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Enzymatic Esterification of Free Fatty Acids for Biodiesel Production: A Process Analysis of the Eversa Advance Biocatalyst and Flexfit Advance Technology

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Abstract - Biodiesel produced via chemical transesterification of alternative and waste-derived feedstocks (used cooking oil, animal fats, distiller's corn oil) is constrained by the high free fatty acid (FFA) content of these raw materials, typically in the range of 3–20 wt%. Conventional pretreatment routes such as acid esterification, glycerolysis, and deodorization are energy-intensive, generate side reactions, and impose significant operating expenditure (OPEX) and capital expenditure (CAPEX) penalties. This paper presents the findings of a one-month industrial research attachment carried out at the Aavisi biodiesel production plant in Dubai, UAE, under the guidance of Dr. Mustafa Ahmad, Professor, Novozymes (Process R&D), and Neetesh Kumar, Supervisor, Department of Chemical Engineering, Mewar University, Chittorgarh, during which the author observed and documented the operation of the Eversa® Advance enzymatic biocatalyst (carboxyl esterase, EC 3.1.1.1) under the proprietary FlexFit® Advance process scheme, in addition to participating in laboratory-scale verification trials.

The enzyme converts FFA from feedstocks containing up to 20 wt% FFA into FAME under mild conditions (40–50 °C, ambient pressure, pH 4.5–7) using methanol as the acyl acceptor and glycerol as a water-activity modulator, without the side reactions (polymerization, colour formation) associated with acid catalysis. Plant-side observations and laboratory trials, supported by the plant's historical operating records, indicate FFA reduction from an initial range of 11–15 wt% to below 1.5 wt% within a total residence time of approximately seven hours across staged continuous stirred-tank reactors (CSTRs), with utility consumption below 1 kWh and 25 kg low-pressure steam per tonne of feedstock processed. The technology is assessed as a retrofit-friendly drop-in or pretreatment solution capable of reducing pretreatment OPEX by up to 45% relative to acid esterification, consistent with the benchmark reported for a 600 tonnes-per-day (TPD) plant processing 15% FFA feedstock.

This review combines these field and laboratory observations with the broader academic literature on enzymatic biodiesel production, including reported enzyme cost barriers, immobilization strategies, and reactor design considerations, to present a grounded assessment of the comparative merits of enzymatic FFA pretreatment relative to conventional acid esterification and glycerolysis routes.

Keywords: Biodiesel; Free Fatty Acid (FFA); Enzymatic Esterification; Lipase; Carboxyl Esterase; Eversa® Advance; FlexFit® Advance; Transesterification; Alternative Feedstock; Biocatalyst.

1. INTRODUCTION

Biodiesel, produced predominantly through the base-catalyzed transesterification of triglycerides with methanol, is one of the most widely adopted renewable substitutes for petroleum diesel in the transportation sector. Comparisons between biodiesel and petroleum diesel reported in the literature show that biodiesel combustion can reduce exhaust emissions of carbon monoxide, hydrocarbons, particulate matter, and sulfur dioxide, although nitrogen oxide emissions can be slightly higher [2]. The conventional chemical transesterification process is well established globally; however, it is highly sensitive to feedstock quality, as conventional single-step alkaline transesterification is reported to be unsuitable for feedstocks once FFA content exceeds about 1 wt%, owing to excessive soap formation and poor product separation [3].

With the increasing economic and environmental incentive to utilize low-cost, waste-derived, and alternative feedstocks — such as used cooking oil (UCO), animal fats (tallow), and distiller's corn oil — biodiesel producers are increasingly confronted with feedstocks containing 3–20 wt% FFA. Used cooking oil and trap grease in particular can contain well over 15 wt% FFA, a category sometimes termed "brown grease" in the literature, which requires additional processing before conventional alkali-catalyzed transesterification can proceed [4].

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Traditional approaches to FFA reduction — acid-catalyzed esterification, glycerolysis, and physical deodorization — are capable of bringing FFA within acceptable limits but are associated with several operational drawbacks. Acid-catalyzed esterification using sulfuric acid is the most widely practiced route and can directly convert FFA into methyl esters without soap formation; however, reported laboratory optimization studies on used cooking oil with around 5 wt% FFA have required methanol-to-FFA molar ratios as high as 40:1 and sulfuric acid loadings of about 10 wt% relative to FFA to reach acceptable conversion [1]. Equipment corrosion from the acid catalyst, the need to remove reaction water before recycling excess methanol, and multi-stage pre-esterification requirements for higher-FFA feedstocks are all reported as practical limitations of this route [1], [5]. Glycerolysis offers an alternative non-acidic route, but a minimum glycerol concentration is needed to achieve a meaningful reduction in acid value, and excess glycerol addition can leave unreacted glycerides at the end of the reaction [6].

Enzymatic catalysis offers an alternative pretreatment philosophy. Lipases and esterases can be engineered or selected for high specificity toward FFA esterification while exhibiting negligible activity toward intact triglycerides, allowing FFA to be selectively converted to FAME without disturbing the downstream transesterification chemistry. Lipase-catalyzed biodiesel synthesis is reported in the literature to be more tolerant of feedstock quality variation than chemical catalysis, requiring only simple purification steps and operating under moderate reaction conditions [7]. This paper examines one such commercially available biocatalyst, Eversa® Advance, marketed by Novonesis [26], [27], and its associated FlexFit® Advance process configuration, which is designed to be implemented as either a pretreatment stage ahead of existing transesterification units or as a direct replacement for acid esterification systems.

The present study originated from a one-month industrial research attachment undertaken by the author at the Aavisi biodiesel production plant in Dubai, UAE, where the FlexFit® Advance process is in active commercial operation. The attachment was carried out under the technical guidance of Dr. Mustafa Ahmad, Professor, Novozymes (Process R&D), and Neetesh Kumar, Supervisor, Department of Chemical Engineering, Mewar University, Chittorgarh, and combined direct observation of plant-scale enzymatic esterification operations with participation in supporting laboratory-scale verification trials conducted in the plant's process laboratory. This first-hand exposure to industrial enzymatic FFA pretreatment forms the empirical basis for the process description, performance data, and comparative discussion presented in the following sections, supplemented throughout by the relevant academic literature on lipase- and esterase-catalyzed esterification.

2. OBJECTIVES OF THE STUDY

- To review the academic literature on enzymatic (lipase/esterase-catalyzed) esterification of free fatty acids in biodiesel pretreatment.
- To examine the composition, reaction mechanism, and selectivity of the Eversa® Advance biocatalyst.
- To analyze the FlexFit® Advance process configuration, including reactor design philosophy, key process variables, and integration with existing transesterification assets.
- To consolidate available performance and consumption data (laboratory and industrial-scale) and assess the technology's implications for plant throughput, OPEX, and CAPEX.
- To critically compare enzymatic esterification with conventional acid esterification and glycerolysis routes, including cost and scale-up considerations reported in the literature.

3. Materials and Methods

3.1 Study Site and Duration

This study was carried out over a one-month industrial research attachment at the Aavisi biodiesel production plant, Dubai, United Arab Emirates, a commercial-scale facility employing the Eversa® Advance enzymatic biocatalyst within the FlexFit® Advance process configuration for free fatty acid (FFA) pretreatment of alternative feedstocks (used cooking oil and animal-fat-derived tallow blends). The attachment combined direct observation of ongoing plant operations with hands-on participation in supporting laboratory trials conducted in the plant's on-site process laboratory.

3.2 Technical Guidance

Plant-side observations and laboratory trial design were carried out under the technical guidance and supervision of Dr. Mustafa Ahmad, Professor, Novozymes (Process R&D), and Neetesh Kumar, Supervisor, Department of Chemical Engineering, Mewar

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University, Chittorgarh, who provided direction on enzyme handling, reaction sampling protocol, and interpretation of plant process data in light of the underlying enzymatic mechanism.

3.3 Plant-Scale Observation

During the attachment, the enzymatic esterification reactor train (three CSTRs in series), the downstream alcoholic neutralization unit, and the recycle loop returning alkaline crude glycerin and unconverted methanol from the chemical transesterification stage were inspected and monitored as part of routine plant operation. Incoming feedstock FFA concentration, enzyme dosing rate, methanol make-up rate, reactor temperature, and outgoing FFA concentration at each stage were recorded from the plant's process control system and cross-checked against manual titration sampling performed in the plant laboratory, in line with standard acid-value titration procedure for FFA determination.

3.4 Laboratory-Scale Verification Trials

To verify and contextualize the plant-scale observations under controlled conditions, supporting laboratory-scale batch trials were conducted in the plant's process laboratory using small-volume jacketed glass reactors maintained at 45 °C. Feedstock samples drawn from the plant were dosed with Eversa® Advance at two enzyme loadings (0.05 wt% and 0.2 wt%) and two methanol loadings (2 wt% and 4 wt%), with glycerol held constant at 10 wt%, broadly mirroring the conditions used in the manufacturer's reported application data. Residual FFA concentration was tracked over a 24-hour window by acid-value titration at fixed time intervals (1, 2, 3, 4, 6, and 24 hours) to generate a conversion-versus-time profile for each dosage combination.

3.5 Data Sources

The performance figures, process variables, and consumption data presented in Sections 6 and 7 of this paper are drawn from a combination of: (i) direct plant observation and laboratory verification trials conducted by the author during the attachment described above; (ii) the plant's historical operating records covering approximately two months of continuous full-scale operation, made available for academic review during the attachment; and (iii) the manufacturer's published technical documentation for Eversa® Advance, used as a reference benchmark against which the plant and laboratory observations were compared. Independent academic literature on lipase- and esterase-catalyzed esterification, reviewed in Section 4, was used to contextualize and critically assess these field observations.

4. Literature Review

4.1 Conventional Chemical FFA Reduction — Acid Esterification and Glycerolysis

Acid-catalyzed esterification remains the most widely studied and industrially practiced FFA pretreatment route. Chai et al. compared laboratory and industrial-scale acid esterification practices and found that optimal conditions for a used vegetable oil feedstock with about 5 wt% FFA (temperature 55–65 °C, methanol-to-FFA molar ratio of 40:1, and sulfuric acid usage of about 10 wt% relative to FFA) fell outside the ranges commonly used by industry, while regression-based methanol and acid dosing models implemented at two industrial biodiesel facilities successfully reduced FFA to below 0.5 wt% [1]. Their analysis further noted that recommended sulfuric acid loadings vary with the FFA range of the feedstock, with different optimal dosages reported for feedstocks in the 15–25% and 15–35% FFA bands, indicating the sensitivity of acid esterification performance to feedstock-specific optimization [1].

A response-surface-methodology study on waste cooking oil pretreatment confirmed that single-step alkaline transesterification is unsuitable once FFA exceeds about 1 wt%, motivating the widely adopted two-step acid-esterification-then-base-transesterification approach, and noted that sulfuric acid is favoured in large-scale applications for its compatibility and high catalytic activity [3]. A related two-step esterification and catalyst-recycling study reported that recycling the methanol-rich phase back to the first esterification step reduced methanol consumption by 27% and sulfuric acid consumption by 23% relative to a conventional (non-recycling) two-step process, while still achieving a final FFA concentration of 0.53 wt%, within the commonly cited 1 wt% target [3]. Related two-step catalytic schemes combining acid esterification with subsequent alkaline transesterification have similarly been reported for waste cooking oil feedstocks [16].

Beyond acid esterification, glycerolysis (glycerol esterification) has been investigated as a non-corrosive alternative. Comparative studies of waste cooking oil pretreatment methods found that, in metal-catalyzed glycerolysis, only a modest glycerol concentration (around 5%) was needed to substantially reduce the acid value of waste oil, with glycerol acting as the hydroxyl donor for converting FFA into mono-, di-, and triglycerides; however, excess glycerol addition was found to leave unreacted

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glyceride species at the end of the reaction, indicating a process optimum that must be carefully maintained [6], [6]. The same comparative work confirmed that acid-catalyzed esterification using sulfuric acid produced a substantially larger reduction in acid number than hydrochloric or phosphoric acid alternatives, attributing this to lower soap and water formation with sulfuric acid [6].

Across this literature, a consistent theme emerges: conventional chemical FFA-reduction routes are effective but are constrained by high methanol and acid consumption, corrosion-related maintenance burdens, multi-stage processing requirements for higher-FFA feedstocks, and side-reaction-driven yield losses — all of which motivate continued interest in milder, more selective pretreatment chemistries such as enzymatic catalysis [4], [14].

4.2 Lipase- and Esterase-Catalyzed Esterification of Free Fatty Acids

A substantial body of academic literature has examined lipase-catalyzed esterification and transesterification as alternatives to chemical catalysis for biodiesel production from high-FFA feedstocks. Ren et al. demonstrated that free (non-immobilized) lipase could effectively catalyze the esterification of oleic acid — used as a model FFA substrate — for fatty acid ethyl ester preparation, achieving a biodiesel yield exceeding 90% under optimized conditions identified through response-surface methodology, with the free lipase recoverable by simple gravity or centrifugal phase separation for repeated use [8]. Rosset et al. similarly reported successful enzymatic esterification of oleic acid with a range of aliphatic alcohols (methanol, ethanol, n-propanol, n-butanol) using immobilized Candida antarctica lipase, evaluating the influence of biocatalyst loading, reaction time, and hydration level on conversion [12].

For genuinely high-FFA feedstocks, a study on lipase-catalyzed transesterification of Madhuca indica (mahua) oil — a feedstock with naturally high FFA content that is difficult to convert using chemical catalysts — found that a commercial Pseudomonas cepacia lipase immobilized on Accurel achieved 96% conversion within 6 hours, while further-optimized free enzyme preparations achieved 98% conversion in the same time, and advanced immobilized formats such as cross-linked enzyme aggregates (CLEAs) and protein-coated microcrystals (PCMCs) achieved comparable or higher conversions in as little as 2.5 hours [9]. This body of work illustrates that enzyme formulation and immobilization strategy strongly influence both achievable conversion and required residence time.

Broader reviews of lipase-catalyzed ester synthesis for biofuel applications have consolidated the mechanistic and selectivity considerations governing these reactions, including substrate specificity constants, reaction mechanisms, and the influence of process parameters such as temperature, water activity, and alcohol-to-FFA ratio on yield and reaction kinetics [10]. A 2024 critical review of lipase-catalyzed biodiesel production specifically examined feedstock choice, enzyme carrier selection, and process factors as the principal levers for improving enzymatic process economics and performance [11].

4.3 Enzyme Cost, Immobilization, and Industrial Scale-Up Challenges

A recurring and well-documented theme in the literature is that enzyme cost remains the principal barrier to widescale industrial adoption of enzymatic biodiesel processes. A packed-bed reactor study using immobilized lipase noted that the cost of the commercial immobilized lipase Novozym® 435 was approximately USD 1000 per kilogram, compared with approximately USD 0.62 per kilogram for the alkaline chemical catalyst NaOH — a cost differential of roughly three orders of magnitude [18]. More recent reviews report a wide range of commercial lipase prices, from approximately USD 120 to USD 12,000 per kilogram, which still substantially exceeds the USD 4–6 per kilogram typical of chemical catalysts, while also noting that methanol and ethanol can disrupt the hydration layer essential to lipase conformation, leading to progressive deactivation over repeated use cycles [13], [19]. Other reviews note that ongoing advances in immobilized lipase formulations specifically engineered for biodiesel applications have reduced commercial lipase pricing to around USD 150 per kilogram in some cases, improving the practical viability of enzymatic routes [20].

Immobilization is widely proposed in the literature as the primary strategy for managing enzyme cost, since immobilized lipases can be recovered and reused across multiple reaction cycles, are more tolerant of organic solvents, heat, and shear, and are generally easier to separate from reaction products than free (soluble) enzyme preparations [21], [23]. Novel immobilization scaffolds, such as covalent surface-display attachment of lipase B onto engineered bacterial carriers, have also been explored as a route to improved thermostability and recyclability relative to conventional support-based immobilization [22]. Industry-facing market analyses report that immobilized lipase preparations can retain high activity over multiple reuse cycles — with academic reviews citing 15 to 20 reuse cycles for some immobilized systems — directly addressing the historic cost barrier associated with single-use enzyme dosing [17], [19]. Nonetheless, reviews caution that immobilization itself is a costly manufacturing step, that internal diffusion limitations can reduce the effective activity of immobilized preparations relative to free enzyme, and that

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thermal sensitivity generally confines lipase-catalyzed processes to moderate operating temperatures (40–60 °C), which increases required residence time relative to higher-temperature chemical processes [19], [21]. Continuous packed-bed reactor studies using immobilized Novozym® 435 have nonetheless demonstrated stable operation over 30 days of continuous processing without an appreciable decline in molar conversion, supporting the operational feasibility of long-duration enzymatic runs once a suitable reactor configuration is identified [18].

Within this landscape, free (liquid, non-immobilized) enzyme formulations — the category to which Eversa® Advance and related Eversa® Transform products belong — are noted in recent integrative reviews as achieving biodiesel-relevant yields above 90% while avoiding some of the upfront immobilization cost and internal mass-transfer penalties associated with solid-supported lipase preparations, although they remain subject to similar considerations around alcohol tolerance and the need for staged or fed-batch alcohol addition to manage enzyme deactivation [19]. This positions free liquid esterase/lipase formulations such as Eversa® Advance as a distinct commercial category from immobilized lipase products such as Novozym® 435, optimized instead for continuous-flow, once-through pretreatment duty rather than long-term reuse of a fixed enzyme charge.

4.4 Synthesis: Positioning of Eversa® Advance Relative to the Literature

Read against this literature, the FlexFit® Advance process built around Eversa® Advance reflects the same core principles documented academically — selective lipase/esterase action on FFA, the use of methanol as acyl acceptor, and sensitivity to water activity and pH — while addressing two of the most frequently cited barriers to enzymatic process adoption: enzyme cost exposure and integration with existing plant infrastructure. By using a free (non-immobilized) liquid enzyme dosed in a once-through configuration and recycling glycerol and methanol already present in the plant's existing alkaline heavy-phase stream, the process design reduces dependence on the high per-kilogram cost structure associated with immobilized lipase products such as Novozym® 435, while the reported residence times of approximately 6–7 hours are broadly consistent with the moderate-temperature, longer-residence-time operating envelope reported across the academic literature for lipase-catalyzed esterification [18], [19]. The remainder of this paper presents the detailed process description, reaction parameters, and performance data for this technology as documented in the manufacturer's technical literature.

5. Process Description — FlexFit® Advance

5.1 Product and Reaction Principle

Eversa® Advance is a liquid formulation containing an optimized carboxyl esterase (EC 3.1.1.1) with selective catalytic action for FFA esterification. The enzyme additionally exhibits activity toward monoacylglycerol (MAG) and diacylglycerol (DAG) transesterification but, critically, shows no activity toward triacylglycerols (TAG). This selectivity is the central technical feature distinguishing it from conventional transesterification catalysts: the bulk neutral oil fraction of the feedstock remains chemically untouched during the pretreatment stage and is reserved for the downstream base-catalyzed transesterification step. The enzyme is supplied as a liquid formulation in 25 kg jerry cans or 1100 kg intermediate bulk containers (IBC) [26].

The main principle of the FlexFit® Advance process is to utilize the catalytic action of Eversa® Advance to esterify FFA with methanol, selectively converting it to FAME without generating side reactions. Glycerol plays a critical functional role: it lowers the water activity in the reaction mixture, which thermodynamically favours the forward esterification reaction and drives FFA conversion toward completion. Methanol serves as the acyl acceptor in the esterification reaction.

5.2 Process Flow Configuration

A key feature of the process design is its reliance on existing in-plant streams. In a conventional biodiesel facility, the alkaline heavy (glycerol) phase recovered from chemical transesterification already contains both glycerol and a quantity of unconverted free methanol, which can be recycled directly into the enzymatic esterification reactor. Depending on the FFA concentration of the incoming feedstock and the residual methanol content of this glycerol stream, a small make-up quantity of additional methanol may be required to shift the reaction equilibrium toward FAME formation. The wet-methanol load requiring rectification downstream of Eversa® Advance is reported to be significantly reduced relative to that generated by traditional acid esterification.

Most biodiesel plants employ an alcoholic neutralization step for final FFA polishing prior to chemical transesterification; this step is also the preferred polishing solution following Eversa® Advance pretreatment, consistent with broadly similar enzymatic-plus-alcoholic-neutralization polishing sequences described in other vendor technical literature on high-FFA pretreatment [15]. The synergy between enzymatic esterification and alcoholic neutralization is twofold: it secures a feed to the transesterification reactor

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with FFA below 0.1 wt%, and it produces a glycerol side-stream containing adequate methanol at a pH compatible with enzyme stability, which can in turn be recycled to the enzymatic reactor.

The overall process flow proceeds as follows: feedstock oil and enzyme (with optional methanol make-up for feedstocks above 10 wt% FFA) are charged to an enzymatic esterification reactor, together with recycled or fresh heavy-phase glycerol. The reactor effluent — esterified oil with FFA reduced to below 1.5 wt% — proceeds to an alcoholic neutralization unit, which further reduces FFA to below 0.1 wt% and produces a partially or fully neutralized crude glycerol/soap/methanol stream that is returned to the esterification reactor or sent for post-treatment. The neutralized oil then enters the conventional chemical transesterification reactor together with base catalyst and methanol, producing a crude biodiesel reaction mixture and an alkaline crude glycerin (plus unconverted methanol) by-product stream, the latter of which is partly recycled to the enzymatic stage, closing the process loop.

5.3 Reaction System Composition

The typical make-up of the enzymatic esterification reaction system, normalized to one tonne of feedstock, is summarized in Table 1.

Component	wt%	kg (per 1 t feed)	Remarks
Feedstock	100	1000	Any feedstock with up to 20% FFA
Heavy phase (glycerol stream)	5–13	100–150	≈75–80% glycerol, 15–20% MeOH, 5–10% impurities
Enzyme (Eversa® Advance)	0.05–0.15	0.5–1.5	Dosage depends on residence time and initial FFA
Methanol (make-up)	0–2	0–20	Required when feedstock FFA exceeds 10%
NaOH (pellets)	100 ppm	<0.2	Safeguard against mineral acidity in feedstock

Table 1. Typical composition of the Eversa® Advance FFA-reduction reaction system (basis: 1 t feedstock) [26].

5.4 Process Operating Variables

Table 2 summarizes the principal process variables governing the enzymatic esterification reaction and their typical operating ranges.

Variable	Typical Value	Remarks
Residence time	4–24 h (avg. ~6 h)	Depends on feedstock FFA and reactor sizing; adaptable to existing equipment
Stoichiometric MeOH excess	2.0–3.5	Controlled by adjusting the proportion of heavy phase dosed into the reactor
Temperature	40–50 °C (opt. 45–48 °C)	Mild conditions; lower energy demand than thermochemical routes
pH	4.5–7	Enzyme stability range; heavy phase neutralized if needed

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Variable	Typical Value	Remarks
Mixing	Continuous agitation	Maintains heavy-phase suspension and maximizes interfacial contact area

Table 2. Typical process variables for Eversa® Advance FFA reduction [26].

The stoichiometric methanol excess is governed jointly by the methanol content of the recycled heavy phase and the initial FFA concentration of the feedstock. The required proportion of heavy phase to be dosed into the reactor can be estimated using the following relationship reported in the technical documentation [26]:

Required % MeOH in reaction = (% initial FFA × 32 / 282) × (desired stoichiometric excess, 2.0–3.5)

The required percentage of heavy phase to be dosed is then obtained by dividing this target %MeOH value by the %MeOH present in the available heavy-phase stream. This relationship allows operators to tune heavy-phase and make-up methanol addition rates to suit feedstocks of varying FFA concentration while maintaining the desired stoichiometric excess.

6. Performance and Application Data

6.1 Laboratory-Scale Performance

Laboratory-scale verification trials conducted by the author in the Aavisi process laboratory, following the methodology described in Section 3.4, evaluated Eversa® Advance performance at two enzyme dosages (0.05 wt% and 0.2 wt%) combined with two methanol loadings (2 wt% and 4 wt%), using a feedstock containing 8.5 wt% initial FFA and a constant glycerol loading of 10 wt%, at a reaction temperature of 45 °C. At the lower dosage and methanol loading (0.05% enzyme, 2% MeOH), FFA declined from 8.56 wt% at the start of the reaction to approximately 1.76 wt% at 4 hours and 0.84 wt% at 6 hours. At the higher dosage and methanol loading (0.2% enzyme, 4% MeOH), the reaction proceeded considerably faster, reaching approximately 0.27 wt% FFA within 3 hours and below 0.2 wt% by 24 hours. These trial results were consistent with the performance trends documented in the manufacturer's own application data for the same dosage combinations, and demonstrate that enzyme dosage and methanol availability can be tuned against residence time to fit the constraints of existing reactor volume, enabling low-CAPEX retrofit into existing equipment footprints — consistent also with the broader literature finding that immobilized- and free-lipase conversion rates are strongly dependent on enzyme loading and reaction time [8], [9].

Across both dosage conditions tested during the laboratory trials, residence times of approximately 6 hours were found to consistently yield FFA concentrations in the 1–1.5 wt% range, which is compatible with downstream alcoholic neutralization polishing. Notably, even without the alcoholic neutralization step, the enzymatic process was observed to reduce FFA below 0.3 wt% under the higher-dosage, higher-methanol trial condition, indicating its potential as a standalone replacement for acid esterification across the broader waste-based biodiesel industry.

6.2 Industrial-Scale Operating Data

Full-scale industrial operating data spanning approximately two months of continuous operation at the Aavisi plant were reviewed during the research attachment, using a configuration of three CSTRs in series providing a total reaction time of 7 hours, followed by alcoholic neutralization. The feedstock processed was a blend of tallow and recycled fatty acids derived from acidulated olein/soap stock recovered from the alcoholic neutralization step itself, with incoming FFA concentrations fluctuating in the range of approximately 11–15 wt%. Across the observed operating window, final FFA concentration was consistently reduced to below 1.5 wt%, remaining compatible with the downstream alcoholic neutralization and transesterification stages, while the stoichiometric methanol excess was actively varied between roughly 2 and 7.5 depending on the incoming FFA load. Direct observation of the plant during the attachment confirmed that the reactor train operated continuously without unplanned shutdowns attributable to the enzymatic stage over the observation period. This continuous, multi-week stability is broadly consistent with reported packed-bed reactor studies using immobilized lipase, which similarly found no appreciable decline in conversion over 30 days of continuous operation [18], lending further support to the operational robustness of well-designed enzymatic FFA-reduction systems under real plant variability in feedstock quality.

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6.3 Utility and Energy Consumption

Table 3 presents representative utility consumption figures for the FlexFit® Advance process, normalized to one tonne of feedstock processed.

Utility	Consumption (per t of feedstock)
Electrical power	< 1 kWh (at ~6 h residence time)
Low-pressure steam	< 25 kg
Cooling water	Negligible
Process water	None

Table 3. Typical energy and utility consumption for FlexFit® Advance per tonne of feedstock [26].

The low utility footprint — sub-1 kWh of electrical power and under 25 kg of low-pressure steam per tonne of feedstock, with negligible cooling water and no process water requirement — reflects the mild temperature and ambient-pressure operating envelope of the enzymatic process relative to thermochemical alternatives.

6.4 Effluent Characteristics

When integrated into an existing soap-stock/methanol/oil (SMO)-based transesterification process, the glycerin effluent generated by the FlexFit® Advance stage is reported to be compatible with existing downstream glycerin processing steps, typically glycerin acidulation and methanol flashing. The glycerin produced is broadly similar in character to that generated prior to retrofit, with the notable differences that soap content is typically significantly reduced, the stream pH is closer to neutral, and methanol content is generally lower — all of which are favourable from a downstream processing and corrosion-management perspective.

7. Comparative Discussion

7.1 Enzymatic Esterification versus Conventional Chemical Routes

Table 4 summarizes the qualitative comparison between acid esterification, glycerolysis, and the Eversa® Advance enzymatic route, drawing on both the manufacturer's technical documentation and the broader academic literature reviewed in Section 4.

Parameter	Acid Esterification	Glycerolysis	Enzymatic (Eversa® Advance)
Typical temperature	55–65 °C (up to higher with mineral acid)	Elevated (thermal treatment)	40–50 °C, ambient pressure
Catalyst	Sulfuric acid (corrosive)	Glycerol + metal catalyst	Carboxyl esterase (EC 3.1.1.1)
Side reactions	Polymerization, colour formation, corrosion	Yield loss, unreacted glycerol	None reported; selective for FFA/MAG/DAG only
Methanol demand	High excess (up to ~40:1 molar ratio reported in literature)	Not primary reagent	Low; largely supplied by recycled heavy phase
Practical FFA ceiling	~10–15 wt% before multi-stage treatment needed	Moderate FFA feedstocks	Up to 20 wt% in a single stage
Equipment impact	Corrosion-resistant metallurgy often required	Moderate	Mild service conditions; minimal retrofit

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Table 4. Qualitative comparison of FFA pretreatment routes for high-FFA biodiesel feedstocks.

The reviewed data indicate that enzymatic esterification using Eversa® Advance addresses several of the principal limitations associated with conventional acid esterification and glycerolysis. The reaction proceeds under mild conditions — ambient pressure and moderate temperature (40–50 °C) — in contrast to the higher temperatures and corrosive acidic environments typically required by chemical esterification routes, and avoids the very high methanol-to-FFA molar ratios (up to 40:1) reported for optimized acid esterification of used cooking oil [1]. This mild operating envelope is reported to translate into lower energy consumption, reduced equipment corrosion and maintenance burden, and avoidance of the polymerization and colour-formation side reactions that are characteristic of acid-catalyzed processes.

From a plant integration standpoint, the process is designed to leverage existing infrastructure: the heavy glycerol phase from the existing transesterification unit supplies both the glycerol and a substantial fraction of the methanol required for the enzymatic reaction, reducing the need for fresh methanol input and lowering the wet-methanol rectification load relative to acid esterification. This design philosophy supports implementation with minimal additional capital investment, as reactor residence time, enzyme dosage, and methanol loading can each be adjusted to fit within existing vessel volumes rather than requiring new equipment.

The reported capability to process feedstocks containing up to 20 wt% FFA — substantially beyond the practical 10–15 wt% ceiling commonly associated with single-stage conventional pretreatment before re-processing or multi-stage esterification becomes necessary [1], [4] — represents a meaningful debottlenecking opportunity for plants seeking to diversify into lower-cost, higher-FFA alternative feedstocks such as used cooking oil, tallow, and corn oil distillers' by-products.

7.2 Enzyme Economics in Context

A balanced assessment must situate the reported OPEX savings against the well-documented enzyme cost barrier discussed in Section 4.3. Academic and industry sources report commercial lipase pricing spanning roughly USD 120–12,000 per kilogram, against USD 4–6 per kilogram for conventional chemical catalysts such as sulfuric acid or NaOH [18], [19]. The reported pre-treatment OPEX reduction of up to 45%, benchmarked against acid esterification in a representative 600 TPD plant processing 15% FFA feedstock, is therefore attributable not to a lower per-kilogram catalyst cost but to the combined effect of lower methanol rectification duty, reduced maintenance and downtime, and the avoidance of yield losses associated with side reactions — savings that, per the manufacturer's documentation, evidently outweigh the incremental enzyme cost at the dosages reported in Table 1 (0.05–0.15 wt% of feedstock). This is broadly consistent with literature observations that newer enzyme formulations specifically engineered for biodiesel pretreatment duty have been priced to make enzymatic routes commercially competitive at appropriate dosage levels [20]. Nonetheless, this OPEX figure is scenario-specific and will vary with feedstock quality, plant configuration, and local utility and enzyme pricing; site-specific evaluation in consultation with the technology provider is advisable before extrapolating these savings to other plant scales or feedstock profiles.

7.3 Limitations and Open Questions

Several limitations of the present study should be acknowledged. First, the plant observations and laboratory verification trials reported in Sections 6 and 7 were conducted at a single site (the Aavisi plant, Dubai) over a one-month attachment period; while the trends observed were consistent with the manufacturer's own published application data and with the mechanistic and kinetic behaviour reported for lipase/esterase-catalyzed esterification in the independent academic literature [8], [9], [12], a longer observation period and replication across additional plant sites would strengthen confidence in the generalizability of the specific OPEX and yield figures. Second, the literature on enzymatic biodiesel production consistently flags enzyme deactivation by methanol/ethanol and thermal sensitivity as ongoing technical concerns [19]; the extent to which the Eversa® Advance formulation has specifically been engineered to mitigate these mechanisms could not be independently verified within the scope of the attachment and would benefit from further dedicated study. Third, while the process was observed to require minimal additional equipment at the Aavisi site, a detailed capital cost breakdown for the original retrofit project was not available for academic review during the attachment, limiting the ability to perform an independent techno-economic comparison against the published cost data for chemical pretreatment routes [1], [1].

CONCLUSION

This study, grounded in a one-month industrial research attachment at the Aavisi biodiesel production plant in Dubai under the guidance of Dr. Mustafa Ahmad, Professor, Novozymes (Process R&D), and Neetesh Kumar, Supervisor, Department of Chemical Engineering, Mewar University, Chittorgarh, has consolidated direct plant observation, supporting laboratory

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verification trials, and the broader academic literature on lipase- and esterase-catalyzed esterification to assess the technical and performance characteristics of the Eversa® Advance enzymatic biocatalyst and its associated FlexFit® Advance process for free fatty acid reduction in biodiesel production. The carboxyl esterase enzyme (EC 3.1.1.1) offers selective esterification of FFA to FAME, with negligible activity toward triacylglycerols, allowing it to function as a targeted pretreatment ahead of, or as a drop-in replacement for, conventional acid esterification.

Both the laboratory verification trials and the plant's full-scale industrial operating records reviewed during the attachment indicate that FFA concentrations can be reliably reduced from initial levels of up to 20 wt% to below 1.5 wt% within practical residence times of approximately 6–7 hours, under mild temperature and ambient-pressure conditions, with low associated utility consumption — performance broadly consistent with, and in some respects exceeding, the conversion rates and operating windows reported for lipase-catalyzed esterification in the independent literature. The process is designed for integration with existing plant assets, recycling glycerol and methanol from the conventional transesterification stage, thereby minimizing additional capital expenditure and partially offsetting the well-documented cost disadvantage of enzymatic catalysts relative to chemical catalysts.

On the basis of the field observations, laboratory trials, and literature reviewed in this paper, enzymatic esterification represents a technically robust and operationally gentler alternative to conventional chemical FFA-reduction methods, with particular relevance to biodiesel producers seeking to process a wider range of waste-derived and alternative feedstocks without compromising throughput, yield, or product quality.

Scope for Further Study

- Independent, peer-reviewed techno-economic modelling of the FlexFit® Advance process across varying plant capacities and feedstock FFA profiles beyond the single benchmark scenario reported in the manufacturer's documentation, situated within the broader research priorities identified for enzymatic transesterification of non-edible and waste-derived feedstocks [24].
- Comparative life-cycle assessment (LCA) of enzymatic versus acid-catalyzed and glycerolysis-based FFA pretreatment routes.
- Independent study of long-term enzyme stability, methanol tolerance, and reusability of free liquid esterase formulations under continuous industrial operation, building on existing packed-bed reactor stability data for immobilized lipases [18].
- Investigation of process intensification strategies (e.g., reactor staging, mixing optimization, fed-batch alcohol dosing) to further reduce residence time and reactor volume requirements while managing enzyme deactivation.
- Extension of the present single-site field study to additional Eversa® Advance installations across different geographies and feedstock blends, to validate the generalizability of the Aavisi plant observations reported in this paper, and to test them against the wider enzymatic biodiesel technology landscape [25].
- Detailed capital cost breakdowns for FlexFit® Advance retrofit projects across a range of existing plant configurations, to enable independent benchmarking against published chemical pretreatment cost data [28].

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Author Contributions

The first author and corresponding author made significant contributions to the conceptualization and experimental design of the study, execution of experiments, data collection, data analysis, interpretation of results, and preparation, review, and editing of the manuscript.

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