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Addressing wastewater challenges in the dairy industry: a focused case study

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Research Article

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Abstract

This Research Paper addresses the hypothesis that wastewater characteristics in the dairy industry vary with product type and operational procedures, and that current treatment methods face limitations in managing such variability. The study examined raw and clean-in-place (CIP) wastewater from a Serbian dairy plant over three years. Physico-chemical and microbiological analyses included pH, protein, fat, carbohydrates, total solids, total microorganisms, *E. coli*, Enterobacteriaceae, chemical oxygen demand (COD), total suspended solids (TSS), nitrogen (N), phosphorus (P), and dissolved oxygen (O_2).Dairy plants produced 0.2–10 L wastewater per litre of milk. Protein content ranged 0.07–0.31 g/100 ml, fat 0.01–0.19 g/100 ml, and carbohydrates up to 1.37%. Total solids were 0.13–2.95%. pH varied from 4.41 to 12.76, affected by lactic fermentation and cleaning agents. COD values (529–12,476 mg/l) indicated strong organic loads. Microbiological counts were highly variable, with *E. coli* up to 10^3 cfu/ml and total microorganisms up to 1×10^8 cfu/ml. Nitrogen ranged 36–104 mg/l and phosphorus reached 10.91 mg/l, sometimes exceeding limits. Principal component analysis (PCA) explained 61.86% of variance, driven by N, pH, P, *E. coli*, Enterobacteriaceae, and oxygen content. Seasonal patterns were identified: higher TSS during spring and summer, and increased microbial loads, COD, and oxygen fluctuations in autumn and winter. The findings demonstrate that dairy wastewater is complex and variable, requiring adaptive treatment strategies. Optimised management, including pH control, nutrient removal, and combined biological and advanced technologies, can improve treatment efficiency, support reuse, and mitigate environmental impact.

In recent decades, it has become evident that our global environment is under severe threat, impacting not only our health but also our prosperity and survival (Habib *et al.,* 2019). Environmental protection requires balancing economic activities with the need to preserve the quality of our natural surroundings (Ajila *et al.,* 2012; Wilson *et al.*, 2015). The food industry plays a crucial role in addressing humanity's environmental impact, particularly due to the significant amounts of energy and water consumed during processing (Compton *et al.,* 2018). Among food processing sectors, the dairy industry stands out for its high water consumption at every stage of production (Yonar *et al.*, 2018). The extensive use of water in the dairy industry generates large quantities of wastewater, which can pose serious environmental risks if not properly managed (Britz *et al.*, 2004; Kolev Slavov, 2017). This wastewater, primarily generated from milk processing activities, can be categorised into various types based on its origin within the dairy industry. Sources include milk reception and storage, pasteurisation and homogenisation, cheese and butter production, and equipment cleaning operations. Each type of wastewater has distinct characteristics depending on the specific process from which it originates. Water is mainly utilised for cleaning production machines and plants, as well as for general hygiene and cleaning of work areas (Kaur, 2021). So, dairy wastewater can be broadly classified into two types: wastewater generated by cleaning and washing production equipment (CIP – Cleaning in Place), and general wastewater, which includes water from washing work surfaces, processing water, and sanitary water (Canut and Pascual, 2008; Su and Jacobsen, 2021; Shi *et al.,* 2021). Both types of wastewater are typically discharged into the sewer system, either in their raw state or after purification at water treatment plants (Shete and Shinkar, 2013). Dairy wastewater is characterised by high levels of organic matter, fats, oils, and grease (FOG), nutrients such as N and P, and fluctuating pH levels. The organic content, often measured as Chemical Oxygen Demand (COD), can range from 1.3 to 7.0 kg COD/m^3 (Karadag *et al.*, 2015). Milk fats and proteins contribute to elevated FOG levels, while cleaning agents can cause variations in pH. This results in water-rich residues of milk, dairy products, and other ingredients, which, in large quantities, can become an environmental problem (Britz *et al.*, 2004; Kolev Slavov, 2017). Treating dairy wastewater is challenging due to its complex and variable composition. To enhance energy and environmental efficiency in dairies, wastewater can be recycled (CIP wastewater) or subjected to biotransformation to produce biogas (total wastewater) (Watkinson *et al.,* 2007; Linclau *et al.,* 2016; Murunga *et al.*, 2016; Buabeng-Baidoo *et al.,* 2017). But the high organic load can overwhelm conventional treatment systems, leading to inefficient pollutant removal. The presence of FOG can cause operational issues, such as clogging and reduced treatment efficiency (Vidal *et al.*, 2000). Fluctuations in wastewater composition and flow rates, often due to batch processing and cleaning cycles, add further complexity to the design and operation of treatment systems. Addressing these challenges requires a combination of physical, chemical, and biological treatment methods, tailored to the specific characteristics of wastewater from each dairy processing facility (Jindal *et al.*, 2019). Most cities in the Republic of Serbia face the problem of wastewater. Only 20 cities have a system for purifying these waters. In the case of discharge of wastewater (with or without purification) into the public sewer system, as in the case of potential biotransformation and recycling of wastewater, there is a need to analyseanalyse and measure the basic parameters over a longer period, to characterise them and select a particular purpose. To analyse wastewater it needs to be properly sampled, transported, and kept in adequate conditions to obtain reproducible results and established standardised procedures for sampling. This study aimed to determine the physico-chemical characteristics of raw wastewater and CIP wastewater for wastewater quality monitoring and to test the hypothesis that the composition and characteristics of wastewater generated by the dairy industry vary significantly with different types of dairy products and operational procedures, and current treatment methods face challenges in managing the wastewater effectively, necessitating optimised and adaptive strategies to mitigate environmental impact. Principal component analysis (PCA) as a powerful tool for analyzing and visualizing data to identify patterns and relationships was applied to the experimental data (used as descriptors) to characterise and differentiate among the observed samples of wastewater and CIP water. This technique helped in understanding the differences and similarities between the samples, ultimately leading to better insights and decision-making.

Material & methods

Sampling

Batches of dairy final raw waste water and CIP waste waters were collected from industrial Serbian dairy processing plant Mekara Subotica over three years. Two types of wastewater are generated during the production of dairy products: CIP wastewater and total wastewater. The total wastewater consists of CIP waste water and water used to clean and rinse floors and eqipment in production plants, and their ratio are about 80:20% in favor of CIP waste water. The main dairy residues in CIP wastewater originate from the production of different types of yogurt and cheese (semi-soft and hard cheeses). Residues in the water used to wash floors and production units consist of impurities that can be found on the floors and equipment being washed. All these together make up residues found in the total wastewater with dairy residues from the CIP wastewater being dominant. The sampling was performed from all sources of wastewater (total wastewater and the CIP wastewater). In order to make the total wastewater samples representative, a manhole was installed on the sewage drain to allow for flow measurement and sampling of wastewater and taking a composite sample proportional to the flow. A 24-hour composite sample is a mixture of individual samples taken over 24 h, proportional to time in the case of constant flow or proportional to flow in case the volume of discharged wastewater varies significantly during sampling time. All equipment used for sampling is used for this purpose only, so as to prevent cross contamination. The equipment was cleaned before each sampling. Sampling of CIP and total wastewater was done by using a peristaltic pump equipped with a hose from environment proof material and container for collecting samples made of resistant plastic. Due to the uneven and erratic flow of waste water, the sampling needs to cover a period of 24 h to obtain a representative sample. The flow peristaltic pump was adjusted to collect sufficient material for all planned analyses (approximately 40 L of water). After collection in a large plastic container, the samples was transferred into the cold chamber or divided into smaller containers and placed in refrigerator until further use and analysis. Storage temperature in a cold chamber or the refrigerator was above freezing and up to a maximum of 8°C. Also, samples were protected from direct light while in the refrigerator. Storage as time interval between sampling (at the end of a 24 -hour sampling period) and the beginning of the analysis was 10 d maximum. The sample volume was 5 L.

Physicochemical and microbiological analysis

The pH of the water samples was determined by a pH meter (ExStickTM, Extech Instruments, USA), calibrated with buffers at pH 4 and 7 before measuring. Soluble solids content (SS) was measured in °Brix with a digital refractometer ATR-BR SCHMIDT-HAENSCH (Germany) Microbiological analysis of waste water was examined by determining a total number of microorganisms (TN) (ISO 4833 1991) *Escherichia coli* (ISO 16 654 2001) and Enterobactericeae (ISO 21 528 2017). Total soluble solids (TSS), volatile solids (VS), total chemical oxygen demand (COD), total Kjeldahl N (TKN) and fat content were determined in accordance with the Standard Methods for the Examination of Water and Wastewater. Protein content measured according to AOAC Official method 991.20. P (ISO 6878 2004), N (ISO 20236 2018), and oxygen (ISO 5814 2012) contents were analysed according to standard methods. Carbohydrates were determined according to FAO (2003).

Statistical analyses

All determinations were made in triplicate, all data was averaged, expressed as means for comparison of the chemical variables such as COD, N, P, O_2, TSS, SV and V), and microbiological analysis (total count of microorganisms, Enterobacteriaceae count and *E.coli* count). Pattern recognition techniques, such as principal component analysis – PCA was applied to the experimental data (used as descriptors) to characterise and differentiate among the observed samples. All data were processed statistically using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA).

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Results and discussion

CIP water proximate analysis

CIP wastewater, originating from cleaning processes within dairy plants, typically contains residues such as fats, proteins, and carbohydrates. These residues are the result of cleaning processing equipment, pipelines, and storage tanks. Determination of the intrinsic characteristics of wastewaters, such as proximate composition is a crucial step to optimise their potential for valorization. CIP wastewater is the main contributor to the final raw total wastewater and their characteristics (80:20% ratios). In that way it is also the main contributor to residues found in the CIP wastewater which originate from the production of different types of yogurts and cheeses as the main products at the production facility. When combined with other wastewater streams, CIP wastewater contributes significantly to the organic load, nutrient content, and overall composition of the final raw wastewater. Understanding this contribution is crucial for developing effective treatment strategies. Dairy residues in the form of fat, sugars, proteins and total solids in CIP water and category of the products from Mlekara Subotica are in Table 1. Different dairy products lead to varying levels and types of residues in CIP wastewater. For instance, the production of cheese results in higher protein and fat residues rather than milk processing and fruit yogurt production, which have higher carbohydrate content due to lactose presence. In the previous study simulated wastewaters was obtained by dissolving full cream-milk powder, and wastewaters, obtained from skimmed-milk powder. The relative contributions of proteins/ sugars/fats expressed in terms of %COD were 17.4/30.5/52.1 for full cream-milk powder-type wastewaters and 34.2/62.8/3.0 for skimmed-milk powder type wastewaters, respectively (Vidal *et al.*, 2000). According to the results, butter production causes a high fat content (0.11-0.19%) and low protein (0.06–0.09%) and carbohydrate levels in CIP water. Milk production results in lower fat and protein content compared to cheese and sour cream. Cheese production generates the highest protein content and a higher total dry matter. Fruit yoghurt production causes the highest total carbohydrate content. Cheese production typically generates CIP water withhigher protein content compared to milk production because the protein in milk is concentrated during cheese production. For example, on 26/01/2021, the protein content was 0.29%, which is typical for cheese, whereas on 18/02/2021, the protein content dropped to 0.08% because it was just milk, which naturally has a lower protein concentration. During the three years of the study the protein content in CIP water ranged from 0.07 to 0.31 g/100 ml, which is on average in accordance with the obtained results of wastewater analysis in other dairy industry plants (Alalam *et al.,* 2021). Cheese and fruit yoghurt are the products associated with protein contents greater than 0.2%, which makes sense because both products tend to have higher protein concentrations compared to milk or butter, for example. The fat content ranged from 0.01 to 0.19 g/100 ml. The presence of fats, oils and fats in milk processing wastewater can cause several problems in local wastewater treatment systems as well as in municipal wastewater treatment plants. Total carbohydrates content in CIP wastewater is up to 1.37%. In the dairy industry, carbohydrates in wastewater largely consist of lactose, which represents a potential source of substrate for the production of hydrogen gas according previous research (Castelló *et al.,* 2009). Soluble organics, soluble and insoluble solids, and trace minerals make up total solids. Total solids in CIP water during monitored period were in the range of 0.13 to 2.95%. CIP wastewater presents unique treatment challenges due to its high organic load, fluctuating pH levels, and presence of cleaning agents that can inhibit biological treatment processes. Pre-treatment methods such as pH adjustment, temperature equalization, and chemical neutralization can help stabilize CIP wastewater before it enters the main treatment process.

Final raw waste water analysis

When combined with other wastewater streams, CIP wastewater contributes significantly to the organic load, nutrient content, and overall composition of the final raw wastewater. Understanding this contribution is crucial for developing effective treatment strategies. The data presented in Table 2 reveals significant variations in wastewater characteristics over the three-year period from January 2021 to December 2023. These fluctuations provide valuable insights into the dynamic nature of the wastewater treatment process and highlight several important trends and concerns. The pH values show extreme fluctuations, ranging from highly acidic (4.41 on 13/10/2023) to strongly alkaline (12.76 on 03/12/2022). The lower pH values are the result of lactic acid fermentation during yoghurt proccessing, which quickly converts lactose in waste water into lactic acid (Shete and Shinkar, 2013). In addition, the pH of wastewater can vary significantly depending on applied cleaning agents. Most common used alkaline CIP chemicals are caustic soda, potassium hydroxide, sodium carbonate, trisodium phosphate. These chemicals have a significant effect on pH rise of final waste water (Britz *et al.*, 2004). This wide pH range poses significant challenges for biological treatment processes, which typically operate optimally within a narrower pH range of 6.5-8.5. The occasional extreme alkaline conditions (pH $>$ 12) are particularly concerning, as they can inhibit microbial activity and potentially lead to precipitation of certain compounds, affecting subsequent treatment efficiency. Microbiological parameters of pathogenic microorganisms and indicators of faecal pollution were determined during the entire period. Enterobactericeae (Ent) and *E. coli* counts showed a decreasing trend over time, with many recent measurements at the minimum detectable level (10 cfu/ml), which is in aaccordance with other authors (Fitzhenry *et al.,* 2018). This pathogen reduction is a positive sign, potentially indicating enhanced disinfection processes. Total microbial counts (TM) exhibit substantial variability, ranging from 10 cfu/ml to 13,000,000 cfu/ml. This variability suggests inconsistent influent characteristics or potential limitations of biological treatment processes. Of particular note are the periodic extremely low counts (10–120 cfu/ml) observed in February and July of each year, which indicate periodic shock loads or the presence of inhibitory substances in the wastewater. Chemical oxygen demand (COD) values fluctuated widely, from 529 mg/l to 12,476 mg/l. The results for chemical oxygen demand (COD) are higher compared to some previous reports (Choudhury *et al.,* 2021; Pathak *et al.,* 2016). In addition, according the COD values of the dairy wastewaters in different countries, Serbia's dairy wastewater records high COD (Jindal *et al.*, 2019; Ekka *et al.,* 2021). This variability in organic load suggests inconsistent influent characteristics, possibly due to changes in

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Table 1. Proximate analysis of CIP wastewaters

Date	Protein content (%)	Fat content (%)	Directly reducing sugars (%)	Total carbohydrates (%)	Total solids (%)	Soluble solids content (refractometric) (%)	Product category
26/01/2021	0.29	0.05	n.d.	0.09	0.3	0.28	Cheese
18/02/2021	0.08	0.02	n.d.	0.5	0.77	0.28	Milk
24/03/2021	0.09	0.12	n.d.	0	0.2	0.2	Butter
12/04/2021	0.11	0.01	n.d.	0.5	0.98	0.95	Sour cream
25/05/2021	0.08	0.01	n.d.	0	0.17	0.14	Milk
13/06/2021	0.09	0.11	n.d.	0.12	0.20	0.18	Butter
01/07/2021	0.07	0.07	n.d.	0	0.18	0.14	Milk
03/08/2021	0.11	0.02	n.d.	0	0.15	0.14	Cheese
12/09/2021	0.11	0.01	0.13	0.5	0.98	0.95	Yoghurt
13/10/2021	0.25	0.12	n.d.	1.23	2.32	2.3	Fruit yoghurt
01/11/2021	0.17	0	n.d.	0	0.49	0.14	Yoghurt
03/12/2021	0.07	0.17	n.d.	0	0.26	0.26	Butter
26/01/2022	0.30	0.06	n.d.	0.10	0.27	0.26	Cheese
18/02/2022	0.07	0.02	n.d.	0.44	0.70	0.33	Milk
24/03/2022	0.08	0.13	n.d.	0.00	0.20	0.20	Butter
12/04/2022	0.10	0.01	n.d.	0.45	0.88	0.82	Sour cream
25/5/2022	0.07	0.01	n.d.	0.00	0.19	0.11	Milk
13/06/2022	0.10	0.11	n.d.	0.10	0.18	0.16	Cheese
01/07/2022	0.08	0.07	n.d.	0.00	0.19	0.17	Sour Cream
03/08/2022	0.11	0.02	n.d.	0.00	0.16	0.14	Yoghurt
12/09/2022	0.11	0.01	n.d.	0.53	1.04	0.99	Yoghurt
13/10/2022	0.29	0.10	n.d.	1.29	2.68	2.53	Fruit Yoghurt
01/11/2022	0.18	0.00	0.10	0.00	0.40	0.14	Cheese
03/12/2022	0.06	0.19	n.d.	0.00	0.30	0.23	Butter
26/01/2023	0.25	0.05	n.d.	0.08	0.26	0.26	Cheese
18/02/2023	0.08	0.01	n.d.	0.36	0.60	0.30	Milk
24/03/2023	0.08	0.15	n.d.	0.00	0.21	0.19	Butter
12/04/2023	0.09	0.01	n.d.	0.36	0.78	0.73	Milk
25/5/2023	0.07	0.01	n.d.	0.00	0.17	0.12	Sour Cream
13/06/2023	0.09	0.10	n.d.	0.11	0.18	0.16	Butter
01/07/2023	0.09	0.07	n.d.	0.00	0.17	0.15	Yoghurt
03/08/2023	0.10	0.02	n.d.	0.00	0.13	0.12	Yoghurt
12/09/2023	0.12	0.01	n.d.	0.43	0.86	0.84	Sour Cream
13/10/2023	0.31	0.11	0.14	1.37	2.95	2.84	Cheese
01/11/2023	0.15	0.00	n.d.	0.00	0.40	0.14	Yoghurt
03/12/2023	0.06	0.18	n.d.	0.00	0.31	0.23	Butter

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Table 2. Physico-chemical and microbiological analysis of wastewater from Mlekara Subotica (2021–2023)

Date	pH value	TM (cfu/ml)	Ent (cfu/ml)	*E.coli* count (cfu/ml)	COD (mg/l)	N (mg/l)	P (mg/l)	O_2 (mg/l)	TSS (g/l)	SV (ml/l)	V (m^3)	Category of product
26/01/2021	8.2	4,900,000	12,000	860	8000	75	3.7	0.1	4	360	19,491	Cheese
18/02/2021	10.8	10	10	10	8530	104	0.3	0	4.9	550	43,160	Milk
24/03/2021	9.2	13,000,000	10	10	2290	89	0.3	0.1	4.6	420	47,312	Butter
12/04/2021	6.8	5,200,000	100	75	680	104	0.3	0	5.2	520	41,373	Sour cream
25/05/2021	7.7	1,200,000	10	10	10,900	42	6.2	0	3.9	460	65,738	Milk
13/06/2021	8.1	3400	10	10	4270	96	2.8	0.1	3.8	360	96,658	Butter
01/07/2021	9.68	120	10	10	8210	76	0.3	0.0	3.6	300	27,489	Milk
03/08/2021	6.96	2,900,000	10	10	10,700	70	2.5	0.1	3.5	330	67,628	Cheese
12/09/2021	6.4	5,200,000	100	75	3740	42	8.1	0.1	3.5	300	1161	Yoghurt
13/10/2021	4.54	6,100,000	11,000	5800	6680	39	2.4	0.1	3.6	270	87,574	Fruit yoghurt
01/11/2021	8.2	4,900,000	12,000	860	6410	81	3.5	0.2	3.7	380	12,318	Yoghurt
03/12/2021	11.76	1400	10	10	6910	56	0.3	0.1	2.6	250	42,475	Butter
26/01/2022	7.60	5,200,000	12,000	995	8606	63	4.42	0.1	3.3	370	21,706	Cheese
18/02/2022	10.48	11	10	10	9931	97	0.33	0.0	5.2	650	43,167	Milk
24/03/2022	10.52	1,050,000	10	10	2184	82	0.33	0.1	4.3	390	39,024	Butter
12/04/2022	6.89	5,800,000	120	80	586	100	0.31	0.0	4.7	420	37,940	Sour cream
25/5/2022	7.13	1,260,000	10	10	10,134	36	6.45	0.0	3. 8	540	57,845	Milk

(*Continued*)

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Table 2. (*Continued.*)

Date	pH value	TM (cfu/ml)	Ent (cfu/ml)	*E.coli* count (cfu/ml)	COD (mg/l)	N (mg/l)	P (mg/l)	O_2 (mg/l)	TSS (g/l)	SV (ml/l)	V (m^3)	Category of product
13/06/2022	7.09	4000	10	10	4734	82	2.82	0.1	3.6	330	105,362	Cheese
01/07/2022	9.61	120	10	10	9684	72	0.25	0.0	4.2	280	22,256	Sour Cream
03/08/2022	5.84	2,440,000	10	10	12,476	82	2.27	0.1	3.4	290	63,851	Yoghurt
12/09/2022	6.08	5,170,000	100	75	3591	42	9.14	0.1	3.9	250	934	Yoghurt
13/10/2022	5.04	6,800,000	10,500	6800	7181	43	2.85	0.1	3.5	220	95,934	Fruit Yoghurt
01/11/2022	9.55	4,090,000	12,100	1025	7350	87	2.94	0.2	4.1	440	10,985	Cheese
03/12/2022	12.76	1600	10	10	7388	59	0.31	0.1	2.5	290	48,203	Butter
26/01/2023	6.97	4,870,000	12,600	825	8453	65	4.36	0.1	3.6	350	21,366	Cheese
18/02/2023	8.55	10	10	10	8714	85	0.39	0.0	5.1	540	48,903	Milk
24/03/2023	12.61	10,100,000	10	10	2590	92	0.38	0.1	4.6	450	39,249	Butter
12/04/2023	7.71	5,225,000	100	90	529	88	0.36	0.0	4.3	360	37,968	Milk
25/5/2023	8.53	1,320,000	10	10	8289	40	5.47	0.0	3.6	600	54,562	Sour Cream
13/06/2023	6.07	3300	10	10	4772	75	3.01	0.1	3.0	280	115,430	Butter
01/07/2023	7.74	120	10	10	7804	79	0.25	0.0	4.9	330	18,062	Yoghurt
03/08/2023	5.04	2,890,000	10	10	11,784	86	2.65	0.1	2.8	290	76,122	Yoghurt
12/09/2023	6.79	4,350,000	90	100	4148	36	10.91	0.1	4.1	300	1099	Sour Cream
13/10/2023	4.41	5,920,000	11,300	6250	8423	49	2.57	0.11	3.4	240	108,719	Cheese
01/11/2023	7.68	4,490,000	10,700	830	8015	75	2.61	0.27	3.5	360	9410	Yoghurt
03/12/2023	12.28	1600	10	10	80,431	55	0.34	0.12	2.5	240	53,217	Butter

Table 3. Limiting values prescribed by Serbian regulation (Rulebook 2012) for wastewater quality classification

	pH value	TM cfu/ml	Ent cfu/ml	COD mgO_2/l	N mg/l	P mg/l	O_2 mg/l
I class	6.5–8	500	200	5	1	0.05	8.5
II class	6.5–8	10,000	400	10	2	0.2	7
III class	6.5–8	100,000	4000	20	8	0.4	5
IV class	6.5–8	750,000	40,000	50	15	1	4
V class	<6.5; >8	>750,000	>40,000	>50	>15	>1	<4

industrial discharges or seasonal effects. The occasional high COD values (>10,000 mg/l) may challenge the following treatment system's capacity and efficiency. Although COD and BOD are related parameters, COD measures the total oxygen demand exerted by all organic matter (both biodegradable and non-biodegradable), whereas BOD specifically reflects the oxygen demand caused by the biodegradable fraction of organic material. In wastewater from the dairy industry, which often contains complex organic compounds such as proteins, fats, and sugars, COD values are typically high, indicating the presence of substantial organic pollution. Literature indicates that the COD/BOD ratio in dairy wastewater often exceeds 2, which suggests that a significant portion of the organic matter is not easily biodegradable and thus would not be readily removed through biological treatment processes alone (Shivsharan *et al.*, 2013).

N concentrations show moderate variability (36–104 mg/l), while P levels exhibit more extreme fluctuations (0.25–10.91 mg/l). Typically, dairy wastewater contains 17–1120 mg /L of N (Tawfik *et al.,* 2008). Probably whey is the most polluting dairy product and source of organic components such as lactose, casein, nitrates and N in waste waters (Carvalho *et al.,* 2013). The sporadic high P concentrations (e.g., 10.91 mg/l on 12/09/2023) may indicate periodic industrial discharges or inefficiencies in P removal processes. These nutrient variations can impact the effectiveness of biological nutrient removal processes and may contribute to eutrophication risks in receiving water bodies if not adequately addressed (Walsh *et al.,* 1994). Dissolved oxygen (O_2) levels are generally low (0–0.27 mg/l), which is typical for raw wastewater. Dairy wastewater characterised by high COD reduces the level of dissolved oxygen (mostly in between 0.0–0.2 mg/l) in discharge waterways (Wang and Serventi, 2019). The total suspended solids (TSS) concentrations show moderate variability (2.5–5.2 g/l), with no clear long-term trend. This suggests relatively consistent performance in solids removal processes.Total suspended solids (TSS) encompass small organic and inorganic particles including fats, oil and grease (FOG), which are measured during the TSS analysis. In dairy wastewater, insoluble solid components are mainly small milk curd particles derived from cheese making. It is critical that a majority of these solids are removed during primary treatment to ensure biological treatment will perform efficiently (Mohammed and Ismail, 2021). In general, aerobic or anaerobic biological treatment processes can be effective in breaking down organic matter. Anaerobic digestion, in particular, can be beneficial for high-strength final wastewater, producing biogas as a byproduct. Membrane filtration (e.g., ultrafiltration, nanofiltration, reverse osmosis) can be employed to remove fine particles and dissolved substances, though they require regular maintenance and management of concentrate streams. Advanced Oxidation Processes (AOPs) can be used to break down complex organic molecules and chemical residues, making them more amenable to biological treatment or direct discharge. Combining different treatment technologies, such as biological treatment followed by membrane filtration or AOPs, can provide a more comprehensive approach to managing final dairy wastewater, making an integrated treatment system (Ji *et al.*, 2020).

Final raw waste water classification

Table 3 lists the limiting values prescribed by domestic regulations in Rulebook Serbia (2012) this regulation sets emission limit values for certain groups or categories of pollutants for technological wastewater before their discharge into public sewerage, and directly into the recipient, water that is discharged from the public sewerage system into the recipient after treatment. Table 4 presents a score table for the physicochemical parameters in wastewater, classifying samples based on their values of pH, total number of microorganisms, COD, P, N, and O_2. The most variable parameters appear to be pH, Enterobacteriaceae and COD. N and P levels seem to be consistently in the highest category (V), which could indicate persistent nutrient loading issues. Dissolved oxygen levels are generally low (mostly V), which is typical for wastewater, but there are some instances of higher levels.

During this period, around 60% of the samples were classified in the V class for pH values. The total number of microorganisms in all samples fell into class I. Enterobacteriaceae count in most samples reached the V class. COD values were predominantly in the V class for all wastewater, except for a few samples taken in the summer, possibly due to variations in production dynamics and lower industrial capacity utilisation. This categorised data provides a simplified view of the water quality trends, making it easier to identify patterns and potential areas of concern in the wastewater treatment process. It could be useful for quickly assessing compliance with different quality thresholds or for identifying periods when certain parameters were outside of desired ranges. For instance, consistently high levels of N and P (both in category V) indicate an opportunity for nutrient recovery technologies. Future wastewater management in the dairy industry could focus on extracting these nutrients for use as fertilizers, aligning with circular economy principles.

PCA analysis

The PCA plot indicated that geometrically close points represent similar patterns, with vector orientations showing trends in variables, and vector lengths proportional to the square of the correlation values (Figure 1). The angles between vectors reflected the degree of correlation between variables. The samples were systematically labeled using an abbreviation of the month in which they were collected (Jan–Dec), combined with the last digit of the corresponding year (2021–2023). The naming convention facilitated

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Table 4. Score table of physicochemical waste water parameters

	pH	TM	Ent	COD	N	P	O_2
26/01/2021	V	I	V	V	V	V	V
18/02/2021	V	I	I	III	V	V	V
24/03/2021	V	I	V	III	V	V	V
12/04/2021	I, II, III, IV	I	V	V	V	V	V
25/05/2021	I, II, III, IV	I	V	III	V	V	III
13/06/2021	V	I	III	III	V	V	V
01/07/2021	V	I	I	III	V	V	V
03/08/2021	I, II, III, IV	I	V	III	V	V	V
12/09/2021	V	I	V	V	V	V	II
13/10/2021	V	I	V	V	V	V	V
01/11/2021	V	I	V	V	V	V	V
03/12/2021	V	I	III	III	V	V	V
26/01/2022	I, II, III, IV	I	V	V	V	V	IV
18/02/2022	V	I	I	II	V	V	V
24/03/2022	V	I	V	III	V	V	V
12/04/2022	I, II, III, IV	I	V	V	V	V	V
25/5/2022	I, II, III, IV	I	V	III	V	V	III
13/06/2022	I, II, III, IV	I	IV	II	V	V	V
01/07/2022	V	I	I	III	V	V	V
03/08/2022	V	I	V	II	V	V	V
12/09/2022	V	I	V	V	V	V	I
13/10/2022	V	I	V	V	V	V	V
01/11/2022	V	I	V	V	V	V	V
03/12/2022	V	I	III	II	V	V	V
26/01/2023	I, II, III, IV	I	V	V	V	V	IV
18/02/2023	V	I	I	II	V	V	V
24/03/2023	V	I	V	II	V	V	V
12/04/2023	I, II, III, IV	I	V	V	V	V	V
25/5/2023	V	I	V	III	V	V	III
13/06/2023	V	I	III	II	V	V	V
01/07/2023	I, II, III, IV	I	I	III	V	V	V
03/08/2023	V	I	V	II	V	V	V
12/09/2023	I, II, III, IV	I	V	V	V	V	I
13/10/2023	V	I	V	V	V	V	V
01/11/2023	I, II, III, IV	I	V	V	V	V	V
03/12/2023	V	I	III	II	V	V	V

identification and chronological tracking of the samples within the dataset. Each sample's temporal context was preserved, allowing for precise analysis of seasonal and annual trends in water quality.

The PCA analysis of the wastewater data revealed that the first three principal components accounted for 61.86% of the total variance across the 11 variables, which included chemical and microbiological analysis data. Specifically, the first component explained 27.33% of the variance, the second 20.48%, and the third 14.05%.

In the first principal component (PC1), N content and pH had negative scores, contributing 14.4% and 13.5% of the variance, respectively. Positive scores were observed for P content at 8.3% (according to correlations), *E. coli* at 17.5%, Enterobacteriaceae at 17.2%, and oxygen content at 16.3%. The second principal component (PC2) showed positive contributions from TM at 25.6%, suspended solids at 12.5%, and N at 14.4%, while chemical oxygen demand (COD) contributed negatively with 24.2%. For the third principal component (PC3), TSS (15.4%) and V (28.4%) had positive influences, whereas pH (12.5%) and O_2 content (15.3%) contributed negatively.

The water quality, as determined through chemical analysis, exhibited significant annual variability, which was clearly reflected in the PCA diagram. Seasonal patterns were observed, with higher TSS levels from February to August, and increased *E. coli*, Enterobacteriaeciae, O_2, and COD levels from September to January. The distinct clustering of samples in the PCA plot highlights the influence of seasonal changes on the chemical composition of the water. Monthly sampling further underscored the temporal shifts in water quality, providing a detailed view of how these variations align with different periods of the year. The observed seasonal patterns, such as higher TSS levels from February to August and increased *E. coli*, Enterobacteriaceae, O_2, and COD levels from September to January, suggest the need for seasonally optimised treatment strategies. Dairy plants may need to adjust their treatment protocols and potentially increase treatment capacity during specific periods of the year.

The monthly analysis of water samples over several years reveals significant seasonal and temporal variations in key chemical parameters, including protein content, fat, total carbohydrates, total solids, and soluble solids content. Elevated concentrations of total carbohydrates, total solids, and soluble solids during the colder months (October to December) suggest potential challenges in maintaining water quality during these periods, which may require adjusted purification protocols. While directly reducing sugars remained consistently low, indicating effective purification, the observed periodic spikes in protein and fat content especially in October and December highlight potential inefficiencies or external factors influencing water quality that warrant further investigation and targeted interventions.

The dataset reveals significant temporal fluctuations in key water quality parameters, including pH, microbial counts (*E. coli* and Enterobacteriaceae), chemical O_2 demand, N, P, and total suspended solids. The pH values exhibit a wide range, from highly acidic (4.41) to strongly alkaline (12.76), indicating potential pollution events or changes in water source composition that could challenge water treatment processes. Periodic spikes in microbial contamination, particularly in *E. coli* and Enterobacteriaceae counts observed in December 2023 and October 2022, underscore the need for targeted interventions during these times to mitigate health risks. Elevated COD levels, especially the peak in December 2023 (80.431 mg/L), reflect substantial organic pollution, necessitating enhanced treatment measures. Additionally, the variability in N, P, TSS, and sediment volume suggests ongoing changes in the water's chemical and particulate composition, which may further complicate purification efforts and require adaptive management strategies to ensure water quality standards are consistently met. The observed seasonal patterns, such as higher TSS levels from February to August and increased *E. coli,* Enterobacteriaceae O_2, and COD levels from September to January, suggest the need for seasonally optimised treatment strategies. Dairy plants may

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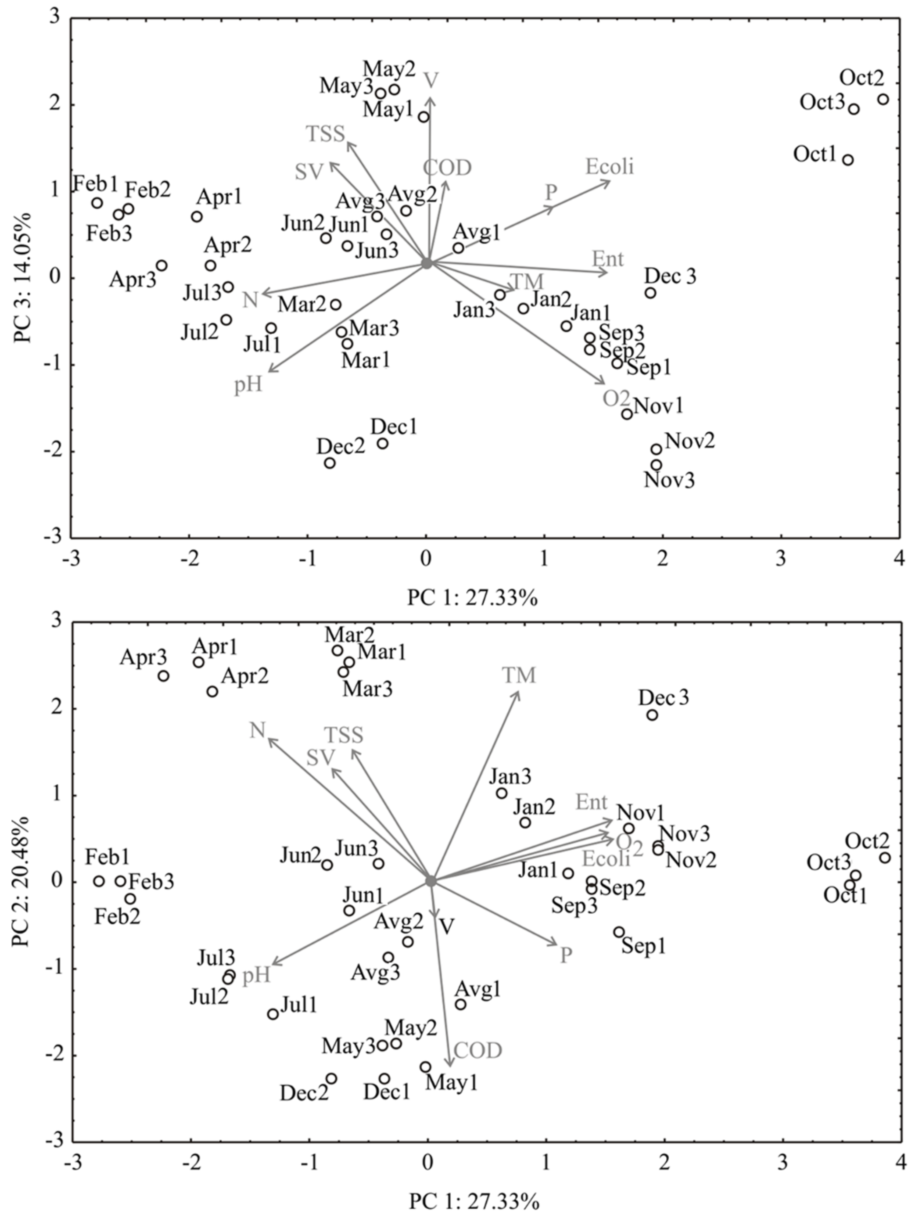

Figure 1. PCA ordination of variables based on component correlations.

need to adjust their treatment protocols and potentially increase treatment capacity during specific periods of the year.

Conclusion

By understanding the relationship between CIP processes, dairy products, and wastewater characteristics, treatment strategies can be optimised to address the specific challenges posed by CIP wastewater and final raw wastewater in dairy plants. This approach not only improves treatment efficiency but also opens up opportunities for resource recovery and water reuse, contributing to more sustainable dairy operations.The extreme fluctuations in pH, organic load, and microbial populations suggest a need for robust and adaptable treatment processes. If a dairy industry switches from one type production to another, adjustments would be needed in both CIP systems and wastewater treatment. Butter production involves separating cream and requires cleaning equipment that handles milk and cream. Cheese production, for example, involves coagulating proteins and removing whey, which requires different cleaning chemicals and processes. The increased whey from cheese production may also demand changes in wastewater treatment systems to manage higher protein and lactose content, potentially using filtration or biological treatment. CIP systems would also need to be tailored for the specific residues and bacteria involved in cheese production. Further investigation into the sources of these variations, particularly the periodic extreme values, is warranted. Additionally, optimisation of pH control and nutrient removal processes could potentially improve overall treatment efficiency and effluent quality. Given the variability in organic load, future wastewater treatment systems in dairy plants should focus on energy-efficient technologies, such as anaerobic digestion coupled with biogas utilisation, to offset treatment costs and reduce the carbon footprint. Moreover, piloting advanced treatment technologies capable of handling such variable wastewater characteristics could be beneficial for long-term process improvement. However, these findings underscore the

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importance of effective treatment processes to address the complex composition and pollution potential of dairy wastewater in Serbia. Detailed temporal data provided by this analysis could be used to develop predictive models for wastewater characteristics. Such models could help plant operators anticipate changes in wastewater composition and proactively adjust treatment processes.

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References

Ahmad T, Aadil RM, Ahmed H, Ur Rahman U, Soares BC, Souza SL, Pimentel TC, Scudino H, Guimarães JT, Esmerino EA, Freitas MQ, Almada RB, Vendramel SMR, Silva MC, and Cruz AG (2019) Treatment and utilisation of dairy industrial waste: a review. *Trends in Food Science and Technology* **88**, 361–372. doi:10.1016/j.tifs.2019.04.003

Ajila C, Brar SK, Verma M and Prasada Rao U (2012) Sustainable solutions for agro processing waste management: an overview. In Malik A and Grohmann E (Eds) *Environmental Protection Strategies for Sustainable Development. Strategies for Sustainability*. Dordrecht: Springer. doi:10.1007/978-94-007-1591-2_3

Alalam S, Ben-Souilah F, Lessard MH, Chamberland J, Perreault V, Pouliot Y, Labrie S and Doyen A (2021) Characterisation of chemical and bacterial compositions of dairy wastewaters. *Dairy* **2**(2), 179–190. doi:10.3390/dairy2020016

American Public Health Association (2005) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association.

Britz TJ, van Schalkwyk C and Hung YT (2004) Treatment of dairy processing wastewaters. In *Handbook of Industrial and Hazardous Wastes Treatment*. CRC Press, pp. 673–705. doi:10.1201/9780203026519

Buabeng-Baidoo E, Mafukidze N, Pal J, Tiwari S, Srinivasan B, Majozi T and Srinivasan R (2017) Study of water reuse opportunities in a large-scale milk processing plant through process integration. *Chemical Engineering Research and Design* **121**, 81–91. doi:10.1016/j.cherd.2017.02.031

Canut A and Pascual A (2008) Pollution prevention in food industries through both cleaning of closed equipment with ozonated water and cleaning in place (CIP) systems. *WIT Transactions on Ecology and the Environment* **111**, 615–625. doi:10.2495/WP080601

Carvalho F, Prazeres AR and Rivas J (2013) Cheese whey wastewater: characterisation and treatment. *Science of the Total Environment* **445**, 385–396. doi:10.1016/j.scitotenv.2012.12.038

Castelló E, Santos CG, Iglesias T, Paolino G, Wenzel J, Borzacconi L and Etchebehere C (2009) Feasibility of biohydrogen production from cheese whey using a UASB reactor: links between microbial community and reactor performance. *International Journal of Hydrogen Energy* **34**(14), 5674–5682. doi:10.1016/j.ijhydene.2009.05.060

Choudhury P, Ray RN, Bandyopadhyay TK, Basak B, Muthuraj M and Bhunia B (2021) Process engineering for stable power recovery from dairy wastewater using microbial fuel cell. *International Journal of Hydrogen Energy* **46**(4), 3171–3182. doi:10.1016/j.ijhydene.2020.06.152

Compton M, Willis S, Rezaie B and Humes K (2018) Food processing industry energy and water consumption in the Pacific northwest. *Innovative Food Science & Emerging Technologies* **47**, 371–383. doi:10.1016/j.ifset.2018.04.001

Ekka B, Dejus S and Juhna T (2021) Case study on the dairy processing industries and their wastewater generation in Latvia. *Journal of Dairy Research* **88**(4), 425–428. doi:10.1017/S0022029921000819

FAO (2003) Methods of food analysis Ch 2 U: FAO Food and nutrition paper 77, 2.3 analytical methods for carbohydrates in foods.

Fitzhenry K, Rowan N, Finnegan W, Zhan X and Clifford E (2018) Microbiological characterisation and impact of suspended solids on pathogen removal from wastewaters in dairy processing factories. *Journal of Dairy Research* **85**(3), 391–395. doi:10.1017/S0022029918000602

Habib MS, Sarkar B, Tayyab M, Saleem MW, Hussain A, Ullah M, Omair M, and Iqbal MW (2019) Large-scale disaster waste management under uncertain environment. *Journal of Cleaner Production* **212**, 200–222. doi:10.1016/j.jclepro.2018.11.154

Ince O (1998) Potential energy production from anaerobic digestion of dairy wastewater. *Journal of Environmental Science and Health, Part A* **33**(6), 1219–1228. doi:10.1080/10934529809376784

ISO 20236 (2018) Water quality – Determination of total organic carbon (TOC), dissolved organic carbon (DOC), total bound nitrogen (TNb) and dissolved bound nitrogen (DNb) after high temperature catalytic oxidative combustion.

Ji S, Ma W, Wei Q, Zhang W, Jiang F and Chen J (2020) Integrated ABR and UASB system for dairy wastewater treatment: engineering design and practice. *Science of the Total Environment* **749**, 142267. doi:10.1016/j.scitotenv.2020.142267

Jindal T, Sinha S, Srivastava A, Mehrotra T and Singh R (2019) A review on the dairy industry waste water characteristics, its impact on environment and treatment possibilities. *Emerging Issues in Ecology and Environmental Science: Case Studies from India*, 73–84. doi:10.1007/978-3-319-99398-0_6

Karadag D, Köroğlu OE, Ozkaya B and Cakmakci M (2015) A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. *Process Biochemistry* **50**(2), 262–271. doi:10.1016/j.procbio.2014.11.005

Kaur N (2021) Different treatment techniques of dairy wastewater. *Groundwater for Sustainable Development* **14**, 100640. doi:10.1016/j.gsd.2021.100640

Kolev Slavov A (2017) General characteristics and treatment possibilities of dairy wastewater–a review. *Food Technology & Biotechnology* **55**(1), 14–28. doi:10.17113/ftb.55.01.17.4520

Linclau E, Ceulemans J, De Sitter K and Cauwenberg P (2016) Water and detergent recovery from rinsing water in an industrial environment. *Water Resources and Industry* **14**, 3–10. doi:10.1016/j.wri.2016.03.001

Mohammed NA and Ismail ZZ (2021) Green sustainable technology for biotreatment of actual dairy wastewater in constructed wetland. *Journal of Chemical Technology and Biotechnology* **96**(5), 1197–1204. doi:10.1002/jctb.6631

Murunga SI, Mbuge DO, Gitau AN, Mutwiwa UN and Wekesa IN (2016) Characterisation of brewery waste water and evaluation of its potential for biogas production. *Agricultural Engineering International Cigr Journal* **18**(3), 308–316.

Pathak U, Das P, Banerjee P and Datta S (2016) Treatment of wastewater from a dairy industry using rice husk as adsorbent: treatment efficiency, isotherm, thermodynamics, and kinetics modelling. *Journal of Thermodynamics* **2016**(1), p. 3746316. doi:10.1155/2016/3746316

Shete BS and Shinkar NP (2013) Dairy industry wastewater sources, characteristics & its effects on environment. *International Journal of Current Engineering and Technology* **3**(5), 1611–1615. http://inpressco.com/category/ijcet

Shi W, Healy MG, Ashekuzzaman SM, Daly K, Leahy JJ and Fenton O (2021) Dairy processing sludge and co-products: a review of present and future re-use pathways in agriculture. *Journal of Cleaner Production* **314**, 128035. doi:10.1016/j.jclepro.2021.128035

Shivsharan VS, Kulkarni SW and Wani M (2013) Physicochemical characterisation of dairy effluents. *International Journal of Life Science and Pharma Research* **2**(2), 182–191.

Srbija Rulebook (2012) Uredba o graničnim vrednostima zagađujućih materija u površinskim i podzemnim vodama i sedimentu i rokovima za njihovo dostizanje.

SRPS EN ISO 21528-2 (2017) Horizontal Method for the Detection and Enumeration of Entero-bacteriaceae - Part 2: Colony-count Technique. Microbiology of the food chain. Geneva, Switzerland International Organization for Standardisation.

SRPS EN ISO 5814 (2012) Water Quality - Determination of Dissolved Oxygen - Electrochemical Probe Method SRPS EN ISO 6878 2008 Water Quality - Determination of Phosphorus - Ammonium Molybdate Spectrometric Method.

SRPS EN ISO. 4833-1 (2014) Horizontal Method for the Enumeration of Microorganisms — Part 1: Colony Count at 30 °C by the Pour Plate Technique. Microbiology of the food chain. Geneva, Switzerland: International Organization for Standar-dization.

SRPS ISO 16649-2 (2008) Microbiology of Food and An-imal Feeding Stuffs. Horizontal method for the enumera-tion of β-glucuronidase-positive

Escherichia coli - Part 2: Colony-count technique at 44 ^{0}C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.

Su Y and Jacobsen C (2021) Treatment of clean in place (CIP) wastewater using microalgae: nutrient upcycling and value-added byproducts production. *Science of the Total Environment* **785**, 147337. doi:10.1016/j.scitotenv.2021.147337

Talaiekhozani A (2019) A review on different aerobic and anaerobic treatment methods in dairy industry wastewater. Goli A, Shamiri A, Khosroyar S, Talaiekhozani A, Sanaye R, Azizi K. A review on different aerobic and anaerobic treatment methods in dairy industry wastewater. *Journal of Environmental Treatment Techniques* **7**(1), 113–141.

Tawfik A, Sobhey M and Badawy M (2008) Treatment of a combined dairy and domestic wastewater in an up-flow anaerobic sludge blanket (UASB) reactor followed by activated sludge (AS system). *Desalination* **227**(1–3), 167–177. doi:10.1016/j.desal.2007.06.023

Vidal G, Carvalho A, Mendez R and Lema JM (2000) Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. *Bioresource Technology* **74**(3), 231–239. doi:10.1016/S0960-8524(00)00015-8

Walsh JL, Ross CC and Valentine GE (1994) Food processing waste. *Water Environment Research* **66**(4), 409–413. doi:10.1002/j.1554-7531.1994.tb00110.x

Wang Y and Serventi L (2019) Sustainability of dairy and soy processing: a review on wastewater recycling. *Journal of Cleaner Production* **237**, 117821. doi:10.1016/j.jclepro.2019.117821

Watkinson AJ, Murby EJ and Costanzo SD (2007) Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. *Water Research* **41**(18), 4164–4176. doi:10.1016/j.watres.2007.04.005

Wilson DC, Rodic L, Modak P, Soos R, Carpintero A, Velis K, Iyer M, and Simonett O (2015) *Global Waste Management Outlook*. United Nations Environment Programme, 2015.

Yonar T, Sivrioğlu Ö and Özengin N (2018) *Physico-chemical Treatment of Dairy Industry Wastewaters: a Review. Technological Approaches for Novel Applications in Dairy Processing*, IntechOpen. p. 179. 10.5772/intechopen.77110