



# Recovery of Enzyme Alkaline Protease from Dairy Waste by Fermentation and Membrane Separation

Ajay V Gawali<sup>1</sup>, Miss. Sapna K. Deotale<sup>2</sup>, Tousif Yunus Shaikh<sup>3</sup>

Department of Chemical Engineering, A.E.C, Chikhli, Maharashtra, India<sup>1,2,3</sup>

**Abstract:** Enzyme Alkaline Protease can be produced by microbial fermentation under optimum conditions of Temperature, pH, agitation, and aeration. In this work it is proposed to use microfiltration ultrafiltration membranes as an alternative methodology to carry out fermentation process producing alkaline protease enzyme and as an alternative method for the cell separation and recovery of alkaline protease from the fermentation broth because it has the potential for energy saving and higher purity. A well-known microfiltration technique for cell separation and diafiltration for enzyme recovery is used. Ultra filtration of 20,000 MWCO is used for purification and concentration of enzyme alkaline protease.

**Keywords:** Biotechnology, Microbial fermentation, Membrane sterilization, Ultrafiltration.

## I. INTRODUCTION

The term fermentation has undergone numerous changes in meaning during the past hundred years. Fermentation may be defined, in a broad sense, as a process in which chemical changes are brought about in an organic substrate (e.g. carbohydrate, fat, protein, hydrocarbon, etc.) through the action of the biochemical catalysis known as 'enzymes' produced by specific type of living microorganisms. In general the fermentation process can be expressed by the following biochemical reaction in presence or absence of oxygen

Micro-organisms + Substrate → New Microorganisms + Fermentation Products

Products of fermentations are, in fact the metabolic products formed during the microbial growth.

fermentation temperature (37 °C), aeration rate (1 vvm i.e. volume of air per volume of medium per min), and reaction time (35 hours). All chemicals used for the fermentation were reagent grade purchased from Sigma Chemical Co. Throughout the fermentation process, the following parameters were monitored: pH, agitation (rpm) and temperature (°C). After 35 hours of fermentation, the reaction was stopped and the biomass was harvested, characterized and stored at 4 °C.

C. Downstream Process:

In this work Microfiltration was used to replace the uneconomical Centrifugation, pre-coat filtration methods for separation and recovery of enzyme alkaline protease as well as clarify broth for further processing.

## II. MATERIALS AND METHODS

A. Upstream Process:

In upstream processing inoculum is prepared from Enzyme Alkaline protease producing species of microorganism was obtained from the Dairy waste and stored at 4°C in nutritive medium for few days. The fermentation medium was prepared using double distilled water and had following composition : NaCl, 5gm/L; Beef extract, 3g/L; Peptone, 5g/L. The pH was adjusted to 9. The nutritive media sterilized by keeping the fermenter (covering all ports with aluminium foils) in autoclave for 1.5 hours under 15 psi. pressure.

B. Fermentation Process:

Inoculation was carried out under aseptic condition and fermentation started in 5 liters fermenter ( Inceltek make) with 2.5 L working volume. The following fermentation conditions were used: agitation rate (110 rpm),

## III. RESULT AND DISCUSSION

A. Enzyme Alkaline Protease Preparation by Fermentation Experiment for preparation of Enzyme Alkaline Protease by fermentation was carried out at the controlled temperature of 37 °C, agitation was provided at the rate 110 rpm, aeration at the rate 1 vvm. The fermentation operation was continued for 35 Hrs. and the pH variation during fermentation was from 9-7. The media can be sterilized by using membranes before fermentation starts which have the following advantages 2.5 L broth obtained at the end of fermentation, which 2.5 L broth obtained at the end of fermentation, which was stored at 4 °C for further processing.

B. Primary Isolation of Cells by microfiltration

The plate and frame microfilter module was used for cell separation. The 1600 ml fermented broth was taken for



primary cell separation 1250 ml permeate was collected and 200 ml retentate was collected. The permeate flux Variation during microfiltration is given in Table 1

After every 5 minute the samples from permeate was collected and labeled as  $P_1, P_2, P_3, P_4$  and  $P_5$  and retentate as  $R_1, R_2, R_3, R_4$  and  $R_5$  as shown in the table 3. The sample from initial broth was labeled as  $F_0$  final permeate as  $P_6$

and final retentate as  $R_6$  It is clear from Table 1 that the permeate flow rate decreases with time and it is due to fouling of membrane. The samples collected above ( $P_1$  to  $P_6, R_1$  to  $R_6$  and  $F_0$ ) are stored at  $4^\circ\text{C}$  and thereafter analyzed

For enzyme protease activity measuring the absorbance at 440 nM ( given below) during Enzyme assay.

TABLE 1 MICROFILTRATION OF FERMENTATION BROTH

Sr. No.	Pressure (bar)		Flow rates (ml/sec)		Time min	Permeate volume collected (ml)
	Feed	Retentate	Permeate	Retentate		
1	0.3	0.1	16/15	192/15	0	0
2	0.3	0.1	12/15	190/15	5	300
3	0.3	0.1	10/15	192/15	10	600
4	0.2	0.0	8/15	190/15	15	710
5	0.2	0.0	10/15	64/15	20	1000
6	0.2	0.0	8/15	50/15	25	1250

TABLE 2 PROTEASE ASSAY OF MICROFILTRATION SAMPLES

Samples	Absorbance at 440nM	Samples	Absorbance at 440nM
$F_0$	0.2174	$F_0$	0.2174
$P_1$	0.1664	$R_1$	0.2191
$P_2$	0.2319	$R_2$	0.2251
$P_3$	0.2333	$R_3$	0.2171
$P_4$	0.2218	$R_4$	0.2450
$P_5$	0.2201	$R_5$	0.2281
$P_6$	0.2253	$R_6$	0.2265

Protein estimation was carried out using Lawrys methods. The dilution factor for protein estimation was 10. The protein estimation of various samples is clear that the protein concentration of the feed is 7567.9182 microgram per ml (i.e. 1600 ml feed contains 2508.669 mg of protein) The protein concentration of final permeate is 1261.4541 microgram per ml (ie. 1250 ml of permeate contains 1576.817 mg of protein). And the final retentate concentration is 1312.1591 microgram per ml ( i.e. 200 ml of retentate contains 262.43 mg. of protein) The material balance for protein if considered across microfiltration operation it is clear that 669.42 mg of protein remained in the hold up volume of microfilter. The protein separation efficiency for microfilter is about 63%. At the end of microfiltration the following concentrations were found. Concentration of Permeate,  $C_p$ , ( $P_5$ ) = 1133.1052 microgram / ml. Concentration of feed,  $C_f$ , ( $R_6$ )= 1312.1591 micro gram /ml., Rejection coefficient for the microfilter,  $R = 1 - (C_p/C_f) = 1 - (1133.1052/1312.1591) = 0.14$  (i.e. 14% Rejection of protein by microfiltration) Final retentate is also contain considerable amount of protein in it and need to be diafiltration of retentate again.

#### C. Activity Calculation:

By the same way activity of all samples is given Table 3. That is activity of sample =  $Y/[C \times 150 / (1000 \times 1000)]$  U/mg.

TABLE 3 ENZYME ACTIVITY OF MICROFILTRATION SAMPLES

Samples	Activity (U/mg)	Samples	Activity (U/mg)
$F_0$	0.924	$F_0$	0.924
$P_1$	0.854	$R_1$	0.998
$P_2$	1.180	$R_2$	0.959
$P_3$	1.458	$R_3$	0.942
$P_4$	1.170	$R_4$	1.059
$P_5$	1.295	$R_5$	0.864
$P_6$	1.190	$R_6$	1.150

#### IV. RECOVERY OF ENZYMES BY DIAFILTRATION

Before starting of diafiltration the microfiltration plate was characterized for pure water permeability was considered as a feed and it filtered by adding 1000 ml of distilled water at the rate of permeate.

The various samples like  $P_1, P_2, P_3, P_4, P_5$  form permeate line and  $F_1, F_2, F_3, F_4, F_5$  from feed tank were collected.  $F_1$  considered as initial feed.  $F_6$  as final combined permeate and  $F_5$  as a final retentate collected and labeled. The results are shown in Table 4.



TABLE 4 DIAFILTRATION OF MICROFILTRATION RETENATE

Sr. No.	Feed Pressure (bar)	Retentate Pressure (bar)	Permeate flow rate (ml/sec.)	Time (min)	Permeate volume collected.
1	0.3	0.1	8/5	4	200 (P <sub>1</sub> )
2	0.3	0.1	8/5	7	400 (P <sub>2</sub> )
3	0.3	0.1	6.5/5	10	600 (P <sub>3</sub> )
4	0.3	0.1	6/5	13	800 (P <sub>4</sub> )
5	0.3	0.1	5/5	16	1000 (P <sub>5</sub> )

Protein estimation of above samples was carried out and it is clear that the protein concentration of the feed is 1312.1591 microgram per ml. (i.e. 200 ml feed contains 262.4 mg of protein) The protein concentration of final permeate is 129.1944 microgram per ml (i.e. 1000 ml of permeate contains 129 mg of protein). And the final retentate concentration is 430.0038 microgram per ml (i.e. 200 ml of retentate contain 86 mg. of protein) The material balance for protein if considered across diafiltration operation it is clear that 47.4 mg of protein remained in the hold up volume of the equipment. The protein separation efficiency for diafiltration is about 50%.

The Figure 1 shows the graph of protein concentration in permeate with time of operation concentration in permeate with time of operation. Initially the permeate was more concentrated but after some time the permeate get diluted due to continuous addition of distilled water to feed and there by to permeate.

## V. CONCLUSIONS

Microfiltration along with diafiltration operation has shown good results for the cell separation and enzyme recovery up to 93 % Ultrafiltration of proper MWCO is very effective for concentration and purification of enzyme solution.

In totality the membrane technique can made operation economical due to repeated use of membranes resulting in higher product recovery due to minimum hold up volume and elimination of contamination. It consumes less power, has low chemical and labour costs and is easy and practical to handle. Thus it has helped the process to achieve higher productivity, making it more reliable and economical.

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