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PARTITIONING AND PURIFICATION OF CARBOXYMETHYL CELLULASE IN AQUEOUS BIPHASIC SYSTEM EXTRACTION

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OUTLINE

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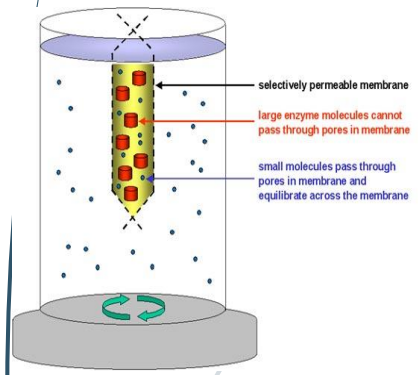
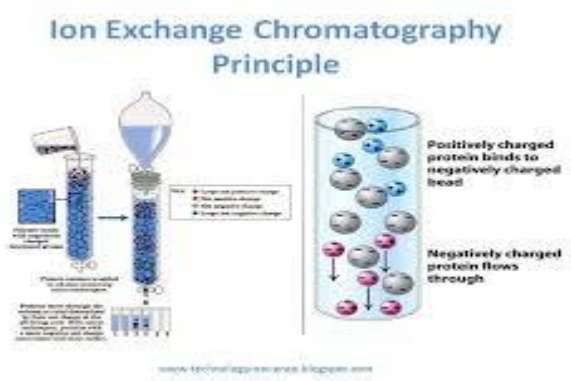
Overview

- Cellulases enzymes are proteins belonging to the family of glycosyl hydrolases and found wide application in food, textile, animal feed, pulp and paper and biofuel industries.
- Commercial cellulase are majorly produced from fungi, however bacterial cellulase in presently gaining recent attention.
- Purification of cellulase is required to gain complete understanding of its properties.
- The application of conventional protein purification techniques have been useful in the downstream processing of cellulase.

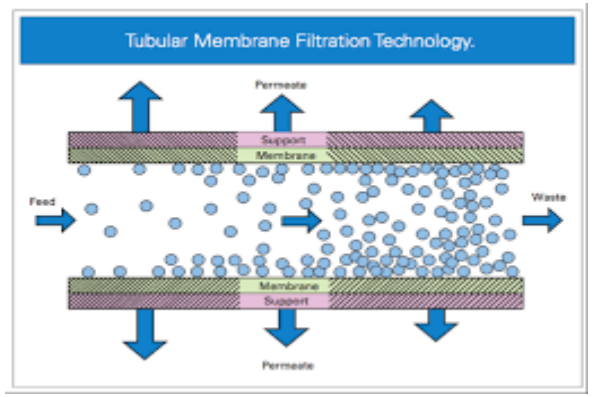
Overview



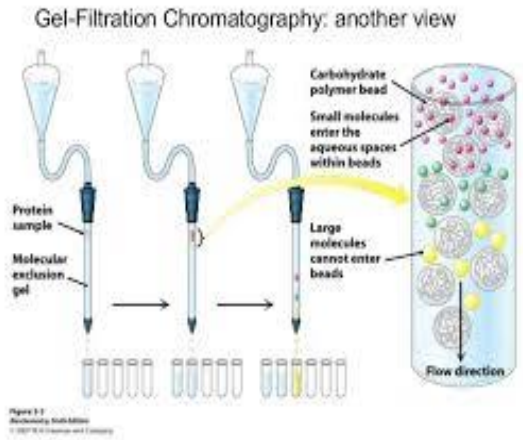
Ammonium sulphate purification



Ion exchange chromatography



Membrane filtration

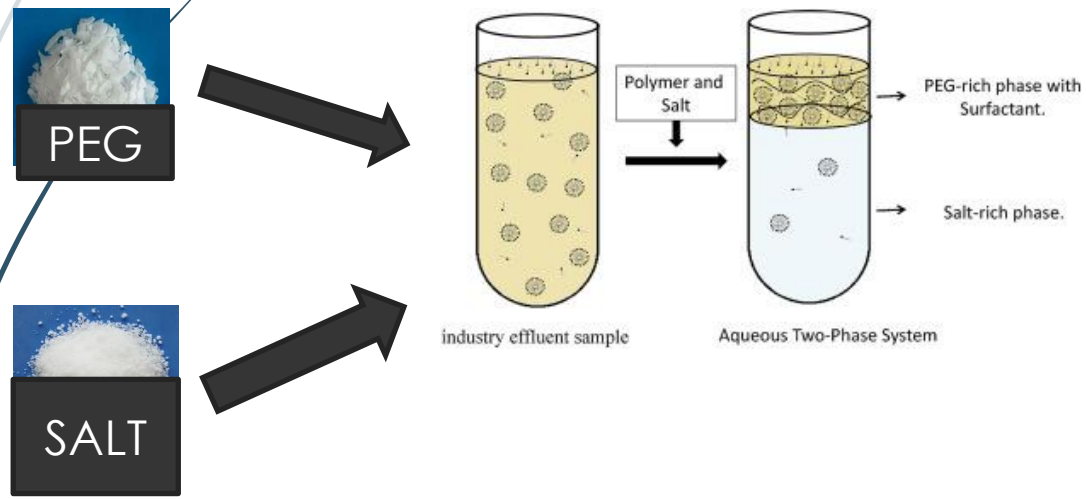


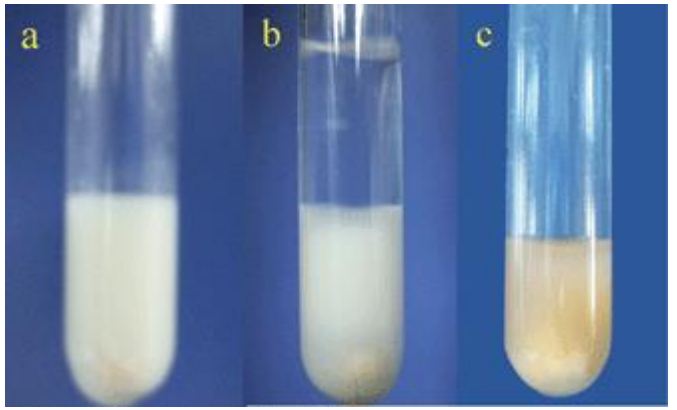
Gel filtration

Overview

- Aqueous biphasic system is a liquid-liquid fractionation technique that enables selective partitioning by extraction, separation and purification of biomaterials such as proteins, membranes, viruses and enzymes from complex mixtures.

- This system is formed by mixing the solutions of two mutually incompatible polymers or polymer/ salt above critical concentrations (Polyethylene glycol).





- The basis of separation is the uneven distribution of biomaterials between two phases, both having high water content.

Overview

- This high water content combined with the low interfacial tension of the system allows non-destructive partitioning of sensitive biomaterials and is often refered as biocompatibility and can also be used in preservation of the enzyme

- Partitioning is governed by numerous factors such as molecular weight PEG (hydrophobicity) pH, crude load, temperature, salt concentration.

- This can be manipulated to achieve desired separation and purification results, which makes ATPS very flexible for the application.

Comparison between conventional purification and Aqueous biphasic system

Conventional purification	Aqueous biphasic system
Laborious	Simple
Special equipment	10 ml falcon tube
Sephadex/salt	Polyethylene glycol/salt
Difficult scale up	Easy scalability
Require multiple steps	Can be done in single step
Enzyme loss	Biocompatible
Longer processing time	Shorter processing time
Expensive	Cheap

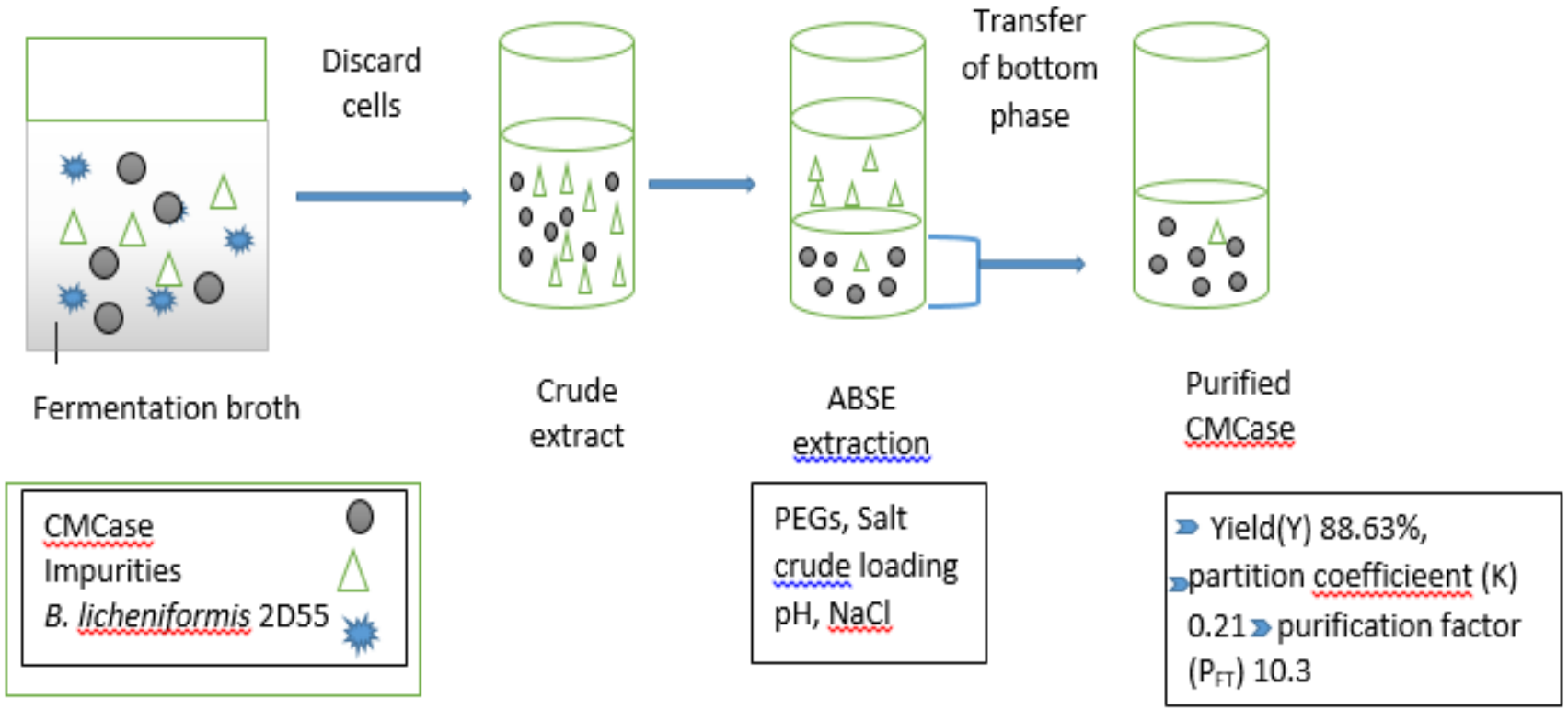
Problem Statement

- Conventional purification are cumbersome and expensive

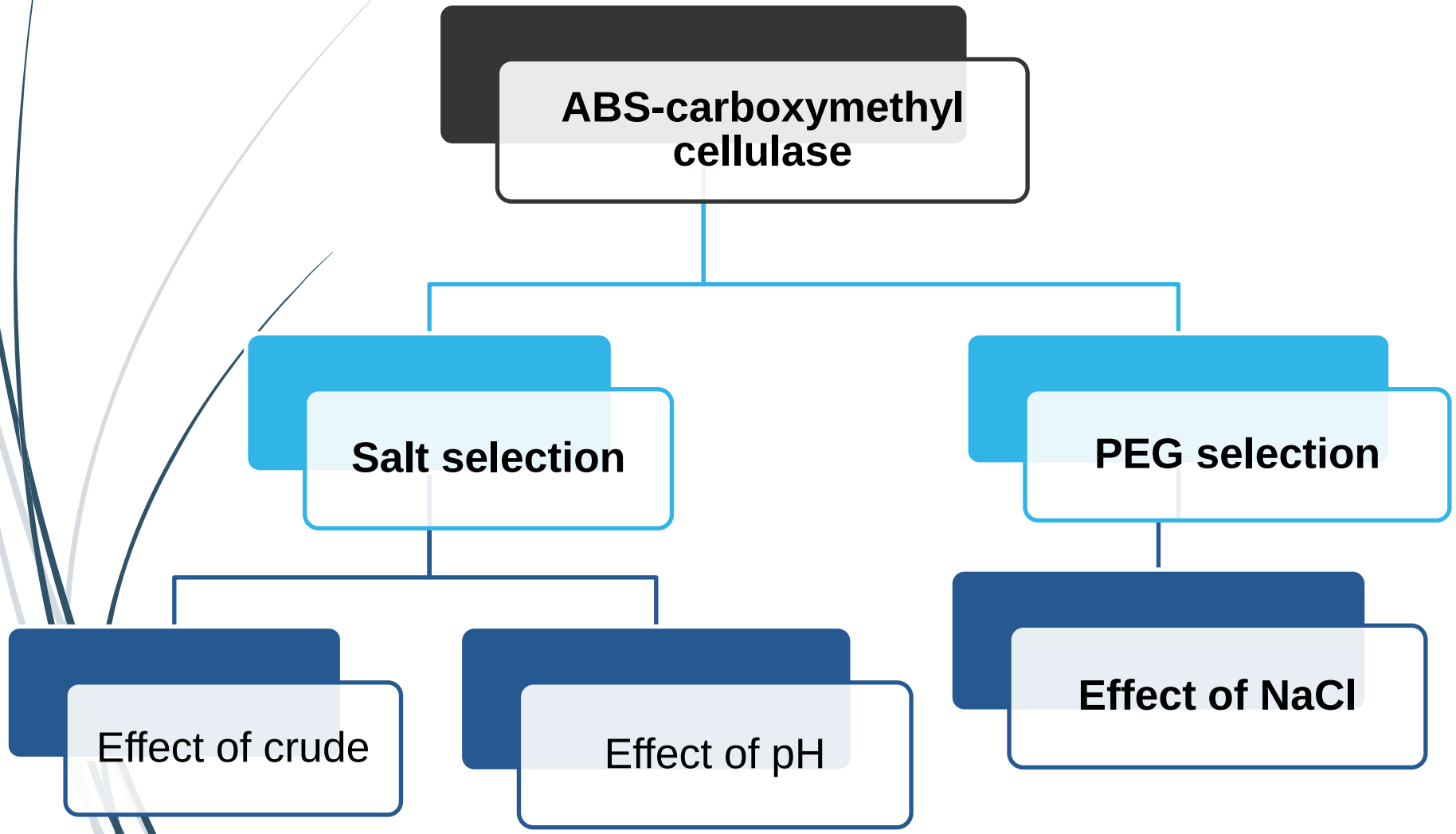
OBJECTIVE

- To optimize aqueous biphasic system parameters for extraction and purification of carboxymethylcellulase

Methodology



Methodology



Analysis

- Patition co-efficient $\mathrm{K} = \frac{A_T}{A_B}$
- Specific activity $\mathrm{SA}\ \left(\frac{U}{mg}\right) = \frac{cellulase\ activity\ \left(\frac{U}{mL}\right)}{Protein\left(\frac{mg}{mL}\right)}$
- Purification factor $\mathrm{P}_{FT} = \frac{SA\ of\ phase\ sample}{SA\ of\ crude\ feed\ stock}$
- Yield (Y) $(\%) = \frac{Enzyme\ activity\ in\ bottom\ phase}{Enzyme\ activity\ in\ crude\ extract}$

RESULTS AND DISCUSSION

Table 1: Selection of various bottom phase (salt) for CMCase stability

Type of bottom phase	Concentration (%, w/w)	Cellulase activity (U/mL)
Phosphate	5	26.55 ± 0.62
	10	25.38 ± 0.56
	20	22.93 ± 0.21
Magnesium sulphate	5	21.53 ± 0.09
	10	19.15 ± 0.31
	20	15.45± 0.18
Ammonium sulphate	5	26.41 ± 0.43
	10	25.06 ± 0.46
	20	21.51 ± 0.28
Sodium citrate	5	28.59 ± 0.21
	10	26.28 ± 0.43
	20	21.49 ± 0.53
Sodium acetate	5	25.55 ± 0.51
	10	23.98 ± 0.31
	20	20.71 ± 0.21

Table 2: Influence of PEG molecular weight on the partitioning behaviour of carboxymethyl cellulasee

PEG MW (g/mol)/	PEG/Citrate composition (%,w/w)	Partition coefficient (K)	Purification factor (P_{FT})	Yield (%)
4000	20.5/15	0.63	1.63	47.11
6000	20.5/15	0.52	3.32	58.89
8000	20.5/15	0.32	6.18	68.72
10,000	20.5/15	0.58	1.96	50.34

Result and Discussion

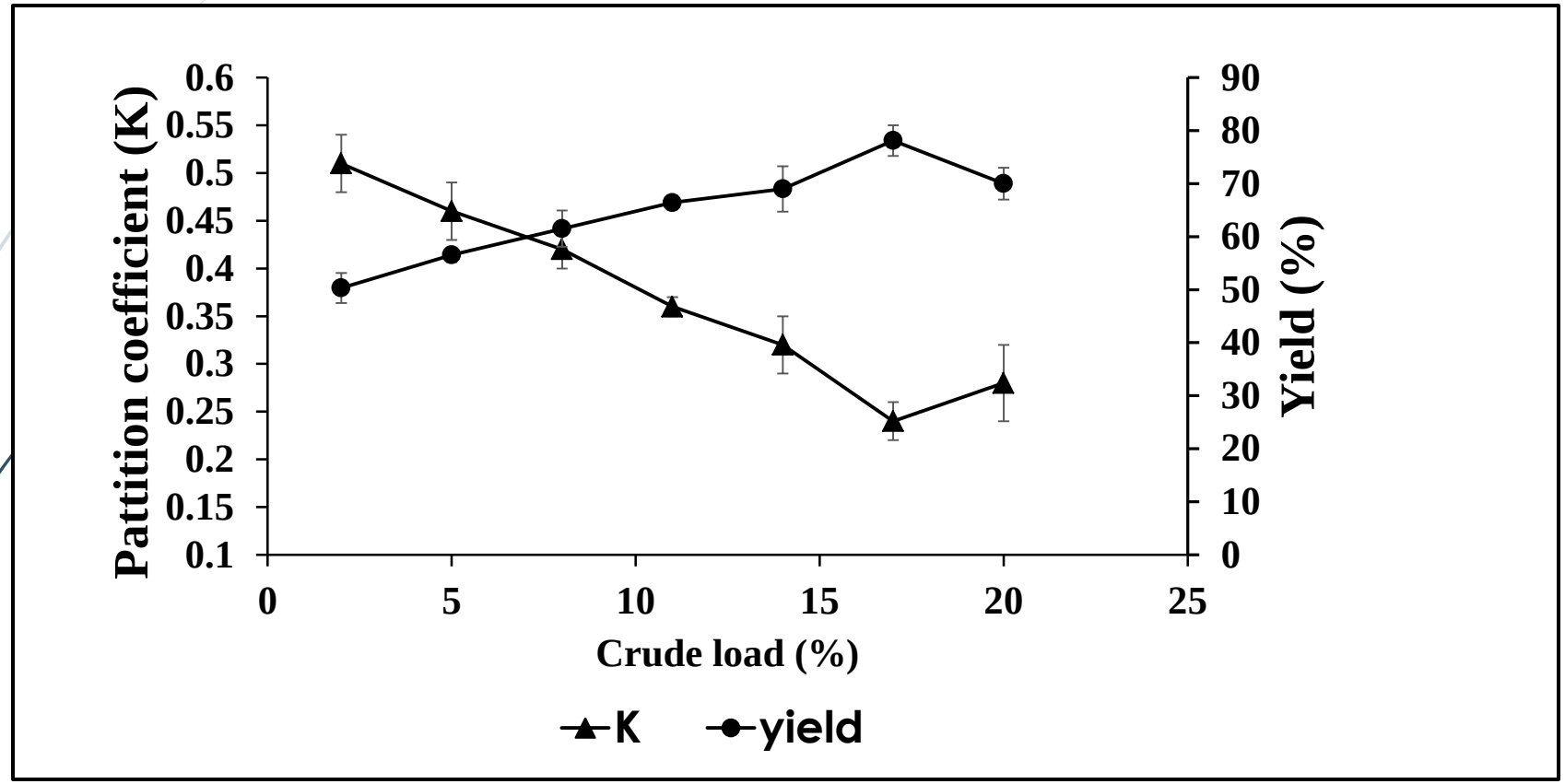
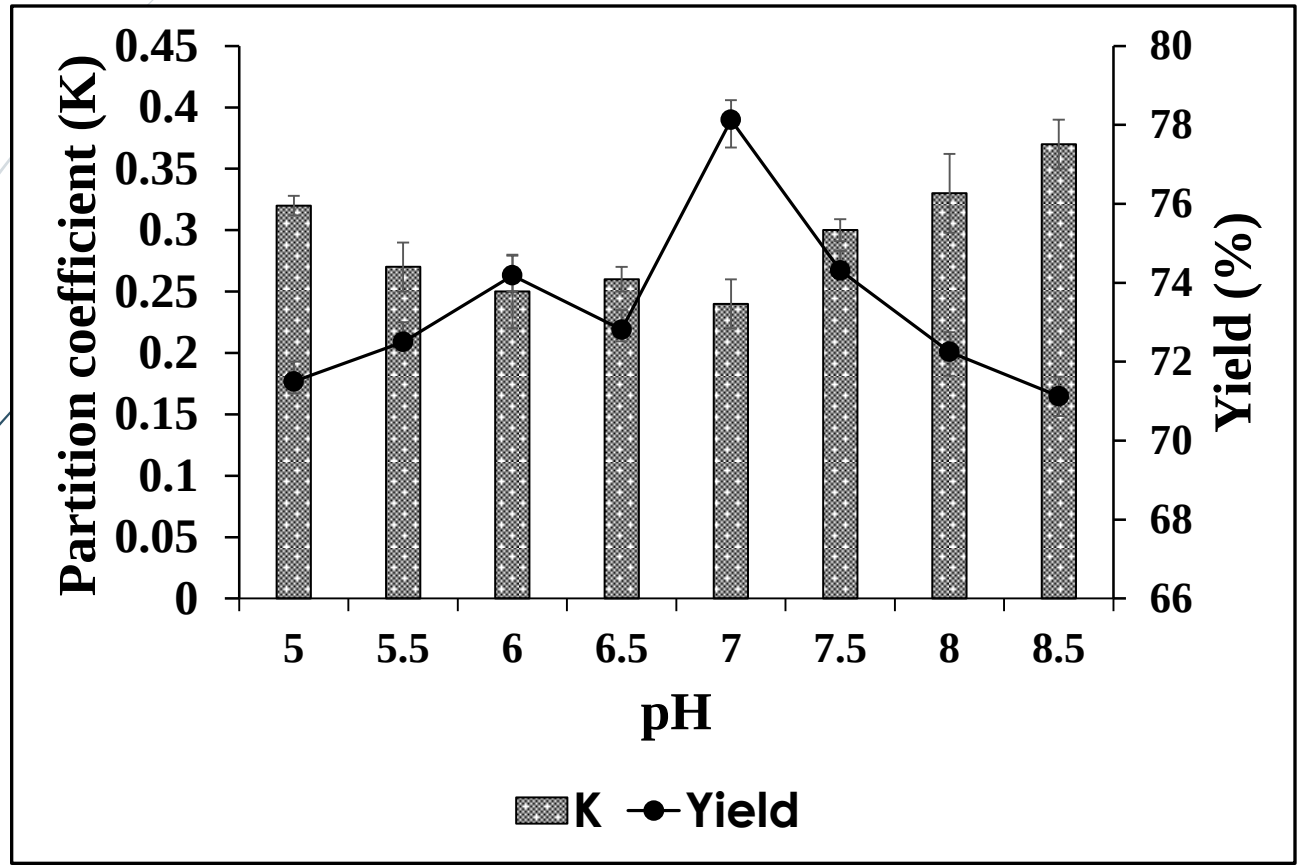


Figure 1: Influence of crude load on the partionning behaviour of cellulase

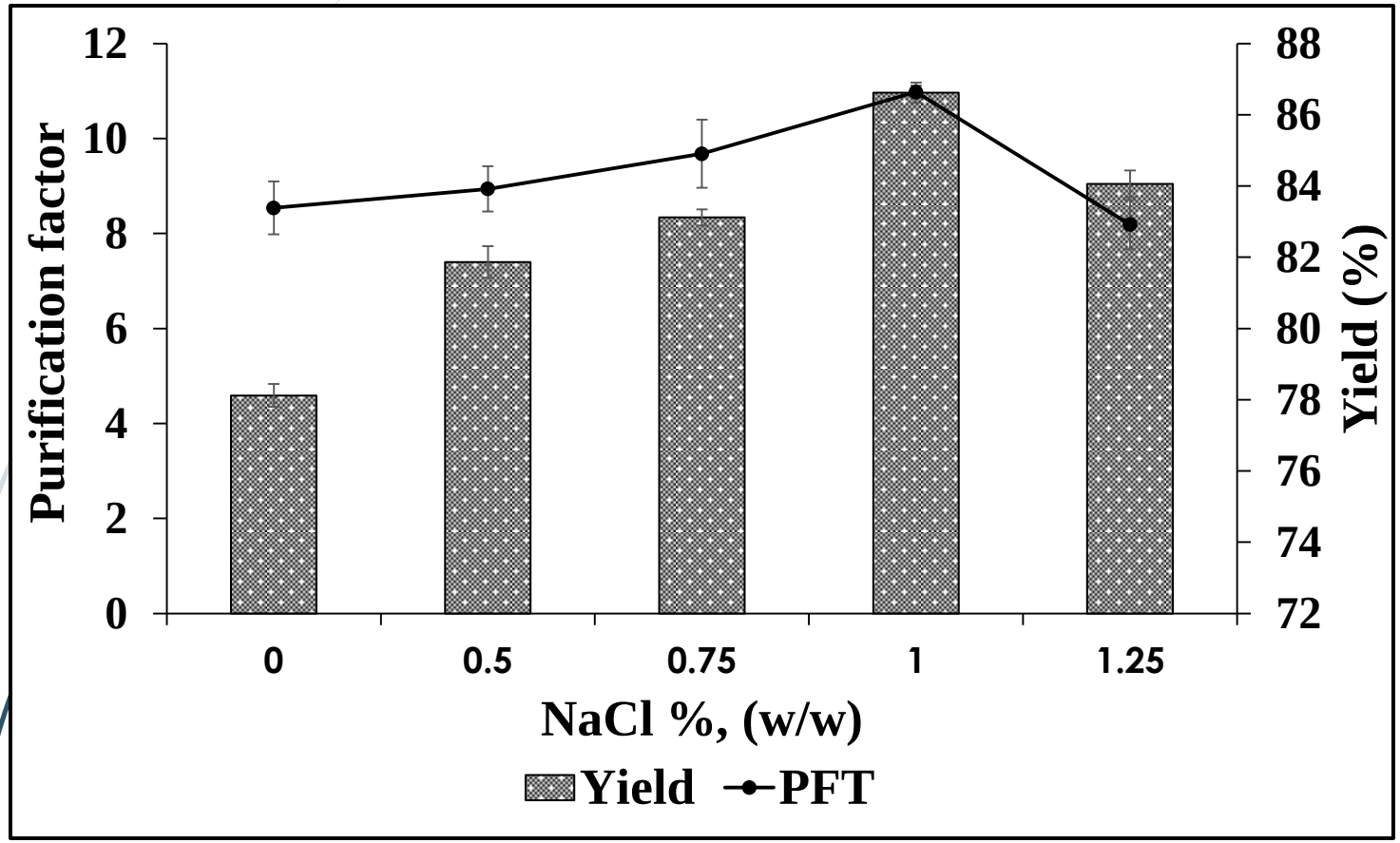
Results and Discussion



In this case, as the pH of ABSE was increased above the pI of cellulase, the cellulase becomes negatively charged which then was attracted to the positively charged PEG to phase there by reducing the yield of cellulase (Ratanapongleka, 2010)

Figure 2: Influence of pH on partitioning behaviour of cellulase

Results and Discussion



Addition of NaCl could cause the disruption of water structure due to greater electrical potential between the PEG and salt phase, thus enhancing the interaction of cellulase with the phase component (Settu *et al.*, 2015).

Figure 3: Inflence of NaCl on the partitioning behaviour of cellulase

Result and Discussion

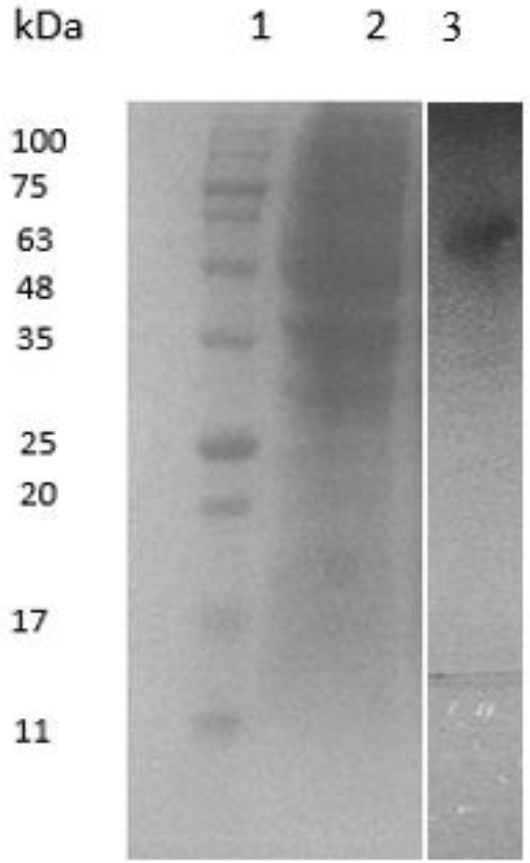


Figure 4: SDS-PAGE analysis of cellulase recovery in PEG8000/citrate ABS

CONCLUSION

- Aqueous biphsic system parameters at 14 % (w/w) crude load , 1 % NaCl, pH 7.0 produced optimum recovery of CMCase at a yield (Y) of 86.62 %, a partition coefficient (K) of 0.21 and purification factor (P_{FT}) of 10.3 from the bottom phase of PEG 8000/sodium citrate

- The results from this study has demonstrated that aqueous biphasis system can be potentially be used to purify carboxymethylcellulase from fermentation broth of *B. licheniformis* 2D55

ACKNOWLEDGMENTS

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