Evaluation of the Applicability of Alkaline Pretreatment for the Development of a Simple Quantification Method of Biodegradable Plastics
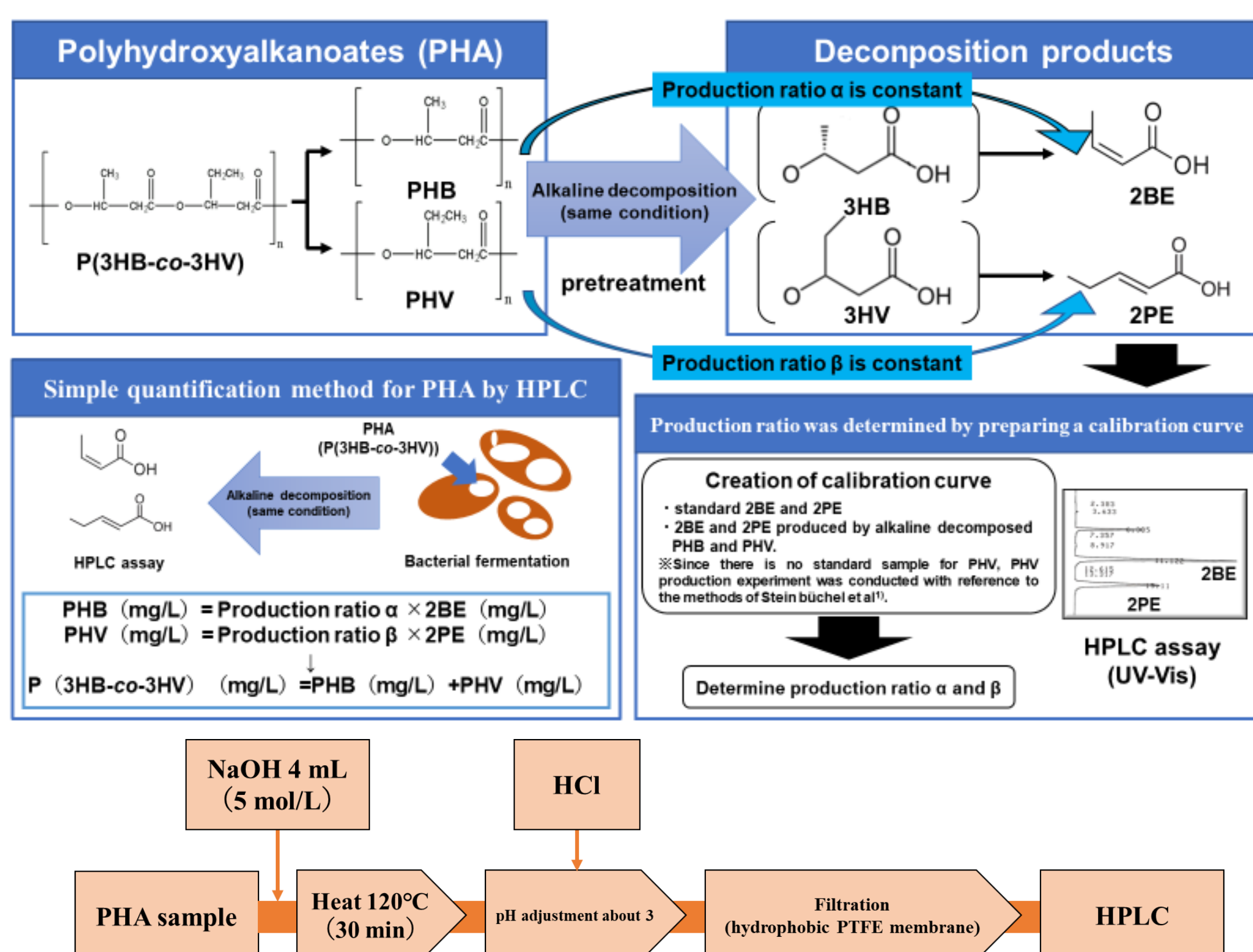
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INTRODUCTION & AIM

Many methods for quantifying polyhydroxyalkanoates (PHA), a type of biodegradable plastic, have been reported. However, pretreatment is required, such as removing various attached components and extracting target components. In recent years, quantification by GC analysis using pretreatment such as methanolysis has become mainstream. However, a more straightforward and safer analysis method is still required. Therefore, in this study, we attempted to develop a simple PHA quantification method using alkaline decomposition with HPLC analysis. PHB analysis uses HPLC (crotonic acid method, Appl. Environ. Microbiol., 46, 1339-1344, 1983).Our suggested method can analyze PHB and P(3HB-*co*-3HV).
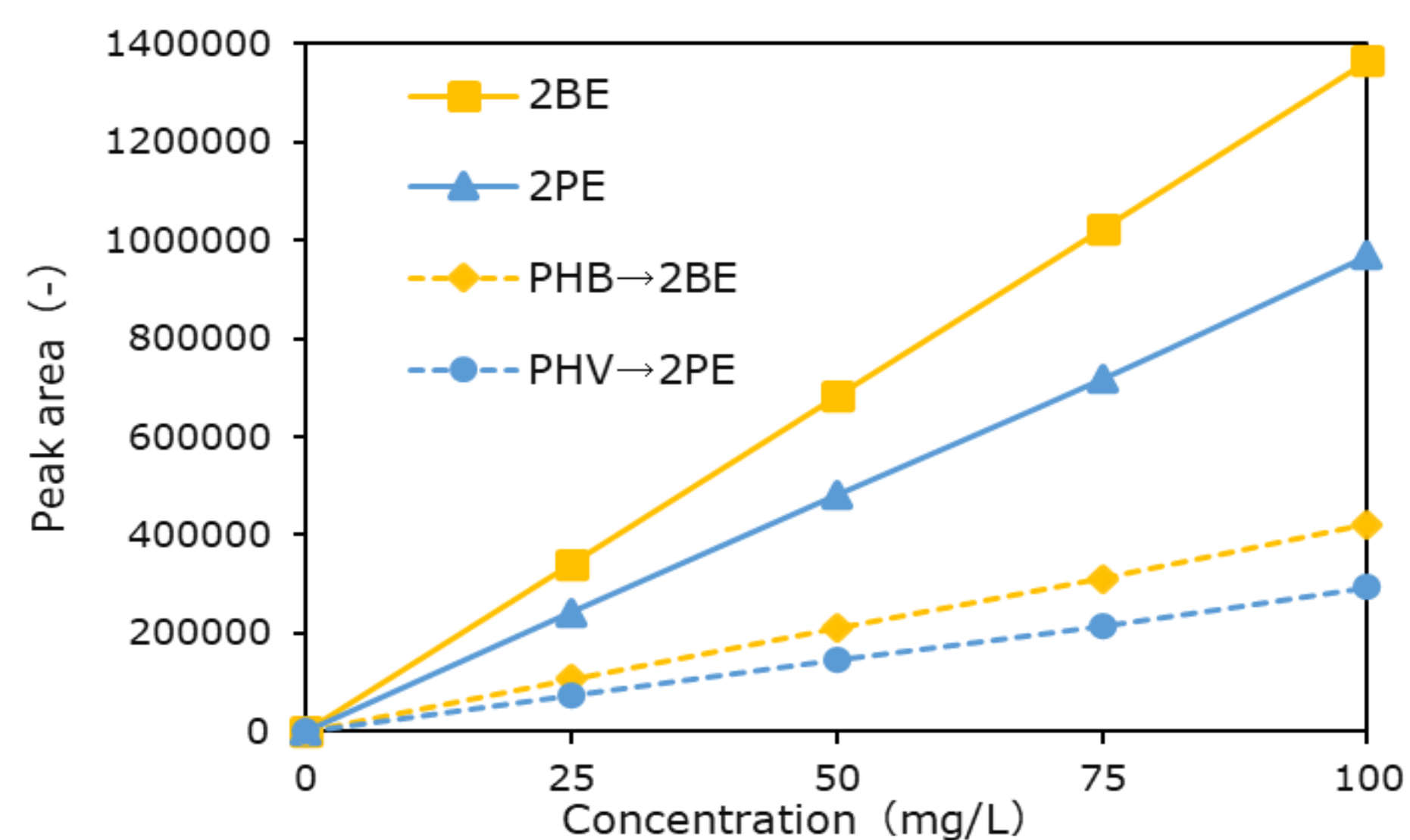
METHOD

NaOH 4 mL (5 mol/L) → PHA sample → Heat 120°C (30 min) → HCl → pH adjustment about 3 → Filtration (hydrophobic PTFE membrane) → HPLC

RESULTS & DISCUSSION

Table. Quantification of standard samples of P (3HB-*co*-3HV) and accuracy of the analysis

Concentrations（mg/L）	Measured concentration（mg/L）	Coefficient of variation（%）
25	25.6±1.23	4.81
50	52.9±1.82	3.44
75	76.5±3.31	4.33
100	104.8±4.23	4.04

The calibration curve of standard 2BE and 2PE and 2BE and 2PE produced by alkaline degradation of PHB and PHV.
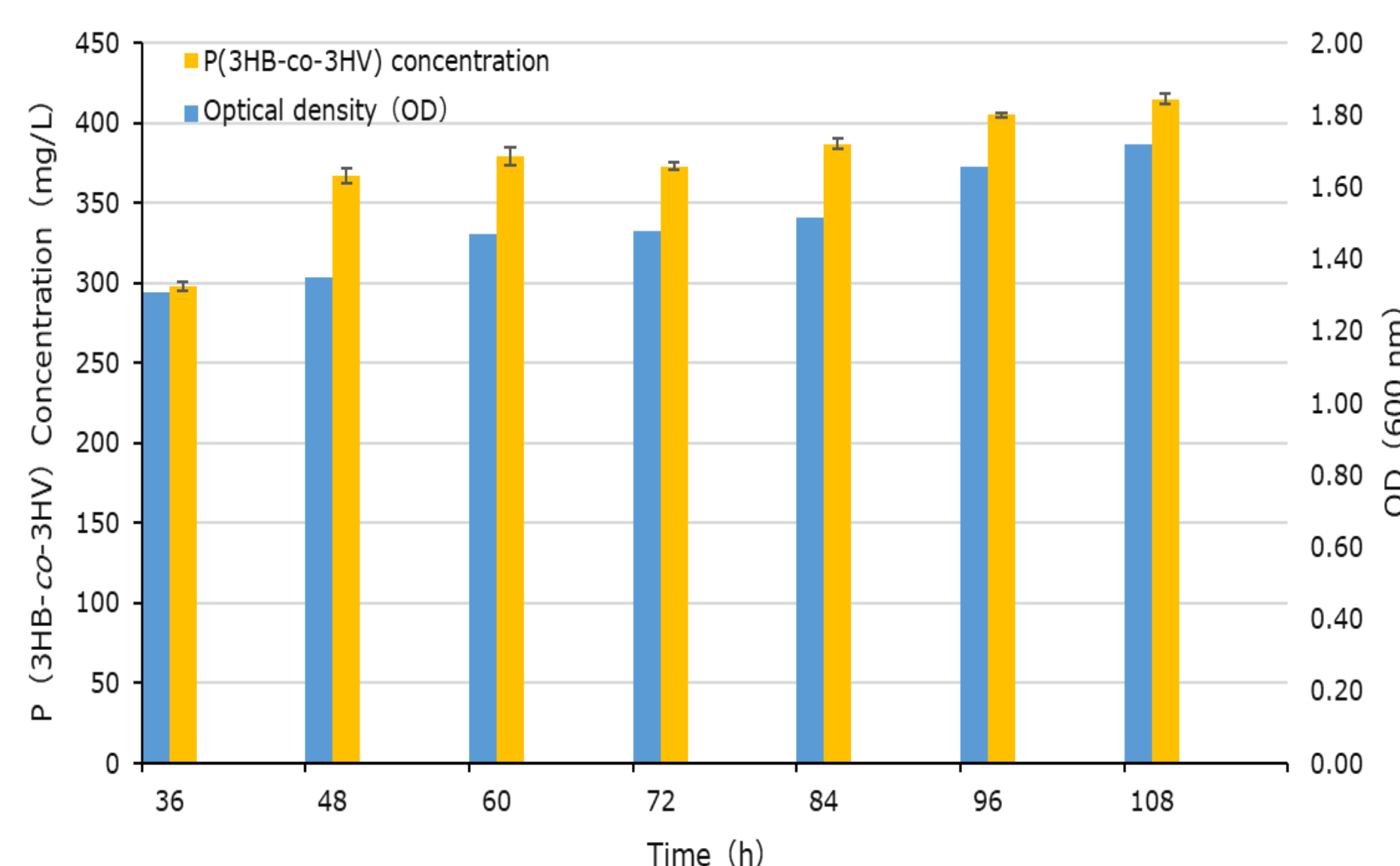
Quantification of P(3HB-*co*-3HV) in microbial biomass. *Bacillus* sp. CYR1 strain (DDBJ accession number LC049103)[3] was used as the PHA-producing bacterium. As a preculture, CYR1 strain was incubated in NB medium (polypeptone 10 g/L, yeast extract 2 g/L, $MgSO_4 \cdot 7H_2O$ 1.0 g/L, pH = 7.0), and conducted shaking culture (30°C, 100 rpm) for 8 h. To obtain P (3HB-*co*-3HV), 8% (v/v) preculture was added into MS medium containing acetic acid (10 g/L) and valeric acid (10 g/L) and conducted shaking culture (30°C, 100 rpm) for 108 h. 20 mL of the culture medium was collected every 12 hours and centrifuged at 10000 r/min for 10 min at 4°C. The pellet was dried to constant weight. Dried cells were pretreated with alkaline decomposition and analyzed by HPLC. Currently, to compare PHA's quantitative properties, we are also conducting GC analysis.

CONCLUSION

The retention times of the standard reagents 2BE and 2PE were 2BE (11.1 min) and 2PE (15.1 min), respectively. The formation of 2BE and 2PE was observed from PHA that had been pretreated with alkaline degradation, and the prepared calibration curve showed linearity. From this, it was shown that the production ratio α of 2BE from PHB and the production ratio β of 2PE from PHV are constant. From the ratio of the slopes of the calibration curve, the production ratios were determined to be α = 3.26 and β = 3.30. From the obtained absorption sensitivity, it was found that 2BE and 2PE, which have double bonds in the structure, can be detected with higher sensitivity than 3HB. From these results, it is considered that short-chain-length PHA can be quantified easily and with high sensitivity by measuring 2BE and 2PE of actual samples by the analysis method of this study.