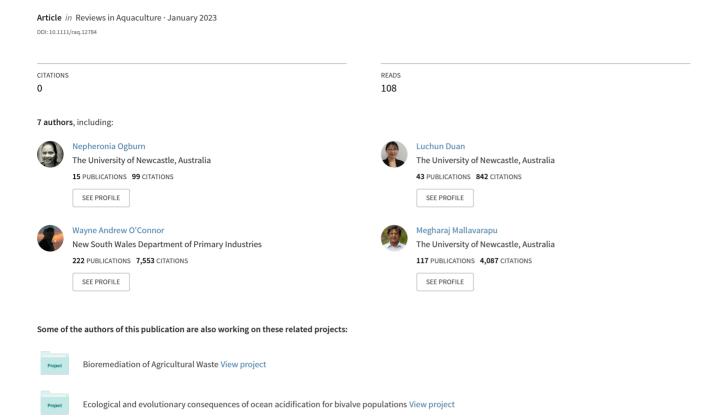
### Agricultural wastes for brine shrimp Artemia production: A review



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#### REVIEW



## Agricultural wastes for brine shrimp Artemia production: A review

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#### **Abstract**

An increasing global population has meant aquaculture, one of the fastest growing food industry sectors, faces significant sustainability challenges as it tries to address the rising global protein demand. In many sectors, production is underpinned by fishmeal as dietary ingredient, but this is a finite resource with competing users from the poultry and livestock industries. Alternatively, some (planktonic) aquatic species, especially brine shrimp Artemia, can be produced using agricultural waste to provide food or biomass to support increasing aquaculture demand. This review investigates research and production of Artemia using agricultural waste. Various systems used for Artemia production in inoculated ponds are analysed and discussed to provide options for environmentally sustainable food systems that can be applied from either an artisanal level in developing countries with a considerable labour force, or in intensive systems in countries with large volumes of under-utilised resources, for example, sugar/alcohol-based waste and inland saline areas. Using agricultural waste, single cell protein production in a separate aerobic digester can be a simple, continuous food source for Artemia to enable daily biomass harvest. This could then be used as a fishmeal replacement or possibly for human consumption to promote a circular economy by remediating waste to produce protein, like a food production mine.

#### KEYWORDS

aerobic digestion, Artemia production, circular economy, single cell protein, waste remediation

## 1 | INTRODUCTION: THE IMPORTANCE OF BRINE SHRIMP PRODUCTION AND UTILISATION IN AQUACULTURE

In recent decades, aquaculture has become the fastest growing major food production sector globally, with production rising yearly at 7.5% since 1970.<sup>1</sup> At the same time, the proportion of wild fish stocks that were being harvested within biologically sustainable levels decreased. As the World's population approaches 10 billion, aquaculture is

increasingly being called upon to fill the gap to feed growing populations.<sup>2,3</sup>

While aquaculture plays a crucial role in global food security, it also faces significant environmental challenges as production intensifies. The reliance on fishmeal, fish oil and other products to feed fish is potentially problematic because decreasing percentages of these feed ingredients are processed from world fisheries production. The poultry and livestock industries also compete for the use of these feed resources. Thus, there is a need to promote the increased use of alternative protein sources in aquaculture and for industry and innovation to scale healthy, sustainable feed alternatives.

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Even if crop-based fishmeal replacement is found, the use of fish oil will likely increase due to the absence of cost-effective alternative lipid sources rich in long-chain polyunsaturated fatty acids (PUFAs), especially to satisfy the requirements of higher trophic level finfish and crustaceans. Production of marine microalgae or bacteria with high PUFA is currently too expensive for use in most aquaculture feeds, compounded by the problem of harvesting them in significant volumes. The potential use of *Artemia* biomass grown using agricultural waste as alternative food source and the different *Artemia*-waste culture systems reported are investigated in this Review.

Aquaculture development is constrained by the reliance on live feeds, which include the brine shrimp, *Artemia*.<sup>7,8</sup> The demand for *Artemia* cysts continues to increase with the expansion of hatchery production, and annual consumption is now estimated at 3500–4000 tonnes to produce over 900 billion crustacean post-larvae and fish fry,<sup>9</sup> with cysts sourced mostly from salt lakes and solar saltworks with no to minimal management capabilities.

Artemia nauplii are the most common live feed used in larviculture of fish and crustaceans because they are easily cultured and are a suitable size for many larvae. Nauplii can be hatched overnight from easily transportable dry cysts (in contrast to most zooplankton used in aquaculture) and they have high nutritional value. Artemia is a continuous, non-selective, particle-filtering organism, with 40%-67% protein according to life stages and feed. Artemia has a high reproductive capacity, and a female can produce up to 300 nauplii or cysts every 4 days, which can quickly grow to adults in 8-14 days, depending on food and/or culture system. With its ability to grow well in extreme conditions, feeding on single cell protein (SCP) food, 7,10 the potential of Artemia biomass production to help address protein food shortage and the dwindling fishmeal supply from marine sources should be further explored, the more since current production is still very limited as presented in this review. This can promote circular economies if, as has been practised for millennia, 11 waste can be used as a resource to produce Artemia. This is becoming increasingly important for the future of aquaculture not only for profitability but also for sustainability in an increasingly environmentally challenged industry.

#### 2 | AIMS

With this review, we aim to:

- 1. Investigate and summarise the use of agricultural wastes and byproducts as a potential food source for *Artemia* farming.
- Examine the direct application of agricultural waste as fertiliser in contrast to indirect use of waste in managed systems, like a flowthrough poultry/livestock and the biofloc system for Artemia production.
- 3. Compare the use of a separate aerobic digester system for *Artemia* production with other waste-*Artemia* production systems and determine where each system is more suited; and
- 4. Assess the potential of remediating high-volume waste, like vinasse or dunder from sugar-based alcohol distilleries, with

Australia as a case study, by utilising the waste as SCP food sources to produce nutritious *Artemia*.

## 3 | HISTORY OF PRODUCTION OF THE BRINE SHRIMP ARTEMIA USING AGRICULTURAL WASTES

Since the early 1960s, when the importance of the brine shrimp, *Artemia*, for aquaculture was first reported, there have been numerous overviews and documents on the biology, production and use of *Artemia*. 7,12,13 With the expansion of aquaculture and ensuing feed shortages, increasing demand for this protein-rich shrimp with a micrometre-thin shell has led to more studies on improved use, sourcing of supplies, alternatives like microencapsulated diets, and growing biomass indoors or in inoculated managed salt ponds. 7

#### 3.1 | Indoor production of Artemia using waste

Indoor high-density culturing of suitable algae as food for Artemia, is mostly uneconomical or limited, so their use can only be considered in locations where algal production is an additional feature of the primary aquaculture activity. The approach to on-growing batches of Artemia to adults in indoor systems, using waste as a food source, was pioneered in the Philippines when scientists from the Artemia Reference Center in Ghent, Belgium, introduced Artemia to the country and encouraged more research on its production and use. The problem of finding a cheap and suitable food for Artemia was overcome by using rice bran as a cheap food source. and growing batches of Artemia adults in air-water-lift-operated recirculating raceways (AWL). 14 This was followed by studies using agricultural by-products and other feeds for Artemia to compare the quality of hatchery food for tiger prawns, Penaeus monodon, 15 to lower the cost of using smaller Artemia nauplii from imported cysts or minimise reliance on microalgae that require huge cultivation space.

A study on the growth response of *Artemia* to various feeding regimes, using *Dunaliella tertiolecta* as food, <sup>16</sup> showed that *Artemia* larvae are voracious feeders and one shrimp can clear 64 ml of water of 6,400,000 cells daily, and that over 10 million cells/shrimp/day can be converted into faecal pellets by adult animals. This continuous filterfeeding capacity makes *Artemia* a great harvester for SCPs that mostly require complex methods or costly equipment to harvest in significant volumes because of their minute size.

The use of SCP other than microalgae has been proven feasible with trials of non-soluble by-products from agricultural crops or food-processing, such as rice bran, corn bran, soybean pellets and lacto-serum. These by-products have the advantage of being widely available and relatively inexpensive. They also provide ideal conditions for the growth of suitable microflora, for example, *Pseudomonas* in rice bran, which constitute an important food source in the diet of *Artemia*.<sup>17</sup>

**TABLE 1** Summary of publications on the use of agricultural waste for *Artemia* culture, emphasising biomass production and development of indoor culture/tank production techniques.

Years	Agricultural waste/by-product used	Country	Culture set-up/technique	Products	References
Indoor/tanl	k culture				
1980	Rice bran	SEAFDEC, Iloilo, Philippines	Fibreglass tanks (A-W-L operated raceways)	Artemia biomass	18
1982	Wheat flour, rice bran and milled rice-fed Artemia pre-adults compared to mussel meat as prawn feed	SEAFDEC, Iloilo, Philippines	Fibreglass tanks	Milled rice and rice fed <i>Artemia</i> juveniles gave better growth as feed for <i>Penaeus monodon</i> postlarvae)	22
1985	Rice bran (compared to microalgae, corn, copra, and soybean diets)	SEAFDEC, Iloilo, Philippines	2-L jars (1000 Artemia nauplii/L)	Artemia (fed 3–5 days) as food for P. monodon post-larvae (PL 10) Proved that Artemia quality (PUFA) follows its diet and can be manipulated	
1987	Mono SCP yeast diet, and mixed diets of yeast and micronised corn-soybean, corn-wheat husks and corn-yeast blended in brine	Ghent, Belgium	300-L culture tanks in high- density (5000–15,000 <i>Artemia</i> larvae/L) flow-through recirculating system	Artemia biomass—2-5 kg live weight per tank, after 14 days Mixed diets are suitable or better alternatives to rice bran	20
1987	Rice bran, microalgae from salt pan, soaked cabbage filtrate, salt pan Spirillum, yeast, and mix of all feed	India	2-L containers with 50 mg/L seawater, stocked with 50 Artemia nauplii/L	A mixed diet gave the best growth for parthenogenetic Tuticorin Artemia; maximum Artemia length of 10.24 mm after 18 days	23
1987	Waste cabbage leaves, cow dung, poultry manure	India	159-L Cement tanks with a 10-cm sun-dried soil base	Best survival after 7 days obtained with mixed waste compared to individual waste	24
1987	Untreated rice bran blended in seawater, chicken manure as fertiliser for algae (vs. fresh Spirulina)	Mexico	Four cement 1 cu m tanks, filled with 375 L seawater, stocked with 1 <i>Artemia</i> nauplii/ml	Artemia biomass growth similar up to Day 10, better growth with rice bran and Spirulina after, then best with Spirulina from Day 15.	25
1987	Dry, 44 μ-sieved defatted rice bran, soybean, yeast lactoserum, Cerophyl and <i>Spirulina</i> homogenised/diluted in seawater	United States	Laboratory screw cap glass tubes (25 $\times$ 200 $\text{mm}^2$ ), with 10-ml media and stocked 5 nauplii	Artemia biomass—Diets of rice bran and Cerophyl most likely to provide best results for large- scale production under condition of uncontrolled bacterial contamination	26
1987	Rice bran, whey powder	United States	430-L air-water-lift (AWL) operated raceways	Artemia biomass—rice bran shows better production of Artemia adults; whey powder gave good results for younger Artemia	27
1987	Dry, micronised feed (in water) of wheat bran versus <i>Ulva</i> (macroalgae)	Portugal	2-L flask inoculated with 1 nauplii/ml	Artemia biomass—Ulva gave better growth, survival, and food conversion efficiency than wheat bran	28
1990-199	2 Enzyme- and heat-treated and chemically treated yeast—fresh and dry	Belgium			21,29
1992	Rice bran	Philippines	Semi flow-through <i>Artemia</i> culture unit	Artemia biomass for feeding fish/ shrimp	19
1994	Cow dung, pig dung, poultry manure and cabbage leaves in various combinations, with rice bran suspension as control	India	10-L tubes, stocked with 100 nauplii/L	Artemia biomass—better growth using experimental diets than rice bran	30
1999	Micronised filtered rice bran and micronised wheat	Australia	5-ton tanks operated as Air- Water-Lift Raceway with filtration system, and partly introducing semi-flow-through	Artemia biomass sold frozen, from an average yield of 12.3 kg/tank for rice bran and 5.3 kg/tank for wheat after 14–15 days of culture	31

TABLE 1 (Continued)

REVIEWS IN Aquaculture

Years	Agricultural waste/by-product used	Country	Culture set-up/technique	Products	References
2000	Rice bran (control), black gram husk, and red gram husk	India	200-ml glass containers stocked with 1000 nauplii/L	Artemia biomass—faster maturity with experimental diets than with control	32
2008	Live and cooked cell wall deficient Saccharomyces cerevisiae	Belgium	Sterile 500-ml glass bottles, each filled with 200-ml filtered autoclaved seawater and stocked with 1000 germ-free <i>Artemia</i> nauplii	Four-day test to check yeast effectiveness	33
2010	Wheat bran (with Dunaliella)	Iran	1000-L AWL tanker in semi flow-through with	Artemia biomass (7116.7 g after 14 days)	34
2010-2011	Dunaliella salina biomass and water (still containing high level of algae cells) after the extraction of carotenoids.	Australia	Closed plastic-moulded (manhole at the top) 32,000 L tanks with a water inlet and filters that retain the Artemia in the tank on the outlet, built near Dunaliella commercial ponds	Frozen biomass: although this is reported as the first super-intensive <i>Artemia</i> rearing system in the World, there is no data given on actual biomass produced	35
2012	Wheat bran, soybean (each with Dunaliella)	Iran	Glass bottles with 6-L saline water with 6000 nauplii	Artemia biomass (4571 to 7018 g after 15 days)	36
2017	Molasses (compared to non-waste sucrose, glucose, corn flour as carbon source)	Bohai Bay, China	10-L plastic cones experiment	Biofloc development for enhanced Artemia production (14-day culture)	37
2018	Vermicompost manure leachate powder (VCL), originating from cow dung, with <i>Dunaliella salina</i> as control, fed singly or in combination with algae	Sari, Iran	1-L cylindroconical glass tubes with 750-ml 33 mg/L water	Possible to use VCL powder only as much as 25% in the diet of Artemia; Best to grow Artemia on algae for small laboratory cultures	38

Abbreviations: PUFA, polyunsaturated fatty acid; SCP, single cell protein.

Agricultural waste and by-products for *Artemia* indoor production have been trailed mostly in small experiments and pilot tank trials to reduce hatchery costs, but no large commercial indoor system has been established. Batch production of *Artemia* in air-water-lift operated raceways is not considered commercially attractive due to cost and limited biomass output. However, improvement of static culture systems has been reported using a simple semi flow-through *Artemia* culture unit for possible integration in marine fish and shellfish hatcheries as the source of a cheap nursery diet and the possibility of producing brine shrimp populations with a uniform size. <sup>19</sup>

A summary of *Artemia* indoor production using agricultural wastes or by-products, collectively referred to in this review as 'waste', is provided in Table 1.The focus is on wastes from agricultural industries. Waste from manufacturing industries or domestic consumption is excluded.

It should be noted that yeast reported as *Artemia* food in Table 1 is not necessarily sourced from industries that produce a significant amount of spent yeast waste, but more commonly in more expensive pure forms like baker's yeast. However, they are included since yeast is sometimes used as a reference diet,<sup>20</sup> aside from being one of the earliest food tested for *Artemia*, or as a substitute for microalgae as live food.<sup>21</sup>

### 3.2 | Outdoor/pond production of *Artemia* using waste

Development of *Artemia* production using agricultural waste has progressed more significantly in outdoor systems and *Artemia* is now cultured in countries where it is not endemic to address the expensive cost of importation and limited supply. There is also increasing interest in ongrown *Artemia*, which had been much less frequently used in aquaculture hatcheries than nauplii. The preference for nauplii is due to their ease of production by simple overnight hatching of widely available and storable cysts.

Pioneering studies were reported in the Philippines to integrate *Artemia* production in salt pond systems.<sup>39</sup> This was followed by a much larger development of an integrated system consisting of the first brackish water flow through salt-fish farm, poultry, and a cattle feedlot. This development integrated a saline waste processing pond for *Artemia* biomass and cyst production.<sup>10,40,41</sup> This closed loop approach to farming, used agricultural wastes from one part of an agricultural landscape as inputs to a subsequent phase, significantly increasing farm productivity and profitability while achieving reductions in greenhouse gas (GHG) emissions over traditional farming systems.<sup>41</sup> This provided an important social contribution through income-generating opportunities for poor rural communities. The estimated total cumulative annual GHG

emission savings from the integrated system amount to 12.9 tonnes CO2-e per head of cattle passed through the feedlot for 300 days. The unitised ratios for the integrated system were: 1 ha sugarcane: 4 head of cattle: 0.13 ha saline waste processing pond that serviced both milkfish and *Artemia* ponds.

Culturing Artemia biomass requires considerable labour and infrastructure, unless the hatchery is situated near an Artemia commercial pond production system aimed primarily at biomass production. The system in the Philippines was developed with this consideration to provide biomass for prawn hatchery or grow-out systems, and poultry feed manufacture<sup>10,40</sup> while relying on labour from marginal communities dependent on over-fished marine resource for their daily food.

The use of sugar-mill effluent as a food source for *Artemia* began in the Philippines from 1988 to 1993, to increase *Artemia* biomass production further to feed Penaeid shrimps directly and later as part of the first documented integrated intensive grouper pond production system supplying the local and export market. <sup>10,42</sup> The integrated grouper-*Artemia* farm provided alternative employment to 300 families in marginal communities to stop illegal fishing and encouraged them to voluntarily engage in mangrove reforestation as soon as some fishermen started earning a year's income within a week.

Sugar mill effluent was traditionally disposed of directly in rivers or the sea, causing severe pollution and months of no catch for local fishermen. However, high volume sugar mill waste, locally referred to as vinesse (or vinasse in other countries), increased cyst production to 30 kg/ha/month and provided *Artemia* biomass standing crops of up to 10 tonnes/ha/day<sup>10</sup> in a one-meter deep pond, enabling daily harvest (done on as needed basis only, so optimal daily harvest is not determined but could feasibly go over 70 kg/ha/day) to feed shrimps in the hatchery or to wean fish larvae. Commercial development in the Philippines stopped in the 1990s.

In more recent years, Vietnam established a commercial *Artemia* production operation, <sup>43</sup> mainly in salt pond systems. However, Southeast Asia's integrated *Artemia* pond operations are not necessarily applicable to developed countries like Australia, where labour cost is high, suitable farming land is either remote or expensive and involves strict approvals for any venture using waste.

An example of an intensive pond production system used mainly for biomass production, like vinasse application in Philippines, is the commercial *Artemia* system in Thailand, which uses 'ami-ami' as feed. 'Ami-ami' is the waste obtained in the industrial production of monosodium glutamate (MSG), a food flavour enhancer commonly used in Asia.<sup>44</sup> MSG is produced by fermentation using a culture of bacteria with carbohydrates sources, like tapioca and molasses. The waste 'ami-ami' is a dark coloured viscous liquid, that is further fermented for weeks to months before being applied to ponds. Production yields in these *Artemia* biomass farms in Thailand reach over 100 kg per ha per day.<sup>45</sup>

'Ami-ami' is similar to an MSG by-product used in a study in the Philippines in the late 1980s, 10,46 although in that study aerobically digested sugar mill vinasse and liquid manure showed significantly superior performance to the MSG by-product and unaerated vinesse or undigested manure and could be used directly after a few days of aeration. This study also showed that growth rates in treatments

where feed rate was based on cell volume calculations were significantly better than those fed according to dry weight basis.

The promising results obtained using vinasse to produce *Artemia* biomass commercially suggest it would be beneficial to explore its application in Australia, where high volumes of waste are produced in the sugarcane and sugar-based alcohol industries.

A summary of *Artemia* pond culture using agricultural wastes or by-products, is provided in Table 2. Design and operation details of various systems can be found in the literature cited.

Figure 1 illustrates the different aquaculture systems where Artemia has been grown and an overview of how agricultural wastes were used.

### 3.3 | Benefits and prospects of indoor and outdoor production of *Artemia* using waste

Depending on culture set-up, the use of Artemia biomass for feeding hatchery post-larvae can result in improved economics, as expenses for cysts and weaning diets can be reduced. Artemia culture done as part of an integrated system produces a multiplier effect on profits, while also reducing carbon footprint.

Large-scale production of good-quality *Artemia* biomass from agricultural waste will benefit the aquaculture and aquarium industry as a live food source, a feed ingredient or fishmeal replacement, in shrimp/prawn broodstock maturation and hatchery production, in fish production, and even benefit other industries like poultry, which relies on fishmeal for feed production. It will also be a potential protein source for human consumption,<sup>27</sup> as has been practised by some communities where natural population of *Artemia* occurs. Furthermore, in Asia, *Artemia* is now used as a major ingredient in *Artemia* omelette in Vietnam,<sup>63</sup> or *Artemia* kebab in Bangladesh (Meezanur Rahman, pers comm. 2022).

The potential for human nutrition is excellent if the fatty acid profile of the *Artemia* can be manipulated after growing them intensively in large amounts using high-volume wastes as a food source, and then enriching them with long-chain PUFA-rich microalgae (or other rich microbial sources) just before harvest, as recommended in a prawn hatchery study. The fatty acid composition of *Artemia* sp. is primarily determined by the food it ingests and the nutritional quality can be improved by dietary manipulation just before feeding the *Artemia* to the consumer. The nutritional value of ongrown *Artemia* can be superior compared to freshly hatched nauplii, which could be affected by unpredictable changes to the natural environment.

Because of their capacity to grow fast and frequently reproduce on SCP produced from agricultural waste and by-products, the potential of *Artemia* as fishmeal replacement is high.

Recently, research and publications on Artemia have generally decreased

Figure 2 summarises the number of publications showing various agricultural wastes to produce *Artemia* in different outdoor pond systems that reached commercial scale or are now ongoing. Countries shown are only those where the development of *Artemia* production

Summary of publications on use of agricultural waste for Artemia culture, with emphasis on biomass production, and evolution of culture techniques and set-up from traditional systems to high-volume waste usage of vinasse for SCP production in outdoor/pond systems. TABLE 2

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Outdoor/pond culture					
1960s	Bird droppings (natural productivity)	San Francisco, United States	Commercial salt pond	Salt and Artemia cysts (former global cyst supplier)	Authors' observation (1980–1984)
1979	Manure for Fertilisation	Philippines	Commercial salt pond	Salt, Artemia (5 kg cysts/ha/month at dry season)	39
1980-1983	Chicken and cow manure as fertiliser	Philippines	Pilot-scale integrated Artemia-salt pond system	Artemia, milkfish, salt Cyst = $7.45 \text{ kg/ha/month}$ Biomass = $154 \text{ g/sq}$ m in dry weight	10,47,48
1980-1985	Chicken manure as fertiliser weekly or as needed	Thailand	Experimental, then scaled up to commercial in Artemia-salt, Artemia-salt-fish, or monoculture of Artemia	Artemia/salt/fish: ave. of 25 kg/ha/per month wet cysts in 1980, shift to biomass increased from 1983, with ~86.4 tonnes total wet biomass in 1984	49
1982	Chicken manure with rice hulls (sacks in ponds)	Vietnam	Experimental salt ponds— 'semi-intensive and static'	6.8 kg dry wt/ha/month Artemia cysts	50
1984	Rich mangrove water supplemented weekly with chicken manure fertiliser	Brazil	Solar Salt Ponds/large Salinas, with few experimental ponds	Salt, Artemia (30,800 kg down to 1240 dry cysts/year by end of declining harvest)	51
1984-1988	Poultry flow-through green water coming from reservoir below the poultry which flows through increasingly saline ponds + aerobically digested cow manure in seawater, for SCP production, as direct food source if microalgae-rich water is insufficient	Philippines	Commercial, 20-ha integrated poultry-flow through system, Artemia inoculated at stocking density of 50/L	Chicken egg, salt, milkfish, shrimp, sea bass, mangrove snapper and <i>Artemia</i> (20 kg dry cysts/ha/month and daily biomass standing crop of 2-7 tonnes/ha, with 1%-5% harvested to feed chicken layers and tiger prawns daily); first record of continuous culture through rainy season using overflow pipes	10,40,41,48
1986–2018	Green water from adjacent ponds; chicken manure (producing Cyanophytes); direct pond supplement: rice bran, chicken manure	Vietnam	First inoculation trial of Artemia in saltworks, commercialised over the years	<ul> <li>Salt; Artemia cyst production</li> <li>Intensive system: 150-250 kg WW/ha/season (3-4 months/season)</li> <li>Extensive system: 50-70 kg WW/ha/season Artemia Biomass production</li> <li>2-4 tonnes WW/ha/month</li> </ul>	7 (to be updated in 2023)45
1987	derivative) Marine bird manure applied monthly as fertiliser	Peru	Experimental evaporator pond	Artemia cysts (wet weight basis: 1.3 kg/ha/day for 13 days) and biomass (wet weight 60 g/cu m for 48 days or 24 g/sq m)	52

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Years	Agricultural waste/by-product used	Country	Culture set-up/ technique	Products	References
1988-1989	Aerobically digested sugar mill vinasse + cow manure in seawater; MSG by- product tested but not commercially used as vinasse gave superior result	Philippines	Commercial 60-ha integrated intensive <i>Artemia</i> salt pond system in a 1000-ha farm, <i>Artemia</i> inoculated at stocking density of 50/L using 1987 Philippine cysts	Salt, milkfish and <i>Artemia</i> (30 kg dry cysts/ha/month and daily standing crop up of 7–10 tonnes/ha, variable harvest (for use in tiger prawn hatchery up to 1% of biomass daily)	10,46
1988-1991	Algal-rich fishpond effluent, chicken manure fertiliser and micronised soy protein supplement	Israel	Large experimental ponds	Artemia: Average 5 kg/1000 m²/day of biomass for few months; Over 2 kg of dry cysts/1000 sq m/month	53
1989-1990; 1992	Dry chicken manure	Bangladesh	1000 m $^2$ salt pond with 45 m $^2$ Artemia culture	1639.9 g (dry wt) of cysts	54
1990-1993	Chicken manure, aerobically digested sugar-mill waste (vinasse)	Philippines	2-ha integrated Artemia- grouper commercial fishpond system	Use of sugar-mill waste-fed <i>Artemia</i> to wean grouper larvae and on grow them for weekly export to Hongkong or to supply local restaurants	10,42
1991	MSG derivative, chicken manure for phytoplankton bloom before stocking Artemia	Thailand	Seasonal solar salt farms	10–20 kg dry weight (dw) cysts and/or 100 to 375 kg wet weight (ww) biomass/ha/month	55
1994	Endemic bird excrements (guano), pond detritus, <i>Ulva lactuca</i> pruned by males	Peru	Commercial salt ponds	Artemia biomass and enriched, frozen Artemia to feed shrimp broodstock in hatcheries.	56
2000-2018		Ecuador	Commercial salt ponds 0.25-ha recirculating ponds	200–300 pounds per hectare per month 4000–18,000 kg A <i>rtemia</i> biomass per month.	
2002	Chicken manure, sieved before aerobic fermentation	Mexico	Experimental ponds	Artemia biomass	57
2002-2003	Mixture of Torula yeasts and micronised soy protein after initial microalgae feed	srae	Experimental interconnected series of four 600-L to 1000-L tank and pilot system of three 2000-L to 5000-L tanks	Artemia biomass, average of 40.28 $\pm$ 4.84 kg m $^{-3}$ (wet weight) in 600 L and 31 kg m $^{-3}$ in 3000-L tanks after 17–20 days	288
2009	Pig manure (PM), rice bran (RB), combined PM + RB, combined PM, and soybean meal and green water (pig manure fertilised)	Vietnam	Experimental ponds, adjacent green water (manure fertilised) with supplemental feeding directly applied or in sacks	Artemia: Average 1.8 and 2.3 tonnes wet weight ha <sup>-1</sup> biomass 1.587 kg/ha, highest biomass harvest in 3-day harvest intervals	59,60

(Continued)

TABLE 2

Years	Agricultural waste/by-product used	Country	Culture set-up/ technique	Products	References
2012-2013	Assorted waste, including chicken manure as fertiliser: micronised rice bran and soya pellets as supplement in Vietnam; MSG waste up to 100 L/ day/ha in Thailand	China, Vietnam, Thailand case studies	60-ha semi-intensive pond cultures in China Asstd. sizes commercial ponds systems in Vietnam and Thailand	Artemia cyst and biomass 1500-3000 kg wet weight/ha/month in Thailand's MSG-waste-fed developed ponds	44
2015	Pig manure (PM) and rice bran (RB– fermented in alcohol yeast) as supplement to green water	Vietnam	Twelve 300-sq m Experimental ponds, with direct feed supplement	Artemia: 27.8–51.9 kg wet weight $ha^{-1}$ cyst; Average 2.2-ton wet weight $ha^{-1}$ biomass	59
2018	MSG derivative	Thailand	Commercial ponds	Artemia biomass production: 4.5 metric tonnes/ha/month  • 75% local use, 25% exported frozen	61,45
2018	Chicken Manure—mainly as fertiliser in 'green water' ponds for supply of phytoplankton, wheat flour, molasses, tapioca, pig manure—carbon source in	Bangladesn Vietnam	Commercial ponds	NA Artemia cyst and biomass	62
	biofloc development				

systems was introduced in managed salt farms or specifically designed set-up, from artisanal to intensive commercial level for local use and import markets. Indoor systems are not included since, until now, there are no known established commercial indoor production systems. One report may cover several years in a given country, indicating the scarcity of publications since the 1980s, especially those focused on high-volume waste utilisation for *Artemia*.

It is important to note that although there may be limited publications available according to when each country conducted trials or research, the potential for commercially producing *Artemia* cysts or biomass profitably is already clearly demonstrated from the reports reviewed. Remediating agricultural waste through the production of nutrient-rich *Artemia* could potentially be an approach to achieving a circular economy that could also help alleviate the problem of protein shortage. The use of *Artemia* as potential fishmeal replacement has been also recommended in a study using algal fed *Artemia* culture in tanks (brine shrimp bioreactors) as part of an integrated marine production system. <sup>64</sup>

### 4 | DIRECT AND INDIRECT USE OF AGRICULTURAL WASTE IN ARTEMIA PRODUCTION

Section 3 has shown different methods of using agricultural wastes in *Artemia* production. A closer look at these systems is presented in this section, but discussion will be limited to outdoor production because there is no documented commercial scale operation of growing *Artemia* biomass in indoor systems yet.

#### 4.1 | Direct addition of waste as fertiliser

Direct addition of agricultural waste, like solid manures, to the *Artemia* production area is usually done as part of the pond preparation to increase the organic matter of the soil and then followed by inorganic fertiliser once the water is deep to promote phytoplankton growth, as discussed in some of the publications using manure as given in Table 2. However, it is difficult to maintain sufficient algal bloom to provide natural food for *Artemia* in the widespread traditional use of chicken manure in *Artemia* ponds as direct food or fertilisation ponds to stimulate algal growth, followed by pumping 'enriched water/green water' to the culture ponds.<sup>60</sup> Hence, inert diets, like rice bran, have been applied in the culture pond as a food supplement.

The problem with relying on the natural productivity of the culture area is that *Artemia* can easily consume natural food and a continuous supply must be provided. In a review on feeding as one of the most important factors affecting *Artemia* production (see Table 2), the inadequacy of traditional methods used in ponds to promote phytoplankton bloom using fertilisers supplementation with cheap agricultural waste products and chicken manure directly applied in the pond was discussed.<sup>62</sup> These methods often result in suboptimal feeding levels and high nutrient pond effluent discharges. Hence, the authors recommend using biofloc technology for *Artemia* production.

#### AGRICULTURAL WASTES AND BY-PRODUCTS FOR ARTEMIA PRODUCTION

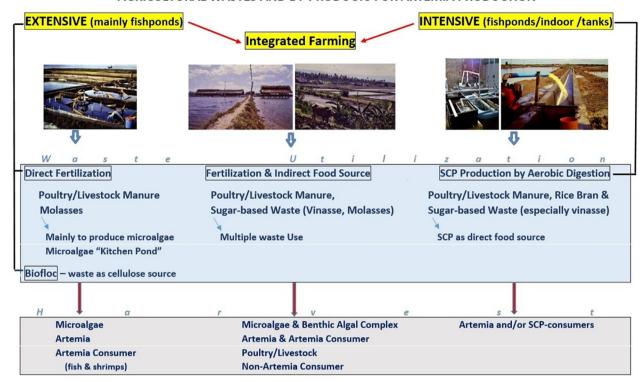


FIGURE 1 Overview of the use of agricultural waste and by-products to produce Artemia in indoor and outdoor systems.

#### 4.2 | Biofloc technology

Biofloc technology is based on the principle of waste nutrients recycling, particularly nitrogen, into microbial biomass that can be used in situ by cultured animals or be harvested and processed into feed ingredients.<sup>65</sup> Heterotrophic microbiota is stimulated to grow by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source in the water, so that the bacteria can assimilate the waste ammonium for new biomass production, eliminating the need for water exchange.

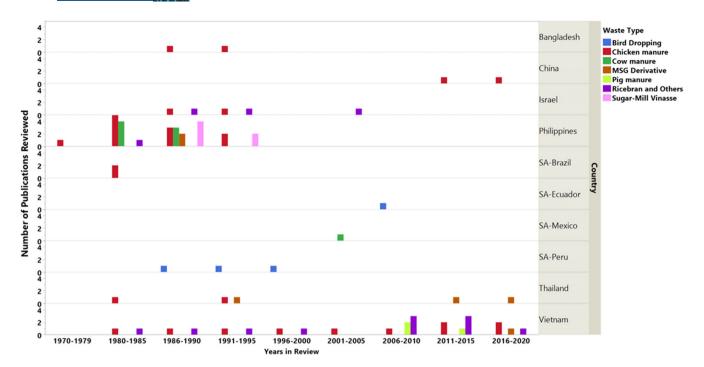
In the review recommending the use of biofloc technology, <sup>62</sup> the formulated feed developed specifically for *Artemia* pond production involved adding tapioca flour or molasses as the carbon source to obtain the ratio C/N ≥10 to stimulate bio-floc development as feed for *Artemia*. Biofloc application is related to using SCP in aquaculture to reduce feed costs by minimising the need for expensive protein components through microbial protein synthesis. <sup>66</sup> To remove the problem of harvesting and processing microbial cells, they tested directly in situ production of SCP in continually mixed and aerated circulated fish ponds, with daily addition of cellulose or cereal meal, like sorghum. The carbon source provided the substrate for SCP, with nitrogen coming from the pellets or ammonium sulphate supplements.

A comprehensive review of Biofloc use for aquaculture applications and the animal food industry summarises the advantage of the technology in minimising consumption and release of water, recycling in situ nutrients and organic matter, reduction in introduced pathogens, introduction and improving the farm biosecurity, enabling aquaculture to further develop an environmentally friendly approach. $^{67}$ 

The increasingly popular approach to managing ponds using biofloc systems in aquaculture is widely discussed. <sup>37,68,69</sup> This involves providing a nutritious food source that promotes higher productivity or higher nutrient in an integrated aquaculture system. These studies followed after it was reported that bacteria could be used as a nutrient source for *Artemia* to compensate for suboptimal algae supply when molasses supplementation resulted in much lower total *Artemia* biomass compared to significant improvement when beneficial bacteria were combined with molasses. <sup>70,71</sup>

Although biofloc development to increase *Artemia* production and improve pond water quality has been well reported, the application may only be suitable for the addition of limited volumes of waste/by-products as carbon source (e.g., molasses), or the use of a more refined source of cellulose, but not for direct addition of high COD-waste, like sugar-mill and distillery vinasse, with at least 30,000–40,000 mg/L COD<sup>46,72</sup> as abundantly found in some countries like Australia.

The lower *Artemia* biomass obtained using molasses supplementation in a biofloc system may be caused by its direct application to the *Artemia* culture without aerobic digestion first to promote SCP growth.<sup>70,71</sup> Direct addition of the cellulose or carbon source to a culture, even in combination with beneficial bacteria, has to be well regulated and best applied to lower COD sources, such as the use of cellulose or cereal meal (e.g., sorghum).<sup>65</sup> However, said materials



**FIGURE 2** Artemia production reports in outdoor pond systems using various agricultural wastes in countries where commercial trials or business has been established.

could be more costly as they have other uses in the livestock, or other developed industries, unlike high-volume waste that are still primarily disposed of or stored long to get remediated and pose an environmental risk if leached.

The disadvantages of the Biofloc system have been summarised in a review,<sup>73</sup> suggesting that producers better adopt a closed management system for biofloc ponds and have a compartmental design—where fish production and microbial proliferation occur in separate spaces—to make management easier. This separate waste processing compartment has already been proven very effective in the saline waste processing pond or aerobic digester designed for intensive *Artemia* production in the Philippines,<sup>10,41</sup> as discussed further in this section.

#### 4.3 | Integrated poultry/livestock Artemia systems

Integrating poultry or livestock into an *Artemia* salt pond system<sup>10,40,41</sup> in flow-through system enabled continuous supply of phytoplankton and other SCPs to feed *Artemia* as well as utilisation of several crop by-products and other waste in feed manufacture for the poultry or livestock. The SCP in this system is produced separately from the *Artemia* pond because the waste goes to a reservoir below the poultry or in the waste sump adjacent to the livestock and flows or is hosed to the *Artemia* pond already aerobically converted to SCP, similar to the digester discussed in the next section. A standing crop of 7 tonnes/ha can be maintained daily in a poultry flow-through/ livestock system, although optimum daily harvest was not determined since harvest was on as needed basis for prawn or poultry supplement. *Artemia* production could even be extended to the wet season

through a simple pipe overflow system design that enables maintenance of higher salinities at 60-80 g/L to exclude predators and competitors that generally start to inhabit the culture ponds in the wet season, as summarised in Table 2.These high biomass production systems result in a multiplier effect on profits.  $^{10,40,48}$ 

#### 4.4 | Aerobic digester system for SCP production

The use of a separate aerobic digester to produce SCP adjacent to the *Artemia* culture, prior to the addition of liquid feed to the *Artemia* pond, <sup>10</sup> makes addition of beneficial bacteria inoculum unnecessary. A readily available SCP sustains continuous high *Artemia* biomass production. Bioremediation of the waste to manageable nutrient levels improved the physico-chemical and biological parameters before adding SCP daily to the *Artemia* culture at regular hourly intervals through regulated faucets and piping. This is shown during preliminary trials in Australia using dunder wastes from bioethanol and rum production when aeration for 3–15 days continued to lower the BOD while increasing SCP in the digested waste used to feed *Artemia*. <sup>74</sup> The production of SCP that can be directly utilised by *Artemia* is a more sustainable way of utilising waste. <sup>10,41</sup>

The use of SCP as a potential solution to the increasing food protein demand in the World has long been recognised, studied, documented or reviewed. Authors roughly define SCP as the dried cells of bacteria, algae, yeast and fungi, rich in proteins and could be used as a dietary supplement after growing using various substrates, mainly agricultural wastes. They are mostly dried because harvesting the tiny cells of 10-micron or below in enormous tonnage is still an expensive process, if not a logistic impossibility, to be used fresh as the protein source.

Among the advantages of growing SCP is as a nutrient supplement for humans because it contains not only protein but carbohydrates, fats, water and other elements, and its requirement for growth is not as limiting as those for animals and plants because it is neither seasonal nor climate dependent. However, SCP production is still minimal and has not risen in proportion to rising protein food demand. Moreover, its production, which usually involves anaerobic fermentation, is relatively costly and capital intensive, harvest is not easy, improvements in quality is required to remove potential toxicants or health hazards for human, its acceptability needs to be increased, and palatability may need to be enhanced. There are suggestions of the need to make genetic improvements in producer organisms.

Production of Artemia using SCP grown in a simpler and cheaper aerobic digestion setup is an attractive alternative to producing SCP directly for human consumption, unless it involves high-value products, as currently used in nutraceutical industries, like the production of nutritious microalgae that makes capital and operational cost sustainable. Biofloc technology for Artemia also uses SCP to create a healthy nutrient balance in ponds; the difference is that the reaction is done directly in the ponds by adding cellulose and bacteria, which is more difficult to control compared to a separate SCP producing aerobic digester. The digester removes the need to closely manage the Artemia culture pond, or add bacteria as required in the biofloc technology. SCP that is pumped daily from the aerobic digester is converted to biomass immediately by Artemia.

Because Artemia is a shrimp and tastes like any prawn or shrimp when cooked, its acceptability may be easier to address, especially in developing countries, like Bangladesh and Vietnam, <sup>63</sup> which now uses Artemia biomass as a replacement for other crustaceans in making omelettes than using SCP as direct human protein source. Analyses of waste can address any concern on safety and the quality of Artemia produced, for example, through metabolomic, nutrient and heavy metal analyses.

A higher standing crop of 10 tonnes/ha was obtained in the intensive system explicitly designed to use high-volume vinasse or washings from a sugar mill in the Philippines. Because *Artemia* harvest to provide live food to a prawn hatchery was on as need basis, optimum daily harvest could not be assessed. Whether the maximum *Artemia* daily standing crop can go higher than 10 tonnes/ha in such a system could not be determined either because vinasse was provided from another island. Feeding was limited by how much waste could be transported.

# 5 | AUSTRALIAN CASE STUDY: POTENTIAL OF USING SUGAR-BASED WASTE FOR ARTEMIA PRODUCTION TO HELP REMEDIATE POLLUTANTS

Sugar-milling, bioethanol and rum production from sugar or molasses, and wine and beer production are significant agricultural industries around the World. An estimated 191.2 million L of pure alcohol are available for consumption from alcoholic beverages in Australia, with 39% contributed by beer, 38.6% by wine and 19.9% by spirits/RTDs.<sup>81</sup> These industries produce a significant volume of waste, often

associated with low pH and high COD. Hence, they must be disposed of properly. The existing process is either to bury the waste in unproductive private lands or to apply a more costly, complex process involving anaerobic and aerobic treatments, requiring additional infrastructure for existing operations. In Southeast Asia, river or coastal waters sometimes become the disposal site of significant amounts of waste, for example, sugar mill washings, with deleterious effects on the environment, including fish kills that can deprive marginal communities of a food source.<sup>82</sup>

Poultry and livestock (feedlot and dairy) also produce high volume wastes that are proven effective in *Artemia* biomass production, as shown in Tables 1 and 2, however, the wastes have other significant use as fertiliser, especially for the horticulture industry in Australia. Manure supply from the biggest poultry producers in Australia is already contracted in bulk for horticulture use (Baiada and Inghams Enterprises, pers. comm., 2018). Livestock manure is often reused to fertilise paddocks or horticulture. These wastes can also be easily dried, packed, stored or transported.

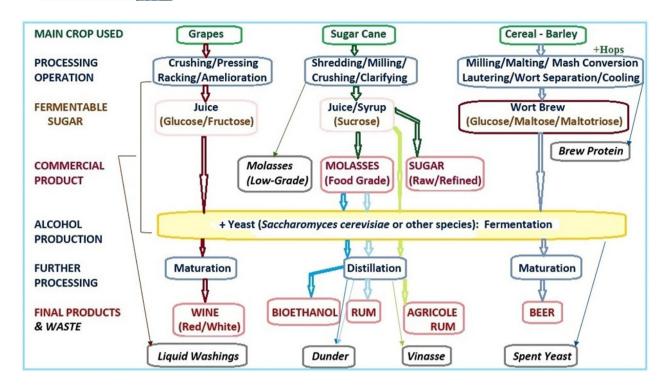
Because the use of high-volume sugar-based vinasse waste for intensive *Artemia* production has only been reported in one commercial salt farm in the Philippines, <sup>10</sup> there are few examples of how sugar and alcohol production-based wastes are currently treated. Figure 3 shows a simplified diagram of where waste can be sourced in Australia's alcohol and sugarcane-based industries, with potential as SCP food source for *Artemia*. Only wastes that pose a challenge for disposal and used as fertiliser or as a livestock food supplement, are included here, such as dunder and vinasse.

For the wine industries, the waste is mainly a collection of washings from various processing activities often directed to a holding/ storage area and allowed to aerobically decompose before use as paddock fertiliser or disposed of on land. This effluent could vary highly from farm to farm. Bulk waste from a crushed grape, known as marc, is not included in this review, rather the focus is on liquid wastes that are harder to store, pack or transport in significant volumes.

In breweries, relatively high protein mash collected after separation from wort brew, and the spent yeast resulting from fermentation, are a potential medium for growing microalgae or as SCP food source for *Artemia* 

In sugar cane-milling, a direct sugar-base that could be used as an SCP medium for *Artemia* is low-grade molasses. However, farmers already buy it to re-fertilise sugar cane or other crop farms or use it in livestock feed preparation. Hence, remediation is not as pressing an issue as other high-volume wastes like vinasse.

In Australia, farms generally refer to the concentrated waste from molasses-based alcohol production as dunder, also known globally as stillage or vinasse. Wilmar Bioethanol, the only Australian company to operate the Biostil process, produces a more concentrated dunder stream, referred to as BioDunder, than molasses-based rum distilleries. This liquid by-product of ethanol contains approximately 30%–40% solids, comprising vegetable matter (yeast biomass) with potassium, sodium, nitrogen, calcium, magnesium, phosphorous and sulphur. It is also useful as liquid fertiliser, although significant volumes are left unused and stored in ponds, awaiting technology for further use.



**FIGURE 3** Simplified diagram of waste sources in the alcohol and sugarcane-based industries (Based on information taken during visits to Australian sugar mills, rum/bioethanol distilleries and beer and wine production facilities).

Distilleries that produce alcohol using fresh sugarcane juice (e.g., Agricole rum) or from heated sugar syrup, raw or dextrose sugar, produce waste vinasse.

In a review of approaches to distillery wastewater, effluent from distilleries or spent wash is considered an extensive soil and water pollutant. Spent wash is described as highly acidic (pH 4.0–4.3) with high rates of biochemical and chemical oxygen demand (BOD: 52–58, COD: 92–100 kg/m³) and suspended solids (2.0–2.5 kg/m³). In Brazil, vinasse is often used in fertilisation and irrigation practices, referred to as fertirrigation, highly which may be linked to adverse environmental outcomes if an excess is applied. Spental process.

Anaerobic digestion is widely accepted as the first step in treating distilleries spent wash or sillage followed by aerobic processing, aimed at getting biogas and reducing the polluting effect.<sup>88</sup> However, in the absence of such complex treatment set-ups in many distilleries, a promising alternative is to use a simple, aerobic-digester to grow SCP that can be used as food to produce intensive *Artemia* biomass and remediate a potential pollutant at a faster rate, <sup>10,46,74</sup> as discussed in the previous section.

Table 3 summarises examples of studies on the treatment of high-volume wastes from the sugar and alcohol industries, focusing on spent wash or vinasse. The table shows that various methods have been used to treat winery, brewery and distillery wastes. However, the problems in treating large volumes of distillery effluent containing recalcitrant compounds, including the difficulty of removing the dark colour with anaerobic treatment, are well documented.<sup>83</sup> Therefore, conventional wastewater treatment methods may not be sufficient for distillery effluent and the suggested treatment is a combination of

processes or multi-stage treatments for both anaerobic and aerobic phases, including a decolouration step, for the effluent to be reused or safely disposed of.

Treatments involving anaerobic and aerobic processes may be more beneficial for companies that invest in infrastructure to treat waste for fuel production (e.g., methane from the anaerobic phase), but not all distilleries are equipped for complex treatment processes that would add to the cost of the operation. Thailand uses the anaerobic treatment of MSG successfully for commercial Artemia production. A more straightforward approach, like direct aerobic digestion of spent wash/vinasse/stillage to produce SCP as food to grow the highly fecund brine shrimp, can be an alternative with economic benefits. Even if bacterial, fungal and phytoremediation treatments are used to produce species with high values, as shown in Table 3, the problem of mass harvesting relatively minute organisms in larger volumes could still pose problems. The microscopic SCP resulting from direct aerobic digestion of relatively concentrated distillery wastes can be easily consumed by Artemia to produce a high quantity of biomass that is easier to harvest, process and transport.

An important consideration in using high-volume sugar-based alcohol waste to produce *Artemia* in Australia is that 5.7 million hectares of land in the country have become unproductive for conventional agriculture due to increasing salinity levels, arising from traditional farming methods introduced by man. This caused a marked deterioration in the quality of surface waters, which led to the establishment of large-scale evaporation basins as an engineering response. 31.89 Although several research and commercial ventures have been conducted using inland saline aquaculture in

**TABLE 3** Some examples of bioremediation studies done on high-volume agricultural wastes, with emphasis on sugar and alcohol-based industries.

	by-products	Country	Industry	studies	References
2002	Molasses distillery wastewater/spent wash	India	Cane molasses-based distillery	Biogas recovery by bio-methanation, followed by multiple effect evaporators and bio- composting	85
2005	18 assorted winery effluent and 13 distillery vinasse	Spain	Winery and distillery	Waste characterisation to determine feasibility for agriculture use	92
2007	Distillery wastewater, with focus on coloured spent wash	India	Distilleries	Toxicity profile, colourants, and treatment of spent wash by anaerobic or aerobic methods (bacterial, fungal, mixed consortia and phytoremediation)	84
2007	Distillery wastewater/spent wash	India	Distilleries	Ozone pre- and post-aerobic treatment versus conventional aerobic Digestion only; thermal/anaerobic pre-treatment, advanced oxidation techniques, ultrasound, ozone to enhance aerobic oxidation	93,94
2008	Wine-related wastewaters	South Africa	Winery	Fungal and enzymatic remediation	95
2008	Spent brewer's yeast	Turkey	Brewery	Induced autolysis at elevated temperature to produce yeast extract	96
2011	Bagasse/grape marc, lees	Spain	Winery	Trim wastes, grape marc, and wine lees for production of lactic acid and bio-surfactants	97
2011	Assorted wastes, with focus on winery wastewater	Australia	Winery	Primary/Secondary/Tertiary Treatment Technologies, including Aerobic and Anaerobic Treatment, use of evaporation ponds and wastewater bioremediation cell (WBC) being trialled for smaller wineries	98
2011	Molasses-based raw industrial effluents	India	Molasses-based industry		99
2012	Distillery wastewater	India	Alcohol distilleries	Need of cost-effective treatment scheme, using bio-methanation as primary step, followed by physicochemical treatment and ending with aerobic treatment. Also need further research on emerging method like enzymatic treatment.	100
2012	Distillery effluent	India	Alcohol distilleries	Phycoremediation using the green microalga Scenedesmus sp	101
2013	Distillery vinasse	Brazil	Sugar-ethanol industries	Fertirrigation, concentration by evaporation, energy production; the effects on soil physical, chemical and biological properties; its influence on seed germination, its use as bio-stimulant and environmental contaminant. Green methods need to be developed	86
2014	Vinasse	Brazil	Sugarcane bio-refineries	Anaerobic digestion of vinasse to produce biogas for electricity or vehicular fuel replacement or alternative to diesel; Biogas in cogeneration to release bagasse for second- generation ethanol production	102
2014	Spent wash	India	Cellulose-based ethanol industry	Anaerobic methane production; reverse osmosis; melanoidin degradation by phycoremediation	103
2014	Vinasse	Brazil	Sugarcane alcohol	Various fungi ( <i>Pleurotus</i> ) cultured in vinasse as supplement feed for <i>Danio rerio</i> fish; nontoxic	104
2015	Vinasse	Brazil	Ethanol distillery	Biodegradation of sugarcane juice vinasse in aerobic and anaerobic conditions	105

REVIEWS IN Aquaculture

TABLE 3 (Continued)

Years	Agricultural waste/ by-products	Country	Industry	Waste production processes/treatment/ studies	References
2016	Vinasse from Sepahan Bio- product Company Isfahan, Iran	Iran/Sweden	Molasses to Ethanol Company	Edible fungi Neurospora intermedia and Aspergillus oryzae has grown in diluted vinasse, to produce 223 g of fungi per litre of vinasse	106
2017	Brewery wastewater	United States	Brewery	COD treatment by continuous flow microbial fuel cell (MFC) treatment system, with no catalyst	107
2019	Vinasse	Brazil	Ethanol from sugarcane or molasses	Ozone treatment + anaerobic digestion (biogas), + aerobic growth of fungi reduces COD by 95%, total removal of phenols and >80% of total N	72

Australia, 31,35,89-91 it has remained mainly on a practical level or an on-and-off commercial venture.

Little has been done to commercialise live food more suited to inland saline areas, except for Dunaliella and Artemia in Western Australia, 35 a commercial operation in South Australia and a pilot operation in Northern Victoria that showed potential for Artemia production and marketing,<sup>31</sup> none of which have resulted in a sustained continuous commercial venture. From the literature, it is apparent that either the species trialled in inland saline areas are unsuitable to the extreme conditions, the set-up or operation is not designed to operate as a highly profitable integrated venture or intensively with optimum feed input- harvest output as has been established in Asia, where distinct wet and dry season affects Artemia production.

Feed studies using waste or microalgae to grow Artemia have shown that failure to sustain continuous production, in conditions where predation is not much of a threat, is often associated with decreasing food availability, as is the case in salinas, 51 and some salt ponds relying on natural productivity, where Artemia production has reduced over the years as nutrients eventually deplete.

With high-volume waste, like vinasse, remaining a pollution challenge to many countries, if not properly treated, the merger of two industries, aquaculture with its highly nutritious all meat brine shrimp Artemia, and agriculture where remediation of high-volume of waste is still an environmental challenge, may prove to be a great opportunity for food production. Liquid waste can provide the much needed SCP and Artemia, the natural harvester that solves the decades-old problem of processing minute protein cells for sustainable continuous protein production. Managed production of Artemia using waste also eases the reliance on approximately 90% of Artemia harvest from inland salt lakes that are under constant threat of drying up due to climate change, as observed in many lakes over past decades.9

Considering that inland saline areas in Australia are spread across a large area in many states where there is likely to be a distillery, winery or brewery with high volumes of waste needing remediation, it would be a worthwhile venture to see how far the production can be optimised if there is a continuous supply of waste available for aerobic digestion to supply food to Artemia in areas where very few other aquaculture species can survive.

#### APPLICABILITY OF VARIOUS SYSTEMS TO UTILISE WASTE FOR ARTEMIA **PRODUCTION**

Systems to produce Artemia using agricultural waste vary according to country and what resources are available.

Table 4 evaluates the different systems of using agricultural waste for Artemia production based on different factors relevant to a sustainable industry. A simplified version of significant factors, patterned after the One-Health Lens approach, 108 is illustrated in Figure 4 to help those needing to decide which system is best suited for their purpose and area of Artemia production. The factors are rated from 0 to 5 based on Table 4.

If the quality of Artemia is enhanced before harvest and toxicants are not shown in the product, there is potential for the use of Artemia in human nutrition, aside from being a candidate to replace fishmeal to address the increased demand for protein sources in the fastgrowing aquaculture industry. A high-volume waste, like rum dunder, is relatively sterile when leaving the distillers, and microbial components that grow in the holding tanks are generally Lactobacilli, which are relatively safe. Some dunder is even reused in the rum operation to minimise water usage, as shown in the microbial ecology of the Bundaberg rum production process. 109

Figure 5 shows the various culture systems using waste to produce Artemia, illustrating a simplified input-output flow as a further guide to deciding on a design to suit different areas and product requirements.

#### **SUMMARY AND CONCLUSIONS**

Many agricultural industries produce significant volumes of wastes that are underutilised, disposed of, or put on land as fertiliser that may cause pollution due to their high nutrients, especially if accumulated over time beyond any possible initial fertilisation benefits. Treating these high-volume wastes with conventional methods for industrial wastewater, involving multiple steps of anaerobic and aerobic treatment processes, would be uneconomical for those traditionally storing

Comparison of various waste remediation techniques using Artemia or involving high-volume wastes: applicability, constraints and potential TABLE 4

Technology criteria	Waste as direct fertiliser	Waste as nutrient source in integrated farming system	Biofloc system	SCP production from aerobic digestion of agricultural waste	Anaerobic or combined anaerobic and aerobic treatment of waste
Waste used	Poultry and livestock	Poultry and livestock (flow- through for enrichment; not using separate digester)	Molasses, tapioca flour, with nitrogen coming from pellet waste or other nitrogen source in the culture	Poultry, livestock, sugar and alcohol production waste—vinasse, dunder and biodunder	MSG derivative (as used in Thailand); anaerobic-aerobic treatment used traditionally to treat vinasse
Intensity of operation	Extensive aquaculture systems	Extensive to intensive	Extensive to semi-intensive	Semi-intensive to intensive	Intensive
Cost	Low	Low to medium (acc. to poultry/livestock intensity)	Low to medium (bacteria + cellulose to balance N)	Low to medium (high-volume waste sourcing if not produced nearby)	High (involves various equipment and materials in different stages)
Labour	Low	High	Low to medium	Low	Medium to high
Optimised water usage/quality	Low	High	High (if C/N/microbial balance maintained)	High (SCP converted to Artemia biomass quickly)	Low to medium
Use of chemicals/ supplement	None	Low to medium (poultry only)	Low to medium	None	High
Management/ operation complexity	Low	Low to medium (mainly poultry management)	Low to medium (balancing chem ratio for SCP in situ production)	Low (automated, SCP pre- produced)	High
Income generation	Low	High	Medium to high	High	High (if fuel and valuable microbes produced) Low as treatment only
Sustainability/ optimised farm system	Low	High	Medium to high	High	Medium
Reducing carbon footprint	Low	High	High	High	Medium (if fuel produced but complex processes may result to increased footprint)
Potential to remediate high volume of waste	Low	Medium to High (indirect remediation through use of waste for poultry/cattle feed)	Low (C/N/microbe balance is hard to maintain, esp. with high nutrient waste)	High (aerobic digestion outside the ponds lowers polluting nutrient and mainly SCP goes to pond)	High if complex operation is offset by fuel and special microbe production

Abbreviations: MSG, monosodium glutamate; SCP, single cell protein.

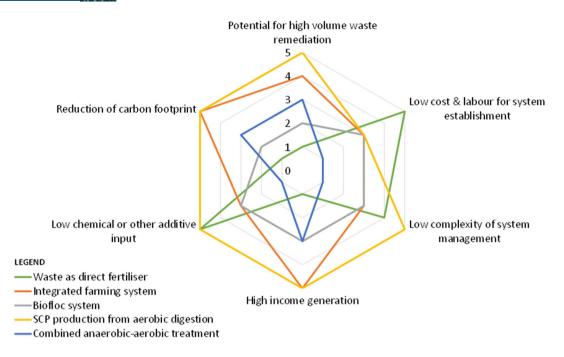


FIGURE 4 Comparative analysis of different waste processing systems for Artemia production using a modified One-Health lens approach. 108

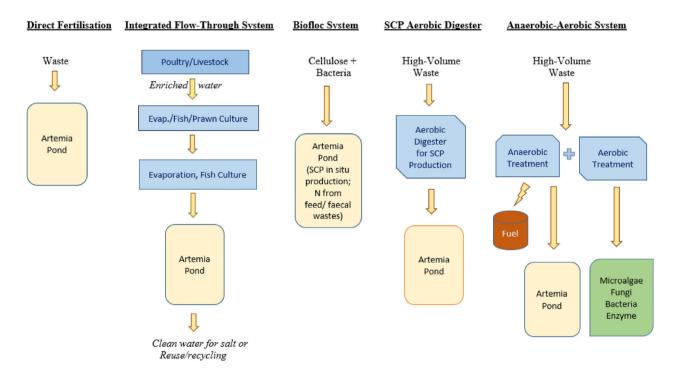


FIGURE 5 Simplified diagram of different outdoor production systems of Artemia and their input-output flow for waste processing.

or holding excess waste in private lands other than what they use for fertilisation. Better ways are needed to remediate waste beyond the nutrient capacity of their crop production areas.

Using sugar and alcohol-based wastes in the intensive production of the brine shrimp, *Artemia*, in indoor or pond production systems is potentially a solution to two major global problems: bioremediation of high-volume agricultural wastes and production of nutritious feed/food to address the global protein shortage. This can minimise

dependence on *Artemia* importation or cut costs in the aquaculture and aquarium industries. Furthermore, the more straightforward process of SCP production by aerobic digestion instead of a more costly and complex method of using multi-stage anaerobic/aerobic processes will boost sustainability in distilleries that are not equipped with specialised waste treatment facilities, and promote a circular economy where waste becomes a resource. Because of the simpler set-up, the system applies to any country.

For less developed countries, where marginal coastal communities rely on harvesting the diminishing resources of the sea for subsistence, an integrated poultry/livestock-*Artemia*-fish/crustacean flow-through system may be an excellent alternative to address socio-economic concerns since the system can provide a multiplier effect on profits and a much-reduced carbon footprint.

The use of multi-stage or complex processes to remediate high-volume waste is best limited to bioremediation aimed at producing high-value products, for example, microalgae with highly specialised use, like the production of biofuels and nutritional supplements where the end product can pay for the cost of treatment. Although there are no publications describing details of the process used in feeding the waste product of MSG production, 'ami-ami' to *Artemia*, Thailand has successfully produced *Artemia* biomass commercially for many years, with daily production of 10–50 kg/ha, equivalent to 340 tonnes/year, <sup>110</sup> which is currently updated in a forthcoming publication to 100 kg (average) per ha of 1.5 m depth pond (Banchong Farm, Chachoengsao, Thailand, pers. comm., 2022).

Large-scale production of good-quality *Artemia* biomass from high-volume waste will benefit the aquaculture and aquarium industry as a source of natural food or feed ingredient in larviculture and in broodstock maturation, or as fishmeal replacement for various industries, and offers a potential protein source for human consumption by enhancement of *Artemia* quality before harvest. Because *Artemia* grow quickly and reproduce well on SCP produced from agricultural waste and by-products, their potential as a fishmeal replacement is also very promising.

Various systems of growing Artemia in inoculated managed ponds have been successfully done in many countries, although the literature on the use of waste is quite limited. Regardless, the options exist to produce Artemia on SCP grown from agricultural wastes that develops a circular economy to help solve the increasing protein food demand in an ever-growing global population.

A merger of agriculture, with its high volume of waste needing remediation, and aquaculture, where fishmeal and live natural food supply is increasingly in demand, could become a protein production mine, with high volume waste as the liquid gold raw input.

#### **AUTHOR CONTRIBUTIONS**

Nepheronia Jumalon Ogburn: Conceptualization; investigation; methodology; visualization; writing – original draft. Luchun Duan: Resources; software; visualization; writing – review and editing. Suresh Ramraj Subashchandrabose: Supervision; writing – review and editing. Patrick Sorgeloos: Resources; writing – review and editing. Wayne O'Connor: Supervision; writing – review and editing. Megharaj Mallavarapu: Supervision; writing – review and editing. Ravi Naidu: Funding acquisition; project administration; resources; writing – review and editing.

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#### **CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

#### **DATA AVAILABILITY STATEMENT**

New data were not created nor analysed in this review, so data sharing is not applicable.

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