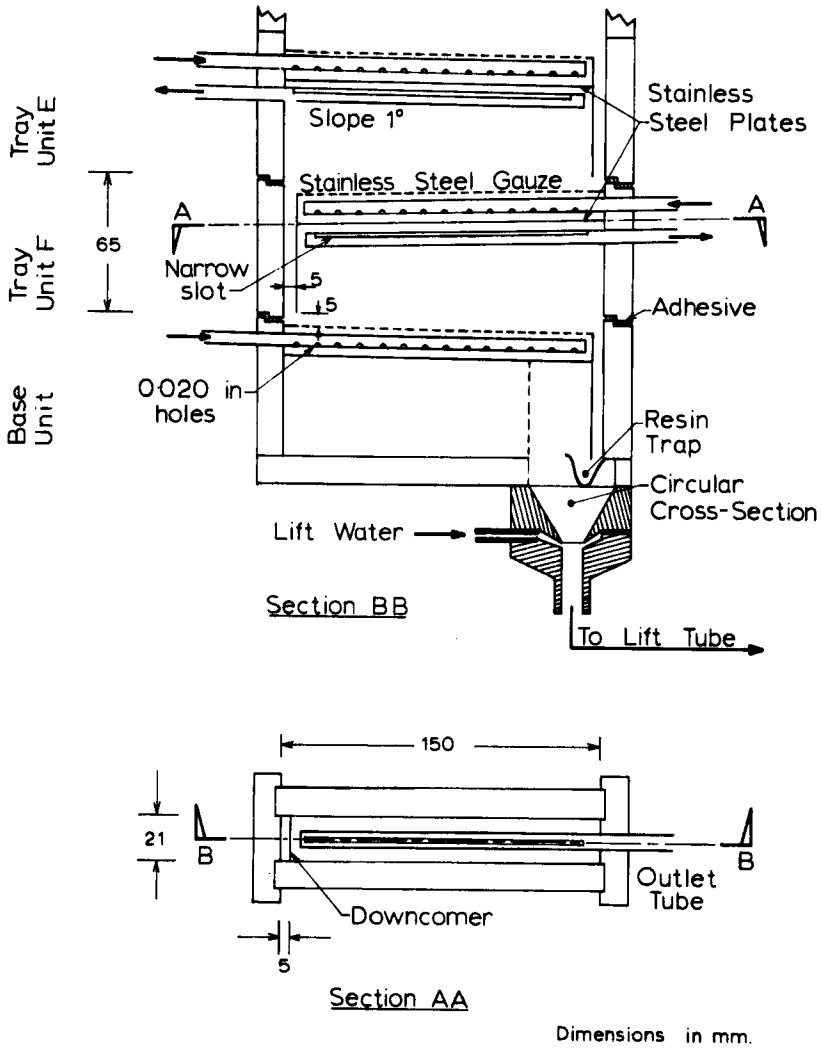

FIGURE 1
Standard Column Units

Flow Control:

The complete apparatus outlined in Fig. 2 consisted of 6 stages (A-F) which, from the top, were wash, sorption, wash, enzyme desorption, regeneration and wash. The resin on the trays of all stages was fluidised by a base buffer which was 0.022 M, pH 7.0 phosphate containing 10^{-4} M ethylene diamine tetra-acetic acid (EDTA). In addition, stage D buffer was 0.25 M in NaCl and stage E was 0.5 M in NaCl. The lift water control line could be fed by base buffer although tap water was usually used. Two wash stages were necessary to remove sufficient NaCl to allow the resin to be re-used effectively.

Each inlet and outlet tube was fitted with a flow meter and an inverted 300 ml bottle pulse suppressor. The inlet suppressors had rubber-tube/screw-clip connectors to control the inlet pulse height.

Ideally, the 12 pump lines would all derive from the same pump and have individual flow rate adjustment. In this investigation lines were selected from two Ismatec 4 channel mp-ge pumps and one 8 channel Masterflex pump, all being peristaltic pumps. Resin movement was greatly assisted by pulsation. Best operation was obtained when the pump pulses in the column outlet line were very largely isolated from the column by the pulse suppressors. The pulses for the column inlets were brought into phase and deliberately sharpened by feeding pump flows through a 6 channel rotary valve which was made in this Laboratory. Optimum pulse heights for each stage could be obtained by means of the pulse height adjustment clamps. Beef liver extract was pumped into the stage B inlet line using a Milton Roy Model 196-26 Instrument Mini Pump.

Materials

Ion Exchange Resin:

The resin used was a diethyl amino ethyl (DEAE) ion exchange cellulose manufactured under the trade name "Protion" by Tasman Vaccine Laboratory, Upper Hutt, New Zealand. This material

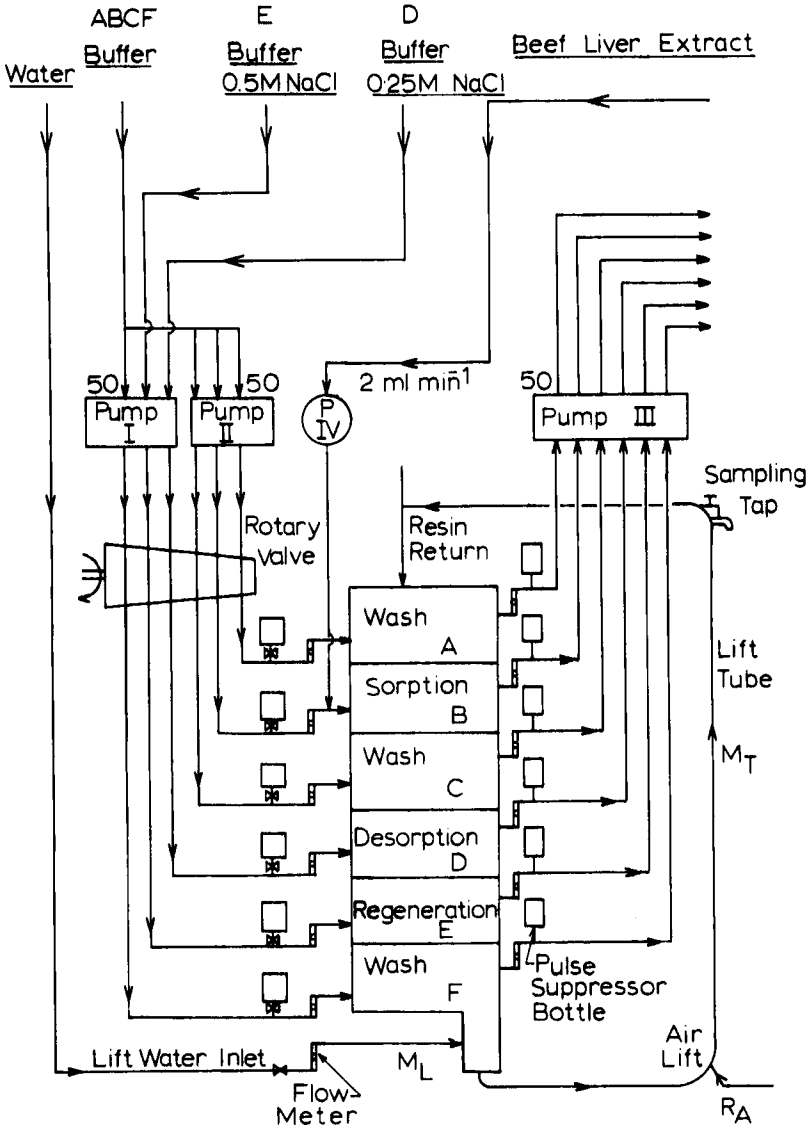

FIGURE 2
General Layout of Process Equipment

was made from 75-150 μm mesh viscose granules. In order to increase the capacity for catalase (molecular weight 250 000) the commercial resin was expanded by Dr. J. Ayers (Massey University, Palmerston North, New Zealand) to allow entry of larger protein molecules. The density of the swollen resin grains, found by specific gravity bottle measurements, was 1.04-1.05 g cm^{-3}, whereas the density of the unexpanded resin grains was about 1.13 g cm^{-3}. Before use the resin was acidified to pH 3, evacuated for 1 hour on a water pump to remove CO_2, washed, conditioned in 0.5 M NaOH, then washed again. This cycle was repeated before conditioning in 0.25 M pH 7 phosphate buffer.

Beef Liver Extract (BLE):

The beef liver extract was prepared for us by Dr. G. Midwinter (Massey University). Beef liver (2.3 kg) was macerated in a 4.5 litre Waring blender with 2.3 litres of buffer for 10 secs. The supernatant was centrifuged at 5000 rpm for 10 min and any lipid filtered off on glass wool. The extract, mostly protein but said to contain bile pigments and some mitochondria, contained about 10 g l^{-1} total protein. It was split into about 250 ml portions and stored in plastic bottles in the deep freeze until needed.

Criteria for Evaluating Equipment Performance

Two parameters were used to compare the quality of the output stream from the catalase desorption section in different experiments.

(i) Y - the yield of catalase is the ratio of the activity of the catalase ejected per minute in the output stream to the activity injected per minute into the column, and

(ii) Z - the specific activity increase of catalase is the ratio of the value of the activity of catalase per unit amount of total protein in the output to the value of the same parameter in the enzyme input stream. Z is thus a measure of the degree of purification of the

enzyme from other proteins. The specific activity would more meaningfully be related to the total mass of non-catalase material, but this would probably require gravimetric analyses which would be too time consuming for routine analysis.

Analytical Methods

Catalase:

Based on accepted definition of enzyme activity, one unit (U) of catalase was taken as that amount which catalyses the decomposition of 1 μmole of hydrogen peroxide per minute under standard conditions. Activities were determined using a conventional technique in which hydrogen peroxide concentrations were calculated from optical density measurements at 240 nm in 10 mm silica cells.

Total Protein:

Direct spectrophotometry at 280 nm enabled rapid analysis of large numbers of samples per day. Absolute values for protein as mass per unit volume were not calculated, only optical densities relative to those of diluted stock beef liver extract (BLE). Interference by low molecular weight non-protein compounds was not significant.

NaCl Concentration in Outlet Lines:

Conductivities were measured using a Philips Conductivity Measuring Bridge. Calibration curves were plotted for the base buffer (usually 0.022 M phosphate pH 7.0 with 10^{-4} M EDTA) to which NaCl had been added to make standards from 0.0 - 0.5 M in NaCl.

Circulation Rates of Resin and Associated Liquids

In order to understand and control resin circulation and salt gradients within the column it is desirable to quantify as far as possible the flows of resin and liquid. These flowrates were

obtained by collecting in a measuring cylinder a timed sample from a sampling valve at the top of the lift tube. The flowrates were calculated from the total volume V_T and the volume of resin V_R which had settled in the cylinder after 3 minutes settling time.

$$\text{Total flowrate} \quad M_T = \frac{V_T \rho_T}{t}$$

$$\text{Resin flowrate} \quad M_R = \frac{V_R \rho_R}{t}$$

where t is the sampling time

ρ_T is the mean density of the total resin/liquid mixture

ρ_R is the mean density of the settled resin volume

The density of all mixtures of resin and liquid was taken to be constant for the purposes of this investigation. This was a reasonable assumption as the density of individual wet resin grains was 1.04 - 1.05 g cm^{-3} and that of the most dense buffer about 1.02 g cm^{-3}.

If M_L was the injected lift water flow rate then the flowrate from the bottom downcomer M_D was calculated from the mass balance

$$M_D = M_T - M_L$$

The flow within the other downcomers in the column will equal M_D if there is no nett transfer of liquid from the slurry to the fluidising liquid in each stage. The 'thickness' of the slurry on the downcomers can be described by the ratio M_R/M_D which cannot exceed unity - the thickness of settled resin.

Although the flowrate of water associated with the resin in the downcomers is most conveniently defined as

$$M_W = M_D - M_R$$

it is in reality greater than this by the amount of water between the grains in settled resin. This amount is equal to $\frac{\nu_w V_R}{t}$

where ν_w is the volume fraction of free water in settled resin, about 0.66. Similarly the true resin flowrate is

$$M'_R = \frac{V_R \rho_{BR}}{t}$$

where ρ_{BR} is the bulk density of resin in a settled resin bed i.e. 1.04 (1-0.66).

RESULTS

Catalase Extractions - Batch Type Fluidised Beds

The maximum efficiency which could be expected for the continuous process would be that of a batch type fluidised bed process. This was assessed by tests using the adsorption cell sketched in Fig. 3. The cell was charged with 4 ml resin and and successive 20 ml aliquots of aqueous solutions as described below. The slurry was fluidised with nitrogen and after each treatment as much liquid as possible was removed from the resin via the bottom tap. The performance of the fluidised bed was

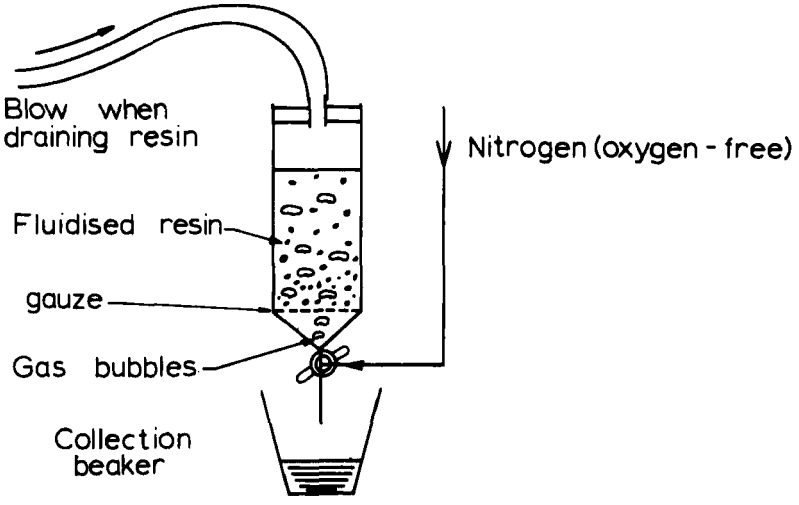

FIGURE 3
Batch Fluidised Bed Extraction Cell. Cross-sectional area 4 cm^2

assessed from determinations of enzyme and total protein in the following five solutions (A-E).

Solution A consisted of three bulked aliquots from the test cell - the first of base buffer to flush the bed, the second from a timed adsorption of beef liver extract, and the third again of base buffer to flush the bed.

B consisted of three bulked aliquots of catalase desorption buffer, the first to condition the resin, the second for the timed desorption, the third to flush the bed.

C consisted of three bulked aliquots of resin regeneration buffer applied in the same way as for solution B.

DE both consisted of three bulked aliquots of base buffer applied as above to prepare the bed for the next experiment.

Material balance studies from 18 experiments gave

Catalase recoveries	101 ± 7%
Total protein recoveries	89 ± 9%

The results of experiments to optimise some extraction parameters are shown in Fig. 4. The values given refer to the catalase recovered in the desorption sample. Table I shows the much greater efficiency of the fine expanded resins. Values are means for two experiments. Table II shows the necessity for at least 2 minutes broth/resin contact time. In some experiments more than 90% of the catalase in the extract was adsorbed on the resin. These experiments enables a suitable selection of conditions for operating the CCCIX apparatus to be made, and indicated that the best performance which could be hoped for would be a yield of about 70% of the available catalase with a specific activity increase of not more than about 3.6 x.

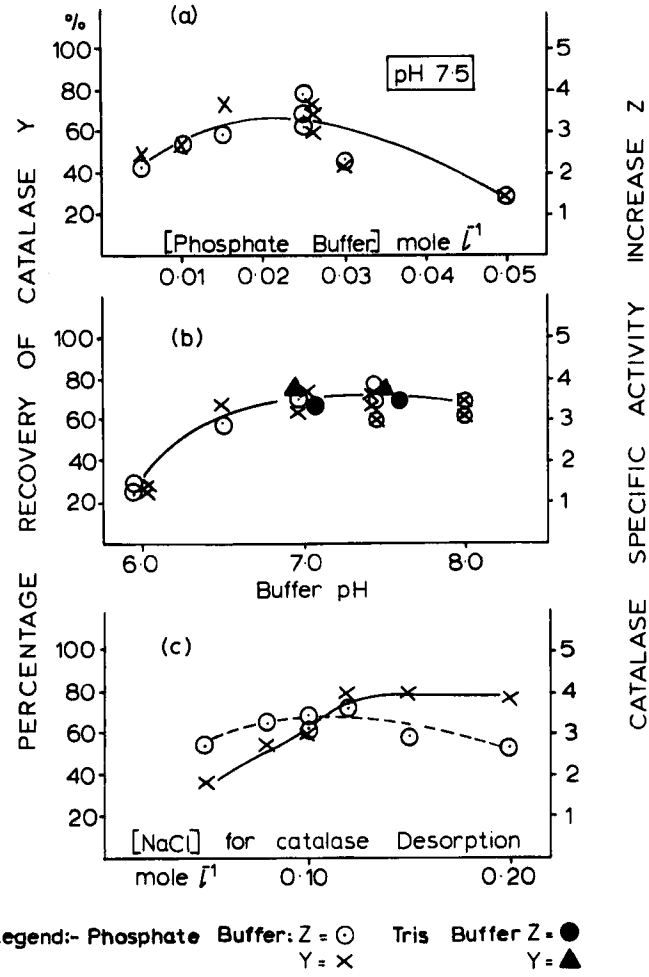

FIGURE 4
Batch Fluidisation Extraction Experiments. Optimisation of extraction conditions (a) Base buffer concentration (b) Buffer pH and (c) Concentration of NaCl for catalase desorption.

Catalase Extraction - CCCIX Equipment

Altogether 54 extraction experiments were carried out during which time many changes were made to experimental variables including - column design and resin type; values of

TABLE I

Batch Fluidised Bed Experiments - Effect of Resin Type

The resin mesh size given below refers to the grade of dry viscose granules used to make the "Protion" resin, and not to the size of the resin or expanded resin particles.

Resin Type	Catalase Yield %	Specific Activity Increase
150-320 μm, not expanded	44	1.8
150-320 expanded	58	2.6
75-150 expanded	62	3.7

TABLE II

Batch Fluidised Bed Experiments - Effect of Broth/Resin Contact Time

Contact time per stage (min)	1	2	4
Catalase recovery %	39	61	69
Specific activity increase Z	2.5	3.7	3.6

air, pump and lift water input flow rates; pulse size, stage and phase; stop/start operation; number of sorption trays and sorption stages; and recycling of the output from the sorption stage. Initially there was no method of predicting whether or not any particular experiment was likely to give a satisfactory result. Eventually it was found that once resin circulation appeared uniform, then a satisfactory result was likely if the following conditions, which could be monitored prior to injecting the liver extract, were obtained

(i) the resin circulation rate was greater than about 10 ml min^{-1}

(ii) the conductivity of the sorption stage outlet was equivalent to a NaCl concentration of less than 0.02 mole $litre^{-1}$ added to the base buffer

(iii) the conductivity of the enzyme desorption stage outlet was equivalent to an NaCl concentration of greater than 0.12 mole $litre^{-1}$ added to the base buffer.

Typical of the best results usually obtainable with the CCCIX equipment are those of Expt. K29 shown in Table III. Although specific activity increases of 3-4 (i.e. comparable with the batch process results) were obtained, catalase recoveries in the desorption stage were not greater than about 15%. The reason

TABLE III

Continuous Extraction Experiments - Output Analyses

Expt K29	Continuous operation	0.5 ml min^{-1} BLE
Expt K47	20:80 sec go:stop with 50% recycle of sorption stage	0.4 ml min^{-1} BLE average flow

	Expt. K29			Expt. K47		
	Protein Recovery %	Catalase Recovery %	Sp.Act. Inc. x	Protein Recovery %	Catalase Recovery %	Sp.Act. Inc. x
Wash	0	-	-	1	-	-
sorption	64	62	0.95	24	12	0.5
wash	12	12	0.95	46	18	0.4
desorption	4	13	3.3	14	52	3.8
regeneration	3	3	1.1	7	14	2.0
wash				-	-	-
Total	83	89		91	98	

is simply that the broth/resin contact time, probably less than 1 minute, was too short. The contact time could not be increased by altering running conditions without causing a breakdown in the uniformity of the resin circulation. It was concluded that this could be done only be changing the dimensions of the column. However short term operation with longer broth/resin contact times was obtained by recycling 50% of the output from the sorption stage or by placing the whole column on a 20:80 second go:stop cycle. The results, typified by Expt. K47 in which both modifications were used, are given in Table III. The catalase recovery increased to 50% while maintaining a specific activity increase of 3.5 to 4. The variation of yields with time over a 4 hour period is shown in Table IV.

TABLE IV

Continuous Extraction Experiments - Time Stability of Extractions

Expt. K47 - 20:80 sec go:stop with 50% recycle of output from the sorption stage

0.4 ml min^{-1} BLE averaged flow.

Time after start hrs	MATERIALS BALANCE		DESORPTION OUTPUT	
	Protein %	Catalase %	Catalase % Recovery	Spec. Act. Increase
1.0	65	54	24	1.9
1.5	72	71	39	3.4
2.0	81	90	49	4.0
2.5	91	105	58	4.0
3.0	92	98	52	3.8
3.5	94	91	43	3.4
4.0	83	88	46	3.6

Comparison with Alternative Systems

Any final assessment of CCCIX applied to enzyme extraction would, of course, be economic. However, at this stage, there are a few comparisons of the technical performance of different methods which are interesting. These are summarised in Table V for the following systems, scaled for 150 ml resin, as used in the CCCIX process.

(i) The CCCIX process as described in this paper.

(ii) A batch reverse flow (BRFIX) system using the same resin and buffer system. The experiments were carried out in the apparatus described in Fig. 3, 1 cycle being achieved every 30 minutes.

(iii) A batch downflow (BDFIX) system using a 50 ml bed (50 mm x 10 cm^2 cross-section) of the same resin eluted at 20 ml min^{-1} with

200 ml beef liver extract diluted 5 x in base buffer

250 ml base buffer (0.0225 M; pH 7.0 phosphate; 10^{-4} M EDTA)

250 ml base buffer, 0.25 M NaCl

250 ml base buffer, 0.50 M NaCl

500 ml base buffer

Cycle time was 2 hours.

(iv) Precipitation processes, supplied by Dr J Ayers of Massey University, Palmerston North, New Zealand.

The table indicates that the CCCIX process

(a) gives specific activity increases comparable with the fluidised bed batch process

(b) gives low yields of only 15% but the reasons for this have been discussed above

(c) gives lower degrees of purification, less concentrated output streams and uses more buffer than the downflow batch process

(d) gives a product comparable in quality with that from a single stage acetone precipitation but not as good as that from a two stage precipitation.

TABLE V

COMPARISON OF SOME CATALASE EXTRACTION PROCESSES
(normalised for systems containing 150 ml resin)

Process	Process Rate $ml\ (BLE)\ hr^{-1}$	% Yield Catalase	Spec.Act. Inc. Z	Output [Cat.] $Ul^{-1} \times 10^5$	Total Buffer Use ml/ml (BLE)	Cat.Extr. Time hr
CCCIX	60 (a)	15	3 - 4	0.7	300	0.2
continuous	24 (b)	45	3 - 4	1.0	150	1.0
BRFIX batch reverse flow	30	90	4	0.1 (c)	670 (c)	0.5
BDFIX	60	25	30	30	40	1.5
batch down flow		75	10	10	40	1.5
Precipitation						
1 stage (d)		48	3			(d)
2 stages (e)		30	14			(e)

Notes: (a) normal operation without attempt to increase contact time.

(b) contact time increased by recycle plus 80% stop cycle.

(c) no attempt made to minimise buffer use - probably could increase output catalase conc. and reduce total buffer use at least 5 x to make them comparable with CCCIX values.

(d) acetone precipitation - time not given, probably 1 - 2 days.

(e) acetone precipitation followed by ammonium sulphate precipitation - time probably several days.

Control of Resin Circulation and Salt Gradient

Control of resin circulation and the salt concentration in different stages was critical for the successful recovery of catalase. Two main factors seemed to govern the resin circulation characteristics. These were the rate at which the resin descended the column, and the rate at which it was removed by the air lift at the bottom. An additional control affecting mainly the salt gradient was the rate of lift water injection at the base of the column as shown in Fig. 2.

Resin was made to move through the column mainly by hydraulic pulses applied to the column. Since pulse irregularities could adversely affect resin circulation, peristaltic pump pulses in column outlet lines were suppressed by the inverted bottle system described earlier. The inlet lines were led to the rotary valve shown so that pulses to the different stages could be synchronised or, although this was not investigated, sequenced, depending on the valve design. Control of both pulse frequency (by speed of rotation of the valve) and pulse height, (by adjustment of the efficiency of the pulse suppressor bottles) was necessary. The sharply peaked pulses obtainable using the rotary valve appeared considerably more effective in fluidising and moving the resin than when non-pulsed flows or flows pulsed by the peristaltic pumps were used.

Having achieved uniform resin movement the next task prior to injecting the beef liver extract was to achieve the salt concentration limits defined earlier. This combination of low salt concentration in the sorption stage and high concentration in the desorption and regeneration stages required minimisation of "interstage liquid leakage" caused by back diffusion or channelling of liquid from a lower stage to a higher one, and by forward movement of excessive amounts of liquid in association with the resin. Thus the resin slurry moving down through the downcomers should be as thick as possible without clogging the column. The thickness of the slurry supplied to the top

of the downcomer depended on the flow rate of fluidising liquid, together with the pulse characteristics of that flow. A minimum flow rate on this equipment of 40 - 50 ml min^{-1} per stage was necessary to avoid stagnation in regions of "dead" resin on the tray gauzes. Further control of slurry thickness was achieved by the lift water injection rate. Accepting that the air lift injection rate controlled the total transfer of resin plus liquid up the lift tube, the thickness of resin slurry descending the downcomers could be increased by increasing the fluid injection rate at the base of the column.

The effects of the above controls are illustrated in Figs 5 and 6. When the injected lift water flow rate M_L is zero the measured total flow up the lift tube M_T will equal M_D, the flow from the downcomer of the last stage. This will equal the flow in each of the other downcomers only if there is perfect hydraulic balance between all stages. Fig. 5 shows the effect of the column control parameters R_A and M_L on the calculated

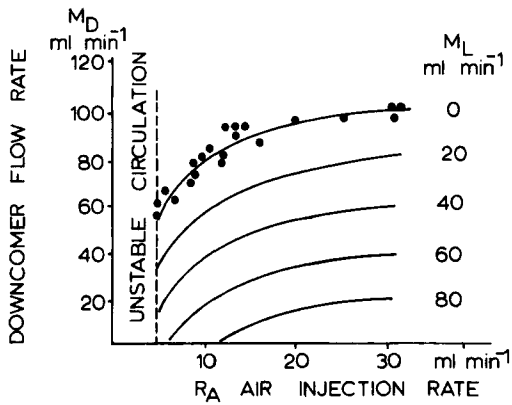

FIGURE 5

Effect of Injected Air and Lift Water Flow Rates on Downcomer Flow Rate. The downcomer flow rate M_D is the measured value M_T (total flow rate) when M_L (the lift water flow rate) is zero, otherwise it is calculated from the relationship $M_D = M_T - M_L$.

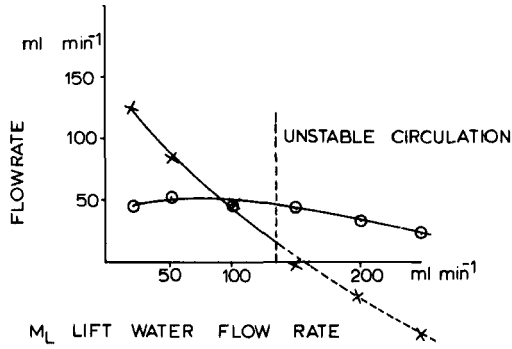

FIGURE 6
Effect of the Lift Water Flow Rate on the Composition of the Downcomer Slurry. These results were obtained with an earlier circular cross-section column and illustrate the general behaviour of CCCIX columns although the absolute values do not apply to the equipment described in this paper. Flow rate of water associated with the resin in the downcomers was calculated from the balance relationship $M_W = M_T - M_L - M_R$. Legend ⊙ M_R Flowrate of resin • × M_W Flowrate of water associated with resin in the downcomers.

downcomer slurry flow rate M_D. Fig. 6 shows the effect of M_L on the individual components M_R and M_W of the downcomer slurry flow rate.

Although the results shown in Fig. 6 were obtained using a circular cross-section column not described in this paper, they illustrate how increasing M_L reduces 'interstage liquid transfer' without reducing resin circulation rates. However when M_L is increased too far M_W approaches zero, channelling of liquid up the downcomers begins, salt concentration gradients deteriorate and resin circulation becomes unstable. An additional factor to be considered is that resin losses via the column output lines increase as M_L increases and also depend on the flow rate of fluidising liquid and on its pulsing characteristics.

Despite careful manipulation of the above factors, it was still found impossible to reproduce at will the required circulation features, and attainment of satisfactory catalase

extractions seemed often to be a matter of chance. The reason for this is that optimum adjustment of M_L requires close matching of the 12 pump lines to maintain a delicate hydraulic balance between stages. This was not possible with the pumps available to us. The 12 lines were distributed over either two or three different peristaltic pumps, the relative speeds of which varied slightly during the day. Furthermore, even on one pump, the smallest flow rate might be 6 to 10% less than the greatest and no method was available for accurately monitoring and matching flows. It is this equipment limitation which finally has caused us to suspend the project at the stage described in this paper. Further work would necessitate better quality pumping equipment or a different equipment design.

DISCUSSION

Substantial reviews of continuous and fluidised bed ion exchange systems were presented in 1969[3,4] with a few others appearing more recently[5,6,7]. The systems considered in these reviews were applied usually to water softening, uranium leaching and effluent purification. The work most closely related to that described in this paper is that of Jones[8], who described a continuous process for the recovery of protein from industrial effluents using the "Vistec" ion exchange cellulose which is very similar to the "Protion" material used for the present work.

Dunnill and Lilly[9] have been developing continuous processes for the extraction and purification of enzymes. They are trying to include all stages of the production, isolation and purification process and to achieve their aim by stringing together commercially available fermentors, homogenisers, filters and other equipments. On the other hand, our work has been centred on a new design of equipment to carry out only one function - the preliminary purification of an enzyme from a proteinaceous broth. Rendell[10] in "The Future of Large Scale

Chromatography" made a comprehensive comparison of many different systems using columns. He concluded that scale-up and continuous output could be best achieved by the staggered operation of multiple columns in parallel; and that systems with flowing solids have problems due to poor packing efficiency and to the practical difficulties of moving solids. Salter[6] noted that it now seems difficult to make a good case in engineering and economic terms for moving packed-bed ion exchange equipment and that to justify continuous systems, it is necessary to examine the prospects of treating unclarified solutions and to minimise capital cost of plant. More attention has been given in recent years to fluidised rather than packed-bed equipment.

The present paper has described the development of one type of continuous fluidised bed equipment, and its application to enzyme extraction from unclarified broths. Given better flow control and some geometry changes it seems likely that a bench scale equipment could be built which would operate reliably giving outputs approaching those attainable by batch fluidised bed techniques. No attempt has been made to investigate ways of decreasing buffer usage, increasing effective resin capacity or optimising tray and downcomer design. This is better done after reliable operation is achieved. The equipment, as described here, could also be adapted for other applications, possibly for continuous processing using enzymes immobilised on particles or resin beds.

If further development of truly continuous fluidised bed processing is desired along the lines investigated here, consideration should be given to the merits of a multiple-column system. In this type it would be more easily possible to optimise the design of each stage individually since many designs for efficient single stage liquid-solid contactors are given in the literature.

GLOSSARY

CCCIX	Continuous counter-current ion exchange
BRFIX	Batch reverse flow ion exchange
BDFIX	Batch down flow ion exchange
BLE	Beef liver extract
Y	yield of catalase - ratio of the activity of the catalase ejected per minute into the output stream to the activity injected per minute into the column
Z	the specific activity increase of catalase - ratio of the value of the activity of catalase per unit amount of total protein in the output, to the value of the same parameter in the input stream
V	volume (ml)
t	time (min)
ρ	density ($g\ ml^{-1}$)
M	mass flow rate ($g\ min^{-1}$)
Subscripts	
D	downcomer slurry
L	lift water
T	total in lift tube
R	'resin' as in a resin bed settled under gravity.
W	water associated with 'resin' in the downcomers

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