We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 9

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

Lin Rulong, Cai Wenxuan, Xing Bingpeng and Ke Xiurong

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52475

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[41-45]. 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 transesterfication 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]..

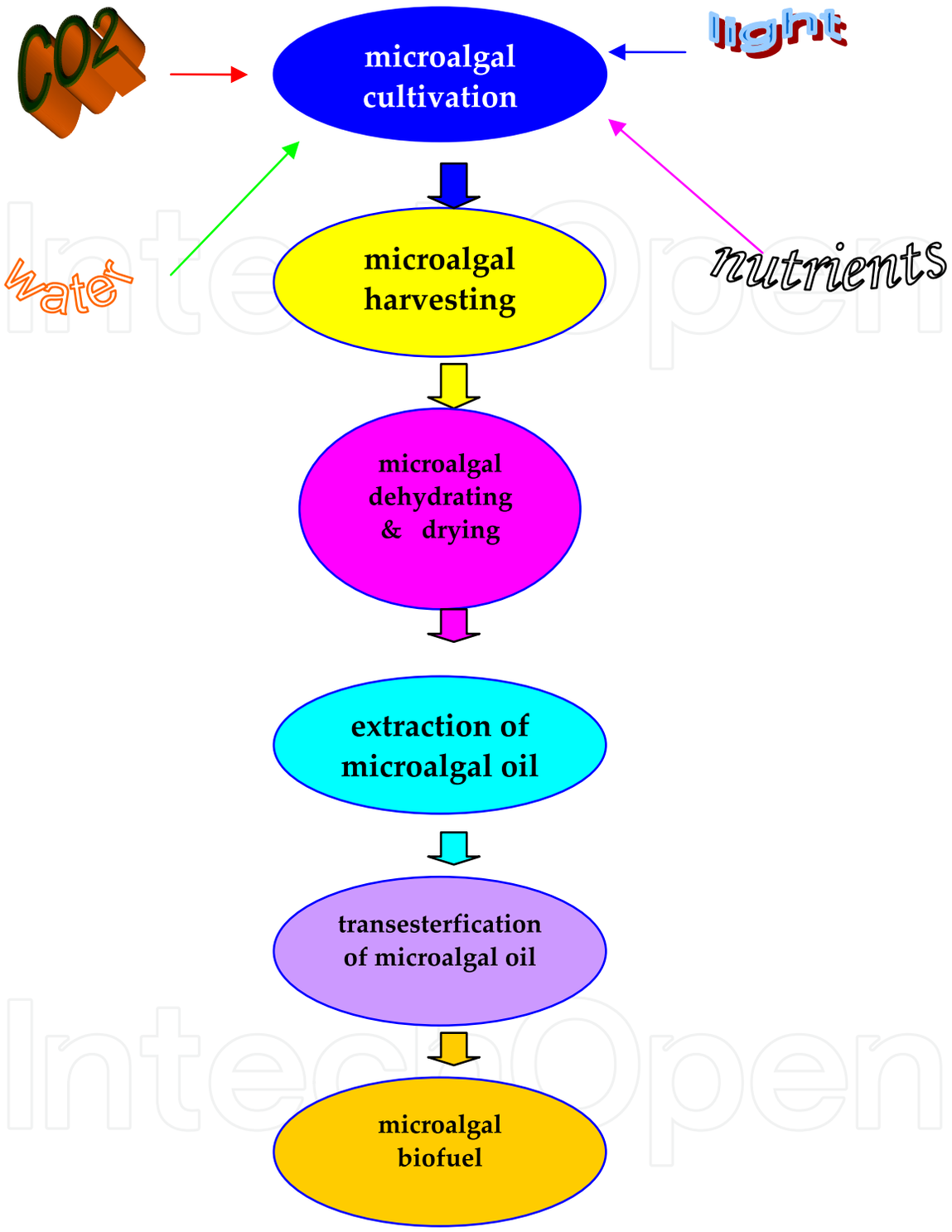

Figure 1. Schematic diagram of biotechnical process of microalgal biofuel preparation

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} (5^6) to reach rapid and high lipid extraction for the marine microalga （*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 factoral 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（*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 25C, 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 intrument 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. Intrument 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} (5^6) 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.

factor / level	A extraction solvent	B extraction tem. ◎	C extraction time min	D extraction cycle times
1	hexane	75	4	1
2	chloroform	100	8	2
3	petroleum ether	125	12	3
4	ethanol	150	16	4
5	acetone	175	20	5

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 ℃, 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 ℃ 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 ammout of microalgal power 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:

$$EI(\%)=100\times A\text{-}S)/A$$

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 microalga. 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: extract temperature, extraction solvent, 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

of a =0.05 was also observed (P =0.035094). 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.

factor / trial no	A extraction solvent	B extraction T ◎	C extraction time min	D cycle times	lipid (%) (alga DW)
1	hexane	75	4	1	2.98
2	hexane	100	8	2	5.76
3	hexane	125	12	3	8.52
4	hexane	150	16	4	15.74
5	hexane	175	20	5	16.15
6	chloroform	75	8	3	10.05
7	chloroform	100	12	4	10.21
8	chloroform	125	16	5	13.37
9	chloroform	150	20	1	13.10
10	chloroform	175	4	2	13.92
11	petroleum ether	75	12	5	5.81
12	petroleum ether	100	16	1	5.44
13	petroleum ether	125	20	2	13.32
14	petroleum ether	150	4	3	10.77
15	petroleum ether	175	8	4	13.45
16	ethanol	75	16	2	12.25
17	ethanol	100	20	3	16.09
18	ethanol	125	4	4	14.87
19	ethanol	150	8	5	15.99
20	ethanol	175	12	1	15.86
21	acetone	75	20	4	10.79
22	acetone	100	4	5	10.47
23	acetone	125	8	1	12.74
24	acetone	150	12	2	13.07
25	acetone	175	16	3	21.36
level I	49.15	41.89	53.01	50.12	∑=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 2. Result and analysis of orthogonal experimentation by ASE method

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

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

Table 3. Variance analysis of orthogonal experimentation by ASE method

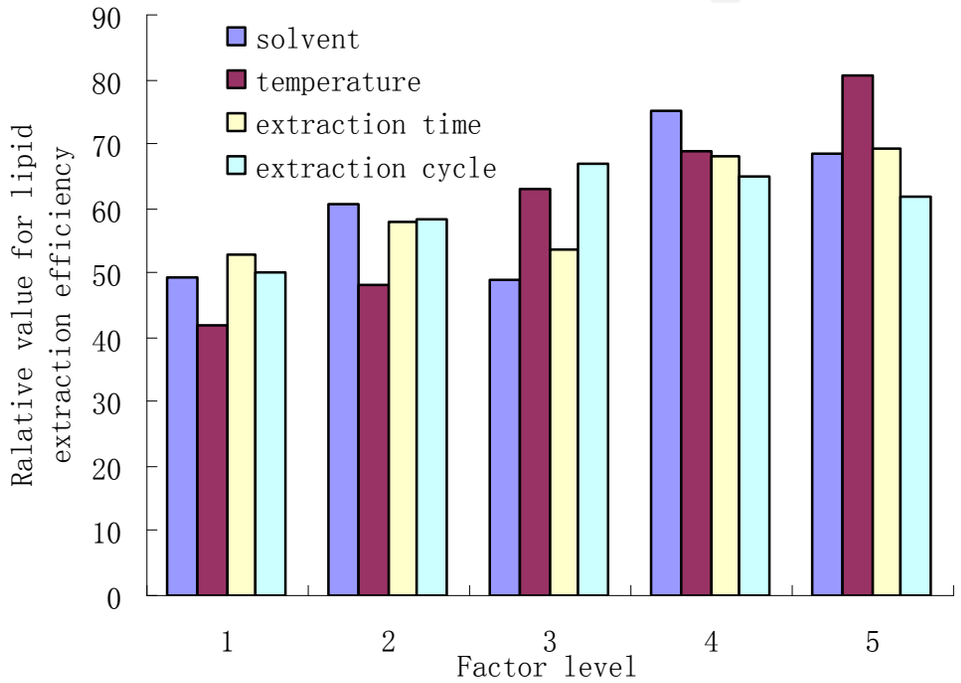

Figure 2. Variation trend of lipid extraction for different factors and levels

Figure 2 also demonstrated the variation trend of extraction effect of microalgal lipid 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 ℃,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

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.

Trial #	ethanol（ASE）				acetone（ASE）			
	Microalgal powder (g.dw)	Extracted lipid(g)	Lipid %based on micro-algal dw	AI%	Microalgal powder (g.dw)	Extracted lipid(g)	Lipid %based on microalgal dw	AI%
1	5.0578	0.9805	19.57	44.19	5.0140	0.9810	19.57	39.08
2	5.0637	1.0356	20.45	47.09	5.0616	1.0694	21.13	43.58
3	5.0504	0.9908	19.62	44.85	5.0521	1.0012	19.82	39.85
4	5.0838	0.9779	19.24	43.75	5.2892	1.0763	20.35	41.42
5	5.0735	0.9739	19.20	43.63	5.0856	1.0658	20.96	43.12
6	/	/	/	/	5.0040	1.0687	21.36	44.19
($\overline{X}$)	5.0658	0.9917	19.62	44.70	5.0844	1.0437	20.53	41.87
(±SD)	0.0131	0.0253	0.50	1.42	0.1048	0.0414	0.73	2.10

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 [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 essencial 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[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) [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 [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[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

extraction parameters set as pressure 1500psi, temperature 140◎, 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) [98]. Zhang *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 10.3Mpa, temperature 70 ◎, time of 10 minutes, extraction cycle number 1 [99] . Pang *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 ◎, time 6-12 minutes, extraction cycle number 1-3[100]. Herrero (2005) studied the extraction of bioactive products for microalga (*Spirulina platensis*).The optimal ASE extraction conditions of antioxidant compounds of the microalga were using ethanol as solvent, temperature 170◎, 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 [101]. 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.

Author details

Lin Rulong, Cai Wenxuan, Xing Bingpeng and Ke Xiurong
Key Laboratory of Global Change and Marine-Atmospheric Chemistry,
Third Institute of Oceanography,State Oceanic Administration, Xiamen, China

Acknowledgement

This study was supported by special research project fundings of China National Marine Public Welfare Industry (grant number200705025 and grant number 200705025).

6. References

[1] Abdus Salam, Hassan Al Mamoon, Md. Basir Ullah, Shah M. Ullah. Measurement of the atmospheric aerosol particle size distribution in a highly polluted mega-city in Southeast Asia (Dhaka-Bangladesh). *Atmospheric Environment.* 2012, 59:338-343.

[2] Jing Liu, Xiaoqian Ma. The analysis on energy and environmental impacts of microalgae-based fuel methanol in China. *Energy Policy,* 2009, 37(4): 1479-1488.

[3] Kakali Mukhopadhyay, Osmo Forssell. An empirical investigation of air pollution from fossil fuel combustion and its impact on health in India during 1973–1974 to 1996–1997. *Ecological Economics,* 2005, 55(2): 235-250.

[4] Xiaoping Wang, Denise L. Mauzerall. Evaluating impacts of air pollution in China on public health: Implications for future air pollution and energy policies. *Atmospheric Environment,* 2006, 40(9): 1706-1721.

[5] Jasmin Honold, Reinhard Beyer, Tobia Lakes, Elke van der Meer. Multiple environmental burdens and neighborhood-related health of city residents. *Journal of Environmental Psychology,* 2012, 32(4): 305-317.

[6] Marilena Kampa,Elias Castanas. Human health effects of air pollution.*Environmental Pollution,* 2008, 151(2): 362-367.

[7] J. Schwartz. Long-Term Effects of Particulate Air Pollution on Human Health. *Encyclopedia of Environmental Health,* 2011, 520-527.

[8] G.D. Thurston. Outdoor Air Pollution: Sources, Atmospheric Transport, and Human Health Effects. *International Encyclopedia of Public Health,* 2008, 700-712.

[9] Kira Matus, Kyung-Min Nam, Noelle E. Selin, Lok N. Lamsal, John M. Reilly, Sergey Paltsev. Health damages from air pollution in China. *Global Environmental Change,* 2012, 22(1): 55-66.

[10] Kenneth Donaldson, William MacNee. Potential mechanisms of adverse pulmonary and cardiovascular effects of particulate air pollution (PM10). *International Journal of Hygiene and Environmental Health,* 2001, 203(5-6): 411-415.

[11] Jane V. Hall, Victor Brajer, Frederick W. Lurmann. Air pollution, health and economic benefits—Lessons from 20 years of analysis. *Ecological Economics,* 2010, 69(12): 2590-2597.

[12] Klara Slezakova, Dionísia Castro & Arlindo Begonha *et. al.* Air pollution from traffic emissions in Oporto, Portugal: Health and environmental implications. *Microchemical Journal,* 2011, 99(1): 51-59.
[13] B.R. Gurjar, A. Jain, A. Sharma, A. & Agarwal, *et. al.*Human health risks in megacities due to air pollution. *Atmospheric Environment,* 2010, 44(36): 4606-4613.
[14] Janet Currie, Matthew Neidell, Johannes F. Schmieder. Air pollution and infant health: Lessons from New Jersey. *Journal of Health Economics,* 2009, 28(3): 688-703.
[15] Leigh A. Beamish, Alvaro R. Osornio-Vargas, Eytan Wine. Air pollution: An environmental factor contributing to intestinal disease. *Journal of Crohn's and Colitis,* 2011, 5(4): 279-286.
[16] Ni Bai, Majid Khazaei, Stephan F. van Eeden, Ismail Laher. The pharmacology of particulate matter air pollution-induced cardiovascular dysfunction. *Pharmacology & Therapeutics,* 2007, 113(1): 16-29.
[17] Mata T M, Martins A A, Caetano N S. Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews, 2010, 14(1): 217-232.
[18] Hossain A B M, Salleh A. Biodiesel fuel production from algae as renewable energy. *American Journal of Biochemistry and Biotechnology,* 2008, 4(3): 250-254.
[19] Song D. H, Fu J J. and Shi D. J. Exploitation of oil-bearing microalgae for biodiesel. *Chinses Journal of Biotechnology,* 2008, 24(3): 341-348.
[20] Cantrell, K.B. and Walker, T.H. 2009. Influence of temperature on growth and peak oil biosynthesis in a carbon-limited medium by pythium irregular. *Journal of the American Oil Chemists Society* 86 (8):791–797.
[21] Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*. 25(3): 294–306.
[22] Cooney, M., Young, G. and Nagle, N. 2009. Extraction of bio-oils from microalgae.*Separation and Purification Reviews.* 38 (4): 291–325.
[23] He hongbo, Yao yisheng; Jiang laien. Research progress of biodiesel preparation. *Anhui Chemical Industry,* 2008, 34(6):7-10.
[24] Evan Stephens, Ian L. Ross, Jan H. Mussgnug, Liam D. Wagner, Michael A. Borowitzka, Clemens Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25(3), 294-306.
[25] Gouveia, L., Oliveira, A.C. 2008. Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology & Biotechnology*, 36(2): 269-274.
[26] Posten, Olaf Kruse, Ben Hankamer. 2010. Future prospects of microalgal biofuel production systems. *Trends in Plant Science*. 15(10): 554-564.
[27] Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., Kruse, O., Hankamer, B. 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research*, 1(1): 20-43.
[28] Wang Y Y, Wang J N, Gu B J. Research progress of biodiesel preparation method. *Modernizing Agriculture,* 2011(3): 40-42.
[29] Robles M A, Gonzalez M P A, Est Eban C L, *et al*. Bio catalysis: Towards ever greener bio diesel production. *Biotechnology Advances,* 2009, 27 (4) : 398-408.
[30] Miao X L, Wu Q Y. Study on preparation of biodiesel from microalgal oil. *Acta Energiae Solaris Sinica*. 2007, 28(2): 219-222.

[31] Fajardo, A.R., Cerdan, L.E., Medina, A.R., Fernandez, F.G.A., Moreno, P.A.G.. and Grima, E.M., 2007. Lipid extraction from the microalga *Phaeodactylum tricornutum*. *European Journal of Lipid Science and Technology.* 109(2): 120–126.

[32] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production:perspectives and advances. *Plant Journal.* 54 (4): 621–639.

[33] Lalman, J.A. and Bagley, D.M. 2004. Extracting long-chain fatty acids from a fermentation medium. *Journal of the American Oil Chemists Society*. 81 (2): 105–110.

[34] Lee, S.J., Yoon, B.D.and Oh, H.M., 1998. Rapid method for the determination of lipidfrom the green alga *Botryococcus braunii. Biotechnology Techniques.* 12 (7): 553–556.

[35] Liu, B.and Zhao, Z. 2007. Biodiesel production by direct methanolysis of oleaginousmicrobial biomass. *Journal of Chemical Technology and Biotechnology.* 82 (8):775–780.

[36] Liu, X.J., Jiang, Y.and Chen, F., 2005. Fatty acid profile of the edible filamentous cyanobacterium Nostoc flagelliforme at different temperatures and developmental stages in liquid suspension culture. *Process Biochemistry.* 40 (1): 371–377.

[37] Molina Grima, E., Robles Medina, A., Gimenez Gimenez, A., Sanchez Perez, J.A.,Garcia Camacho, F.and Garcia Sanchez, J.L., 1994. Comparison between extraction of lipids and fatty-acids from microalgal biomass. *Journal of the American Oil Chemists Society*. 71 (9): 955–959.

[38] Rittmann, B.E. 2008. Opportunities for renewable bioenergy using microorganisms.*Biotechnology and Bioengineering*. 100 (2): 203–212.

[39] Sheng, J., Vannela, R.and Rittmann,B.E. 2011. Evaluation of methods to extract and quantify lipids from *Synechocystis* PCC 6803. *Bioresource Technology.* 102: 1697–1703.

[40] Xu, H., Miao, X.L. and Wu, Q.Y. 2006. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *Journal of Biotechnology*. 126 (4): 499–507.

[41] Doan, T.T.Y., Balasubramanian Sivaloganathan B. and Obbard J.P. 2011. Screening of marine microalgae for biodiesel feedstock. *Biomass and Bioenergy*. 35: 2534-2544.

[42] Glacio S. Araujo, Leonardo J.B.L. Matos, Luciana R.B. Gonçalves, Fabiano A.N. Fernandes, Wladimir R.L. Farias. 2011. Bioprospecting for oil producing microalgal strains: Evaluation of oil and biomass production for ten microalgal strains. *Bioresource Technology*. 102(8): 5248-5250.

[43] Griffiths, M.J., Harrison, S.T.L. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21(5), 493-507.

[44] T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, F. Bux. 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology*. 102(1): 57-70.

[45] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR.2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol Bioeng*. 102(1):100-112.

[46] Guido Breuer, Packo P. Lamers, Dirk E. Martens, René B. Draaisma, René H. Wijffels. 2012. The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation

in nine microalgae strains. *Bioresource Technology*. http://dx.doi.org/10.1016/j.biortech.2012.08.003.

[47] Lv, J.M., Cheng, L.H., Xu, X.H., Zhang, L., Chen, H.L. 2010. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. *Bioresource Technology*, 101(17): 6797-804.

[48] Pal, D., Khozin-Goldberg, I., Cohen, Z., Boussiba, S. 2011. The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp. *Applied Microbiology and Biotechnology*, 90(4): 1429-41.

[49] Ramasamy Praveenkumar, Kalifulla Shameera, Gopalakrishnan Mahalakshmi, Mohammad Abdulkader Akbarsha, Nooruddin Thajuddin. 2012. Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga Chlorella sp., BUM11008: Evaluation for biodiesel production. *Biomass and Bioenergy.* 37: 60-66.

[50] Santos, A.M., Janssen, M., Lamers, P.P., Evers, W.A., Wijffels, R.H. 2012. Growth of oil accumulating microalga *Neochloris oleoabundans* under alkaline-saline conditions. *Bioresource Technology*. 104: 593-599.

[51] Takagi, M., Karseno, Yoshida, T. 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *Journal of Bioscience and Bioengineering*, 101(3), 223-226.

[52] Nasrin Moazami, Alireza Ashori, Reza Ranjbar, Mehrnoush Tangestani, Roghieh Eghtesadi, Ali Orlando Jorquera, Asher Kiperstok, Emerson A. Sales, Marcelo Embiruçu, Maria L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology.* 101(4): 1406-1413.

[53] Probir Das, Siti Sarah Aziz, Jeffrey Philip Obbard. 2011. Two phase microalgae growth in the open system for enhanced lipid productivity. *Renewable Energy*. 36(9): 2524-2528.

[54] Sheykhi Nejad. 2012. Large-scale biodiesel production using microalgae biomass of *Nannochloropsis*. *Biomass and Bioenergy*. 39: 449-453.

[55] Eleonora Sforza, Alberto Bertucco, Tomas Morosinotto, Giorgio M. Giacometti. Photobioreactors for microalgal growth and oil production with *Nannochloropsis salina*: From lab-scale experiments to large-scale design. *Chem.Eng.Res.Des.*(2011), doi:10.1016/j.cherd.2011.12.002

[56] Niels-Henrik Norsker, Maria J. Barbosa, Marian H. Vermuë, René H. Wijffels. 2011. Microalgal production — A close look at the economics. *Biotechnology Advances.* 29(1): 24-27.

[57] Orlando Jorquera, Asher Kiperstok, Emerson A. Sales, Marcelo Embiruçu, Maria L. Ghirardi. 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology.* 101(4): 1406-1413.

[58] Pruvost, J., Van Vooren, G., Le Gouic, B., Couzinet-Mossion, A., Legrand, J. 2011. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. *Bioresource Technology*, 102(1), 150-158.

[59] E. Sevigné Itoiz, C. Fuentes-Grünewald, C.M. Gasol, E. Garcés, E. Alacid, S. Rossi, J. Rieradevall. 2012. Energy balance and environmental impact analysis of marine

microalgal biomass production for biodiesel generation in a photobioreactor pilot plant. *Biomass and Bioenergy*. 39: 324-335.

[60] H. De la Hoz Siegler, W.C. McCaffrey, R.E. Burrell, A. Ben-Zvi. 2012. Optimization of microalgal productivity using an adaptive, non-linear model based strategy. *Bioresource Technology*.104: 537-546.

[61] Jianhua Fan, Jianke Huang, Yuanguang Li, Feifei Han, Jun Wang, Xinwu Li, Weiliang Wang, Shulan Li. 2012. Sequential heterotrophy–dilution–photoinduction cultivation for efficient microalgal biomass and lipid production. *Bioresource Technology*. 112: 206-211.

[62] Evan S. Beach, Matthew J. Eckelman, Zheng Cui, Laura Brentner, Julie B. Zimmerman. 2012. Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans.BioresourceTechnology*.http://dx.doi.org/10.1016/j.biortech.2012.06.012.

[63] Dong-Geol Kim, Hyun-Joon La, Chi-Yong Ahn, Yong-Ha Park, Hee-Mock Oh. 2011. Harvest of Scenedesmus sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. *Bioresource Technology*. 102(3): 3163-3168.

[64] Hongli Zheng, Zhen Gao, Jilong Yin, Xiaohong Tang, Xiaojun Ji, He Huang. 2012. Harvesting of microalgae by flocculation with poly (γ-glutamic acid). *Bioresource Technology*. 112: 212-220.

[65] Richard M. Knuckey, Malcolm R. Brown, René Robert, Dion M.F. Frampton. 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquacultural Engineering*. 35(3): 300-313.

[66] Zechen Wu, Yi Zhu, Weiya Huang, Chengwu Zhang, Tao Li, Yuanming Zhang, Aifen Li. 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource Technology*. 110: 496-502.

[67] Andrew K. Lee, David M. Lewis, Peter J. Ashman. 2012. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energyrequirements.*Biomass.andBioenergy*.http://dx.doi.org/10.1016/j.biombioe.2012.06.034.

[68] Dang-Thuan Tran, Kuei-Ling Yeh, Ching-Lung Chen, Jo-Shu Chang. 2012. Enzymatic transesterification of microalgal oil from Chlorella vulgaris ESP-31 for biodiesel synthesis using immobilized Burkholderia lipase. *Bioresource Technology.* 108: 119-127.

[69] Jing-Qi Lai, Zhang-Li Hu, Peng-Wei Wang, Zhen Yang. 2012. Enzymatic production of microalgal biodiesel in ionic liquid [BMIm][PF6]. *Fuel.* 95:329-333.

[70] Yuchi Han, Qinxue Wen, Zhiqiang Chen, Pengfei Li. 2011. Review of Methods Used for Microalgal Lipid-Content Analysis. *Energy Procedia*. 12: 944-950.

[71] Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37 (8), 911–917.

[72] Certik M , Andrasi P, Sajbidor J. Effect of extraction methods on lipid yield and fatty acid composition of lipid classes containing γ-2linolenic acid extracted from fungi. *JAOCS* ,1996 ,73 (3):357-365.

[73] Molina G E ,Robles M A ,Gimenez A , et al. Comparision between extraction of lipid and fatty acids from microalgal biomass. *JAOCS* , 1994 ,71 (9): 955-959.

[74] Tran, H.L., Hong, S.J.and Lee, C.G. 2009. Evaluation of extraction methods for recovery of fatty acids from *Botryococcus braunii* LB 572 and *Synechocystis sp.* PCC 6803. *Biotechnology and Bioprocess Engineering.* 14 (2):187–192.

[75] Richterb B E, Ezzell J L, Felix W D, et al. Comparison of accelerated solvent extraction with conventional solvent extraction for organic phosphorus pesticides and herbicides. *LC/ GC*, 1995, (13):390-398.

[76] Mou S F. Principle and application of accelerated solvent extraction. *Environmental chemistry*. 2001, 20(3):299-300.

[77] H. Evangelaras, E. Kolaiti, C. Koukouvinos. Robust parameter design: Optimization of combined array approach with orthogonal arrays.J*ournal of Statistical Planning and Inference.*2006, 136(10):3698-3709.

[78] Yamini Y, Saleh A, Khajeh M. Orthogonal array design for the optimization of supercritical carbon dioxide extraction of platinum(IV) and rhenium(VII) from a solid matrix using cyanex301. *Separation and Purification Technology,* 2008, 61(1): 109-114.

[79] Georgiou S D. Orthogonal designs for computer experiments. *Journal of Statistical Planning and Inference*, 2011, 141(4): 1519-1525.

[80] Abud-Archila M., D.G., Vázquez-Mandujano MA, Ruiz-Cabrera, *et.al*. Optimization of osmotic dehydration of yam bean (Pachyrhizus erosus) using an orthogonal experimental design. *Journal of Food Engineering*, 2008,84(3): 413-419.

[81] http://www.dionex.com/en-us/products/sample-preparation/ase/instruments/lp-81379.html

[82] Schafer R B, Mueller R, Brack W, *et al*. Determination of 10 particle-associated multi class polar and semi-polar pesticides from small streams using accelerated solvent extraction. *Chemosphere*, 2008, 70(11): 1952-1960.

[83] Schafer, K. 1998. Accelerated solvent extraction of lipids for determining thefatty acid composition of biological material. *Analytica Chimica Acta*. 358 (1):69–77.

[84] Reid A M, Brougham C A, Fogarty A M, *et al.* Accelerated solvent-based extraction and enrichment of selected plasticisers and 4-nonylphenol, and extraction of tin from organotin sources in sediments, sludges and leachate soils. *Analytica Chimica Acta,* 2009,634 (2): 197-204.

[85] Zhu X L, Cai J B, Yang J, Su Q D. Determination of Organophosphate Pesticide Residues in Soil by Accelerated Solvent Extraction-Gas Chromatographyic. *Chinese Journal of Analytical Chemistry*. 2005, 33(6):821-824.

[86] Wang L, Mou Y L, Li X C. Determination of organ-phosphorus pesticide in sea sediment by accelerated solvent extraction-gas chromatography /mass spectrometry. *Chinese Journal of Health Laboratory Technology.* 2007, 17(5):769-771.

[87] Gong Y L, Sun W L, Wang S Q, Shen B. A Comparative Study on Extraction of Organic Matters in Source Rocks by Accelerated Solvent Extraction and Soxhlet Extraction. *Rock and Mineral Analysis.* 2009, 28(5):416-422.

[88] Chang C Y, Wang Y F, Ge B K, Liu C. Detection of organic chlorine pesticide residues in fruit and vegetables by using accelerated solvent extraction (ASE) method. *Port Health Control.* 2004, 96(6):25-26.

[89] DIONEX. Extraction of hydrocarbon pollutants in soil using accelerated solvent extraction (ASE) technique. *Environmental Chemistry.* 2009, 28(6):957-958.

[90] DIONEX. Extraction of PCBs in fish using accelerated solvent extraction (ASE) technique. *Environmental Chemistry.* 2008, 27(5):315-316.

[91] Wang P, Zhang Q H, Wang Y W, *et al*. Evaluation of soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. *Analytica Chimica Acta,* 663(1):43-48.

[92] Zhao H X, Wang L P, Qiu Y M, *et al*. Simultaneous determination of three residual barbiturates in pork using accelerated solvent extraction and gas chromatography–mass spectrometry[J]. *Journal of Chromatography* B, 2006, 840(2):139-145.

[93] Hu B Z, Song W H, Xie L P, *et al.*Determination of 33 pesticides in tea using accelerated solvent extraction/gel permeation chromatography and solid phase extraction/gas chromatography-mass spectrometry. *Chinese Journal of Chromatography,* 2008,26(1):22-28.

[94] Zheng C X, Li C E. Accelerated Solvent Extraction Technology in Traditional Chinese Medicine Active Components Analysis. *Chinese Journal of Medicinal Guide.* 2010(10):1820-1821.

[95] Zhao H Q, Chen J H, Guo X C, Zhen X L, Li X C,Wang X R. Fast extraction of alkaloids in Coptis chinensis franch by accelerated solvent extraction. *Chinese Journal of Analysis Laboratory.* 2008, 27(11):5-8.

[96] Chen J H, Li W L, Yang B J, *et al*. Determination of four major saponins in the seeds of Aesculus chinensis Bunge using accelerated solvent extraction followed by high-performance liquid chromatography and electrospray-time of flight mass spectrometry. *Analytica Chimica Acta,* 2007,596(2):273-280.

[97] Chen J H, Wang F M,Liu J, et al. Analysis of alkaloids in Coptis chinensis Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Analytica Chimica Acta,* 2008, 613(2):184-195.

[98] Song W B, Dai Y C, Xu M, Li X G, Yu J. Determination of Ginsenosides in Ginseng by ASE-SPE-LC-UV method. *Modern Scientific Instruments.* 2009,(6):104-108.

[99] Zhang Y, Wu H M, Yu J W, Zhan S L, Wang J Q. Extraction of total flavonoid from orange peel by accelerated solvent extraction. *Food Science and Technology.* 2007,(11):213-215.

[100] Pang X A, Liu W J, Sun H Z, Jin Y X, Wan Y, Ma L, Kong X Y. Application of uniform design to optimize sweet almond oil extraction by accelerated solvent extraction process. *Food and Nutrition in China.* 2007, (4):43-45.

[101] Herrero M, Pedro J, Senorans J. Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga. *Food Chemistry,* 2005, 93(3):417-423.