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Optimalization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

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Additional information is available at the end of the chapter

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1. Introduction

Since the fossil fuel crisis broke out in the nineteen seventies with the continual rise in fossil fuel prices, the mankind has been searching for renewable energy for consumption. For the past decades, atmospheric pollutions that involve with using fossil fuel have resulted in many severe problems of environment and human health[1-16]. Therefore, exploitation and utilization of clean and renewable energy have become the strategic consideration for many countries. Biofuel, as an alternative fuel, is recently attracting increasing attention[17-20]. Microalgae grow in aquatic environments and use light and carbon dioxide to create biomass and have been recognized as potentially good material sources for biofuel production. Microalgae possess several aspects of advantages for development of clean green energy, i.e. they have short growth period and are easy to cultivate and reproduce to large biomass. Controlled culture conditions of microalgae could trigger high lipid content of microalgae which could be used for lipid extraction, in turn, by further transesterification reaction, for preparation and production of biofuel with excellent characteristics. Therefore, utilization of the microalgal lipid for producing biofuel has promising future[21-27]. It should be noticed that obtaining lipid is a prerequisite for the production of microalgal fuel. More and more investigations have showed that microalgae are potentially good biomass materials for development of clean green energy[28-40].

As shown in figure 1, there are six major steps for biofuel preparation and production from initiating microalgal cultivation to biofuel products. Each of six steps involves with crucial techniques and methods in order to achieve high production of biofuel. For example, during
microalgal cultivation, it is very important to screening and selecting fine microalgal strains with higher oil content for reproduction and amplification of algal cells[43-45]. In addition, investigating on controlled culture conditions that could improve oil accumulation of microalgal cells is necessary for the increase in biofuel production[46-51]. Usually, open ponds are used in microalgal cultivation with the less expense, simple facilities and operation. However, its disadvantages are associated with such issues as culture contamination, difficulties in regulation and control of culture conditions(like temperature and light control), lower productivity and so on[52-54]. Closed system such as photobioreactors(PBRS) are as well used for microalgal culture. These are highly-automated clear piping systems, which allow the operator to control nutrients, light, temperature and contamination for high productivity. But such facilities require expensive investment[55-59]. While heterotrophic culture and amplification of algal cells by using fermentation tanks can obtain highly-concentrated algal cells for high productivity effects, such facilities are also involved in high investment cost[60-61]. Microalgal harvesting is another crucial technique for entire biofuel production process. Since microalgal cells are so tiny (only micron order of magnitude in size) that it is quite difficult for effective microalgal cell harvesting. Taking cost and energy efficiencies into consideration, a relative simple and feasible method, flocculation of microalgal cells by changing pH value of culture medium or using certain eco-friendly chemical and biological flocculants like ferric chloride and chitosan, could be adapted in harvesting microalgal biomass. During flocculation, the dispersed microalgal cells can aggregate and form larger conjugates with higher sedimentation rate. Moreover, those methods allows the cycle reuse of the flocculated medium, thereby contributing to the economic cultivation and harvest of microalgae[62-66]. Harvested microalgal cells can further be made in form of powers through the process of dehydrating and drying. Optimized treatment conditions regarding extraction and transesterification reaction of microalgal oils, which need to be further developed and explored, as well play an important role in the effective biofuel target products[67-70].

Due to miniature and hardiness of microalgal cells, it is usually difficult for the extraction of microalgal lipid component which often requires the operation of special treatment (such as cell wall breaking, pressurizing and heating etc.) to achieve more complete extraction effect. Traditionally, there could be several methods used in extraction of microalgal lipid to get information on lipid content of biological samples, for instance, the Soxhlet extraction method by using organic solvents for biological sample treatment and sample-heating treatment with some strong inorganic acids and so on.[71-74]. In spite of simplicity and easiness regarding those methods, obvious disadvantages are time-consuming for the analysis and operational treatment. Moreover, a considerable amount of organic solvents or other acid substances, which could involve with human health problem and the pollution of the environment, are often used in a sample-treating process. Supercritical CO2 extraction method for extracting biological sample lipid is quite effective, but it requires expensive equipment to complete sample analysis[75-76].
In order to consume less toxic, less amount of organic solvents and obtain higher algal lipid yield result, it is crucial to adopt appropriate methods and conditions for lipid extraction of microalgal materials. Accelerated solvent extraction (ASE) is one of the best methods for extraction of microalgal lipid with a small amount of organic solvents needed in extraction treatment. In this investigation, we carried out the experimental
study on optimalization of extraction conditions for increasing microalgal lipid yield by using accelerated solvent extraction method based on applying an orthogonal array design (OAD). Experimental factors including extraction solvent (hexane, chloroform, petroleum ether, ethanol, acetone), temperature (75-175 °C), time (4–20 min) and extraction cycle number (1–5) at five-levels were studied in 25 trials by OAD_{25} (5⁵) to reach rapid and high lipid extraction for the marine microalga (\textit{Nannochloropsis oculata} Droop).

The objectives of this study were:

1) to determine which factors might have more significant effects than the others on the extraction of microalgal lipid;
2) to obtain the optimum level of each tested factor; and
3) to determine a best combination of the 4 tested factors with 5 factorial levels to be used as increasing extraction efficiency for microalgal lipid yield.

2. Materials and methods

2.1. Cultivation and treatment of microalgal species for experiment study

The strain of marine microalga (\textit{Nannochloropsis oculata} Droop) was from our laboratory storage and used for batch culture step by step to sufficient quantity of algal cells. The microalga was cultivated with general enriched seawater f/2-Si medium designed for growing coastal marine algae (Guillard and Ryther 1962). The microalga was grown under regulated and controlled conditions (water temper 25°C, light intensity 5000lux, salinity 30‰, PH 7.8) and harvested during log growth phase. Microalgal cells from collection liquid were condensed by a centrifugal treatment process and desalinated after two times of distilled water washing and centrifugal treatment and prepared in form of microalgal powder by using freeze drying process for extraction of microalgal lipid.

2.2. Chemical reagents and instrument used in the experiment

All chemicals and reagents used in this experimental study were analytical or research grade without further purification and from Xiamen Luyin Chemical Company. Instrument accelerated solvent extractor ASE 100 (Dionex) was used for extraction of microalgal lipid.

2.3. Experimental designation of ASE method for the extraction of microalgal lipid

Four factors with five levels each were designed for their effects to be investigated on the extraction of microalgal lipid with orthogonal array design. An orthogonal array table OAD_{25} (5⁵) was used for designing ASE experiment of microalgal lipid extraction. Experimental designation for different factors and levels influencing the extraction of microalgal lipid was arranged in following table 1.
Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

<table>
<thead>
<tr>
<th>factor</th>
<th>level</th>
<th>A extraction solvent</th>
<th>B extraction tem.</th>
<th>C extraction time (min)</th>
<th>D extraction cycle times</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>hexane</td>
<td>75</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>chloroform</td>
<td>100</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>petroleum ether</td>
<td>125</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>ethanol</td>
<td>150</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>acetone</td>
<td>175</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1. Designation of factors and levels for lipid extraction by ASE method

2.4. Operational method of ASE extraction and instrument analytical conditions

Extraction operation process of microalgal lipid and parameter settings: Appropriate amount of about 5g (5.120 ± 0.076g) of microalgae powder samples was put into 34ml extraction pool of the instrument. The extraction pressure value was constant at 1500psi. Based on combination of different factors and levels of five types of different extraction solvents (hexane, chloroform, petroleum ether, ethanol, acetone), extraction temperature range from 75~175 °C, extraction time range from 4~20 min, extraction cycle number for 1~5 times, corresponding operational treatment of ASE microalgal lipid extraction was adopted according to 4 factors and 5 levels of orthogonal experiment set (refer to Table2). Other relevant extraction parameters were constantly set as 60% of flush volume and 90s of purge time for microalgal lipid extraction. Microalgal lipid extracted was steam-dried by a rotary evaporator and further dried via N2 gas blowing process and finally dried at 100 °C for 2 hours. The final microalgal lipid quantity extracted for different ASE operation was expressed as lipid % based on algal dry weight.

2.5. Conventional Soxhlet extraction method for microalgal lipid

The extraction of microalgal lipid was concurrently conducted by conventional classic Soxhlet and using same extraction solvents to compare extraction efficiency with ASE method. Appropriate amount of microalgae powder samples mixed with quartz sand particles was ground in a mortar and then transferred to extraction cylinder of the extractor. Solvent extraction included the process with 18 hours of Static extraction and 6 hours of dynamic extraction to reach a thorough extraction. The final microalgal lipid quantity extracted was expressed as lipid % based on algal dry weight.

2.6. Calculation of extraction efficiency increase based on ASE and Soxhlet methods for microalgal lipid

Extraction efficiency increase (EI%) was calculated by formula below:

\[ EI(\%) = 100 \times \frac{A - S}{A} \]
A and S respectively represent the lipid amount (gram) of microalga extracted by the methods of ASE and Soxhlet.

2.7. Data analysis and treatment

The arrangement of importance of the four factors to the extraction of microalgal lipid were evaluated according to the effectiveness of each factor through the calculation of ranges (R value) (determined from the difference between the maximal and minimal lipid content (%) within the five levels of each factor), that means, the factor with the most effectiveness (i.e., with the largest range of R value) to the extraction of microalgal lipid is considered as the most important factor, the factor with the lest effectiveness (i.e., with the smallest range of R value) to the extraction of microalgal lipid is considered as the lest important factor. Analysis of variance (ANOVA) was conducted to test the significance of the effects of the four factors on the extraction of microalgal lipid by using statistical software SPSS 15.0. In all analyses, the level of significance was set at a P-value of 0.05.

3. Results

3.1. The orthogonal experiment result and analysis

The orthogonal experiment result and analysis based on 4 factors and 5 levels was shown in Table 2 for ASE extraction of microalgal lipid and the associated variance analysis result shown in Table 3. Variation trend of extraction efficiency of microalgal lipid was shown in Figure 2 for different extraction operations with various factor level values.

The experimental results indicated that: by using different extraction solvents and various combinations of different extraction operations, lipid content (%) had the apparent difference (range between 2.98%~21.36%). This suggests that different extraction treatments on microalgal cells result in the difference in lipid yield of the microalgae. Solvents chloroform, hexane and petroleum ether had normally poor extraction effect on microalgal cells, and the anhydrous ethanol and acetone were good extraction solvents for microalgal lipid. Calculation results of range of R value based on table 2 test experiment reflect the size of the corresponding factor effect. Compared to those factors with smaller R value, the factors with greater R value are generally significant factors to make remarkable influence on lipid extraction of microalgal cells since more difference of lipid yield occurs at the different levels of those factors. Our experimental results showed that, the R values caused by the extraction solvent, extraction temperature, extraction time and extraction cycles were respectively 25.91, 38.85, 16.44 and 16.67. Therefore, according to the size of the R values, the significance of test factors for accelerated solvent extraction (ASE) of microalgal lipid may be arranged as: extraction solvent, extraction temperature, extraction cycle, extraction time. Variance analysis of the results of accelerated solvent extraction (ASE) processing experiment data further indicated (Table 3), extraction effect of temperature on the microalgal lipid was significant (P =0.000515), followed by significant extraction effect of solvents (P =0.003855).The significant extraction effect of extraction time at significant level
Optimalization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

Comparatively, the effect of extraction cycles on microalgal lipid was relatively small and it was not significant ($P = 0.081996$) at the significance level set for $a = 0.05$.

<table>
<thead>
<tr>
<th>trial no</th>
<th>extraction solvent</th>
<th>extraction T min</th>
<th>cycle times</th>
<th>lipid (%) (alga DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hexane</td>
<td>75</td>
<td>4</td>
<td>1</td>
</tr>
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<td>2</td>
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<td>8</td>
<td>2</td>
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<td>hexane</td>
<td>125</td>
<td>12</td>
<td>3</td>
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<tr>
<td>4</td>
<td>hexane</td>
<td>150</td>
<td>16</td>
<td>4</td>
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<tr>
<td>5</td>
<td>hexane</td>
<td>175</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>chloroform</td>
<td>75</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>chloroform</td>
<td>100</td>
<td>12</td>
<td>4</td>
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<tr>
<td>8</td>
<td>chloroform</td>
<td>125</td>
<td>16</td>
<td>5</td>
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<tr>
<td>9</td>
<td>chloroform</td>
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<td>20</td>
<td>1</td>
</tr>
<tr>
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<td>chloroform</td>
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</tr>
<tr>
<td>11</td>
<td>petroleum ether</td>
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<td>5</td>
</tr>
<tr>
<td>12</td>
<td>petroleum ether</td>
<td>100</td>
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<td>petroleum ether</td>
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<td>3</td>
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<td>petroleum ether</td>
<td>175</td>
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<td>4</td>
</tr>
<tr>
<td>16</td>
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<td>ethanol</td>
<td>125</td>
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<td>20</td>
<td>ethanol</td>
<td>175</td>
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<td>acetone</td>
<td>100</td>
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<td>5</td>
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<tr>
<td>23</td>
<td>acetone</td>
<td>125</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>acetone</td>
<td>150</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>acetone</td>
<td>175</td>
<td>16</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Result and analysis of orthogonal experimentation by ASE method

| level I  | 49.15 | 41.89 | 53.01 | 50.12 | $\sum=302.09$ |
| level II | 60.65 | 47.97 | 58.00 | 58.33 |              |
| level III| 48.80 | 62.82 | 53.47 | 66.79 |              |
| level VI | 75.06 | 68.68 | 68.17 | 65.06 |              |
| level V  | 68.43 | 80.74 | 69.45 | 61.80 |              |
| Value R  | 25.91 | 38.85 | 16.44 | 16.67 |              |
Table 3. Variance analysis of orthogonal experimentation by ASE method

<table>
<thead>
<tr>
<th>source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. solvent</td>
<td>108.117</td>
<td>4</td>
<td>27.029</td>
<td>9.564</td>
<td>0.003855</td>
<td>***</td>
</tr>
<tr>
<td>B. temperature</td>
<td>197.100</td>
<td>4</td>
<td>49.275</td>
<td>17.435</td>
<td>0.000515</td>
<td>***</td>
</tr>
<tr>
<td>C. time</td>
<td>50.116</td>
<td>4</td>
<td>12.529</td>
<td>4.433</td>
<td>0.035094</td>
<td>**</td>
</tr>
<tr>
<td>D. cycle</td>
<td>34.878</td>
<td>4</td>
<td>8.719</td>
<td>3.085</td>
<td>0.081996</td>
<td></td>
</tr>
<tr>
<td>error</td>
<td>22.610</td>
<td>8</td>
<td>2.826</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>sum</td>
<td>412.820</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of squares; df = degrees of freedom; MS = mean squares

Figure 2 also demonstrated the variation trend of lipid extraction for different factors and levels of operational conditions based on ASE method. For extraction solvents, ethanol and acetone had the best extraction effect for microalgal lipid extraction, followed by hexane and chloroform and solvent petroleum had the poorest extraction effect for microalgal lipid. The lipid yield raised with an increase in extraction temperature or extraction time and reached the maximum at a temperature of 175 °C, extraction time of 16min and 3 extraction cycles. Therefore for operational simplicity, it was not necessary for extraction process of microalgal lipid to take more than 16min and 3 extraction cycles.

Taking into consideration the optimal extraction effect for lipid yield of microalgal cells based on the R values of orthogonal experimental data and the results of variance analysis of factors and levels, the best operational parameters for ASE method are: using extraction solvents of ethanol or acetone, extraction temperature of 175°, extraction time of 16min and 3 extraction cycles, which resulted in the highest lipid production. For the health and cost...
Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

consideration, it is more preferable for using ethanol in lipid extraction operation in due to its relatively less toxicity and price.

3.2. Validation of optimal ASE extraction conditions and comparison of extraction effectiveness

Based on the results of orthogonal experiment and data analysis, it was observed that optimized ASE treatment for the extraction of microalgal lipid was using ethanol or acetone as solvents with other operational parameters such as 1500psi of extraction pressure, 175° of extraction temperature, 16 minutes of extraction time and three extraction cycle. To evaluate the stability and superiority of optimized ASE treatment effect for the extraction of microalgal lipid, a comparison was made between the optimized ASE treatment and conventional Soxhlet method for the extraction effectiveness of microalgal lipid. The results were shown in Table 4.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Microalgal powder (g,dw)</th>
<th>Extracted lipid (g)</th>
<th>Lipid %based on micro-algal dw</th>
<th>AI%</th>
<th>Microalgal powder (g,dw)</th>
<th>Extracted lipid (g)</th>
<th>Lipid %based on micro-algal dw</th>
<th>AI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0578</td>
<td>0.9805</td>
<td>19.57</td>
<td>44.19</td>
<td>5.0140</td>
<td>0.9810</td>
<td>19.57</td>
<td>44.19</td>
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<tr>
<td>2</td>
<td>5.0637</td>
<td>1.0356</td>
<td>20.45</td>
<td>47.09</td>
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<td>3</td>
<td>5.0504</td>
<td>0.9908</td>
<td>19.62</td>
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<td>5.0521</td>
<td>1.0012</td>
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<td>43.95</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>5.0735</td>
<td>0.9739</td>
<td>19.20</td>
<td>43.63</td>
<td>5.0856</td>
<td>1.0658</td>
<td>20.96</td>
<td>43.12</td>
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<tr>
<td>6</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>5.0040</td>
<td>1.0687</td>
<td>21.36</td>
<td>44.19</td>
</tr>
<tr>
<td>(X)</td>
<td>5.0658</td>
<td>0.9917</td>
<td>19.62</td>
<td>44.70</td>
<td>5.0844</td>
<td>1.0437</td>
<td>20.53</td>
<td>41.87</td>
</tr>
<tr>
<td>(±SD)</td>
<td>0.0131</td>
<td>0.0253</td>
<td>0.50</td>
<td>1.42</td>
<td>0.0108</td>
<td>0.0414</td>
<td>0.73</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Note: For conventional Soxhlet method, 1.0 gram of microalgal powder was used for lipid extraction using same extraction solvents. Microalgal yields were respectively 0.1082 gram with calculated lipid % as 10.82% (based on algal dry weight) for using ethanol extraction and 0.1192 gram with calculated lipid % as 11.92% (based on algal dry weight) for using acetone extraction.

Table 4. Validation of optimal ASE extraction conditions and comparison of extraction effectiveness

Table four results clearly showed that the fluctuation of lipid extraction yield was very small and extraction effect was quite stable for optimum processing conditions of accelerated solvent extraction (ASE) for microalgal lipid extraction. Not only were the extraction process time and extraction solvent volume considerably saved, but also lipid extraction effectiveness were greatly improved in ASE method. Compared with conventional Soxhlet extraction method, ASE method with ethanol as extraction solvent, extraction efficiency could increase 43.63-47.09% (mean ± SD 44.70 ± 1.42%). For using acetone as the extraction solvent extraction efficiency of ASE method could increase 39.08-44.19% (mean ± SD to 41.87 ± 2.10%). Therefore, adopting the optimum processing conditions of ASE method for microalgal lipid extraction can reach maximum microalgal lipid yield and its lipid extraction efficiency is obviously higher than conventional Soxhlet extraction method.
4. Discussion

The orthogonal array design is a useful experiment methodology, especially for multi-factor experiment and analysis. It can provide useful and sufficient information for accessing and evaluating main factors and the optimum combination of factor levels for target parameter as less experimental trials as possible\textsuperscript{[77-80]}. ASE method is approved for use by the U.S. EPA and CLP Program and a good one for extracts in treating many different samples. Extractions that normally take hours can be done in minutes using Accelerated Solvent Extraction (ASE). Compared to techniques like Soxhlet and sonication, ASE generates results in a fraction of the time. In addition to speed, ASE offers a lower cost per sample than other techniques by reducing solvent consumption by up to 90%. Relatively less extraction time, reduction in solvent consumption and wide range of application are the essential advantages of ASE method. By using conventional liquid solvents at elevated temperatures and pressures, ASE increases the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, and elevated pressure keeps the solvent below its boiling point, thus enabling safe and rapid extractions. Although ASE uses the same aqueous and organic solvents as traditional extraction methods, it uses them more efficiently. ASE can be used to replace Soxhlet, sonication, wrist shaking, and other extraction techniques typically used\textsuperscript{[81]}

Because the ASE method requires relatively simple equipment with many aspects of advantages such as higher degree of automation, good safety, less solvent consumption, fast-complete extraction and high efficiency, it has been widely applied in analyzing and testing various types of samples from different sources. For instance, it can be used to detect the extracts from the water, soil, sediment, minerals, chemical products, biological samples (vegetables, fruits, meat, fish, plants) and other harmful substances (such as various pesticides, hydrocarbons, chemicals and the like)\textsuperscript{[82-93]}. ASE technology is very important and helpful for environmental protection and human health. For determination and evaluation of bio-active components from animals and plants, especially for separation, extraction and purification of Chinese traditional herb medicines, ASE also played an important role\textsuperscript{[94-97]}. For example, ASE method has been applied for extraction of phenolic acid compound salvia, volatile oil from Mu Xiang, almond oil from plants, saponin from Ginseng. Many relevant studies indicate that target product yield and extraction efficiency are higher by using ASE method than conventional types of extraction techniques\textsuperscript{[98-100]}. Our present study also showed extraction efficiency for microalgal lipid has an increase of 39.08-47.09\% by using ASE method, compared with conventional Soxhlet extraction method. This suggest that ASE technology has the wide applicability in different fields of sample extraction.

Due to different features and characteristics with extract of the target products, the application of ASE method should depend on the actual situations and determine appropriate parameter settings for extraction processing in order to obtain the practical optimal extraction results. For example, using methanol as solvent extraction with
Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

extraction parameters set as pressure 1500 psi, temperature 140°C, time 5 minutes, extraction cycle number 2, ginseng saponins could reach the maximum extraction amount, which was 25.88-58.68% of higher than other conventional extraction methods (such as immersion method, ultrasonic method, homogenization, mechanical vibration method)\(^9\). Zhang \textit{et al} (2007) reported the results of extraction of flavonoid compounds in citrus peels using optimum ASE operational conditions and showed that maximum extraction rate of target products was obtained with using 80% ethanol as solvent, pressure 10.3 Mpa, temperature 70°C, time of 10 minutes, extraction cycle number 1 \(^9\). Pang \textit{et al} (2007) used uniform experimental design method and reported extraction of almond kernel oil with the optimum ASE process operations. The results showed that the maximum amount of oil extraction was obtained by using acetone: hexane (1:3) as solvent, the temperature of 120-140°C, time 6-12 minutes, extraction cycle number 1-3\(^9\). Herrero (2005) studied the extraction of bioactive products for microalgae (\textit{Spirulina platensis}). The optimal ASE extraction conditions of antioxidant compounds of the microalgae were using ethanol as solvent, temperature 170°C, time 3-9 minutes, which resulted in target product extraction as high as 19.7% (based on algal dry weight) compared to only 2.94-8.22% of antioxidant compound extraction by using the other three types of solvents (hexane, petroleum ether, water) in the same extraction conditions \(^1\). This suggests that extraction parameter setting for target product extraction is crucial for the extraction result of applying ASE method. In summary, determination of the optimal extraction conditions should depend on different samples, target products and the experimental designs in application of ASE method.

Our present study was involved with the extraction of microalgal lipid by using ASE method. This study clearly showed that an increase of temperature and pressure during the extraction process could greatly enhance the solvent penetration and diffusion capacity, thereby result in a rapid extraction of microalgal lipid components. Compared with the conventional Soxhlet extraction method, ASE method with the optimal operational conditions could significantly improve the microalgal lipid extraction and raise 39.08-47.09% of lipid extraction efficiency. For a consideration of security and practicability, using ethanol or acetone with the lowest toxicity as extraction solvents is another advantage for sample treatment. Therefore, using ASE method with the optimization of extraction conditions is suitable for the rapid and efficient extraction of microalgal lipid.

5. Conclusion

Our findings in the present investigation demonstrated that accelerated solvent extraction method (ASE) based on the orthogonal array design is an effective approach for the extraction and determination of lipid content in biological microalgal samples. This study also demonstrated that the application of multiple-factor and level experimental design based on Taguchi’s orthogonal array could determine the optimal extraction operation and obtain maximal yield for lipid extraction of microalgae.
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6. References


Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design


Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design

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Optimization of Extraction Conditions for Increasing Microalgal Lipid Yield by Using Accelerated Solvent Extraction Method (ASE) Based on the Orthogonal Array Design


