

# Acrylamide synthesis using immobilized cells of *R. rhodochrous* PA-34

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## Introduction

Acrylamide finds its application as coagulator, soil conditioner, and stock additive for paper treatment and in leather and textile industry (Jallageas 1980). It can be synthesized both chemically and enzymatically. The chemical process for acrylamide synthesis involves hydration of acrylonitrile in the presence of copper as catalyst. This method has some disadvantages, e.g. rate of acrylic acid formation is higher than acrylamide, by product formation and high-energy inputs (Yamada 1996). Enzymatic synthesis of acrylamide involves nitrile hydratase (Nhase) mediated hydration of acrylonitrile to acrylamide, a process operating under optimum pH conditions and does not produce any byproducts. A lot of research on acrylamide synthesis has been carried out using free cells of different microbes (Nagasawa 1993, Yamada 1996, Raj 2006). In a few reports immobilized cells have also been used for conversion of acrylonitrile to acrylamide (Graham 2000, Fradet 1985, Bui 1982, Mersinger 2005) but most of these studies were preliminary. In the present communication, immobilization of an earlier reported nitrile degrading bacterium *R. rhodochrous* PA-34 (Bhalla 1992) in polyacrylamide gel and its application in conversion of acrylonitrile to acrylamide at one litre scale in a partitioned fed batch reactor is being reported.

## Materials and Methods

*Rhodococcus rhodochrous* PA-34 was from Nippon Mining Co., Ltd, Japan (now Japan Energy Corporation) and was cultured as described previously by Raj (2006). The cells were harvested and suspended in 0.1 M potassium phosphate buffer (pH 7.0) such that it contained 22 mg dcw/ml.

### Assay for Nhase activity

The Nhase activity was assayed following the procedures reported earlier (Hijort 1990, Raj 2006). One Unit of Nhase activity was defined as the amount of enzyme, which converts one  $\mu$ mole of acrylonitrile to acrylamide per min under the assay conditions.

### Standardization of concentration of various parameters for entrapment of *R. rhodochrous* PA-34 cells in polyacrylamide gel

*R. rhodochrous* PA-34 cells were immobilized in polyacrylamide gel as described by Kierstan (1985). To standardize the polyacrylamide entrapment gel method for the immobilization of *R. rhodochrous* PA-34 cells, some parameters effecting gel strength, porosity and cell concentration were varied (Table 1) and Nhase activity was assayed.

### Optimization of reaction conditions for conversion of acrylonitrile to acrylamide using polyacrylamide entrapped cells of *R. rhodochrous* PA-34

The reaction conditions for conversion of acrylonitrile to acrylamide with immobilized cells were optimized as mentioned in Table 2. The complete conversion of 8 % acrylonitrile (w/v) to acrylamide was followed up to 4 h under the optimized conditions.

## Synthesis of acrylamide at one litre scale

The conversion of acrylonitrile to acrylamide was carried out (at one litre scale) at 10°C in a partitioned fed batch reactor. In this reactor nylon net bag was used centrifugally to partition the immobilized discs to avoid their breakage during agitation (Fig 1). The initial reaction mixture consisted of 428 ml of 0.1 M potassium phosphate buffer (pH 7.5), 8 % acrylonitrile (w/v) and resting cells (1.12 g dcw). The substrate (8 % w/v or 1500 mM acrylonitrile) was added at an interval of 3 h. The water consumed during the hydration of acrylonitrile to acrylamide was periodically compensated. The amount of acrylonitrile, acrylamide and acrylic acid (if any) in the reaction mixture was analyzed at an interval of three year by gas chromatography as described previously (J. Raj et al., 2006). The nylon bag containing the polyacrylamide immobilized *R. rhodochrous* PA-34 cells was taken out from the reaction vessel, washed with buffer and used for the next cycle of reaction. This process was repeated three times and the residual Nhase activity was tested after at every cycle.

## Results and Discussion

For the entrapment of *R. rhodochrous* PA-34 cells in polyacrylamide gel, different concentrations of acrylamide, bisacrylamide, ammonium persulphate, TEMED, cells were used and the optimized values/concentrations after assaying the Nhase activity are shown in Table 1. These optimized parameters were used to immobilize the *R. rhodochrous* PA-34 cells for further studies. The immobilized cells were cut into the disc of 1 cm diameter (0.5 cm thick) and one disc was equivalent to 0.70 mg dcw.

S.No	Parameter	Range tested	Optimum value
1	Acrylamide	4-20 % (w/v)	12.5 (w/v)
2	Bisacrylamide	0.2- 1.0 % (w/v)	0.6 % (w/v)
3	Ammonium persulphate	0.1 – 0.5 % (w/v)	0.2 % (w/v)
4	TEMED	0.2 – 1.0 % (w/v)	0.4 % (v/v)
5	Cells (1 ml = 22 mg dcw)	4 –20 % (v/v)	8 % (v/v)

**Table 1: Standardized parameters for the entrapment of *R. rhodochrous* PA-34 cells in polyacrylamide gel**

S.No	Parameter	Range tested	Optimum value
1	0.1 M potassium phosphate buffer pH	6.0-8.5	7.5
2	Temperature	10-50 ° C	10°C
3	Cells concentration (mg dcw/ml)	0.28- 1.62 mg dcw	1.12 mg dcw
4	Substrate (acrylonitrile)	2-20 % (w/v)	8 % (w/v)

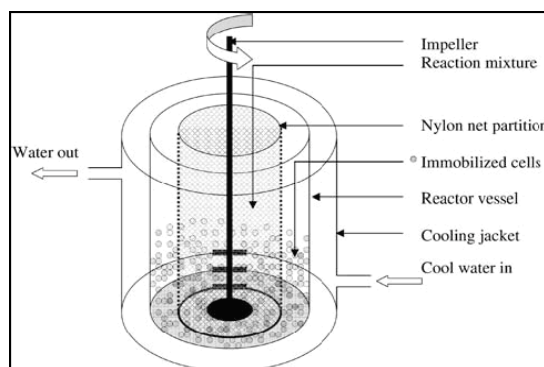
**Table 2: Standardized reaction parameters for the conversion of acrylonitrile to acrylamide using polyacrylamide entrapped cells of *R. rhodochrous* PA-34**

### Optimization of reaction conditions for conversion of acrylonitrile to acrylamide using polyacrylamide entrapped cells of *R. rhodochrous* PA-34

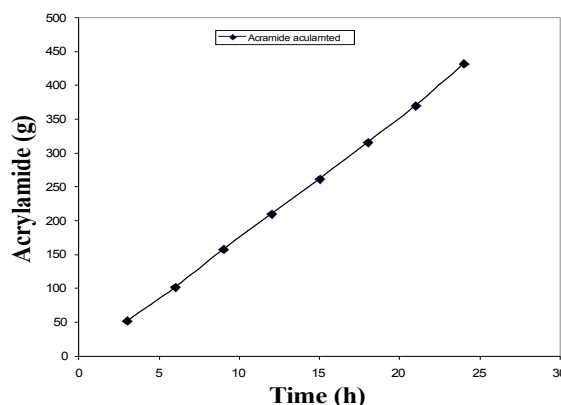
The optimum pH of the reaction with immobilized cells was 7.5 (Table 2) while free cells had exhibited maximum activity at 7.0 pH (Raj 2006). This change in optimum pH by 0.5 unit upon

immobilization of *R. rhodochrous* PA-34 can be attributed to partitioning of hydrogen ions in the polyacrylamide matrix. The conversion of acrylonitrile to acrylamide was carried out 10 to 50°C (Table 2) with polyacrylamide entrapped cell and maximum transformation was recorded at 10°C. Above 10°C, acrylic acid was also detected in the reaction. It has been reported earlier that at temperature above 10°C, amidases get activated and convert amide to acids (Nagasawa 1993; Raj 2006). Generally at higher temperatures (>30°C) problems associated with loss of acrylonitrile and polymerization of acrylamide become significant (Nagasawa 1993).

To optimize the concentration of substrate and biocatalyst (immobilized cells of *R. rhodochrous* PA-34) for this reaction, concentration of acrylonitrile (2-20% w/v) and amount of immobilized cells (equivalent to 0.4-2.0 mg dcw/ml reaction mixture) was varied. The maximum Nhas activity (6.85 U/mg dcw) was observed using 8% acrylonitrile (w/v) and biocatalyst equivalent to 1.12 mg dcw/ml reaction mixture (Table 2). The conversion of 8% acrylonitrile (w/v) to acrylamide using polyacrylamide entrapped cells of *R. rhodochrous* PA-34 (1.12 mg dcw/ml) was followed up to 4 hours under the optimized conditions and GC analysis of the reaction mixture showed complete conversion of 8% acrylonitrile (w/v) to acrylamide in 3 h. Therefore, substrate feed at the rate of 8% was added after every 3 h in this fed batch reaction



**Fig. 2 Schematic diagram of the reaction vessel used for the bioconversion of acrylonitrile to acrylamide by the polyacrylamide immobilized cells of *R. rhodochrous* PA-34**



**Fig 3: Acrylamide accumulated at 1 litre scale using polyacrylamide entrapped cells of *R. rhodochrous* PA-34**

### Acrylamide Synthesis at 1 liter scale

The conversion of acrylonitrile to acrylamide was carried out at one liter scale in a partitioned fed batch reactor up to 24 h. After 24 h, samples could not be withdrawn due to formation of acrylamide crystals. The reaction mixture was diluted with distilled water, decanted from the reaction vessel and was kept overnight at 0-4°C for crystallization of acrylamide. The acrylamide so recovered was dried, weighed and its GC and HPLC analysis showed that it was free from acrylic acid. A maximum accumulation of 432 g/l of acrylamide was recorded with 100% conversion of acrylonitrile in 24 h (Fig 3). The acrylamide produced was crystalline white in color and the residual Nhas activity in the polyacrylamide entrapped cells was 95 %, which showed the stabilization of the biocatalyst after immobilization. The polyacrylamide entrapped cells of *R. rhodochrous* PA-34 were recycled for the three cycles and a total of 1217 g acrylamide was accumulated i.e. 405 (first recycle), 210 (second recycle) and 170 (third recycle).

## Conclusion

*R. rhodochrous* PA-34 emerged as potential catalyst for acrylamide synthesis. The polyacrylamide entrapped cells (1.12 mg dcw/ml) of *R. rhodochrous* PA-34 completely converted 8 % (w/v) acrylonitrile to acrylamide at 10°C in 0.1 M potassium phosphate buffer (pH 7.5). The polyacrylamide entrapped cells were recycled up to four cycles of reactions in a partitioned fed batch reactor and were successfully recovered for the next cycle of reaction without any breakage. The design of a partitioned fed batch reactor used in the present studies may be of interest and relevance to those who are involved in scale up of biocatalytic reaction using immobilized whole cells.

## Acknowledgments

We acknowledge the financial support from University Grants Commission (UGC), New Delhi, India to Jog Raj.

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