Process Biochemistry 51 (2016) 1664–1673

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Kinetic and thermodynamic characteristics of ultrasound-assisted extraction for recovery of paclitaxel from biomass

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ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 18 July 2016
Accepted 10 August 2016
Available online 11 August 2016

Keywords:
Paclitaxel
Ultrasound-assisted extraction
Kinetics
Thermodynamics

ABSTRACT

This study developed an ultrasound-assisted extraction (UAE) process that can efficiently extract the anticancer agent paclitaxel from biomass. Most of the paclitaxel (~99%) was recovered from biomass by extracting once under the optimum ultrasound power (380 W) and operation time (10 min) for UAE. The kinetic analysis results revealed that the hyperbolic model was the most suitable with the highest accuracy among the four kinetic models considered. Significant correlations were found between the paclitaxel yield and the scalable ultrasonic process parameters of power density (P/V) and energy density (E/V). The thermodynamic analysis showed that the enthalpy change (ΔH^0) and entropy change (ΔS^0) were both positive, while the Gibbs free energy change (ΔG^0) was negative and decreased when increasing the temperature and ultrasound power. Thus, the extraction was more feasible when using a higher temperature and higher ultrasound power. The results indicated that the UAE process for recovering paclitaxel from biomass was endothermic, irreversible, and spontaneous.

1. Introduction

Paclitaxel is a widely used anticancer agent found in the bark of the yew tree, and has been approved by the FDA as a treatment for ovarian cancer, breast cancer, head and neck cancer, Kaposi's sarcoma, and non-small cell lung cancer [1]. Thus, the demand for this drug is expected to increase steadily; its indications are expanding continuously, and clinical tests for combined prescriptions with other therapies are currently being conducted [2,3]. Among the main methods of paclitaxel production, including plant cell cultures, semi-synthesis, and direct extraction from the yew tree, plant cell cultures enable a stable mass production of consistent quality in a bioreactor without the influence of external factors, such as climate or the environment [4–7].

Since most of the paclitaxel produced by a plant cell culture is contained in the plant cells (biomass) [8], efficient extraction of paclitaxel from the biomass is important to increase recovery [9–11]. According to previous studies, the typical methods of paclitaxel extraction from biomass include conventional solvent extraction (CSE) and microwave-assisted extraction (MAE), where CSE is most commonly used [9–13]. However, CSE requires a long extraction time with large amounts of organic solvents and only a low extraction efficiency. Even under optimum extraction conditions, CSE requires four extraction times to recover most of the paclitaxel (>98%) [1]. Meanwhile, MAE, which uses microwave energy and an organic solvent, has a higher and faster paclitaxel recovery rate than CSE, requiring only one extraction cycle under optimum extraction conditions for most of the paclitaxel to be recovered (~99%) [13]. Notwithstanding, while the number of extraction and time can be reduced when using microwave energy, the MAE apparatus and equipment are relatively expensive and their operation relatively difficult, especially in the industrial processing of bioactive substances for commercial purposes [14]. Therefore, a highly efficient extraction method is needed that can reduce equipment and energy costs with an improved process convenience and feasibility. Ultrasound-assisted extraction (UAE) has recently been explored for more efficient recovery and separation of bioactive compounds from biomass [15–17]. Demonstrating a high extraction efficiency and low energy and solvent consumption, UAE is a potentially powerful alternative method for research studies and commercial processes. The mechanism of UAE is attributed to mechanical, cavitation, and thermal efficacies that can disrupt cell walls and enhance mass transfer across cell membranes [18]. Thus, the anticipated effects include enhanced efficiencies, simplified processes, and reduced equipment and energy costs [19]. UAE has already been applied to improve the extraction efficiency of bioactive substances, such as phenolic compounds [20], antioxidants [21], and oleuropein [22] from plant materials.

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http://dx.doi.org/10.1016/j.procbio.2016.08.012
1359-5113/

Nomenclature

b	Washing coefficient of the unsteady diffusion model
b'	Washing coefficient of the film model
C_e	Concentration of extracted paclitaxel at equilibrium (mg/mL)
C_{se}	Concentration of paclitaxel at equilibrium in the biomass (mg/mL)
C_t	Concentration of extracted paclitaxel at any time (mg/mL)
E_a	Activation energy (kJ/mol)
ΔG^0	Gibbs free energy change (kJ/mol)
h	Initial extraction rate (mg/mL min)
ΔH^0	Enthalpy change (kJ/mol)
k_0	Pre-exponential factor (mL/mg min)
k_2	Second-order extraction rate constant (mL/mg min)
k_u	Slow extraction coefficient of the unsteady diffusion model (min^{-1})
k'_u	Slow extraction coefficient of the film model (min^{-1})
K_1	Initial extraction rate (min^{-1})
K_2	Constant related to the maximum extraction yield (min^{-1})
K_e	Equilibrium constant (C_e/C_{se})
n	Number of experimental runs
q	Extraction yield
q_e	Content of paclitaxel initially present in the biomass
q_0	Maximum concentration of paclitaxel in the solvent at temperature (K) (mg/mL)
q_s	Concentration of paclitaxel in the liquid extract during extraction (mg/mL)
q_T	Yield of paclitaxel in the extract at equilibrium at T_d (°C)
q_{T0}	Yield of paclitaxel in the extract at equilibrium at 0 °C
q_w	Content of paclitaxel in the biomass during extraction
r^2	Coefficient of determination
R	Gas constant (8.314 J/mol K)
ΔS^0	Entropy change (kJ/mol K)
t	Time (min)
T	Absolute temperature (K)
T_d	Celsius temperature (°C)

Greek letters

γ	Temperature extraction coefficient

Subscript

RMSD	Root mean square deviation

However, most previous extraction studies have focused on optimizing the experimental conditions or process factors and qualitative description of their effects, with very little quantitative analysis of the kinetics and thermodynamics of UAE, which is important to understand the feasibility and nature of the extraction process. Furthermore, UAE has not yet been applied to the recovery of paclitaxel from a plant cell culture.

Therefore, this study systematically investigated the characteristics of UAE for the recovery of paclitaxel from biomass in terms of the extraction kinetics and thermodynamics in order to provide useful information on the UAE process. In particular, the thermodynamic parameters, such as the standard Gibbs free energy change, standard enthalpy change, and standard entropy change, were investigated to evaluate the characteristics of the UAE process.

2. Materials and methods

2.1. Plant materials

The plant cell culture medium used in the study was prepared using a cell line from the leaves of *Taxus chinensis*. A suspension of cells originating from *T. chinensis* was maintained in darkness at 24.0 °C with stirring at 150 rpm. The suspension cells were cultured in a modified Gamborg's B5 medium supplemented with 30 g/L sucrose, 10 μM naphthalene acetic acid, 0.2 μM 6-benzylamino purine, 1 g/L casein hydrolysate, and 1 g/L 2-(*N*-morpholino) ethanesulfonic acid. The cell cultures were transferred to a fresh medium every 2 weeks. During the prolonged culture for production purposes, 4 μM $AgNO_3$ was added at the initiation of the culture as an elicitor, while 1 and 2% (w/v) maltose were added to the medium on day 7 and 21, respectively [23]. Following the cultivation, the plant cells were recovered using a decanter (CA150 Clarifying Decanter; Westfalia, Germany) and high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa-Laval, Sweden). The recovered plant cells, hereinafter referred to as the biomass, were provided by Samyang Biopharm Company, South Korea.

2.2. Paclitaxel analysis

The dried residue was redissolved in methanol for a quantitative analysis using an HPLC system (Waters, USA) with a Capcell Pak C_{18} column (250 mm × 4.6 mm; Shiseido, Japan). The elution was performed based on a gradient using a distilled water–acetonitrile mixture varying from 65:35 to 35:65 within 40 min (flow rate = 1.0 mL/min). The injection volume was 20 μL, and the effluent was monitored at 227 nm using a UV detector [9]. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich and used as the standard. Each sample was analyzed in triplicate.

2.3. Conventional solvent extraction (CSE)

The biomass from the plant cell cultures was mixed with methanol and stirred at room temperature for 30 min [12]. The mixture was filtered under a vacuum in a Buchner funnel through filter paper (150 mm, Whatman), where methanol was preferably added to the biomass at a ratio of 1:1 (mL/g, v/w). Extraction was repeated four times with new methanol. Each methanol extract was collected, pooled, concentrated and dried at 40 °C under a vacuum (760 mmHg) for HPLC analysis.

2.4. Ultrasound-assisted extraction (UAE)

Fig. 1 shows a schematic diagram of the UAE process. The UAE experiments were performed with two 40 kHz ultrasonic cleaners, one with a maximum output power of 250 W (UC-10, Jeiotech, Korea) and the other of 530 W (JAC-4020, KODO, Korea). The reactor size and working volume were 100 and 30 mL, respectively. The biomass was extracted once using methanol at a ratio of 1:1 (w/v) and the possibility of paclitaxel degradation at high temperatures (>50 °C) was considered [24]. The mixture was stirred using ultrasonic cleaners at different temperatures (25, 30, 35, 40, and 45 °C), ultrasound powers (80, 180, 250, 380, and 530 W), and operation times (1, 2, 4, 6, 8, 10, 12, 15, and 30 min) with a stirring speed of 500 rpm. After the extraction, the mixture was filtered under a vacuum using filter paper (150 mm, Whatman). The filtrate (methanol extract) was collected, concentrated using a concentrator (CCA-1100, EYELA, Japan), and then completely dried in a vacuum (40 °C,