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Multiobjective Harvest-Time Optimisation of Microalgal Biodiesel Production: Balancing Methyl-Ester Output, Conversion Yield and Cultivation Time

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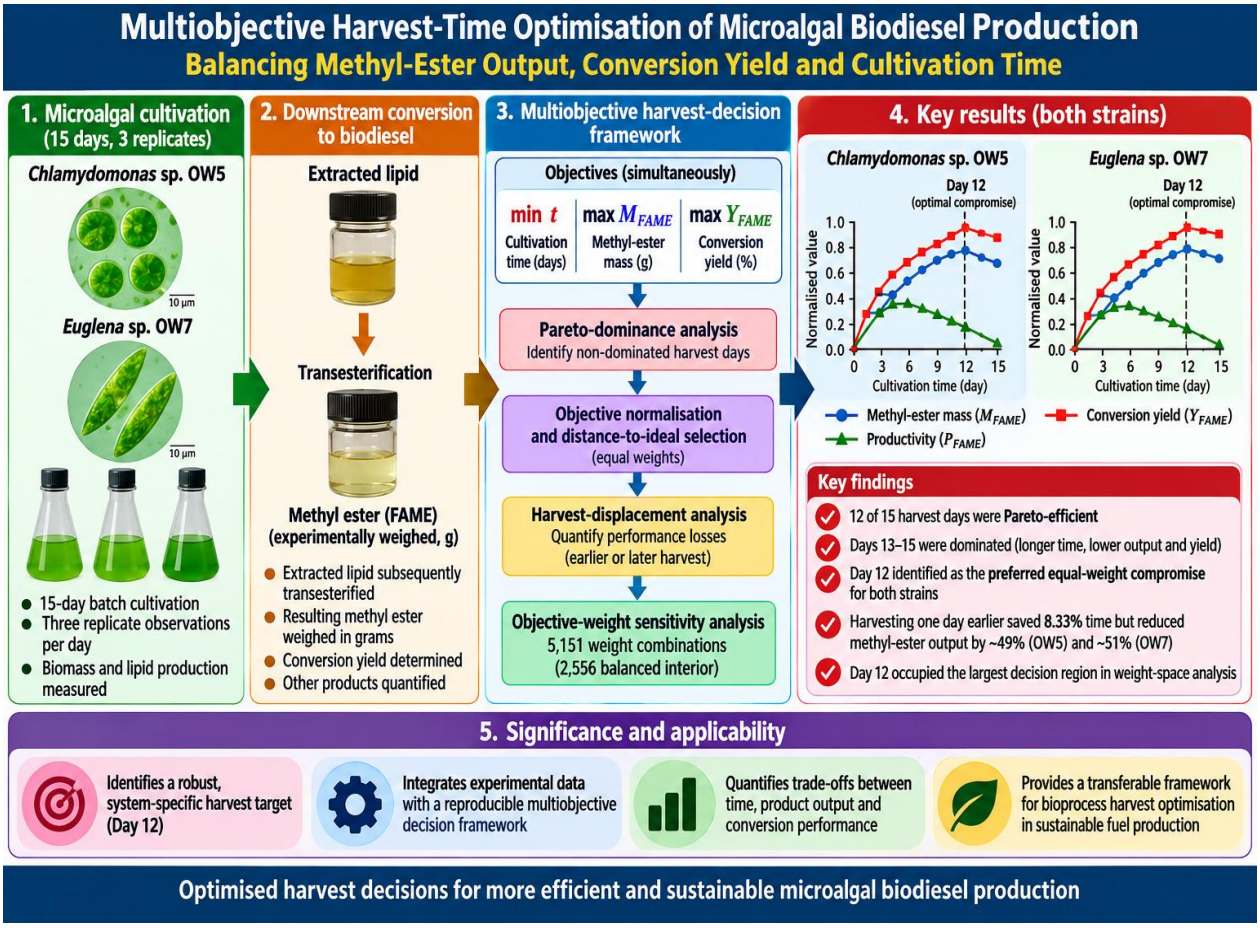
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Highlights

- Harvest timing was formulated as a multiobjective microalgal bioprocess decision.
- Twelve of 15 candidate harvest days remained Pareto-efficient for both strains.
- Day 12 provided the minimum equal-weight ideal-point distance in both strains.
- One-day earlier harvesting reduced methyl-ester output by approximately 49–51%.
- Day 12 remained robust across objective weights but was not a universal optimum.

Graphical Abstract



Abstract

Harvest timing directly influences reactor occupancy, cultivation efficiency and downstream product performance, yet microalgal biodiesel studies frequently identify harvest points from individual production maxima rather than explicit multiobjective decision criteria. This study developed time-resolved harvest-decision framework for *Chlamydomonas* sp. OW5 and *Euglena* sp. OW7 by integrating cultivation duration, experimentally measured methyl-ester output and conversion yield. Fifteen candidate cultivation days were evaluated using Pareto-dominance screening, normalized distance-to-ideal compromise analysis, harvest-displacement penalties and exhaustive objective-weight sensitivity. Days 1–12 were Pareto-efficient for both strains,

reflecting trade-offs between shorter cultivation and improved product performance, whereas Days 13–15 were dominated because prolonged cultivation increased process duration while reducing methyl-ester output and conversion yield. Equal-weight ideal-point analysis identified Day 12 as preferred compromise for both strains, with minimum distance of 0.4536. Harvesting one day earlier reduced cultivation duration by 8.33% but decreased methyl-ester output by 48.99% in *Chlamydomonas* and *Euglena* (50.97%), demonstrating substantial production penalty for limited time saving. Across 5,151 objective-weight combinations, Day 12 was selected in 71.7% and 70.3% of cases for *Chlamydomonas* and *Euglena*; these frequencies increased to 81.8% and 80.8% within the balanced interior weight space. Strong prioritisation of cultivation time nevertheless shifted the preferred solution toward earlier harvest states. Despite quantitative differences in absolute methyl-ester output and conversion performance, both strains therefore converged on the same decision structure. Day 12 represents robust, preference-dependent and system-specific compromise rather than universal microalgal harvest optimum. The framework provides transferable process-engineering approach for distinguishing production maxima, Pareto-efficient alternatives and robust operational harvest targets.

Keywords: Microalgal biodiesel; Harvest-time optimisation; Multiobjective optimisation; Pareto efficiency; Methyl-ester output; Conversion yield; Ideal-point analysis; Process decision-making

1. Introduction

Microalgal biodiesel synthesis is a time-dependent bioprocess characterised by the progression of biomass buildup, intracellular lipid accessibility, and downstream fuel conversion potential throughout the cultivation period. The conditions of cultivation significantly influence biomass and lipid production, while harvesting serves as a crucial connection between upstream biological generation and downstream lipid extraction and conversion [1, 2]. Therefore, the length of cultivation should not be seen only as a conclusive phase of experimentation or a timeframe for sampling. Viewed through the lens of process engineering, it denotes an operational decision variable that establishes when accumulated biological resources are moved from cultivation to downstream processing.

The timing of this change can provide critical effects on process performance. Early harvesting may shorten the cultivation period and reactor occupancy, but it also risks terminating the culture before fully utilising its output potential. In contrast, increasing cultivation is not necessarily favourable if lipid availability or the performance of downstream products has already stabilised or begun to worsen [3, 4]. Consequently, harvest time has been identified as a significant predictor of microalgal lipid output and the fatty-acid attributes essential to eventual biodiesel generation [5, 6]. Consequently, the engineering challenge is not only the length of time a culture may be sustained but also the appropriate moment for termination in accordance with the advancing product-performance trajectory.

The date of harvest is commonly evaluated using distinct biological or production indicators, including biomass levels, lipid concentration, lipid generation, or biodiesel yield. Even though these measures are instructive on their own, selecting a harvest state from a singular maximum may result in a partial understanding of process performance [7, 8]. The lipid titre indicates the volume of lipid accessible for each unit of culture, whereas the mass of methyl ester produced from transesterification signifies a concrete downstream product. The conversion yield of methyl esters offers a relative assessment of conversion effectiveness, whereas the length of cultivation indicates the time needed to achieve a certain process status [9, 10]. Thus, these signs relate to distinct elements of the cultivation-to-product trajectory and should not be presumed in advance to signify the same favoured harvest decision.

This differentiation assumes special relevance when the success of farming is clearly associated with the generation of biodiesel. Microalgal cultivation creates a source of biomass and intracellular lipids that may be used in later processing. After lipid extraction, transesterification alters compatible lipid elements into fatty-acid methyl esters (FAMEs), where the feedstock properties, catalyst system, and reaction parameters impact conversion efficacy [11, 12, 13, 14]. Focusing just on lipid accumulation does not adequately capture the whole quantity of methyl ester that is eventually generated. The mass of methyl esters assessed by experimentation and the percentage yield of conversion are notably beneficial complementing indicators, with the former indicating total product yield and the latter illustrating relative conversion effectiveness. A harvest condition can therefore be enticing due to its ability to yield a larger amount, enhance conversion efficiency, decrease cultivation time, or present an acceptable trade-off among these factors.

An extra methodological distinction is essential when associating lipid availability with later methyl-ester synthesis. The lipid content is quantified based on culture volume (g L^{-1}), whereas the methyl-ester yield, obtained by experimentation, is shown as a definitive mass (g). Consequently, the time-resolved paths may be evaluated to establish if greater lipid availability is linked to elevated methyl-ester output, yet any numerical similarity between the two variables must not be interpreted as direct physical mass closure without a unified processed-volume or mass framework. Thus, the connection is primarily instructive as a cultivation-to-conversion link rather than as a straight material balance.

These aspects imply that harvest selection is intrinsically compatible with a multi-objective decision structure. Simultaneously assessing culture length, absolute methyl-ester production, and conversion efficacy may lead to the necessity of sacrificing performance in one category to increase another. Pareto analysis furnishes a structured method for pinpointing such trade-offs. A harvest state is classified as dominated when another state meets or exceeds its performance in all objectives and excels in at least one, whereas non-dominated states are those where progress in one objective cannot occur without detriment to another. The use of multiobjective strategies in microalgal bioprocessing has increased, as a single performance optimum cannot adequately represent conflicting productivity metrics [21, 22, 23].

However, Pareto efficiency alone fails to specify the favoured option for operation. An extensive array of growing days might stay non-dominated, as early harvesting presents a time benefit, while late harvesting improves product performance. Consequently, an additional decision layer is essential to discern technically achievable trade-offs from an operational compromise. Standardising the competing objectives allows for their evaluation on a shared scale, while

distance-to-ideal analysis determines how closely each option aligns with a theoretical state that integrates the optimal values of all objectives. Objective weighting can thereafter signify different process priorities, and systematic weight sensitivity can evaluate if the preferred harvest state is consistent throughout an extensive preference range or reliant on a narrowly articulated weighting system. Recognising the contrast between Pareto efficiency and compromise selection is crucial for multiobjective engineering optimisation [26, 27].

Despite extensive research on microalgal culture, harvesting, lipid extraction, and transesterification, much of the academic focus has been on enhancing specific unit operations or optimising particular biological and fuel-production responses [28, 29]. Harvest time is deemed critical for lipid synthesis and composition [30], and multiobjective methods have shown their efficacy in tackling conflicting reactions in microalgal processes [21]. Yet, focus has been disproportionately small regarding the treatment of culture termination as an empirically determined multi-objective decision that concurrently assesses cultivation period, overall downstream methyl-ester production, and conversion performance. This results in a crucial analytical deficiency in recognising when a production response peaks and evaluating whether that timing signifies a valid operational harvest.

The novelty of the present study lies in addressing this gap through an integrated harvest-decision framework based on time-resolved experimental trajectories. Rather than defining harvest suitability from an isolated production maximum, the framework combines Pareto-dominance screening, normalized distance-to-ideal compromise analysis, quantitative assessment of premature and delayed harvest displacement, and exhaustive objective-weight sensitivity. This analytical hierarchy explicitly distinguishes a production maximum from a Pareto-efficient

harvest state and, subsequently, from a robust compromise harvest target. Accordingly, the objectives were to: (i) characterise the time-dependent relationship between lipid availability, absolute methyl-ester output and conversion performance; (ii) formulate cultivation duration, experimentally measured methyl-ester mass and conversion yield as competing harvest-decision objectives; (iii) identify Pareto-efficient and dominated cultivation days; (iv) determine a distance-to-ideal compromise and quantify the performance consequences of premature and delayed harvesting; and (v) evaluate the robustness of harvest-day selection across alternative objective priorities. The resulting approach is intended to provide a process-engineering basis for harvest planning without assuming that an experimentally observed production maximum necessarily constitutes a universal optimum.

2. Materials and Methods

2.1 Experimental dataset

The experimental dataset included time-resolved culture and subsequent methyl-ester production data for *Chlamydomonas* sp. OW5 and *Euglena* sp. OW7 gathered throughout a 15-day cultivation duration. For every day of culture, three trial observations were accessible for the assessed production variables and were preserved as the foundational experimental observations. The information encompassed culture responses detailing biomass and lipid generation with subsequent measures acquired after lipid extraction and transesterification. The extracted lipid underwent transesterification, and the resultant methyl-ester fraction was then recovered and measured in grams. The associated amounts of methyl ester and other products, together with

their documented percentage yields, were preserved for future data integrity evaluation and the formulation of the harvest-decision dataset.

2.2 Definition and dependency classification of harvest-performance variables

Harvest-performance variables were classified according to their measurement status, dimensional basis and mathematical dependency before multiobjective analysis (Table 1). Cultivation time, t(d), represented the temporal decision variable. Lipid titre, L (g L^{-1}), represented lipid availability within the cultivated biomass. Following transesterification, methyl-ester mass, M_{FAME} (g), represented the experimentally weighed absolute methyl-ester output, whereas M_0 (g) represented the experimentally reported mass of the accompanying other-product fraction. Methyl-ester conversion yield, Y_{FAME} (%), was expressed from the relative contribution of methyl ester to the combined measured product fractions. Methyl-ester productivity, P_{FAME} (g d^{-1}), was calculated as M_{FAME}/t and was therefore treated as a derived time-normalised performance indicator rather than an independent baseline objective.

The complementary percentage yield of the other-product fraction was retained for data-integrity assessment but excluded from the optimisation objective set because it contains no independent decision information when expressed as the complement of Y_{FAME}. Similarly, P_{FAME} was not simultaneously optimised with both M_{FAME} and t, thereby avoiding duplication of mathematically dependent information.

A dimensional distinction was also maintained between lipid availability and downstream product output. Although L and M_{FAME} were compared temporally, lipid titre is expressed per

unit culture volume (g L^{-1}), whereas methyl-ester output is an absolute experimentally measured mass (g). Consequently, they were **not equated as a physical mass balance or used to infer conversion efficiency directly.** Such reconciliation would require a documented common processed-volume or mass basis. Lipid titre was therefore retained as a contextual feedstock-state variable, while the baseline harvest optimisation used cultivation time, methyl-ester output and conversion yield as the decision objectives.

Table 1: Experimental and derived variables used in the multiobjective harvest-decision framework

Variable	Symbol (unit)	Data status	Decision role	Treatment in optimisation
Cultivation time	t (d)	Experimental design variable	Independent decision dimension	**Minimise**
Methyl-ester mass	M_{FAME} (g)	Experimentally weighed after transesterification	Primary production response	**Maximise**
Methyl-ester yield	Y_{FAME} (%)	Derived conversion ratioa^{a}	Conversion-performance criterion	**Maximise**
Lipid titre	Lg L^{-1}	Cultivation response	Feedstock-state/context variable	Not included as an independent Pareto objective
Methyl-ester productivity	P_{FAME} gd^{-1}	Derived: $M_{FAME/t}$	Time-normalised secondary metric	Diagnostic/sensitivity metric
Other-product mass	M_0 (g)	Experimentally reported product fraction	Input to conversion-yield calculation	Not an optimisation objective
Other-product yield	Y_0 (%)	Complementary derived fraction	Quality-control variable	Not an optimisation objective
Biomass concentration	X g L^{-1}	Cultivation response	Upstream contextual variable	Excluded from baseline Pareto objective set

Notes: A

[a]$Y_{Fame}=100M_{FAME}/(M_{FAME}+M_0)$. The baseline multiobjective problem was formulated as mint, maxM_{FAME}, and maxY_{FAME}. Y_{FAME} was retained as a conversion-performance criterion despite its algebraic dependence on product masses and was therefore not interpreted as statistically independent. P_{FAME} was not simultaneously introduced as an additional baseline objective because it is derived from M_{FAME} and t. Lipid titre was retained as process context rather than an additional optimisation dimension. Lipid titre (gL^{-1} and methyl-ester mass (g) were not interpreted as a direct physical mass balance because their dimensional bases differ.

2.3 Construction of the time-resolved harvest-decision matrix

A time-resolved harvest-decision matrix was constructed separately for *Chlamydomonas* sp. OW5 and *Euglena* sp. OW7 across the 15 candidate cultivation days (Table 2). For each strain and cultivation day, the three trial observations were consolidated as the daily mean ± standard deviation (SD) for the measured variables. The resulting matrix aligned cultivation time with lipid titre, experimentally weighed methyl-ester mass and methyl-ester conversion yield, thereby providing a common temporal basis for subsequent harvest-decision analysis. Original trial-level observations were retained during data processing to preserve traceability between the experimental measurements and the aggregated daily states.

To provide an additional time-normalised indicator of product formation, methyl-ester productivity at cultivation day t, P_{FAMEt} ($g\ d^{-1}$), was calculated using equation (1) shown below:

$$P_{FAME,t} = M_{FAME,t}/t$$

where $M_{FAME,\ t}$ is the experimentally measured methyl-ester mass (g) at cultivation day tt, and tt is cultivation duration (d). $P_{FAME,t}$, was explicitly classified as a derived, time-normalised performance metric, rather than an independent experimental response. Because it is

mathematically determined by methyl-ester mass and cultivation time, it was used for descriptive and diagnostic interpretation but was not included simultaneously with both M_{FAME} and t in the baseline multiobjective optimisation. This prevented mathematical dependency from introducing redundant weighting into the harvest-decision framework.

2.4 Multiobjective harvest-decision formulation

Harvest selection was formulated as a three-objective decision problem in which each experimentally evaluated cultivation day represented a candidate harvest state. The baseline optimisation simultaneously sought to minimise cultivation duration while maximising absolute methyl-ester output and methyl-ester conversion yield:

$$\mathrm{Min}_{t},\ \mathrm{max} M_{FAME,max}\ Y_{FAME}$$

where t is cultivation duration (d), M_{FAME} is the experimentally weighed methyl-ester mass (g), and Y_{FAME} is the methyl-ester conversion yield (%). This formulation explicitly represents the operational trade-off between terminating cultivation earlier and allowing additional cultivation time to improve downstream product performance. No predetermined harvest day was imposed before the multiobjective analysis.

Lipid titre, L (gL^{-1}), was retained as a feedstock-state variable describing the lipid availability associated with each cultivation state rather than introduced as an additional baseline optimisation objective. This treatment avoided over-representing closely related production information within the objective space, because lipid availability provides upstream context for the subsequently measured methyl-ester output but does not constitute an independent

downstream decision endpoint. Moreover, lipid titre and methyl-ester mass have different dimensional bases and were therefore not treated as directly interchangeable quantities.

Similarly, the derived time-normalised methyl-ester productivity, P_{FAME}, was excluded from the baseline objective set because it is mathematically determined by M_{FAME} and t. The resulting three-objective formulation therefore preserved distinct decision information while limiting redundancy and potential double-counting among dependent performance variables.

2.5 Pareto-dominance and non-dominated-set analysis

Pareto-dominance analysis was applied to distinguish harvest states that represented defensible multiobjective trade-offs from those that were inferior to another experimentally evaluated cultivation day. Each candidate harvest day was represented by the three-objective vector comprising cultivation duration, methyl-ester mass and methyl-ester conversion yield, with cultivation time minimised and both product-performance variables maximised. For two candidate harvest days i and j, harvest day ii was defined as dominating harvest day jj when ii was no worse than **j** across all three objectives and strictly better in at least one objective**.** Accordingly, dominance required

$$ti \leq tj, \quad M_{FAME,I} \geq M_{FAME,j} \quad Y_{FAME,i,} \geq Y_{FAME,j}$$

with at least one of these inequalities being strict. A harvest state was classified as Pareto-efficient (non-dominated) when no other candidate day satisfied these dominance conditions

against it. Conversely, a state was classified as dominated when at least one alternative harvest day provided equal or superior performance for every objective while improving at least one.

Each strain underwent independent Pareto categorization during all 15 days of experimental culture. Notably, no weighting, composite score, or arbitrary performance criterion was established during this stage. Consequently, Pareto analysis acted only as an objective assessment technique reliant on the directional preferences designated to the three choice variables. The non-dominated set produced depicted practical time–production–conversion trade-offs, rather than a ranking of harvest days or the determination of a single optimum. The process of compromise selection was later performed as a distinct analytical step.

2.6 Objective normalisation and distance-to-ideal compromise selection

Given that cultivation length, methyl-ester mass, and conversion yield were represented in various units and numerical scales, the three objectives were standardized to dimensionless desirability scores before conducting compromise analysis. Each strain underwent independent normalization throughout all 15 proposed harvest days, where increased normalized values signified enhanced performance.

For the minimisation objective, cultivation time was normalized as

$$S_{t,i}=[t_{max}-t_i]/\ [t_{max}-t_{min}]$$

whereas each maximisation objective j, corresponding to M_{FAME} or Y_{FAME}, was normalized as

$$s_{IJ}=[f_{ij}-f_{jmin}]\ /\ [f_{j,max}\ -f_{j,min}]$$

The resulting scores ranged from 0 to 1, with 1 representing the most desirable observed value for an individual objective. The ideal normalized decision state was therefore represented by (1,1,1).

The compromise performance of candidate harvest day i was quantified using its weighted Euclidean distance from this ideal state:

$$D_i = \sqrt{\sum_j w_j (1 - s_{ij})^2},$$

where w_j denotes the relative weight assigned to objective j, subject to $\sum_j w_j = 1$. The baseline analysis assigned equal importance to cultivation-time desirability, methyl-ester output and conversion yield:

$$w_t = w_m = w_Y = 1/3$$

The candidate selection days were arranged in ascending order based on Di, where the smallest value signifies the optimal equal-weight compromise solution. This label was purposefully set apart from a universal biological standard: it denotes the candidate status that is closest to the intended ideal position according to the provided objective criteria, normalization approach, and weighting assumptions. The strength against different objective criteria was thus investigated individually by a thorough weight-sensitivity study.

2.7 Harvest-displacement and performance-loss analysis

Following identification of the preferred equal-weight compromise state, harvest-displacement analysis was performed to quantify the consequences of harvesting earlier or later than the selected reference day. Day 12 was used as the reference state because it produced the minimum

equal-weight distance-to-ideal for both strains. For each product-performance criterion j, the percentage performance loss at candidate harvest day tt was calculated as

$$Loss_{j,t} = \{[f^*_j - f_{j,t}] / f^*_j\} \times 100$$

where f^*_j is the value of criterion j at the Day-12 reference state and $f_{j,t}$ is its value at the displaced harvest day. Losses were calculated separately for methyl-ester mass and conversion yield.

For harvest states preceding Day 12, absolute time saving was calculated as 12−t days, with percentage time saving expressed relative to the 12-day reference duration. These values were evaluated alongside the corresponding losses in methyl-ester output and conversion performance. For post-Day-12 states, additional cultivation time was expressed as t−12 days and considered together with the associated product-performance losses. This analysis therefore distinguished premature-harvest trade-offs from delayed-harvest penalties. All reductions are reported as performance losses or harvest-displacement penalties, rather than economic costs, because monetary, energy and environmental consequences were outside the defined decision framework.

2.8 Objective-weight sensitivity and robustness analysis

The robustness of harvest-day selection to alternative decision priorities was evaluated by systematically varying the relative weights assigned to cultivation-time desirability (w_t), methyl-ester output (w_m) and conversion yield (w_Y). All weights were constrained to be non-negative and to satisfy

$w_t + w_M + w_Y = 1$.

The complete three-objective simplex was enumerated at 0.01 weight increments, producing 5,151 unique weight combinations. For every combination, the weighted distance-to-ideal was recalculated for all candidate harvest days, and the day producing the minimum distance was recorded as the preferred compromise under that specific weighting scheme. Selection frequency was subsequently expressed as the proportion of evaluated weight combinations for which each harvest day was preferred.

A second analysis examined a balanced interior weight space in which

$w_t, w_M, w_Y \geq 0.10$

This restriction ensured that each objective retained a meaningful minimum contribution to the decision while excluding extreme boundary preferences approaching effective single-objective optimisation. The constrained simplex contained 2,556 weight combinations, again evaluated at 0.01 increments. Robustness was assessed from the persistence of the selected harvest day across the complete and balanced-interior weight spaces. These frequencies represent deterministic coverage of the specified objective-weight space and were not interpreted as probabilities of a particular harvest day being optimal.

2.9 Statistical treatment and inferential boundaries

Trial-level observations were preserved during data reconciliation prior to the computation of daily summary statistics. For every strain and day of culture, the measured responses were compiled as mean ± standard deviation (SD) from three experimental observations. No further

inferential biological importance was assigned to the optimisation results. Pareto classification, normalised objective scores, distance-to-ideal metrics, harvest-displacement penalties, and objective-weight selection frequencies were regarded as deterministic decision-analysis results obtained from the experimental dataset, rather than as inferential biological statistics. Consequently, weight-space frequencies characterise resilience across assessed choice inclinations rather than sample probabilities or population-wide confidence levels. Software titles or version details were not given in instances where they were undocumented.

3. RESULTS AND DISCUSSION

3.1 Temporal progression from lipid availability to methyl-ester output

The time-resolved profiles demonstrated a synchronised yet strain-dependent advancement from lipid accessibility to methyl-ester synthesis (Table 2; Figure 1). Throughout the 15-day culture duration, lipid concentration (L), empirically assessed methyl-ester mass (M_{FAME}), methyl-ester conversion efficiency (Y_{FAME}), and time-adjusted methyl-ester productivity (P_{FAME}) escalated to a shared apex on Day 12, subsequently diminishing in the post-peak phase. This advancement is mechanistically significant, as the generation of microalgal biodiesel relies on both lipid accumulation and the subsequent transformation of the isolated lipid fraction into fatty-acid methyl esters. The circumstances of cultivation and subsequent extraction/transesterification might thus affect the ultimate generation of methyl esters [31, 32].

In the case of Chlamydomonas sp. OW5, the lipid concentration rose from 0.797 g L^{-1} on Day 8 to 2.510 g L^{-1} on Day 12, whereas M_{FAME} escalated from 0.333 ± 0.006 g to 2.143 ± 0.006 g

(Table 2; Figure 1). During the same period, Y_{FAME} rose from 41.84 ± 0.12% to 85.39 ± 0.18%, whereas the average P_{FAME} escalated from 0.0417 to 0.1786 g d^{-1}. Euglena sp. OW7 exhibited a consistent temporal trend, but with reduced total lipid and methyl-ester levels. During the period from Day 8 to Day 12, LL rose from 0.350 to 1.467 g L^{-1}, whereas MFAME grew from 0.170 g to 1.380 ± 0.010 g. Simultaneously, Y_{FAME} rose from 48.57% to 94.09 ± 0.34%, while the average P_{FAME} escalated from 0.0213 to 0.1150 g d^{-1}. Consequently, the Day-12 condition distinguished the two production phenotypes: Chlamydomonas produced a higher total methyl-ester output, whereas Euglena attained a superior conversion yield. This differentiation illustrates why the volume of product and conversion efficiency need not be regarded as synonymous metrics of biodiesel-production efficacy.

The simultaneous rises in L and M_{FAME} align with the function of stored microalgal lipids as a substrate for transesterification. However, enhanced lipid availability does not inherently ensure a corresponding proportional rise in methyl-ester conversion, as lipid accumulation is contingent upon both strain and cultivation conditions, while extraction and transesterification parameters further affect FAME yield and conversion efficacy. The timing of harvesting is therefore significant: earlier studies have shown that the harvest period might influence the lipid output and fatty-acid profiles of microalgae pertinent to future biodiesel synthesis [35, 36].

A significant turnaround ensued after the Day-12 peak. By Day 15, the MFAME levels diminished to 0.430 ± 0.010 g in Chlamydomonas and 0.157 ± 0.006 g in Euglena, even after three further days of culture. Consequently, Y_{FAME} decreased to 65.48 ± 0.29% and 75.79 ± 0.69%, respectively. The simultaneous declines in lipid availability and P_{FAME} after the peak suggest that prolonging culture failed to maintain the productive condition reached on Day 12.

This behaviour aligns with the comprehensive notion that microalgal lipid synthesis is variable and heavily influenced by physiological and cultural factors, rather than being a static characteristic of the strain.

The temporal trajectories together indicate that Day 12 serves as the recognised common peak for L, M_{FAME}, Y_{FAME}, and P_{FAME} in both strains. Significantly, this description peak does not yet correspond to an ideal harvesting period. A justifiable harvest choice must also evaluate if the increases in methyl-ester production and conversion efficiency warrant the extra cultivation duration needed to achieve that outcome. This differentiation between a recorded output peak and a multi-faceted harvesting preference underpins the ensuing choice analysis.

Table 2: Time-resolved lipid and methyl-ester performance of ***Chlamydomonas*** sp. OW5 and ***Euglena*** sp. OW7 across candidate harvest days

Strain	**Day**	**L(gL^{-1})**	**M_{FAME} (g)**	**Y_{FAME} (%)**	**P_{FAME}(gL^{-1})**
Chlamydomonas **sp. OW5**	1	0.020 ± 0.000	0.002 ± 0.000	10.00 ± 0.00	0.0020 ± 0.0000
	2	0.050 ± 0.000	0.007 ± 0.000	14.00 ± 0.00	0.0035 ± 0.0000
	3	0.090 ± 0.000	0.020 ± 0.000	22.22 ± 0.00	0.0067 ± 0.0000
	4	0.170 ± 0.000	0.040 ± 0.000	23.53 ± 0.00	0.0100 ± 0.0000
	5	0.303 ± 0.006	0.090 ± 0.000	29.68 ± 0.56	0.0180 ± 0.0000
	6	0.443 ± 0.006	0.150 ± 0.000	33.84 ± 0.44	0.0250 ± 0.0000
	7	0.650 ± 0.010	0.240 ± 0.000	37.12 ± 0.33	0.0343 ± 0.0000
	8	0.797 ± 0.012	0.333 ± 0.006	41.84 ± 0.12	0.0417 ± 0.0007
	9	0.987 ± 0.006	0.493 ± 0.012	50.00 ± 0.88	0.0548 ± 0.0013

Strain	Day	L(gL^{-1})	M_{FAME} (g)	Y_{FAME} (%)	P_{FAME}(gL^{-1})
	10	1.173 ± 0.072	0.770 ± 0.044	65.64 ± 0.39	0.0770 ± 0.0044
	11	1.443 ± 0.006	1.093 ± 0.006	75.75 ± 0.10	0.0994 ± 0.0005
	12	**2.510 ± 0.010**	**2.143 ± 0.006**	**85.39 ± 0.18**	**0.1786 ± 0.0005**
	13	1.663 ± 0.012	1.303 ± 0.012	78.36 ± 0.15	0.1003 ± 0.0009
	14	1.247 ± 0.015	0.903 ± 0.012	72.46 ± 0.30	0.0645 ± 0.0008
	15	0.657 ± 0.015	0.430 ± 0.010	65.48 ± 0.29	0.0287 ± 0.0007
Euglena **sp. OW7**	1	0.005 ± 0.001	0.001 ± 0.000	10.89 ± 5.00	0.0006 ± 0.0003
	2	0.020 ± 0.000	0.002 ± 0.000	10.00 ± 0.00	0.0010 ± 0.0000
	3	0.050 ± 0.000	0.008 ± 0.000	16.00 ± 0.00	0.0027 ± 0.0000
	4	0.100 ± 0.000	0.020 ± 0.000	20.00 ± 0.00	0.0050 ± 0.0000
	5	0.150 ± 0.000	0.050 ± 0.000	33.33 ± 0.00	0.0100 ± 0.0000
	6	0.210 ± 0.000	0.080 ± 0.000	38.10 ± 0.00	0.0133 ± 0.0000
	7	0.260 ± 0.000	0.120 ± 0.000	46.15 ± 0.00	0.0171 ± 0.0000
	8	0.350 ± 0.000	0.170 ± 0.000	48.57 ± 0.00	0.0213 ± 0.0000
	9	0.477 ± 0.006	0.280 ± 0.000	58.75 ± 0.72	0.0311 ± 0.0000
	10	0.593 ± 0.006	0.470 ± 0.000	79.22 ± 0.77	0.0470 ± 0.0000
	11	0.780 ± 0.010	0.677 ± 0.006	86.76 ± 0.59	0.0615 ± 0.0005
	12	**1.467 ± 0.015**	**1.380 ± 0.010**	**94.09 ± 0.34**	**0.1150 ± 0.0008**
	13	0.927 ± 0.006	0.797 ± 0.006	85.97 ± 0.09	0.0613 ± 0.0004
	14	0.653 ± 0.006	0.523 ± 0.006	80.10 ± 0.17	0.0374 ± 0.0004

Strain	Day	L(gL^{-1})	M_{FAME} (g)	Y_{FAME} (%)	P_{FAME}(gL^{-1})
	15	0.207 ± 0.006	0.157 ± 0.006	75.79 ± 0.69	0.0104 ± 0.0004

Notes: Values are mean ± SD from three trial/replicate observations. L, lipid titre; M_{FAME}, experimentally weighed methyl-ester mass following transesterification; Y_{FAME}, calculated methyl-ester conversion yield; P_{FAME}, time-normalised methyl-ester productivity calculated as M_{FAME} /t. Day 12 is shown in bold because it represents the observed maximum of all four displayed performance indicators in both strains. This descriptive maximum is **not itself used to define the multiobjective optimum**; harvest-day preference is established subsequently through Pareto dominance and compromise analysis.

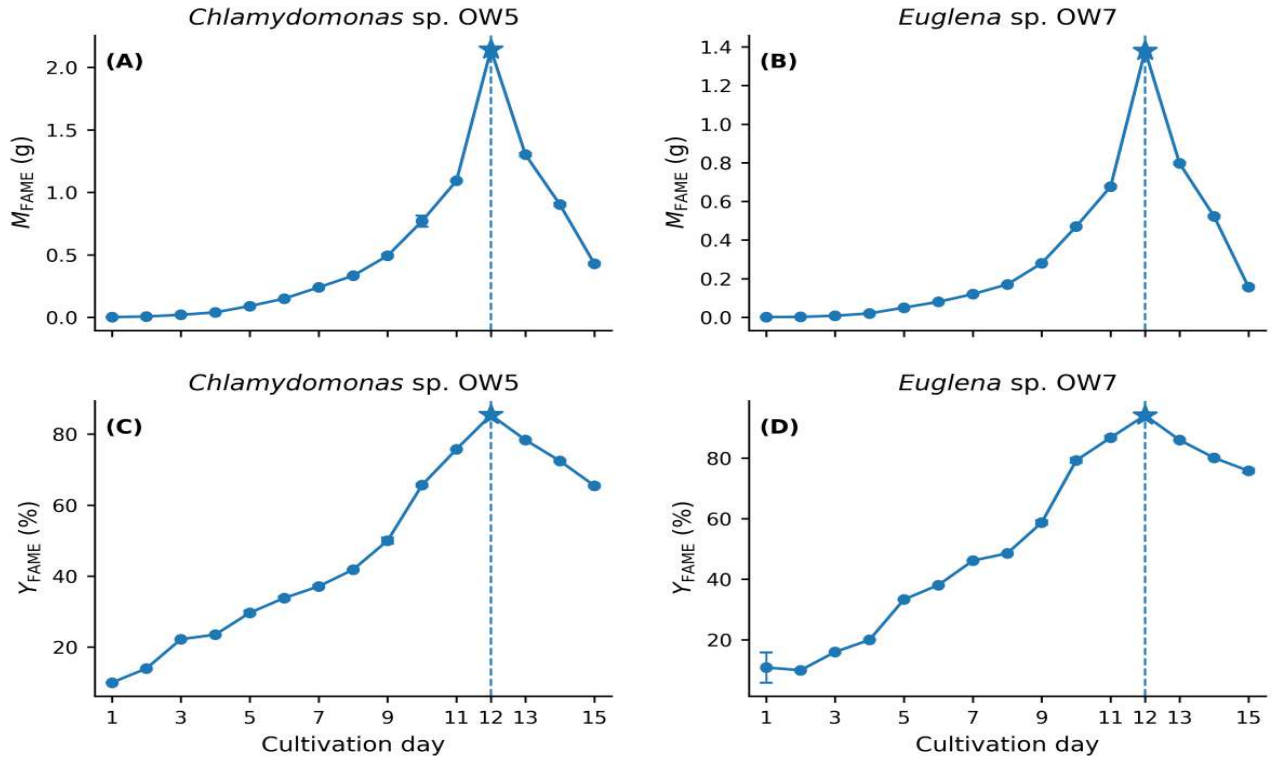


Figure 1: Time-resolved progression of methyl-ester output and conversion yield across candidate harvest days

Time-resolved changes in experimentally weighed methyl-ester mass (M_{FAME}) and calculated methyl-ester conversion yield (Y_{FAME}) during 15-day cultivation of (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7. Both performance indicators increased progressively toward Day 12 and declined thereafter. The vertical dashed line identifies Day 12, the common observed maximum for methyl-ester output and conversion yield in both strains. The figure describes the temporal production trajectory; identification of the preferred harvest compromise was subsequently established through Pareto screening, normalized distance-to-ideal analysis, and objective-weight sensitivity rather than from the observed maxima alone.

3.2 Lipid availability and absolute methyl-ester output

The association of lipid accessibility with the experimentally determined methyl-ester mass delineates a strong process-level relationship between the culture and conversion phases (Figure 2). Across all strains, M_{FAME} showed a steady ascent in association with lipid titre (L) during the pre-peak culture period, resulting in a well-defined arrangement by Day 12. In Chlamydomonas sp. OW5, the advancement shifted from very low lipid and methyl-ester quantities during early development to a lipid concentration of 2.510 g L^{-1} and 2.143 ± 0.006 g of methyl ester by the twelfth day. Euglena types. OW7 demonstrated a reliable directional association, reaching a lipid content of 1.467 g L^{-1} and 1.380 ± 0.010 g of methyl ester by Day 12. The heightened lipid content seen on Day 12 in Chlamydomonas was linked to an enhanced total methyl-ester synthesis, while the lower lipid supply in Euglena caused a decline in methyl-ester output. These conclusions are consistent with the fundamental purpose of retained microalgal lipids as the material that is later altered into fatty-acid methyl esters by transesterification [38]. It is vital that the association shown in Figure 2 be not seen as a direct mass balance or conversion ratio. The

volumetric quantification of lipid content was expressed in grams per litre (g L^{-1}), whereas MFAME signifies the methyl-ester product assessed in grams.

As a result, L (g L^{-1}) is not equivalent to M_{FAME} (g) when assessed on a direct dimensional basis. Despite the two variables demonstrating similar numerical patterns, their distinct dimensional foundations hinder the interpretation of their numerical disparity, ratio, or perceived closeness as indicators of mass closure. Creating a physical lipid-to-FAME mass balance necessitates the relevant processed culture or extraction volume along with a standardised mass foundation. This certification is especially significant, as the manufacture of microalgal biodiesel encompasses unique phases of growth, lipid extraction, and transesterification, all of which might influence the quantity of FAME finally produced.

The path following Day 12 improves the understanding as a temporal connection instead of a fixed proportional one. Following Day 12, both lipid titre and MF diminished simultaneously in both strains, resulting in a deviation of MFAME from the shared peak seen in Figure 2. On Day 15, Chlamydomonas maintained a lipid concentration of 0.657 g L^{-1} and 0.430 ± 0.010 g of methyl ester, but Euglena's figures decreased to 0.207 g L^{-1} and 0.157 ± 0.006 g, respectively. This type of synchronised motion is biologically plausible, as the potential for biodiesel correlates with the quantity and characteristics of stored lipids, and the final conversion stage is also affected by lipid composition and transesterification variables. Consequently, Figure 2 substantiates a robust correlation between lipid availability and methyl ester yield throughout the culture progression, without asserting dimensional mass closure. Day 12 exemplifies the acknowledged peak of both variables; nonetheless, this correlation alone fails to determine the

optimal harvest schedule, as it overlooks the culture duration necessities and conversion yield targets that will be analysed in the next multiobjective research.

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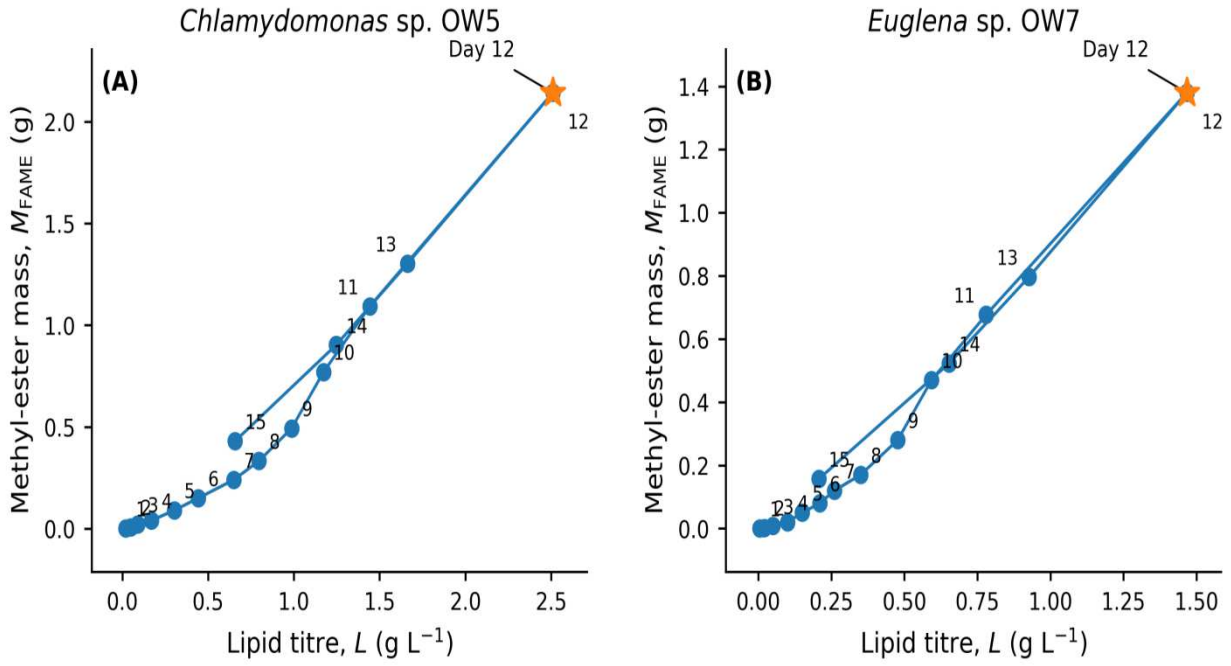


Figure 2: Lipid availability versus experimentally measured methyl-ester mass across candidate harvest days Relationship between lipid availability and experimentally measured methyl-ester mass across candidate harvest days for (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7. Points represent daily mean states and connecting lines preserve their chronological sequence from Day 1 to Day 15. Day 12 is highlighted as the common observed maximum for both lipid titre and methyl-ester mass. The post-Day-12 reversal illustrates that extending cultivation beyond the production maximum did not increase methyl-ester output, providing experimental context for the subsequent multiobjective harvest-time analysis.

3.3 Why single-objective peak extraction is insufficient for harvest selection

The standardised objective trajectories indicate a significant difference between determining a production peak and choosing a harvest moment (Figure 3). When analysed separately, the production-related reactions significantly align with Day 12 in both strains. The normalised methyl-ester production (s_m) and conversion yield (s_Y) steadily rose during the pre-peak cultivation phase, peaking on Day 12. Conversely, the normalised desirability of culture time (s_t) diminished consistently as cultivation advanced, since earlier harvesting is favoured when the duration of cultivation is considered a factor to minimise. As a result, the three choice dimensions fail to pinpoint a shared optimal condition: extending the cultivation period to Day 12 enhances methyl-ester output and conversion efficiency; however, it concurrently undermines the benefits linked to an earlier conclusion of the process.

This differentiation reveals a constraint of traditional single-objective peak extraction. If harvest selection relied just on M_{FAME}, Day 12 would be chosen, as both strains achieved their maximum empirically determined methyl-ester mass on that date. The identical result would be derived from Y_{FAME}, as the conversion yield similarly attained its peak on Day 12. Nevertheless, these individual peaks fail to indicate whether the performance improvement justifies the additional cultivation duration required to reach Day 12. This matter pertains to microalgal biodiesel systems since the period of culture is a crucial aspect of operational processes, with biomass and lipid yield being significantly influenced by cultivation circumstances and physiological status.

This discrepancy is especially apparent in Figure 3. In the case of Chlamydomonas sp. OW5 and Euglena sp. OW7, the shift from prior harvest conditions to Day 12 led to a rise in sm and sY, accompanied by a steady reduction in st. Therefore, Day 12 excels in the production targets, although achieving this status demands the longest growing time among the pre-peak alternatives. Harvesting earlier results in reduced methyl-ester output and conversion yield while preserving increased temporal desirability. This finding indicates a legitimate choice trade-off rather than the commonly referenced biomass-lipid antagonism in microalgal production. Multiobjective methodologies are relevant when the simultaneous maximisation of process responses is unattainable under one operational preference; recent studies on microalgal optimisation have similarly utilised Pareto-based approaches to tackle competing productivity objectives rather than limiting process performance to a single maximum response.

The suggestion is that Day 12 should not be labelled the ideal harvest simply because it encompasses the identified production maxima. Harvesting on Day 11 ends culture one day earlier, whereas Days 10, 9, and 8 increasingly conserve cultivation time; nonetheless, whether these efficiencies justify their reduced M_{fame} and Y_{fame} cannot be discerned from the analysis of a solitary response. In contrast, continuing culture beyond Day 12 provides no similar output benefit, as both production parameters eventually decrease. This separation of pre-peak trade-offs and post-peak degradation delineates the conceptual move from descriptive production analysis to formal harvest optimisation. The harvest-selection problem is reformulated as three simultaneous objectives:

Min_t, $maxM_{FAME}$, $maxY_{FAME}$

The appropriate question is therefore not simply **"**On which day is methyl-ester production highest?"**,** but rather "Which harvest states remain defensible when cultivation time, absolute methyl-ester output and conversion yield are considered simultaneously?" The Pareto-dominance analysis in the following section addresses this question without imposing an arbitrary preference among the three objectives.

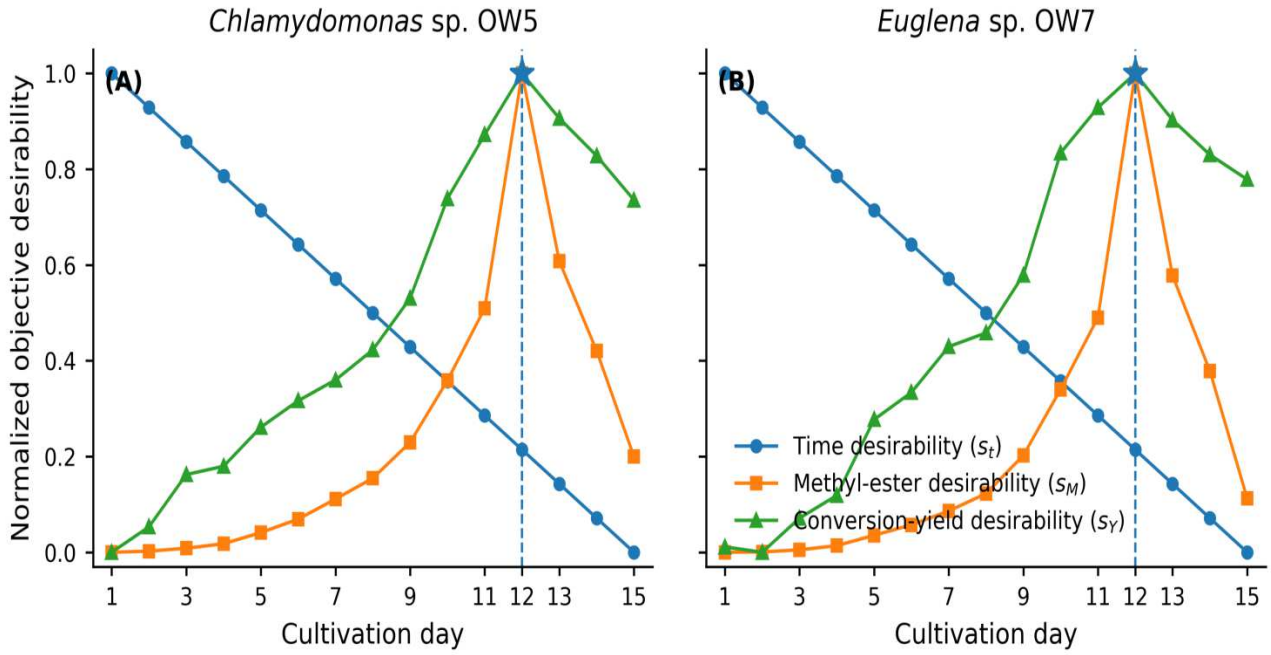


Figure 3: **Normalized objective trajectories reveal the temporal trade-off between cultivation time, methyl-ester output and conversion yield**

The figure shows normalized desirability trajectories for cultivation time (s_t), methyl-ester mass (s_M), and methyl-ester conversion yield (s_Y) for (A) ***Chlamydomonas*** sp. OW5 and (B) ***Euglena*** sp. OW7. Increasing cultivation duration progressively reduces time desirability, whereas methyl-ester and conversion-yield desirabilities generally increase toward Day 12 before declining. The dashed vertical line identifies Day 12, where sM=sY=1 for both

strains. The opposing trajectories establish the explicit time–production–conversion trade-off underlying the subsequent Pareto and compromise analyses.

3.4 Pareto-efficient and dominated harvest states

The application of Pareto-dominance analysis drastically modified the comprehension of time-resolved production profiles. According to the core three-objective model—minimisation of cultivation time (t) in conjunction with maximisation of the experimentally quantified methyl-ester mass (MFAME) and the calculated conversion yield (Y_{FAME})—Days 1–12 were Pareto efficient for both *Chlamydomonas* sp. OW5 and *Euglena* sp. OW7, while Days 13–15 were Pareto dominated (Table 3; Figure 4). This conclusion reveals that the recorded production apex on Day 12 does not rule out earlier harvesting options from consideration when the period of cultivation is distinctly included in the decision-making equation.

A harvest state is identified as Pareto-dominated when another candidate day performs at least comparably in all three objectives and outperforms it in at least one. On the other hand, a non-dominated state cannot achieve improvement in one target without sacrificing progress in at least one other objective. This notion is distinctly applicable for bioprocess optimisation when competing objectives impede a sole response maximum from embodying the full choice challenge. In microalgal biodiesel systems, multiobjective frameworks have similarly been implemented to reconcile competing performance indicators instead of depending on isolated peaks [21].

The Pareto efficiency achieved in Days 1–12 resulted mostly from the opposing orientation of cultivation time concerning the production aims. Although previous eras usually generated lesser amounts of M_{FAME} and Y_{FAME}, they also needed a briefer cultivation period. To exemplify,

shifting from a previous harvest condition to Day 12 steadily augmented methyl-ester output and conversion rates; however, this enhancement was only possible by lengthening the culture period. Thus, Day 12 failed to overshadow the preceding states because its advantageous production performance was counterbalanced by a deficiency in the time-reduction target. In the same vein, the earlier nations failed to excel on Day 12, as their time advantage was hindered by lesser production and conversion capabilities. Therefore, the Days 1–12 set indicates a succession of non-dominated time-production trade-offs, rather than twelve harvest periods of equal desirability. This perspective is consistent with the core goal of Pareto analysis: identifying choices in which advancement in one criterion necessitates a sacrifice in another, therefore safeguarding the decision-making scope before preferences are enforced [21].

A significantly different state arose on Day 12. Days 13 to 15 exhibited Pareto dominance in both strains, as extended culture time led to a decline rather than an enhancement in the two production goals (Table 3; Figure 4). On Day 13, an extra culture day was necessary compared to Day 12, resulting in reduced M_{FAME} and Y_{FAME} yields. The drawback became more apparent on Days 14 and 15. As a result, Day 12 exhibited an earlier occurrence, an increased methyl-ester production, and a superior conversion yield compared to all post-peak conditions. No compensatory benefit was present for maintaining Days 13–15 inside the foundational aim framework. This sharply contrasts with Days 1–11, where a shorter cultivation period resulted in a significant opposing advantage.

The outcome is significant since Pareto efficiency must not be mistaken for optimality or hierarchy. A Pareto-efficient condition is one where no evident alternative excels unequivocally across all goals. Multi-criteria methodologies are beneficial because they require balancing

multiple relevant criteria in decision-making processes, rather than relying exclusively on a single response variable [48]. Consequently, the extensive Pareto set derived here does not designate Days 1–12 as a comparable harvest period, nor does it confirm Day 12 as the singularly favoured option.

Pareto screening yields the manuscript's initial significant optimisation outcome: it divides the cultivation timeline into a pre-peak decision zone (Days 1–12), where time and production are authentically conflicting goals, and a post-peak dominated area (Days 13–15), where further cultivation offers no advantageous return based on the evaluated objectives. The pertinent inquiry is not which days are technically non-dominated, but rather which element of this extensive Pareto-efficient collection offers the most justifiable compromise among cultivation duration, methyl-ester production, and conversion efficiency. This differentiation drives the examination of distance to the ideal in the next section.

Table 3: Pareto-dominance classification of candidate harvest days under the production–conversion–time decision framework

Strain	Harvest day	Pareto status	Dominated by earlier harvest state?
Chlamydomonas **sp. OW5**	1	Non-dominated	No
	2	Non-dominated	No
	3	Non-dominated	No
	4	Non-dominated	No

Strain	Harvest day	Pareto status	Dominated by earlier harvest state?
	5	Non-dominated	No
	6	Non-dominated	No
	7	Non-dominated	No
	8	Non-dominated	No
	9	Non-dominated	No
	10	Non-dominated	No
	11	Non-dominated	No
	12	**Non-dominated**	**No**
	13	**Dominated**	**Yes**
	14	**Dominated**	**Yes**
	15	**Dominated**	**Yes**
Euglena **sp. OW7**	1	Non-dominated	No
	2	Non-dominated	No
	3	Non-dominated	No
	4	Non-dominated	No
	5	Non-dominated	No
	6	Non-dominated	No
	7	Non-dominated	No
	8	Non-dominated	No
	9	Non-dominated	No
	10	Non-dominated	No
	11	Non-dominated	No
	12	**Non-dominated**	**No**

Strain	Harvest day	Pareto status	Dominated by earlier harvest state?
	13	**Dominated**	**Yes**
	14	**Dominated**	**Yes**
	15	**Dominated**	**Yes**

Notes: Pareto dominance was evaluated using the baseline objective vector comprising minimisation of cultivation time (t), maximisation of experimentally weighed methyl-ester mass (M_{FAME}, and maximisation of calculated methyl-ester conversion yield (Y_{FAME}). A harvest state was classified as dominated when another candidate state was no worse across all three objectives and strictly better in at least one objective. Accordingly, Days 1–12 constitute the non-dominated candidate set for both strains, whereas Days 13–15 are dominated by an earlier harvest state. **Non-dominated status does not itself identify a unique optimum**; selection of the preferred compromise from the Pareto-efficient set is subsequently resolved using normalized distance-to-ideal analysis

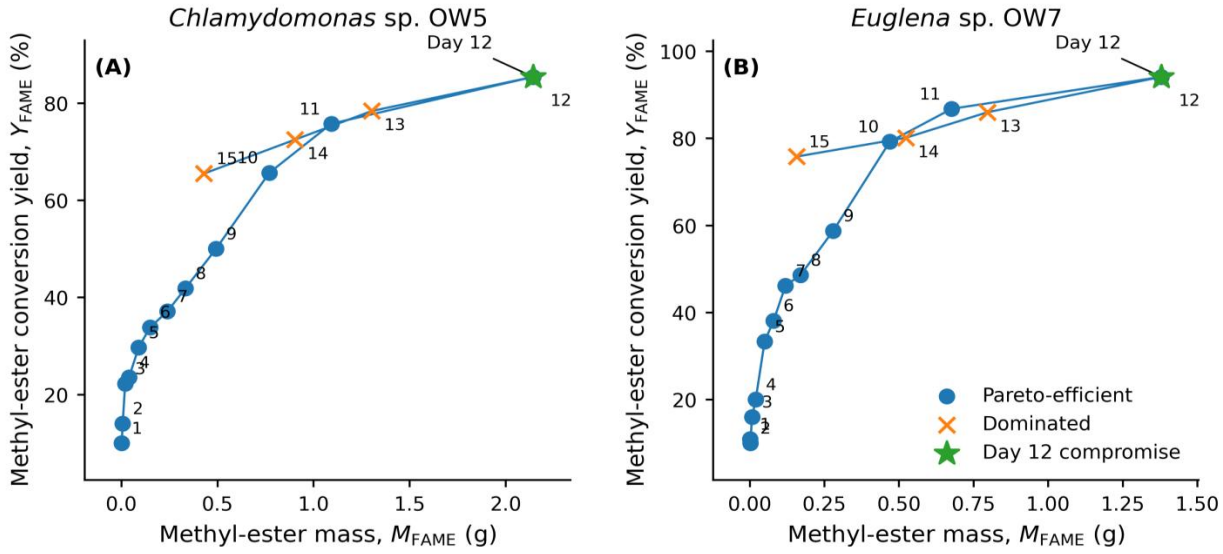


Figure 4: Pareto-efficient and dominated harvest states under the production–conversion–time decision framework. Pareto classification of candidate harvest states for (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7 under simultaneous minimisation of cultivation time and maximisation of methyl-ester mass

(M_{FAME}) and conversion yield (Y_{FAME}). Days 1–12 remained Pareto-efficient because improvements in production and/or conversion required additional cultivation time, whereas Days 13–15 were dominated by earlier harvest states. Day 12 is highlighted as the subsequent robust compromise target identified through distance-to-ideal and objective-weight sensitivity analyses. Connecting lines indicate chronological progression and do not represent fitted relationships. This figure makes the important distinction that Pareto-efficient does not mean equally preferred: Days 1–12 constitute the admissible trade-off set, while the subsequent analyses determine which state provides the strongest compromise

3.5 Ideal-point compromise identifies a common harvest target

The Pareto screening indicated that Days 1–12 were non-dominated, although this comprehensive Pareto set did not determine which option produced the most balanced mix of cultivation time, methyl-ester production, and conversion efficiency. Consequently, the normalised ideal-point analysis established a supplementary choice layer by measuring the geometric nearness of each harvest state to an imagined solution that amalgamates peak time desirability, maximum M_{FAME}, and maximum Y_{FAME} (Table 4; Figure 5). This divergence has significance since Pareto efficiency acknowledges justifiable alternatives yet fails to evaluate them based on their comprehensive compromises.

Applying equal weighting (wt=wM=wY=1/3), Day 12 achieved the minimal distance-to-ideal metric across both strains (D12=0.4536), revealing a unified balanced harvest goal despite differing absolute output levels. Within Chlamydomonas sp. OW5, Day 11 represented the subsequent closest condition (Di=0.5056), trailed by Day 10 (Di=0.5457). The same arrangement was noted in Euglena sp. OW7, where Di=0.5092 for Day 11 and Di=0.5415 for Day 10 (Table

4). As a result, the compromise environment steadily approached Day 12 in the late pre-peak timeframe rather than revealing a sharp, isolated maximum.

The determination of Day 12 is clearly articulated through the normalised goal architecture. Even if the appeal for culture duration was lessened (st=0.2143), Day 12 simultaneously reached s_m=1.000 and s_Y=1.000 across both strains. Previous states held a time benefit while still being farther distant from the optimal production coordinates. Day 11 saw an increase in time desirability to s_t=0.2857s; however, the normalised methyl-ester production fell to 0.5097 in Chlamydomonas and 0.4902 in Euglena. Thus, the little progress in time desirability was insufficient, when equally weighted, to offset the displacement caused by the two production goals.

This interpretation, based on compromise, is more advantageous than choosing a harvest day exclusively based on the highest value of a single answer. The manufacture of microalgal biodiesel encompasses cultivation, biomass and lipid creation, and subsequent conversion; hence, the efficacy of the process relies on interrelated rather than independent operational parameters. Ideal-point analysis does not eradicate trade-offs; rather, it clarifies the preference framework by assessing all normalised objectives on a unified decision scale.

Significantly, the distance ranking illustrates why Pareto efficiency and favoured compromises are not equivalent. The initial twelve days were Pareto-efficient, although their deviations from the optimal condition varied somewhat. In contrast, Day 13 was positioned fourth by geometric distance in both strains, even though it was Pareto-dominated, demonstrating that scalar closeness should not supplant dominance evaluation. The two evaluations are therefore synergistic: Pareto analysis delineates the non-dominated decision realm, while ideal-point

analysis refines that extensive realm into a harmonised preference based on designated weights. Consequently, Day 12 is identified as the favoured equal-weight solution, rather than a universal ideal. The necessity for a follow-up weight-sensitivity study arises to see if this preference persists when the relative significance of cultivation duration, yield, and conversion is altered.

Table 4: Normalized objective performance and distance-to-ideal compromise ranking of candidate harvest days

Strain	**Day**	S_t	S_M	S_Y	**Di**	**Rank**
Chlamydomonas **sp. OW5**	1	1.0000	0.0000	0.0000	0.8165	15
	2	0.9286	0.0023	0.0531	0.7952	14
	3	0.8571	0.0084	0.1623	0.7540	12
	4	0.7857	0.0178	0.1797	0.7492	11
	5	0.7143	0.0411	0.2612	0.7182	10
	6	0.6429	0.0692	0.3164	0.6980	9
	7	0.5714	0.1112	0.3599	0.6792	8
	8	0.5000	0.1547	0.4226	0.6578	7
	9	0.4286	0.2295	0.5305	0.6166	5
	10	0.3571	0.3587	0.7385	0.5457	3
	11	0.2857	0.5097	0.8720	0.5056	2
	12	**0.2143**	**1.0000**	**1.0000**	**0.4536**	**1**

Strain	Day	S_t	S_M	S_Y	Di	Rank
	13	0.1429	0.6077	0.9061	0.5469	4
	14	0.0714	0.4208	0.8279	0.6395	6
	15	0.0000	0.1998	0.7356	0.7550	13
Euglena **sp. OW7**	1	1.0000	0.0000	0.0112	0.8122	14
	2	0.9286	0.0007	0.0000	0.8171	15
	3	0.8571	0.0051	0.0713	0.7900	13
	4	0.7857	0.0138	0.1188	0.7734	11
	5	0.7143	0.0355	0.2772	0.7149	10
	6	0.6429	0.0572	0.3337	0.6974	9
	7	0.5714	0.0862	0.4294	0.6691	8
	8	0.5000	0.1225	0.4581	0.6614	7
	9	0.4286	0.2023	0.5790	0.6162	5
	10	0.3571	0.3403	0.8341	0.5415	3
	11	0.2857	0.4902	0.9286	0.5092	2
	12	**0.2143**	**1.0000**	**1.0000**	**0.4536**	**1**
	13	0.1429	0.5777	0.9027	0.5546	4
	14	0.0714	0.3788	0.8302	0.6521	6
	15	0.0000	0.1129	0.7786	0.7819	12

Notes: S_t, S_M, and S_Y denote normalized desirability scores for cultivation time, methyl-ester mass, and calculated methyl-ester conversion yield, respectively. Cultivation time was treated as a minimisation objective, whereas methyl-ester mass and conversion yield were treated as maximisation objectives. Normalization was performed on a 0–1 scale, with larger values indicating greater desirability.

For cultivation time,

$S_t = t_{max} - t / t_{max} - t_{min}$

whereas benefit objectives were normalized as

$s_j = f_j - f_{j,min} / f_{j,max} - f_{j,min}$.

Under equal weighting (wt=wM=wY=1/3), distance from the ideal point (1,1,1) was calculated as

$D_i = \sqrt{1/3(1-s_t)^2 + 1/3(1-s_M)^2 + 1/3(1-S_Y)^2}$

Candidate harvest days were ranked in ascending order of DiD_i, such that a smaller value represents a closer compromise to the ideal multiobjective state

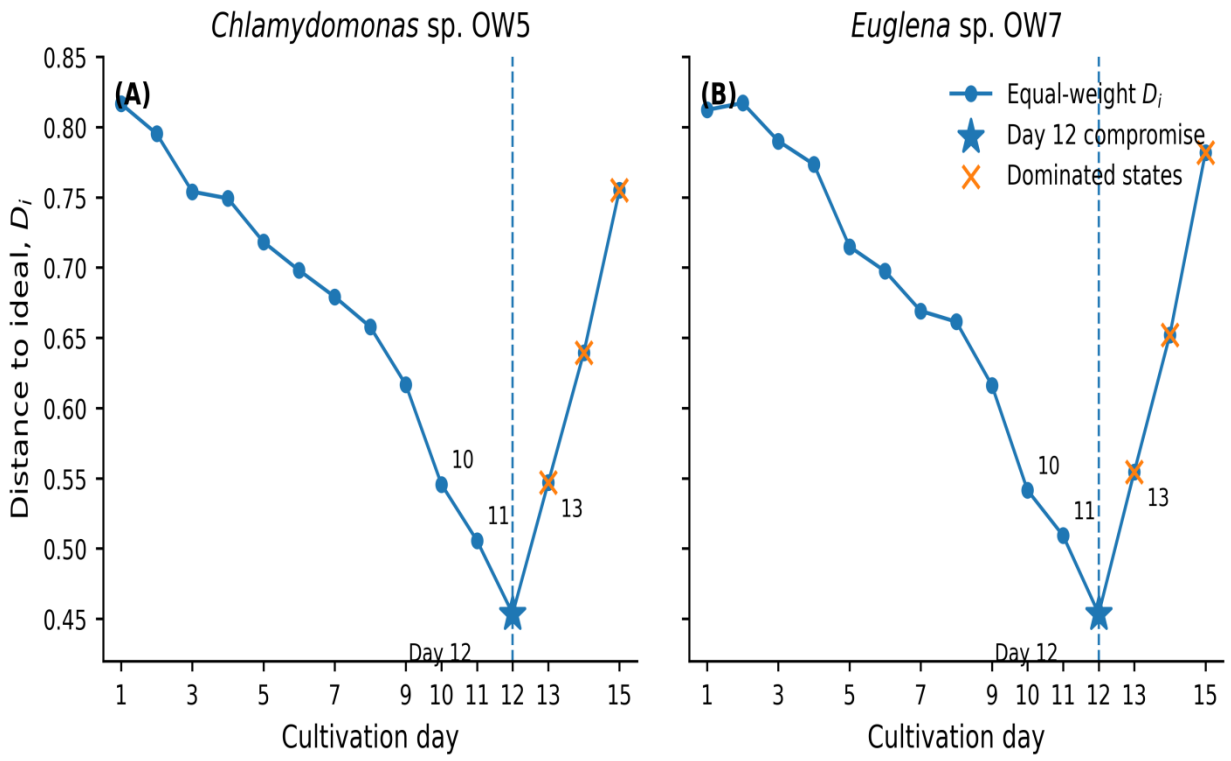


Figure 5: Distance-to-ideal compromise landscape across candidate harvest days

Note: Equal-weight distance-to-ideal (D_i) across candidate harvest days for (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7 under simultaneous consideration of cultivation-time desirability, methyl-ester output and conversion yield. Lower D_i indicates closer proximity to the ideal multiobjective state. Day 12 produced the minimum distance in both strains (D_i=0.4536) and therefore represents the preferred equal-weight compromise. Days 10 and 11 approach the compromise minimum from the pre-peak region, whereas Days 13–15 move progressively away from the ideal and are Pareto-dominated. The dashed vertical line identifies Day 12, while crosses distinguish dominated post-peak harvest states.

3.6 Asymmetric consequences of premature and delayed harvesting

The tangible relevance of the Day-12 compromise was shown when harvest displacement was conveyed in terms of the output maximum (Figure 6). The investigation uncovered a marked disparity between premature and postponed harvesting: early harvesting offered a temporal advantage in culture but adversely affected methyl-ester quality, whereas delayed harvesting increased cultivation time and concurrently lowered product effectiveness. This differentiation holds special significance in process engineering since the length of cultivation is an operational variable, and the cultivation and harvesting of microalgae are critical restrictions in biodiesel production systems [28].

The most robust evidence was gathered for the nearest pre-peak alternative. The decision to harvest Chlamydomonas sp. OW5 on Day 11 instead of Day 12 resulted in a culture duration decrease of one day, which corresponds to an 8.33% time efficiency gain, but caused a 48.99% drop in M_{FAME} and an 11.29% reduction in Y_{FAME}. The relevant trade-off was rather elevated for the total methyl-ester yield in Euglena sp. OW7: the same 8.33% savings in cultivation time led to a 50.97% drop in MFAME and a 7.80% contraction in Y_{FAME}. As a result, around half of the

Day-12 methyl-ester mass was relinquished to end cultivation just one day ahead. This imbalanced exchange clarifies why Day 11, despite its Pareto efficiency and status as the second-ranked equal-weight compromise, could not achieve performance comparable to Day 12.

The sanction escalated with increasingly premature harvests. On Day 10, a 2-day decrease in culture (16.67%) correlated with MFAME losses of 64.07% in Chlamydomonas and 65.94% in Euglena. Collecting the crop four days sooner, on Day 8, preserved 33.33% of the cultivation time compared to Day 12, although it decreased methyl-ester yield by 84.45% and 87.68%, respectively. Prior harvest conditions thus continued to be valid Pareto alternatives since they provided genuine time savings, although at the cost of progressively greater reductions in recoverable methyl-ester production. This underscores the wider acknowledgement that the efficacy of microalgal biodiesel cannot be assessed in isolation from cultivation and harvesting choices [51, 52].

The post-Day-12 area exhibited markedly distinct decision-making patterns. Delaying the harvest to Day 13 added another culture day, but M_{FAME} dropped by 39.19% in Chlamydomonas and 42.27% in Euglena. At the same time, Y_{FAME} dropped by 8.24% and 8.63%, respectively. On Day 15, the losses of methyl esters amounted to 79.94% and 88.65%, respectively. In contrast to early harvesting, delayed harvesting yielded no beneficial enhancement in the evaluated criteria. This finding statistically elucidates the Pareto categorisation of Days 13–15 as prevailing conditions. The engineering differentiation may thus be articulated as early harvest = temporal advantage + product-performance compromise, whereas delayed harvest = extended cultivation duration + product-performance compromise.

This imbalance is more insightful than merely recognising Day 12 as the peak. It illustrates the differing implications of departures on each side of Day 12. Premature harvesting may be justified when the culture period is prioritised, whereas delayed harvesting is not endorsed in the current three-objective framework due to the adverse effects on both M_{FAME} and Y_{FAME} from extended cultivation times. Consequently, the displacement analysis reinforces Day 12 as a solid compromise objective while circumventing the unfounded interpretation of Days 10–12 or 11–12 as a comparable multi-day ideal harvest period.

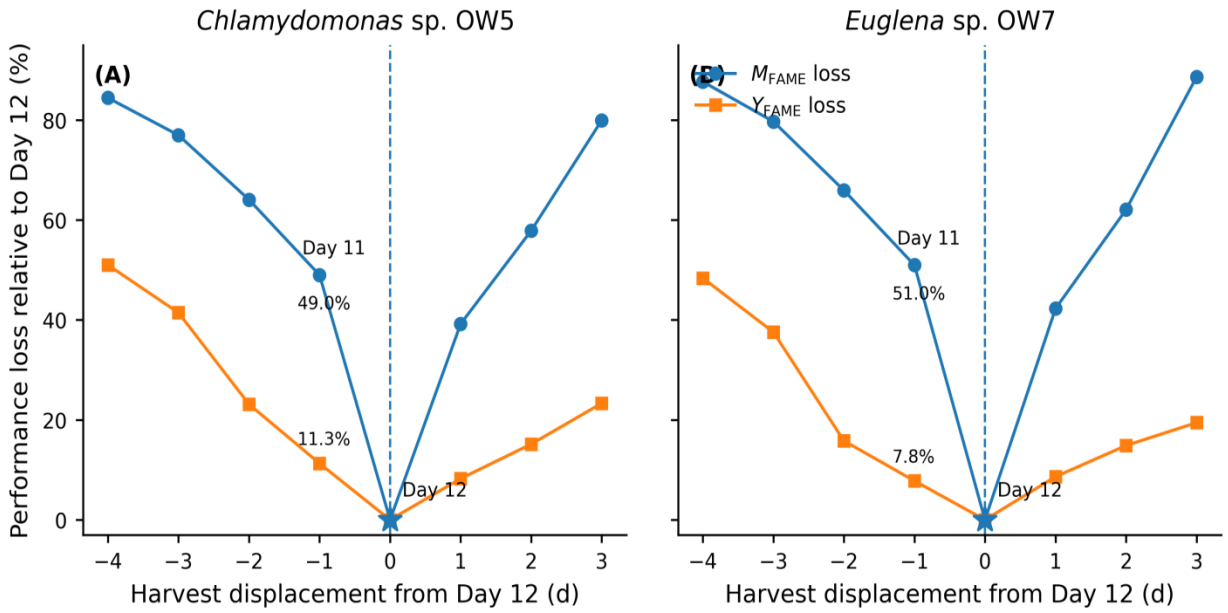


Figure 6: Harvest-displacement penalties relative to the Day 12 compromise target**:** Harvest-displacement penalties in methyl-ester mass (M_{FAME}) and conversion yield (Y_{FAME}) relative to the Day 12 reference state for (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7. Negative

displacement denotes premature harvesting, whereas positive displacement denotes delayed harvesting. Day 11, the nearest pre-peak alternative, reduced cultivation duration by one day (8.3%) but incurred methyl-ester mass losses of **49.0%** and **51.0%** in *Chlamydomonas* and *Euglena*, respectively, together with conversion-yield losses of **11.3%** and **7.8%**. Delaying harvest beyond Day 12 similarly increased cultivation duration while reducing both performance responses. The asymmetric displacement profiles therefore distinguish earlier time-saving alternatives from the post-peak penalty region and support Day 12 as the robust conditional compromise rather than an arbitrarily defined multi-day harvest window.

3.7 Objective-weight sensitivity and robustness of harvest selection

The equal-weight ideal-point analysis determined that Day 12 is the favoured compromise for both strains; nevertheless, a decision solution lacks robustness if it relies solely on a singular arbitrary weighting method. The sensitivity of objective weights was assessed throughout the entire three-objective simplex to ascertain if the chosen harvest day remained consistent as the relative priorities attributed to cultivation time (w_t), methyl-ester output (w_m), and conversion yield (w_Y) were altered (Table 5; Figure 7). This type of sensitivity analysis is crucial in multiobjective optimisation, as the ultimate compromise may hinge on the prioritisation of certain process criteria.

Throughout the whole weight space, consisting of 5,151 combinations assessed at 0.01 increments constrained by wt+wM+wY=1, Day 12 encompassed the most extensive choice area for both strains. It was chosen in 71.7% of all weight configurations for *Chlamydomonas* sp. OW5 and 70.3% for *Euglena* sp. OW7 (Table 5; Figure 7). The resemblance of these frequencies is remarkable due to the significant differences in absolute methyl-ester synthesis and conversion characteristics among the strains. Nonetheless, over almost seventy percent of the complete

preference spectrum, the benefits obtained by persisting with cultivation until Day 12 were adequate to surpass the corresponding decline in temporal appeal.

The strength of Day 12 was further amplified when attention was confined to the balanced internal choice space, delineated by w_t, w_m, $w_Y \geq 0.10$. From the total of 2,556 combinations, Day 12 was preferred in 81.8% of cases for Chlamydomonas and 80.8% for Euglena. By excluding extreme border preferences, the frequency of the Day-12 solution rose by almost ten percentage points in both strains. This implies that Day 12 is especially stable when all three objectives preserve substantial decision significance, instead than allowing one criteria to gain exclusive precedence.

Notably, Day 12 did not remain unchanged in relation to objective preference. Elevating the importance of cultivation-time attractiveness redirected parts of the choice space toward more premature harvest scenarios. In the distinctly analyzed time-priority context $(w_t,w_m,w_Y)=(0.60,0.20,0.20)$, Day 5 was designated for both strains (Table 5). On the other hand, the balanced (1/3,1/3,1/3), production-priority (0.20,0.60,0.20), conversion-priority (0.20,0.20,0.60), time-leaning (0.50,0.25,0.25), and production-leaning (0.25,0.50,0.25) scenarios consistently maintained Day 12. The choice of earlier harvest selection, when supported by robust time weighting, does not represent instability in the optimization framework; it rather illustrates the intended result of intentionally modifying the decision maker's priorities. The purpose of multiobjective optimization is to highlight trade-offs that depend on preferences rather than hiding them in a unified performance measure [53].

The sensitivity landscape together offers more compelling evidence than the equal-weight solution by itself. Day 12 is not globally ideal or mathematically unavoidable; nevertheless, it is

favoured throughout the majority of the assessed weight space and over eighty percent of the balanced interior. It is thus best understood as a strong compromise harvest goal within the current aim framework. Prioritising culture length allows earlier harvest states to be viable options, maintaining the differentiation between a solid operational trade-off and a universal biological ideal.

Table 5: Robustness of harvest-day selection under alternative objective-weight priorities

Weighting analysis / scenario	**W_t**	**W_m**	**W_Y**	*Chlamydomonas* **sp. OW5**	*Euglena* **sp. OW7**
A. Exhaustive objective-weight analysis				**Day 12 selection**	**Day 12 selection**
Full simplex, n=5,151	0–1	0–1	0–1	**71.66%**	**70.26%**
Interior simplex, n=2,556	≥0.10	≥0.10	≥0.10	**81.77%**	**80.75%**
B. Representative priority scenarios				**Selected day**	**Selected day**
Balanced	0.333	0.333	0.333	**12**	**12**
Time priority	0.600	0.200	0.200	**5**	**5**
Production priority	0.200	0.600	0.200	**12**	**12**
Conversion priority	0.200	0.200	0.600	**12**	**12**
Time leaning	0.500	0.250	0.250	**12**	**12**
Production leaning	0.250	0.500	0.250	**12**	**12**

Notes: w_t, w_m, and w_Y denote the weights assigned to cultivation-time desirability, methyl-ester mass, and calculated methyl-ester conversion yield, respectively, with wt+wM+wY=1. The full simplex comprised all **5,151**

weight combinations generated at 0.01 increments. The restricted interior simplex comprised **2,556** combinations for which each objective received a weight of at least 0.10, thereby excluding extreme or near-zero objective priorities. For each combination, the candidate harvest day giving the minimum weighted distance to the ideal point was selected. Percentages in section A represent the proportion of evaluated weight combinations for which Day 12 was selected.

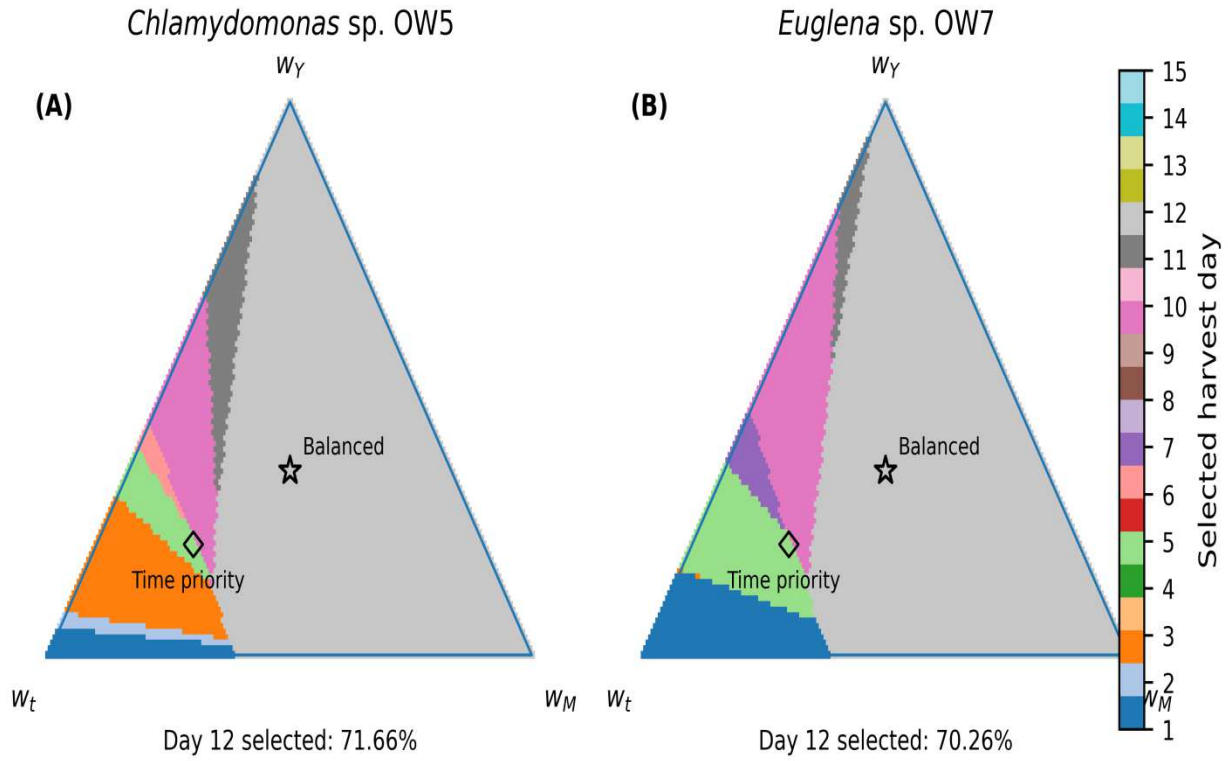


Figure 7: Objective-weight sensitivity maps for harvest-day selection in (A) *Chlamydomonas* sp. OW5 and (B) *Euglena* sp. OW7 across the complete three-objective weight simplex. The vertices represent exclusive prioritisation of cultivation-time desirability (wt), methyl-ester output (w_M), and conversion yield (w_Y), with wt+wM+wY=1. Each point represents one of **5,151** weight combinations evaluated at 0.01 increments, and the mapped region identifies the harvest day producing the minimum weighted distance to the ideal state. Day 12 occupied the largest decision region, being selected for **71.66%** and **70.26%** of the complete weight

space for *Chlamydomonas* and *Euglena*, respectively. Under the restricted interior weight space (wt, wM, wY≥0.10; n=2,556), Day 12 selection increased to **81.77%** and **80.75%**, respectively. The balanced solution retained Day 12 in both strains, whereas sufficiently strong prioritisation of cultivation time shifted the preferred compromise toward earlier harvest states. The maps therefore demonstrate that Day 12 is a **robust but preference-dependent compromise rather than an invariant or universal optimum**.

3.8 Comparative harvest-decision landscapes of the two strains

Although there were quantitative discrepancies in lipid availability, absolute methyl-ester output, and conversion yield between Chlamydomonas sp. OW5 and Euglena sp. OW7, their harvest-decision landscapes were surprisingly alike in shape. The methyl-ester mass for Chlamydomonas on Day 12 was notably larger (2.143 ± 0.006 g), whereas Euglena had a superior conversion yield (94.09 ± 0.34% compared to 85.39 ± 0.18% for Chlamydomonas). These distinctions showcase certain production qualities and point out the importance of not relying on a single criterion for comparing the two strains. The differences in lipid accumulation and resulting biodiesel performance across microalgal strains are recognised, since these variations are determined by physiological parameters, culture settings, and lipid composition [54, 55].

Regardless of these statistical distinctions, both strains established a uniform core decision topology. Days 1 through 12 maintained Pareto efficiency, Days 13 to 15 were surpassed, and Day 12 yielded the smallest equal-weight distance to the ideal point (D12=0.453), with the objective-weight sensitivity analysis predominantly indicating Day 12 for much of the analysed preference space. The inner robustness frequencies exhibited a close alignment—81.8% for

Chlamydomonas and 80.8% for Euglena. Therefore, quantitatively diverse production routes aligned on the same date jeopardise the harvest.

This convergence is shown by the comparative time structure of the goals rather than the equivalence of their absolute sizes. The optimal-point model standardised the objective trajectories of each strain prior to determining the compromise distance. Thus, the choice analysis examined the position of each strain in relation to its respective achievable ranges for cultivation-time preference, methyl-ester production, and conversion efficiency, rather than solely favouring the strain with the highest absolute value. Both systems, M_{FAME} and Y_{FAME}, exhibited enhancement until Day 12, although temporal desirability steadily diminished. Following Day 12, both production outputs declined despite the extended cultivation period. The collective arrangement of these temporal alterations produced analogous Pareto and compromise frameworks despite variations in their absolute production values.

The displacement analysis offers additional proof of this structural resemblance. Collecting on Day 11 instead of Day 12 decreased methyl-ester production by 48.99% in Chlamydomonas and 50.97% in Euglena, corresponding to an identical 8.33% decrease in culture time. In contrast, postponing the harvest to Day 13 resulted in an extra day of culture, although the methyl-ester yield fell by 39.19% and 42.27%, respectively. The intensity of these reactions varied, although their decision-making trajectory was notably uniform.

Thus, the mutual Day-12 compromise need not be construed as proof that the two strains are physiologically identical or that one is categorically better. Chlamydomonas exhibited a higher total methyl-ester production, but Euglena demonstrated a superior conversion efficiency at the current experimental parameters. The primary outcome of process engineering is that different

production phenotypes can align on the same harvest choice if their normalised temporal trade-off frameworks are comparable. Harvest optimisation should thus be grounded in the framework of pertinent choice goals instead of relying on cross-strain comparisons of a single maximum response.

3.9 Engineering implications, limitations and transferability

The primary engineering consequence of the current research is that harvest time should be considered a distinct multiobjective process choice instead of only the calendar date linked to a singular peak response. In the examined batch systems, Day 12 yielded the optimal balance of culture time, total methyl-ester production, and conversion efficiency, whereas extending cultivation to Days 13–15 did not present any advantageous returns within the assessed objectives. This differentiation is functionally significant, as culture and harvesting are critical elements of microalgal biodiesel production systems, and erroneous harvesting choices might jeopardise overall process efficacy.

From a batch-scheduling viewpoint, the findings suggest that prolonging cultivation over the productive peak should not be considered operationally neutral. Given the current circumstances, postponed harvesting concurrently used more cultivation time and decreased M_{FAME} and Y_{FAME}. Circumventing this post-peak phase might enhance reactor scheduling by liberating cultivation capacity sooner instead of prolonging batches once the observed output benefit has vanished. On

the other hand, early harvesting constitutes a legitimate technical compromise: reactor utilisation is diminished; however, the resultant time efficiency must be balanced against significant decreases in methyl-ester production. The roughly 49–51% loss of methyl esters linked to harvesting just one day prior to the Day-12 objective demonstrates that choosing harvest time solely based on scheduling convenience may be similarly deceptive.

The framework also offers techno-economic or life-cycle benchmark groundwork for future online or at-line harvest decision-making help. By utilising proper process measures, regularly revised indicators of biomass and lipid conditions, as well as product-related reactions, these elements may be integrated into a dynamic multiobjective decision framework for techno-economic or life-cycle benchmarks, where prospective harvest states are reviewed throughout the cultivation process. Developments in observation, modelling, and data-informed governance are increasingly allowing for more adaptive management of microalgal farming processes [21, 58, 59, 60, 61]. This expansion would need established connections between quantifiable online data and the resulting methyl-ester responses employed here; hence, it is a future application rather than a capacity shown by the existing experiment.

Various restrictions establish the correct confines of interpretation. At the outset, the optimisation addressed just three goals: period of culture, mass of methyl ester, and yield of conversion. The analysis did not consider the financial implications, energy expenditure, environmental effects, workforce requirements, energy harvesting, or downstream processing demands. As a result, the outcomes signify an optimisation of process performance and must not be construed as a techno-economic or life-cycle benchmark. The inclusion of TEA or LCA

objectives can change the optimal compromise and create an additional, broader decision dilemma.

Secondly, the temporal search domain was confined to 15 empirically assessed daily harvest conditions. Daily sampling fails to determine if a more advantageous compromise exists between successive days, such as between Days 11 and 12, so the analysis can only pinpoint the most substantiated target at the experimental temporal resolution. Subsequent research employing abbreviated sample intervals in the transition zone may enhance the harvest objective. The experimental dataset included three trial trajectories for each condition; hence, the decision landscape must be understood in light of the replication and variability indicated by those observations, rather than as a universally applicable optimisation principle.

Ultimately, Day 12 is tailored to the unique system. It encapsulates the strains, culture parameters, reactor setup, analytical methodologies, and proposed temporal domain assessed in this study. Alterations in light conditions, nutritional accessibility, thermal variations, inoculum concentration, reactor fluid dynamics, culture volume, or post-processing methods may modify the temporal pathways and hence displace the Pareto set and jeopardise the solution. The Day-12 objective should not be immediately applied to different microalgal strains or reactor dimensions without experimental adjustment. The decision framework itself is more transferable: identify experimentally validated candidate harvest states, differentiate between non-dominated and dominated options, measure compromise distance, assess displacement penalties, and examine the stability of the chosen state in relation to shifting objective priorities. This paradigm preserves the engineering significance of the current research while refraining from asserting an unsubstantiated universal ideal harvest time.

4. Conclusion

This research identifies harvest time as a multifaceted process-engineering choice instead of only a singular peak-selection issue. Fifteen days of culture were experimentally evaluated by concurrently reducing cultivation time and enhancing the experimentally determined methyl-ester output (M_{FAME}) and conversion yield (Y_{FAME}). Despite Day 12 aligning with the recorded peaks of the primary production responses in both *Chlamydomonas* sp. OW5 and *Euglena* sp. OW7, the decision analysis revealed that only identifying these peaks is inadequate for determining a justifiable harvest objective.

Pareto screening conceptually narrowed the decision space by designating Days 1–12 as non-dominated states, illustrating authentic trade-offs between reduced cultivation duration and enhanced product performance, while Days 13–15 were deemed Pareto-dominated due to the fact that extended cultivation time resulted in declines in both methyl-ester output and conversion yield. Equal-weight ideal-point analysis later determined Day 12 as the nearest compromise condition for both strains (D12=0.4536). The practical importance was shown by harvest-displacement analysis: harvesting just one day earlier saves 8.33% of culture time while diminishing MFAME by 48.99% in *Chlamydomonas* and 50.97% in *Euglena*. Postponed harvesting was evidently more detrimental, as it concurrently extended the culture period and diminished product efficacy. A weight-sensitivity study indicated that Day 12 was consistently chosen within 71.7–70.3% of the whole weight spectrum and 81.8–80.8% of the balanced

interior for *Chlamydomonas* and *Euglena*, respectively. Day 12 needs to be seen as a strong, preference-based compromise within the assessed framework, rather than a universal ideal for microalgal harvesting.

The findings are, however, constrained by the limits of the experimental and analytical framework. The assessment of harvest states occurred at a daily resolution across just 15 candidate days, hindering the discovery of potentially advantageous sub-daily or intermediate harvest intervals. The dataset included three experimental trajectories, and the optimisation excluded considerations of economic cost, energy requirements, environmental effects, downstream processing intensity, or scale-up uncertainty. Furthermore, the recognised compromise pertains only to the examined strains, culture parameters, reactor setup, and intended formulation.

Subsequent research should thus utilise enhanced temporal resolution during the Day 11–12 transition, augment independent experimental replication, and corroborate the decision framework across various strains, operational settings, and reactor dimensions. Integrating uncertainty-aware optimisation with empirically validated economic, energy, and environmental goals would enhance the framework from process-performance optimisation to comprehensive sustainable bioprocess decision-making. The significant contribution of this research is not Day 12 per se, but rather the analytical framework that differentiates Pareto-efficient harvesting options from a resilient operational compromise.

Declaration of competing interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Abideen. A. Adekanmi: Conceptualisation, Design, Investigation, Methodology, Writing the original draft, final Review & Editing

Emmanuel. O. Garuba: Conceptualisation, Investigation, Methodology, Review & Editing, Supervision

Nurudeen A. Azeez: Methodology, Review & Editing, Supervision, Review & Editing

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