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Effect of long chain fatty acids on biogas production and biochemical kinetics in anaerobic bioreactors: A review

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ABSTRACT

Long Chain Fatty Acids (LCFAs) are the primary intermediate byproduct of the lipid (fats, oils, and greases) degradation process; if they are accumulated in high concentrations, they can cause failure or reduce the performance of anaerobic bioreactors due to sludge flotation issues, biochemical kinetics problems for soluble substrates, inhibition of microbial activity, and inefficient biogas recovery. Understanding the biochemical kinetics of anaerobic bioreactors requires consideration of the entire process, including microbe growth, substrate degradation, and product synthesis. Biochemical kinetics of anaerobic treatment is the study of polymer biodegradation rates of insoluble organic matter in wastewater, which is the mechanism of bond breaking and bond formation in biochemical reactions. Consequently, biochemical kinetics allow for the design of both desired and undesirable reaction phases. Understanding the reasons of increasing LCFA and VFA allows a bioreactor design to predict pH reduction, foaming, and VFA accumulation. Foaming can reduce the bioreactor's active volume. As a result, it has the potential to increase methane production from waste containing high quantities of substrate. Meanwhile, hydrodynamics, mass transfer phenomena between phases, biochemical reaction kinetics, and heat transfer all play important roles in the development of technical-scale bioreactors, bioprocess mechanisms, and the performance of anaerobic bioreactors for waste treatment. These aspects are analyzed comprehensively within a bioreactor system. The kinetic parameters acquired are utilized to design, operate, and optimize anaerobic bioreactors for wastewater treatment on a technical scale.

1. Introduction

The study of anaerobic process kinetics helps us comprehend the velocity of organic matter breakdown, which is catalyzed by anaerobic microbes. Kinetic models make it easier to analyze organic matter degradation velocity and determine kinetic parameters for designing, operating, and optimizing anaerobic bioreactors for wastewater treatment [1].

The hydrolysis of organic polymers such as protein, carbohydrate, and lipid produces amino acids, simple sugars, fatty acids, and alcohols. Long chain fatty acids (LCFAs) are the primary intermediate consequence of lipid degradation, and their accumulation in anaerobic bireactors has been linked to issues with sludge flotation, biomass washout, and microbial activity inhibition [2].

Lipids, commonly referred to as fats, oils, and greases, are a substantial component of organic matter in waste and wastewater from food processing businesses, slaughterhouses, dairy industries, and fat refineries. Anaerobic bioreactors can process organic wastes, municipal, agricultural, and industrial wastewater, animal excrements, and plant residues [3]. Anaerobic bioreactors are the most suitable method for the treatment of effluents containing high concentrations of organic carbon, such as palm oil mill wastewater, slaughterhouse wastewater, baker's yeast factory effluent, and cattle manure [4, 5,6,1].

This research investigates the effect of LCFAs on biochemical kinetics of insoluble organic solids on wastewater treatment in anaerobic bioreactors.

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2. Biochemical Reaction in Anaerobic Bioreactor

Specific growth rate (μ), endogenous decay coefficient (kd), maximum substrate utilization rate per unit mass of microorganisms (k), maximum cell yield (Y), and half-velocity constant are biokinetic coefficients that are used in the design of an anaerobic bioreactor [7,8].

Figure 1 depicts the process of hydrolysis, acidogenesis, acetogenesis, and methanogenesis in an anaerobic bioreactor for organic materials. The anaerobic bioreactor of organic matter is a complex biochemical process that involves numerous intermediary chemicals and reactions, each of which is mediated by a unique enzyme or catalyst. Organic molecules are broken down in the first anaerobic bioreactor stage, liquefaction, by extracellular enzymes produced by hydrolytic bacteria [6,9].

Anaerobic transformation of organic wastes involves various bacterial groups, including hydrolyzing, acidifying, acetogenic, and methanogenic bacteria that produce CO_2 and CH_4. The bioreactor has three phases: (a) the solid phase, which consists of bioparticles made of inert support material as well as active and non-active attached biomass (biofilm); (b) the liquid phase, which contains substrates, products, enzymes, ions, and active and non-active suspended biomass; and (c) the gas phase, which is a mixture of gaseous fermentation products. Biochemical reactions are believed to take place only in the bioreactor [10].

The nutrient requirements for wastewater treatment were found to be lower than what is typically reported in the literature for C:N:P ratios of 100:5:1 for aerobic treatment and 250:5:1 for anaerobic treatment [11]. According to Rajeshwari et al. (2000), the ideal C:N:P ratio for biological therapy is 100:2.5:0.5. The highest needed ratio, as described in the literature, ranges from 250:5:1 to 500:5:1, depending on the level of loading or COD influent concentrations. If the C/N ratio is high, there is a risk of nutrient insufficiency, and a limited buffering capacity results in a more sensitive process, whereas a high nitrogen concentration may cause ammonia inhibition issues. Mixing carbohydrate-rich wastes with nitrogen-rich wastes can improve their digestibility [81,49,60,79].

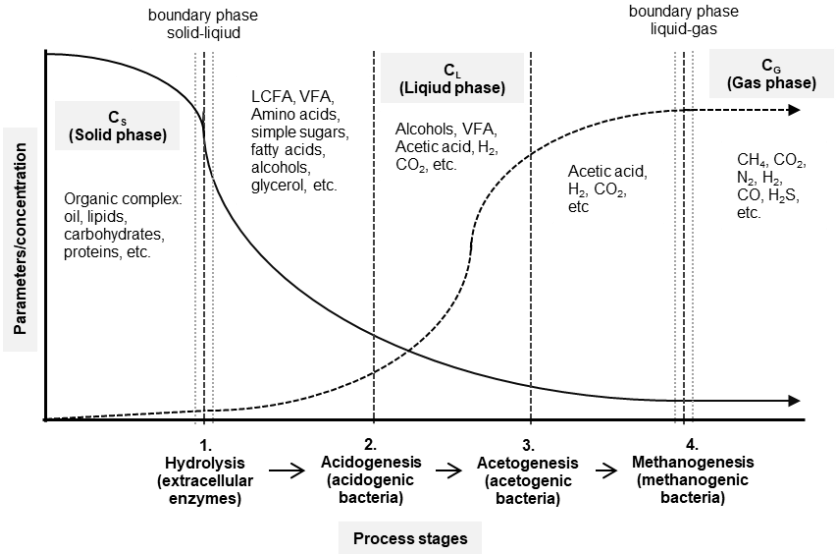

Fig. 1. Anaerobic bioreactor complex diagram

Substrate composition, crystallinity, porosity, particle size, surface area, structural features, and homogeneity are all factors that influence anaerobic bioreactor processes [12]. However, the treatment efficacy of these bioreactors is dependent on variables such as wastewater composition, temperature, and pH pH influences enzymatic activity and digester effectiveness. Acid-forming bacteria have adequate enzymatic activity above pH 5.0, but methane-forming bacteria have no good enzymatic activity below pH 6.2. Most anaerobic bacteria, particularly methane-forming bacteria, thrive in the pH range of 6.8 to 7.2 [13,14].

Anaerobic bioreactors require tight anaerobic conditions (ORP <-200 mV) and rely on coordinated microbial activity to convert organic material into CO_2 and CH_4. Regardless of the subsequent processes, hydrolysis is widely regarded as rate-limiting [15,16].

2.1. Hydrolysis

Hydrolysis of polymerized organic molecules, such as carbohydrates, proteins, and fats, converts them to soluble monomers and dimers, such as monosugars, amino acids, and fatty acids. This stage of the methane biodegradation process involves extracellular enzymes from the hydrolase family (amylases, proteases, and lipases) produced by appropriate strains of hydrolyzing bacteria. The hydrolysis of rarely decomposable polymers, such as cellulose and cellucottons, is thought to be a stage that inhibits the pace of waste biochemical decomposition. Only 50% of organic molecules in solid waste are biodegraded. The remaining chemicals stay in their original condition due to a lack of enzymes involved in their biodegradation [17].

The hydrolysis of lipids represents the rate-limiting step in the overall anaerobic biodegradation process, but significant and detrimental inhibition occurs with LCFAs. The rate of hydrolysis depends on particle size, pH, enzyme production, enzyme diffusion and adsorption, substrate concentration, and temperature [22] .

2.2. Acidogenesis

Acidogenesis refers to the fermentation of amino acids and simple sugars, as well as the anaerobic oxidation of long chain fatty acids (LCFA) and alcohols by acid-forming bacteria. Aside from carbon dioxide, water, and hydrogen, acetic, propionic, butyric, and valeric acids will accumulate. Butyric and valeric acids are particularly important for protein-rich compounds since a variety of amino acids are reduced to these fatty acids [23,24,25].

Acidogenesis may be bidirectional due to the effects of different populations of bacteria. This process can be separated into two types: hydrogenation and dehydrogenation. The basic cascade of transformations includes acetates, CO_2, and H_2, with other acidogenesis products playing minor roles. As a result of these modifications, methanogenesis may be able to exploit the new products as both substrates and energy sources. The bacteria responds to a rise in hydrogen content in the solution by accumulating electrons through molecules such as lactate, ethanol, propionate, butyrate, and highly volatile fatty acids. Methanogenic bacteria cannot consume the new products directly; instead, they must be transformed by obligatory bacteria that produce hydrogen in a process known as acetogenesis. Ammonia and hydrogen sulfide, which give this phase of the process a powerful disagreeable odor, are also acidogenesis products [26,27].

Proteases, which act as exo-enzymes, hydrolyze proteins to produce amino acids. The amino acids can be rapidly absorbed by diffusion through cell walls and membranes. This procedure does not limit the rate of future reactions [28].

Lipids are esters composed of fatty acids and glycerine, a three-valenced alcohol. Lipase enzymes have previously hydrolyzed these molecules. Glycerine can be employed in

anabolic processes and is partially transformed into lesser alcohols (catabolism). Acidogenic bacteria cannot consume the fatty acids, thus they are expelled. Most facultative anaerobic bacteria use exo-enzymes to break down polymeric hydrocarbons into monomers (glucose and other sugars) [29,27].

Arrangement of some key catabolic anaerobic reactions for oxidation (electron-donating reaction) and respiration (electron-accepting reaction) [98]. Glycerol is reduced through acidogenesis, while LCFA is degraded to acetate, H_2, and CO_2 via β-oxidation (syntrophic acetogenesis) [30].

Acidogenic bacteria carry out biological reactions [28] , as follows:

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + 2\,H_2 \quad (1)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2\,CO_2 + 2\,H_2 \quad (2)$$

$$C_6H_{12}O_6 + 2\,H_2 \longrightarrow 2\,CH_3CH_2COOH + 2\,H_2 \quad (3)$$

$$C_6H_{12}O_6 + 2\,H_2O \longrightarrow 2\,CH_3COOH + 2\,CO_2 + 4\,H_2 \quad (4)$$

2.3. Acetogenesis

Acetogenesis is the anaerobic oxidation of intermediates such as volatile fatty acids (mainly propionic and butyric acid, but not acetic acid) to acetic acid and hydrogen by acetogenic bacteria. The collection of hydrogen must be avoided since hydrogen inhibits this sub-process. As a result, hydrogen-using and acetogenic bacteria coexist in dense agglomerations [31].

Even under ideal conditions such low dissolved hydrogen concentrations, acetogenic bacteria grow slowly, with a minimum doubling time of 1.5 to 4 days Only a portion of acetate is produced immediately during fermentation. The majority of it is generated through synthetic processes [16].

Acetogenic bacteria perform β-Oxidation, a biological reaction known as synthrophic reactions [28,27], as follows: Butyrate acetogenesis:

$$CH_3CH_2CH_2COOH + 2\,H_2O \longrightarrow 2\,CH_3COOH + H^+ + 2\,H_2$$
$$\Delta G° = 48{,}1\ \text{kJ mol}^{-1} \quad (5)$$

Propionate acetogenesis:

$$CH_3CH_2COOH + 3\,H_2O \longrightarrow CH_3COOH + H^+ + HCO_3^- + 3\,H_2$$
$$\Delta G° = 76{,}1\ \text{kJ mol}^{-1} \quad (6)$$

Ethanol acetogenesis:

$$CH_3CH_2OH + H_2O \longrightarrow CH_3COOH + H^+ + 2\,H_2$$
$$\Delta G° = 9{,}6\ \text{kJ mol}^{-1} \quad (7)$$

$$CH_3CHOH\,COO^- + 2\,H_2O \longrightarrow CH_3COOH + H^+ + HCO_3^- + 2\,H_2$$
$$\Delta G° = -4{,}2\ \text{kJ mol}^{-1} \quad (8)$$

Because of the action of acidogenic bacteria, hydrogen inhibition during the acidogenic stage is considered. In addition, hydrogen inhibition in the acetogenic phase has been provided to explain why this reaction is blocked at high hydrogen partial pressures. In addition to hydrogen inhibition, acetate inhibition of the butyrate-degrading step and inhibitions caused by intermediate products, such as propionate and butyrate, on the methanogenic step are considered [32,33]. Figure 2 depicts the issues created by the accumulation of LCFA and VFA in the anaerobic bioreactor.

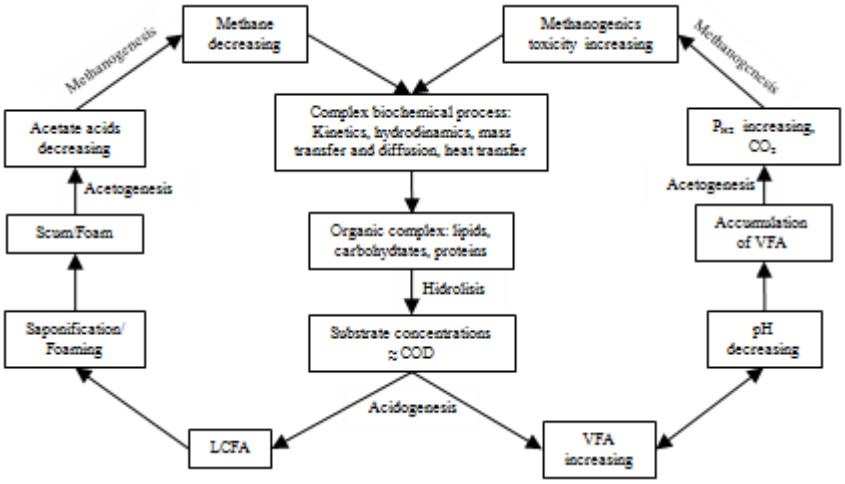

Fig. 2. Problems caused by the accumulation of LCFA and VFA

2.4. Methanogenesis

The final phase in an anaerobic bioreactor is methanogenesis. Methanogens are strictly anaerobic archaea that can be classified into two types: (1) hydrogenophilic or hydrogenotrophic species, which produce methane by reducing CO_2 with H_2 as an electron donor, and (2) acetoclastic or acetotrophic methanogens, which produce methane by decarboxylating acetate. Methanogens can create methane from a limited variety of different substrates, including methanol, methylamines, and formate. Hydrogenotrophic methanogens create methane by converting CO_2 into formyl, methenyl, and methyl intermediates in the presence of particular coenzymes. Although the acetoclastic pathway accounts for about 70% of the methane produced during anaerobic processes [34,35,28].

In this step of the process, methane is created from substrates that are results of previous phases, such as acetic acid, H_2, CO_2, and formate, as well as methanol, methylamine, or dimethyl sulphide. Although just a few bacteria can create methane from acetic acid, heterotrophic methane bacteria convert acetic acid, resulting in the bulk of CH_4 produced during methane digestion [36,37]. The slow biodegradation of LCFAs and their accumulation in anaerobic bioreactors inhibit methanogenic activity and biogas production [38].

Only 30% of the methane produced in this process is from CO_2 reduction by autotrophic methane bacteria. During this

process, H2 is depleted, allowing acid bacteria to proliferate and produce short-chain organic acids in the acidification phase, resulting in insufficient H_2 generation in the acetogenic phase. According to Griffin et al. [39], Karakashev et al. [24], and Ziemiński and Frac [37], such conversions may result in CO2-rich gas, as only a small portion is converted to methane [39,24,37].

Process biochemical reaction CH_4 production by methanogenic bacteria (syntrophic reactions) [28], as follows:

Hydrogenotrophic methanogenesis:

$$CO_2 + H_2O + 4\,H_2 \longrightarrow CH_4 + 3\,H_2O \quad \Delta G° = -135{,}6 \text{ kJ mol}^{-1} \tag{9}$$

Acetoclastic methanogeneis:

$$CH_3COOH \longrightarrow CH_4 + CO_2 \quad \Delta G° = -31{,}0 \text{ kJ mol}^{-1} \tag{10}$$

Furthermore, it processes the biochemical respiration reaction [28], as follows:

$$2\,CH_3CHOH\,COO^- + SO_4^{2-} \longrightarrow 2\,CH_3COOH + 2\,HCO_3^- + 2\,H_2 + S^{2-} \quad \Delta G° = -1120{,}5 \text{ kJ mol}^{-1} \tag{11}$$

$$CH_3COOH + SO_4^{2-} \longrightarrow 2\,HCO_3^- + H^+ + S^{2-} \quad \Delta G° = -47{,}6 \text{ kJ mol}^{-1} \tag{12}$$

$$4\,H_2 + SO_4^{2-} \longrightarrow 2\,H_2O + S^{2-} \quad \Delta G° = -38.1 \text{ kJ mol}^{-1} \tag{13}$$

$$2\,NO_3^- + 5\,H_2 + 2\,H^+ \longrightarrow N_2 + 6\,H_2O \tag{14}$$

3. Long Chain Fatty Acids

Long-chain fatty acid (LCFA) decomposition occurs in syntrophic communities of anaerobic bacteria and methanogenic archaea. To work optimally, these syntrophic communities must be aggregated in compact aggregates, which is typically challenging to do with fat and lipid-containing wastewaters [40].

LCFAs are the primary intermediate byproduct of the lipid degradation process, and their accumulation in anaerobic bioreactors has been linked to sludge flotation, biomass washout, and microbial activity inhibition [2,41], substrate and product transport limitation, sludge flotation, digester foaming, and pipe and pump blockages [42].

Several investigations have demonstrated that LCFA inhibition was reversible and was linked to physical transport limitation effects. After this proof, the permanent cell damage caused by LCFA adsorption was disregarded, and new technological options for high-rate anaerobic treatment of lipid-containing wastewater developed [43,44,45,2].

Triglycerides and free LCFA are the most common types of lipids found in wastewater. Several metabolic processes contribute to lipid breakdown under anaerobic circumstances. Extracellular lipases hydrolyze triglycerides, producing glycerol and LCFA. Glycerol is reduced through acidogenesis, while LCFA are degraded to acetate, H2, and CO2 via β-oxidation (syntrophic acetogenesis) [30,46]. When compared to carbohydrates and proteins, lipids are a very promising substrate for anaerobic bioreactors in terms of methane production, and thus they could be considered a potential energy source [26,47,30].

Table 1 shows the treatment of lipid and LCFA-containing wastewater in various anaerobic bioreactors, as well as the technologies used for the anaerobic treatment of oily effluents. The primary mechanisms of LCFA toxicity are adsorption onto microorganism cell walls, which inhibits transport phenomena, as well as acute toxicity on microbial activity of both acetoclastic and hydrogenotrophic methanogens [26,48,29,46].

3.1. Substrate concentration

Microorganisms require substrates for three primary purposes: to synthesize new cells, to synthesize extracellular products, and to meet energy requirements for cell maintenance [49].

In general, use the term "substrate" to refer to degradable COD [50,23]. It is critical to distinguish between accessible degradable (substrate) and total input COD, as a significant portion of the input COD may be anaerobically nonbiodegradable. Lipids (fats, oils, and greases) are a substantial component of domestic wastewater organic matter, accounting for 25-35% of total COD in raw wastewater [42,46].

After hydrolysis, the LCFA conserves more than 90% of the chemical oxygen demand (COD) of lipids.The predicted biomass/substrate yield for fat conversion is 0.038 g VSS (g COD)-1, while values for proteins and carbohydrates are 0.2 and 0.35 g VSS (g COD)-1, respectively [51,40].

3.2. Saponification, foaming and scum

Saponification is the hydrolysis reaction that occurs between a lipid and an alkali, producing LCFA salt and releasing glycerol. Glycerol is readily biodegraded into volatile fatty acids (VFA), which are subsequently transformed into biogas [52,53,54]. Lipid-containing wastes are promising substrates for biogas production due to their high methane yield potential [52,53,54].

Extracellular lipases produced by microorganisms hydrolyze lipids under anaerobic circumstances, converting them to LCFA and glycerol. LCFA are degraded through β-oxidation to produce acetate and hydrogen, which are then transformed into biogas. A batch research revealed that lipid concentration-induced inhibition was connected to hydrolysis rate, although it was also reversible [48,52,53,54].

Tabel 1: Treatment of wastewater with lipids and LCFA in various anaerobic bioreactors

Bioreactor	Wastewater	HRT, (day)	Temp. (ºC)	$COD_{Inf.}$, (mg/L)	$COD_{Rem.}$, (%)	Biogas Production (Nm^3/kg $COD_{rem.}$)	Refrences
UASB	Slaughterhouse Wastewater	24	30	4,175	90	0.34	[55]
MABR	POME	3-10	-	16,000	87.4-95.3	0.32-0.42	[56]
Fluidized-Bed	Extracted Sunflower Flour	4.5-20	15-19	11,300	69-95.9	0.32	[57]
UASB	Slaughterhouse Wastewater	2-7	30-33	6,037	75	0.3	[58]

Tabel 1: Treatment of wastewater with lipids and LCFA in various anaerobic bioreactors (continued)

Bioreactor	Wastewater	HRT, (day)	Temp. (ºC)	$COD_{Inf.}$, (mg/L)	$COD_{Rem.,}$. (%)	Biogas Production (Nm^3/kg $COD_{rem.}$)	Refrences
AFBR	Leachate	1	35	10,000-50,000 (OLR 2.5-37 kg COD/m^3.d)	80-90	0.50-0.52 L/g COD_{rem}	[59]
UASFF	POME	1-6	38	5,260-3,472.5	80.6-98.6	0.287-0.348	[9]
UASB	Food-Processing Wastewater	5	35	27-52 (OLR (gCOD/L.d)	94-98	0.24-0.32 (L CH_4/g COD)	[60]
UASB		2.5-1.25	35	1.3-8.0 (OLR (g COD/L.d)	84-89	0.24-0.48 (L CH_4/g COD)	
PBR+UASB		2.5-1.25	35	1.3-4.2 (OLR (g COD/L.d)	86-90	0.18-0.42 (L CH_4/g COD)	
Anaerobic Digestion	Food Waste	10-12	40-55	9,800	83	119-223 L CH_4/kg $sCOD_{degraded}$	[47]
UASFF	POME	3-1.5	38	1.8-23.2 (OLR (g COD/L.d)	89-97	0.31-0.35 (L CH_4/g COD)	[61]
UASFF	Dairy Wastewater	3-4 (36-48 jam)	36	50,000-70,000	97.5	3.6-3.75 L/d	[62]
UAF	Slaughterhouse Wastewater	24-48	35	6,196.75	85	-	[1]
MAS	POME	400.6-5.7	70 (55 bars)	18,302-43,500	94.8-96.5	0.25-0.58	[63]
Anaerobic	Leachate	40	35	24,840	94	-	[64]
Anaerobic Bioreactor	POME	14-6.5	35-45	15,000–66,000	70-65	0.35 m^3 CH_4/kgCOD	[65]
AHR	Pharmaceutical Wastewater (Penicillin-G Unit)	30-3 h	30-35	32,256 (OLR 3.20-16.05 kg COD/m^3.d)	91.25-68	1.2-8.7 L/d	[66]
Anaerobic Digestion (Batch)	Cow Dung	10	53	2,200	48.5	0.15 L/kgVS	[67]
AFBR	Dairy Wastewater	1-5.5	Ambient	39,000 (OLR 24-4.4 kg COD/m^3.d)	24.2-82.1	0.07-0.18 L CH4/g COD_{added}	[68]
Semi Continuous Anaerobic Digester (36 L)	Cow Dung	50.0 - 10.0	28.7 – 29.1	VS (% TS) 69.42	OLR: 1.31 g VS/L/day	77.32 L/kg VS removal	[69]
(UASB + AF)	Synthetic dairy wastewater	0.75-3	35 (Mesophilic)	300 to 600	98±1	1.2±0.4 L/d	[70]
(UASB + AF)	Synthetic dairy wastewater	3-5	15 (Psychrophilic)	300 to 600	91±4	1.0±0.4 L/d	[70]

HAIB : Horizontal-Flow Anaerobic Immobilized Biomass ; UASB : Upflow Anaerobic Sludge Blanket; UAF : Upflow Anaerobic Filter; MABR : Modified Anaerobic Baffled Reactor; POME : Palm Oil Mill Effluent; UASFF : Up-Flow Anaerobic Sludge Fixed Film; MAS : Membrane Anaerobic System; ABR : Anaerobic Baffled Reactor; AHR : Anaerobic Hybrid Reactor; UAF-B : Upflow Anaerobic Fixed-Bed; AFBR : Anaerobic Fluidized Bed Reactor

Surface-active agents, often known as surfactants, are large organic compounds that are either chemically generated or created by microorganisms. Surfactants are marginally soluble in water, resulting in foaming in wastewater treatment plants. A typical urban trash contains 1-20 mg/L of feed surfactants, which might rise if the anaerobic bioreactor is overloaded [58]. Anaerobic foaming is generated by filamentous bacteria, which can live and even proliferate in anaerobic mesophilic environments despite being obligate aerobes [36,8]. The identified several parameters that could potentially contribute to foaming in anaerobic bioreactors, which are filamentous microorganisms, accumulation of VFAs, and inadequate mixing of the bioreactors, fats/oil/grease (FOG) and feed sludge quality, bioreactor feeding regime, excessive grease and scum in bioreactor feed, temperature fluctuation. Non-biological factors influencing foaming in anaerobic bioreactors include organic loading rate (OLR), mixing, and the primary/activated sludge solids ratio [73,72,74].

Foaming is seriously unpleasant and can result in the loss of active bioreactor volume, structural damage, leakage, damage to the gas-handling system, and a subsequent drop in biogas output. In general, foaming in anaerobic bioreactors reduces gas generation by up to 40% [75]. Foam generation and accumulation in anaerobic bioreactors causes a wide range of operational issues, including pump clogging, gas collection pipe fouling, gas mixing device blockage, a loss of effective bioreactor volume, and a decrease in both biogas production and volatile solids removal [72].

Saponification, foaming, and scum in anaerobic biological treatment of wastewaters can cause clogging, flotation, mass transfer problems for soluble substrates, reduction of sludge methanogenic activity and methane production [76,77], poor accessibility to microorganisms, and inhibiting properties of LCFA, inefficient biogas recovery creates dead zones and increases electricity generation expenses, resulting in a 20-50% loss of biogas production [71,78].

3.3. Volatile fatty acids (VFA)

Several investigations have also observed reactor failure or underperformance due to pH lowering produced by excessive VFA accumulation in the anaerobic treatment system [79,80].

Volatile acids are organic acids that are commonly referred to as volatile fatty acids (VFA). They can vary in length but are typically low molecular weight (MW) chemicals that dissolve in water and sludge. The seven most prevalent

fatty acids found in anaerobic bioreactors are formic acid, aceticacid, propionic acid, butyric acid, valeric acid, iso-valeric acid, and caproicacid. In bioreactors, total VFA concentrations typically range between 50 and 300 mg/L for the aforementioned acids. Acetic acid is the most abundant acid, accounting for over 85% of the volatile acids in an anaerobic bioreactor [71].

4. Biochemical Kinetics Model

Several studies have been carried out to evaluate kinetic parameters and model equations for anaerobic bioreactors [56,81,5,82], which are all based on the Monod kinetic model and the revised kinetic model [83,84]. Kinetic coefficients are utilized to control biological treatment processes, and models for organic matter and nutrient removal, as well as microbiological development, are anticipated and estimated [8].

Microbial growth, substrate degradation, and product production are the processes that need to be studied in order to analyze the biochemical kinetics of an anaerobic bioreactor. The kinetic equations for this mechanism are as follows: Chen and Hashimoto [84], Barthakur et al., [85], Faisal and Unno [56], and Zinatizadeh et al. [9].

The maximum specific rate of growth of biomass (max), saturation coefficient, decay coefficient, and yield coefficient are some of the important information that can be obtained by using kinetic modeling [7,8].

Hydrolysis, a first step solubilization of solid and/or oil/grease, is believed to be a first order reaction in terms of the concentration of hydrolyzable substrate S (mass/volume) as:

$$\frac{dS_h}{dt} = K_h(S - S_h) \tag{15}$$

Where Sh is the hydrolyzed substrate concentration (mass/volume) and Kh is the hydrolysis rate coefficient (s−1). Kinetics of transporting hydrolyzed substrate into granules (dependent on the amount of active biomass (X) as the biomass consumes the substrate delivered into the granules):

$$\frac{-dS_h}{dt} = k(S_h - S_g)X \tag{16}$$

$$-\frac{dS_h}{dt} = k(S_h - S_g)X = kS_hX \tag{17}$$

Where k is the hydrolyzed substrate transport rate coefficient (s^{-1}):

$$S_h = \frac{K_hS}{kX + K_h} \tag{18}$$

4.1. Monod's model

The Monod model is one of the many mathematical models used to describe kinetics, and it is the most widely used one [8]. Kinetic of cell growth on hydrolyzed substrate, Monod's equation:

$$\mu = \frac{\mu_m S_h}{K_s + S_h} \tag{19}$$

Where K_s is the half-saturation constant with respect to hydrolyzed substrate (mass/volume). Upon substitution of the value of S_h from equation (17), equation (18) becomes:

$$\frac{\mu}{\mu_m} = \frac{S}{\left(K_skX / K_h\right) + K_s + S} \tag{20}$$

A material balance on cell concentration in bioreactor can be expressed as:

$$Q_0X - QX_e + V_R\mu X_R - V_Rk_dX_R = \frac{V_RdX}{dt} \tag{21}$$

Where XR and Xe represent the concentration of microorganisms in the bioreactor and effluent, respectively, and VR and Q represent the bioreactor volume and wastewater flow rate. Under steady-state conditions of continuous digestion (dX/dt = 0), and the assumption that the concentration of microorganisms in the water, X0 = 0, and the endogenous metabolism or death rate is insignificant relative to the growth rate (kd << μ), then:

$$\mu = \frac{QX_e}{V_RX_R} = \frac{1}{SRT} \tag{22}$$

Volumetric substrate removal rate F (mass/volume/time) may be expressed as:

$$F = \frac{S_0 - S}{SRT} \tag{23}$$

and with the assumption that the microbial growth is negligible in a short period of time:

$$X = Y_x(S_0 - S) \tag{24}$$

By use of Equation (23), Equation (19) can be rearranged as:

$$\frac{\mu_m}{\mu} = A\frac{S_0 - S}{S} + \frac{K_s}{S} + 1 \tag{25}$$

Where

$$A = K_skY / K_h$$

Table 2. displays the kinetic coefficients found in the current research investigation at the three temperatures using the monod model

Substrate	Bioreactor Type	COD_{inf} (mg/L)	Parameter Kinetics A	K_s (g/L)	μ_m (per day)	References
Cattle Waste	UASB	49,700	0.640	0.300	0.250	[86]
Acetic acid	UASB	1,140	0.000	0.300	0.440	[77]
Propionic acid	UASB	5,520	0.000	0.250	0.274	[77]
Dairy Manure	UASB	82,200	0.751	0.280	0.450	[77]
POME	MABR	16,000	0.329	0.313	0.304	[56]
ice-cream wastewater	CSTR	5500	0.0131	0.4028	0.7844	[87]
Textile Wastewater	UASB	4,214	0.125	>4000	0.105	[88]
POME	UASFF	34,750	0.738	0.982	0.207	[9]
POME	ABSR	53,500	0.024	203.433	0.524	[89]

Table 2. displays the kinetic coefficients found in the current research investigation at the three temperatures using the monod model (comtinued)

Substrate	Bioreactor Type	COD_{inf} (mg/L)	Parameter Kinetics A	K_s (g/L)	μ_m (per day)	References
Pharmaceutical wastewater	Hybrid-UASB	> 1830	0.0175	2.63	0,5618	[66]
Industrial waste (2,4 dichlorophenol)	UASB	3000	0.780	0.560	0,213	[90]
Weak industrial waste	UASB	54.33±704.55	0.680	0.189	0.008	[91]
Dairy wastewater	AnIMBR	4612–6663	0.2022–0.427 mg/mg	4.612-6.663	0.0334–0.1095	[7]

AnIMBR: anaerobic immersed membrane bioreactor; UASB: upflow anaerobic sludge blanket; ABR: anaerobic baffled reactor; MABR: modified anaerobic baffled reactor; UASFF: upflow anaerobic sludge fixed film; CSTR: continuously stirred tank reactor.

4.2. Modified stover-kincannon model

For biofilm bioreactors such as rotating biological contactors and biological filters, the substrate consumption rate is expressed as a function of the organic loading rate using a monomolecular kinetic model [92,88,1]. The updated Stover-Kincannon model equations are as follows:

$$\frac{dS}{dt} = \frac{R_{max} x\left(QxS_0 / V\right)}{K_B + \left(QxS_0 / V\right)} \tag{26}$$

Where dS/dt is defined in Equation (27):

$$\frac{dS}{dt} = \frac{Q}{V} x\left(S_0 - S\right) \tag{27}$$

Equation (15) obtained from linearization of equation (27) as follows:

$$\frac{V}{Qx\left(S_0 - S\right)} = \frac{K_B}{R_{max}} \frac{V}{QxS_0} + \frac{1}{R_{max}} \tag{28}$$

Table 3 shows the kinetic coefficients derived in the current investigation at the three temperatures using the Stover-Kincannon model.

Table 3. Kinetic constant comparison in the modified stover kincannon model

Substrate	Bioreactor Type	COD_{inf}(mg/L)	HRT (day)	Parameter Kinetics μ_{max} (mg/L.d)	K_B (g/l.d)	References
Soybean Wastewater	AF	7,520-11,450	1-1.45	83.3	85.5	[92]
Molasses	AHR	2,000-15,000	0.5-2	83.3	186.23	[93]
Textile Wastewater	UASB	4,214	0.25-4.16	7.501	8.211	[88]
Poultry Slaughterhouse	SASBR SGBR	1,600-9,100	36-48	121.48-164.40	130.28-177.21	[94]
Milk Permeate Wastewater	AMBBR	55,200	27.56-1.97	89.3	102.3	[95]
Slaughterhouse wastewater	UAF (20°C)	6,000-6,500	24-48	5.22	5.09	[1]
Slaughterhouse wastewater	UAF (27.5°C)	6,000-6,500	24-48	17.12	19.75	[1]
Slaughterhouse wastewater	UAF (35°C)	6,000-6,500	24-48	99.01	120.88	[1]
Municipal wastewater	UASB	2,190–2,688	5-24 (h)	1.996	1.536	[96]
Pharmaceutical wastewater	AHR	4,000-4,500	0.125-1.25	108.69	115,66	[66]
Weak industrial waste	UASB	54.33±704.55	345.6-21.6	1.502	2.924	[91]

AF: anaerobic filter; AHR: anaerobic hybrid reactor;UASB: upflow anaerobic sludge blanket; SASBR: Static anaerobic sludge Granular Bed Reactor (SGBR); UAF: upflow anaerobic filter; AMBBR: Anaerobic Moving Bed Biofilm Reactor.

4.3. Grau-second-order model

Equation (29) represents the general equation from the Grau kinetic model [97,88,1]:

$$-\frac{dS}{dt} = k_s X \left(\frac{S}{S_0}\right)^2 \tag{29}$$

If equation (29) is integrated and then linearized, equation (30) will be obtained:

$$\frac{S_0 - \theta_H}{S_0 - S} = \theta_H - \frac{S_0}{k_s . X} \tag{30}$$

Considering that the second term in the right side of equation (30) is a constant, equation (31) is obtained:

$$\frac{S_0 - \theta_H}{S_0 - S} = n.\theta_H + m \tag{31}$$

Knowing that organic matter removal efficiency is equal to $(S_0\text{-}S)/S_0$ and is expressed as E, equation (21) can be re-written as shown in equation (32):

$$\frac{\theta_H}{E} = m + n.\theta_H \tag{32}$$

Table 4 shows the kinetic coefficients derived in the current research investigation at the three temperatures using the Grau Second Order Model

Tabel 4. Comparison of kinetic constants in the grau second order model

Substrate	Bioreactor Type	COD_{inf} (mg/L)	HRT (day)	Parameter Kinetics			References
				k_s (g/L)	m (per day)	n	
Molasses	AHR	2,000-15,000	0.5-2.0	10.81	0.033	1.192	[93]
Textile Wastewater	UASB	4,214	0.25-4.16	0.337	0.562	1.095	[88]
Slaughterhouse wastewater	UAF (20°C)	6,000-6,500	24-48	0.89	1.10	1.03	[1]
Slaughterhouse wastewater	UAF (27.5°C)	6,000-6,500	24-48	5.31	0.35	1.15	[1]
Slaughterhouse wastewater	UAF (35°C)	6,000-6,500	24-48	15.72	0.06	1.22	[1]
Pharmaceutical wastewater	AHR	4,000-4,500	0.125 - 1.25	-	0.031	1.067	[66]
Weak industrial waste	UASB	54.33±704.55	345.6 - 21.6	0.583	0.168	2.023	[91]

AHR: anaerobic hybrid reactor;UASB: upflow anaerobic sludge blanket; UAF: upflow anaerobic filter.

5. Conclusions

The acidogenesis phase (acidogenic bacteria) produces intermediate products (fatty acids) from amino acids, simple sugars, VFAs, and alcohol from the hydrolysis process (extracellular enzymes). While LCFAs are the primary intermediate byproduct of the lipids (fats, oils, and greases) biodegradation process, their accumulation in anaerobic bioreactors has been linked to issues with sludge flotation, biomass washout, and microbial activity inhibition. When compared to carbs and proteins, lipids are a very promising substrate for anaerobic bioreactor methane generation, and hence they could be considered a viable energy source. Triglycerides and LCFAs make up the majority of lipids found in wastewater. Many portions of COD are hydrolyzed lipids derived from LCFAs. In the first phase of hydrolysis, the first step, the solubilization of solid and/or oil/grease, is thought to be a first-order reaction in terms of hydrolyzed substrate concentration. To analyze the biochemical kinetics of an anaerobic bioreactor, the entire process, including microbe growth, substrate breakdown, and product synthesis, must be considered. It leads to a comprehension of a sequence of primary reaction steps, including the mechanism of bond breaking and bond formation in chemical reactions, as well as the assessment of energy and product stability. As a result, biochemical kinetics allows for the design of both desired and undesirable reaction phases. Biochemical kinetics of anaerobic treatment is the study of the polymer degradation rate of insoluble organic matter in wastewater, and kinetic parameters are utilized on a technical scale to build, operate, and optimize anaerobic bioreactors for wastewater treatment.

Nomenclature

A biokinetic parameter Chen and Hashimoto equation
F volumetric substrate removal rate (g/L per day)
k transportation rate constant into the granule (per day)
K apparent reaction rate constant (L CH_4/g COD day)
K_B saturation value constant (g (L per day))
K_h hydrolysis rate constant (per day)
K_s half-velocity constant (g COD/L)
m So/(ks x X) (d^{-1})
n Grau model Constant (dimensionless)
Q volumetric feed flow rate (L/day)
R_{max} maximum substrate removal rate (mg COD (L per day)
S effluent substrate concentration (g COD/L)
S_0 influent substrate concentration (g COD/L)
S_h hydrolyzed substrate concentration (g COD/L)
t hydraulic retention time (day)
ΔT temperature change of bioreactor (K d^{-1}),
V volume of the reactor (L)
X biomass concentration (mg/L)
X_e effluent VSS concentration (mg/L)
X_w mass fraction of water (%),
X_{ch} mass fraction of carbohydrates (%),
X_{pr} mass fraction of proteins (%),
X_{li} mass fraction of lipids (%),
Y yield coefficient (g VSS g COD^{-1})
Y_x growth yield constant (g VSS/g CODremoved day)
μ specific microbial growth rate (per day)
μ_m maximum specific microbial growth rate (per day)
θ_H hydraulic retention time (HRT) (day)

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