Expert Review

Pharmaceutical BIOPROCESSING

Omega-3 Hydrochloric Synthetic Biology Manufacturing, Carbon Source Materials Comparison and Growth Classification

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Abstract

When cultured with different carbon sources: glucose, pure and crude glycerol, Aurantiochytrium limacinum, a marine heterotrophic protist/microalga, produced interesting yields of docosahexaenoic acid (DHA). A thorough investigation in a lab-scale fermenter enabled the characterization and comparison of the growth kinetic parameters associated with each carbon source. Artificial Marine Medium (AMM) containing glucose, pure and crude glycerol produced comparable biomass yields. The net growth rates (0.10-0.12 h1), biomass (0.7-0.8 g cells/g Substrate), and product yields (0.14-0.15 g DHA/g cells), as well as DHA productivity, were all comparable when the three carbon sources were used. To avoid an environmental problem caused by an excess of by-product, viable potential applications to valorise crude glycerol are envisaged.

Keywords: Biodiesel • DHA • Fatty acids • PUFA • Omega-3 fatty acids • Microalgae • Bioprocess

Introduction

The potential to cultivate oleaginous microorganisms using crude glycerol has increased in popularity, primarily due to the fact that cultivation costs are lower. As a consequence of the boom in biofuels, the requirement to valorise glycerol as a co-product of biodiesel has also emerged. The contaminants in crude glycerol vary according to the feedstock and biodiesel manufacturing process. Methanol and soap are the most common, but high salinity also prevents many potential applications of crude glycerol.Most of the time, the catalyst used in the process causes salinity [1]. Traditionally, the level of removal of contaminants determined whether the glycerol was purified to a technical- or pharma-grade (pure) glycerol. The pharmaceutical, food, and cosmetic industries have been the primary applications for purified glycerol. However, its widespread purification costs have rendered its use uneconomic. As a result, a lot of raw glycerol needs to be used up or disposed of as industrial waste [2].

The following marine heterotrophic microalgae family was chosen: thraustochytrids. Triacylglycerol's with a high concentration of long-chain polyunsaturated fatty acids (PUFAs), particularly DHA and docosapentaenoic acid (DPA), are found in a large number of thraustochytrids strains. The separation and purification of DHA is made simpler by its high DHA ratio and lower amounts of structurally related PUFAs than in other species. The primary strain used in this study was Aurantiochytrium limacinum (A. limacinum) SR21 (formerly Schizochytrium limacinum SR21).The ability of thraustochytrids, specifically A. limacinum, to synthesize DHA is mentioned in a number of publications. Many of this focus on the strain's ability to isolate, identify, and produce DHA .These examinations depend on cup culture tests, which give strain central data, however offer not many bits of knowledge into the bioprocess advancement potential. A. limacinum can successfully grow and produce DHA using pure glycerol as a carbon source, as demonstrated by other studies conducted in lab-scale fermenters and flask cultures. Assays using crude glycerol were used in a small number of attempts. When making

use of crude glycerol, contaminants like methanol and soap, for example, can have a negative impact on the final DHA productivity [3].

It is necessary to benchmark crude glycerol as a viable source for medium- to large-scale production at this point. On the one hand, the same strain and culture conditions are required for a comparison with two common carbon sources: glucose and glycerol. Their suitability must be evaluated due to the difference in purchase price. However, A. limacinum must be fully characterized kinetically in a fermenter or bioreactor. This is necessary for the successful development of batch, fed-batch, or continuous processes and has never been reported in the literature.

Discussion

When evaluating various carbon sources, growth yield is an essential parameter. The results of this comparison show that the three carbon sources yielded similarly [4, 5]. The highest growth yield was achieved with crude glycerol. Several Tran's esterification impurities (unreacted feedstock, catalyst, and other by-products) are present in crude glycerol. Glycerol typically accounts for 75% to 85% of its weight (83 percent in our study's sample).Utilized cooking oils—the unreacted feedstock—may include some nutrients, resulting in increased yield. Nitrogen source plays a significant part in development and an absence of nitrogen can decrease the carbon source yield. Natural nitrogen sources are liked in thraustochytrids developments .In this study, yeast extract and tryptone were chosen as well as the usual choices for the bibliographic references. Development rates and yields are not impacted by various upsides of the liking consistent. However, when designing continuous cultures, KS becomes an essential parameter [6]. However, it is necessary to take into account the distinct cell cycles that take place in a continuous reactor's steady state and batch states. Where primarily vegetative cells are present, Ks values that are measured by a chemostat in a steady state may exhibit significant variations. We are able to confirm that A. limacinum has a metabolic preference for glucose when these values are taken into consideration.Nonetheless, crude glycerol and glycerol—surprisingly—are perfectly acceptable alternatives for the creation of a bioprocess.When lipids and DHA need to be produced on a large scale, the option may be determined by the cost of the nutrients and their availability[7,8].

When striving for high productivities, the product yield is critical. Glycerol-based fermentations produced a slightly higher DHA yield (YP/X) in this study. Around 0.15 g DHA/g cells were obtained from pure and crude glycerol. In the meantime, glucose cultures produced 0.14 g/g on average. Data from triplicate cultures (biomass curve) and duplicated DHA analysis for yield determination are shown in DHA content was monitored throughout the cultivation. The values of various repetitions were used to calculate the standard deviation. There is a clear link between the state of cell growth and the accumulation of lipids and DHA within the cell. As the cells entered the exponential growth phase, the initial yield of DHA (below 5% of cell weight) decreases; Fatty substances (TG) are basically an energy sink, among other physiological and utilitarian uses. Cells beginning their dramatic development utilize the inside energy put away in their TG to create zoospores, diminishing the generally speaking DHA yield. The microorganism then tends to re-accumulate lipids, including DHA, when it enters the vegetative state. This observation is consistent with the literature, which indicates that the formation of zoospores requires a lot of energy.

A mixture of 10 g/L glucose and AMM was used to grow the cultures. To ensure identical growth conditions, triplicate cultures were monitored for pH, temperature, percentage of dissolved oxygen, and agitation (RPM).A summary of the measured growth kinetics parameters. After the exponential growth phase was finished, samples were taken out and processed: triglyceride methylation to fatty acid methyl esters (FAMEs), cell lysis, and lipid extraction [9, 10].

Results

After that, a HRGC 7890GC (Agilent technologies, Germany) with a flame ionization detector and a Supercool SPTM-2380 (60 m 0.25 mm 0.20 m) column was used to collect and examine the chloroform phase. Helium was utilized as transporter gas. The injector and detector were set to 250 °C in temperature. By comparing the retention times to those of standard fatty acids (Sigma-Aldrich, Madrid, Spain), the fatty acids were identified.

A calibration curve consisting of eight different DHA standard concentrations was used to determine the linearity, accuracy, and repeatability of the overall DHA quantification

method (sample processing and HRGC-FID) analysis, which was successful in validating the method.

The DHA yield that was obtained is shown in as the ratio of the DHA that was measured using GC-FID to the dry biomass that was used. Growth media containing 10 g/L of pure glycerol or 10 g/L of total glycerol when using crude glycerol (calculated with the 83% purity as analysed) was used in glycerol-based cultures, as described in Section 3.1.Similar to glucose cultures, each culture had four replicates for controlling operating parameters.DHA was measured by analysing biomass samples taken at the end of the batch.

Conclusions

A. limacinum was successfully cultured in a batch bioreactor to fully characterise growth kinetics parameters with glucose, pure and crude glycerol. A. limacinum was found to be a suitable microorganism for producing DHA from any of the three carbon sources. A. limacinum has emerged as a promising industrial microorganism for the production of lipids and PUFAs. The findings demonstrated that an industrial by-product, such as crude glycerol, could be used as a carbon source in a bioprocess of this type. A. limacinum exhibited robust behaviour, with comparable performance when glucose and pure or crude glycerol were used. When compared to fish oil, algae-oil produces DHA with no contaminants (due to bio magnification of oceanic persistent organic pollutants (POPs). Nonetheless, further investigation is required.

Acknowledgement

None

Conflict of Interest

None

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