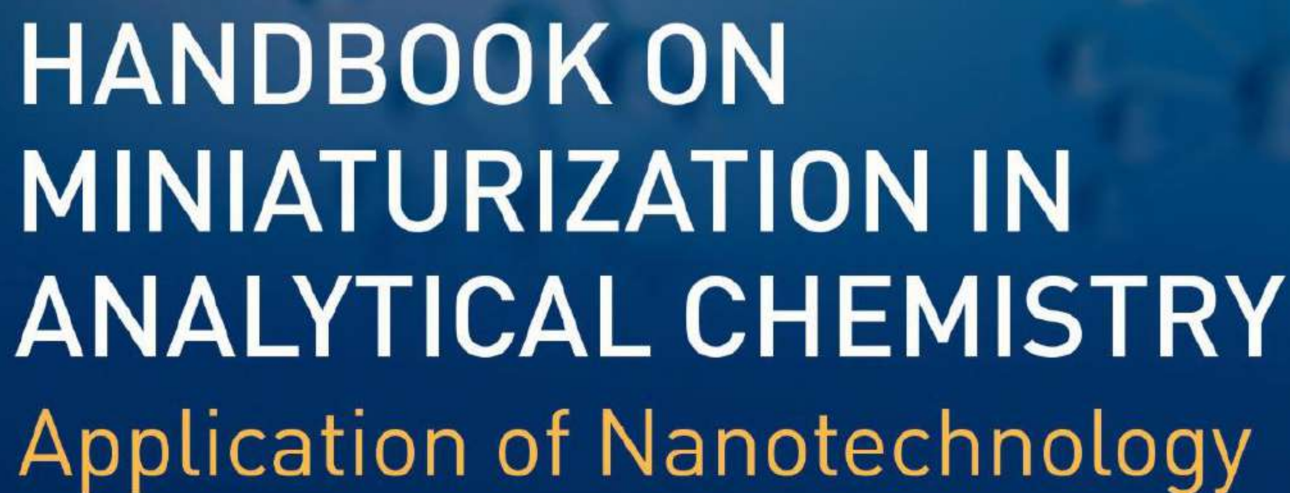
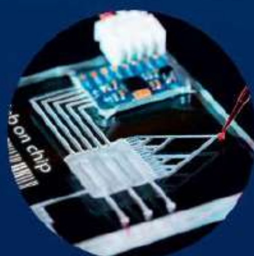


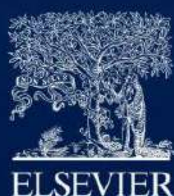
HANDBOOK ON MINIATURIZATION IN ANALYTICAL CHEMISTRY

Application of Nanotechnology



Edited by

Chaudhery Mustansar Hussain



ELSEVIER

Copyrighted material

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

Copyright © 2020 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-819763-9

For information on all Elsevier publications
visit our website at https://www.elsevier.com/books-and-journals

Publisher: Susan Dennis
Acquisitions Editor: Kathryn Eryilmaz
Editorial Project Manager: Lena Sparks
Production Project Manager: Kumar Anbazhagan
Cover Designer: Matthew Limbert

Typeset by SPi Global, India

Copyrighted material

Contents

Contributors xiii
Preface xvii
About the editor xix

1. **Era of nano-lab-on-a-chip (LOC) technology**
Rüstem Keçili, Fatemeh Ghorbani-Bidkorbeh, Ibrahim Dolak, and Chaudhery Mustansar Hussain

1 Introduction 1
2 Lab-on-a-chip technology 2
2.1 Paper-based LOC systems 4
2.2 Centrifugal LOC systems 4
2.3 Droplet-based LOC systems 4
2.4 Digital LOC systems 5
2.5 Surface acoustic wave-based LOC systems 5
3 Applications of LOC technology 5
3.1 LOC platforms for biological applications 5
3.2 LOC platforms for environmental applications 7
3.3 LOC platforms for food applications 9
4 Conclusions 11
References 12

Section A
Sample preparation stage of analysis

2. **Microfluidics in lipid extraction**
Ninad Mehendale, S.P. Jeevan Kumar, Naresh Kumar Mani, Surajbhan Sevda, Sunandan Naha, Swati Sharma, and Vijay Kumar Garlapati

1 Introduction to biodiesel production from lipids 21
2 Positive attributes of microfluidics over existing technologies 22
3 Ideal characteristics of microfluidic devices 23
4 Design of microfluidic devices in lipid extractions from microalgae/oleaginous microbes 24
4.1 Reservoir with filter design 24
4.2 Lab-on-a-disc design 26
4.3 Bead-packed microchannel design for lipid extraction 27
4.4 U-shaped trap split-channel design 28
4.5 Confined impinging jet mixer design 29

Copyrighted material

5 Lipid extractions from microalgae and oleaginous microorganisms 29
6 Future prospects 30
7 Conclusion 30
Websites 32
References 32

3. Microfluidic strategies for extraction and preconcentration of proteins and peptides

Monica Araya-Farias, Szymon Dziomba, and N. Thuy Tran

1 Introduction 36
2 Electrokinetic preconcentration 38
2.1 Field-amplified sample injection 38
2.2 Isotachophoresis 39
2.3 Isoelectric focusing 47
2.4 Multidimensional analysis 48
3 Preconcentration on solid supports 50
3.1 Methodology based on molecular recognition mechanism 50
3.2 Monoliths 51
3.3 Beads 58
3.4 Membranes 60
3.5 Emerging microfluidic strategies for protein preconcentration 66
4 Conclusion and futures perspectives 68
References 68

4. The role of magnetic nanomaterials in miniaturized sample preparation techniques

Amandha Kaiser da Silva, Thiago Gomes Ricci, Ana Lúcia de Toffoli, Edvaldo Vasconcelos Soares Maciel, Carlos Eduardo Domingues Nazario, and Fernando Mauro Lanças

1 Introduction 78
2 Magnetic nanomaterial synthesis 80
2.1 Coprecipitation 82
2.2 Hydrothermal synthesis 82
2.3 Microemulsion 82
2.4 Thermal decomposition 83
2.5 Sonochemical 84
3 Surface functionalization of magnetic nanomaterials: Coatings 84
3.1 Silica 86
3.2 Surfactants 87
3.3 Ionic liquids 87
3.4 Polymers 88
3.5 Molecularly imprinted polymers 88
3.6 Carbon and derivatives 88
4 Application of magnetic nanomaterials in sample preparation 89
5 Conclusion 94

Copyrighted material

References 95
Further reading 98

5. Miniaturized methods of sample preparation
Dorota Wianowska, Marta Gil, and Malgorzata Olszowy

1 Introduction 99
2 Solid-phase microextraction techniques 100
2.1 On-fiber SPME 101
2.2 Solid-phase dynamic extraction 103
2.3 Matrix solid-phase dispersion 103
2.4 Sea sand disruption method 105
2.5 Stir bar sorptive extraction 105
2.6 Microextraction in packed syringe 106
2.7 QuEChERS 108
3 Liquid-phase microextraction techniques 109
3.1 Single-drop microextraction 110
3.2 Hollow-fiber liquid phase microextraction 112
3.3 Electromembrane extraction 113
3.4 Dispersive liquid-liquid microextraction 114
3.5 Supported liquid extraction 116
3.6 Cloud point extraction 117
3.7 Salt-assisted liquid-liquid extraction 118
4 Conclusions 118
Websites 120
References 120

Section B
Separation/identification stage of analysis

6. Lab-on-chip for chromatographic techniques
Yiğitcan Sümbelli, Rüstem Keçili, Fatemeh Ghorbani-Bidkorbeh, and Chaudhery Mustansar Hussain

1 Introduction 129
2 Lab-on-chip and chromatography-on-chip 130
3 Liquid chromatography on-chip 130
4 Gas chromatography-on-chip 132
5 Conclusions 134
References 135

7. Lab on a chip: A versatile integration with spectroscopic techniques
Merve Eryılmaz, Ender Yıldırım, and Uğur Tamer

1 Introduction 139
2 Biomolecular spectroscopy 140

Copyrighted material

2.1 Raman spectroscopy and fluorescence spectroscopy 140
2.2 Surface-enhanced Raman spectroscopy 141
3 Functionality of lab-on-a-chip devices 142
4 Applications of LOC-Raman/SERS 143
5 Applications of fluorescence spectroscopy 147
Website 150
References 150

Section C
Detection stage of analysis

8. Contactless conductivity detection on lab-on-a-chip devices: A simple, inexpensive, and powerful analytical tool for microfluidic applications

Kemilly M.P. Pinheiro, Kariolanda C.A. Rezende, Lucas C. Duarte, Gerson F. Duarte-Junior, and Wendell K.T. Coltro

1 Introduction 155
2 Principle and theory of C^4D 156
2.1 Electronic circuit for C^4D detectors 158
2.2 Cell geometry and electrode design 158
3 Microsystems and portability 159
4 Fabrication of sensing electrodes 159
4.1 Metal electrodes 160
4.2 Nonmetallic electrodes 162
5 Applications 164
5.1 Bioanalytical studies 166
5.2 Clinical applications 170
5.3 Environmental applications 170
5.4 Forensics 172
5.5 Other applications 173
6 Conclusions 174
Acknowledgments 174
References 175

9. Multidimensional sensors: Classification, nanoprobes, and microfluidics

Caroline Y. Nakiri Nicoliche, Osvaldo N. Oliveira, Jr., and Renato S. Lima

1 Introduction 185
2 Classification 186
2.1 Type A: Heterogeneous probe/sample interactions 188
2.2 Type B: Multichannel outputs 194
2.3 Type C: Heterogeneous probe/sample interactions and multichannel outputs 198

Copyrighted material

3 **Nanomaterials for high-performance multidimensional sensors** 202
3.1 Improving the sensitivity and recognition ability 202
3.2 Lab-on-a-nanomaterial 204
3.3 Single probe and measurement 206
4 **Microfluidic platforms** 210
5 **Conclusion and outlook** 213
Websites 215
Acknowledgments 215
References 215

10. Miniaturized microfluidic heuristics for the detection of polluting molecules in the environment

Hardik Ramesh Singhal, Naresh Kumar Mani, Amogh Kodgi, Ninad Mehendale, Swati Sharma, and Vijay Kumar Garlapati

1 **Introduction: Environmental pollutants** 221
1.1 Heavy metals 222
1.2 Pesticides 222
1.3 Organophosphorus compounds 222
1.4 Xenobiotics 222
2 **Existing detection strategies for environment pollutants and their drawbacks** 223
2.1 Detection of heavy metals 223
2.2 Detection of xenobiotics 223
2.3 Detection of pesticides 224
3 **Positive attributes of microfluidics for better detection of environmental pollutants** 224
4 **Designs for detection** 226
4.1 Colorimetric approach 227
4.2 Electrochemical approach 229
4.3 Other optical methods 230
5 **Future scope** 231
6 **Conclusion** 232
References 232

Section D
Important aspects and challenges

11. Pushing the limits of analytical characterization tools: How much is too much?

Kundan Kumar and Anirban Chowdhury

1 **Introduction** 239
2 **Theories involving crystallite and grain size measurements** 242
2.1 Issues related to the measurements and analyses of crystallite and grain sizes 244

Copyrighted material

2.2 Can X-ray diffraction become "a limiting tool" for phase analyses? 248
3 Fundamentals of differential scanning calorimetry 254
3.1 Measurement issues with the order of phase transition using DSC and/or DTA 256
3.2 Measurement and interpretation issues with glass transition 257
3.3 Probing crystallization and associated phase transition events using DSC and/or DTA 266
4 Conclusion 268
References 269

12. Future of the modern age of analytical chemistry: Nanominiaturization

Özlem Biçen Ünlüer, Fatemeh Ghorbani-Bidkorbeh, Rüstem Keçili, and Chaudhery Mustansar Hussain

1 Introduction 277
2 Categorization of miniaturized systems 279
3 Miniaturization of separation techniques 281
3.1 Miniaturization of liquid chromatography systems 281
3.2 Miniaturization of gas chromatography systems 283
3.3 Miniaturization of capillary electrophoresis systems 285
4 Miniaturization of detection techniques 286
4.1 Atomic and molecular spectrometry-based detection systems 286
4.2 Mass spectrometry-based detection systems 288
4.3 Electrochemical detection systems 289
5 Conclusions 290
References 290

13. Nanobiotechnology approaches for miniaturized diagnostics

Vivek B. Borse, Aditya N. Konwar, and Rohit Srivastava

1 Introduction 297
2 Nanobiotechnology 298
3 Nanobiosensors 299
3.1 Types of nanomaterial 302
3.2 Type of biomaterial 307
4 Techniques for the conjugation of biomaterials with nanomaterials 309
5 Nanobiotechnology-based diagnostic devices 310
5.1 Principle and mechanism of signal detection in nanobiotechnology-based diagnostic devices 312
5.2 Commercialized nanobiotechnology-based miniaturized diagnostic devices 313

Copyrighted material

6 Advantages and disadvantages of nanobiotechnology-based diagnosis 314
Acknowledgment 326
Conflict of interests 326
References 326

Index 335

Copyrighted material

Chapter 2

Microfluidics in lipid extraction

Ninad Mehendale[a], S.P. Jeevan Kumar[b,c], Naresh Kumar Mani[d], Surajbhan Sevda[e], Sunandan Naha[e], Swati Sharma[f], and Vijay Kumar Garlapati[f]

[a]Department of Electronics, K. J. Somaiya College of Engineering, Mumbai, Maharashtra, India, [b]Agricultural & Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India, [c]ICAR-Indian Institute of Seed Science, Mau, Uttar Pradesh, India, [d]Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, India, [e]Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam, India, [f]Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh, India

1. Introduction to biodiesel production from lipids

Vegetable oils have been widely tested to synthesize biodiesel. However, due to the exorbitant cost, the food versus fuel dilemma, and the requirement for acreage, the need to look for alternative substrates has become necessary [1]. To overcome the problems associated with vegetable oils, tree-based oils (TBOs) such as *Jatropha curcas*, *Pongamia pinnata*, *Simarouba glauca*, jojoba, etc. are now being used [2, 3]. Nevertheless, these TBOs are nonremunerative and it is expensive to maintain the plantations. Besides, they yield very low amount of lipids, which is a major setback for their efficient use as an alternative to biodiesel. To herald new options, oleaginous microbes are a promising option to consider for biodiesel production, as these are endowed with higher lipid productivity coupled with a desirable lipid combination using inexpensive carbon and nitrogen substrates. Moreover, they have no need for acreage, they are low maintenance, and there is no land versus fuel dilemma [4, 5]. Oleaginous microbes are defined as microbes that can synthesize 20% of lipid content on a dry weight basis using various inexpensive feedstocks [6]. These lipids are similar to vegetable oils and have great potential as an alternative substrate for biodiesel production. Biodiesel production from microbial lipids consists of several steps such as cultivation of oleaginous strains, lipid extraction (lipid synthesis is mostly intracellular), transesterification of lipids to fatty acid methyl esters (FAME), and purification of biodiesel (FAME). Every step in biodiesel production is very important and attention must be paid to the commercial viability of the process. Among oleaginous microbes, microalgae lipid extraction

Handbook on Miniaturization in Analytical Chemistry. https://doi.org/10.1016/B978-0-12-819763-9.00002-7
Copyright © 2020 Elsevier Inc. All rights reserved.

Copyrighted material

comprises dewatering, aggregation of algal biomass, drying, and lipid extraction with green solvents [7].

Unlike microalgae, fungi and yeast biomass are readily accessible from nature; hence, they can easily be separated by filtration followed by drying and lipid extraction using green solvents and techniques. Lipid extraction is an important step that determines the quality of biodiesel [8, 9]. Since lipids are amphipathic in nature, optimization of the solvent system and the technique used are crucial parameters [10]. Furthermore, to minimize the amount of solvent and time used in the process, microfluidics can be deployed that have the potential to carry out the process in less time with reduced solvent usage [11]. Therefore optimization of the microfluidics method for each strain would be highly desirable for the development of a viable approach [6].

2. Positive attributes of microfluidics over existing technologies

The major pitfalls in existing microalgal bioproduct/biofuel research include high cost, labor intensity, low throughput, time consumption, and poor control over cell manipulation [12, 13]. Microfluidic systems leverage artificial tubular channels or reactors of micron size and rely on small volume manipulation in the range of microliters to picoliters, thereby reducing the large quantity of sample required or consumed. They consist of a polymer device made up of polydimethylsiloxane (PDMS) and drive devices. This combination enables researchers to automatize, miniaturize, and parallelize their study. Additionally, microfluidic systems offer researchers the opportunity to continuously monitor microbial cells in microenvironments with high-precision control at the single cell level, and open an avenue to perform high-throughput culture screening as well as lipid separation, biomass analysis, and cultivation [14–17] (Fig. 2.1). Because of their micron-scale dimension, microfabricated devices offer advantages like laminar flow [18], rapid diffusion [19], and thermal transport [20].

The last decade has seen a renewed importance in utilizing microfluidics in processes involved in industrial production [21, 22]. Additionally, recent developments in microfluidic technology have enabled us to categorize these

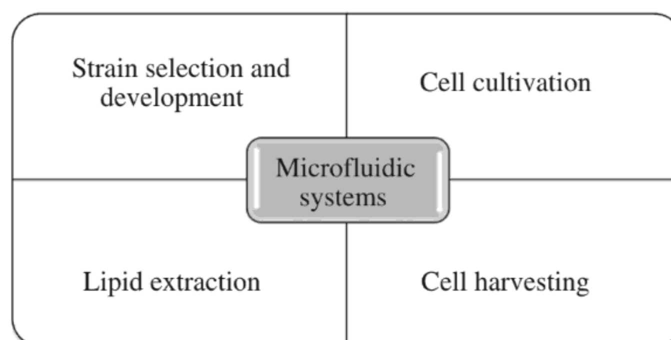


FIG. 2.1 Applications of microfluidic systems.

Copyrighted material

processes into microdetection, microseparation, microculturing, microdroplets, and microreaction. A wide gamut of research has already transformed a simple PDMS-based microfluidic device into a lipid separation unit. Berger et al. successfully separated chloroform from a lipid and oil suspension with an extraction efficiency of 93.5%–97.9% [23]. Interestingly, a two-layer microfluidic chip was developed by Bang et al. to extract lipids by the solid-phase extraction method, which also shows a very high recovery rate (95%–100%) [24].

"Oils" secreted from microbes are generating considerable interest and can be an ideal option for biofuel production. Most of the microorganisms, including yeast, microalgae, bacteria, and fungi, can be deployed for accumulating oils [25]. Roughly 2–8 million microalgal species are present in our ecosystem; however, only a small number of species have been reported so far [26, 27]. It is necessary to identify strains with better stress tolerance and yield. Conventionally, the microalgal samples collected from different locations are further identified through light microscopy and molecular biology techniques with bulky instruments in high-end laboratory settings.

Lim et al. demonstrated an energy-efficient and less labor-intensive way of cultivating *Chlamydomonas reinhardtii*, and analyzed lipid accumulation as well as separation using a microfluidic platform. Because of the controlled environment and reaction system, lipid separation efficiency is found to be much greater than in traditional bulk experiments [28]. Besides lipid extraction, Eu et al. designed a microfluidic device to monitor the cytotoxic response of microalgal cells and their lipid production under the influence of nitrogen [29].

Undoubtedly, by integrating cell culture, lipid production, and lipid extraction in a single microfluidic system a robust and multiplexed device for biomass products can be created. Kwak et al. used an intriguing approach by deploying an eight-chamber microfluidic device to carry out multiple processes from various microbial strains. Strikingly, lipid extraction efficiency was amenable with the traditional Bligh-Dyer method [30]. Bensalem et al. applied pulsed electric fields and mechanical stresses using microfluidic systems as a pretreatment method to enhance lipid extraction [31]. Another remarkable way of simplifying the lipid extraction procedure was proven with the assistance of superabsorbent polymers and microsystems. Interestingly, this method witnessed an increase in extraction rates [24]. With such diverse applications and benefits, certain microfluidic systems can be a potential alternative to conventional cell culture reaction systems, and considered as an unprecedented tool in current biomass analysis, lipid extraction, strain selection, and biofuel research.

3. Ideal characteristics of microfluidic devices

The rule of thumb for microfluidic devices relies on the manipulation of samples by decreasing or downsizing the device lengths (preferably micron scale) and also possessing the following characteristics. First, surface phenomena, including surface charge and capillary forces, dominate gravitational forces, which

Copyrighted material

leads to passive actuation of samples. Second, fluid flow will always be laminar (due to low Reynolds number) offering a reliable liquid/liquid interface analysis and also generating large concentration gradients at single cell resolution. Third, smaller dimensions enable researchers to utilize less reagent or assay volume (picoliter) as well as manipulate monophase and multiphase systems in a single device with the scope for massive parallelization in resource-limited settings. Because of their advantages like portability, better sensitivity, low cost, faster analysis time, and less sample and space consumption, different microfluidic platforms (pressure driven, centrifugal, electrokinetic, and acoustic-based) have outweighed conventional bulk experiments for bio(chemical) and industrial assays [32, 33].

4. Design of microfluidic devices in lipid extractions from microalgae/oleaginous microbes

The production of biofuels from microalgae or oleaginous microbes should be economically viable. Conventionally used benchtop methods for culturing microalgae or oleaginous microbes, collecting accumulated lipids, and extracting lipids are often time-consuming and tedious processes.

Development of microfluidic platforms over the last two decades has helped in the extraction of lipids from numerous microalgae species with high productivity. Microfluidics also provides a platform for quantitative analysis of the extracted lipids. Numerous integrated microfluidic platforms have been developed to efficiently perform multiple steps on a single chip, from culturing microalgae to the extraction of lipids. In this section, we will discuss some of these commonly used microfluidic designs for lipid extraction from microbes and microalgae.

4.1 Reservoir with filter design

In this type of lipid extractor, cell growth and lipid collection are carried out in two separate chambers. A microfluidic filter connects these two chambers. The microfluidic filter blocks cell debris and allows only produced lipids to go through. Fig. 2.2 represents a simple design in this approach by Lim et al. [28]. Fig. 2.2 shows the complete setup for the collection of lipids from microalgae. The syringe pump first seeds the microalgae into the cell chamber along with media. After seeding, culture is allowed to grow for 4 days. Next, during the next 4 days of the induction period, the syringe pump is used to feed nitrogen-deficient media. Finally, solvents (ethanol and isopropanol) are pumped for the extraction of lipids.

The debris from microalgae are separated from lipids using a rectangular pillar filter. Here, any kind of microfluidic filter should work but preferably one that does not clog because it will allow the user to reuse the microfabricated device. To improve the efficiency of collected lipids, multiple reservoir and filter designs can be connected in a circular manner, as shown in Fig. 2.3. Kwak et al. [30] used a

Copyrighted material

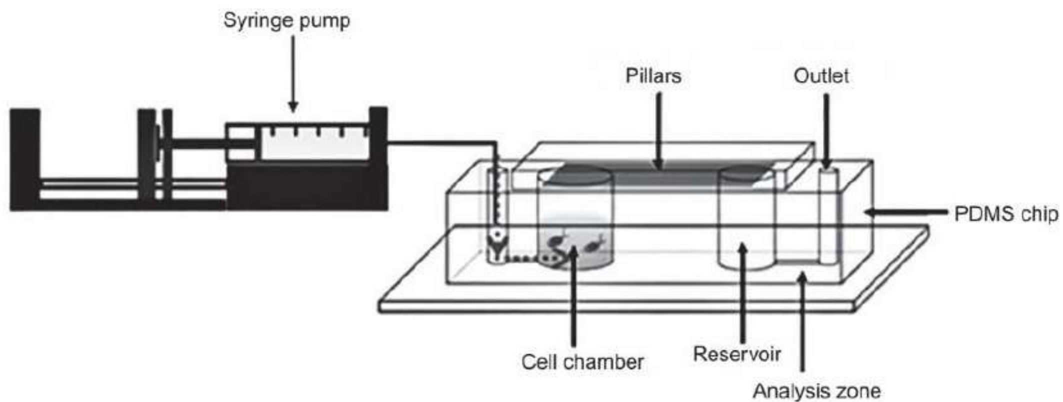


FIG. 2.2 Schematic representation of the reservoir with filter design. The syringe pump is used to load buffer, lysing solution, and lipid extraction solution into the microfluidic chip. The cell chamber is used to culture microalgae. The pillar filter is used to separate debris from lipid. The reservoir is used for accumulation of lipid. The analysis zone is used to analyze lipid quality. The device is based on two-level lithography and bottom and top glass sealing.

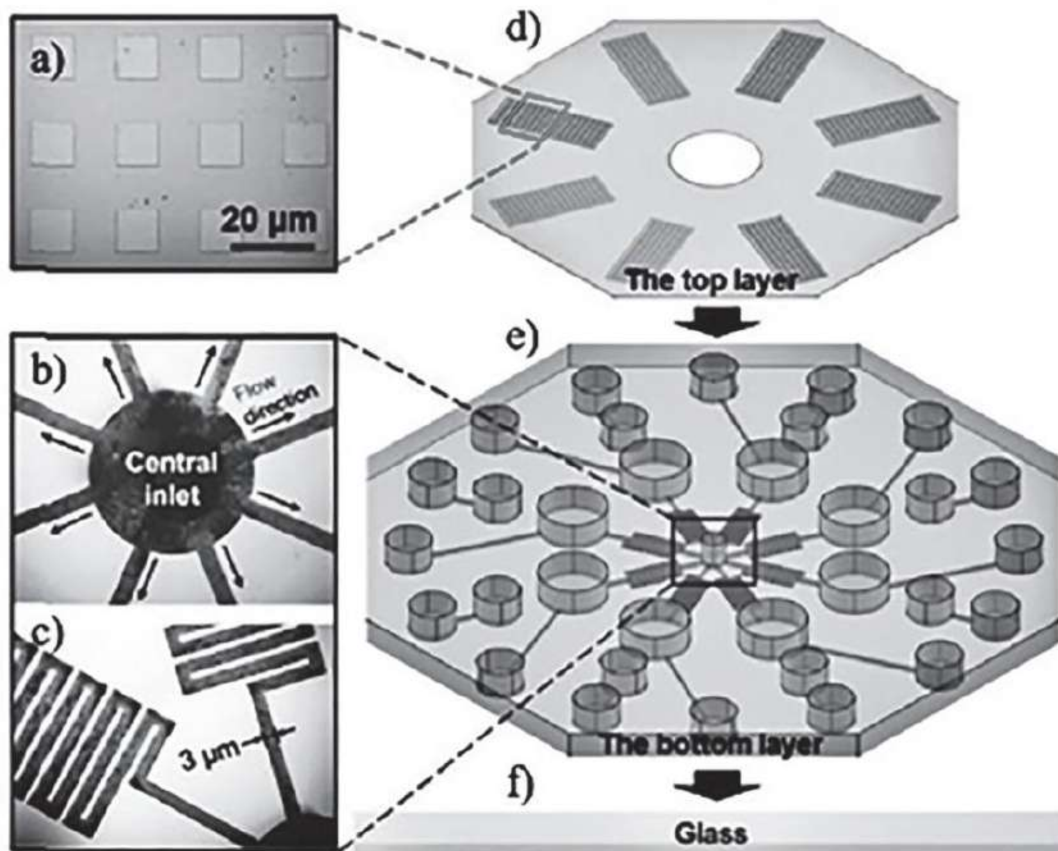


FIG. 2.3 Concentrically arranged reservoir and filter design. (A) A rectangular pillar filter with 10 μm pillars. (B) Common central inlet for inserting different media using a syringe pump. (C) Coregulated channel to prevent cell leakage. (D) The top layer of the device with filter pillars. (E) PDMS chip with multiple reservoirs and channels connecting them. (F) Bottom glass for analysis. *(Modified and adopted from H.S. Kwak, J.Y.H. Kim, S.C. Na, N.L. Jeon, S.J. Sim, Multiplex microfluidic system integrating sequential operations of microalgal lipid production, Analyst 141 (2016) 1218–1225.)*

Copyrighted material

concentric design with a central inlet and eight chips around the inlet (Fig. 2.3B). The top glass has a rectangular pillar filter array with 10 μm gaps. Circular and linear alignment with the reservoir is critical in such a design. The 3 μm corrugated channel is used to connect the central inlet with the culturing reservoir (Fig. 2.3B). The groove in the channel increases the path length and hydrodynamic resistance. Thus the corrugated channel helps to prevent cell leakage. The cell chambers are 8 mm in diameter and 10 mm deep. The output lipid collection chamber is 4 mm in diameter and 10 mm in height.

Another major advantage of using multiple reservoirs with filter design is to optimize the culturing conditions such as temperature, salt concentration, starvation, etc. In that case, instead of a common inlet, there could be one inlet per reservoir. Alignment and two-level lithography are the two major drawbacks of the reservoir and filter design.

4.2 Lab-on-a-disc design

In lab-on-a-disc designs, sequential steps of lipid extraction from microalgae are automated with the help of centrifugal force and valves. Fig. 2.4 shows one such design, where carbon valves are irradiated using a laser.

As shown in Fig. 2.4, lab-on-a-disc has five sections for lipid extraction and quantification from microalgae. There are chambers for sample inlet, lipid extraction, and collection. Reagents, dyes, and waste are also stored in the same disc. The disc is fabricated by hot embossing four layers that contain flow channel, inlets, valving polycarbonate (PC) film, and vent holes. The carbon dot

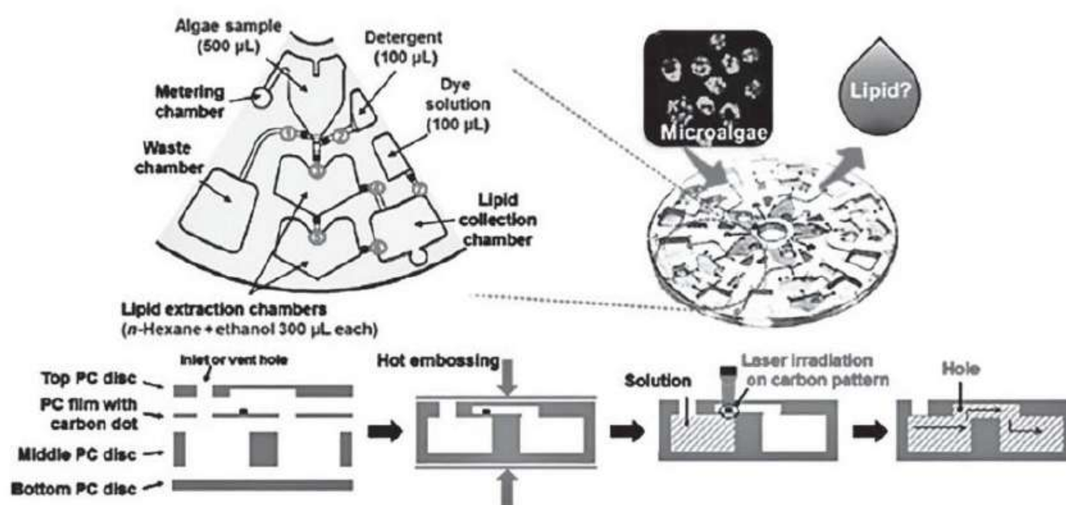


FIG. 2.4 Schematic illustration of a lab-on-disc platform for lipid extraction using microalgae. Four layers of polycarbonate (PC) disc are hot embossed along with a carbon pattern for valving purposes. The holes are drilled using CNC machining. Laser irradiation on a carbon pattern is used to open up the valve. Once the valve is open the liquid is carried to the next chamber because of centrifugal force. *(Modified and adopted from Y. Kim, S.N. Jeong, B. Kim, D.P. Kim, Y.K. Cho, Rapid and automated quantification of microalgal lipids on a spinning disc, Anal. Chem. 87(15) (2015) 7865–7871.)*

Copyrighted material

in PC film is burnt using laser irradiation, which allows the sample to flow through. A 500 μL algae sample is loaded into the chip. Spin speeds of 6000 and 3000 rpm are used alternatively along with shaking (6 Hz at an angle of 5 degrees) and 13 valves are used to extract lipid from microalgae within 13 min. This method is based on mixing lysate and microalgae samples.

The valving and the speed of rotation help in achieving timely operations for lipid extraction purposes. Though the method is very fast (13 min), it requires four layers of complex fabrication and a rotating mechanism with vibration support.

4.3 Bead-packed microchannel design for lipid extraction

In this kind of design, microbeads are packed into the microchannels such that the debris of lysed microalgae are blocked and only lipid is extracted from the lipid collection outlet. Sun et al. designed a similar microfluidic chip [34] as shown in Fig. 2.5. An isometric view of the bead-packed microchannel is shown in Fig. 2.5A. The bead-packing hole is used to load the 15 μm beads inside the microchannels. The lysed samples are fed to the chip using a syringe pump, and lipid is collected at the lipid collection outlet while the bead-packing hole is kept blocked. Fig. 2.5B shows a cross-sectional view of the channel. The channel is 200 μm high and has a 100 μm flat channel with a total channel width of 300 μm. The idea behind this design is a simple chip that can filter microalgae

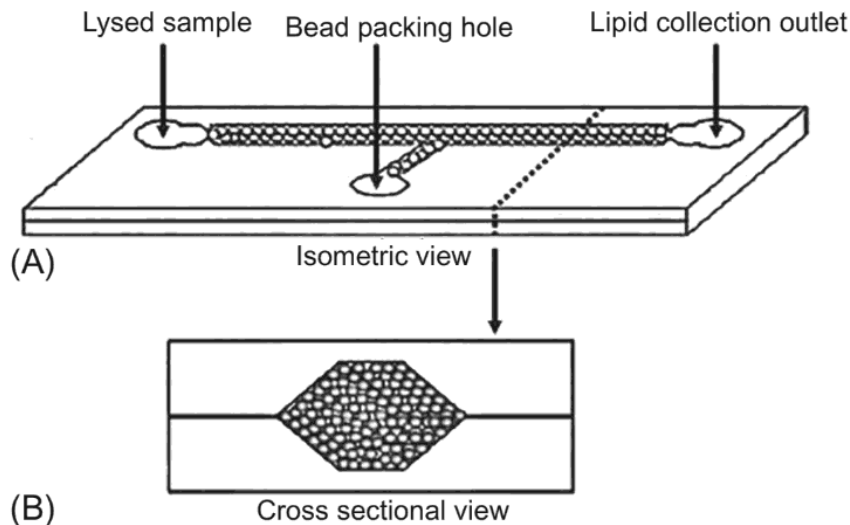


FIG. 2.5 Design schematic of bead-packed microfluidic device. (A) Isometric view of the microfluidic device. The device has a lysed sample inlet, bead-packing hole, and lipid collection outlet. The bead-packing hole is only used initially to load the bead into the channels and remains blocked during lipid extraction. (B) Cross-sectional view of the channel showing the hexagonal structure. The channel height is 200 μm, the flat channel width is 100 μm, and the total channel width is 300 μm. Beads are 15 μm. *(Modified and adapted from T. Sun, S. Pawlowski, M.E. Johnson, Highly efficient microscale purification of glycerophospholipids by microfluidic cell lysis and lipid extraction for lipidomics profiling, Anal. Chem. 83(17) (2011) 6628–6634.)*

Copyrighted material

debris and allow the user to control the gaps between the beads using a bead-packing hole. This design lacks the on-chip cell lysing facilities and also need to utilize injection with valves to pump the sample and cell lysing reagents.

4.4 U-shaped trap split-channel design

The U-shaped trap is useful when the user needs to observe lipid production and accumulation inside microalgae. A single U-shaped trap with four pillars was designed by Bodénès et al. [35]. Fig. 2.6 shows a schematic representation of the U-shaped trap split-channel design. The split channel helps to improve throughput. The design involves four light-blocking layers of fabrication and valving at suitable light wavelengths. The second layer is the cycle-controlled layer that allows light and dark cycles by pneumatically controlling deionized (DI) water and black dye simultaneously. The third layer is a gradient layer that controls light intensity using black dye and DI water. The last layer consists of the microalgae culture layer with U-shaped traps with a 77 μm radius of curvature for pumping the culture media. Although the proposed design allows the high-throughput screening of lipid production, it is difficult to achieve perfect alignment of the four layers. Also, multiple external units of syringe pumps are needed to perform the experiment.

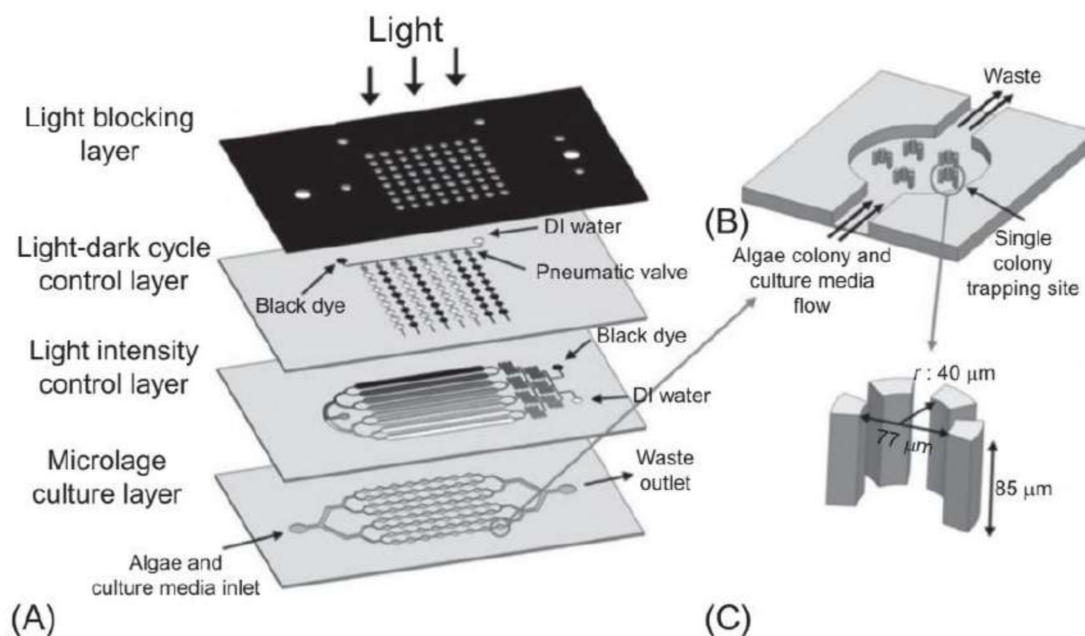


FIG. 2.6 High-throughput microfluidic U-shaped trap to monitor microalgal lipid production and accumulation. (A) The design layers of the microalgal culture monitoring chip. The four layers were composed of a light blocking layer, cycle-controlled layer, light intensity-controlled layer, and microalgae culture layer. (B) Enlarged view of a single culture compartment with multiple U-shaped trapping size. (C) A single microalgae trapping site composed of four micropillars. *(Modified and adapted from P. Bodénès, H.Y. Wang, T.H. Lee, H.Y. Chen, C.Y. Wang, Microfluidic techniques for enhancing biofuel and biorefinery industry based on microalgae, Biotechnol. Biofuels 12(1) (2019) 33.)*

Copyrighted material

4.5 Confined impinging jet mixer design

The confined impinging jet mixer design is used to burst the cell using shear force to extract lipids from it. Tseng et al. [36] designed a lipid extraction method using a microfluidic-confined impinging jet mixer. Fig. 2.7 shows a schematic representation of a confined impinging jet mixer where organic solvent and lipid-rich microbial suspension are mixed in the central round chamber. At the exit, due to confinement, the oleaginous cells experience a high shear force that eventually breaks them apart, and lipid is extracted. Though it is a very simple design, it depends greatly on shear force for its operation. The design lacks the provision for filtering the cell debris, which allows the passage of oleaginous cells into the lipid stream.

5. Lipid extractions from microalgae and oleaginous microorganisms

The lipid extraction process is an important step, which incurs 90% of energy as per the life cycle assessment of the microalgal biodiesel process [7]. Moreover, in the conventional lipid extraction process, a solvent system such as chloroform:methanol has been widely used, which requires a huge amount of solvent and time. To address these issues, microfluidic-based lipid extraction is highly desirable because of the miniaturization of the equipment, highly efficiency solvent usage, and the process can be performed quickly.

Lim et al. [28] developed an in situ double-layered PDMS device for lipid extraction from *C. reinhardtii*. In this setup a microcolumn with micropillars (made of PDMS) was used not only for lipid extraction but also to retain the microalgae cells in the bioreactor for the extraction process. Unlike conventional solvent systems (chloroform and methanol), in this study, ethanol and isopropanol were used, since the chloroform solvent can easily absorb chloroform [3, 37].

Pertinent to the efficiency of the lipid extraction process, isopropanol and ethanol have shown to be less efficient than chloroform and methanol solvents.

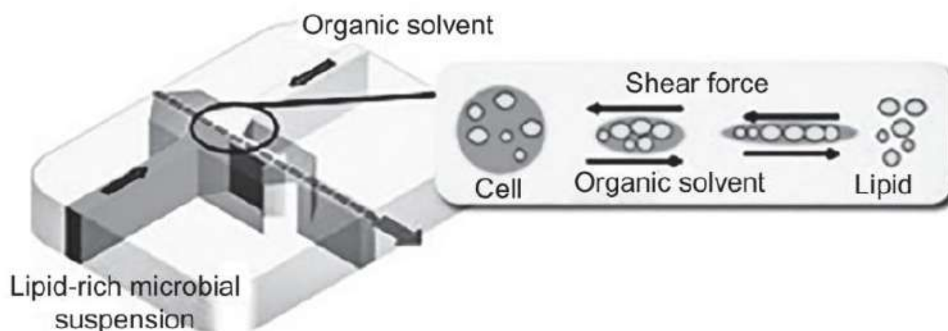


FIG. 2.7 Schematic of the confined impinging jet mixer [36]. The algae from the left and solvent from the right are mixed in the central chamber. The mixed sample then exits from the confining outlet.

Copyrighted material

Besides, the composition of extracted lipids showed that isopropanol and ethanol result in higher polyunsaturated fatty acids (C18:3) than chloroform:methanol, which showed a higher content of saturated fatty acids (C16:0, C20:0). This study showed the potential of microfluidics to integrate various steps like cultivation of microalgae, estimation of lipids (electroporation), and the lipid extraction process. However, the major drawbacks for effective implementation are the incompatibility of polymers studied for reaction and extraction conditions, upscaling of microfluidic cell concentration, and the cell lysis process. The common polymers studied for molding microchannels using poly(methyl methacrylate) and PDMS are nonresistant and react with NaOH, particularly in the transesterification process. To address these problems, future studies of microfluidics should envisage to use of inert materials such as ceramics [38], silicon, and glass [39, 40] for microalgal extraction and transesterification processes.

Recent research attempts at utilizing microfluidics for lipid extraction are summarized in Table 2.1.

6. Future prospects

Microfluidic intervention lipid extraction technology is capable of solving existing algal-biorefinery problems and helps in the upgradation of the lipid-based biofuel sector by addressing biofuel substrate depletion. Microfluidic intrusion in lipid extraction has been greatly enhanced through research into the development of compatible molding polymers, which can withstand extraction conditions. The compatibility issue has been studied through the utilization of inert materials (silicon, glass, ceramics) to develop microfluidic devices for better utilization in lipid extraction [35, 44]. Moreover, better lipid extraction efficiency is possible by studying the emulsion (due to the presence of hydrophilic solutions and organic solvents)-related problems by mainly focusing on reducing the contact area between the hydrophilic and hydrophobic phases of the lipid extraction system. Microfluidic-based lipid extraction has to be practiced on pilot-scale studies, which paves the way for industrial exploitation of microfluidics in the biorefinery sector [6, 45].

7. Conclusion

The better utilization of algal and oleaginous microbial lipids alleviates the current scenario of substrate scarcity in the biorefinery sector. Many researchers are working actively for better utilization of rheology-based microfluidics in microalgal technology and oleaginous biorefinery for enhanced results from the lipid extraction process. The profound knowledge attained through the extensive intrusion of microfluidics in proteomic research has to be applied to the development of better microfluidic devices for lipid extraction. The successful development of better microfluidic devices for lipid extraction helps to reduce the cost of algal- and oleaginous-based biofuels. More research is anticipated to

Copyrighted material

TABLE 2.1 Research attempts at lipid extraction through microfluidics technologies.

Source of microbial lipid	Microfluidic device characteristics	Enhanced yield/array of compounds	Any other relevant data	References
Adenocarcinomic human alveolar basal epithelial cells (A549)	Three-layered microfluidic device [two polydimethylsiloxane (PDMS) layers were attached to an indium-tin oxide-coated glass] for cell capture	> 90% cell occupancy with single-cell occupancy of ~25%	Not suitable for single-celled organisms	[41]
Sulfurospirillum barnesii strain SES-3 cells	T-shaped channel, 300 μm W × 200 μm D, Schott Boro float glass based	Six major classes of phospholipid	Suitable for subsequent lipidomics analysis	[34]
Chlamydomonas reinhardtii CC503 (cw92 mt+), CC124 (mt- agg-1 nit1 nit2), and CC4348 (BAF-J5; cw15 arg7-7 nit1 nit2 sta6-1::ARG7)	PDMS-based two-layer device (top layer has a microchannel filled with an array of square micropillars (5 μm H × 100 μm^2 CSA × 10 μm of interspacing) and bottom layer is a cell-capturing chamber)	Enhanced lipid extractions yielded by using 70% aqueous isopropanol	Suitable for a broad range of microbial sources	[28]
Phaeodactylum tricornutum in F/2 medium	2D-hydrofocusing microfluidic chip was fabricated (total volumetric flow of ~220 μL/min) and attained a 260 mm/s typical cell velocity	Real-time measurement of photosynthetic efficiency (as Fv/Fm) and lipid accumulation (as Nile Red fluorescence)	Suitable for lipid extraction with stressed algal systems	[42]
Chlorella vulgaris	Fabrication of dielectrophoresis device	The operating conditions seem to be 1.4–2.95 mS/cm conductivity, 1–20 MHz operating frequency, and a crossover frequency of 11 wt% lipid content	Suitable for sorting and harvesting of high lipid content	[43]

Copyrighted material

develop an integrated process of lipid extraction coupled with online monitoring of extracted lipid analysis.

Websites

https://microfluidics-master.fr/
https://ufluidix.com/circle/microfluidic-companies/
https://web.uvic.ca/~kelvira/
https://www.elveflow.com/microfluidic-tutorials/microfluidic-reviews-and-tutorials/microfluidics-for-dna-analysis-pcr/

References

[1] S.J. Kumar, R. Banerjee, Enhanced lipid extraction from oleaginous yeast biomass using ultrasound assisted extraction: a greener and scalable process, Ultrason. Sonochem. 52 (2019) 25–32.

[2] L.K. Gujjala, S.J. Kumar, B. Talukdar, A. Dash, S. Kumar, K.C. Sherpa, R. Banerjee, Biodiesel from oleaginous microbes: opportunities and challenges, Biofuels 10 (1) (2019) 45–59.

[3] T.S. Vinothkumar, K. Deivanayagam, A. Ganesh, D. Kumar, Influence of different organic solvents on degree of swelling of poly(dimethyl siloxane)-based sealer, J. Conserv. Dent. 14 (2) (2011) 156.

[4] S.J. Kumar, R. Banerjee, Optimization of lipid enriched biomass production from oleaginous fungus using response surface methodology, Indian J. Exp. Biol. 51 (2013) 979–983.

[5] S.J. Kumar, L.K.S. Gujjala, A. Dash, B. Talukdar, R. Banerjee, Biodiesel production from lignocellulosic biomass using oleaginous microbes, in: Lignocellulosic Biomass Production and Industrial Applications, John Wiley & Sons Inc., New York, 2017, pp. 65–92.

[6] R. Banerjee, S.J. Kumar, N. Mehendale, S. Sevda, V.K. Garlapati, Intervention of microfluidics in biofuel and bioenergy sectors: technological considerations and future prospects, Renew. Sustain. Energy Rev. 101 (2019) 548–558.

[7] S.J. Kumar, V.K. Garlapati, A. Dash, P. Scholz, R. Banerjee, Sustainable green solvents and techniques for lipid extraction from microalgae: a review, Algal Res. 21 (2017) 138–147.

[8] V.K. Garlapati, R. Banerjee, Evolutionary and swarm intelligence-based approaches for optimization of lipase extraction from fermented broth, Eng. Life Sci. 10 (3) (2010) 265–273.

[9] A. Kumari, P. Mahapatra, V.K. Garlapati, R. Banerjee, Enzymatic transesterification of Jatropha oil, Biotechnol. Biofuels 2 (1) (2009) 1.

[10] V.K. Garlapati, R. Banerjee, Optimization of lipase production using differential evolution, Biotechnol. Bioprocess Eng. 15 (2) (2010) 254–260.

[11] C.M. Hussain, Nanomaterials in Chromatography: Current Trends in Chromatographic Research Technology and Techniques, Elsevier, 2018.

[12] A. Han, H. Hou, L. Li, H.S. Kim, P. de Figueiredo, Microfabricated devices in microbial bioenergy sciences, Trends Biotechnol. 31 (2013) 225–232.

[13] T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, F. Bux, Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production, Bioresour. Technol. 102 (2011) 57–70.

[14] A. Grünberger, W. Wiechert, D. Kohlheyer, Single-cell microfluidics: opportunity for bioprocess development. Curr. Opin. Biotechnol. 29 (2014) 15–23, https://doi.org/10.1016/j.copbio.2014.02.008.

Copyrighted material

[15] G.B. Salieb-Beugelaar, G. Simone, A. Arora, A. Philippi, A. Manz, Latest developments in microfluidic cell biology and analysis systems. Anal. Chem. 82 (2010) 4848–4864, https://doi.org/10.1021/ac1009707.

[16] L. Weng, F. Ellett, J. Edd, K.H.K. Wong, K. Uygun, D. Irimia, S.L. Stott, M. Toner, A highly-occupied, single-cell trapping microarray for determination of cell membrane permeability, Lab Chip 17 (2017) 4077–4088.

[17] G.M. Whitesides, What comes next? Lab Chip 11 (2011) 191–193, https://doi.org/10.1039/C0LC90101F.

[18] C.L. Hansen, E. Skordalakes, J.M. Berger, S.R. Quake, A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16531–16536.

[19] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D.E. Ingber, G.M. Whitesides, Subcellular positioning of small molecules, Nature 411 (2001) 1016.

[20] A.R. Jafek, S. Harbertson, H. Brady, R. Samuel, B.K. Gale, Instrumentation for xPCR incorporating qPCR and HRMA, Anal. Chem. 90 (2018) 7190–7196.

[21] A. Miled, J. Greener, Recent advancements towards full-system microfluidics. Sensors (Basel) 17 (2017) https://doi.org/10.3390/s17081707.

[22] H.S. Santana, M.G.M. Lopes, J.L. Silva, O.P. Taranto, Application of microfluidics in process intensification, Int. J. Chem. React. Eng. 16 (2018) 20180038.

[23] S. Berger, J. Stawikowska, D. van Swaay, A. deMello, Continuous suspension of lipids in oil by the selective removal of chloroform via microfluidic membrane separation, Ind. Eng. Chem. Res. 53 (2014) 9256–9261.

[24] G. Bang, Y.H. Kim, J. Yoon, Y.J. Yu, S. Chung, J.A. Kim, On-chip lipid extraction using superabsorbent polymers for mass spectrometry, Anal. Chem. 89 (2017) 13365–13373.

[25] Y. Ma, Z. Gao, Q. Wang, Y. Liu, Biodiesels from microbial oils: opportunity and challenges, Bioresour. Technol. 263 (2018) 631–641.

[26] S.-F. Han, W.-B. Jin, R.-J. Tu, W.-M. Wu, Biofuel production from microalgae as feedstock: current status and potential, Crit. Rev. Biotechnol. 35 (2015) 255–268.

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

[28] H.S. Lim, J.Y.H. Kim, H.S. Kwak, S.J. Sim, Integrated microfluidic platform for multiple processes from microalgal culture to lipid extraction, Anal. Chem. 86 (2014) 8585–8592.

[29] Y.J. Eu, H.S. Park, D.P. Kim, J. Wook Hong, A microfluidic perfusion platform for cultivation and screening study of motile microalgal cells, Biomicrofluidics 8 (2014) 024113.

[30] H.S. Kwak, J.Y.H. Kim, S.C. Na, N.L. Jeon, S.J. Sim, Multiplex microfluidic system integrating sequential operations of microalgal lipid production, Analyst 141 (2016) 1218–1225.

[31] S. Bensalem, F. Lopes, P. Bodénès, D. Pareau, O. Français, B. Le Pioufle, Understanding the mechanisms of lipid extraction from microalga *Chlamydomonas reinhardtii* after electrical field solicitations and mechanical stress within a microfluidic device, Bioresour. Technol. 257 (2018) 129–136.

[32] N. Convery, N. Gadegaard, 30 Years of microfluidics, Micro Nano Eng. 2 (2019) 76–91.

[33] D. Mark, S. Haeberle, G. Roth, F. von Stetten, R. Zengerle, Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications, Chem. Soc. Rev. 39 (2010) 1153.

[34] T. Sun, S. Pawlowski, M.E. Johnson, Highly efficient microscale purification of glycerophospholipids by microfluidic cell lysis and lipid extraction for lipidomics profiling, Anal. Chem. 83 (17) (2011) 6628–6634.

Copyrighted material

[35] P. Bodénès, H.Y. Wang, T.H. Lee, H.Y. Chen, C.Y. Wang, Microfluidic techniques for enhancing biofuel and biorefinery industry based on microalgae, Biotechnol. Biofuels 12 (1) (2019) 33.

[36] Y.H. Tseng, S.K. Mohanty, J.D. McLennan, L.F. Pease III, Algal lipid extraction using confined impinging jet mixers, Chem. Eng. Sci. X 1 (2019) 100002.

[37] J.N. Lee, C. Park, G.M. Whitesides, Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices, Anal. Chem. 75 (23) (2003) 6544–6554.

[38] J.N. Schianti, M.R. Gongora-Rubio, T.A. Horoiwa, N.N.P. Cerize, A.M. Oliveira, Water-in-diesel nanoemulsion by LTCC microfluidic devices, in: 2014 IEEE 9th IberoAmerican Congress on Sensors, IEEE, 2014, pp. 1–4.

[39] X. Cheng, M.D. Ooms, D. Sinton, Biomass-to-biocrude on a chip via hydrothermal liquefaction of algae, Lab Chip 16 (2) (2016) 256–260.

[40] C. Iliescu, H. Taylor, M. Avram, J. Miao, S. Franssila, A practical guide for the fabrication of microfluidic devices using glass and silicon, Biomicrofluidics 6 (1) (2012) 016505.

[41] W. Xie, D. Gao, F. Jin, Y. Jiang, H. Liu, Study of phospholipids in single cells using an integrated microfluidic device combined with matrix-assisted laser desorption/ionization mass spectrometry, Anal. Chem. 87 (14) (2015) 7052–7059.

[42] R.A. Erickson, R. Jimenez, Microfluidic cytometer for high-throughput measurement of photosynthetic characteristics and lipid accumulation in individual algal cells, Lab Chip 13 (15) (2013) 2893–2901.

[43] Y.L. Deng, J.S. Chang, Y.J. Juang, Separation of microalgae with different lipid contents by dielectrophoresis, Bioresour. Technol. 135 (2013) 137–141.

[44] J. Sengupta, C.M. Hussain, Graphene and its derivatives for Analytical Lab on Chip platforms, Trends Anal. Chem. 114 (2019) 326–337.

[45] C.M. Hussain, Handbook of Nanomaterials in Analytical Chemistry: Modern Trends in Analysis, Elsevier, 2019.

Copyrighted material