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BIOPOL – Udvinding af biopolymer fra spildevand

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Forord

Formål med projektet

Hovedformålet med projektet ”BIOPOL - Udvinding af biopolymer fra spildevand” har været at undersøge, udvikle og evaluere potentialet for produktion af biopolymerer til bioplastproduktion fra industri- og byspildevand. Dette er blevet realiseret via laboratorie eksperimenter og pilotforsøg, hvorigennem de teknologiske muligheder for fuldskala bioplastanlæg er blevet afdækket.

Projektet er støttet gennem miljøministeriets Miljøteknologisk Udviklings- og Demonstrations Program (MUDP).

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Konklusion og sammenfatning

Formålet med projektet ”BIOPOL - Udvinding af biopolymer fra spildevand” har været at undersøge, udvikle og evaluere potentialet for produktion af biopolymer til bioplastproduktion fra industri- og byspildevand. For at afdække, hvordan den bedste ressourceudnyttelse opnås, er resultaterne fra projektet anvendt til at sammenligne CO_2-belastningen for fossil-, fødevare- og spildevandsbaserede polymerer, samt sammenligne CO_2-belastningen ved hhv. biogas- og biopolymerproduktion fra spildevand.

Biopolymererne, polyhydroxyalkanoater (PHA), kan under de rette betingelser produceres af de naturligt forekommende bakterier i et renseanlæg. På baggrund af et 9 måneder langt pilotforsøg med spildevand fra KMC, der producerer kartoffelbaserede ingredienser, er der i projektet udviklet en proces til produktion af biopolymer, der kan opskaleres til kommerciel skala.

PHA'en produceres gennem en proces, der er udviklet og patenteret af Veolia (Cella™-processen). Processen består af 4 trin, hvor spildevandet fermenteres til let-omsætteligt kulstof, og de bakterier, der lagrer PHA, favoriseres og opformeres ved at de udsættes for skiftevis substratoverskud og -underskud. Den producerede PHA ekstraheres og oprenses endelig til en biopolymer, der har egenskaber, som er sammenlignelige med kommercielt tilgængeligt PHA.

Spildevandet fra KMC udmærker sig ved at have et højt indhold af VFA (flygtige fede syrer), hvilket giver et højt PHA-potentiale og overflødiggør fermenteringsprocessen. Over 9 måneder blev der udført 15 akkumuleringer i pilotforsøg, og der blev produceret 7,5 kg PHA. Resultaterne viste, at det var muligt at opformere biomasse med et højt akkumuleringspotentiale, der i løbet af forsøget steg fra 0,3 g PHA/g VSS til 0,7 g PHA/g VSS, samtidig med at der blev opretholdt en stabil og effektiv renseproces af spildevandet. Som sidegevinst opnåede slammet en markant forbedring af slamegenskaberne, der har været ringe som følge af dårlige bundfældningsegenskaber.

Der er blevet udviklet et målrettet design til KMC, og biopolymerproduktion vil kunne realiseres på basis af det eksisterende industrirenseanlæg ved blot at tilføje en 200 m^3 beluftet tank.

For at undersøge biopolymer potentialet i byspildevand blev der udført PHA-akkumuleringsforsøg i laboratorieskala med aktiv slam prøver fra luftningstankene på Marselisborg Renseanlæg (MR) og Billund Biorefinery (BBR). Bakterierne i byspildevand fra MR og BBR opnåede et PHA-indhold i biomassen på henholdsvis 0,26 g PHA/g VSS og 0,18 g PHA/g VSS. Det antages, at PHA-produktionen kan øges til 0,4-0,5 g PHA/g VSS ved optimering af spildevandsprocesserne på MR og BBR, for at sikre en favorisering af de PHA akkumulerende bakterier.For at få en kommerciel interessant PHA-oprensningsproces, er det nødvendigt at opnå koncentrationer på mere end 0,4 g PHA/g VSS.

På baggrund af de realiserede udbytter vurderes det, at potentialet for PHA-produktion på de tre anlæg er 450 t PHA/år for KMC, 200 t PHA/år for BBR og 1.000 t PHA/år for MR (2.300 t PHA/år i det ny MR (500.000 PE)), hvilket tilsammen er en årlig produktion på 1.650 t PHA. Fuldskala biopolymer produktion vil kræve ombygning og driftsændringer på alle tre anlæg. BBR modtager kildesorteret affald og organisk industriaffald, der kan indgå i processen med en årlig PHA-produktion på 150-200 t PHA/år.

CO_2-belastningerne for PHA produktion fra hhv. spildevand, fossile kilder og fødevarer er blevet sammenlignet. Her ses det, at PHA fra spildevand har en markant lavere CO_2-belastning (1,42 g CO_2/g PHA) end både de fødevare- (3,44 g CO_2/g PHA for sojabønner) og fossilt-baserede (1,9 g CO_2/g PP) biopolymerer. Dette skyldes kombinationen af, at råvaren i spildevandsbaseret bioplast antages ikke at have en CO_2-belastning, da det er et affaldsprodukt, samt at der anvendes en råvare, der ikke behøver den energikrævende fermenteringsproces. PHA produceret fra spildevand er således et fornybart alternativ til allerede eksisterende biopolymere med sammenlignelige egenskaber. Yderligere har det markant lavere CO_2-belastning end de nuværende konventionelle plastprodukter.

Conclusion and summary

The purpose of this project "BIOPOL- Production of biopolymer from wastewater" was to examine, develop and evaluate the potential for production of biopolymer for bioplastic production from industrial and domestic wastewater. Furthermore, the CO_2-load for fossil based, food based and wastewater based plastic polymer has been compared. In order to uncover the best use of resources, an evaluation of the CO_2-load from biogas production and biopolymer production from wastewater has been made.

The biopolymer polyhydroxyalkanoate (PHA) can at the right conditions be produced by the natural inherent bacteria in a wastewater treatment plant. Based on 9 months of pilot experiments with wastewater from KMC, where products based on potatoes are produced, a biopolymer production that can be up-scaled to commercial scale was developed.

PHA is produced from wastewater through a process that has been developed and patented by Veolia (Cella^{TM}-process). The process consists of 4 steps (PE1-PE4); The wastewater is fermented to readily biodegradable carbon and microorganisms storing PHA are favoured by exposure to alternating feast and famine conditions. The produced PHA is extracted and purified to a biopolymer comparable to commercial available PHA.

The wastewater from KMC was chosen, since it has a high level of VFA (volatile fatty acids) which facilitate a high PHA-potential and eliminates the initial fermentation process. During the 9 months of pilot experiments 15 accumulation-trials were completed and 7.5 kg PHA was produced. The results showed that it was possible to enrich the PHA accumulation potential of the biomass, which increased from 0.3 g PHA/g VSS to 0.7 g PHA/g VSS, while maintaining a stable and efficient wastewater treatment process and improving the normally poor sludge settling properties. For a commercial interesting purification process, the PHA concentration needs to be above 0.4 g PHA/g VSS.

A specific process design was developed for KMCs wastewater treatment plant and PHA production can be achieved by addition of a 200 m^3 aerated process tank.

Samples from the process tanks from Marselisborg wastewater treatment plant (MR) and Billund Biorefinery (BBR) were used to examine the biopolymer potential of domestic wastewater. The samples were tested for PHA-accumulation at laboratory scale. The bacteria in domestic wastewater from MR and BBR obtained a PHA-concentration in the biomass of respectively 0.26 g PHA/g VSS and 0.18 g PHA/g VSS. It is assumed that the PHA-production can be increased to 0.4-0.5 g PHA/g VSS if the existing processes at MR and BBR were optimised.

Based on the experimental results, the assessed potential of PHA-production for the three plants are; KMC: 450 t PHA/y, BBR: 200 t PHA/y and MR: 850 t PHA/y (2,300 t PHA/y for the new Marselisborg wastewater treatment plant). It will demand a certain degree of reconstruction for all three plants, before PHA production is possible. BBR receives organic household waste and organic industrial waste which could be integrated in the process resulting in a production of 150-200 t PHA/y.

The CO_2-footprint from PHA-production from wastewater, fossil sources and food were compared. The PHA from wastewater showed to have a significant lower CO_2-footprint (1.42 g CO_2/g PHA) than food- (3.44 g CO_2/g PHA for soybeans) and fossil based (1.9 g CO_2/g PP) biopolymers, due to the assumption that wastewater has no CO_2-footprint along with the use of a raw material where the energy demanding fermentation process isn't needed. PHA based on wastewater is therefore a renewable alternative to the existing conventional plastic products that have a significant higher CO_2-footprint.

1. Introduktion

1.1 Baggrund for projektet

Plast er i dag et uundværligt materiale, der bruges i en lang række forskellige anvendelser. Den udbredte brug af plast medfører dog en række miljømæssige udfordringer, såsom forurening med plast i naturen, lav genanvendelighed og udledning af giftige forbindelser ved forkert afbrænding.

Konventionel 1. generations plast fremstilles fra ikke-fornybare oliebaserede produkter, og udgjorde i 2015 99% af den anvendte plast (European Bioplastics 2016, Plastics Europe 2016). Biobaseret plast er et fornybart alternativ, der fremstilles af biologiske råvarer (kaldet 2. generations plast) eller restprodukter (kaldet 3. generations plast). 3. generations plast er at foretrække, da det ikke påvirker fødevareproduktion eller -priser. En eventuel realisering af 3. generations plast vil medføre et paradigmeskifte ikke kun inden for plastindustrien, men også inden for ressourcegenvinding fra restprodukter og for miljøet.

Polyhydroxyalkanoater (PHA) er en type biopolymer, der kan anvendes til produktion af 3. generations plast ud fra spildevand. Under de rette betingelser kan PHA produceres af de naturligt forekommende bakterier i et renseanlæg i en kvalitet, der er sammenlignelig med det PHA, der i dag er tilgængelig på markedet.

Kommerciel produktion af biopolymerer fra spildevand foregår ikke i dag, men pilotforsøg på spildevand fra en hollandsk sukkerfabrik har vist, at PHA kan udvindes fra spildevand samtidig med, at spildevandet renses til udledningskvalitet (Anterrieu et al., 2014). Tilsvarende resultater er opnået i laboratorieforsøg med byspildevand (Morgan-Sagastume et al., 2014).

Formålet med dette projekt har været at udvikle en proces til produktion af PHA baseret på industri- og byspildevand, der kan opskaleres til kommerciel skala, samt at sammenligne CO_2-belastningen ved hhv. energi- og PHA-produktion fra spildevand. Ydermere er CO_2-belastningen for PHA produceret fra spildevand også sammenlignet med biopolymer produceret fra fødevarer og fossile kilder.

1.2 Overordnet projektindhold

I projektet "BIOPOL – Udvinding af biopolymer fra spildevand" er potentialet for kombineret PHA-produktion og spildevandsrensning fra både industrielt- og byspildevand undersøgt gennem hhv. laboratorieforsøg med spildevand fra Billund Biorefinery (BBR) og Marselisborg Renseanlæg (MR) samt gennem et 9 måneder langt pilotforsøg med spildevand fra KMC, der fra kartoffelstivelse producerer derivater til fødevareindustrien. Spildevand fra de tre renseanlæg betragtes som separate cases, og en nærmere beskrivelse findes nedenfor:

Case 1: Produktion af biopolymerer i pilotskala fra spildevand fra KMC

KMC i Brande omdanner kartofler til kartoffelmel og derivater af stivelse til fødevareindustrien. Spildevandet fra KMC's produktion udmærker sig ved at have et højt indhold af flygtige fede syrer (VFA), der er spildevandsbakteriernes byggesten i produktionen af PHA.

Foruden at have en spildevandssammensætning, der er ideel i forhold til produktion af PHA, så har KMC også et ønske om at forbedre deres spildevandsproces, der har haft udfordringer med dårlige slamegenskaber samt problemer med at overholde udledningskravene. Renseprocessen med PHA-produktion forventes at overholde udledningskravene samtidig med, at der produceres PHA. Spildevandssammensætningen fra KMC varierer betydeligt hen over året, og over de 9 måneders pilotdrift var det dermed muligt at udvikle PHAproduktionsprocessen og kortlægge dens stabilitet over tid ift. varierende indløbskoncentrationer og spildevandssammensætning.

Case 2: Evaluering af biopolymerpotentialet fra spildevand på MR
Marselisborg Renseanlæg er et traditionelt 2-trins anlæg med primær fældning, hvor der høstes kulstof til biogasproduktion. Renseanlægget har effektiv bundbeluftning og drives som et Bio-denitro anlæg. I 2014 blev der etableret separat rejektvandsrensning på renseanlægget, der i 2015 var belastet med knap 190.000 PE(BOD), hvoraf ca. 40 % kommer fra byens industrier.

Anlægget ejes af Aarhus Vand, der står for at skulle bygge et nyt anlæg på ca. 500.000 PE som samlet erstatning for MR, Viby Renseanlæg og Åby Renseanlæg.

Case 3: Evaluering af biopolymerpotentialet fra spildevand på BBR
Billund Biorefinery (BBR), der er et energiproducerede renseanlæg i Grindsted, har en kapacitet på 50.000 PE og repræsenterer den nyeste teknologi indenfor spildevandsbehandling. Foruden spildevand behandler BBR også organisk husholdningsaffald, der omdannes til biogas, som anvendes til varme- og el-produktion.

2. Biopolymerproduktion fra spildevand

2.1 Biopolymerer og bioplast

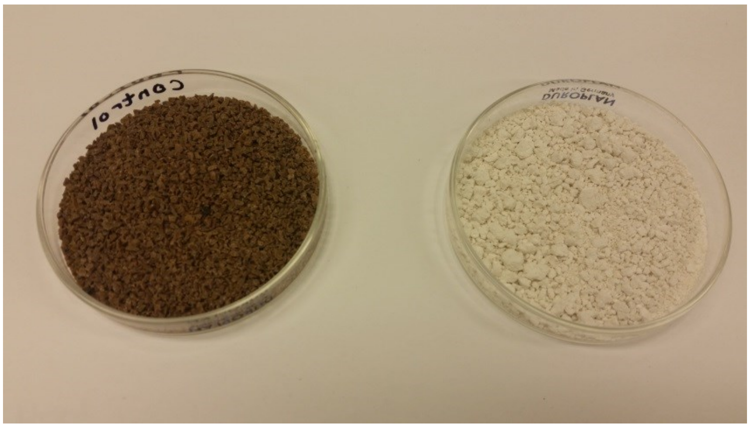
Bioplast fremstilles udelukkende af fornybare biopolymerer, der produceres fra biologiske materialer, og i dag baseres produktionen af bioplast hovedsagligt på kulhydratrige højtydende og robuste afgrøder såsom majs og sukkerrør. Størstedelen af produktionen i 2016 foregik i Asien (43%) og Europa (27%) (European Bioplastics 2016), og bioplasten anvendes bl.a. til produktion af emballage (FIGUR 1).

FIGUR 1: Forskellige biobaserede plast-produkter (Nova Institute, 2016)

Mange biopolymerer er bionedbrydelige, hvilket betyder, at de omdannes i naturen til biologisk materiale, vand og CO_2. Hvorvidt plast er bionedbrydeligt er uafhængigt af plastens oprindelse og handler udelukkende om, om plasten nedbrydes indenfor rammerne (tid, temperatur, betingelser, grad af nedbrydelse) angivet i internationale standarder (såsom ISO 7827, ASTM D55226 og OECD301). Et produkt er bionedbrydeligt i henhold til OECD 301 seriens definition, når mere end 70% er nedbrudt efter 28 dage i et aerobt vandigt miljø. Den endelige udformning af plasten har dog også betydning for, om produktet i praksis er bionedbrydeligt, hvilket fx betyder, at en bioplast, der er bionedbrydelig i sin oprindelige form, godt kan falde udenfor kriterierne for bionedbrydelighed, hvis den fx anvendes til et produkt med en høj godstykkelse.

2.1.1 Biopolymerer fra spildevand

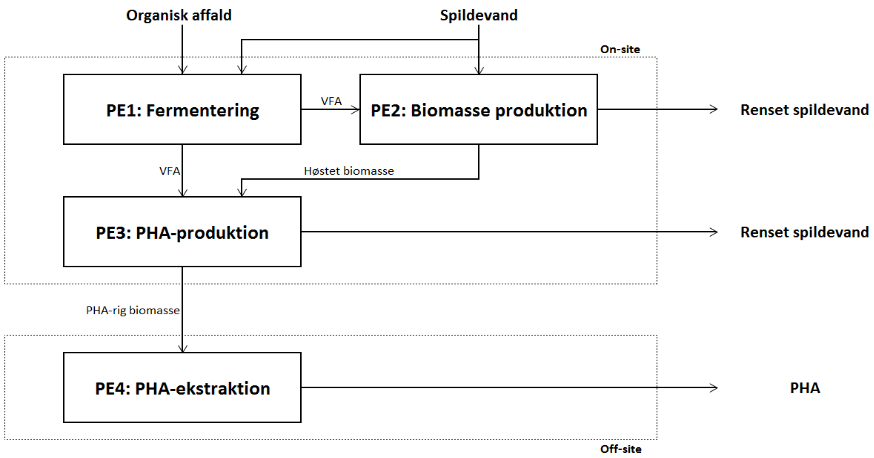
PHA er en fællesbetegnelse for en gruppe bionedbrydelige biopolymerer, der bl.a. kan fremstilles fra spildevand. De kan anvendes til at fremstille både hårde og bløde bio-baserede og -nedbrydelige plastprodukter. Produktion af biopolymerer fra spildevand muliggør, at renseanlæg omdannes fra at være en slutbehandling af affaldsprodukter til at være materialeforædlende bioraffinaderier, der producerer fornybare højværdiprodukter. I Leeuwarden i Holland er der udført forsøg i pilotskala, som har vist, at det er muligt at producere biopolymerer fra spildevand (Dutch Water sector, 2015).



FIGUR 2: PHA-rig biomasse og ekstraheret PHA

PHA fra spildevand produceres af flere naturligt forekommende bakterier i et spildevandsanlæg. Bakterierne anvender PHA til oplagring af energi og kulstof, samtidig med at spildevandet renses. For at sikre maksimal PHA-udbytte skal spildevandsprocessen foregå under betingelser, der favoriserer tilstedeværelsen af de PHA-oplagrende bakterier samt deres produktion af PHA, hvilket opnås gennem faser med hhv. høje og lave substratmængder kaldet fest-faste faser (Serafim *et al.*, 2008). Derudover har den organiske belastning (OLR) og slamalderen (SRT) betydning for PHA-udbyttet, og samtidig skal processen foregå under betingelser, der tillader en stabil spildevandsrensningsproces.

Veolia har udviklet og patenteret Cella^{TM}-processen med henblik på at producere PHA fra spildevand. Processen er opdelt i 4 procesenheder (PE1-PE4), hvilket ses nedenfor. Her ses et overordnet proces flow diagram for Cella^{TM}-processen sammen med en kort beskrivelse af de enkelte procesenheder.



FIGUR 3: Overordnet proces flow diagram for PHA-produktionen

- **PE1: Fermentering**. Her omdannes organisk materiale (fx spildevand, spildevandsslam eller husholdningsaffald) til fermenteringsprodukter såsom flygtige fede syrer. For organiske materialer med et højt indhold af flygtige fede syrer vil dette trin ikke være nødvendigt.

- **PE2: Biomasseproduktion**. Her fremmes betingelserne for en biomasse med en øget evne til at lagre PHA gennem skiftende fest- og faste-betingelser, hvor der er hhv. substratover og -underskud.

- **PE3: PHA-produktion**. Her produceres PHA'en af biomassen beriget i PE2 gennem anvendelse af en fødestrøm med et højt indhold af flygtige fede syrer.

- **PE4: PHA-ekstraktion.** Her ekstraheres den producerede PHA fra overskydende biomasse. Typisk er potentialet i et enkelt renseanlæg ikke stort nok til at levere mængderne, der kræves for at PHA-ekstraktionen er rentabel, hvorfor det anbefales at placere PE4 som et centralt anlæg, der ekstraherer PHA fra overskudsbiomasse fra flere renseanlæg.

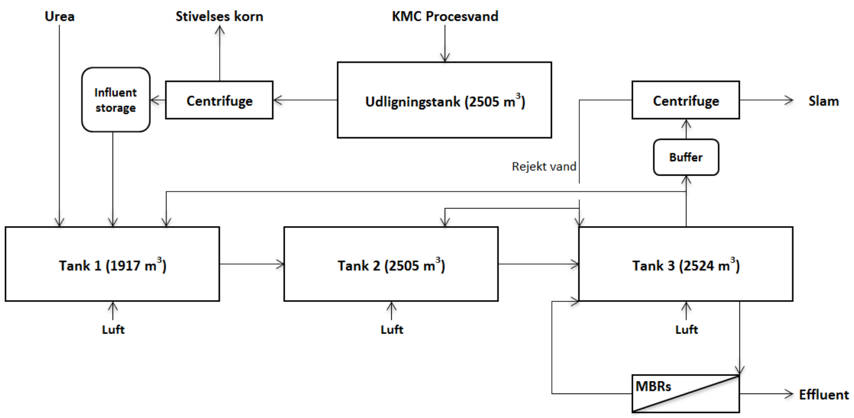
Som tidligere nævnt er Cella™-processen testet i pilotskala med spildevand i Holland (Dutch Water sector, 2015), og derudover er der tidligere udført laboratorieforsøg med spildevand fra KMC (De Grazia, 2014) med henblik på at finde de ideelle driftsbetingelser for det pilotforsøg, der blev udført i nærværende projekt.

3. Resultater og diskussion

3.1 Biopolymerproduktion på KMC's Renseanlæg

Spildevand fra KMC blev anvendt til pilotforsøg fra marts-december 2015. Spildevandet blev udvalgt, da det på forhånd blev betragtet som den ideelle fødestrøm til PHA-produktion grundet et højt VFA-indhold. Dette betyder, at spildevandet har et højt PHA-potentiale, der kan realiseres uden forbehandling (PE1). Derudover oplevede KMC udfordringer med dårlige slamegenskaber i deres eksisterende renseanlæg. De dårlige slamegenskaber kommer til udtryk i form af meget højt slamvolumenindeks (SVI), der i perioder er målt til over 700 mL/g. SVI beskriver slammets bundfældningsegenskaber, og for at opnå en velfungerende rensningsproces anbefales SVI-værdier <180 mL/g. Ydermere blev der observeret problemer med at reducere det organiske indhold i spildevandet tilfredsstillende. De PHA-producerende bakterier er kendetegnet ved at have højere densitet end typisk slam, og det var derfor forventet, at introduktionen af Cella^{TM}-processen foruden produktion af PHA også ville give en biomasse med bedre slamegenskaber og dermed også en mere robust vandrensningsproces med tilfredsstillende rensegrader.

For at imødekomme udfordringerne med dårlige slamegenskaber og utilfredsstillende rensegrader har KMC installeret membranfiltrering til slutbehandling af det rensede spildevand. Det eksisterende renseanlæg er et aktivt slam-anlæg, der opererer med en slamalder på 45 dage. Et overordnet proces flow diagram for anlægget er illustreret nedenfor.



FIGUR 4: Opbygningen af KMC's eksisterende renseanlæg

3.1.1 Pilotforsøg hos KMC

Selve pilotforsøget blev udført hos KMC (FIGUR 5) med en opstartsbiomasse fra tre forskellige spildevandsanlæg (Brande, Sjölunda, Malmö) som inoculum med det selvsamme spildevand, som det eksisterende renseanlæg på KMC behandler. Formålet med forsøget var at udvikle og optimere Cella^{TM}-processen samt opnå driftsdata baseret på et varierende råmateriale, der muliggør opskallering og udvikling af en fuldskala proces til kommerciel produktion af PHA-rig biomasse.

FIGUR 5: Udstyret anvendt til forsøget hos KMC. Containeren til højre indeholdt PE2, containeren i midten PE3, og containeren til venstre indeholdt tørringsudstyr.

I de første 2 måneder blev der anvendt ubehandlet spildevand fra KMC. Uafhængigt af pilotforsøget, blev der herefter installeret dekantere på KMCs renseanlæg til fjernelse af stivelseskorn, som ikke blev omsat i det eksisterende spildevandsanlæg. Dekanteren blev installeret, således stivelseskornene blev fjernet inden spildevandsrensningen og dermed ikke optog kapacitet i anlægget. Den resterende del af forsøget blev derfor udført med centrat fra dekanteren. Middelsammensætningerne og de tilhørende standardafvigelser for spildevandet er angivet i TABEL 1 nedenfor.

TABEL 1: Sammensætningen af KMC-spildevand under pilotforsøget

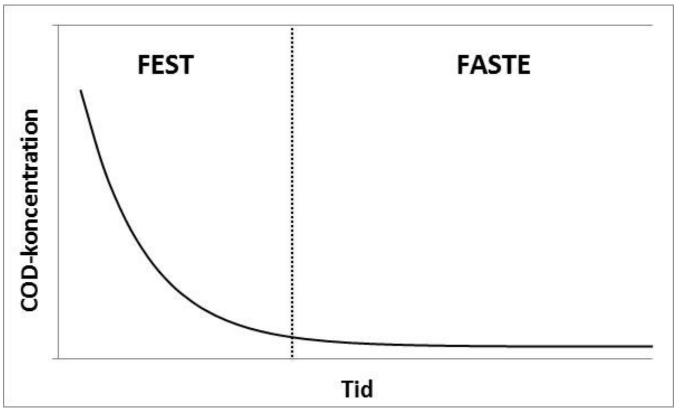
	Enhed	Værdi
Total COD-indhold	[g COD/L]	9,7 ±1,5
SCOD	[g COD/L]	9,5 ±1,5
VFA-indhold	[% of SCOD]	73 ±10
Temperatur	[°C]	14 – 24,1
pH	[-]	4,8 – 6,5

Spildevandet fra KMC har et højt indhold af VFA (73% af det opløste COD), hvorfor PE1: Forbehandling ikke var nødvendig.

PE2: Spildevandsbehandling foregik i en 500L Sequencing Batch Reactor (SBR). Betingelserne i SBR'en svarede til hvad der opleves i en ideel Plug Flow Reactor (PFR), hvor indholdet bevæger sig gennem reaktorens forskellige områder, der svarer til de forskellige batch-faser i SBR'en. Resultaterne kan derfor opskalleres til både SBR og PFR reaktor-typer. SBR'en blev opereret med 7,5 timers cyklustid, der bestod af:

- Fyldningsfase (2,5 minut)
- Reaktionsfase (415 minutter)
- Bundfældningsfase (30 min)
- Udtømningsfase (2,5 minut)

I fyldningsfasen tilsættes recirkuleret biomasse med spildevand, hvorved der opnås en høj substrat mængde (fest betingelser). Løbende omsættes COD'en og ved at tilbageholde spildevand, kan der opnås substrat-begrænsede betingelser (faste) for biomassen, som illustreret på FIGUR 6 nedenfor.



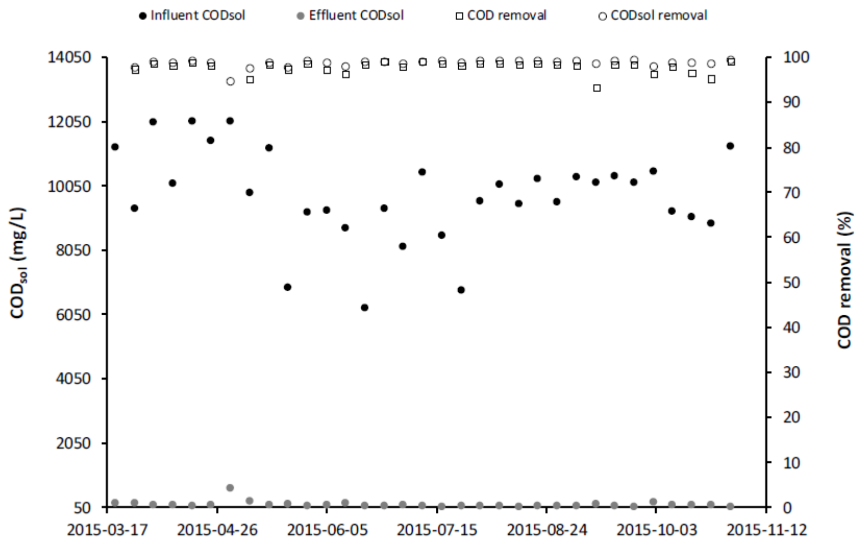
FIGUR 6: COD koncentration i SBR'en som funktion af tiden.

Mikro- og makronærringsstoffer blev tilsat reaktoren for at sikre optimalt forhold mellem COD, P samt N, og overskydende biomasse blev dagligt overført til en 160 L omrørt tank.

PE3: PHA-akkumulering foregik som en fed-batch proces, hvor spildevand blev tilsat pulsvis med henblik på at sikre konstant substratoverskud. Processen blev udført i en 550 L reaktor, hvorfra der blev recirkuleret til en 120 L bundfældningstank. Den PHA-rige biomasse blev afvandet med centrifuge til 15-20%TS og tørret ved 70°C i 24 timer. PHA blev efterfølgende ekstraheret hos AnoxKaldnes i Lund.

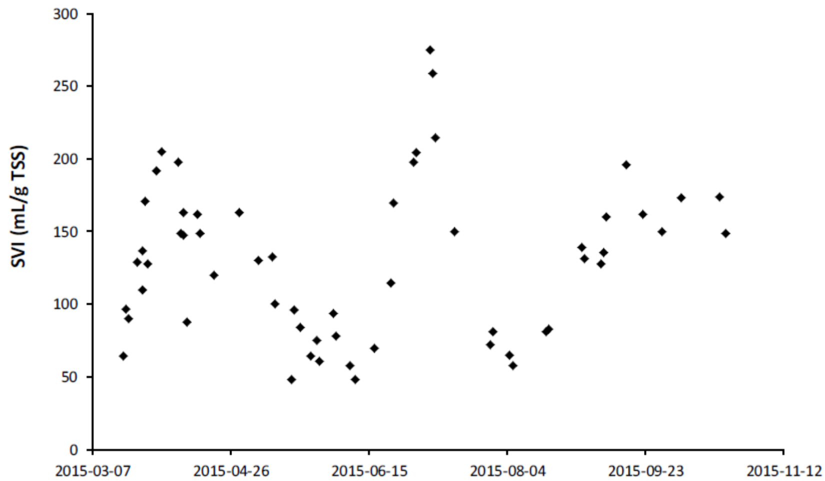
3.1.2 Resultater fra forsøget hos KMC

Pilotforsøget forløb stabilt og der blev kun observeret mindre stop undervejs grundet mindre tekniske nedbrud. På FIGUR 7 er omsætningen af COD gennem pilotforsøget angivet (middel omsætning af COD = 98 ±1,1 %). Her ses det, at der opnåedes stabile omsætningsgrader, der tillader, at det rensede spildevand ville kunne udledes uden den eksisterende membranfiltrering.



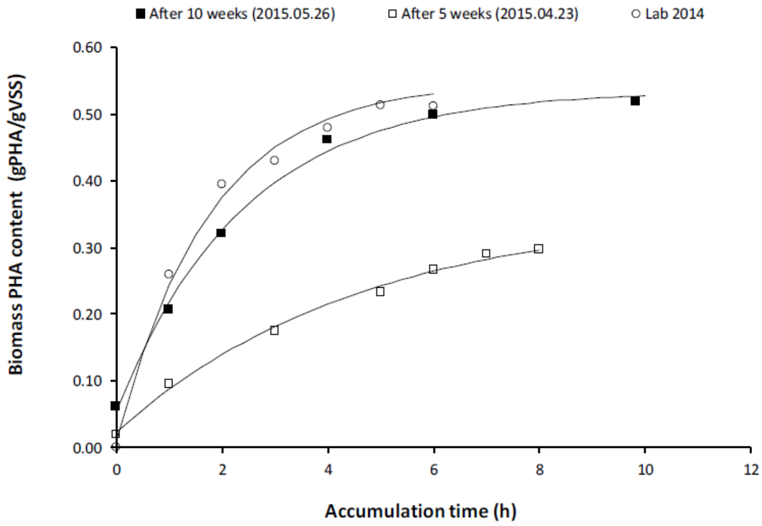
FIGUR 7: COD-indhold i KMC's spildevand før (sorte cirkler) og efter (grå cirkler) spildevandsrensning i pilotanlægget samt COD-omsætning (hvide cirkler og firkanter)

Foruden at producere biopolymer er formålet med eksperimenterne med Cella^{TM}-processen også at undersøge muligheden for at forbedre slamegenskaberne på KMC's renseanlæg. På FIGUR 8 nedenfor ses udviklingen i slamvolumenindeks i pilotanlægget, der beskriver slammets bundfældningsegenskaber. I forhold til det eksisterende renseanlæg, hvor der er observeret SVI-værdier på over 700 mL/g TSS, blev der observeret en markant forbedring, og gennem størstedelen af forsøget blev der målt SVI-værdier lavere end de 180 mL/g TSS, som der typisk anbefales for god bundfældning.



FIGUR 8: Udviklingen i slamvolumenindeks af slammet i pilotanlægget i løbet af pilotforsøget

PE3: PHA-akkumulering foregik med spildevand fra KMC, som fødestrøm til fed-batch-processen. For at afdække akkumuleringspotentialet af den opbyggede biomasse i PE2 blev der også udført laboratorieforsøg med anvendelse af syntetisk substrat i form af eddikesyre, der giver det maksimale PHA-akkumuleringspotentiale af biomassen. På FIGUR 9 er PHA-udbytterne opnået på KMC's spildevand (hvide og sorte firkanter) sammenlignet med udbyttet, der blev opnået med eddikesyre (hvide cirkler). Gennem pilotforsøget med KMC's spildevand blev der observeret en betydelig forøgelse i udbyttet fra 0,3 til 0,5 g PHA/g VSS. For at opnå en kommerciel interessant oprensningsproces er det nødvendigt at opnå koncentrationer højere end 0,4 g PHA/g VSS, hvilket dermed betyder, at spildevandet på KMC's renseanlæg har tilstrækkeligt PHA-udbyttepotentiale til, at det er kommercielt interessant. Forsøg med eddikesyre som substrat indikerede, at der med den opbyggede biomasse i pilotanlægget kan opnås endnu højere udbytter gennem videre optimering af processen (*Lab 2014*, FIGUR 9), og i november 2015 blev der udført endnu et laboratorieforsøg, hvor man opnåede et udbytte på 0,7 g PHA/g VSS.



FIGUR 9: PHA-udbytterne opnået med KMC's biomasse og spildevand efter 5 (hvide firkanter) og 10 ugers pilotforsøg (sorte firkanter) og PHA-udbyttet opnået med KMC biomasse og eddikesyre (hvide cirkler).

Samlet set blev der udført 15 akkumuleringer i pilotforsøget, og der blev produceret 7,5 kg PHA.[1]

Se Bilag 1 for detaljeret beskrivelse af pilotforsøget..

3.1.3 Fuldskala PHA-produktion på KMC

I TABEL 2 ses procesbetingelserne for pilotforsøget, der danner grundlag for designet af fuldskala PHA-produktion på KMC.

TABEL 2: Driftsbetingelser for og resultater fra pilotforsøget hos KMC

	Enhed	Værdi
Organisk belastning	[g COD/L/d]	2,2 ±0,4
Hydraulisk belastning	[d]	4,5 ±0,6
Slamalder	[d]	7,4 ±1,1
Temperatur	[°C]	19-36
pH	-	4,2-8,9
COD-fjernelse	[%]	98 ±1,1
COD-indhold i udledning	[mg SCOD/L]	124 ±103

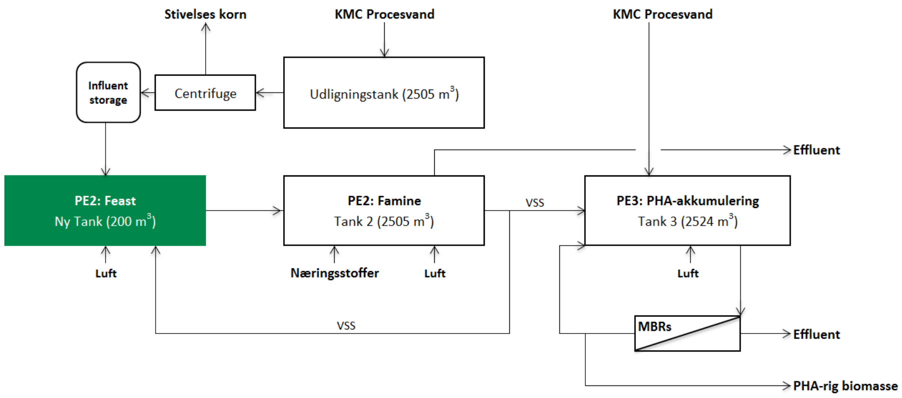
[1] De specifikke typer af PHA-polymerer produceret fra KMC's spildevand var PHBV, 3HV og 3HB, mens forsøgene med eddikesyre som substrat førte til produktion af PHB-polymeren. Fælles for alle PHA-polymererne er, at de har egenskaber, der er sammenlignelige med kommercielt tilgængeligt PHBV.

Som det fremgår af Tabel 2, blev der opereret med en slamalder på kun 7 dage i pilotforsøget, hvilket førte til en markant forbedring af slamegenskaberne ift. de observerede slamegenskaber i det eksisterende renseanlæg, der opererer med en slamalder på 45 dage. Lang opholdstid kan medføre over-oxidation og ødelæggelse af flokkene i det aktive slam, hvilket resulterer i en biomasse med dårlige separationsegenskaber. Den reducerede slamalder nedsætter ligeledes det nødvendige procesvolumen i spildevandsanlægget med 2.300 m^3 fra 2.400 m^3 4.700 m^3 og øger biomasseudbyttet til 0,26 kg VSS/kg COD fjernet. En sammenligning mellem driftsbetingelserne for pilotanlægget (Cella^{TM}-processen) og KMC's eksisterende renseanlæg kan findes i Tabel 3 nedenfor. Der blev i pilotforsøget observeret en markant forbedring af slamegenskaberne i form af en reduktion af slamvolumenindekset, og derudover blev der opnået en reduktion i iltforbruget på 25 % som følge af et øget biomasseudbytte, hvilket medfører en markant reduktion i elforbruget til driften af renseanlægget.

TABEL 3: Sammenligning af driftsresultaterne for KMC's eksisterende renseanlæg og Cella^{TM}-processen.

	Enhed	KMC renseanlæg	Cella^{TM}-processen
SRT	Dage	45	7
Volumen	m3	7.000	2.500 + 200 (PE2) 2.500 (PE3)
Biomasseudbytte	kg VSS/kg COD fjernet	0,11	0,26
Iltforbrug	kg O2/kg COD fjernet	0,84	0,63
SVI	mL/g TSS	>200	<170

Baseret på PHA-udbyttet på 0,35 kg PHA/kg COD opnået i pilotforsøget og de 404 m^3 spildevand/døgn vurderes det, at der på KMC's renseanlæg med nuværende produktion kan produceres 450 tons PHA/år. På FIGUR 10 nedenfor er det illustreret, hvordan spildevandsrensning og PHA-produktion kunne integreres hos KMC. Designet er udviklet med udgangspunkt i de i forvejen tilgængelige tanke og udstyr på renseanlægget. Som nævnt tidligere så har spildevandet fra KMC et højt VFA indhold (> 70% af COD), hvorfor det ikke er nødvendigt med et forbehandlingstrin (PE1).



FIGUR 10: Konceptuel proces flow diagram for produktion af PHA-rig biomasse hos KMC i fuldskala.

PE2 – fastefasen og PE3 – PHA-akkumulering kan begge foregå i eksisterende tanke, mens PE2 - festfasen anbefales adskilt fra PE2-fastefasen, hvorfor det er nødvendigt med en ny tank på 200 m^3. Formålet med PE2 – festfasen er at fjerne COD, og det anbefales, at processen udføres i enten en Sequencing batch reactor (SBR) eller Plug Flow reactor (PFR). PFR'en udmærker sig ved at være en kontinuert proces, hvor recirkuleret biomasse blandes med spildevand fra KMC. Som navnet antyder, opnås et plug flow gennem reaktoren, og COD omsættes løbende gennem reaktoren. Der afgår ikke tid til fyldning og tømning af reaktoren, som det er tilfældet for SBR'en. SBR'en betragtes til gengæld som en fleksibel løsning, som kan håndtere variationerne i spildevandet fra KMC gennem justering af F/M-forholdet og opholdstiden.

Driften af PE2 – fastefasen vil afhænge af designet af PE2 – festfasen. Såfremt festfasen designes som en SBR, så skal faste-fasen ligeledes opereres i cykler, hvor volumenet skiftevis anvendes til aerob behandling efterfulgt af biomasse-bundfældning og dekantering af renset spildevand. Kvaliteten af det rensede vand forventes at være indenfor udledningskravene. Ved PFR design af festfasen vil fastefasen ligeledes opereres som en kontinuert proces, og en efterfølgende separationsproces vil være nødvendigt. Processen vil fungere som en konventionel aktiv-slam proces med intern slam-recirkulering.

De forventede designbetingelser for både SBR- og PFR-reaktoren er angivet i TABEL 4 nedenfor.

TABEL 4: Forventede procesbetingelser for fuldskala PE2-proces hos KMC

Fest-fase		**SBR**	**PFR**
SRT = HRT	Minutter	30 - 45	
SOLR	KgCOD/kgSS/dag	~9	
Volumen	m^3	200 – 250	150 – 200
Fest-cykler	Antal/døgn	~30	~45
COD-fjernelse	%	>98	
Faste-fase		**SBR**	**Kontinuert**
Volumen	m^3	~2.300	
COD:N:P	kgCOD:kgN:kgP	100 : 4.5 : 0.6	
Slamproduktion	kgVSS/dag	1235	
Samlet proces			
SRT	Dage	7	
HRT	Dage	5	
OLR	$kgCOD/d/m^3$	~2	
Elforbrug			
-Fest	kW	~25	
-Faste	kW	~35 - 50	
Nitrogen (Urea)	kgN/d	215	
Fosfor (H_3PO_4)	kgP/d	10	

PE3: PHA akkumuleringen kunne udføres i KMC's eksisterende tanke ved brug af den ene af de aerobe tanke (2 eller 3) (se side 14), og MBR-enheder kunne bruges til biomasse separation.

Gennem pilotforsøget blev der tilsat både makro- (N og P) og mikro-næringsstoffer (K,Fe, Cu og Zn) for at sikre optimale vækstbetingelser. I et kommercielt anlæg vil teknisk kvalitet af makronæringsstofferne blive anvendt, der indeholder urenheder i form af bl.a. mikronæringsstoffer, hvilket kan reducere mængden af mikro-næringsstoffer, som skal tilsættes.

Se Bilag 2 for yderligere beskrivelse af fuldskalaintegrering af PHA-produktion på KMC's renseanlæg.

3.2 Biopolymerproduktion fra byspildevand

VFA-indholdet i typisk råspildevand fra husstande er højt nok til, at spildevandsslam kan akkumulere PHA, og der kan normalt opnås 5-15% PHA-koncentrationer i spildevandsslam uden nogen procesjustering eller optimering for PHA-produktion.

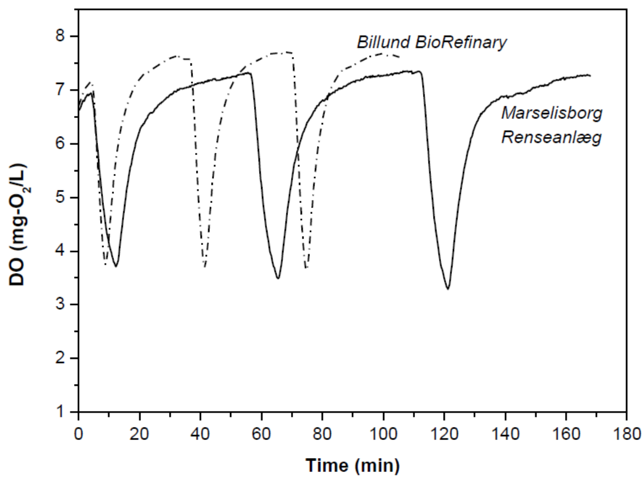
3.2.1 Laboratorieforsøg med slam fra MR og BBR

PHA-akkumuleringspotentialet for aktivt slam fra hhv. MR og BBR blev testet gennem laboratorieforsøg hos AnoxKaldnes i Lund på slamprøver udtaget fra renseanlæggenes beluftningstanke. Der blev ikke fortaget nogen form for justering af driften af de to renseanlæg i forbindelse med prøveudtagningen, og resultatet af forsøgene beskriver således potentialet ved den nuværende drift og er dermed basis for overvejelser for, hvordan Cella^{TM}–processen kan implementeres i fuldskala på anlægget.

De udtagne slamprøver blev anvendt til PHA-akkumuleringsforsøg, der foregik i et 2L reaktor fed-batch system. En substratblanding af eddikesyre, NH_4Cl og KH_2PO_4 og NaOH (tilsat for at justere pH til 5,3) blev anvendt som substrat i doser efter feed-on-demand. Allylthiourea (10 mg/L) blev tilsat for at inhibere nitrifikationen. Forsøget bestod af:

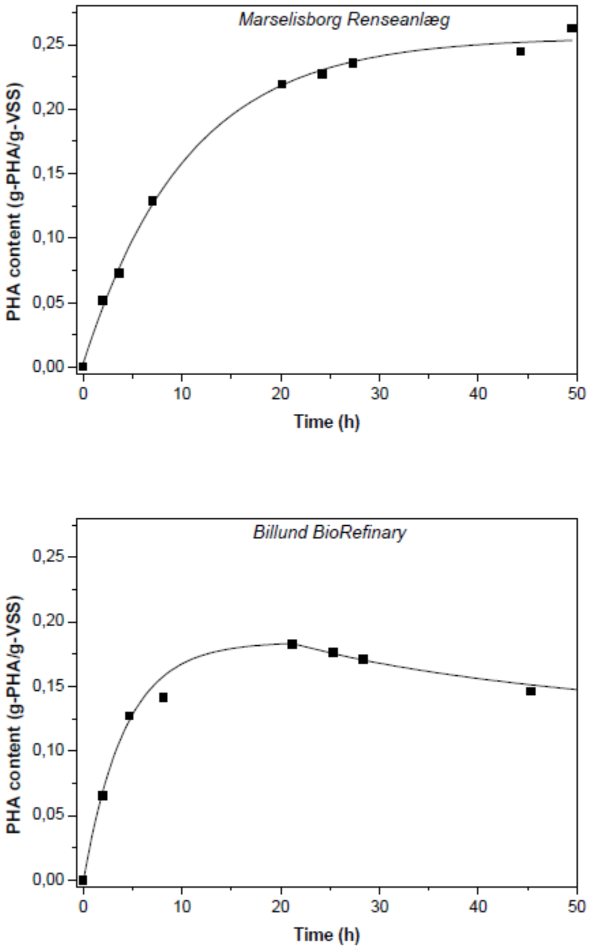
- Pre-akklimatisering med fest-betingelser efterfulgt af en fastefase, der blev gentaget 3 gange. Samlet tid 2-3 timer. Denne pre-akklimatisering udføres for at sikre sig, at de forskellige slamprøver er blevet behandlet ens inden akkumuleringstesten og Pre-akklimatiseringen er altså en slags "nulstilling" af slamprøven.
- Efterfølgende akkumuleringsfase af 2 dages varighed.

På FIGUR 11 nedenfor er profilen for opløst ilt (DO) illustreret for pre-akklimatiseringsprocessen.



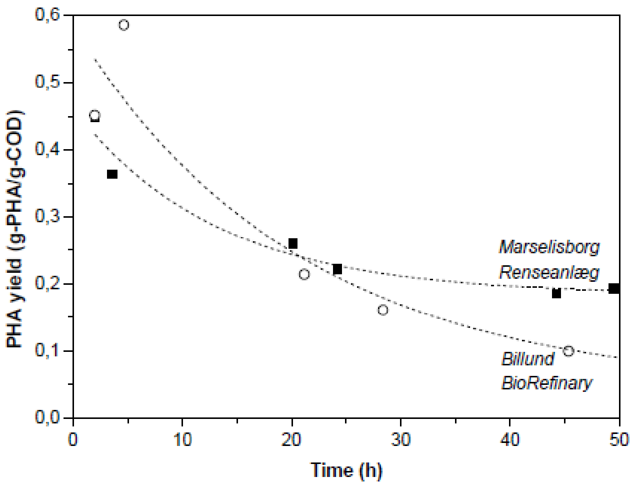
FIGUR 11: Opløst ilt-profil (DO) for pre-akklimatiseringsprocessen for slam fra Billund BioRefinery (BBR) og Marseliborg Renseanlæg (MR).

DO blev målt online og udviklede sig som forventet. Offline målinger af VFA og COD bekræftede, at der kun foregik begrænset akkumulering af COD i væskefasen. DO-trends viste stabil performance. Et lavt DO-niveau (0,5-1 mg O_2/L) i de første 20 timer af forsøget med MR-biomasse kan dog have betydet, at der har været iltbegrænsning, hvilket kan have påvirket det endelige PHA-udbytte, der er bestemt med termo-graviometrisk analyse ud fra massetabet mellem 250-310 °C. Til sammenligning blev test på BBR-slam udført med en DO-koncentration over 2 mg O_2/L. De opnåede PHA-indhold for hhv. BBR- og MR-slam er illustreret på FIGUR 12 nedenfor.



FIGUR 12: PHA-indhold for MR (øverst) og BBR (nederst) som funktion af tiden.

For MR blev der efter 48 timer opnået et PHA-indhold i biomassen på 26%. For BBR blev den maksimale koncentration på 18% opnået efter 24 timer, og i de resterende timer faldt udbyttet. Årsagen hertil skyldes bakteriernes forbrug af PHA, hvilket også kommer til udtryk i det målte PHA-udbytte illustreret i FIGUR 13. Her ses det, at BBR starter ud med et højere udbytte end MR, men efter 24 timer krydser de to kurver hinanden som følge af konsumeringen af PHA i BBR-slammet. Resultatet tyder på, at akklimatiseringen ikke har været tilstrækkelig for BBR, der kun havde 2 timers akklimatisering og ikke 3 timer, som det var tilfældet for MR.



FIGUR 13: PHA-udbyttet som funktion af tiden.

I TABEL 5 nedenfor er PHA-indholdet angivet for spildevandsslammet fra BBR og MR sammen med de teoretiske mængder af PHA, der potentielt kunne produceres på renseanlæggene, som de fremstår i dag. De opnåede koncentrationer ligger over de typiske udbytter opnået fra byspildevand, og andre studier har vist, at man gennem optimering af spildevandsprocessen kan øge udbytterne til 40-50% (Bengtson et al 2012). Pilotforsøg med byspildevand har ydermere bekræftet, at spildevand kan renses for både organisk indhold og kvælstof samtidig med, at der produceres PHA (Morgan-Sagastume et al. 2015, 2014).

TABEL 5: PHA-potentialet for BBR og MR.

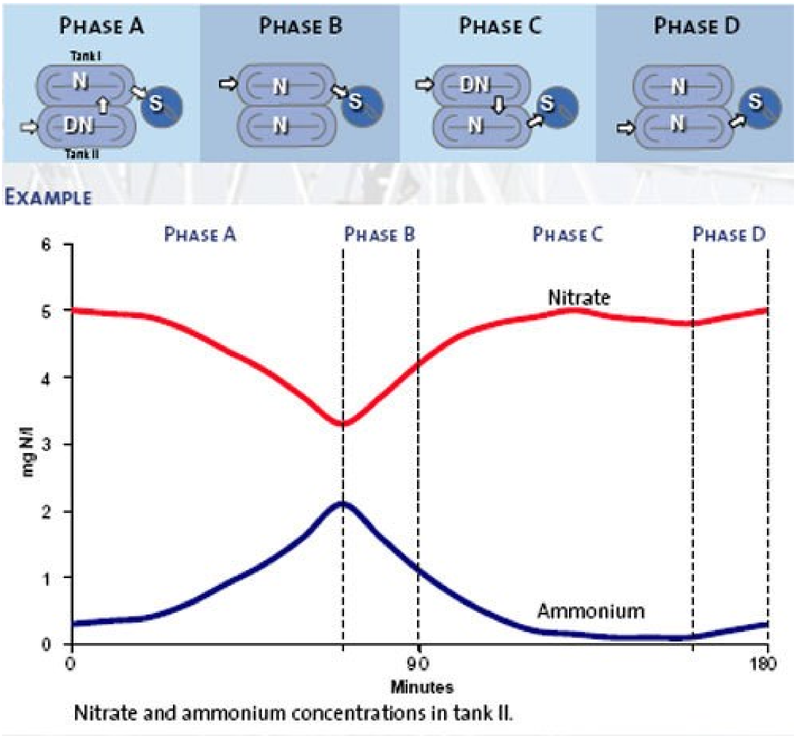
<table>
<tr><th></th><th>Størrelse
[PE]</th><th>PHA-indhold
[g PHA/g VSS]</th><th>PHA-potentiale
[t PHA/år]</th><th>PHA-potentiale
[kg PHA/PE/år]</th></tr>
<tr><td>BBR</td><td>50.000</td><td>18%</td><td>200</td><td>4</td></tr>
<tr><td>MR i dag</td><td>190.000[2]</td><td rowspan="2">26%</td><td>850</td><td>4,5</td></tr>
<tr><td>MR nyt</td><td>500.000</td><td>2.300</td><td>4,6</td></tr>
</table>

Se Bilag 3 for yderligere beskrivelse af laboratorietests af slamprøver fra BBR og MR.

3.2.2 Fuldskala PHA-produktion på renseanlæg

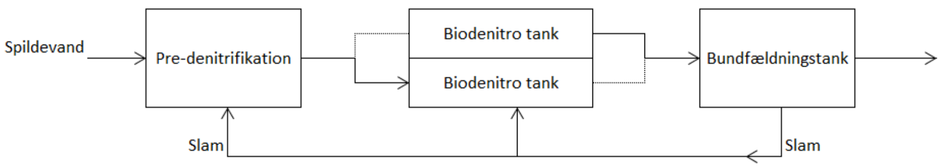
Integrering af PHA-produktion i fuldskala på MR og BBR vil kunne realiseres ved forholdsvis få anlægsændringer. MR og BBR er begge biodenitro renseanlæg, der består af flere par aktiv slam tanke, der opereres med faser med skiftende ilt-betingelser, samt en bundfældningstank. På FIGUR 14 nedenfor er eksempler på driften under de forskellige faser illustreret sammen med koncentrationerne af nitrat og ammonium i tank II.

[2] BI5-belastning i 2015



FIGUR 14: Eksempel på driften af og koncentrationsudviklingen i tank II i Biodenitro-processen.

Systemet har en meget stor fortyndingseffekt af næringsstofferne i spildevandet, og biomassen oplever således ikke skiftende fest-fase-betingelser, der normalt anvendes i Cella™-processen med henblik på at øge PHA-udbyttet og -koncentrationen. For at opnå et højere PHA-udbytte kunne der med fordel introduceres en anoxisk fest-zone (PE2-festfasen) til omdannelse af nitrat i et separat volumen opstrøms inden biodenitro-tankene, hvor recirkuleret biomasse blandes med spildevand (FIGUR 15). De anoxiske betingelser i denitrifikationen kan fint integreres med fest-betingelser, og laboratorieforsøg har vist, at dette bidrager til selektion af biomasse med potentiale for PHA-produktion (Anterrieu et al., 2014).



FIGUR 15: Eksempel på introduktion af en pre-denitrifikation, hvor biomassen oplever fest-betingelser (PE2-fest).

Fjernelse af suspenderet stof gennem enten primærfældning eller forfiltrering af spildevandet vil også hjælpe til at øge udbyttet af PHA i slammet, da det suspenderede stof, der kan fjernes, før procestankene blot fortynder den aktive biomasse og dermed reducerer PHA-koncentrationen. Fjernelsen af kolloid COD, der ofte er svært omsætteligt, og dermed ligger som en baggrunds-substratmængde, vil også gøre det muligt at få endnu skarpere adskillelse mellem fest- og fastefaserne i PE2. Biomassen, der fjernes gennem forfiltrering eller primærfældning, kunne fermenteres til VFA (PE1) og anvendes som fødestrøm i PHA-akkumuleringsprocessen (PE3) eller anvendes til biogasproduktion som antaget i CO_2-belastningsvurderingen i afsnit 4.2.

En af udfordringerne ved PHA-produktion i spildevandsanlæg er valg af slamalderen (SRT). På den ene side ønskes en kort SRT for at opnå et højt slamudbytte, et reduceret iltforbrug og en forøgelse af mængden af aktive celler og dermed også potentialet for PHA-produktion. Omvendt ønskes en lang SRT for at sikre tilstrækkelig mængde nitrifikanter til optimal kvælstoffjernelse. Dette kunne eksempelvis opnås gennem anvendelse af aktivt slam i kombination med nitrifikanter i biofilm på bærere (Moving Bed Biofilm Reaktor teknologien, MBBR), der tilbageholdes, hvorved der opnås en lang SRT for nitrifikanterne.

3.2.3 Fødestrømme til PHA-produktion på BBR

BBR behandler foruden spildevand også kildesorteret organisk affald (KOD) og organisk affald fra industrier, der omdannes til biogas gennem anaerob udrådning og termisk forbehandling. Det organiske affald har et højt indhold af RBCOD (letomsætteligt COD som fx flygtige organiske syrer, korte alkoholer og simple kulhydrater) og egner sig således godt som substrat til PHA-akkumuleringsprocessen (PE3), og man ville kunne øge PHA-produktionen på BBR ved at inkludere denne substratstrøm i en PHA-produktion. PHA-potentialet af det organiske affald blev i projektet undersøgt ved akkumuleringsforsøg med to forskellige affaldsstrømme, der går til rådnetankene på BBR. Disse affaldsstrømme udmærkede sig begge ved at have et højt RBCOD-indhold:

1. Blanding af husholdningsaffald og overskudsslam efter pulpning.
2. Blanding af husholdningsaffald, overskudsslam og industriaffald efter hygiejnisering.

Karakteristika for de to strømme kan findes i Tabel 6 nedenfor. Begge strømme viste sig at have et overskud af mikronæringsstoffer, hvilket medfører et øget PHA-udbytte som følge af biomassevækst under PHA akkumuleringen (Bengtson et al 2008, Morgan-Sagastume 2015, 2011).

TABEL 6: Organisk indhold i affaldsstrømme undersøgt som substratkilder til PHA akkumulering (PE3).

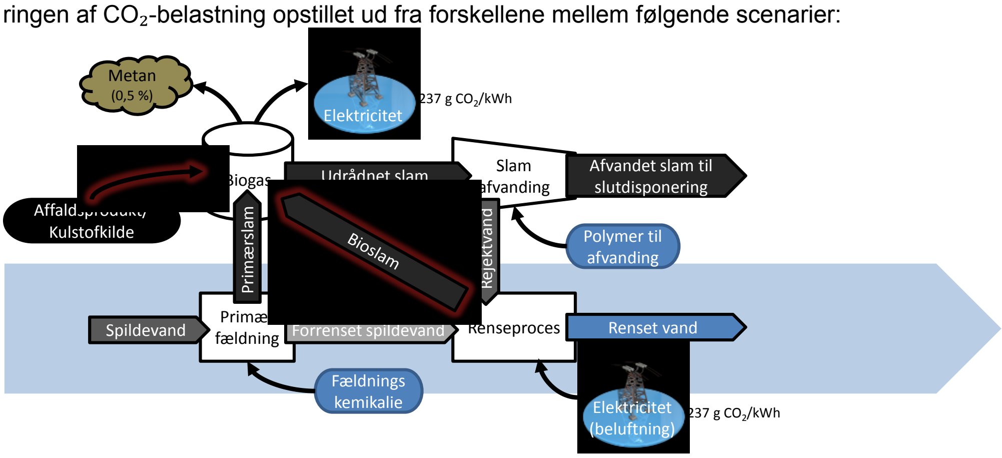
	SCOD [g COD/L]	VFA indhold [g COD/g COD]	VFA konc [g COD/L]	Total ferm produkter [g COD/g COD]
1	38	0,28	10,6	0,40
2	23	0,41	9,4	0,52

Det vurderes ud fra målingerne, at indholdet af fermenteringsprodukter fra det organiske affald kunne øges til 80-90% gennem acidogen fermentering (PE1), hvorved der ville kunne produceres yderligere 150-200 tons PHA/år, hvis strømmene blev anvendt som føde til PHA-produktion i høstet biomasse (PE3).

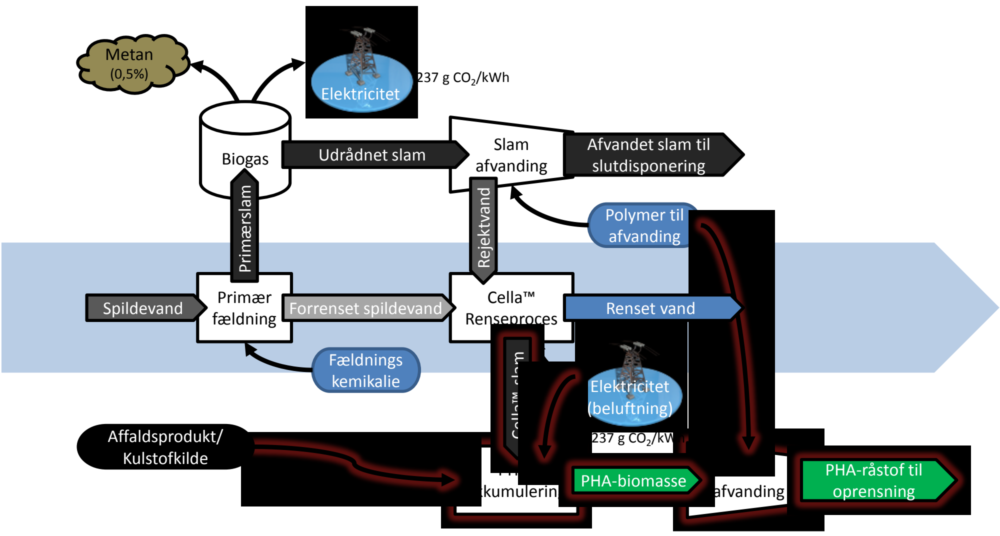
Se Bilag 3 for yderligere beskrivelse af undersøgelsen af affaldsstrømmene.

4. CO_2-belastning

Kulstoffet i det producerede bioslam fra de deltagende renseanlæg nyttiggøres i dag ved udrådning i rådnetanke til produktion af biogas, som i en gasmotor udnyttes til produktion af el og varme på MR og BBR. Bioslammet fra KMC udrådnes ligeledes til metan, dog på gyllebaserede biogasanlæg. I nærværende projekt undersøges mulighederne for at anvende bioslam og kulstof til produktion af biopolymerer til bioplastproduktion sammenlignet med traditionel spildevandsrensning, hvor spildevandsslammet anvendes til produktion af biogas. Dermed er vurderingen af CO_2-belastning opstillet ud fra forskellene mellem følgende scenarier:



FIGUR 16: Scenarie 1 - Spildevandsrensning af 100.000 PE med produktion af biogas ud fra primær slam, bioslam og et koncentreret letomsætteligt affaldsprodukt. Rød kant viser forskelle mellem scenarie 1 og 2.



FIGUR 17: Scenarie 2 - Spildevandsrensning af 100.000 PE med produktion af biogas ud fra primær slam, hvor bioslam og et koncentreret letomsætteligt affaldsprodukt anvendes til produktion af biopolymer (PHA). Rød kant viser forskelle mellem scenarie 1 og 2.

Formålet med denne vurdering af CO_2-belastningen er at klarlægge forskellene mellem disse to scenarier. Dette sammenlignes slutteligt med produktion af biopolymer baseret på fødevarer og fossil baseret plast med tilsvarende egenskaber.

Den samlede CO_2-belastning for Cella™-processens produktion af PHA-beriget biomasse til bioplast, afhænger i høj grad af det enkelte renseanlægs opbygning og de lokale forhold. Det er derfor valgt at begrænse denne CO_2-belastningsvurdering til en sammenligning med drift af renseanlægget på konventionel vis, hvor overskudsslammet fra den biologiske rensning udnyttes til produktion af biogas. Analysen gennemføres i første omgang frem til, den PHA-berigede biomasse forlader renseanlægget, men suppleres med overslagsmæssige vurderinger af CO_2-belastningen fra transport frem til et ekstraktionsanlæg, og en endelig sammenligning med CO_2-belastningen for et sammenligneligt fossil-baseret plast.

4.1 Opdeling i procestrin i belastningsvurderingen

Belastningsvurderingen tager udgangspunkt i en opdeling i følgende trin:

1. PE2: Produktion af biomasse med potentiale for produktion af PHA, som alternativ til produktion af biogas ud fra samme biomasse.
2. PE3: Produktion af PHA ved at berige den producerede biomasse med let omsætteligt kulstof, som alternativ til biogasproduktion ud fra samme mængde kulstof.
3. PE4:Transport og oprensning af PHA-beriget biomasse til plastgranulat.

Disse 3 trin sammenlignes med hhv. gængs bioplastproduktion, produceret ved industriel fermentering af fødevarer og produktion af konventionel fossil-baseret plast. Subfacit opgøres efter hvert trin og samles til sidst i en opsummering.

4.2 Cella™ – Trin 1 – Opformering af PHA-akkumulerende biomasse (PE2)

4.2.1 Forudsætninger

4.2.1.1 Kemikalieforbrug

Det forudsættes at Cella™-processen etableres på renseanlæg med overskud af kulstof, der i udgangspunktet ikke kræver tilførsel af en ekstern kulstofkilde for at få den primære renseproces til at forløbe.

Cella™-driftsformen, hvor biomassen udsættes for anaerobe forhold og høj tilgængelighed af let omsætteligt kulstof, vil give gode betingelser for Bio-P bakterier (Polyfosfat Akkumulerende Organismer, eller PAO), som er en undergruppe af PHA-lagrende organismer. Høj forekomst af PAO kan tænkes at give udfordringer, ved at afgive fosfor under en anaerob PHA-akkumuleringsproces. Da det ikke har været muligt at afgøre endeligt om Bio-P kan kombineres med PHA-akkumulering, og altså afgøre om forbruget af fældningskemikalie *reduceres* eller *øges*, forudsættes det her at forbruget af fældningskemikalier er uændret.

Der forudsættes at forbruget af polymer er uændret ved Cella™-drift, da pilot-forsøgene udført på KMC resulterede i slam med gode bundfældningsegenskaber.

4.2.2 CO_2-beregning

Der regnes med følgende CO_2-faktorer:

Elektricitet:	237 g CO_2/kWh[3]
Metan:	25 g CO_2/g CH_4[4]

4.2.3 Biogasproduktion

[3] Energinet.dk tal for 2015, opgjort efter Energikvalitetsmetoden.

[4] www.IPCC.ch

Den forventede biogasproduktion beregnes ud fra Krügers interne dimensioneringsstandard for biogasproduktion. Et resume er vist nedenfor (TABEL 7). Af sammenligningshensyn er beregningen baseret på en belastning på 100.000 PE. Der regnes med et tab af metan til omgivelserne på 0,5% af den producerede mængde, ud fra en forudsætning om, at der er gjort en fokuseret indsats for at begrænse metan-tabet, i form af katalysator på gasmotor og overdækning af slamlagertank. Den udledte CO_2-mængde ved biogasudnyttelsen regnes som neutral, da den stammer fra fornybare kilder. Den producerede varme forudsættes anvendt til opvarmning af biogas-processen, og bidrager således ikke til CO_2-belastningen. I grove træk må det forventes at der ikke er nogen væsentlig forskel i varmeoverskud ved udnyttelse af bioslam til Cella™-processen, i forhold til udnyttelse til biogas-produktion, da bioslam er mindre energirigt end primærslam, og altså producerer mindre varme, men kræver ca. samme grad af opvarmning.

TABEL 7: Beregning af forskel i biogasproduktion ved anvendelse af bioslam til Cella™, kontra biogas.

Biogasproduktion			
Belastning	PE	100.000	
Princip		2-trins	
Produktion af bioslam	t TS/år	1.180	
Bioslam anvendelse		Cella™	Biogas
Biogas produceret	1.000 Nm^3 CH_4/år	313	487
- heraf tab til atmosfæren	1.000 Nm^3 CH_4/år	1,6	2,4
El-produktion	MWh/år	1.156	1.794
CO_2-balance, trin 1 (PE2)	t CO_2/år	-248	-385

Den potentielle miljøgevinst ved udnyttelse af bioslam til biogasproduktion i stedet for biopolymerproduktion, differencen på de to ovenstående scenarier, er således **-137 ton CO_2/år for 2-trins 100.000 PE anlæg.**

Da det er forudsat at kemikalieforbruget er uændret, svarer forskellen i de to scenarier til den mængde CO_2 det koster i mistet el-produktion ved i stedet at anvende bioslammet til PHA-produktion fremfor til biogas og deraf el-produktion.

4.3 Cella™ – Trin 2 – akkumulering af PHA (PE3)

Ved akkumuleringen af PHA blandes den opformerede PHA-akkumulerende biomasse med rigelige mængder let omsætteligt COD, under beluftning og omrøring.

4.3.1 Kulstofkilde

Det forudsættes, at kulstofkilden anvendt til berigelse af biomasse med PHA, har karakter af restprodukt (spildevand/affald), og altså har en CO_2-belastning svarende til nul. Hvis det ønskes at producere PHA ud fra ikke-restprodukter, vil disse råvares CO_2-belastning skulle indgå i beregningen. På Billund BioRefinery blev 2 potentielle kulstofkilder undersøgt for indhold af letomsætteligt COD. Resultatet af disse undersøgelser er vist i
TABEL 6. For en blanding af kildesorteret organisk dagrenovation og returslam, efter pulpning, blev der fundet i alt 10.600 mg VFA-COD/l. For en blanding af kildesorteret organisk dagrenovation, returslam og industriaffald, efter hygiejnisering, blev der fundet i alt 9.430 mg VFA-COD/l. Ved efterfølgende beregninger er det derfor forudsat, at der anvendes en kulstofkilde med et indhold af letomsætteligt kulstof på 10.000 mg VFA-COD/l.

Den potentielle gasproduktion ud fra denne kulstofkilde, som modregnes i den samlede CO_2-belasting, beregnes ud fra normtallet 0,35 Nm³/ CH_4 pr. kg VFA-COD.

4.3.2 Iltforbrug ved akkumulering

Det forventes, at Cella™-processen kun vil være interessant på større anlæg, hvor der allerede er gjort en indsats for at effektivisere anlæggets procesbeluftning. Det forudsættes derfor, at beluftningen er forholdsvis energieffektiv, med et iltudbytte til spildevand på 3 kg O_2/kWh.

I forbindelse med pilotforsøg på KMC, blev det fundet, at den øgede slamproduktion, som resultat af at kulstof integreres i slammet, fremfor at blive oxideret til CO_2, som ved konventionel rensning, reducerede iltbehovet til 0,63 kg O_2/kg COD, en besparelse i forhold til iltbehovet for konventionel rensning, som er bestemt til 0,84 kg O_2/kg COD. Energiforbruget bliver således 0,21 kWh/kg COD anvendt til PHA-akkumulering, svarende til 49,8 g CO_2/kg COD.

4.3.3 Biogasproduktion

I Scenarie 1, hvor alt spildevandsslammet anvendes til biogasproduktion, ville denne kulstofkilde i stedet blive anvendt til produktion af biogas, hvor det forudsættes, at 1 kg letomsætteligt COD omsættes fuldstændigt til 0,35 Nm^3 CH_4. Ved anvendelse på gasmotor produceres 3,7 kWh/Nm^3 CH_4, således at energiproduktionen bliver 1,3 kWh/kg COD anvendt til biogasproduktion, svarende til en CO_2-besparelse på 305 g CO_2/kg COD – heri skal modregnes CO_2-belastning fra læk af metan, svarende til 29,2 g CO_2/kg COD, med et slutresultat på 276 g CO_2/kg COD anvendt til biogas.

4.3.4 Omrøring og volumenkrav

Udover omrøringseffekt fra beluftningen, forudsættes det, at akkumuleringstanken holdes omrørt med en effekt på 3 W/m³. Denne forudsætning er gjort ud fra et konkret eksempel på en nyanlagt rådnetank, der holdes omrørt med en effekt på 1 W/m³. Da der her er tale om en væsentligt kortere opholdstid, er det valgt at øge effekten med en faktor 3. Som det fremgår af TABEL 8, udgør omrøringen, selv ved denne høje effekt, en meget lille del af det totale energiforbrug.

Ved PHA-akkumuleringsforsøg for slam fra Marselisborg Renseanlæg og Billund BioRefinery, blev det fundet at PHA-akkumuleringen var højest i starten af en akkumuleringsfase, og aftog derefter gradvist. Langt størstedelen af PHA-potentialet blev opnået indenfor forsøgets første 10 timer, hvorefter kurven for PHA-indhold i slammet fladede ud for Marselisborg-slammet, og begyndte at falde for slammet fra Billund (FIGUR 12). Det er derfor valgt at beregne akkumuleringstankvolumenet ud fra en opholdstid på **10 timer.** Da bedre konditioneret slam må forventes at akkumulere hurtigere, vurderes denne antagelse at være relativ konservativ.
Ved en returslamskoncentration på 15 g SS/l og en koncentration af let omsætteligt COD på 10.000 mg VFA-COD/l, giver dette en volumenudnyttelse på ca. 2400 kg bioslam pr. år pr. m³. Tankvolumen rundes op til nærmeste 50 m³.

4.3.5 PHA-akkumulering

På Leeuwarden Renseanlæg (Holland) er det lykkedes ud fra byspildevand at producere biomasse med et potentiale for PHA-akkumulering på 0,4 – 0,5 g PHA/g VSS. Ved pilotforsøget på KMC blev der fundet et potentiale på 0,5 – 0,7 g PHA/g VSS. Ved berigningsforsøg med slam fra Marselisborg Renseanlæg og Billund BioRefinery, blev der fundet potentialer på 26% for slam fra Marselisborg Renseanlæg, og 18% for slam fra Billund BioRefinery. Både BBR og MRs potentiale ligger over de 5-15% som var forventet i almindeligt spildevandsslam,, uden nogen særlig tilpasning. Dette kan til dels tilskrives Bio-P-bakterier, som netop anvender PHA som energidepot.

I det følgende er der valgt at anvende et PHA-akkumuleringspotentiale på **40% af VSS,** da dette er cut-off værdien for, at det er økonomisk rentabelt at integrere PHA-akkumulering i fuldskala. Forsøg i Leeuwarden, Holland viser mulighed for at opnå et endnu bedre potentiale ved optimering af renseanlæggene med henblik på PHA-akkumulering.

For at bestemme mængden af VSS i bioslam, tages udgangspunkt i foreløbige tal for Rådnetanksdatabasen, der samler flere års erfaringer fra en række danske renseanlæg. Disse viser et gennemsnit på 69% VSS.

Ud fra pilotforsøgene på KMC forventes et PHA-udbytte på **0,35 g PHA/g COD**.

Forskellen ved at anvende kulstofkilden til PHA-akkumulering fremfor biogasproduktion, er ud fra afsnit 0 og 4.3.3 opgjort til 326 g CO_2/kg COD anvendt.

TABEL 8: CO_2-belastning for biomasse beriget med PHA ved Cella™ -processen.

PHA-akkumulering		
Belastning		**100.000 PE**
Princip		**2-trins**
COD-forbrug	ton/år	931
PHA-produktion	ton/år	326
Krævet tankvolumen	m^3	200
CO_2-belastning ved PHA-akkumulering frem-for biogas		
– Forbrug til beluftning	MWh/år	196
- Mistet biogaspotentiale	MWh/år	1210
- Forbrug til omrøring	MWh/år	5
I alt	**MWh/år**	**1411**
CO_2-balance, energiforbrug til proces	**t CO_2/år**	**47,6**
CO_2-balance, mistet biogas-produktion	**t CO_2/år**	**286,8**
CO_2-balance, undgået læk af CH_4	**t CO_2/år**	**-27,2**
CO_2-balance, trin 2	**t CO_2/år**	**307,3**
CO_2-balance, Cella™-trin 1	**g/g PHA**	**0,29**
CO_2-balance, Cella™-trin 2	**g/g PHA**	**0,94**
CO_2-balance for Cella™ -produktion af PHA-beriget biomasse ifht. biogasproduktion.	**g/g PHA**	**1,23**

Udfra opsummeringen i tabel 8 vil det altså koste **1,23 g CO_2 at producere 1 g PHA** i et 100.000 PE renseanlæg, i forhold til at udnytte slam og kulstof til biogasproduktion. Dette er tilfældet for både 1-trins og 2-trins anlæg[5], hvor alt det producerede slam i et 1-trinsanlæg anvendes til PHA-produktion og der deraf mistes mere el-produktion end ved 2-trinsanlæg (221 ton CO_2/år). Til gengæld produceres mere PHA pr. PE, så bundlinjen i g CO_2/g PHA ender med at være ens. Den primære indvirkning på CO_2-balancen stammer fra mistet energiproduktion ved anvendelse af slam og kulstofkilde til biogasproduktion (som i stedet bruges til biopolymerproduktion). Energiforbrug til selve Cella™-processen bidrager kun med 47,6/326 = 0,15 g/g PHA..

[5] 1-trins anlæg: Renseanlæg uden primærfældning eller for-filtrering.
2-trins anlæg: Renseanlæg med primærfældning eller for-filtrering

4.4 Trin 3 – Transport + oprensning (PE4) af PHA-beriget biomasse til granulat

4.4.1 Transport

Transportbidraget til den endelige CO_2-belastning afhænger i høj grad af afstand og transportform. Der tages udgangspunkt i, at det PHA-berigede slam afvandes til et tørstofindhold på 25% inden transport. Dette tørstofindhold er valgt ud fra en forventning om gode slamafvandingsegenskaber. Den samlede slammængde efter PHA-berigelse, er den tilførte mængde bioslam (1180 tons) tillagt den indbyggede PHA-mængde (326 tons), i alt 1506 tons TS. Den samlede slammængde til transport er således 1506 tons TS, der ved 25% TS svarer til 6024 tons slam. For at illustrere energiforbruget til transport, er valgt et scenarie, hvor slammet først transporteres 50 km på vej, og herefter omlades til jernbane, og transporteres yderligere 500 km (TABEL 9).

TABEL 9: Transport af afvandet slam (25% TS) til centralt oparbejdningsanlæg

	Slammængde [t/år]	Afstand [km]	CO_2-faktor [g CO_2/ton*km]	CO_2-belastning [t CO_2/år]
Vejtransport	6.024	50	139,8	42,1
Jernbanetransport	6.024	500	15,6	47
Sum				**89,1**

For den beregnede produktion på 326 t PHA/år, svarer dette til et bidrag til en CO_2-belastning på **0,27 g/g PHA,** således at den samlede belastning af råvaren leveret til fabrikken er **1,49 g/g PHA.**

4.4.2 Oprensning

For beregning af CO_2-belastning fra oprensningen af PHA fra biomassen, sammenlignes med en opgørelse af belastningen fra oprensningen af PHA fra industriel fermentering med fødevarer som kulstofkilde (glukose og sojabønneolie) [Akiyama *et al*, 2003].
Her blev det fundet, at oprensningen, afhængigt af anvendt kulstofkilde og procesparametre, forventedes at have en CO_2-belastning i området 0,65-1 g/g PHA. Uden nærmere kendskab til den faktiske oprensningsproces, må det dog kunne forventes at CO_2-bidrag fra elektricitet og andre effektiviseringer har bragt dette CO_2-bidrag ned siden 2003, hvor undersøgelsen blev udarbejdet. Sammenlignet med PHA produceret ved industriel fermentering af glukose og sojabønneolie, har den producerede PHA-rige biomasse ved brug af spildevand et lavere indhold af PHA, men da det væsentlige energiforbrug sker ved tørring af produktet, og biomassen produceret på renseanlæg allerede er afvandet til 25% TS, må det kunne forventes at CO_2-bidraget fra oprensningen er ret lav ifht. den totale CO_2-belastning.
Det forudsættes derfor, at CO_2-belastningen ved oprensningen af PHA ligger i samme niveau for spildevandsbaseret og fødevarebaseret PHA-produktion, svarende til et bidrag til den endelige CO_2-belastning på **1 g/g PHA.**

4.5 Opsummering

Den endelige CO_2-belastning for Cella™-produktion af PHA ud fra spildevand opsummeres som vist i TABEL 10:

TABEL 10: Opsummering af CO_2-belastning for Cella™-baseret PHA fra spildevand

	Enhed	Spildevandsbaseret
Råvare	g CO_2/g PHA	0
Cella™ u. energiproduktion	g CO_2/g PHA	0,15
Transport	g CO_2/g PHA	0,27
Oprensning	g CO_2/g PHA	1
Sum	**g CO_2/g PHA**	**1,42**

Til sammenligning opsummeres produktion af bioplast ved industriel fermentering af fødevarer således af *Akiyama et al, 2003:*

TABEL 11: Opsummering af CO_2-belastning for PHA fremstillet ved industriel fermentering, baseret på sojabønneolie eller glukose [Akiyama et al, 2003].

	Enhed	Industriel (sojabønneolie)	Industriel (glukose)
Råvare	g CO_2/g PHA	-3,26	-2,77
Fermentering	g CO_2/g PHA	2,37	2,25
Oprensning	g CO_2/g PHA	0,66	1
Sum	**g CO_2/g PHA**	**-0,24**	**0,48**

Det fremgår af TABEL 11, at råvaren beregnes med negativt CO_2-belastning. Dette er med afsæt i den mængde CO_2 planterne har optaget fra atmosfæren under vækstfasen. Det er i denne vurdering valgt ikke at regne CO_2-optaget med, da det færdige produkt tænkes anvendt som bionedbrydelig plast, hvorved den oplagrede mængde CO_2 igen frigives til atmosfæren inden for en overskuelig periode. Efter samme princip fratrækkes bidrag fra CO_2 udledt fra selve fermenteringsprocessen.

For at opdele råvarens CO_2-belastning i CO_2-optag under vækstfasen, og CO_2 udledt i forbindelse med dyrkning, er anvendt tal fra råvareanalysen i *Akiyama et al, 2003.* Her er det fundet at for hvert kg råvare, fordeles optag, belastning fra dyrkning og belastning fra forarbejdning, som vist i TABEL 12:

TABEL 12: CO_2-belastning opgjort for råvare [Akiyama et al, 2003].

	Enhed	Sojabønneolie	Glukose
Vækst	g CO_2/g Råvare	-2,84	-1,47
Dyrkning	g CO_2/g Råvare	0,19	0,15
Forarbejdning	g CO_2/g Råvare	0,12	0,35
Sum	**g CO_2/g Råvare**	**-2,52**	**-0,97**

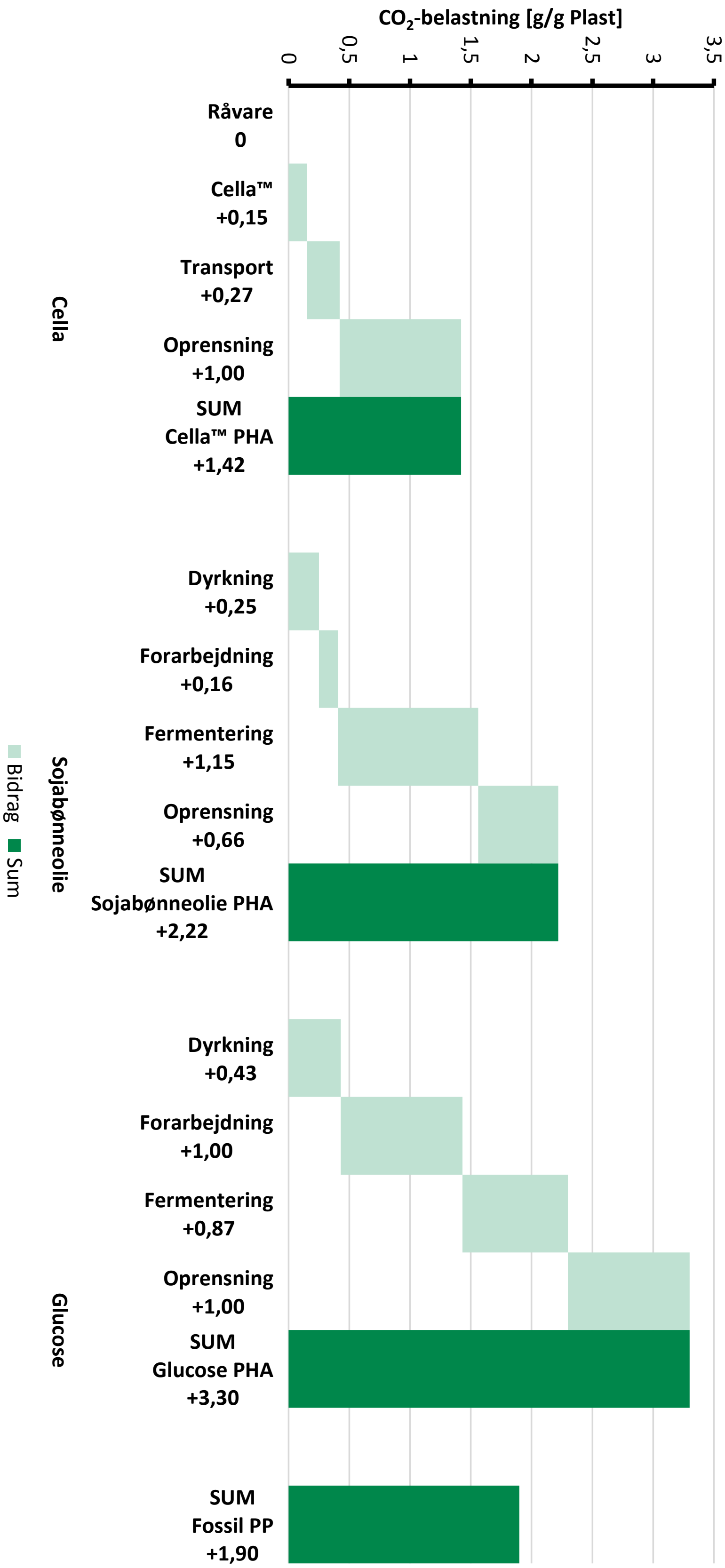
For at omregne dette til g CO_2/g PHA, ganges tallene i TABEL 12 med forholdet mellem summen i TABEL 11, og CO_2-belastningen fra råvaren i TABEL 11, som svarer til råvareforbruget pr. kg PHA. Således er råvareforbruget fundet til at være 1,29 g Sojabønneolie pr. g PHA og 2,86 g glukose pr. g PHA. Her efter kan tallene i TABEL 12 omregnes, og indsættes i stedet for ”Råvare”, som vist i TABEL 13, hvor bidrag fra CO_2-respiration under fermenteringen, jf. opgørelse i *Akiyama et al, 2003*, ligeledes er fratrukket:

TABEL 13: Opsummering af CO_2-belastning for PHA fremstillet ved industriel fermentering, baseret på sojabønneolie eller glukose, faregnet CO_2-optag i vækstfasen. Efter [Akiyama et al, 2003].

	Enhed	**Industriel (sojabønneolie)**	**Industriel (glukose)**
Råvare: Dyrkning	g CO_2/g PHA	0,25	0,43
Råvare: Forarbejdning	g CO_2/g PHA	0.16	1,00
Fermentering total	*g CO_2/g PHA*	*2,37*	*2,25*
- CO2-respiration	*g CO_2/g PHA*	*-1,22*	*-1,38*
Fermentering u. resp.	g CO_2/g PHA	1,15	0,87
Oprensning	g CO_2/g PHA	0,66	1
Sum	**g CO_2/g PHA**	**2,22**	**3,3**

Til sammenligning er der i Akiyama et al. (2003) angivet et CO_2-belastning for plasten Polypropylen (PP), som beskrives at have egenskaber sammenlignelige med PHA produceret ud fra fossil kilde på **1,9 g CO_2/g PP.**

Sammenligningen af disse 4 forskellige plast-produktioner er vist grafisk som FIGUR 18. Spildevandsbaseret PHA har en lavere CO_2-belastning end fossil-baseret plastik med tilsvarende egenskaber (PP). Ved sammenligning af Cella™-produceret PHA ud fra spildevand med industrielt produceret PHA fra soja eller glukose er CO_2-belastningen markant lavere pr. g PHA end ved brug af spildevand ift. anvendelse af fødevarer i produktionen (FIGUR 18).



FIGUR 18: Sammenligning af CO_2-belastning for 3 forskellige fremstillingsprocesser for PHA, samt fossilt baseret plastik med tilsvarende egenskaber.

5. Perspektivering

Resultaterne fra nærværende studie viser, at biopolymerproduktion fra spildevand vil kunne realiseres i fuld skala med forholdsvis små anlægsændringer på både anlæg til byspildevand og industrielt spildevand.

Et fuldskala-ekstraktionsanlæg til ekstraktion af biopolymerer fra spildevandsslam eksisterer på nuværende tidspunkt ikke, og det vurderes at kræve ca. 5.000 t PHA/år for at være økonomisk rentabelt. Renseanlæggene i dette projekt alene, er vurderet til at kunne producere omkring 1.650 t PHA/år tilsammen, og det ser derfor ud til at være basis for et fuldskala ekstraktionsanlæg til ekstraktion af biopolymerer fra spildevandsslam fra danske renseanlæg

Bioplast produceret i dag baseres enten på fødevarer eller fra råvarer, der er dyrket på landbrugsarealer, som kunne være anvendt til fødevareproduktion, og det er først med introduktionen af 3. generations plast, at fødevare- og bioplastproduktion afkobles. Derudover viser sammenligningen af CO_2-belastningerne beregnet i dette projekt, at bioplast baseret på fødevarer har en højere CO_2-belastning end fossilt baseret plast.

PHA-produktion fra spildevand foregår endnu ikke på kommerciel basis, hvilket skyldes, at bioplasten ikke kan produceres til en pris, der kan konkurrere med konventionel fossil-baseret plast. I øvrigt eksisterer der ikke et støtteregime, der kan understøtte udbredelsen af bioplast og kompensere for den øgede produktionspris. I Danmark eksisterer der imidlertid et støtteregime for biogasproduktion fra spildevandsslam på renseanlæg. Ydermere har de nuværende lave oliepriser (FIGUR 19), der dikterer prisen på fossilbaseret plast gjort det endnu mindre attraktivt at producere biobaseret og -nedbrydeligt plast.

FIGUR 19: Prisudviklingen for en tønde Brent-olie i USD. Kilde: *Quandl.com*

På verdensplan bliver der produceret mere end 300 millioner tons plast, og omkring 40% af plasten anvendes til engangsemballage (Plastics Europe 2016), hvor biobaseret og bionedbrydeligt plast baseret på PHA med fordel kunne anvendes. I Danmark er anvendelsen af biobaseret plast dog ganske begrænset, og der fokuseres i højere grad på genanvendelse af den producerede plast end på introduktionen af biobaseret og -nedbrydeligt plast, der ikke er egnet til genanvendelse. Derudover anvendes ikke-genavendt plast til energiproduktion i Danmark, hvorfor bionedbrydeligheden kun er af væsentlig betydning i forhold til plast, der ender i naturen. I en global kontekst er bionedbrydeligheden dog essentiel, da plast hovedsagligt deponeres på lossepladser i langt de fleste lande. Det skal bemærkes, at blot fordi en plast klassificeres som bionedbrydelig i henhold til en specifik standard, så er det ikke sikkert, at den i praksis vil blive udsat for de rette betingelser i naturen, der medfører, at den reelt bliver fuldstændig nedbrudt.

I Danmark blev der i 2015 behandlet spildevand med en samlet belastning på 7,3 mio PE (Vand i Tal 2016), der potentielt kunne anvendes til produktion af 33.000 t PHA/år. Dette ville medføre en CO_2-besparelse på 16.000 t CO_2/år, hvis det udelukkende erstattede PP. CO_2-besparelsen svarer til den samlede CO_2-belastning i 2013 for omkring 2.400 personer i Danmark (European Energy Agency 2013).

Veolia har netop afsluttet et Biopolymerprojekt i Holland kaldet ”PHARIO”. På basis af resultaterne i dette projekt undersøger STOWA (Den hollandske brancheforening for vandselskaber) muligheden for at opføre verdens første fuldskala demonstrationsanlæg til udvinding og ekstraktion af biopolymer fra spildevand.

6. Forkortelser

3HB	3-hydroxybutyrate
3HV	3-hydroxyvalerate
BBR	Billund Biorefinery
COD	Kemisk iltforbrug (Chemical Oxygen Demand)
DO	Opløst ilt (dissolved oxygen)
HRT	Hydraulisk belastning (Hydraulic loading rate)
IFAS	Integrated Fixed-Film Activated Sludge
KOD	Kildesorteret organisk affald
MBR	Membran
MR	Marselisborg Renseanlæg
OLR	Organisk belastning (Organic load rate)
PAP	Polyhydroxyalkanoat akkumulerings potentiale
PAO	Polyphosphat-Akkumulerende-Organismer
PFR	Plug Flow Reactor
PHA	Polyhydroxyalkanoater
PHB	Poly(3-hydroxybutyric acid)
PHBV	Poly(3-hydroxybuturic-co-3-hydroxyvaleric acid)
SBR	Sequencing Batch reactor
SCOD	Opløst Kemisk iltforbrug (Soluble Chemical Oxygen Demand)
SRT	Slamalder (Sludge Retention time)
TSS	Total suspenderet stof
VFA	Flygtige fede syrer (volatile fatty acids)
VSS	Suspenderet stofs glødetab (Volatile Suspended Solids)

7. Bilag

Bilag 1:
BIOPOLYMER PRODUCTION INTEGRATED TO PROCESS WATER MANAGEMENT AT KMC WATER TREATMENT PLANT PILOT PROTOTYPE OPERATION - MARCH 17 - NOVEMBER 4 2015. BIOPOL WP1 - Activity 1.1

Bilag 2:
STRATEGIES FOR INTEGRATING CELLA™ TECHNOLOGIES AT THE KMC PROCESS WATER EFFLUENT TREATMENT PLANT BIOPOL WP2

Bilag 3:
Biopolymer-producing biomass and feedstock from municipal wastewater treatment. Potential for volatile fatty acids and polyhydroxyalkanoates at Marselisborg Renseanlæg and Billund BioRefinery. BIOPOL WP1 (Activities 1.2 & 1.3) and WP2 (Activities 2.1 & 2.2)

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BIOPOLYMER PRODUCTION INTEGRATED TO PROCESS WATER MANAGEMENT AT KMC WATER TREATMENT PLANT

PILOT PROTOTYPE OPERATION - MARCH 17-NOVEMBER 4 2015

BIOPOL WP1 - Activity 1.1

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WATER TECHNOLOGIES

Contents

1. Executive summary

AnoxKaldnes Cella™ Technologies from Veolia include process elements that integrate polyhydroxyalkanoate (PHA) biopolymer production with wastewater treatment and residuals management services. Built on a foundation of more than a decade of fundamental and practical development, case studies for the commercial integration of PHA production with services of municipal and industrial waste management are being explored within local stakeholder networks. Within such networks, regional circular economies can be formed based on value chains producing renewable resources like biopolymers of PHA. The project BIOPOL has included such a case study, as presented in this report.

In Q4 of 2014 the project BIOPOL for renewable resource recovery was granted to Krüger A/S, KMC, Billund Vand A/S, and Aarhus Vand A/S as partners. The BIOPOL project has focused on the production of PHA biopolymers from wastewater in Denmark. BIOPOL was under the initiative of the Danish Ministry of the Environment, Environmental Technology Development and Demonstration Programme - MUDP. AnoxKaldnes was subcontracted by Krüger A/S to provide technical expertise and to pilot Cella™ technologies on-site at KMC in Brande.

KMC is a Danish industrial manufacturer of modified potato-based starch food ingredients. At the KMC production facilities in Brande, a process effluent stream (400 m^3/d) is generated and treated to remove chemical oxygen demand (COD). Due to a relatively high content of acetate, this process water effluent was identified as a potential feedstock for PHA production. A laboratory study conducted by AnoxKaldnes during 2014 demonstrated the treatability and applicability of this process water effluent as feedstock for the production of raw-material biomass for PHA production. In BIOPOL, these outcomes were reproduced at pilot-scale on site. Furthermore, an evaluation was made of the integration of Cella™ Technologies to the routine and requirements of KMC process effluent water quality management.

The pilot scale demonstration confirmed the practical feasibility of producing excess activated sludge biomass with increased PHA-storage capacity. We found that stable activated sludge treatment can be accomplished in a process volume that is 36% smaller than that used today by KMC for the activated sludge treatment. This outcome suggests that the full-scale KMC activated sludge process can be made to be more efficient and that the surplus produced biomass can be harvested as a raw material for PHA production. The piloting prototype has produced PHAs with polymer characteristics that are comparable with respect to PHA that is available on the market today. Polymers available on the market today are produced by means of more expensive pure-culture technologies. The on-site work supports the previous laboratory scale work in suggesting that KMC could be a future stakeholder in potential regional economies of biobased polymer value chains.

The on-site Cella™ technologies pilot plant comprised two main process elements (PEs). The principal PE was a sequencing batch reactor (SBR) for the treatment of KMC process water effluent and production of functional biomass with enhanced PHA-storage capacity. The second PE serviced the production of a PHA-rich biomass by exploiting the biomass harvested from the first PE. These Cella™ PEs were operated as activated sludge processes in parallel to the full-scale KMC wastewater treatment operations from March until November 2015. The PEs were monitored based on standard methods for bioprocesses and water quality engineering. The PHA polymers were also recovered and characterized following AnoxKaldnes in-house and proprietary methods in a pilot recovery PE located in Lund, Sweden.

KMC process water was characterized by variations in COD levels (9.7±1.5 gCOD/L), of which 98% was soluble. Generally, nitrogen levels were low with respect to COD for biological wastewater treatment (14±8 mgN/L; 0 mgNH_4^+-N/L); phosphorus (43±16 mgP/L; 18±5 gPO_4^{3-}-P/L) levels were sufficient. From 57 to 90% of the soluble COD was present as volatile fatty acids (VFAs), mainly as acetate (6.3±1.2 gHAc-COD/L). KMC process water effluent was with relatively high and variable concentrations of chloride (3827±931 mgCl^-/L) and sulphate (581±371 mg$SO4^{2-}$/L).

The pilot-scale wastewater treatment process was operated with an organic loading rate (OLR=2.2±0.4 gCOD/L/d), hydraulic retention time (HRT=4.5±0.6 d) and sludge retention time (SRT=7.4±1.1 d) based on the experience of the previous laboratory study in 2014. These conditions provided for enough capacity for the system to handle the relatively high concentrations of KMC process water COD while ensuring for the production of a biomass with stable and good settling characteristics (sludge volume index, SVI<170 mL/gTSS). The feast environment generated for the biomass for every influent sequence generated high-enough selection pressure to yield a biomass with significant PHA storing capacity. A feast-to-aerobic cycle length ratio below 0.12 (maximum feast length of 45 min) in the process along with microbial community analysis indicated that the feast-famine selection strategy successfully enriched for biomass with PHA storage capacity. Significant storage capacity was confirmed in campaigns of PHA accumulation with the surplus biomass.

The average COD removal efficiency was 98±1%. Treatment performance was stable during the whole operation period despite the significant variations in the influent COD concentration and water quality. The average treated water effluent was with 124±103 mg solubleCOD/L and 0.17±0.15 gTSS/L. Macro and micronutrients were provided to the influent as necessary supplements for stable biological treatment. A COD:N:P ratio of at least 100:3.5:0.5 (mass basis) was targeted. A stable activated sludge biomass developed and was maintained in the activated sludge process throughout the monitoring period.

Using KMC process water and acetate as feedstocks for PHA production, an average PHA accumulation of 50 % gPHA/gVSS was attained. From the perspective of PHA production, KMC process effluent was confirmed to be a suitable feedstock for the production of biomass with enhanced PHA-storage capacity. Due to uncertainties in the chemical content of the wastewater and observed variations in quality, we considered that the process effluent, as it is today, is less than ideal as a feedstock for PHA accumulation in the surplus biomass. Notwithstanding, the pilot results clearly indicated that KMC process water effluent may be efficiently treated in an activated sludge process and with substantially less process volume than that is being used in the full-scale plant today.

At the time of this report writing (December 2015), the pilot on-site operations entered a final phase, in which a PHA production mode will be pursued using acetate and/or a mixture of acetate/propionate in order to continue evaluating stability and quality of polymers produced with KMC biomass. The KMC biomass has exhibited a maximum PHA content of 70% (gPHA/gVSS; Fig. A.1).

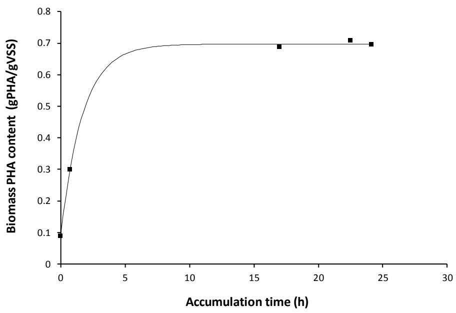

Fig.A1. PHA storage potential of KMC biomass from Cella™ pilot process operations (30°C, acetate)

2. AnoxKaldnes Cella™ Technologies

AnoxKaldnes Cella™ Technologies include bioprocess technologies and process solutions that integrate CellaPol™ biopolymer production with water quality and residuals management (Fig. 1).

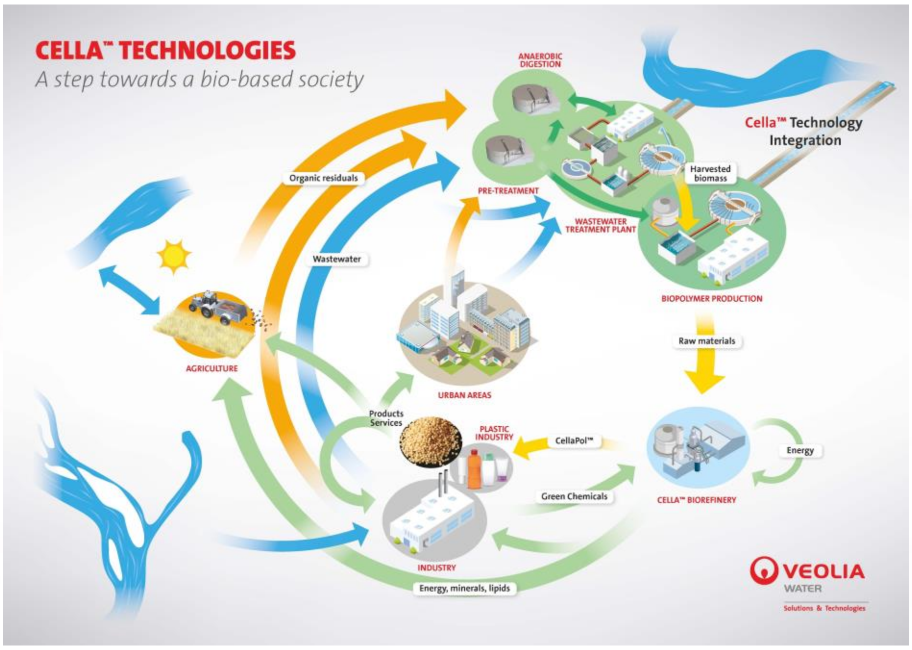

Fig. 1. Cella™ Technologies facilitate a bio-base society by sourcing raw materials regionally based on waste and residual management

AnoxKaldnes Cella™ Technologies have been championed since 2002 by a growing transdisciplinary team of twelve research scientists and engineers (4 Ph.D., 1 Licentiate, 7 M.Sc.), with support from VERI (Veolia Recherche et Innovation) and Veolia Corporate. AnoxKaldnes has assimilated more than 70 person-years of knowledge and know-how that is being applied towards first practical commercial solutions for biopolymer production from wastewater treatment and residuals management services.

The biopolymers produced by Cella™ Technologies are biobased and biodegradable from the family of polyhydroxyalkanoates (PHAs) with the trademark of CellaPol™. PHAs are produced by many species of naturally occurring bacteria, which can be made to thrive in biological systems such as activated sludge processes that are commonly used for the treatment of wastewater.

Cella™ Technologies are based on open mixed microbial cultures and encompass four Process Elements (PEs) that can be integrated into waste residuals and wastewater management services based on different strategies depending on the type of already existing infrastructure, the water quality objectives, and residuals mass flows (Fig. 2):

- PE1 sources volatile fatty acids (VFAs) from the fermentation of residual organic streams process water, or wastewater,
- PE2 produces functional biomass with PHA-storage capacity from the biological treatment for water quality improvement,
- PE3 accumulates PHAs in the biomass produced by PE2 utilizing as feedstock of VFA-rich streams, or the VFAs produced in PE1, and
- PE4 recovers CellaPol™ from the PHA-rich biomass produced in PE3.

CellaPol™ PHAs can be compounded into bioplastics that have properties comparable to those of conventional plastics that are produced from non-renewable fossil fuels. CellaPol™ biopolymers are produced using residual streams such as industrial process water, wastewater, waste activated sludge, municipal organic wastes, and other industrial or agricultural wastes or by-products.

Cella™ Technologies are backed up currently by a total of 13 patent applications, which are at various stages in the process towards international filing and registrations.

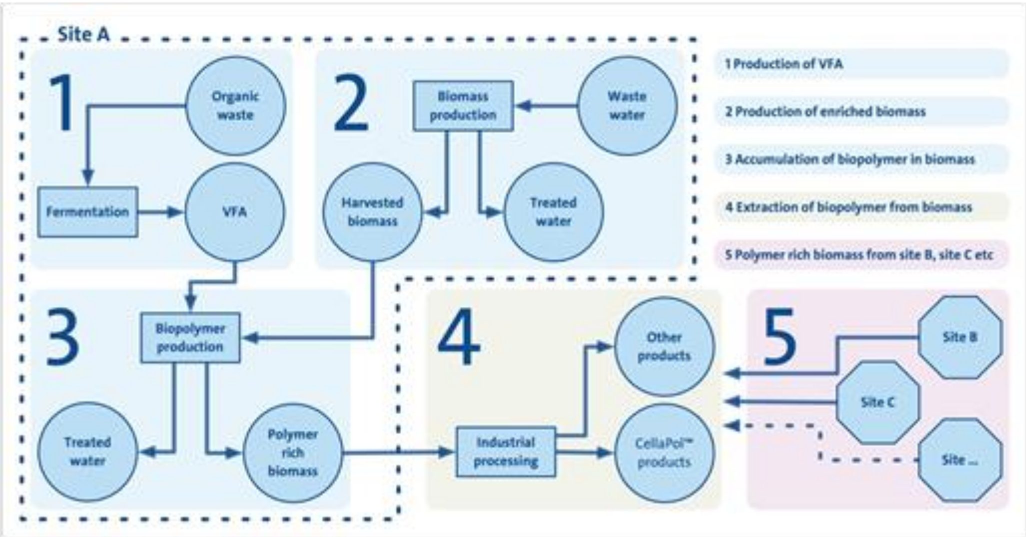

Fig. 2. Process elements (PEs) for the integration of Cella™ Technologies to waste and residuals management

3. Cella™ Technologies pilot prototype at KMC - Introduction

3.1 Cella™ Technologies: from bench-scale testing to piloting prototypes

The development of Cella™ Technologies started in 2002 with initial proof-of-concept laboratory testing at AnoxKaldnes. Positive results lead to a subsequent decade of built up experience with successful experience in the operation of laboratory bench-scale processes. Studies were focused on gaining realistic experience with opportunities and challenges for PHA production as a side-benefit from essential needs for the treatment of wastewater and waste organic residuals.

The first pilot-scale facility to benchmark production of PHA-rich biomass and PHA polymers from the treatment of industrial residuals was commissioned by AnoxKaldnes in 2008 in Lund, Sweden. A diary industry effluent was used for the testing platform that operated continuously for the process development from 2008 until 2011.

By 2011 AnoxKaldnes took a significant step in the development of Cella™ Technologies by designing, commissioning and installing two pilot process prototypes for field operation under real life challenges of site specific flows, and water quality variations:

- One pilot facility was directed to evaluate the integration of PHA production with the treatment of a food-industry effluent and operated from 2010 to 2013 at the Ellinge WWTP in Eslöv, Sweden.
- The second pilot facility was used to establish the working feasibility of using municipal wastewater treatment as a PE2 in a collaborative effort with Veolia Recherche et Innovation (VERI) and AQUIRIS and operated from 2011 to 2014 at the Brussels North WWTP in Belgium (Morgan-Sagastume *et al*., 2014, 2015).

From 2013 to 2014, first pilot facility was moved to the Netherlands and was operated in Leeuwarden. Here we expanded the understanding of PHA accumulating biomass production by establishing principles for carbon and nitrogen water quality management for municipal wastewater with integration to the material flows for regional PHA value chains.

Laboratory and pilot scale systems have generally applied the so-called feast-famine principle as an bioprocess environmental pressure for the enrichment of PHA storing organisms in an activated sludge biomass. Feast is a short period during which some of the process biomass is disposed to higher concentrations of readily biodegradable COD. Feast is a means to promote PHA storage activity by capable microorganisms. Famine is a longer time period during which the bacteria with stored PHAs survive. The stored PHA is an energy and carbon reserve for bacterial grow and maintenance when external sources of organic carbon is scarce. By subjecting the biomass to periodic feast-famine cycles, those microorganisms that can store PHA gain a selective advantage to grow and eventually become more dominant in the biomass. The biomass becomes in this way enriched in mass with PHA storing microorganisms.

Current efforts towards the commercialization of Cella™ Technologies target the development of regional value chain scenarios for the integration of PHA production with services of municipal and industrial waste management services within a local stakeholder network. The goal is to facilitate with such technology development an opportunity for regional economic growth within circular bioeconomies of a biobased society.

As part of the progression in the commercial development, and technology benchmarking and implementation, next to BIOPOL, other important Cella™ initiatives have been taking place in 2015 in the Netherlands and Australia:

- PHARIO project (PHA from wastewater treatment, Dutch Topconsortium voor Kennis- en Innovatie Biobased Economy TKI-BBE) is a cooperation between local Dutch business, Dutch Water Boards and Veolia. AnoxKaldnes piloting facility and surplus activated sludge from a full-scale municipal wastewater treatment plant (Bath, The Netherlands) are being used for regular production of PHA with the purpose of evaluating the regional opportunities in the raw material flows, products and product quality. These activities are focused on setting the corner stone in technology, stakeholder involvement, and regional developments towards a scaled-up demonstration project with production capacity of 100 tPHA/yr.
- Australian Research Council-Linkage programme (University of Queensland) is a cooperation between Norske Skog, University of Queensland and Veolia. PHA-based wood plastic composites are being developed as part of a whole new resource management scheme for the pulp and paper industry. The flow scheme of the next generation pulp mill, with technology unit processes to yield bio-composites is being evaluated in theory and practice.

3.2 BIOPOL – A project funded by the Danish Ministry of the Environment

With the accumulated experience of process development and technology prototyping of Cella™ Technologies within Veolia Water Technologies, in 2014 a project proposal for resource recovery was granted to Krüger A/S, KMC, Billund Vand A/S, and Aarhus Vand A/S as partners of the BIOPOL project focused on the production of biopolymers from wastewater. The BIOPOL project is partially financed by the Danish Ministry of the Environment as part of the Environmental Technology Development and Demonstration

Programme (MUDP). AnoxKaldnes was subcontracted by Krüger A/S to provide technical expertise for technology pilot prototyping, laboratory testing and evaluation of results.

The BIOPOL project runs over a period of 16 months (January 2015 – April 2016), and it comprises the following activities for which AnoxKaldnes has been responsible:

- Pilot prototyping of Cella™ Technologies during 6 months
- Investigation of biopolymer production potential of municipal wastewater sludge biomass at Marselisborg municipal wastewater treatment plant (Aarhus) and Billund BioRefinery (Billund)
- Evaluation of the potential for full-scale integration of Cella™ Technologies at KMC and municipal wastewater treatment plants.

3.3 Background to piloting work at KMC

KMC is a Danish manufacturer of potato-based food ingredients, namely modified starch, with headquarters located in the central part of Jutland, in Brande, and with a number of production sites in Denmark. At KMC's starch production facilities in Brande, native potato starch is modified for applications in the food industry. These starch production facilities generate a residual process water effluent with an average flow of 400 m3/d. The effluent is treated to remove chemical oxygen demand (COD) by activated sludge biological treatment before final discharge to the municipal sewer.

Due to the relatively high content of acetate, the process water effluent was identified as a feedstock for PHA production. Therefore, in parallel to the application for the BIOPOL project led by Krüger A/S and with the possibility for pilot prototyping of Cella Technologies on site at KMC treatment plant, AnoxKaldnes conducted a preliminary benchmark study. In the preliminary study applicability of KMC process effluent as feedstock for the production of functional biomass for PHA production in lab-scale PE2 reactors was demonstrated. This study was part of the Master's thesis of Giulia De Grazia as a collaborative work between AnoxKaldnes and Sapienza University of Rome (De Grazia, 2014).

As part of the benchmark study, two lab-scale sequencing batch reactors (SBRs) were operated under a feast-famine regime using as feedstock KMC process effluent (COD=13 g/L) in order to enrich biomass for PHA-storing organisms while meeting water quality objectives for the treated the process water effluent. One SBR was operated at 15°C and the other one at 25°C. Both SBRs were operated with a hydraulic retention time (HRT) of 1.2 d, a solids retention time (SRT) of 6 d, and an organic loading rate (OLR) of 1.7 gCOD/L/d. The SBR influent was maintained with a COD:N:P ratio of 200:7:1 (mass basis). The excess functional biomass from the SBRs was harvested and the PHA accumulation potential was repeatedly tested in batch using acetate as substrate at 15, 20, 25 and 30°C. An additional PHA batch accumulation was performed with KMC process water effluent as feedstock.

The main outcomes from the lab-scale benchmark study were as follows:

- Both SBRs at 15 and 25°C achieved a consistent removal of COD with higher than 98% during the 125 days of operation
- Similar microbial cultures dominated by Pseudomonas spp. and Arenibacter spp. were enriched at both temperatures providing good treatment performance, and the cultures adapted well to sudden temperature shifts when transferred from enrichment to accumulation bioprocess environments.
- A strong selection for PHA-storing organisms was achieved in the SBRs by a feast-famine selection strategy.
- The harvested biomass from the SBRs displayed a consistent capacity for PHA accumulation potential, accumulating up to 65% (gPHA/gVSS) within 16-24 h with acetate as substrate irrespective of enrichment and accumulation process temperatures.
- The kinetics of PHA production and COD consumption were dependent on the accumulation temperature rather than on the enrichment temperature.
- Higher accumulation temperatures resulted in higher kinetics of PHA production and COD consumption following an Arrhenius model.
- Higher PHA yields (gCOD/gCOD) were obtained at lower temperatures for the accumulation process

These results were presented to KMC in March, 2015 as part of an internal workshop. The results were further used as technical reference for defining operating conditions for the pilot Cella™ demonstration at the KMC treatment plant for purposes of BIOPOL.

4. Cella™ Techonologies demonstration for BIOPOL

4.1 Overall objective

To demonstrate the integration of PHA production with the treatment of KMC process water effluent within the realistic context of actual daily and seasonal variations in effluent water quality and using process and methods of Cella™ Technologies.

4.2 Specific objectives

- To evaluate the treatment performance and stability of an activated sludge process fed with KMC process water effluent with feast-famine selection operations,
- To demonstrate the enrichment of surplus activated sludge biomass with PHA accumulation potential due to feast-famine selection,
- To quantify the PHA accumulation potential of the biomass harvested over an extended period of process operations,
- To assess the recovery of CellaPol™ from PHA produced with biomass used to treat KMC process water effluent, and
- To characterise the quality of the PHAs recovered from the pilot scale KMC activated sludge biomass.

4.3 Piloting prototype and its operation

In order to meet the specific objectives, the Cella™ process element prototypes PE2 and PE3 were operated on-site at KMC from March until November 2015. PE2 treated KMC process effluent and produced functional biomass with PHA-accumulation potential, or PAP. PE3 produced a PHA-rich biomass by exploiting the PAP of the harvested surplus biomass from PE2 when fed with KMC process effluent or other VFA rich feedstocks. The piloting operations were representative of the full-scale operations. Further, the Cella™ prototype process element PE4, located in Lund, for the polymer recovery was used for the polymer recovery from PHA-rich biomass.

PE2 was placed in one container and comprised a sequencing batch reactor (V=500L; operating V=400-460 L) and one well-mixed tank (V=500 L) for surplus (harvested) biomass storage. PE2 was fed with KMC process effluent directly from a feed-holding tank (V=160 L), which was fed directly from the KMC treatment plant equalization tank. In mid-May, after approximately 60 days of operation, the influent to the PE2 was shifted to centrifuged KMC process effluent from the equalization tank. KMC started removing remaining starch from the effluent of the equalization tank by installing a full-scale cylindro-conical bowl-scroll centrifuge. In order to ensure a fresh and representative influent feed to PE2, the feed-holding tank volume (approximately 100L) was continuously flushed with fresh KMC process effluent. The retention time in the feed delivery system after centrifugation from the equalization tank to the SBR, including piping, pumping and feed-holding tank, was approximately 24 h. The SBR cycle treating KMC process effluent operated under aerobic feast-famine with three 8-h cycles per day. Each cycle included feeding (2.5 min with aeration and mixing except during the first min), reaction (aeration and mixing; 360 min), settling (30 min), and effluent discharge under quiescent conditions (2 min). During additional 85 min at the end of the reaction phase, aeration was interrupted for approximately 30 min to log data for calculating oxygen respiration rates and then restored to log data for the estimation of the oxygen transfer coefficient and finalise the famine phase. The total aerobic time (feast and famine) averaged 415 min. Mixing in the SBR was provided by a paddle stirring shaft, and aeration was provided via coarse-bubble diffusers. The SBR was operated with SRT control through biomass wasting, with maintained total suspended solids (TSS) concentrations, in order to sustain a stable specific OLR. Surplus biomass was wasted (harvested) from the SBR once per day as a single discharge during one the of the SBR cycles (no mixing; 3 min). PE2 was operated without pH control and with all the seasonal temperature variations of the full scale process. However, a minimum process operating temperature of 15°C was ensured. The operation of PE2 was automated to a large extent via a PLC controller. Dissolved oxygen (DO) levels, pH and temperature in the SBR were continuously measured and logged. Macro and micronutrients were provided to the SBR to supplement KMC process water effluent limitations. Additions ensured sufficient nutrient availability for microbial metabolism and stable biological treatment. Nitrogen (N) and phosphorus (P) were provided in a solution of NH_4Cl (~60 g NH_4^+-N/L) and KH_2PO_4 (~2.5 g PO_4^{3-}-P/L). Micronutrients were provided in two separate solutions: one with $CoCl_2 \cdot 6H_2O$ (25 mg/L), $CuSO_4 \cdot 5H_2O$ (450 mg/L), $MnCl_2 \cdot 4H_2O$ (354 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (20 mg/L), H_3BO_3 (300 mg/L), and a second one with $FeSO_4 \cdot 7H_2O$ (1000 mg/L) and $ZnCl_2$ (200 mg/L). The average activated sludge operating conditions during the period of operation are summarised in Table 1. Note that the SBR pilot reactor operation is analogous to continuous flow plug flow or tanks in series process models. Therefore results of the SBR process operations are translatable to other activated sludge process configurations.

PE3 was divided into two containers and consisted of an accumulation reactor vessel (V=550 L, operating V=400 L) with recirculation to a settling tank (V=120 L) and downstream biomass thickening units (settler and diffused air floatation) in one container, and a dewatering centrifuge (perforated drum CEPA TZ4), and a drying oven (Binder FP240) in a second container. PHA accumulations with PE2 biomass were conducted in a closed, fed-batch mode using a feed-on-demand approach. Peak substrate concentrations of 200 mg COD/L were applied. These closed fed-batch accumulations were thus with process volume increase due to feedstock additions. For each accumulation, three batches of harvested (wasted) biomass from PE2 (approximately 3x55 L=166 L) were used in total, including one batch of fresh biomass harvested on the same day when the accumulation was started. All the accumulations were controlled by respirometry. A total of 18 PHA accumulations were conducted in the pilot facility, 16 of which were fed with KMC process effluent and 2 with acetate. The acetic acid feedstock was at 9 gCOD/L (pH=5.6) and nutrients were added in the form of NH_4Cl and K_2HPO_4 to a COD:N:P ratio of 100:1:0.05 (mass basis). The accumulations were terminated at different times ranging from 7 to 20 h. Four additional batch accumulations with PE2 biomass and acetate and KMC process effluent were conducted at lab-scale at AnoxKaldnes headquarters in Lund, Sweden. The lab-scale data were used to evaluate further biomass response and compare the biomass storage capacity at different time points during the operation of the pilot prototype.

The thickened PHA-rich biomass was dewatered and processed according to published methods (Werker *et al*., 2012, Werker *et al*. 2014): pH adjustment below 6, centrifugation to approximately 15-20% dry solids, and drying (70°C, 24 h). PHA was extracted from some batches of dried PHA-rich biomass after granulation following the methods published elsewhere (Werket *et al*. 2014). The PHA extraction was conducted in the above mentioned PE4 piloting facility at AnoxKaldnes headquarters in Lund, Sweden.

4.4 Analytical techniques

Mixed-liquor grab samples during the SBR cycles and accumulations were taken in order to characterise the process dynamics. Total concentrations were determined from well-mixed grab samples and soluble (S) concentrations were obtained after filtering with 1.6 µm pore-size glass-fibre filters (Munktell). COD, N, and P were measured by spectrophotometry using different Hach Lange™ kits depending on concentrations: LCK014, 114 or 414 for COD; LCK138 or 238 for total N; LCK303 or 304 for ammonium N; LCK 339 for nitrate N; LCK 341-342 for nitrite N; LCK 349 and 350 for total P and orthophosphate P; LCK353 for sulphate; and LCK311 for chloride. The content of metals and other trace elements (Na, K, Ca, Fe, Mg, Mn, Al, As, Ba, Pb, B, Cd, Co, Cu, Mo, Ni, Se, Ag, S, Sn, Zn) was determined in mixed liquor and liquid supernatant samples by an accredited laboratory (Eurofins) using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Biomass separability at the end of the reaction phase of the SBR (PE2) was assessed often by measuring the sludge volume index (30 min SVI). Biomass from the SBR was evaluated by microscopy and samples for microbial community analyses were taken at different points during the operation period. The abundance of filamentous bacteria was recorded based on the Jenkins *et al*. (1993) scoring system. The microbial community in selected samples over operating time was analysed based on new-generation 16S rRNA amplicon sequencing targeting the bacterial variable region 1-3 using MiSeq (Illumina) and was conducted by DNASense ApS (Aalborg, Denmark).

VFAs were analysed by gas chromatography (GC) with helium as carrier gas (Perkin-Elmer, Clarus 400, split injector 1:20, flame ionization detector at 250°C, capillary column Elite-FFAP 30 m, 0.32 mmID, 0.25 µm df) on filtered (0.45 µm) samples, previously stored at 4°C and transported to Lund for analysis. Samples of 0.9 mL were acidified with 0.1 mL 25% formic acid containing 3 g acrylic acid/L as internal standard. The injector temperature was 240°C, and the column's temperature was held at 85°C for 0.5 min and then raised to 105 °C (25°C/min), then to 200°C (7 °C/min), and then to 240°C (20 °C/min) and maintained for 1 min. TSS and volatile suspended solids (VSS) were measured according to Standard Methods (APHA, 1998).

Biomass PHA content was assessed by thermo-gravimetric analysis (Q500 TA Instruments) (Werker *et al*., 2012). Briefly, dried PHA-rich-biomass (2 and 10 mg) was heated in N_2 up to 550 ºC. First, temperature was raised to 105 ºC (10 ºC/min and held for 10 minutes) and moisture loss was assessed. Then, the temperature was raised to 550 ºC (10 ºC/min) and weight loss assessed. At 550 ºC, the oven atmosphere was changed from N2 to air. Ash content was assessed based on weight loss at 550 ºC in air over 30 min, and weight losses and weight loss rate changes as a function of temperature were analysed. For PHA contents greater than 3%, a well-defined peak of weight loss is resolvable. The polymer's molecular weight and thermal stability by rheology were also determined as per Werker *et al*. (2012) protocols.

Table 1. Operating conditions in the pilot prototype from March to November 2015. Average and standard deviations reported for weekly average values (*n*=32)

Parameter	Range or Average ± Standard Deviation
pH	4.2-8.9
Temperature (°C)	19-36°C
HRT (d)	4.5±0.6
SRT (d)	7.4±1.1
OLR (gCOD/L/d)	2.2±0.4
Specific OLR (gCOD/vVSS/d)	0.6±0.1
TSS (g/L)	5.1±1.0
VSS (g/L)	4.1±0.8
Load SCOD:SN:SP	200:9:1.2

5. Cella™ pilot treatment performance

5.1 Overall operating approach

The results presented and discussed in this report are based on operations of the pilot prototype over 230 days (7 months) continuous operations from March until October 2015. During this period, wastewater treatment performance indicators were routinely monitored, and the biomass PHA accumulation potential and polymer properties were regularly evaluated. Although the pilot plant was continuously operated without any major shut-down, a few intermittent interruptions occurred. These interruptions related the malfunctioning of pumps, PLC, DO meter, plant energy shutdown, feed- and nutrient-delivery failure. Interruptions lasted no longer than 2-4 days and, what is more, they were not observed to have affected treatment performance. However, a process operation break is anticipated to be a perturbation in the biomass enrichment. The data reporting corresponds periods of representative stable operations.

Nutrient addition to the SBR was based on the influent water variable COD levels. The addition of N and P was tuned into the influent COD loading was aimed at a set point of COD:N:P ratio of at least 100:3.5:0.5 (mass basis), based on the previous laboratory experience. Nitrogen additions were made ensuring N levels lower than 3-7 mgNH_4^+-N/L and 15-20 mg (NH_4^+-N + NO_3^--N + NO_2^--N)/L in the treated effluent. During the first 188 days of operation, macro and micronutrients were added with the feed at the start of the feast phase. During the last 42 days of operation, nutrients were added at the end of the feast phase, as a known strategy to add additional selection pressure to the enrichment biomass.

5.2 Start-up and initial operation

PE2 was inoculated with a mixture of municipal activated sludge from three different full-scale municipal wastewater treatment plants (WWTP) for reasons of convenience and ease of surplus sludge availability (20 L return activated sludge from Brande WWTP; 10 L of mixed liquor from Sjölunda, Lund WWTP, and 5 L of mixed liquor from Klagshamn, Malmö WWTP).

SBR operation was started on March 17, 2015 targeting an OLR of 1 g/L/d during 4 days, after which a full OLR of approximately 2 gCOD/L/d was applied as the process operating target.

After one week of operation, the COD and SCOD removals in the SBR were 98% and the treated effluent contained an average COD and SCOD concentrations of 252 and 177 mg/L, respectively. The typical DO trends of oxygen demand reflecting a stable biomass feast-famine cycle response were established quickly after the initial operating cycles and days.

5.3 KMC process water effluent characterization

KMC process effluent water used as influent to the pilot system was characterised based on grab samples from the feed-holding tank. A comparison of water quality parameters was made between those measured in the influent to the pilot and those measured in grab samples from the equalization tank that were taken by the KMC treatment plant staff. A good agreement was found between the independently analysed parameters indicating that the feed to the piloting system was typical of the water quality of the influent to the full-scale WWTP.

The pH of KMC process effluent was slightly acidic and ranged between 4.8 and 6.5, whereas the temperature varied seasonally from 14.0 to 24.1 °C (Fig. 3A).

The average concentrations of total and soluble COD (COD and SCOD) in the water influent to the piloting facility were 9.7±1.5 gCOD/L (*n*=65) and 9.5±1.6 gCOD/L (*n*=80), respectively. Therefore, 98% of the total incoming COD was of a soluble nature. *Soluble* was defined based on 1.6-μm filtration. Variations in the concentration and quality of the COD were detected to occur on a daily and weekly basis (Figs. 4 and 5). These variations were likely as a response to upstream operations in the factory production campaigns. Most of the total COD was soluble and mainly in the form of volatile fatty acids (VFAs). The VFA fraction with respect to SCOD varied from 57 to 90% (73±10% gVFA-COD/gSCOD; *n*=30). The dominant influent VFA was acetate (6.3±1.2 gHAc-COD/L; *n*=35), and the fraction of acetic acid with respect to total VFA and ethanol COD ranged from 84 to 100% (94±4%; *n*=35). The fraction of acetic acid with respect to VFA and ethanol COD was slightly lower during the summer months (Fig. 5). Butyrate was the second largest component to the VFA-COD in the influent, accounting from 2 and up to 14% of the VFA+ethanol COD when the acetate COD to VFA+ethanol COD ratio was low. During April and May, some minor amounts of ethanol (37-90 mgCOD/L) were also detected in the influent.

The KMC process water effluent total and soluble N concentrations averaged 14±8 mgN/L (*n*=12) and 9±6 mgN/L (*n*=24), respectively, and the total and soluble P concentrations averaged 43±16 mgP/L (*n*=16) and 40±14 mgP/L (*n*=30), respectively. Nitrogen was limiting with respect to COD and requirements of biological treatment. Nitrogen concentrations were typically undetectable levels of NH_4^+-N. Approximately 45% of the SP corresponded to PO_4^{3-}-P concentrations averaging 18±5 mgPO_4^{3-}-P/L (*n*=37). KMC process effluent water quality was furthermore characterised by high and variable concentrations of chloride (3827±931 mgCl^-/L; *n*=22) and sulphate (581±371 mgSO4^{2-}/L; *n*=19).

The average total COD:N:P composition of the wastewater was 100:0:0.19 on a mass basis as SCOD: NH_4^+-N: PO_4^{3-}-P. The relatively low levels of NH_4^+-N and PO_4^{3-}-P made the addition of nutrients for biological treatment necessary in order to ensure nutrient availability in a controlled fashion.

The concentrations of total COD, SO_4^-, Cl^-, N and P in the water influent to the piloting facility with the associated daily-to-weekly variations were similar to those typically measured in the equalization tank for the KMC full-scale treatment plant (Figs. 4). Therefore, the pilot system was operated with influent variations as experienced for the full scale activated sludge process.

5.4 Operating conditions and COD removal efficiency

The SBR was operated sufficiently high specific OLRs and medium retention times (Table 1) that were reckoned to provide for enough capacity for the system to handle the relatively high concentrations of KMC process effluent (9.7±1.5 gCOD/L; *n*=65). At the same time, the organic loading conditions were selected for operations to ensure for the production of a biomass with good settling characteristics and with selection pressure for a surplus biomas with significant PHA accumulating potential.

The temperature of the mixed liquor in the SBR was slightly higher than that of KMC process effluent in the full-scale equalization tank (Fig. 3). This difference was probably due to heating from pumping equipment and the containerized bioprocess. The pH was not controlled, but remained within the range of 7.8 and 8.8 during the feast-famine cycles in the SBR and during the whole operation period (Fig. 3). The trends in pH during cycles were in agreement with expectations due to the process loading and removal of acetic acid.

The weekly average removal efficiencies of COD and SCOD were 98±1% (*n*=31) and 99±1% (*n*=32), respectively (Fig. 6). The COD removal was stable during the whole operation period despite the significant variations in influent water quality (Fig. 4). The effluent concentrations of SCOD and TSS were stable and averaged 124±103 mgSCOD/L (*n*=32) and 0.17±0.15 gTSS/L (*n*=32), respectively.

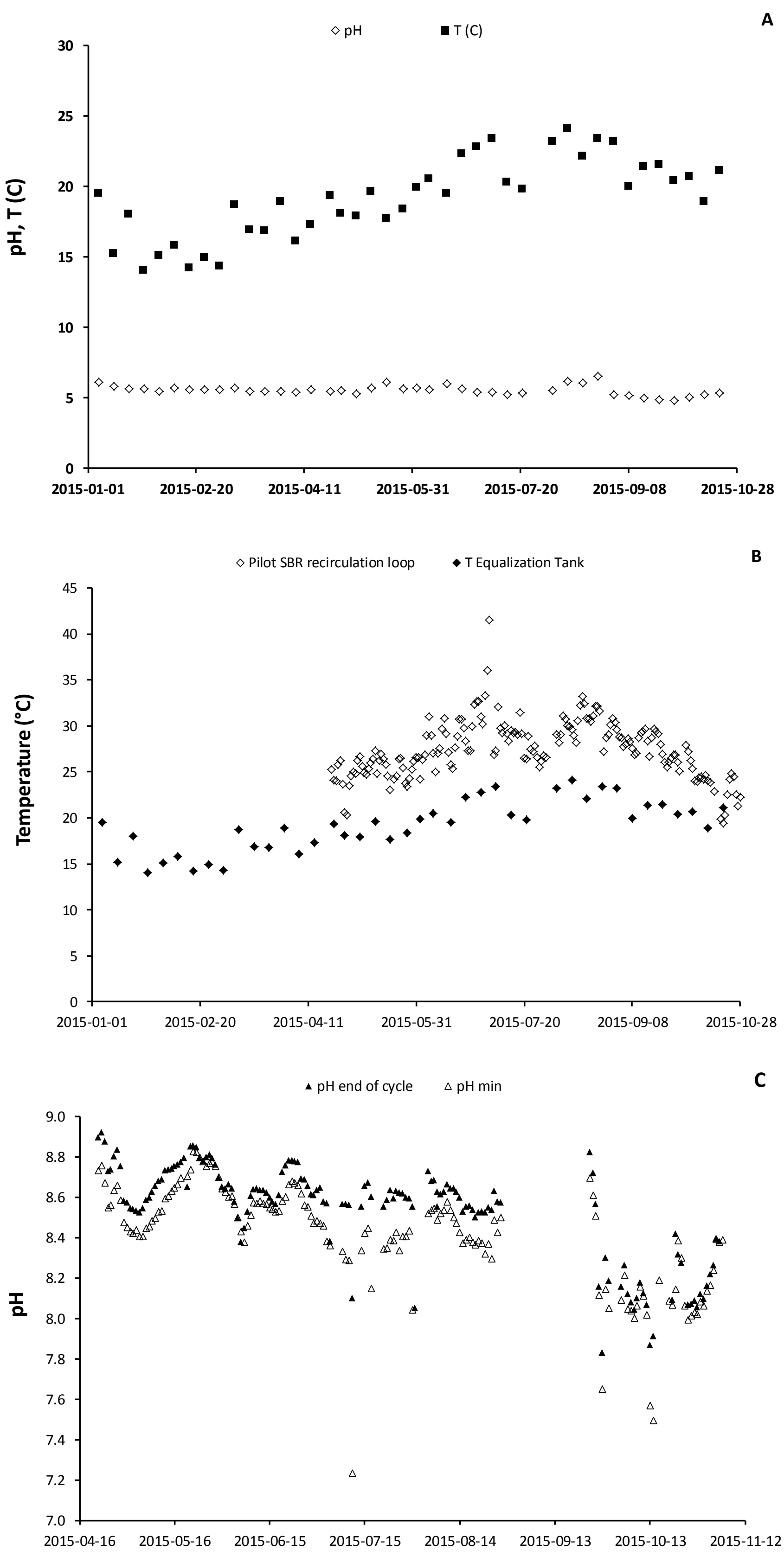

Fig. 3. Temperature and pH in KMC process effluent as measured in the equalization tank during 2015 (KMC's Drift Journal) (A) and in the mixed liquor of the pilot process measured in the recirculation loop (average of 3 cycles reported) (B, C)

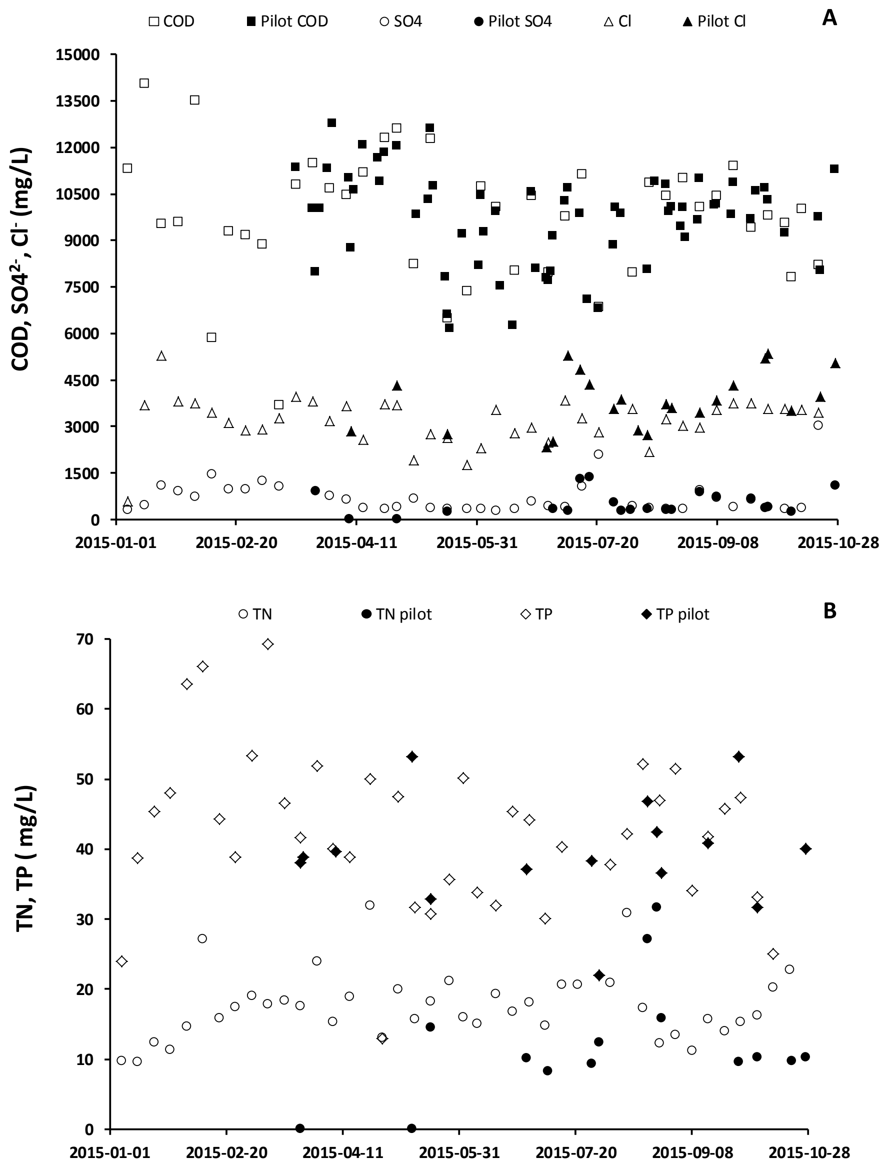

Fig. 4. COD, Cl^- and SO_4^- (A) and total N and total P (B) concentrations in KMC process water effluent as measured in the influent to the pilot prototype and in the full-scale equalization tank during 2015

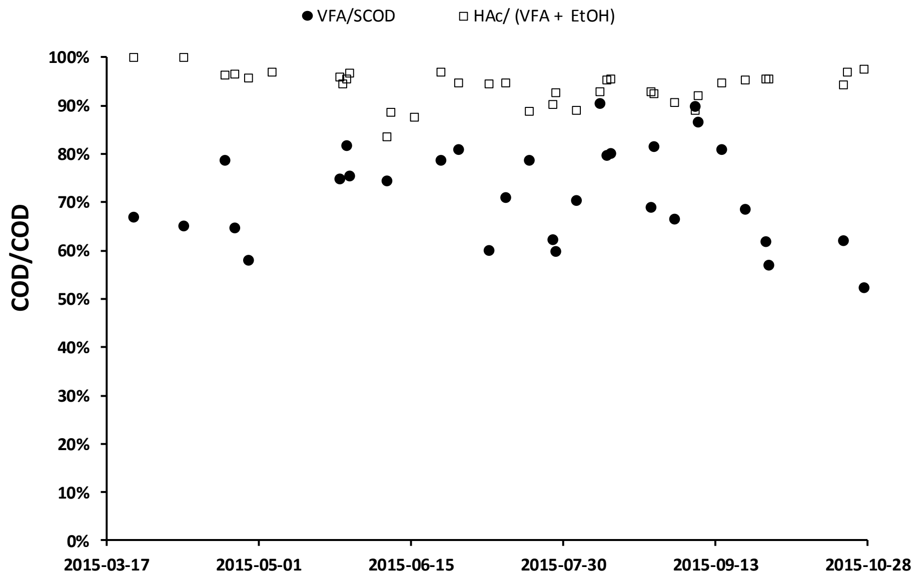

Fig. 5. COD quality in KMC process effluent as percent of acetic acid COD with respect to SCOD and with respect to the total VFA and ethanol COD as measured in the influent to the pilot process over time

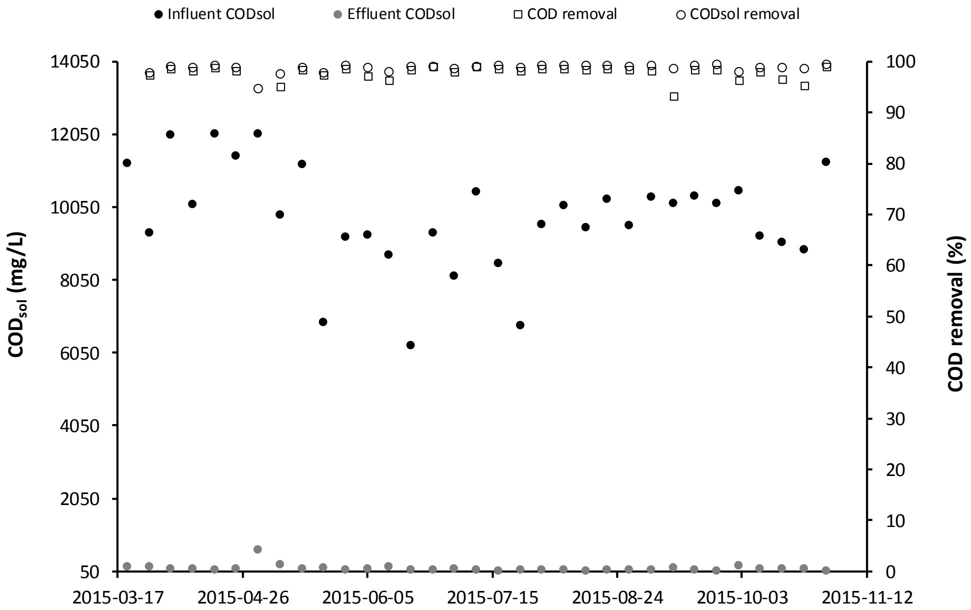

Fig. 6. Weekly average influent and effluent SCOD concentrations and COD removal efficiencies in the pilot process over time

5.5 Nutrient addition and biomass production

Macronutrient (N and P) addition was performed considering the extant COD and soluble P levels in the KMC influent process water effluent (Fig. 5). Additions were made to provide nutrient availability for stable microbial growth during the biological treatment. N and P additions were controlled manually by ensuring that a COD:N:P ratio of at least 100:3.5:0.5 (mass basis) was achieved, and by targeting as low levels of NH_4^+-N, NO_3^--N, and NO_2^--N as possible in the treated effluent. Effluent characterization was based on routine grab sampling. The relatively low levels of NH_4^+-N, NO_3^--N, and NO_2^--N in the effluent helped to ensure that N was not significantly nitrified nor added in excess as to yield high effluent ammonium N levels. The average N levels in the treated effluent were 1.0±2.0 mgNH_4^+-N/L (*n*=67), 15±21 mgNO_3^--N/L (*n*=56), and 13±28 mgNO_2^--N/L with the variability associated with periods in the routine optimization. The pilot PE2 raw influent was N deficient and most of the NH_4^+-N added was removed (SN removal = 94±6%; *n*=30). On the other hand, the PE2 raw influent was with variable levels of PO_4^{3-}-P (10-31 mg PO_4^{3-}-P/L) and P addition aimed at topping up the SP in the influent. The added phosphate P was in excess as suggested by the 79±11% (*n*=22) SP treatment removal efficiency. Notwithstanding, the average mass load ratio of SCOD:SN:SP applied during the whole operating period was 200:9:1.2. Given the variable nature of the raw influent water quality, the control of N and P addition based on the loaded COD became an important factor ensuring stable treatment performance and good biomass morphology for gravity separation.

The addition of micronutrients (metals and trace elements) aimed at assuring no trace element limitation for biological treatment that may be expected with some industrial process waters. The levels of metals and trace elements in the pilot plant were similar to those from the lab-scale experiments. However, differences in composition of the biomass was found for the KMC tank 3 activated sludge (Table 2). The KMC biomass was interpreted to be with some degree of micronutrient deficiency when compared to the biomass produced in the lab and at pilot scale. This perspective of a degree of deficiency was made with respect to the typical average content of micronutrient in bacteria as reported from the literature (Table 2). KMC activated sludge was potentially deficient in Fe, Cu, Mn, and Mo, which are all important trace elements for microbial activity. A trace element deficiency may negatively influence treatment performance as well as biomass morphology.

At the OLR (2.2±0.4 gCOD/L/d) and SRT (7.4±1.1 d) applied, the pilot process produced an average of 239±56 gVSS/d (*n*=32) as waste activated sludge with enhanced PHA-accumulation potential. This WAS biomass was periodically harvested and used for assessing the biomass PHA accumulation capacity. The average biomass yield based on WAS produced was 0.26±0.06 gVSS/$gCOD_{removed}$.

Table 2. Operating conditions in the pilot process from March to November 2015

Element	Lab SBR (15°C)	Lab SBR (25°C)	Bacteria (Metcalf & Eddy;)	Bacteria (Tchobanoglous, 1975)	Pilot SBR	KMC Tank 3
	% (g/gTSS)					
Al	0.081	0.036		0.01	0.004	1.428
As	0	0			0	0
Ba	0.002	0.004			0.002	0.005
Pb					0	0
B					0.002	0.002
Fe	0.087	0.120	0.2	0.2	0.118	0.037
Cd	0	0			0	0
Ca	0.59	0.82	0.5	1.4	2.552	2.105
K	0.77	0.87	1	1.5	0.716	0.286
Co	0	0			0	0
Cu	0.007	0.010		0.01	0.015	0.000
Mg	0.12	0.15	0.5	0.54	0.150	0.090
Mn	0.007	0.009		0.01	0.007	0.002
Mo	0.0003	0.0003			0.0004	0.000
Na	6.8	4.4	1	1.3	2.385	0
Ni	0	0			0	0

5.6 Activated sludge characteristics

The biomass characteristics in the pilot process were monitored based on microscopic observations and SVI measurements. An increase in SVI up to 200 mL/gTSS was observed during the first two weeks after startup, but the SVI, despite some variation, remained below 170 mL/gTSS for most of the operating period (Fig. 7). Accordingly, for most of the operating period, the biomass settled well and separability allowed for effluent discharge with low effluent suspended solids.

One exception with SVI values above 260 mL/gTSS occurred during early July. This upset was interpreted to be most likely due to a disruption in feeding and mixed liquor overheating from the pilot process recirculation pumping. Overall, when the mixed liquor temperature was above 30°C, SVIs drifted to be above 200 mL/gTSS.

Conversely, when the mixed liquor temperatures were below 25°C, the SVIs were below 100 mL/gTSS. Within the temperature range of 25 and 30°C, no correlation between SVI and temperature was directly apparent.

The mixed liquor TSS and VSS ranged from 3.3-7.4 gTSS/L and 2.6-5.6 gVSS/L, respectively (Fig. 8). The lowest values were caused by biomass losses due to overflows from an event of a malfunctioning level controller during the summer months.

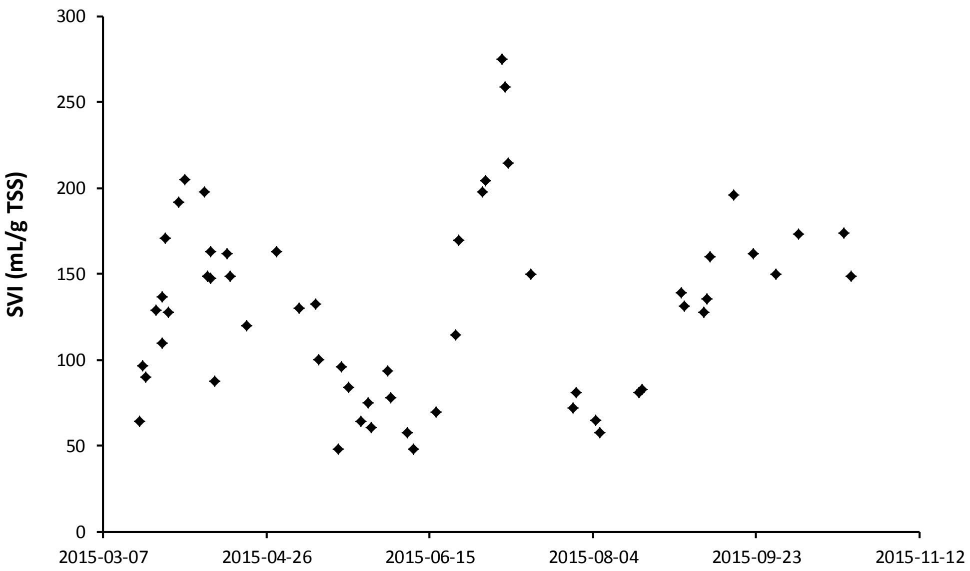

Fig. 7. SVI of the mixed liquor from the pilot process over time

The microscopic evaluation of the mixed liquor indicated that a healthy activated sludge biomass developed and was maintained in during the operating period. By healthy we mean that the biomass was composed mostly of flocs with a low abundance of filamentous bacteria (<1.5-2) and with low abundance of free-swimming bacterial growth (Fig. 9). Protozoa were in good abundance and dominated mostly by free-swimming and stalked ciliates. Sporadic observations of metazoan (rotifers and nematodes) were made. Metazoa tend to increase in abundance with longer SRTs and therefore we consider that their minor presence may have been due to some biofilm development on protected surfaces areas of the pilot SBR tank volume. Floc morphology was irregular, diffused with open structure, crosslinked by filaments. The flocs were with medium compactness, although some very compact round areas could be seen. The highest SVI values (Fig. 7) corresponded to observations of less compact flocs in the mixed liquor. Also, predominantly pin-point flocs with a more open and less compact structure were observed initially in the few weeks after start-up, coinciding with initial high SVIs.

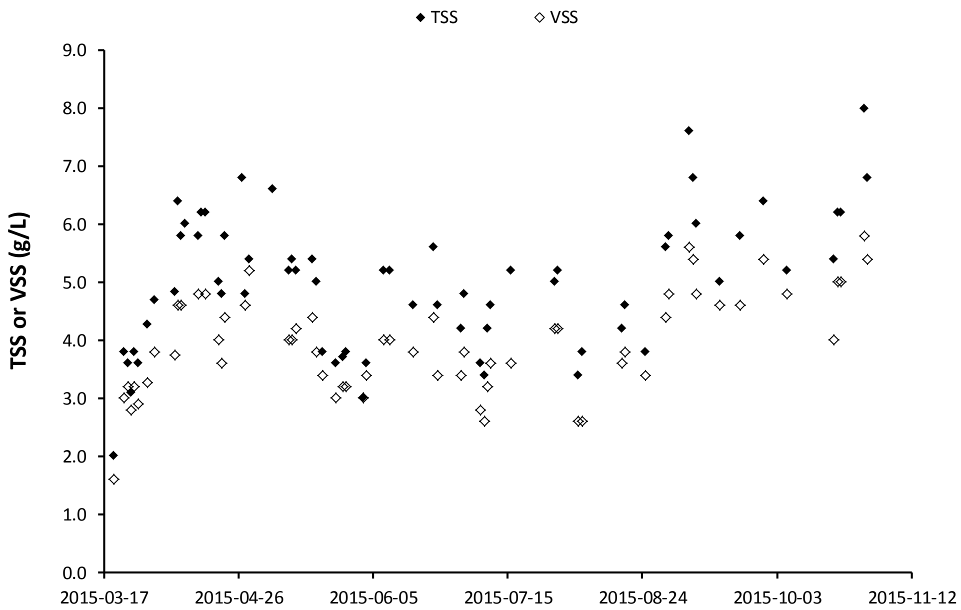

Fig. 8. Mixed liquor TSS and VSS levels in the pilot process over time

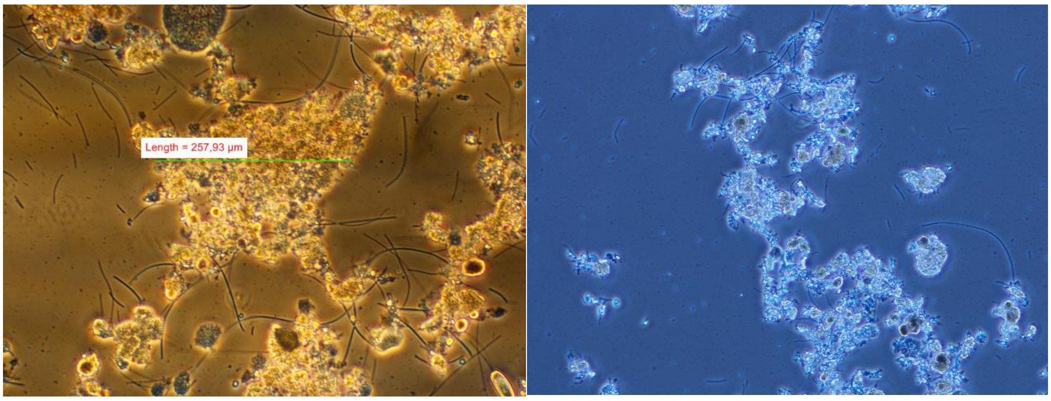

Fig. 9. Micrographs of typical activated sludge biomass with irregular, open floc structure and medium compactness (Samples from May 8 and 28, 2015).

The microbial community analysis indicated that the operating conditions in the pilot bioprocess selected for a stable microbial community after 100 days of operation, and this supports the potential for enrichment of a biomass providing robust water quality improvement and with demonstrated significant PHA accumulation potential. The community structure changed significantly from that of the inoculum biomass during the first 50 days of operation (2015.05.08) and after July 2015, a more stable microbial community developed. Although after July 2015, the microbial community was similar in terms of the types of bacteria present, the relative abundance of individual genera was dynamic. Overall, the community structure in the mixed liquor from startup until the end of October 2015 changed from a diverse community in the inoculum, to a highly enriched community (two genera) in

April/May 2015, and settled finally with a more diverse but distinct microbial community by the end of October 2015. The changes in community structure and relative abundance may be in response to variations in the incoming raw KMC process effluent as discussed above. The microbial community structure that developed after 100 days of operation was dominated by *Actinobacteria* (among others, unidentified from the *Propionibacteriaceae* family, *Ornithinimicrobium*, *Microlunatus*), *Proteobacteria* (among others, *Azoarcus*, unidentified from the *Rhodobacteraceae* and *Phyllobacteriaceae* families) and unidentified *Bacteroidetes*. This microbial community was distinctly different from the microbial community of the activated sludge present in KMC Tank 1 during the same time. The KMC full scale activated sludge was dominated by *Chloroflexi* (P2CN44), *Proteobacteria* (among others, *Shinella, Azospirillum, Paracoccus*).

5.7 Feast-famine cycle trends

The PE2 feast-famine biomass response was continuously monitored based on dissolved oxygen (DO) concentration profiles. In addition, the PHA-storage response, COD removal and nutrient levels were monitored periodically in more detailed periodic cycle study evaluations.

A feast-famine response by the biomass was achieved since the process start-up. The feast response was characterized by a decrease in DO levels (high respiration rates) concomitant with COD removal and PHA storage (Fig. 10), whereas the famine response started with an increase in DO demarking a decrease in biomass respiration rates. Thus biomass respiration rates decreased from a maximum feast level (minimum DO values achieved during feast) to a relatively low famine respiration rate as indicated by a constant high DO value until the end of the SBR reaction phase. During famine, the stored PHA was metabolized (Fig. 10). Ammonium N and phosphate P were used during the feast and the start of the famine, and some level of nitrification was observed during the start of the famine with the formation of NO_3^--N and NO_2^--N, which were later consumed during the later part of the famine (Fig. 10). The feast respiration is stimulated by presence of easily degradable COD whereas famine respiration levels ensue when this added COD becomes consumed. Respiration levels are also due to nitrification activity, the removal of slowly degradable COD, and the metabolism of feast-stored PHA during famine. During most of the pioot operations, the minimum feast DO levels were ≥ 0.5 mgDO/L. During the feast, the SCOD uptake rates averaged 329±79 mg SCOD/gVSS/h (*n*=8) and on average 92±0.03% (*n*=8) of the SCOD in the influent was removed during the feast. This consumed COD during feast corresponded to VFAs and remaining unidentified easily degradable SCOD present in the influent.

The level of selective pressure for PHA-storing bacteria established during feast-famine can be evaluated based on the feast-to-aerobic cycle length ratio. Feast-to-aerobic cycle ratios below 0.25 are considered to provide selective pressure for biomass with increased PHA-storage capacity (e.g., Dionisi *et al*. 2006). For the pilot process, the feast-to-aerobic-cycle length ratios remained below 0.12 (maximum feast length of 45 min) during the operating period (Fig. 11), and this suggested good and stable enrichment conditions for PHA-storing microorganisms. The feast-famine regime and PHA storage capacity of the biomass was maintained when nutrient addition was conducted at the start of the famine instead of at the start of the feast.

Some variability in cycle-to-cycle performance was indicated by the variations in the feast-to-aerobic-cycle length ratios (Fig. 11) and SVIs (Fig. 7) over the course of the whole operating period. Although no direct correlative factor for these variations was identified, the changes in raw influent water quality (Figs. 4 and 5), including temperature, pH, and organic composition are anticipated to require adaptations of the mixed liquor biomass. Trends in mixed liquor TSS (Fig. 8) levels will also influence the time required in feast for COD removal.

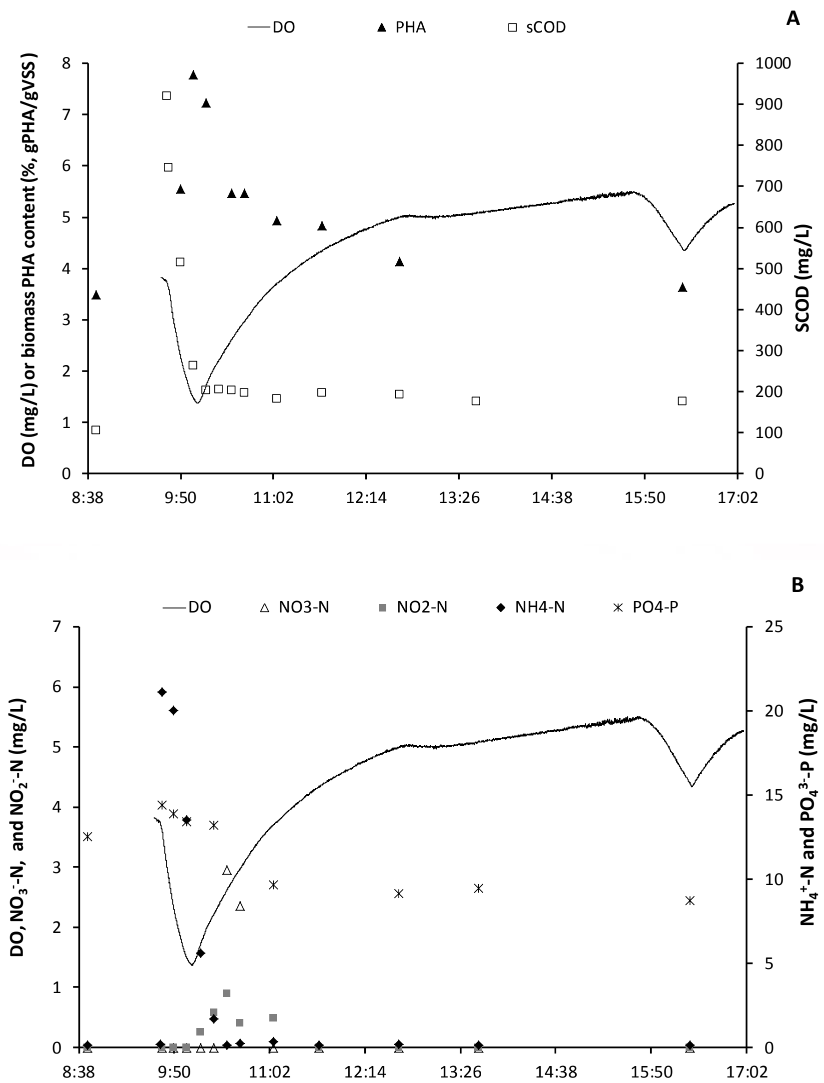

Fig. 10. Characteristic feast-famine cycles with nutrient addition at the start of the feast (2015.06.24). DO profiles with respect to COD and biomass PHA content profiles (A), and DO profiles with respect to N and P profiles (B).

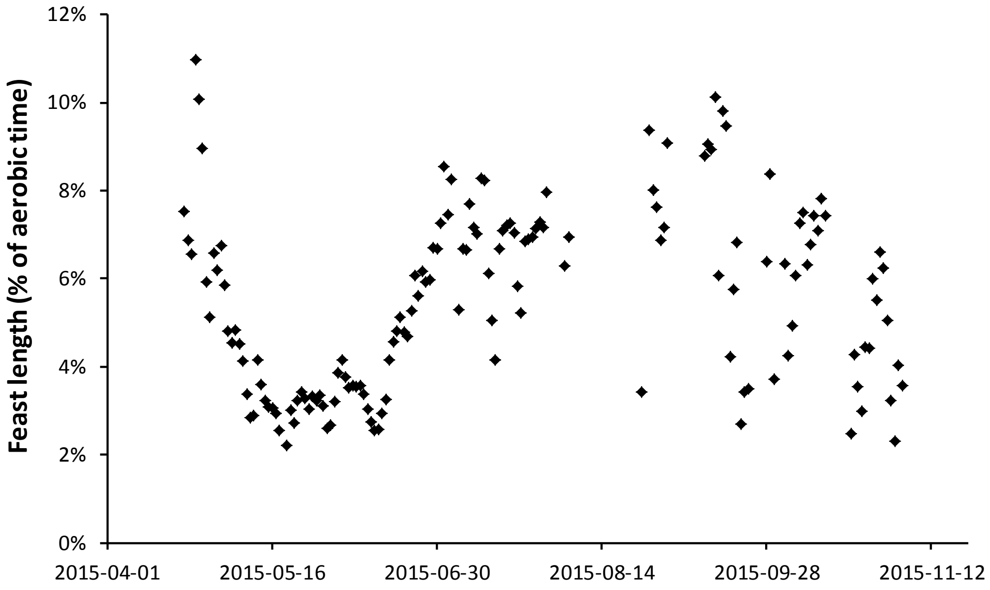

Fig. 11. Average cycle feast length as a percent of the aerobic (feast-famine) time per cycle (415 min) during the operating period

6. PHA-accumulation potential of the enriched activated sludge biomass

The fed-batch PHA accumulations conducted during the whole operating period were intended to monitor for PHA-accumulation potential of the pilot biomass. The produced PHA-rich biomass was also characterized as were the recovered polymers. Although most of the accumulations were conducted using KMC process effluent as feedstock, acetate was also used on selected occasions as a reference feedstock with 100% VFA-COD. Acetate had been successfully used in previous lab-scale PHA accumulations with biomass enriched with KMC process effluent. KMC process effluent had also been successfully used as substrate for PHA accumulation for the same biomass (De Grazia, 2014). These positive lab-scale outcomes were now to be demonstrated at pilot scale.

After five weeks from start-up, the biomass already presented a signficant PHA-accumulation potential (PAP) of 0.30 gPHA/gVSS after 8 hours of accumulation time. A higher and faster PAP response of 0.5 gPHA/gVSS was achieved within 10 weeks of operation. These levels after 10 weeks of pilot operation were similar to the PAP response observed in the biomass enriched at lab-scale (Fig. 12).

The pilot process maintained an enrichment for PAP and COD-removal capacity during the whole pilot operating period. Using KMC process effluent as feedstock for PHA production, an average PHA accumulation potential of 0.49±0.04 (*n*=5) gPHA/gVSS was measured (Fig. 13). The typical PHA-accumulations were characterized by an exponential asymptotic trend of increase in PHA content in the biomass over time, generally achieving saturation after 7 to 10 hours (Fig. 13). Observed variability in PAP response from May to September 2015 in terms of PHA-storage kinetics and biomass PHA content was interpreted to be due to underlying variations in the quality of KMC process effluent in combination with biomass variations due to operation disturbances (e.g., recirculation pump overheating in July, 2015). Two strategies were tested for enhancing the biomass PAP response. One strategy aimed at achieving a physiological acclimation of the biomass to possible variations in KMC process effluent water quality, and a second strategy aimed at enhancing selection pressure in PE2 with the timing of influent N dosing. Neither of these strategies were found to exert a measureable influence. Therefore, we considered that without further direct specific insight into the KMC process effluent water quality that this stream was a less than ideal feedstock for PE3 PHA production when compared to a well-defined VFA rich feedstock.

KMC process effluent water quality was interpreted to have influenced the PAP response in ways that could be explicitly identified based on the collected body of data. Lab- and pilot-scale PHA accumulations using acetate as feedstock performed consistently resulted in a better PAP response than those using evaluations made using KMC process effluent as feedstock (Fig. 13). It should be clear that the outcomes of PAP with KMC process effluent as PE3 feedstock were nevertheless significant. However, we concluded that a process optimisation strategy may need to be further developed in order to realize the full extant PAP of the biomass with this industrial feedstock in a PE3.

Specifically, variations in the ratios of acetate COD to VFA+ethanol COD and VFA-COD to SCOD (Fig. 5) in KMC process effluent are anticipated to exert influence on the accumulation process. The PHA storage yields with KMC process effluent were relatively constant during the accumulations and ranged within 0.30 and 0.50 $gPHA/gCOD_{removed}$. The active biomass growth yields tended to increase during the accumulation and these ranged from zero up to 0.25 $gVSS_{active}/gCOD_{removed}$, and remained low as expected due the growth-limiting levels of nitrogen in the KMC process effluent. The COD treatment performance in the accumulation reactor can be estimated to range between 75 and 94%, which was dependent on the level of PHA saturation in the biomass. Tuning the accumulation for effective COD removal requires additional elements in the process monitoring and control that were not implemented as part of the present investigation.

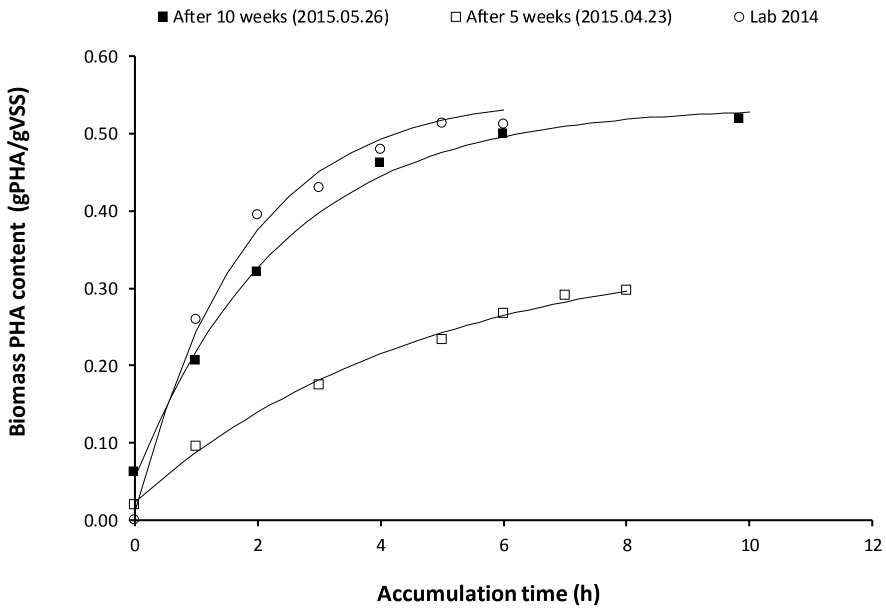

Fig. 12. PHA-accumulation response of biomass enriched with KMC process effluent upon addition of KMC process effluent as feedstock. Results for the pilot process biomass are shown after 5 and 10 weeks of operation, and from a lab-scale PHA accumulation in 2014.

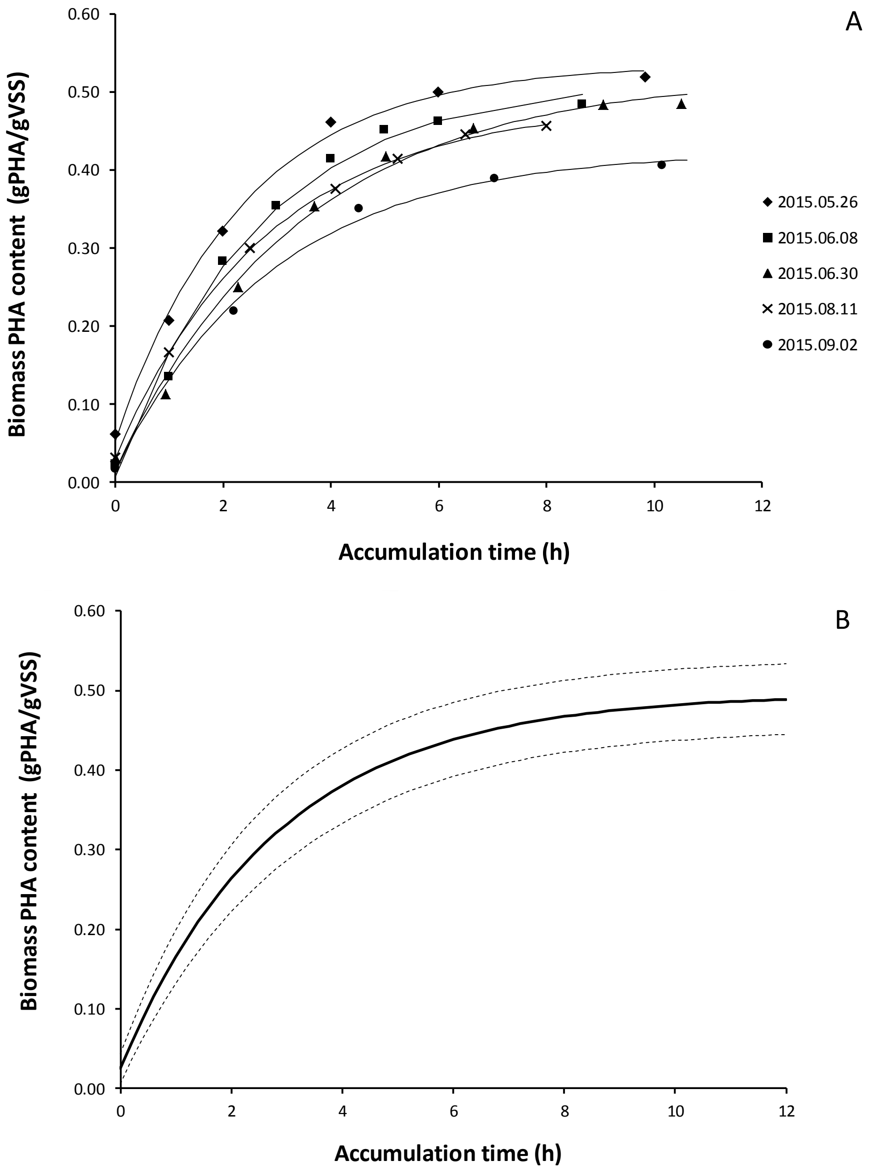

Fig. 13. PHA-accumulation response of pilot process biomass using as feedstock KMC process effluent at different points during the operating period (A) and as an average (solid line) with standard deviations (dotted lines) (B)

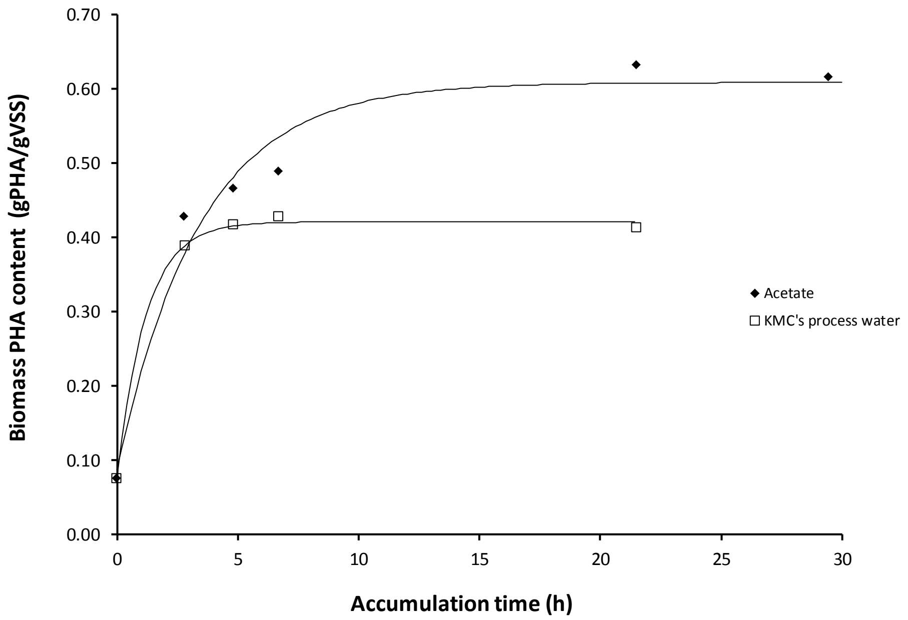

Fig. 14. Comparative lab-scale PHA accumulations with PE2 biomass (2015.05.20) using as feedstocks KMC process effluent and acetate

7. PHA polymer characterization

The PE3 pilot accumulations nominally produced around 0.5 kg of intracellular PHA. Selected batches of PHA-rich biomass were further processed for complete PHA recovery by green-solvent extraction. Recovered polymer quality was characterized.

KMC process effluent yielded PHA co-polymer blends of poly(3-hydroxybutyric-co-3-hydroxyvaleric acid) or PHBV. The 3-hydroxyvalerate (3HV) content was estimated to be on average 2% (mass) based on GC analyses. These 3-hydroxybutyrate (3HB) dominated co-polymers were with a weight average molar mass of 573±60 kDa (*n*=6) and a high thermal stability (Decomposition temperature, T_d = 286°C). When acetate was used as substrate, a homopolymer, of poly(3-hydroxybutyric acid) or PHB, was produced for which the weight average molar mass was similarly high and within 500 and 594 kDa and the thermal stability similarly high with of T_d = 286°C. Other assessments based on plate-plate melt rheology and thermal gravimetric analysis suggested a polymer product of commercial quality. Overall, the recovered PHBV and PHB had properties comparable to a similar grade of commercially available factory grade PHBV made in China, made from pure culture fermentation and with refined feedstocks as carbon source.

8. Conclusions

The operation of the Cella™ Technologies pilot prototype at KMC Treatment Plant demonstrated that the enrichment and production of biomass with PHA accumulation potential can be achieved under simultaneous water quality management of the KMC process water effluent.

The activated sludge biomass developed by feast-famine conditions was able to remove 98% of the incoming COD from KMC process effluent and presented good settling separability. Treatment performance was consistently maintained despite the variations in KMC process effluent COD quality and strength, temperature, and pH.

The pilot evaluations demonstrated the practical feasibility of producing excess activated sludge biomass with increased PHA-storage capacity. In an extended outlook for biological wastewater treatment, this implies that “waste” activated sludge (WAS) can be transformed into a harvested useful biomass with an increased value for resource recovery in residuals management schemes of bio-resource value chains.

From the holistic perspective of PHA production, KMC process effluent is a good feedstock for the production of PE2 biomass with enhanced PHA-storage capacity. The use of the process effluent as a PE3 feedstock requires further examination if the full extent in the developed capacity of the biomass PHA accumulation potential of the biomass is to be exploited. From a COD treatment perspective, KMC process effluent can be efficiently treated in PE2 and PE3 unit processes.

Based on the evaluated performance, the last months of operation of the pilot prototype will be dedicated to achieving a production mode of at least 2 batches of PHA-rich biomass per month using acetate and/or a mixture of acetate/propionate in order to continue evaluating product stability and quality. The piloting prototype has produced PHAs with polymer quality similar to commercial grade materials. It is noteworthy to realise that a water quality management service can yield products similar to current commercial polymer production methods based on more expensive pure-culture technologies.

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STRATEGIES FOR INTEGRATING CELLA™ TECHNOLOGIES AT THE KMC PROCESS WATER EFFLUENT TREATMENT PLANT

BIOPOL WP2

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CONTENTS

1. Executive Summary

KMC in Brande, Denmark, produces high-quality, potato-based food ingredients. The production results in a daily process water effluent of 400 m^3 and this flow is high in organic matter measured as soluble chemical oxygen demand (sCOD). The effluent sCOD is required to be removed before discharge to the local sewer. An on-site activated sludge process water treatment plant has been in operation for many years with ongoing challenges in meeting final effluent discharge quality objectives. In this report outcomes of a pilot investigation are provided that suggest KMC could establish a more robust process water treatment system while enjoying an opportunity to become a raw material supplier within regional biopolymer value chains.

Currently, the KMC treatment plant is operated with a long sludge retention time (SRT) of about 45 days and with three aerobic activated sludge tanks operated in series (total volume of 7000 m^3). Final effluent biomass separation is by means of five parallel membrane-filtration units, which currently operate for, and with, the challenges of poor biomass separation. A low separation efficiency reduces the plant treatment capacity and, this has led to arrangements wherein fractions of KMC process water effluent is routinely transported off-site for management by other regional biological wastewater treatment facilities. Within the project BIOPOL, Veolia Water Technologies AB have evaluated the activated sludge process at KMC at pilot scale. The primary focus has been to assess the potential for KMC to use process water effluent as a raw material for biopolymer production based on Cella™ Technologies. In so doing, we have also established insights for the treatment plant strategy towards more reliable process water treatment operations and performance.

Cella™ Technologies pilot facilities were used to benchmark both an efficient means of effluent COD management, and a route for procuring raw materials in the form of a surplus biomass as a value added raw material for polyhydroxyalkanoate (PHA) production. PHAs are biopolymers of increasing interest as platform chemicals for the biochemical and bioplastics industries. During 2014, a laboratory-scale preliminary investigation successfully demonstrated that KMC process water effluent was a good feedstock for a biopolymer value chain while still meeting the COD removal requirements (De Grazia, 2014). These positive laboratory outcomes were reproduced on-site this year now at pilot scale in Brande, as part of the project BIOPOL.

Based on both laboratory and pilot operation outcomes and experience, we now confirm that KMC water quality management could be a biopolymer-production opportunity in the order of 450 tons of PHA per year. We have found that production of a functional PHA-storing biomass achieves greater than 98% COD removal from KMC process water effluent and that this biomass is readily separated after treatment by a gravity unit process. Two of the existing KMC activated sludge volumes are believed to be in excess for the needs of water quality improvement, and we estimate that oxygen requirements for treatment may be reduced by 25%. Thus, the existing process operations at KMC treatment plant have the opportunity to be simplified and the existing infrastructure may be re-directed to an opportunity of PHA as a renewable resource made out of the factory effluent.

From the pilot-scale investigation, it was suggested that KMC process water effluent could be managed within a significantly reduced process volume and with a well-performing settleable activated sludge that exhibited a PHA accumulation potential of up to 70% gPHA/gVSS. In contrast to the current full-scale WWTP operations at KMC, the Cella™ pilot prototype was operated based on outcomes from the preliminary laboratory-scale investigation with feast and famine cycles and with a significantly shorter SRT of about 7 days. Feast and famine cycles selected for an easy-to-separate functional biomass while consistently removing greater than 98% of the factory process water effluent COD.

An activated sludge biomass with good separation characteristics is anticipated to be necessary should KMC wish to become independent from needs of off-site effluent management and in order to be able to expand on the current factory production capacity. A reduction in process SRT from the current 45 days to 7 days would allow for treatment of the process effluent flow in one main process volume of about 2300 m^3 (about 36% of the current volume from 3 tanks at KMC treatment plant). The main process volume would establish a famine zone for the biomass. Engineering of a full-scale integration of the pilot-scale study feast operation may be accomplished by means of an adjunct process selector volume (~200 m^3) that is operated in series with the main aerobic (famine) volume. The feast and famine zones to which the biomass are subjected to, during process effluent treatment, contribute to the selection of a PHA-storing biomass and, as well, we believe to a biomass with good separability. Furthermore, the manner of combination of feast and famine volumes are understood to be advantageous in the economy of the process aeration and the costs of an upgrade based on a desire to utilize the existing infrastructure to as great an extent as possible.

The shorter 7 day SRT will produce more biomass and this increase in biomass production yield is advantageous for reducing process energy demands (aeration) and for the utilization of the biomass as a value-added, renewable resource, raw material. In order to operate at a 7 day SRT, a COD-to-macronutrients requirement of 100:4.5:0.6 (COD:N:P) is estimated. By reducing the process SRT, the sludge production yield increases to an estimated value of 0.26

kgVSS produced per kgCOD removed with consequent savings in aeration demand of the process (0.63 kgO_2 consumed per kgCOD removed expected). Currently, it is estimated that KMC operates with a biomass yield of 0.11 kgVSS produced per kg COD removed, and, thus, the amount of oxygen necessary to accomplish COD removal is in the order of 0.84 $kgO_2/kgCOD$. In this way, we predict a 25 percent reduction in oxygen demand for the wastewater treatment.

Further associated benefits are understood to be possible from the integration of Cella™ Technologies to the KMC process water effluent management. We experienced a biomass at pilot scale with good separability exhibiting an SVI of less than 170 mL/gTSS. Both the famine volume as well as the feast volume may be operated as sequencing batch reactors (SBRs). If the main famine SBR serves also as the settler for the final effluent discharge, then other on-site infrastructure (settler and membrane units) may be directed to other purposes such as for an on-site activity of PHA production in the surplus biomass. Based on the experience at pilot scale it should be possible to produce an activated sludge from the process effluent water treatment that enables KMC to meet final effluent discharge standards from simple gravity separation of the process effluent from the biomass.

The anticipated KMC excess activated sludge (about 450 tonVSS/year) could be made to be a valuable resource raw material for a PHA production value chain (Figure E1). PHA production using 450 tonVSS/year as raw material requires about 1300 tonCOD/year. Other KMC residual streams (e.g., starch residuals, fruit juice) are produced in large amounts during the starch modification processing steps and these are all potential additional feedstocks to be evaluated as substrate for PHA production. A PHA accumulation unit process could be performed on-site at KMC treatment plant by exploiting already existing infrastructure that is suggested not to be essential for the needs of the process water treatment based on the experience from the present investigation. KMC could become further involved with other local stakeholders within the regional landscape in suppling and processing of biomass and carbon-rich streams as raw materials for locally bio-based economic developments.

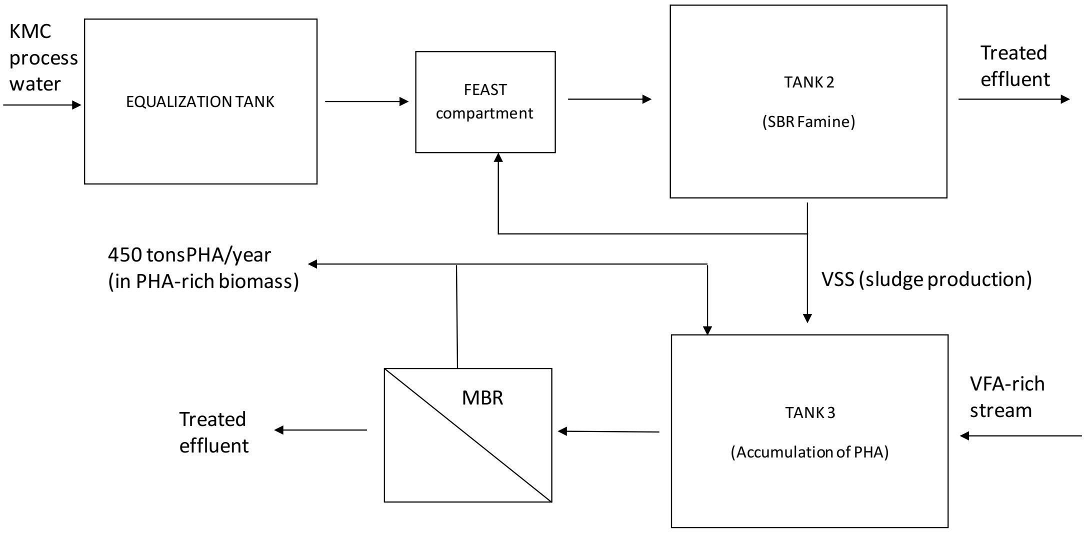


Figure E1. Envisioned potential operation of KMC treatment plant for the treatment of process water and production of a value-added PHAs with Cella™ Technologies.

2. Introduction

In this report we present strategies for the integration of Cella™ Technologies into the existing facilities of the KMC process water treatment plant. The process water can be treated while at the same time be used for the production of biomass with enhanced storage capacity of polyhydroxyalkanoate (PHA) biopolymers. Process water treatment requires the removal of factory effluent organic content, which is typically measured as chemical oxygen demand (COD). Based on laboratory tests (De Grazia, 2014) and on the pilot-scale prototyping of Cella™ Technologies with KMC process water effluent treatment (BIOPOL WP1), it was found that the COD of KMC process water effluent:

i) is biodegradable (> 98% COD removal) by an acclimated activated sludge that is disposed to cyclic feast and famine environmental conditions, and as a result,

ii) produces a functional activated sludge biomass with satisfactory settling properties (SVI < 200mL/g) and enhanced PHA-production potential.

Cella™ technologies comprise in this case the operation of two sequential biological process elements (PEs): one for biomass production (PE2) and one for PHA accumulation (PE3). Both process elements are generally aerobic activated sludge treatment units where the soluble COD (sCOD) of the process water is consumed biologically. In PE2, the influent sCOD (KMC process water effluent after suspended solids separation) is partially consumed by biological oxidation into carbon dioxide and partially consumed and converted into functional biomass with enhanced PHA storage capacity. PE3 exploits this produced functional biomass from PE2 for sCOD conversion primarily into intracellular PHA and to carbon dioxide.

An integration strategy of Cella™ Technologies at KMC treatment plant could entail the operation of both PE2 and PE3 with approximately equal volumetric distribution of KMC process water effluent between these two process elements. Nevertheless, from the operating experience of the pilot prototype, the available COD best avails for the production of biomass in PE2. The pilot prototype accumulation process (PE3) has shown the ability to remove COD from KMC process water effluent and produce significant amounts of intracellular PHA. Reliable use of the KMC process effluent as a PE3 feedstock requires a deeper understanding of the COD quality and of measures to compensate for quality variations that were experienced in this study. PE2 operations were robust in spite of such variations wherein the COD removal capacity remained >98% with good biomass settling characteristics throughout (SVI < 200 mL/g). Moreover, the excess waste activated sludge produced in PE2 was a harvested functional biomass, characterized by an enhanced and stable PHA accumulation potential (PAP) of up to 70 % gPHA/gVSS). If the Cella™ Technologies integration strategies (Section 4) were to be considered as an upgrade option for KMC treatment plant, the excess biomass produced will have the potential to be harvested as feedstock for eventual PE3 PHA production (Section 5), instead of being “wasted”, as is the practice currently today.

3. KMC process water effluent treatment plant configuration

3.1. General description of current treatment process

KMC starch-modification activities today discharge a yearly process effluent flow of 150 ML (10^6 L) with an associated COD of around 1500 tons of COD per year. KMC plans to increase production of modified starch by 2020 to an increased yearly effluent of about 200 ML. Information reported in this section are derived from the KMC Drift Journal (Appendix1) or from personal communications from KMC technical direction and the operators of the treatment plant.

Today, the process effluent COD treatment is accomplished in three aerobic activated sludge tanks operating in series (Tank1, Tank2 and Tank3). This process train is followed by a membrane system (MBR) for solids separation from the effluent residual (Figure 1). The total aerated tank volume available for the activated sludge process is about 7000 m^3. The factory final effluent needs to meet final effluent water quality criteria (Table 1) as defined by the local municipality before factory effluent can be discharged to the municipal sewage system.

Daily factory process effluent flows and water quality are variable. Variations depend on the production campaigns in terms of specific products, modification process chemistry, and time frame. The treatment plant headworks therefore employ an equalization tank with a working volume of 2500 m^3. The equalization volume buffers the biological process from peak flows and transients in the process factory effluent water quality.

The factory process water effluent suspended solids are removed before the biological process. Suspended solids are for the most part residual starch particles coming from the starch-modification facilities. Suspended solids removal is by settling and centrifugation. The collected starch residuals (around 60 wet tons per month) are stored in containers and transported as a feedstock to external biogas production facilities.

The task of process effluent COD removal currently produces excess activated sludge, a biomass that needs to be separated from the final effluent and disposed of. The activated sludge wastage is controlled by maintaining the total suspended solids (TSS) concentration in Tank3 within 5 and 6 gTSS/L. When the concentration of TSS in Tank3 exceeds the upper limit, a defined volume of mixed liquor suspended solids (MLSS) is centrifuged, collected and transported offsite as a feedstock for digestion and biogas production. The reject water exiting the centrifuge is recirculated back to either Tank2 or Tank3.

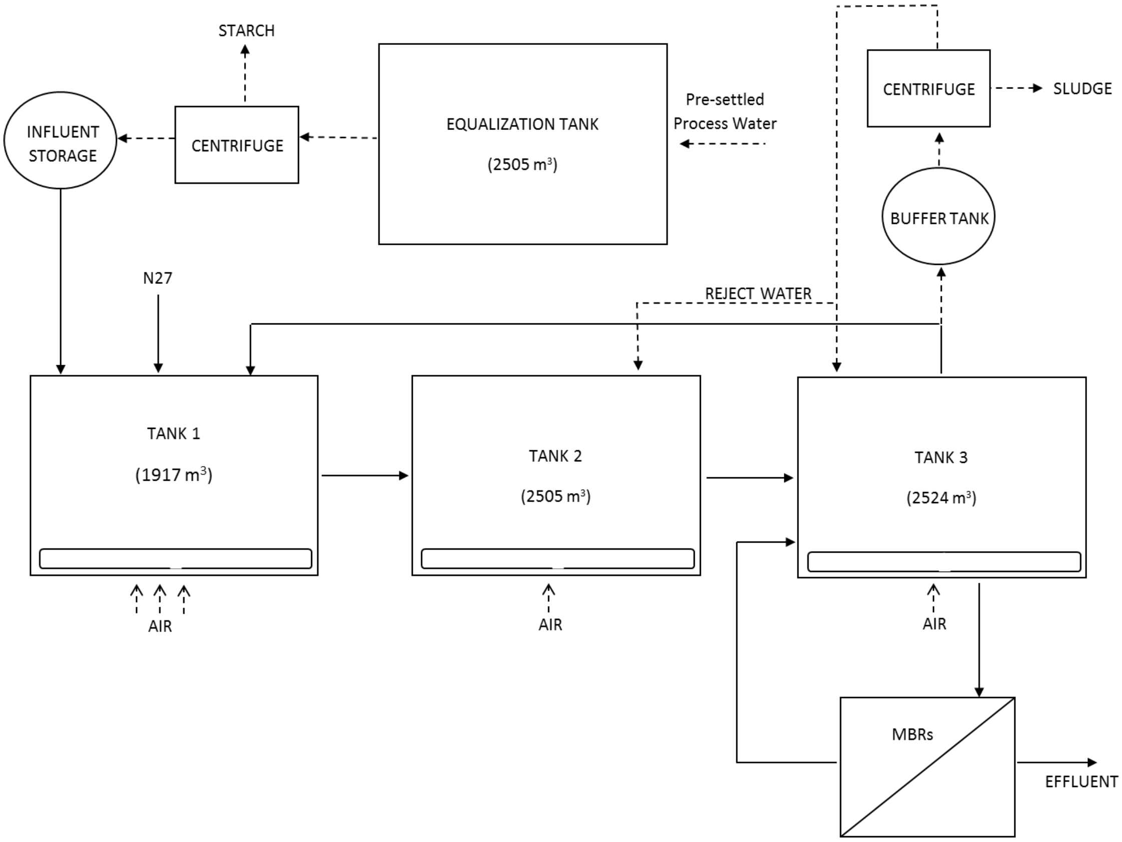


Figure 1. Current configuration (December 2015) of the KMC process water effluent treatment plant. Dashed lines represent semi-continuous flows

Table 1. Effluent water discharge limits imposed to KMC treatment plant

FLOW LIMITS	average	maximum
m^3/h	16	40
m^3/d	410	550
m^3/year	125000	150000
CHEMICAL LIMITS	kg/d	kg/m^3
t-COD	700	1.25
t-Nitrogen	20	0.035
t-Phosphorus	10	0.02
SS	165	0.3
Chloride	2200	4

3.2. Main inputs to the treatment process

From January to September 2015, an average of 404 m^3/d of KMC process water effluent flow has been fed to Tank1. The KMC treatment plant operates with energy and chemical inputs in three categories (Table 2):

i) chemicals associated with the process water effluent,

ii) extra chemicals for process operation and maintenance, and

iii) energy for process operation.

In Table 2, the main inputs to the treatment plant that are relevant for biological treatment control and plant maintenance have been identified.

Table 2. Main inputs for KMC treatment plant operation and maintenance.

	VOLUMETRIC FLOW	CONCENTRATION	MASS FLOW	
ASSOCIATED WITH THE PROCESS WATER	m^3/d	kg/m^3	kg/d	Appendix 1
t-COD	404	9.7	3920	
s-COD	404	9.5	3840	
s-Nitrogen	404	0.01	4	
s-Phosphorus	404	0.04	16	
Phosphate-P	404	0.018	7	
EXTRA CHEMICALS	L/d	kg/m^3	kg/d	Appendix 2-3-4
N27	50 - 80	345	17 - 30	N addition
Polyaluminum Chloride	120 - 200	30-40%	n/a	P precipitation
Struktol J650	2 - 4	n/a	n/a	Foam killer
Hydrochloric Acid	30 - 100	30%	n/a	Membrane cleaning
INSTALLED POWER	**Tank1**	**Tank2**	**Tank3**	
	kW	kW	kW	
Air Blowers	110+18+13	36	45	
Air distribution	fine bubbles	coarse bubbles	coarse bubbles	Appendix 5-6
Mixing	n/a	22	22	

The principal chemicals involved in the biological treatment of COD are nitrogen and phosphorus (macronutrients). Some macronutrients are associated with the process water but they also need to be added separately. Nitrogen and phosphorus are essential for microbial biomass metabolism and growth. The macronutrient loading target to the treatment plant has been understood to be a ratio of 100:5:1 (COD:N:P) with respect to the COD loading and on a mass basis.

A comparison between the available COD-to-macronutrient levels in the process water with the target values was made (Table 3). This comparison shows that KMC process water effluent is deficient in both N and P for biological COD removal. An external source of nitrogen, in the form of urea (N27, Appendix 2), and in amounts as indicated to us by the plant operators (Table 2), is added to Tank1. Nevertheless, the amount of urea delivered was estimated to be insufficient to reach the understood COD:N target ratio of 100:5 (Table 3). Furthermore, no additional phosphorus is added, as part of the routine operations, even though the COD:P levels in the process water effluent are below the target values for biological treatment. As shown in Table 2, a precipitant agent (Appendix 3) is used for removing what appears to be excess phosphorus from the treated effluent and comply with the effluent discharge limits.

Energy consumption in an aerobic biological treatment plant for COD removal is mainly associated with blower operations for air supply. Since most COD removal is currently achieved within Tank1, most aeration power has been installed in this reactor (Table 2). In Tank1 no mixing is performed because of the mixing energy generated from the 110 kW of installed blowers. Blowers are connected to a submersible fine bubble distribution system (Appendix 5). It has been reported by the operators that due to issues of stability of operation (liquid entering the motor) provided by the submersible aerators currently in place in Tank1, other air delivery systems (e.g. bottom aeration membranes) are being evaluated (18 + 13 kW blowers).

Tank2 and Tank3 also consume power for aeration and mixing (Table 2). The same types of aeration/mixing systems (coarse bubbles) are used in both tanks (Appendix 6). Due to the long treatment process SRT and the fact that most soluble COD is removed in Tank1, mixing and aeration of the mixed liquor in Tank2 and Tank3 most probably achieves some level of aerobic digestion, resulting in internal process cycling of nitrogen and phosphorus.

Table 3. A comparison between the level of macronutrients available in the KMC raw process water effluent and after N addition with the target values for plant operation.

	AVAILABLE	KMC TARGET
LEVEL OF MACRONUTRIENTS	kg/kg	kg/kg
s-COD : s-N	100 : 0,1	100 : 5
s-COD : s-P	100 : 0,4	
s-COD : PO_4 - P	100 : 0,18	100 : 1
AFTER N27 ADDITION	kg/kg	kg/kg
s-COD : s-N	100 : 0,9	100 : 5

3.3. Evaluation of operation and performance

The operation of KMC treatment plant may be characterized by the large inventory of activated sludge solids (TSS), the long sludge retention time (SRT), and the large tank volumes (Table 4). This combination is similar to the operation of an *extended aeration* process configuration. An extended aeration process configuration is usually applied for municipal wastewater treatment where reduction of both COD and nitrogen (nitrification) is required and low sludge production is preferred. Generally, these systems are operated at long SRTs (> 30 days), and this can ensure low effluent COD concentrations provided that enough oxygen and nutrients are supplied. The waste sludge produced as part of the COD removal process is generally with some level of aerobic digestion and such aerobic stabilization is typified by a relatively low organic content of the TSS, expressed as VSS/TSS, where VSS is the volatile suspended solids concentration. The activated sludge VSS/TSS is presently indicative of extended aeration with VSS/TSS between 65 and 75 percent.

Table 4. Some parameters that currently characterize the KMC treatment plant

		Tank1	Tank2	Tank3	Overall
SRT	days				45
HRT	days				17
Volume	m^3	1917	2505	2524	6946
TSS	$kgTSS/m^3$	2.6	3.3	5.4	
TSS	tons TSS				27
VSS/TSS	%				65-75

In an extended aeration configuration, the sludge production yields are lower because of the long SRTs and the oxygen requirements for COD removal are generally higher than for conventional activated sludge systems (WPCF Manual Practice No.8, Water Pollution Control Federation). From a COD mass balance of a biological treatment process, one can derive a relationship between oxygen consumption (-ΔO2), overall COD removed (-ΔS) and sludge production (ΔX):

$$(-\Delta O_2) = (-\Delta S) - (\Delta X) \qquad 1$$

Only a part of the treatment process influent COD removed (-ΔS) relates to oxygen consumption ($-\Delta O_2$) because some COD is converted into biomass (ΔX).

The estimated average sludge production yield for the KMC treatment plant during 2015 from operation data was 0.11 kgVSS produced per kgCOD removed. Assuming a COD conversion factor for the biomass of 1.42 kgCOD per kgVSS, an oxygen consumption yield (kgO_2 consumed per kgCOD removed) of 0.84 is estimated based on equation 1. The mass of oxygen required to remove the COD, together with the efficiency of its distribution into the liquid phase, determine the overall cost of aeration operational expenses.

Currently, 60 to 80 % of the influent COD is removed in Tank1. This estimate is based on grab samples that were taken in order to characterize the full-scale process during the parallel pilot operations. Since Tank2 and Tank3 were found to be negligibly loaded with soluble COD, these two tanks are interpreted to operate at endogenous respiration levels. Extended aeration at endogenous respiration rates is a unit process of sludge digestion.

3.4. Key aspects identified to affect plant operation and performance

From the observations of the full-scale process operations juxtaposed to the pilot plant demonstration experience, SRT was understood to significantly affect the KMC process water treatment plant performance and OPEX.

The characteristics of the biomass produced and contained in the process volumes is, at least in part, related to SRT. The mixed liquor suspended biomass morphology is characterized by diffuse, small flocs (Figure 2), with relatively high amounts of extracellular polymeric substance (EPS). One probable causative factor for this characteristic biomass is the very long SRT (Wanner, 1994). Long SRTs may induce over oxidation and destruction of activated sludge flocs leading to the production of biomass with poor separation characteristics. A history of poor biomass quality for separation has been experienced at KMC with sludge volume indices (SVIs) of greater than 700 mL/g reported. Poor biomass separation characteristics affect plant treatment capacity, operation and OPEX. A counter measure has been to install membrane filtration (MBR), due to poor gravity separation, with associated CAPEX and OPEX costs. The MBR efficiency in filtration determines the daily effluent flow and this can constrain the capacity for process water flow. In addition, high EPS levels can also be indicative of nutrient limitation during COD removal.

Unnecessarily high OPEX costs are suspected to be associated with energy and maintenance of three aerated tanks, with a high mixed liquor sludge inventory, coupled to the currently applied long SRT. We interpret that approximately one third of the process volume achieves the goal of influent COD removal, while the remaining two thirds of the process volume aerobically digests a fraction of the excess biomass into inert solids and CO_2. Therefore, in the current operations, a lot of effort, energy, and expenses are devoted to challenges of biomass management.

Based on these observations of the current full scale process configuration and operations, and alongside experience and outcomes of the piloting plant operations, an integration strategy was established for Cella™ Technologies at KMC. In the first instance this integration strategy is not so much about PHA production but, rather, more about optimal and economic biological process water treatment. What we can construe is that KMC should be able to treat the factory process effluent more robustly while producing a biomass with significantly better morphology. Improved morphology is with respect to a flocculating biomass that can be readily separated from the treatment process effluent by gravity settling. The benefit as we see it is in the first instance primarily related to the business of effluent water quality management. However, the opportunity becomes that the surplus biomass becomes easier to manage, and what is more, the surplus biomass promises to be a raw material for PHA production. This latter economic and resource supply opportunity can be explored at leisure after the fact of more pressing needs for readily implementable process configuration and operation improvements.

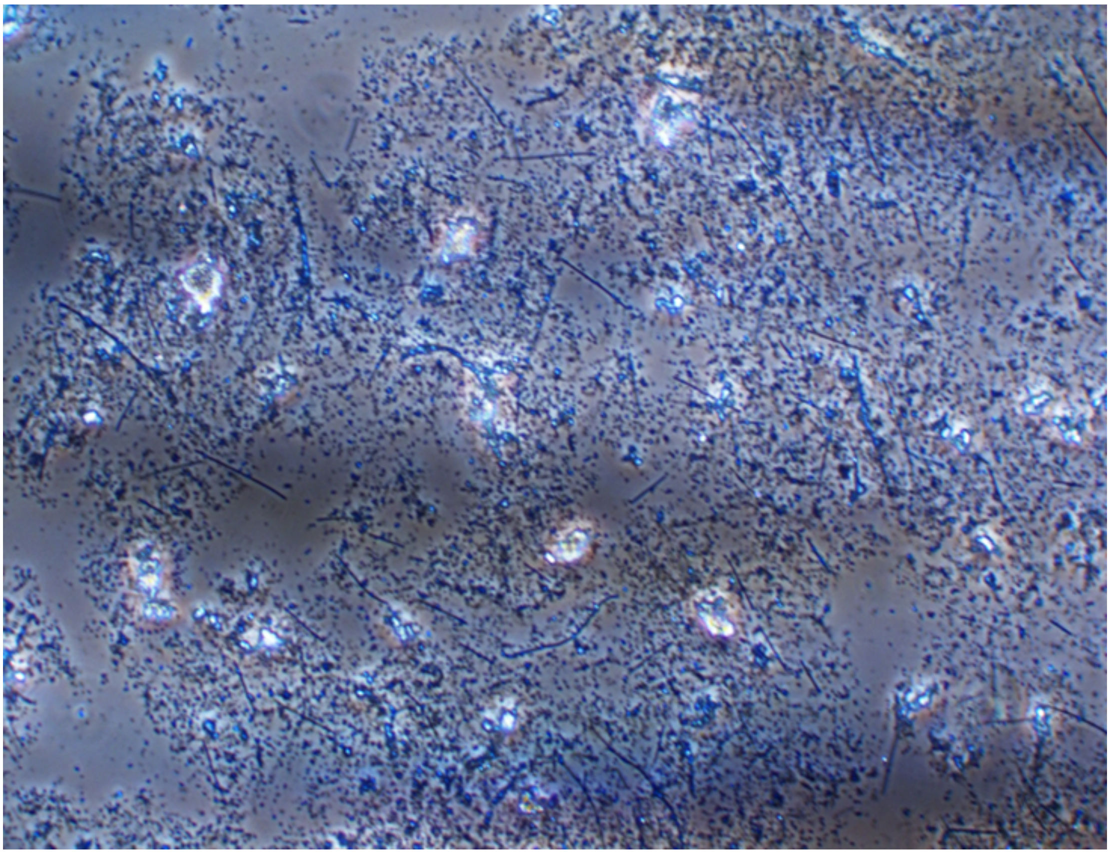


Figure 2. Picture (100x) of biomass harvested from Tank1 at KMC treatment plant (March 2015)

4. Integration Strategies of Cella™ Technologies at KMC

In the following sections considerations are made for parameters and process configurations for the integration of Cella™ Technologies for COD removal and biomass production into the existing facilities of the KMC process water effluent treatment plant. Estimations of the process design parameters are based on average (January to September 2015) COD concentration data, COD removal rates (pilot prototype operations), and KMC plans to increase production in the future (2020 plan). As noted in the previous section, this integration strategy is, in the first instance, a recommendation for improved reliability and economy of the water quality management. This recommendation is made with the objective to make use to as great an extent as possible of the existing infrastructure. As a bonus to the improvement of the water quality management and economy, we anticipated based on the piloting experience that the surplus biomass from the treatment plant can be a raw material for PHA based biopolymer production value chains.

4.1. Rationale and essential conditions for technology integration

In contrast to the current KMC treatment plant configuration, the Cella™ strategy for the removal of the COD from the KMC process water effluent employs the engineering of feast-famine process environments and the production of excess biomass at relatively shorter SRT. In the pilot operations, this operating strategy allowed for COD removal (> 98%) *in just one process volume* while selecting for a settleable PHA-storing biomass (BIOPOL WP1).

A shorter SRT biomass comes with the advantage of producing a heterotrophic biomass with higher active content (higher VSS/TSS ratio), and this results in faster specific COD removal rates when compared to a long SRT biomass. Moreover, for KMC, reducing the SRT and the treatment process volume comes with an increased cost effectiveness in operations due to a decreased treatment oxygen demand, and an anticipated reduction in maintenance. In order to reduce the SRT, supply of both macronutrients (nitrogen and phosphorus), in addition to air for oxygen need to be provided in step with the COD removal rates and excess biomass ("sludge") production yields.

SRT reduction with control of macronutrients and oxygen supply represent necessary conditions, but these in themselves do not guarantee for good activated sludge floc morphology with good settleability. Our anticipation is that, selectors used historically in the wastewater treatment for improving floc morphology for good separation, will in parallel ensure the production of excess biomass with significant PHA accumulating potential. Therefore, by attention to the bioprocess engineering towards a goal of improved biomass separability, one also achieves a benefit of producing a functional raw material for PHA based value chains.

The characteristics of the KMC process water effluent (high strength and rich in easily biodegradable COD) is classically one for which a selector is recommended due to a risk of producing a bulking activated sludge biomass with predominant filamentous microorganisms. The growth and proliferation of filamentous organisms is known to be promoted when operating single-stage complete mix activated sludge systems where low COD effluent levels are maintained in the aerobic reactor (Metcalf and Eddy, third edition, 537).

A well-known approach that is reported in the literature for the prevention and control of filamentous microorganism proliferation is the use of a separate compartment (selector) where influent wastewater and recirculated biomass are combined at high food to microorganism ratios (F/M). This selector configuration is in itself a feast environment, and the downstream maintenance of the biomass provides for a famine environment. Thus the bioprocess engineering for enrichment of a PHA accumulation biomass is congruent to the interests of KMC to improve the process for selecting biomass with a better floc morphology.

Therefore, the benefit of creating feast and famine conditions in the KMC COD removal process is double because it provides for the selection of an easy-to-separate activated sludge biomass with high PHA accumulation potential. These outcomes were demonstrated in practice in WP1 with the pilot plant operations for the project BIOPOL.

4.2. Integration of PE2 feast and famine with KMC process effluent treatment

Feast and famine conditions can be achieved as part of the operations of a sequencing batch reactor (SBR). An SBR configuration is an excellent and convenient unit process for establishing principles of treatment at laboratory and pilot scales. SBRs can also be effective and economic in full-scale unit processes. Notwithstanding, there can be reasons to seek to achieve the same principles in a full scale bioprocess design by means other than a classical SBR unit process design.

Process information obtained in SBR experiments has analogy to what biomass experience in ideal plug-flow continuous-flow bioprocess designs. Therefore, the data that has been gathered to date by AnoxKaldnes at bench and pilot scales about bioprocess and treatment of KMC factory effluent can be applied towards a spectrum of possible scaled up process configurations. Given that KMC already has vested interested with existing treatment volumes and equipment, it is of advantage to apply upgrades for an improvement by using the existing infrastructure as much as possible.

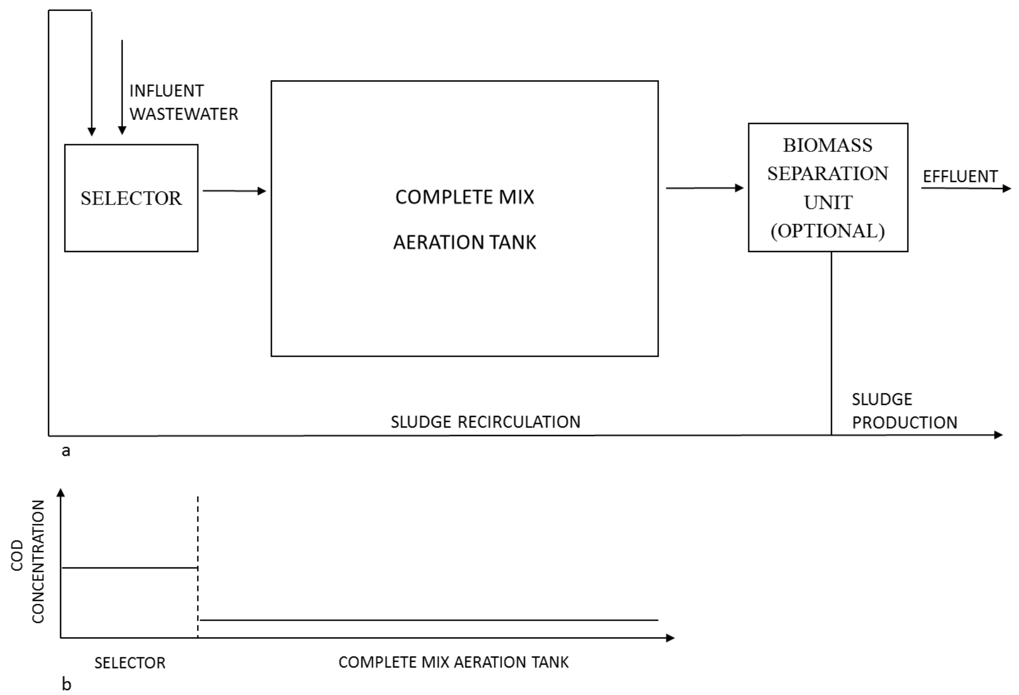
As a means to maintain the control over both feast and famine environments while also paying attention to needs for improved overall CAPEX and OPEX for the upgrade and operations, we recommend separate process volumes of the selector (feast) and the biomass maintenance (famine) environments. A biomass maintenance volume already exists and can be selected from one of the three existing treatment volumes. Biomass separation from the treated effluent can be undertaken in the selected maintenance volume, or with a downstream separation unit process. A viable upgrade to the KMC process water treatment is envisioned to be either continuous or semi-continuous flow (Figures 3 to 5).

The purpose of the feast selector is to remove readily biodegradable COD. In all our practical work to date, we have evaluated the operation and performance of an aerobic feast process volume. Sufficient biomass and air are mixed with the effluent from the equalization tank (Figure 1) such that a selector volume effluent is with significantly reduced sCOD concentration (Figures 3 to 5). What has been out of the scope of the current laboratory and piloting work to date is a known potential to also achieve feast selection anaerobically (Bengtsson 2009). The emphasis on an aerobic selector to date was drawn from the interests to demonstrate the production of a robust activated sludge based on aerobic treatment and in contrast to the current experience at KMC. Both aerobic and anaerobic selector options are considered as viable integration strategies and process upgrade options for KMC. Laboratory and pilot operating data that has been gathered to date can be used towards the design and specification of an aerobic selector. Historical research and in-house know-how at AnoxKaldnes for anaerobic selection could be applied towards a conceptual design, but some form of practical testing of principles of the biology are to be recommended in this particular case.

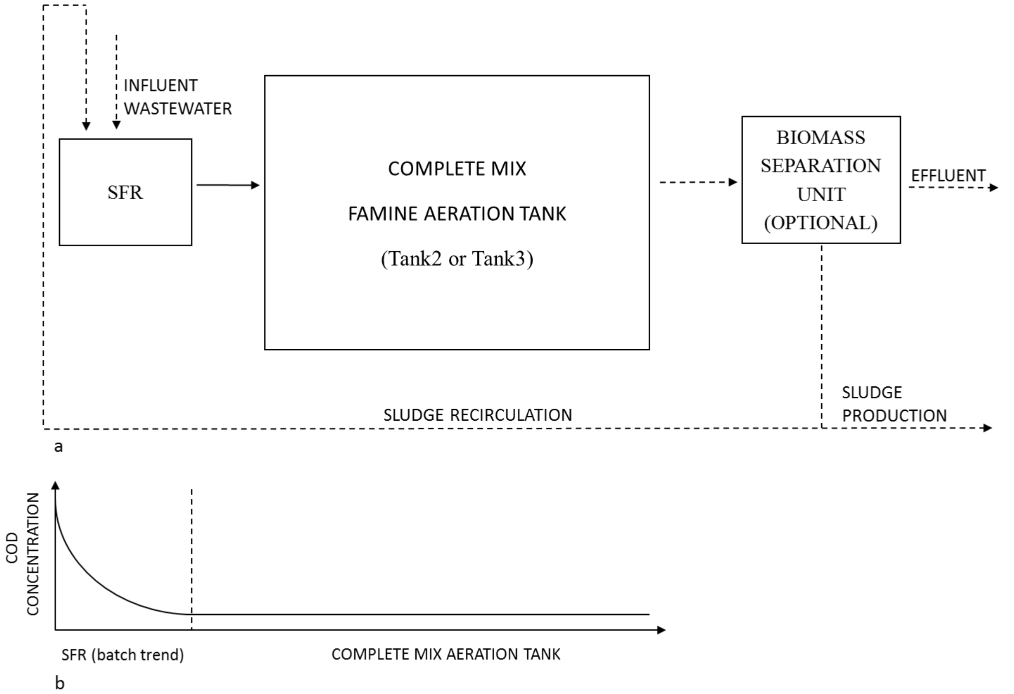
Naturally, an anaerobic selector implementation could help to further save considerably on aeration costs because the initial stage of sCOD removal requires only mixing energy. Practical testing would inform on projections for additional savings while also confirming the formation of a biomass with stable morphology and reliable treatment with anaerobic selection. Such a biomass can be similarly functional with respect to potential for PHA accumulation.

Even an aerobic feast selector is nevertheless anticipated to significantly reduce aeration CAPEX and OPEX of the overall treatment plant compared to the situation today. Conditions of aerobic biomass digestion currently applied are to be avoided. The selector volume for aerobic feast represents a small vessel of 5 to 10% of the overall process volume. The main process biomass maintenance volume would be one of the existing tanks (Tank2 or Tank3) at KMC. Therefore, the total aeration volume will be substantially reduced. The strategy of the external feast selector allows for more optimal distribution of power demands such that costs, if based on peak consumption, are kept lower. Influent process water and recirculated biomass are mixed together to achieve the desired organic loading rate and to induce feast conditions repeatedly for fractions of the biomass at any given time.

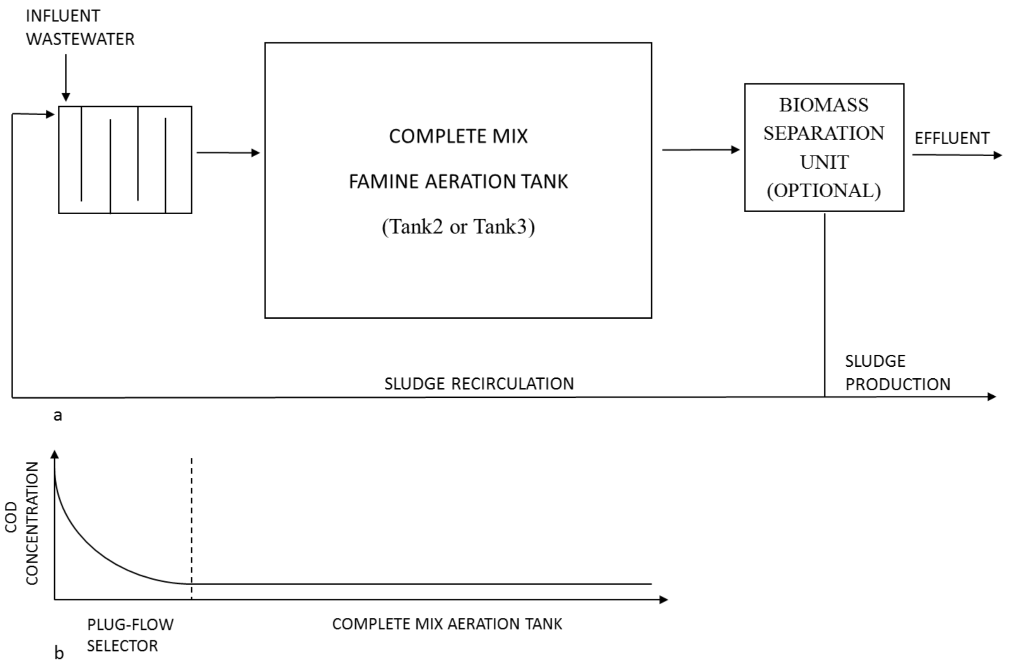
A conventional selector (Figure 3) operates continuously under completely-mix conditions and not necessarily with full COD removal. With interests to establish distinct environments of feast and famine, the objective of the selector in this case will be to remove all the input readily biodegradable COD (RBCOD). To this end, we propose process configurations with either the integration of a sequencing feast selector (Figure 4) with semi-continuous influent from the equalization tank, or a continuous plug-flow selector (Figure 5). Biomass separation is recommended to be by gravity settling. By incorporating some form of sequence operation to the process, the maintenance (famine) tank could be employed for collection, decanting and final effluent discharge. Alternatively, biomass and process effluent from the maintenance volume may be separated continuously, or semi-continuously in a downstream unit process. The ideas of aerobic and anaerobic selection are presented further in more detail below.



Figures 3. Configuration (a) and COD profiles (b) of a biological treatment plant with selector strategy for sludge bulking control. In this configuration the selector operates under complete-mix conditions.



Figures 4. Configuration (a) and COD profiles (b) of KMC process effluent treatment with semi-continuous flows characterized by a sequencing feast reactor (high F/M) and a famine reactor volume (low F/M). Dash lines (a) represent semi-continuous flows.



Figures 5. Configuration (a) and COD profiles (b) of KMC process effluent treatment with continuous flows characterized by a selector feast reactor (high F/M) and a famine reactor volume (low F/M).

4.3. Fully-aerobic selector integration strategy

Two possible process configurations have been considered towards an upgrade and improvement of the current status of the KMC process water treatment process. These considerations are independent of ideas of PHA production opportunities. Based on the piloting outcomes, we find that PHA value chains are an added bonus to anyway necessary steps towards improving treatment reliability, process capacity, and biomass morphology. For an aerobic selector the flows to the treatment plant can be semi-continuous or continuous (Figures 4 and 5). Figure 4 illustrates a sequencing feast reactor (SFR) and Figure 5 depicts a plug-flow selector (PFS).

The SFR (Figure 4a) represents a batch feast volume wherein influent KMC process water effluent from the equalization tank and recirculated biomass from the famine reactor are mixed together at selected intervals. The timing and operation of the batch feast cycles may be driven with feedback control from the biomass response to the organic loading. Therefore, the SFR is considered to be a flexible solution to accommodate variations in the influent water quality. The timing of feast cycles is constrained in part by the requisite daily volume of process water to be treated. COD concentrations for each SFR cycle may be expected to follow the trend in time similar to trends in distance for ideal plug flow (Figure 4b). However, our experience has been that COD removal rates for biomass stimulated into feast will follow zero order kinetics. This observation suggests that the time for COD removal by first order kinetics is conservative. Biomass respiration rate attenuation after the removal of COD for one influent batch is a means to respond in timing from one sequencing batch feast cycle to the next. For each cycle, the SFR volume is discharged to the maintenance volume and a new batch of process water effluent from the equalization tank is mixed with a selected volume of recirculated mixed liquor. The SFR configuration offers flexibility where both the applied F/M ratio and/or length of every feast cycle can be tuned in step with external factors such as factory operation peaks, or seasonal temperature changes. Because of a sequential operation, some time is taken, before and after every feast cycle, for discharge from SFR to the maintenance volume, and for pumping and flow of influent and mixed liquor to the SFR. The opportunity for flexibility in an SFR configuration are enjoyed in part because a significant equalization tank volume is already part of the KMC process water treatment system. A sequencing operation also makes it natural to consider the use of the maintenance volume doubly as a settler. In this case, treated volume is collected in the maintenance tank and periodically the maintenance suspended solids are allowed to settle so that the collected effluent can be decanted and discharged to the local sewer.

A PFS configuration is meant to achieve the same outcome as the SFR but now with continuous flow of process effluent from the equalization tank (Figure 5a). This strategy entails a process volume designed so as to ensure a plug-flow hydraulics from influent to effluent. Recirculated biomass and influent process water are continuously provided at the confluence of the PFR influent, and the influent COD is consumed in transport through the volume, which is analogous to time of incubation in the SFR batch cycles (Figure 5b). The PFR volume specification will need to be conservative in order to accommodate influent variations that require more residence time for treatment. Thus the SFR offers potential for flexibility in time of incubation which the PFR must in principle achieve with additional volume. For both the SFR and PFR, detailed design considerations are required for maximizing the use of gravity flows such that pumping CAPEX and OPEX costs can be trimmed as best as possible.

The operation of the maintenance or “famine” process volume may be adapted as mentioned to the SFR or PFR configuration. A sequencing famine mode of operation assumes that the maintenance volume is used in cycles of aerobic famine followed by periods of biomass settling and effluent decanting. A defined routine is necessary for mixed liquor “harvesting” and activated sludge retention time control (SRT), internal settling, and effluent withdrawal for hydraulic retention time control (HRT). The process effluent water quality is anticipated to be able to meet the municipal discharge standards based on the pilot plant operating experience for BIOPOL. The available MBR units could nevertheless still be used if necessary to polish the effluent suspended solids concentration. Alternatively, the MBR infrastructure and the unused tank volumes could be utilized in the future for PHA production in the harvested activated sludge.

A continuous mode of the maintenance volume operation for famine would require the implementation of a separation unit process downstream of the famine volume. This separation unit process would function as for conventional activated sludge with an internal return activated sludge flow. While it may be considered that the MBR units could serve the function of biomass separation from the process effluent, MBR separation may not necessarily select for a settling biomass floc morphology.

4.4. Anaerobic selector integration strategy

Selectors that stimulate a feast response in a biomass can be aerobic or anaerobic. Anaerobic feast selection tends to favour a more specialized kind of PHA storing microorganisms, known as GAOs or glycogen accumulating microorganisms. Therefore, the process configuration need not to change, but the conditions of the selector are changed from oxygen supply to no-oxygen supply.

AnoxKaldnes has been at forefront of leading academic research concerning the selection of GAO mixed cultures in process water treatment and the production of PHA in such harvested biomass (Bengtsson, 2008; 2009; 2010). In general, during the anaerobic feast (high F/M ratio), GAOs are able to utilize internal glycogen as a source of energy instead of oxygen to take up the sCOD and store PHAs. During aerobic famine, external sCOD is no longer available and internally stored PHA is utilized for growth, glycogen replenishment and cell maintenance. Based on our experience, overall sludge production yields and mass of oxygen required for the GAO biomass production are expected to be similar to those estimated for the aerobic feast and famine configuration. However, the GAO famine aerobic respiration demand is more steady in time and this means that the peak aeration demands may be expected to be less when compared to aerobic feast and famine selection. Notwithstanding, we have compensated for peak energy demands with aerobic feast by disposing only a portion of the biomass to feast at any given time in the SFR and PFR configurations. Therefore, in the end there may not be an aeration energy demand advantage with anaerobic-aerobic selection methods. These considerations required more detailed quantitative evaluation and ideally direct experimental evaluation of the anaerobic selection if that route seems to offer distinct CAPEX and OPEX benefits to the process upgrade.

An element of uncertainty for the success of a GAO strategy is due to the factory process effluent containing 40 to 50% of unidentified organic composition. Since the BIOPOL pilot undertaking was an effort of technology demonstration, and since we knew the non-VFA sCOD in the process effluent was aerobically readily degradable, we selected the approach most likely to succeed in demonstration and most like the current water treatment process employed by KMC today. The GAO approach is noted as a potential alternate route that is anyway compatible with the upgrade recommendations. However, confirmation of the GAO approach would require a separate effort of validation. Such a validation could be readily accomplished with laboratory scale testing in a similar fashion to the thesis work and research contributions of Giulia Degrazia.

4.5. *Fully-aerobic strategy: process operation and fundamental inputs*

The recommended upgrade route for KMC requires the consideration of the flows through the feast and famine process volumes (Table 5) and of the external inputs (chemicals and energy) required to accomplish the sCOD removal water quality objectives (Table 6). Based on an expected future influent process water flow (500 m^3/d) and associated COD removal rates (BIOPOL WP1), a volume of approximately 2300 m^3 is estimated to be necessary to accomplish targets of COD removal performance (> 98%). The feast compartment is estimated in preliminary evaluations to be an additional process volume of between 150 and

250 m^3. The selector will need to be built but the famine process volume (2300 m^3) is part of the existing treatment infrastructure. Either Tank2 or Tank3 could be utilized as the famine process volume.

Both laboratory and pilot studies have shown average sludge production yield of 0.26 kgVSS produced per kgCOD removed (BIOPOL WP1). From equation 1, an oxidation yield of 0.63 kgO_2 consumed per kgCOD removed was estimated.

The time required for each feast cycle to be completed (feast HRT) is expected to be less than one hour (BIOPOL WP1) and with variations due to seasonal temperature and campaign production shifts. Operating the famine stage with an SFR selector may be accomplished without an external biomass separation unit if the interruption in time for settling and decanting can be managed along with storage and release from the famine process volume. The volume of the SBR famine tank will vary during the operation between a minimum of about 1800 m^3 and a maximum of about 2300 m^3 before mixed liquor settling and events of effluent discharge. These ideas would also apply to the PFR if flow was periodically interrupted for volume management in the famine maintenance vessel. With or without semi continuous or pseudo-continuous flow, an external downstream separation unit process step can be used but for reasons cited above, the MBR is not recommended for this purpose if there is to be a selection pressure for a flocculating activated sludge morphology.

Macronutrients (as nitrogen and phosphorus) are required for the sCOD biological treatment with biomass production due to a nutrient limitation in the factory process effluent. A recommended target for operations on a mass balance is a treatment process COD:N:P of 100:4.5:0.6 based on the pilot demonstration mass balance results.

We also recognize that the factory process effluent is limiting for biological treatment with respect to trace nutrients, as for example, K, Fe, Cu, and Zn (BIOPOL WP1) among others. In the present work, nutrient additions were made with scientific grades of chemicals and a separate trace nutrient cocktail was employed. One may anticipate that at full scale, technical grade supplies of N and P will contain carry over of trace nutrients as impurities in quantities to support stable biological treatment. However, we recommend that the trace nutrient loading to the process be evaluated explicitly as we have experienced with some industrial process waters that a trace nutrient deficiency can upset the process operations.

For the feast selector, we estimate a requirement for a bottom aeration system (25 kW, based on design of a 5-meter tank depth) similar to the one currently tested (13 kW) in Tank1 at KMC treatment plant (no mixing necessary). The famine process volume (Tank2 or Tank3) will require blower power for the aeration of between 35 and 50 kW depending on the effective oxygen transfer efficiency. Detailed evaluation of a design and upgrade would need to include process information of oxygen transfer efficiencies of the air distribution systems employed today in Tank2 and Tank3 (Appendix 6). Nevertheless, maintenance volume famine oxygen requirements (AOR, Table 6) have been estimated. Measurable and distributed levels of dissolved oxygen greater than 1 kg/m^3 are recommended for the feast and famine unit processes in order to avoid kinetic limitation in the aerobic microbial activity.

Table 5. Operating conditions of the proposed feast and famine compartments and of the overall PE2 process

FEAST STAGE			
OPERATION		**SFR**	**PLUG-FLOW**
SRT=HRT	minutes	30 - 45	30 - 45
SOLR	kgCOD/kgVSS/d	~ 9	~ 9
Volume	m^3	200 - 250	150 - 200
Feast cycles	number/d	~ 30	~ 45
Biomass recirculation	m^3/d	6500	6500
Biomass recirculation	mode	batch	continous
COD flow	m^3/d	500	500
COD removal	%	>98	>98
DO	kO_2/m^3	>1	>1
FAMINE STAGE			
OPERATION		**SBR**	**CONTINOUS**
Volume	m^3	~ 2300	~ 2300
COD:N:P	kgCOD:kgN:kgP	100:4.5:0.6	100:4.5:0.6
Sludge production	kgVSS/d	1235	1235
DO	kO_2/m^3	>1	>1
OVERALL PROCESS			
HYDRAULIC			
SRT	days	7	7
HRT	days	5	5
OLR	kgCOD/d/m^3	~ 2	~ 2

Table 6. Chemical and energy inputs necessary for the operation of KMC treatment plant under the proposed PE2 configuration

FEAST STAGE			
INPUTS			
AOR	kgO_2/d	~1050	
COD mass	kgCOD/d	4750	
Air blowers	kW	~ 25	fine bubble bottom aeration
FAMINE STAGE			
INPUTS			
AOR	kgO2/d	~2000	
Air blowers	kW	35-50	fine or coarse bubble system
extra Nitrogen	kgN/d	215	N27 possible source
extra Phosporous	kgP/d	10	H_3PO_4 possible source

4.6. *Win-win for KMC with a Cella™ Technologies approach*

Currently, the COD removal and influent capacity of the KMC treatment plant is mainly regulated by the biomass separation unit (MBRs) efficiency. Poor biomass separation characteristics cause frequent interruptions of the filtration units for cleaning and maintenance limiting the effluent production and thus the influent flow that the plant can receive. As consequence, process water effluent is periodically transported off-site to other regional treatment facilities.

Biomass selection by feast and famine as a means for COD removal was found to improve the biomass morphology towards gravity separation. In so doing, we found that stable activated sludge treatment can be accomplished in just 36 % of the process volume used today by KMC for the activated sludge treatment. This outcome suggests that the full-scale KMC activated sludge process can be made to be more efficient and that the surplus produced biomass can be readily harvested as a raw material for PHA production. Tank volumes and even the existing MBR infrastructure could be redirected to an opportunity for biopolymer production as a renewable resource. These biopolymers could be used in applications that support the local agricultural growers that feed the KMC starch modification plant. The surplus volumes could also be applied to support goals of factory production increases.

The proposed Cella™ strategy approach operates with a relatively shorter SRT in reduced volume while still achieving almost complete influent COD removal (> 98%). Removing the current COD loading in a selector plus the famine maintenance tank is estimated to free up approximately 4500 m^3 process volume with all the associated OPEX. It is understood that there are ongoing discussions at KMC in the consideration of if equalization tank capacity increase with an extra 6000 m^3 volume, which would help to improve process stability. The BIOPOL pilot process trials suggested that influent COD variabilities did not result in process upset; however, increasing the equalization volume capacity, even by reusing one of the "retired" tank volumes, could help to simplify the process control and operations by further balancing the influent water quality. The harvested biomass which is projected to be 1235 kgVSS/d in 2020, from the service of process effluent COD removal, will have capacity to accumulate PHA (up to 0.7 kgPHA/kgVSS) and this capacity could be exploited instead of the "sludge" being disposed of as a waste by-product.

At shorter SRTs, the sludge production yields will increase from the current estimated levels of 0.11 to an expected amount of 0.26 kgVSS produced per kgCOD removed. Increased sludge production means lower overall oxygen demand (Equation 1), from a currently estimated 0.84 down to a predicted 0.63 kgO_2 consumed per kgCOD removed. Therefore SRT control is expected benefit KMC with 25% energy saving in process operations. With this benefit comes more surplus "sludge" but such sludge is not a waste but a harvested raw material if it is of value. Since the biomass is produced with significant PHA accumulation potential, the savings in energy come with a further benefit of a raw material, produced from the factory residuals, with potential value in PHA bio based economies. In order to reduce the SRT and the process aeration demand, appropriate amounts of balanced macronutrients need to be delivered without limitation to the biological process.

As mentioned, the famine reactor could operate under intermittent SBR mode for effluent production and biomass retention. Such configuration is an attractive case scenario for KMC because gravity settling adds an extra positive pressure to maintain a healthy biomass floc morphology. Physical as well as suitable environmental pressures of specific organic loading rate, and the distribution of the organic loading work in combination. An MBR unit process is not recommended for the upgrade if an easy to separate biomass is to be produced. It is the feast (high F/M) and famine (low F/M) cycles in combination with the method of separation that shape the community structure and community morphology.

4.7. KMC and a sidestream of PHA from process effluent management

Operation of the KMC process effluent treatment as proposed from the results of this study will result in a functional PHA-storing biomass as a by-product meeting effluent discharge water quality objectives. Based on a process effluent flow of 500 m^3 and a harvested biomass production yield of 0.26 kgVSS produced per kgCOD removed, about 450 tons of VSS functional biomass can be harvested per year. KMC could exploit this functionality along with other regional stakeholders to produce and promote PHA value chains.

KMC harvested biomass fed with a volatile fatty acid (VFA) rich stream will accumulate PHA up to 0.7 kgPHA/kgVSS. The KMC process water effluent is a viable feedstock for such PHA accumulation. However we experienced variations in this "KMC feedstock" for PHA accumulation that seemed to influence the performance in the biomass response when compared to other more well-defined VFA rich feedstocks. We suspect that variation and a current poor understanding of the non-VFA fraction of the KMC effluent to be implicated. Therefore, our current recommendation is to consider the KMC effluent as a resource for biomass production and other fermentable organic residuals as a source of feedstock for PHA accumulation (BIOPOL WP1).

We estimate, from laboratory and pilot experience in BIOPOL, that we may expect an overall accumulation yield of 0.35 (kgPHA/kgCOD) and polymer contents conservatively over 0.5 (kgPHA/kgVSS). KMC harvested biomass could therefore be a source of at least 450 tons of PHA per year with a supply of a VFA rich feedstock providing 1300 tons of COD per year.

Additional carbon sources of carbon may be garnered as feedstock organic residuals (Jesper Jensen, personal communication) as part of the potato processing steps and these could be converted into PHA accumulation feedstocks. These additional sources include, starch residuals (800 tons per year), fruit juice (225.000 tons per year), and mos-mix (28.000 tons per year). All three streams would require fermentation to hydrolyse and convert the organic content into a suitable VFA rich feedstock. All streams are with potential in terms of a quantity of supply, but all three need evaluation to confirm their suitability in quality towards yielding a raw material feedstock for PHA production. The KMC residual starch stream has already been evaluated at AnoxKaldnes in a preliminary study, and full fermentation to mainly propionic and acetic acid was observed.

The PHA accumulation “PE3” infrastructure could be achieved conceivably within the existing KMC process infrastructure after a recommended upgrade for “biomass production”. A remaining available aerobic tanks (Tank2 or Tank3) provides for the main accumulation volume and the MBR units would provide for biomass separation during and after the accumulation process. Considerations of the VFA feedstock pre-treatment supply and an eventual business model require deepened consideration within a well-defined regional material flow and economic evaluation of the business opportunities. For example, KMC could be a link within a material flow and economic value chain with other regional stakeholders wherein KMCs principle role is to be a biomass raw material supplier. Efforts to define the business and the most logical material flow require more discussion of context with respect to KMC, its shareholder interests, and the regional landscape opportunities for stimulating circular economies.

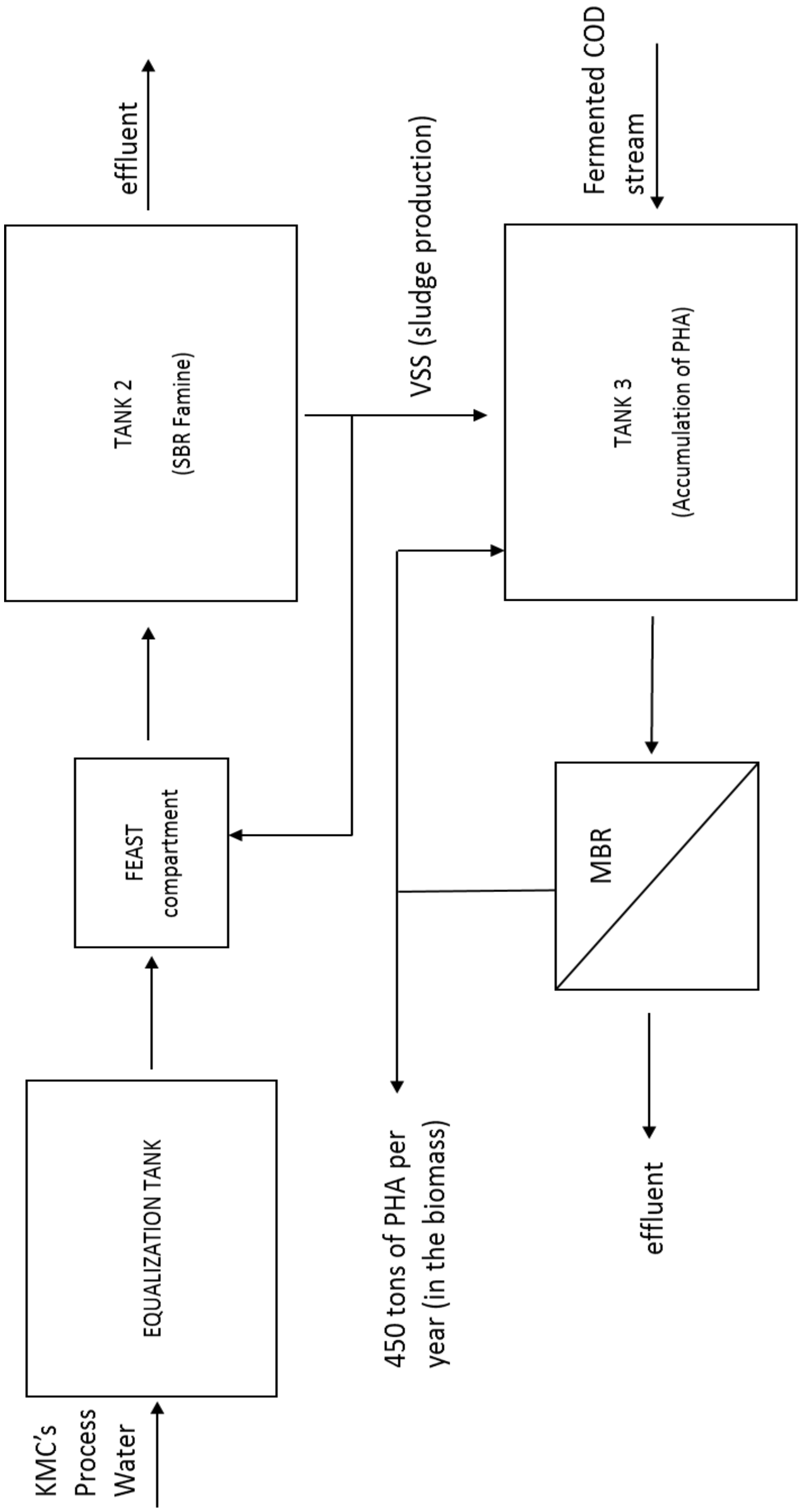


Figure 6. Conceptual process flow diagram illustrating the integration of functional biomass production (PE2) and PHA production (PE3) with KMC process water effluent water quality management activities.

5. Conclusions

Laboratory experiments (De Grazia, 2014) and recent piloting demonstration (BIOPOL, WP1) have shown similar and stable treatment performance of the KMC process water effluent. Services in KMC process effluent water quality management were further shown to offer opportunity in the production of a functional biomass. The biomass is functional because it is enriched with populations of bacteria that can accumulate PHA with a potential of up to 70% gPHA/gVSS.

The KMC process water effluent treatment facility is currently challenged in capacity due the formation of an activated sludge biomass that exhibits a poor separation morphology. As a consequence, five parallel MBR units are being used with associated costs of operation and maintenance. In contrast, we found that final effluent COD water quality objectives could be met while producing a biomass with stable and good separability by gravity settling. This kind of floc morphology produced was based on environmental and physical selection pressures including feast and famine, a relatively short SRT, and gravity settling for biomass separation and retention in the process.

Feast and famine conditions furthermore are conditions which are known to select for the PHA storing phenotype in mixed culture systems. Therefore, an upgrade to the KMC process water treatment system is recommended wherein a selector provides for feast conditions in an upstream process volume of about 200m^3. In conjunction to and upstream feast, one of the existing tanks on site can be used to maintain the biomass in conditions of famine. Feast can be aerobic or anaerobic but an anaerobic selection has not been verified as part of the scope of BIOPOL. Aerobic feast conditions have been proven to work such that two of the existing 3 tank volumes may be redirected to other purposes, such as for PHA accumulation. If a stable floc-forming biomass morphology with low SVI is desired, then we recommend that gravity based separation be used as the principal strategy to retain the biomass in the process. The MBRs may still serve in final effluent polishing or they could serve in an eventual PHA production unit process on site given the projected saved volume for treatment as determined in the present study.

We consider that either continuous or semi-continuous effluent flow from the equalization tank volumes could be accommodated in an upgraded biological process configuration. The most appropriate solution will depend on a number of important engineering details of the existing infrastructure, and KMC specific requirements with regard to present and future planned water quality management needs. These considerations require a more detailed engineering and costing exercise based on the concepts proposed in this report, alongside input from additional discussion and deliberation that may hopefully be stimulated from the BIOPOL study for all stakeholders involved.

A significant SRT reduction from the current 45 days to a target 7 days is recommended as this will provide savings in OPEX (aeration costs) while improving the efficacy of producing a functional biomass. Towards this end, macro-nutrients (nitrogen and phosphorus, with COD:N:P of 100:4.5:0.6) and oxygen must be delivered in appropriate amounts and locations to support a healthy biomass. The external selector volume design provides for a means to mitigate aeration CAPEX and OPEX for the biological process while applying selection pressure on the microbial community. It was estimated that SRT reduction will significantly reduce the overall process oxygen consumption by 25%.

Micro-nutrient demands were also examined as part of the study and here it is also recommended to establish that sufficient trace elements are being supplied (directly or indirectly) for stable microbial activity. Reliable delivery of appropriate levels of macronutrients may require specific methods and controls to be implemented given the pilot experience of KMC process effluent organic load and quality variations.

In all of this, we were exited to find that the KMC process water effluent could be reliably treated in about 2300 m^3 process volume instead of the currently used 7000 m^3. Two of the three currently available aerobic process volumes as well as the MBRs could be redirected to future opportunities in renewable resource generation from the KMC residuals management activities. Savings in the current water quality management infrastructure also provide an opportunity for investment in the future.

From the demand of factory effluent organic discharge management, we estimate that KMC could harvest about 450 tons of active biomass. A measured PAP (PHA accumulation potential) for the pilot plant harvested biomass was more than 50 and up to 70% (gPHA/gVSS). This performance suggests that the harvested biomass could yield from 450 and up to in the order of 1000 tons of PHA per year. This performance is based on the assumption of no active biomass growth during the accumulation process. Greater productions are possible with active growth pending the supply of feedstock. What is more is that the freed infrastructure from a proposed process upgrade provides for necessary elements to support a PHA production activity. PHA production requires both an active biomass as well as a VFA rich feedstock as input raw materials. The sourcing and fermentation of regional organic residuals towards furnishing the biomass with a feedstock for PHA production requires further evaluation that were beyond the scope of the present study. KMC may have suitable organic residuals already (starch, the fruit-juice, and mos-mix residual streams) but other available regional agro-industrial sources could also provide synergies worth serious business consideration. The motivation to explore an eventual participation in a PHA value may be understandably difficult to grab hold of with conviction in the short term. Notwithstanding, the incentive to improve the functioning and economy of the process effluent water quality management does stand on its own feet. In the best of possible worlds, an improvement to solve one problem could open the doors to an unexpected world of opportunities for KMC and its shareholders.

6. References

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Biopolymer-producing biomass and feedstock from municipal wastewater treatment

Potential for volatile fatty acids and polyhydroxyalkanoates
at Marselisborg Renseanlæg and Billund BioRefinery

BIOPOL WP1 (Activities 1.2 & 1.3) and WP2 (Activities 2.1 & 2.2)

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Summary

Production of biodegradable polymers in the form of polyhydroxyalkanoates (PHAs) by activated sludge treating wastewater means that excess activated sludge biomass becomes a value-added by-product that can be harvested from a wastewater treatment plant (WWTP). The harvested biomass can be made to accumulate significant levels of biopolymers and the biopolymer-rich biomass then becomes a raw material in the value chain towards bioplastics and/or fine chemicals. In this manner, a sludge disposal burden may be turned into a renewable resource opportunity. Facilitating this renewable resource opportunity is a goal embodied by Veolia with Cella™ Technologies.

In this report, the background regarding integration of the production of PHA by the AnoxKaldnes Cella™ Technologies with municipal wastewater treatment is presented. This is followed by the BIOPOL assessment of the PHA accumulation potential of two biomass grab samples taken from Marselisborg Renseanlæg and Billund BioRefinery. In addition we have characterized two potential feedstocks for PHA production that are available at the Billund BioRefinery. The outcomes are then considered with recommended strategies for value chain development from an integration of PHA production at the two WWTPs.

At current full-scale operation of the Dutch WWTP in Bath, surplus activated sludge is produced that can accumulate 40-50 % of its dry weight as PHA (40-50 % g-PHA/g-VSS). As part of the on-going project PHARIO, this biomass is being used for the regular production of PHA with the purpose of evaluating the raw material and raw material compounding for motivating investment in regional PHA based bioplastic value chains. The full-scale activated sludge is able to convert feedstocks containing volatile fatty acids into PHAs in a consistent and robust way.

Over the past decade, Veolia in Sweden have progressively and repeatedly strengthened a proof-of-concept and deepened fundamental insight in PHA value chain integration to municipal wastewater treatment infrastructure at laboratory and pilot-scale operation. Practical evaluations have been conducted at pilot scale at Brussels North WWTP (Belgium) 2011-2014 and at Leeuwarden WWTP (the Netherlands) 2013-2014. At Leeuwarden WWTP, it was demonstrated that municipal wastewater treatment and production of a PHA production biomass can be integrated with robust biological nitrogen removal by nitrification and denitrification. Biomass from wastewater treatment can be a raw material towards facilitating regional value chains worth thousands of tons of PHA per year.

As part of the present BIOPOL investigation, activated sludge samples from Marselisborg Renseanlæg (MR) and Billund BioRefinery (BBR) were assessed for PHA accumulating potential or (PAP). The PAP, measured with units of weight PHA accumulated per gram of activated sludge biomass (volatile suspended solids or VSS, g-PHA/g-VSS) gives an indication of the biomass capacity to produce PHA. A value of 40 % PAP represents a significant and commercially relevant level and a value of 15 % PAP is a typical background level for activated sludge. The observed PAPs were 26 % g-PHA/g-VSS and 18 % g-PHA/g-VSS for MR and BBR, respectively.

The observed PAP with the biomass from MR and BBR are typical of activated sludge where there is little to no applied selection pressure for the PHA storing phenotype from the bioprocess. The outcomes indicate that these two biomass sources require specific attention to the bioprocess environment during pre-denitrification should it be of interest to produce a biomass with PAP exceeding the required 40 % g-PHA/g-VSS. We know from a pedigree of experience that such an increase is achievable in WWTPs that are analogous to MR and BBR. It was estimated that MR and BBR could be

sources of biomass as raw material for the production of in the order of 1 000 and 200 tons-PHA/year, respectively.

For the Biodenitro™ processes at MR and BBR, one effective way to promote PAP in the biomass would be to create zones where the biomass is exposed to more well-defined ‘feast’ conditions with higher concentrations of RBCOD during pre-denitrification. The return activated sludge and influent could, for example, be mixed in an anoxic ‘feast’ zone under a relatively short retention time. This could be achieved either in a separate zone within the carrousel reactors, or in a separate volume up-stream of the main reactor volume associated with the already existing influent distributor. Thus, the improvements in bioprocesses that are necessary for valorizing the surplus biomass do not have to involve complicated and expensive upgrades. They require subtler attention to the history of environments that the bacteria in the process are repeatedly disposed to.

An evaluation of possible WWTP process modifications is recommended given a next step to realize a biomass supply for PHA production. A simple starting point in this step would be an on-paper techno-economic evaluation on the influence of an upgrade strategy. In such an evaluation, the process would be modelled in order to evaluate conditions for establishing a ‘feast’ zone while also ensuring robust wastewater treatment performance. Pending those outcomes as a go/no-go gate, practical testing would follow as a confirmation before any eventual process implementation.

Two digester streams at BBR were evaluated for potential as future feedstocks for PHA production. One of the streams was the mixture of household waste and surplus sludge after pulping. The other stream was the mixture of household waste, surplus sludge and industrial waste after hygienization. These two streams were both found to have relatively high concentrations of soluble COD, 38 and 23 g-COD/L, respectively, of which 28 % and 41 %, respectively, were in form of VFAs. The soluble fractions from both streams have potential to become feedstocks for PHA production but the levels of VFAs in these streams need to be further increased. To this end the controlled evaluation of fermentation of these organic residual sources is a recommended first step. It was estimated that if the fraction of VFAs is improved by fermentation, in the order of 150 to 250 tons-PHA per year could be produced from these sources given an available harvested biomass.

Table of content

1. Introduction

In this report, the background for conveying insight and understanding for the integration of the production of polyhydroxyalkanoate (PHA) biopolymers by the Cella™ Technologies with municipal wastewater treatment is first presented. This background information is followed by results of practical testing for the PHA accumulation potential of biomass (activated sludge) grab samples taken from Marselisborg Renseanlæg and Billund BioRefinery. In addition we have characterized two potential feedstocks streams for PHA production that are available at the Billund BioRefinery.

Production of biodegradable polymers in the form of PHAs by biomass treating wastewater means that surplus activated sludge becomes a valuable by-product that can be harvested from a biological wastewater treatment process. The harvested biomass can be made to accumulate significant levels of biopolymers and the biopolymer-rich biomass then becomes a raw material resource in the value chain towards bioplastics and/or fine chemicals. In this manner a sludge disposal burden of wasted activated sludge may be turned into a renewable resource opportunity in the form of a functional *harvested* activated sludge. PHA biopolymers can be accumulated and then recovered from biomass and converted into biodegradable plastics of commercial value. Such bioplastics may be applied in a broad spectrum of practical commercial applications and services.

Production of PHA from services of wastewater treatment can be a part of an overall biorefinery concept involving biological and chemical treatment process elements (PE1-4) comprising (Figure 1):

PE1: Optional pretreatment by acidogenic fermentation in order to convert organic matter into readily biodegradable chemical oxygen demand (RBCOD) fermentation products such as volatile fatty acids (VFAs).

PE2: Removal of contamination from the wastewater that results in the concurrent production of a biomass with significant PHA accumulation potential (PAP).

PE3: Accumulation of PHA in the surplus biomass from PE2 by using a waste or residual process stream rich in RBCOD as feedstock.

PE4: Recovery and purification of the PHAs, alongside lipids and energy from the PHA-rich biomass produced in PE3.

The main substrates that are typically used for mixed culture (activated sludge) PHA synthesis are VFAs. In some cases there are already streams with VFAs available (such as at KMC in Brande) and in such cases, acidogenic fermentation in not needed. The VFA-rich stream is used as a feedstock "raw material" in PE3 or in both PE2 and PE3.

When an open mixed culture is used for production of PHA the process needs to be engineered and operated in such a way as to favor the natural enrichment of PHA storing bacteria and repress the growth of non-PHA-storing bacteria in the biomass. The most well-studied enrichment method is "feast and famine" selection, where the biomass repeatedly experiences short periods with substrate excess(feast) where the bacteria can store some of the substrate as PHA. The stored PHA can then be used for growth in the famine phase where no external substrate is available. When the biomass is repeatedly exposed to cycles of feast and famine conditions the non-PHA-storing bacteria in the biomass are out-competed by the PHA-storing bacteria in the biomass (Bengtsson et al. 2008a, 2012; Werker et al. 2011b).

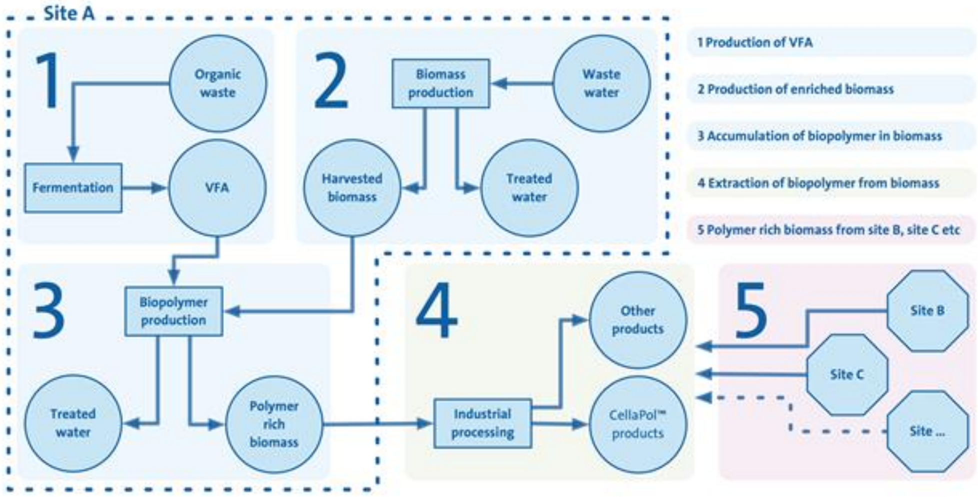


Figure 1. Process elements (PE1 to PE4) generating value chain opportunities from activities in wastewater and residuals management services.

The substrate for the bacteria can either be a VFA rich stream (such as with the pilot demonstration at KMCs wastewater treatment plant in Brande) or a municipal wastewater (as is being demonstrated in the Dutch project PHARIO). In the case of a municipal wastewater, no fermentation is needed and the readily biodegradable COD fraction of the municipal wastewater can be used to stimulate the enrichment of a PHA storing activated sludge biomass.

In PE2 the PHA content in the biomass is typically always well below the levels required for economic recovery of the PHA. Generally for PHA recovery, levels of PHA in the biomass should be greater than 40 percent (gPHA/gVSS). Therefore, the biomass that is produced in PE2 needs to be made to be saturated with PHA. PHA saturation is accomplished by exposing the biomass to a prolonged feast period in a PE3. Once the biomass in PE3 becomes saturated with PHA, the polymer in the biomass is stabilized and dried before the polymer is then recovered from the residual biomass (Werker et al. 2012).

Veolia Water Technologies AB (AnoxKaldnes) has been investigating and finding wide spread opportunities of PHA production in conjunction to municipal wastewater treatment services. The focus of these investigations has been on integration of the Cella™ Technologies within existing infrastructure and material management frameworks, while maintaining existing standards in water quality and solid waste management. Strategies for integrating PHA production into existing treatment infrastructure and procedures are tuned case-by-case.

The objective with the present investigation was to make an initial evaluation of bioprocess strategy and the opportunity for integration of PHA production in wastewater treatment at two Danish municipal wastewater treatment plants (WWTPs), namely Marselisborg Renseanlæg and Billund BioRefinery. These WWTPs were examined as part of the BIOPOL project Work Package 1.

In this report, knowledge and practical experience in PHA production from municipal wastewater treatment is reviewed (Section 2). Against this background, results of the activated sludge biomass accumulation potential assessments from Marselisborg Renseanlæg and Billund BioRefinery at laboratory-scale are presented (Section 3). In order to address a potential opportunity for PHA production feedstock raw material supply, two residual streams from Billund BioRefinary were evaluated (Section 4). The results and discussion drawn from Sections 2 to 4 and then considered in a context of recommended strategies for value chain development from an integration of PHA production at the two WWTPs (Section 5).

2. PHA production with municipal wastewater treatment

Our fundamental and practical experience to date suggests the technical feasibility to produce a biomass with significant PHA storing capacity from not only the treatment of industrial wastewaters but also the treatment of municipal wastewaters.

It is well established in the research literature that it is feasible to enrich for a PHA-accumulating biomass using high concentrations of VFAs, such as synthetic substrates or some fermented industrial process waters. By extension we found that even the relatively low levels of RBCOD present in municipal wastewater may be sufficient to drive a selection pressure favoring PHA-storing microorganisms given that the bioprocess unit steps are appropriately designed and operated (Bengtsson et al., 2012). Furthermore, we found that in such cases, RBCOD other than VFAs can adequately contribute to PHA storage in the enrichment PE2 biomass production. The principles of enrichment using municipal wastewater form part of Veolia intellectual property developments (Bengtsson et al., 2012) and these findings of processes and methods have continued to be confirmed and demonstrated at pilot-scale (Section 2.2).

Process optimization for generating functional activated sludge can be integrated to existing wastewater treatment plant infrastructure and water quality demands. To date, we have experience of integrating the biological processes of carbon and nitrogen removal. Here it is assumed that phosphorus removal is achieved by chemical addition. Processes of enhanced biological phosphorus removal (EBPR) may also be possible, but these would require further and more detailed consideration on a case-by-case basis.

Notwithstanding the specific case-to-case details, we find that there exists an untapped opportunity for many municipal wastewater treatment facilities to be involved in renewable resource value chains as a provider of functional biomass (PE2) and/or sources of RBCOD for PHA-production (PE1). Therefore, all municipal WWTPs are natural hubs for organic carbon that can be converted into PHA feedstock raw materials as functional biomass and/or volatile fatty acid rich streams.

The goal of integrating the utilization of VFAs with harvested biomass from water treatment is to produce a biomass with PHA contents in excess of 40% (g-PHA/g-VSS). This content of 40% is a threshold level where we estimate the polymer recovery (PE4) economy becomes economically sustainable. However, the objective of producing and recovering PHA from biomass and VFA sources at the WWTP has much wider scope because it involves a synergistic route of regional resource management. The surplus activated sludge from the WWT facility does not need to be minimized, dewatered and disposed of as a burden at the bottom of the value chain pyramid. The biomass can be a value added raw material to be further processed and refined to higher levels in a resource value pyramid. The PHA biopolymers can even come back full circle cradle to grave back to the WWT facility for biogas production.

In the material flow of PHA-rich biomass production, nitrogen and phosphorus in the activated sludge are not released back into the wastewater treatment line, as it is the case today for anaerobic digestion of waste activated sludge. Keeping the nutrients in the residual biomass relieves the WWTP of return nutrient loading to the headworks. Every kilogram of ammonia nitrogen released into the digestate from biogas production, costs the wastewater treatment plant between 1.2 (ANITA Mox) and 3 (conventional neglecting carbon demand) kWh of energy in post treatment. Therefore, taking the nitrogen out of the water in the biomass, and keeping it out, offers benefits in the energy balance of the municipal WWTP.

The biomass organic material after PHA extraction can still be utilized directly as an energy source but more directly by incineration or pyrolysis. The phosphorus in the ash may be recovered. Lipids from the biomass can be also recovered as a platform chemical for industry. Therefore, producing and recovering PHA in conjunction with wastewater treatment is part of the ambition to more systematically create biobased economies from residuals as products from the essential services of water quality management.

2.1 Factors influencing enrichment for PAP

Generally, most activated sludge will exhibit some ability to accumulate PHA when fed with RBCOD like VFAs and when these VFAs are fed during an accumulation process in a controlled manner. We define PHA accumulation potential (PAP) as the maximum possible fraction of PHA in a biomass at the end of a standardized reference accumulation process (g-PHA/g-VSS). For WWTPs that run under typical operating conditions, a background PAP for the activated sludge is nominally between 5 and 15 %. However, given the opportunity to tune conditions for the biomass enrichment in a treatment process, biomass from municipal wastewater treatment may accumulate in the order of 40-50 % of the final organic mass (Bengtsson et al., 2012). Since such a level of PAP makes for a viable PHA recovery economy based on our experience, municipal WWTPs harvesting such biomass may therefore become raw material suppliers in regionally driven PHA value chains bioeconomies.

2.1.1. Readily biodegradable organic matter

Typical domestic household wastewater contains relatively low levels of RBCOD. Since it is the RBCOD that stimulates the biomass to store PHA, the peak concentration of RBCOD a biomass is exposed to, is an important factor for selective enrichment of PHA-storing organisms in the biomass. Engineering of the contact zones of peak RBCOD concentration in a municipal WWTP can be simplified in some cases by higher influent RBCOD levels. Contributions to the influent RBCOD may be influenced on a case by case basis due to the length and type of sewer distribution system, as well as the COD contributions from other regional inputs. Such contributions may include, for instance, industrial wastewaters originating from dairy or other food-processing industries. Likewise, if reject water from sludge management, containing higher levels of RBCOD, is being sent to the main treatment line that may also be used to positively affect the enrichment pressures on the biomass.

2.1.2. Short or long SRT for the functional biomass production

Generally longer SRTs are used for municipal activated sludge processes as means to maintain sufficient populations of nitrifying organisms and/or to reduce sludge production. For producing a biomass with PAP, a shorter SRT for the heterotrophic biomass has advantages due to a higher fraction of active cells in the biomass which ultimately may lead to a higher PAP. Short SRTs can also mean greater biomass yield and lower oxygen demands. Thus, a shorter SRT can lead to a higher PAP and increase the amount of biomass available as a resource for PHA production while contributing at the same time to treatment performance by N and P assimilation.

If a short SRT is used for the heterotrophic bacteria then some means to maintain nitrifiers in the process is still required. To this end, for example, we found that an integrated fixed-film activated sludge (IFAS) process upgrade can be used to satisfy the combined needs in a long SRT for robust ammonia removal by the nitrifiers while maintaining a short SRT for the PHA producing heterotrophs. To this end both at pilot and at full-scale practical experience we see that biological nitrogen removal municipal wastewater treatment systems are very well-suited to be biomass suppliers for regional biopolymer (PHA) based value chains.

2.1.3. Creating feast conditions in the treatment process

Feast conditions, where the biomass is exposed to relatively high peak levels of readily biodegradable COD, stimulates storage of PHA and favors the enrichment of PHA-storing organisms in the biomass. Existing selector volumes can explicitly achieve feast conditions but our internal research and development results suggest that separate selectors are not essential to the process of enrichment for PAP. This means that many existing WWTPs can be readily adapted to promote for a surplus biomass with significant PAP. The implementation of the technology may require some form of process optimization to the main waterline of the municipal WWTP but we have found that the scope for a simple integration into existing process infrastructure is broad. Over the past years during pilot investigations, we have discovered process and methods to influence the activated sludge composition and physiological state to enhance the PAP of a biomass produced from treating a municipal wastewater.

2.1.4. Primary treatment

Primary treatment is considered to be an advantage since otherwise, influent solids that are not degraded in the process (fibers, inert organic solids, etc.), and that end up associated with the biomass solids detract from the harvested "functional" biomass *quality* since the active fraction of the biomass may become "diluted out". Furthermore, without primary treatment, more colloidal COD that is degraded more slowly becomes available to the biomass and this COD can challenge the extent to which the biomass will be subjected to a defined period of famine conditions within the existing process volumes. Nevertheless, famine can still be achieved in a side stream as part of the biomass return (Werker et al., 2011b). Additionally, we find that the manner in which famine is applied to the biomass can have significant influence on the expressed PAP during the accumulation process. In this sense, primary treatment is advantageous but not absolutely required from the perspective of generating a biomass with high PAP.

Initiatives to achieve advanced primary treatment that include fiber removal with novel filtration technologies are complementary to the production of a biomass with high PAP. Improved primary treatment removes organic solids from the influent making the task of achieving a good feast and famine regime and enrichment for PAP in the main line more straight forward to engineer. The solids that are captured in the advanced primary treatment further reduce the oxygen demand in the wastewater treatment. These solids can be fermented that thereby provide a good side stream source of VFAs for use in a PHA accumulation process and/or for biogas.

2.1.5. Biological nitrogen and phosphorus removal

We have found that the objective of producing a biomass with PAP will fit very well with the flow scheme of biological nitrogen removal, especially in bioprocess designs employing the strategy of pre-denitrification. The anoxic conditions of pre-denitrification can be made to support a feast on influent RBCOD and we have proven in practical field work (Section 2.2 and 2.3) and in laboratory research and development (Anterrieu et al., 2014) that an anoxic feast contributes to the robust selection of a biomass with PAP.

Thus, a feast stimulation for PAP enrichment can be either under aerobic or anoxic conditions. Although anaerobic feast conditions for a municipal wastewater influent will favor polyphosphate-accumulating organisms (PAOs) as part of the EBPR process, PAOs are a subpopulation of the PHA-storing organisms that are not required for achieving high PAP. On the contrary, PAOs in the biomass may release P to the water during the PHA accumulation process and thereby require further consideration to the overall P management. Therefore, integration of PHA production with EBPR, albeit technically feasible, entails specific challenges for further practical and fundamental evaluations.

2.2 Pilot-scale PHA production in municipal wastewater treatment

Proof-of-concept and establishment of fundamental knowledge in PHA production in municipal wastewater treatment was conducted at laboratory scale (Bengtsson et al., 2012). The enrichment of biomass able to accumulate substantial levels PHA while treating municipal wastewater was subsequently confirmed and demonstrated at pilot-scale at Brussels North wastewater treatment plant during 2011 to 2014 (Morgan-Sagastume et al., 2015, 2014). At pilot-scale, PHA production and integration in a more practical and realistic scenario was developed and benchmarked. In this case, wastewater treatment was mainly for RBCOD removal with some nutrient removal by biomass assimilation. At this demonstration site, a VFA-rich stream was also produced by fermenting excess solids from the full-scale WWTP and it was utilized as substrate for the PHA accumulation. Furthermore, we developed and implemented a novel process control strategy for automated PHA accumulation under continuous feeding applicable to wastewater feedstocks.

In a following pilot operation campaign during 2013 to 2014 at the Leeuwarden WWTP demo site in the Netherlands, we prototyped a process for enrichment of biomass with PAP with simultaneous nitrogen removal. The working hypothesis was that wastewater RBCOD may be utilized during an anoxic feast phase for PHA storage and denitrification while nitrate is reduced to N_2. Under subsequent aerobic conditions, ammonia should be nitrified to nitrate and the stored PHA used for growth and maintenance by the PHA-storing organisms. The enrichment reactor was an IFAS system with K5 biofilm carriers (50 % volumetric filling degree). The purpose of the biofilm carriers was to provide a long SRT suitable for the development of a robust nitrifying community, while enrichment for PHA-storing biomass would occur in the suspended biomass in the bulk phase mixed liquor. Indeed we confirmed the validity of this working hypothesis within a realistic scenario of municipal wastewater treatment.

With the two-fold purpose to enhance the PAP of the biomass produced in the process and to obtain improved nitrogen removal, a recent AnoxKaldnes invention was applied (Werker et al., 2014a). According to one alternative of this process, fractions of biomass are periodically transferred from the main treatment system to a secondary reactor in which the biomass is exposed to anoxic famine conditions before being returned to the main treatment system. By so doing, biomass is exposed to a secondary perturbation of famine that conditions the biomass to increase its capacity for PHA accumulation in the subsequent biopolymer accumulation stage. Since this stage is operating under anoxic conditions, it also serves a treatment purpose of endogenous post-denitrification which contributes to nitrate removal.

The pilot prototyping at Leeuwarden WWTP, during 170 days of operation and monitoring, demonstrated for the first time that a municipal wastewater treatment process can be purposefully engineered and used for the production of a biomass with significant PAP while, at the same time, maintaining robust biological nitrogen removal performance with nitrification and denitrification. Biomass with PAP of 49 % (g-PHA/g-VSS) was obtained (Figure 2) which is well above the 40 % level that is considered by AnoxKaldnes as a threshold for an economically viable down-stream polymer recovery. The PAP was confirmed using a fermented agro-industry centrate as the accumulation substrate. The biomass was produced treating the municipal wastewater from the Leeuwarden WWTP which is highly dominated by domestic household wastewater. Therefore, the results of this study are generally applicable and enrichment for PAP could be readily achieved in the treatment of most municipal wastewaters.

Contributions of industrial wastewaters, with higher levels of RBCOD, are generally expected to be of further benefit to the degree of enrichment.

Despite the fact that optimization of the wastewater treatment performance of the pilot prototype at Leeuwarden WWTP was not of primary focus and a relatively high organic loading rate was applied, significant reductions of organic matter and nutrients were obtained, as indicated by a 75 % reduction in soluble chemical oxygen demand and 83 % reduction in total soluble nitrogen (Figure 3).

Periodic presence of significant levels of cellulosic fibres in the biomass (up to 28 % of dry weight) suggested the potential advantage of primary treatment on biomass quality with respect to achieving maximum PHA content, increasing biomass settleability and improving down-stream biopolymer recovery. Up-stream separation of fibres and recovery of cellulose are further understood to offer synergistic value for PHA production alongside other renewable raw materials recovery. A broader scheme of renewable resource recovery is part of a Dutch national strategic agenda that we also contribute to in the Dutch project PHARIO.

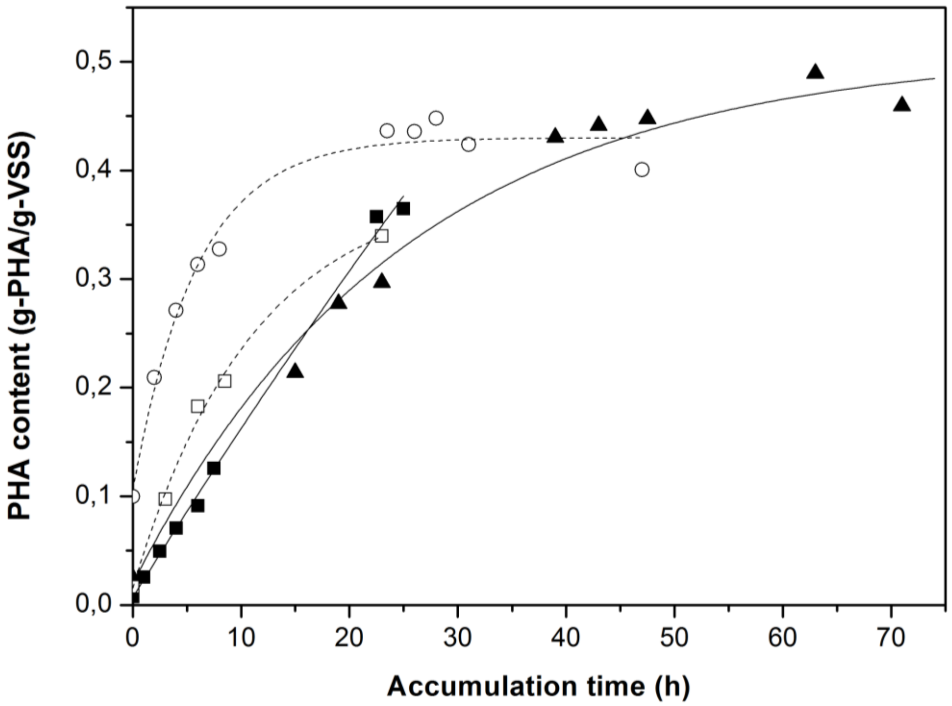


Figure 2. Replicate PAP evaluations for Leeuwarden Cella™ biomass using a reference substrate based on acetate (■ and ▲) and fermented agro-industry centrate (□ and ○) as feedstocks.

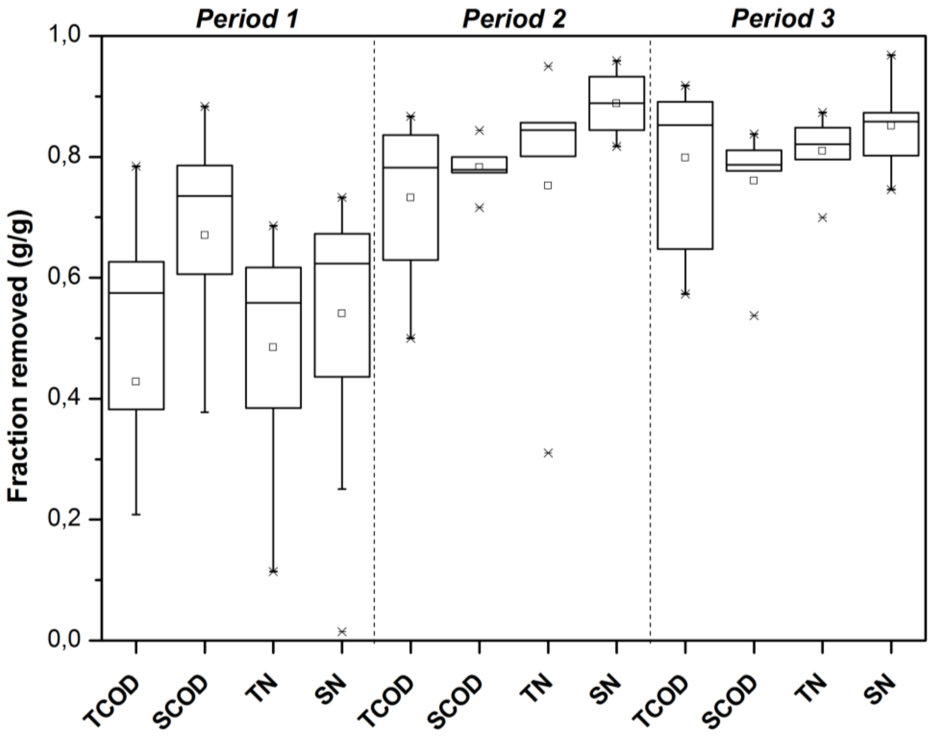


Figure 3. Removal efficiencies of COD and total N in three operational periods where Period represents a process of pre-denitrification-nitrification and Period 3 represents the same process with an additional element of post-denitrification.

2.3 Enrichment of PAP in full-scale municipal wastewater treatment

At present, as part of the project PHARIO (PHA from wastewater treatment, Dutch Topconsortium voor Kennis- en Innovatie Biobased Economy TKI-BBE), AnoxKaldnes piloting facility and surplus activated sludge from the full-scale municipal wastewater treatment plant of Bath (The Netherlands) are being used for the regular production of PHA with the purpose of evaluating the raw material and raw material compounding for motivating investment in regional PHA based bioplastic value chains.

In AnoxKaldnes research and development contributions in collaboration with the Dutch Water Boards, the WWTP in Bath in the southern Netherlands was identified as one promising candidate for producing a biomass with PAP based on a number of bioprocess features:

1. Wastewater is conveyed to Bath WWTP via long pressure lines. This influent delivery generally promotes higher influent RBCOD (readily biodegradable COD) levels for stimulating a “feast” response in the activated sludge,
2. Initial contact between influent wastewater and the return activated sludge creates conditions of a high specific loading (or feast) in the anoxic pre-denitrification zone,
3. Primary treatment of the raw influent reduces the non-RBCOD loading to the biomass making for stringent conditions of “COD famine” during aerobic nitrification, and also within the post-denitrifying zones of the process train,
4. Bath treats wastewater for approximately 500 000 PE and generates surplus activated sludge as resource for about 2 500 tPHA/yr.

In the first benchmarking experiments, a PHA-rich-biomass was produced from the Bath WWTP biomass using fermented sludge centrate produced at the Brussels piloting facility as VFA feedstock. The key outcomes from these practical tests were a proof of concept and are summarized below.

Biomass was collected from Bath WWTP and transported to the AnoxKaldnes/Veolia pilot site located at the Brussels North wastewater treatment facility and PHA accumulation (Werker et al., 2011a) was performed with either a synthetic medium or the fermented sludge centrate. The carbon source used was either acetic acid, a simple VFA mixture of acetic and propionic acids (68 %/32 % of HAc/HPr on COD basis) or centrate produced from fermentation of a mixture of primary and secondary solids. Fermented sludge centrate is a VFA source to be readily valorised in practice and thus this was a relevant feedstock to gain experience with. In selected cases, phosphorus content of the fermented centrate feedstock was reduced by precipitation with $FeCl_3$ and gravity separation.

Accumulation of PHA with synthetic media containing VFAs as feedstock resulted in PHA contents after 24 h of 50 and 46 % (g-PHA/g-VSS), with standard (acetate) and mixed (acetate/propionate) VFAs respectively (Figure 4). In these accumulations, nitrogen and phosphorus were maintained at limiting levels. The trends suggested in replicate trials that the inherent biomass PAP_{24} was greater than 40 % g-PHA/g-VSS and even with potential to be in excess of 50 % g-PHA/g-VSS. These PAP levels may be considered to be exceptional and well within the range supporting economic viability for the PHA recovery from biomass.

Fermented sludge centrate promoted more rapid accumulation kinetics in the Bath activated sludge (3 to 5 times faster) in comparison to the accumulation assessments using the synthetic VFA media (Figure 5). At the same time, the PAP of the biomass in these trials was lower than with the synthetic media (30 – 40 % g-PHA/g-VSS). The lower observed PAP were in part attributed to the presence of nutrients. However, a lower PAP may nevertheless be accompanied by a greater absolute production of PHA in the accumulation process due to the potential to engineer a process of combined biomass growth with PHA accumulation (Valentino et al., 2015; Werker et al., 2014b).

Presence of nutrients in these streams of available VFA requires consideration in the overall mass balance of the residuals management. To this end we evaluated the influence of removal of phosphorus by chemical precipitation in advance of the accumulation process. We found that a consistently greater PAP was obtained by the biomass if we pre-treated the fermented sludge to reduce phosphorus content of the centrate. In these tests, a PAP between 40 and 50 % g-PHA/g-VSS with the surplus Bath activate sludge was obtained (Figure 5). More consistent results have been experienced with phosphorus limitation, but phosphorus supply is nevertheless important to the biomass response in PHA accumulation.

Biomass with a PHA content of at least 40 % g-PHA/g-VSS is considered to be a commercially viable resource from the perspective of a PHA recovery process. Thus, the potential for PHA production with the biomass from Bath WWTP is significant even if we understand opportunities for further improvement. These findings are practical full-scale proof-of-concept of what has been shown to be feasible as part of well-controlled laboratory investigations (Anterrieu et al., 2014; Bengtsson et al., 2012). A biomass selection strategy of anoxic-feast with aerobic-famine, representing conditions that may be readily created in the normal course of biological nitrogen removal, may be tuned to generate a surplus biomass with significant PAP.

We also know from recent in-house R&D developments, that the PAP of a biomass produced on municipal wastewater for PHA production can be made to be more robust and in excess of 50 % g-PHA/g-VSS by selective influence on the biomass history created during periods of feast and famine in the wastewater treatment (Werker et al., 2014a). Therefore, we believe that while the biomass from some wastewater treatment plants such as Bath WWTP can already be used for industrial demonstration of PHA production, further simple and already known optimisation steps for PHA production are within a short reach to implement.

Bath WWTP receives an influent flow of 112 207 m^3/day and its activated sludge process removes on average 29 tons-COD/day (2012 figures). These numbers suggest the ability to produce and harvest in the order of 7 tons-VSS/d of functional active biomass, which indicates that the plant could be a source of supply at full scale of between 1 700 and 2 500 tons-PHA per year. Such a level is sufficient to influence regional economic developments in the economy of a PHA-recovery biorefinery, and to stimulate significant local growth in biobased networks providing products and services built on renewable resources.

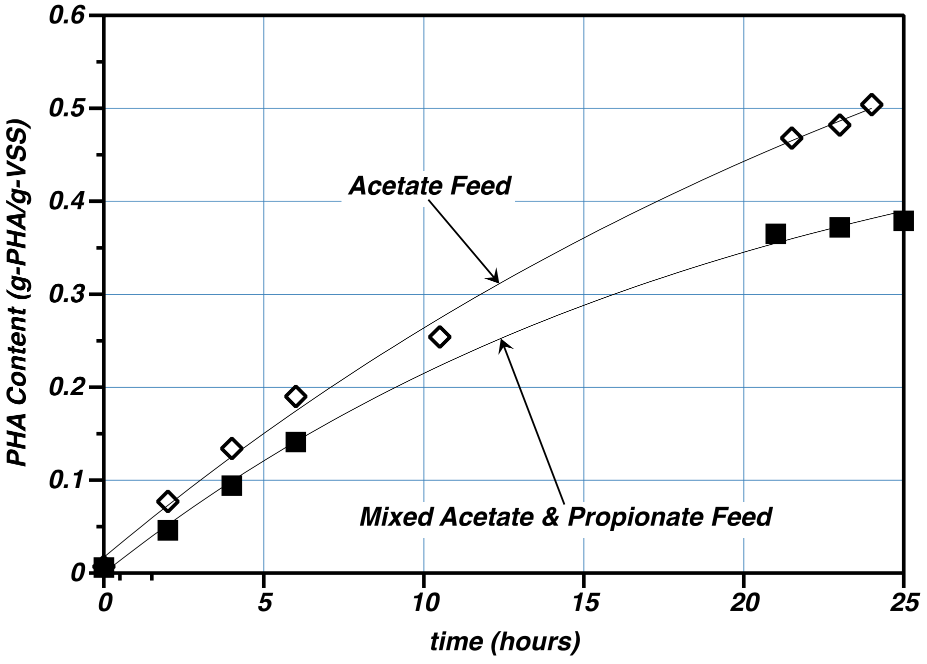


Figure 4. Summarized pilot scale PHA accumulation results with RWZI Bath biomass for PAP assessments using synthetic media for VFA feedstock.

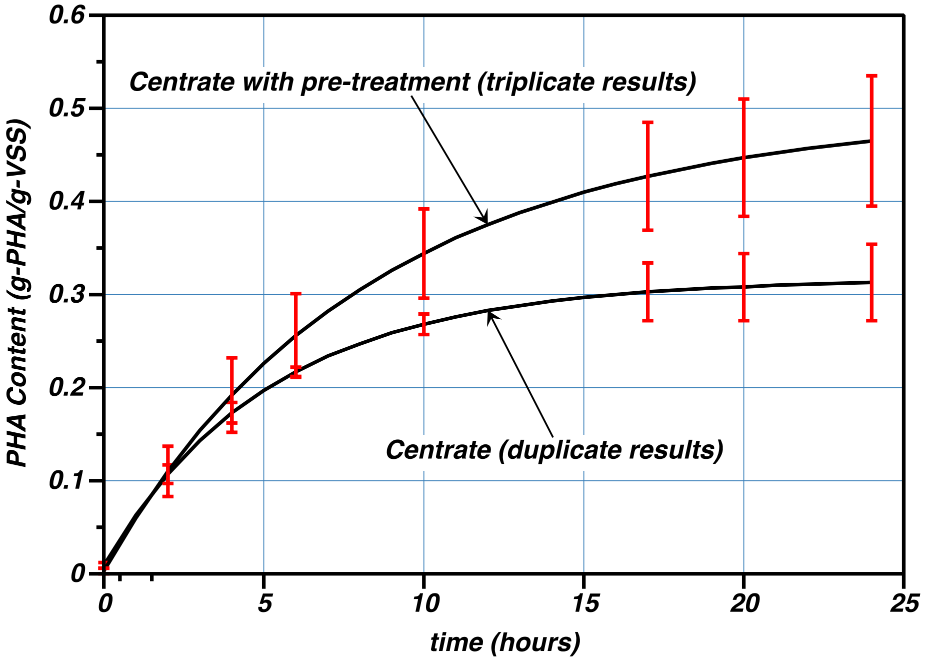


Figure 5. Summarized pilot scale PHA accumulation results with RWZI Bath biomass for replicate PAP assessments using fermented sludge centrate for VFA feedstock, with and without pre-treatment for reducing feedstock phosphorus content.

3. PHA accumulation potential in biomass from Marselisborg Renseanlæg and Billund BioRefinery

As part of this BIOPOL investigation, activated sludge samples from two Danish WWTPs, namely Marselisborg Renseanlæg (MR) and Billund BioRefinery (BBR) were assessed for PHA accumulation potential (PAP) to provide the status based on the current conditions of operation. The goal was to use these results as a foundation in consideration for making recommendations for the steps and strategies for the integration of Cella™ Technologies with these and/or other similar plants.

3.1 Materials and methods

Activated sludge was collected (2015-08-31) from the aerated reactors of the Biodenitro™ processes at the municipal WWTP Marselisborg Renseanlæg (Aarhus, Denmark) and at Billund BioRefinery (Grindsted, Denmark). During the period of the sampling, only one of the two parallel Biodenitro™ treatment lanes at Billund BioRefinery was in operation and the volumetric loading rates were thereby twice the normal ones at this plant. The sludge was stored refrigerated overnight (6°C) and tested for PHA accumulation potential the following day.

3.1.1. PHA accumulation potential

The activated sludge samples (1.6 L) were incubated in a PLC controlled fed-batch feed-on-demand reactor system. The temperature of the mantled 2-L reactors was controlled at 24°C (± 1°C). Mixing was provided with magnetic stirrer and the reactors were aerated through a glass membrane diffuser. Disolved oxygen (DO) as well as pH were monitored continuously. A substrate of acetic acid (100 g/L) with NH_4Cl and KH_2PO_4 (COD:N:P = 100:1:0.05 on mass basis) was dosed in pulses based on a well-established respiration feed-on-demand control (Werker et al., 2011a) with feed pulses generating about 100-115 mg-COD/L peak concentration. The trends in DO signal were used to indicate depletion of substrate which triggered the feed pump to add timed doses of substrate. pH of the substrate was regulated to 5.3 by addition of NaOH. Allylthiourea (10 mg/L) was added to both activated sludge samples in order to inhibit nitrification for the laboratory based evaluation (note that we do not add allylthiourea in pilot scale work).

An acclimation of the sludge to the substrate was conducted according to a patent pending method before each accumulation test in order to establish the same biomass history prior to accumulation conditions. The substrate for acclimation was the same as for accumulation. The pre-acclimation consisted of a feast period with an initial reactor substrate concentration of 50 mg COD/L followed of a three times longer famine period. This procedure was repeated three times and lasted, in total, for 2 h. The DO signal was used to monitor the feast and the famine periods. The accumulation tests were started immediately after the acclimation phase.

The PHA accumulation tests were for 2 days and grab samples were taken taken at selected time points to monitor biomass PHA content and water quality trends (COD, VFA and ammonium) during the accumulation.

3.1.2. Analytical methods

Soluble COD (SCOD), soluble total nitrogen (SN), soluble total phosphorus (SP) and ammonium were analyzed on filtered (1.6 µm) samples with HachLange spectrophotometric tests (LCK 114, 138, 350 and 303). Total and volatile suspended solid (TSS and VSS) were analysed at the start and end of the tests according to standard methods (APHA, 1998). The method for VFA analysis is detailed in Section 4.1.

The biomass samples for PHA content analyses were stabilized by addition of sulfuric acid to pH 2 and centrifuged at 3500×g for 5 min. The supernatant was decanted and the pellet was resuspended and washed with deionized water and centrifuged again before the supernatant was withdrawn and the pellet dried overnight at 90°C.

The dried biomass samples were analyzed for PHA content following a previously reported method (Werker et al., 2012) based on thermogravimetric analysis (TGA) using a TGA Q500 (TA instruments). A mass of 2-5 mg of powder sample was loaded into the instrument and analyzed using the following program: (1) N_2 atmosphere, (2) Ramp 10°C/min to 100°C, (3) Isothermal for 10 minutes, (4) Ramp 10°C/min to 550°C, (5) Isothermal for 2 minutes, (6) Change atmosphere to synthetic air and (7) Isothermal for 30 minutes.

The polymer content was measured from the integration of the rate of weight loss with respect to temperature (dW/dT) in the range 250°C to 310°C.

3.2 PAP assessment outcomes

The response from the biomass samples to the feast and famine cycles during the acclimation phase was as expected. The respirometric trends indicated storage during substrate presence (feast) followed by substrate absence (famine). The specific substrate uptake rates were similar for the two biomasses at 147 and 169 mg-COD/g-VSS/h for MR and BBR, respectively. There was no significant difference in the respiration response to the three consecutive pulses in either of the tests (Figure 6).

The phase of biomass acclimation to the substrate was followed by the PHA accumulations which lasted about 2 days. The respirometric profiles during accumulation were typical for a feed-on-demand strategy (Figure 7). Furthermore, the DO trends indicated stable performance of the assessments with no interruption of the feeding trends in any of the accumulations. The offline measurements of COD and VFA confirmed the stable and controlled operation during the accumulations since there was no build-up of VFA and only a minor build-up of other COD in both tests. Such minor build-up of COD (400 mg COD/L) is normal and was interpreted to be due to release of soluble biomass products and/or compounds related to cell lysis. The relatively low DO level in the test with MR biomass during the first 20 h (0.5-1.0 mg O_2/L) could have influenced rate PHA production due to oxygen limitation. Nevertheless, the final PHA content measured in the biomass is considered to have been representative of biomass saturation due to the asymptotic trends for the exaggerated accumulation time applied. The DO concentration in the test with BBR biomass was maintained above 2 mg/L throughout the accumulation.

The PHA content in the biomass from MR increased progressively to finally reach 26 % g-PHA/g-VSS after 50 h. Although the PHA content increased until the end of the test, the incremental increase in PHA content was only 4 % g-PHA/g-VSS during the last 30 h of the accumulation test. Thus, 26 % was considered to be the maximum PHA extant accumulation potential for this biomass (Figure 8). The initial PHA yield was 0.45 g-PHA-COD/g-COD which is within the range typically observed. The yield decreased over the course of the accumulation which is also usually observed (Figure 9). The nominal yield over 50 h of accumulation was 0.19 g PHA-COD/g COD.

The PHA content in the accumulation with biomass from BBR reached an estimated maximum after 21 h of accumulation with 18 % g-PHA/g-VSS. The decrease in biomass PHA content towards the end of the test was due to an onset consumption of the stored PHA. The reactor concentration of PHA decreased from 1460 mg after 21 h to 1130 after 50.5 h (a decrease by 23 %). The initial PHA storage rate was higher in the biomass from BBR biomass compared to the biomass from MR (Figure 8). The initial PHA yield was the same for both biomasses and the PHA yield reached a maximum value after 4.5 h in the BBR biomass with a yield of 0.58 g PHA COD/g COD which was significant higher than the PHA yield in the MR sludge (Figure 9). The fact that the maximum yield was not observed from the start suggests that further acclimation may benefit this particular biomass. At the end of the accumulation with BBR biomass, the PHA yield decreased and this value was also influenced by the PHA consumption observed.

The observed PAP with the biomass from Marselisborg Renseanlæg and Billund BioRefinery are typical of activated sludge where there is little to no applied selection pressure for the PHA storing phenotype from the bioprocess. Thus the bioprocess needs attention towards engineering environments to specifically promote enrichment. Based on principles that are applied by AnoxKaldnes at pilot-scale and that also exist at Bath WWTP, sufficient and significantly high PAP could be readily achieved in biological treatment of conventional domestic wastewater. Strategies for engineering optimization of the processes of Marselisborg and BBR in order to enhance the PAP are discussed in Section 5 of this report.

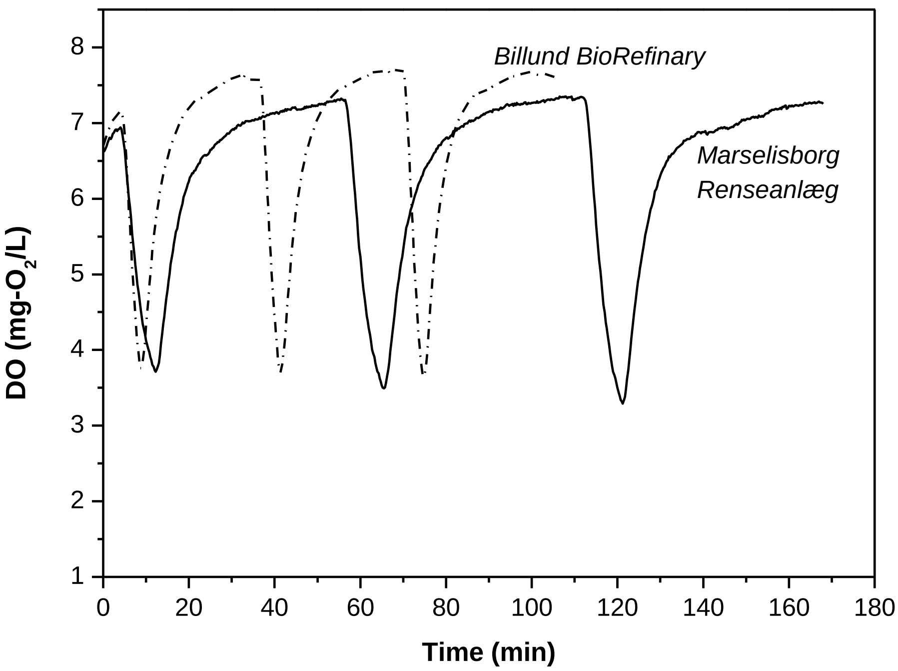


Figure 6. DO concentrations under the pre-acclimation phase for the biomasses from Marselisborg Renseanlæg and Billund BioRefinery.

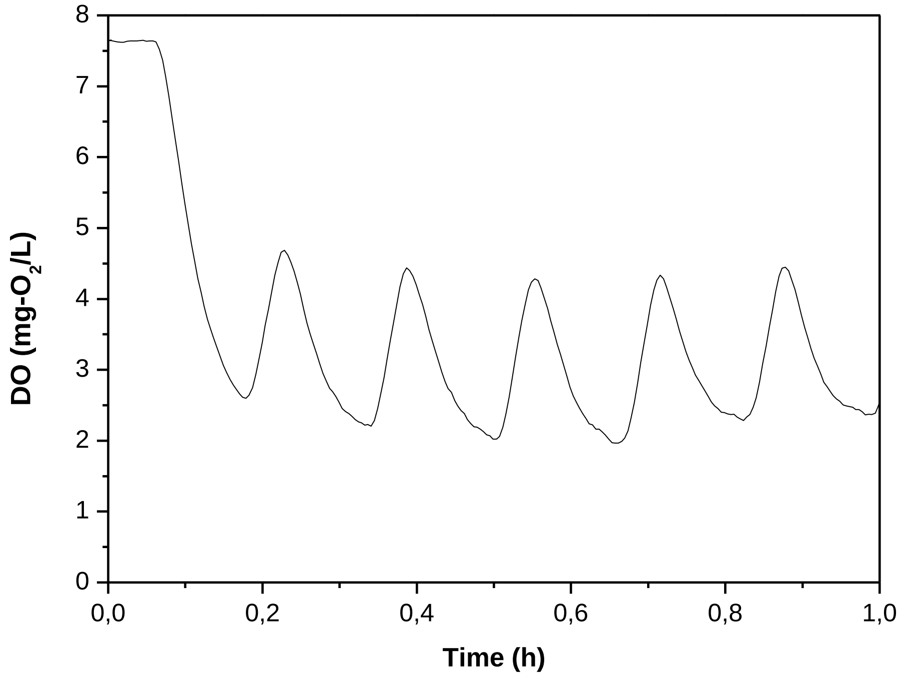
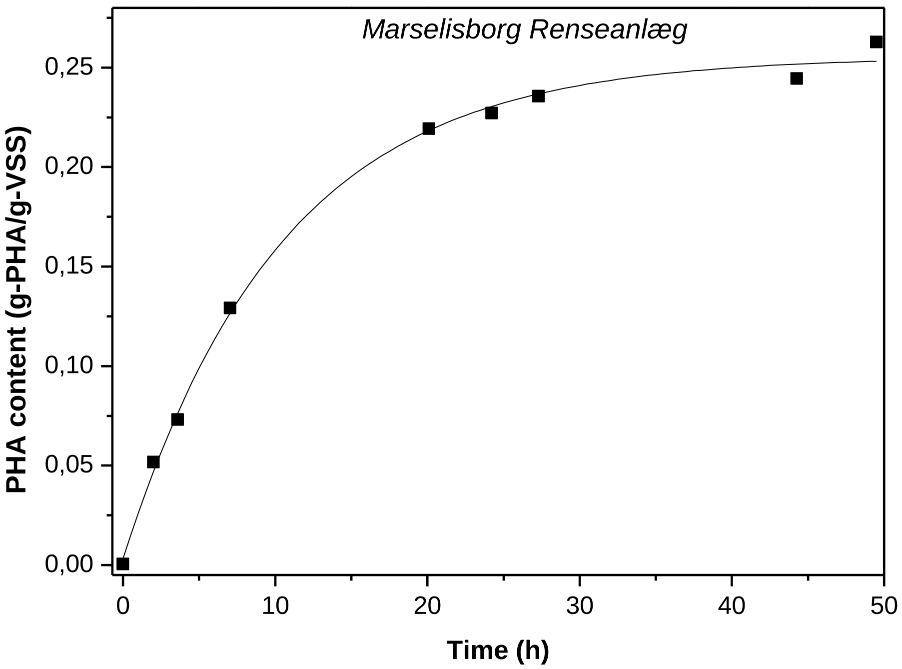


Figure 7. Typical DO concentration curve during the accumulation phases.



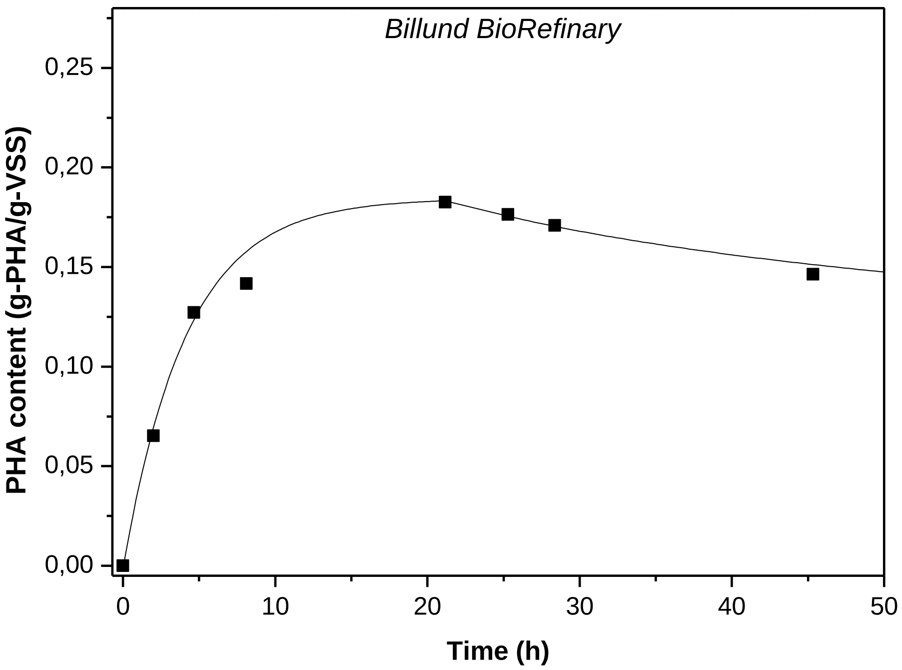


Figure 8. Biomass PHA content during the accumulation test with activated sludge from Marselisborg Renseanlæg and Billund BioRefinary.

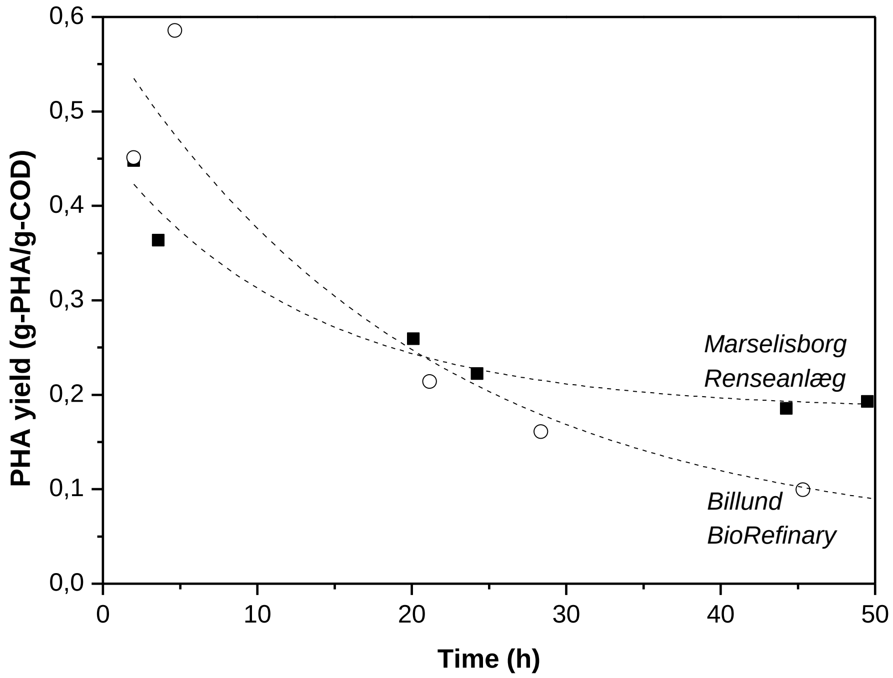


Figure 9. PHA yield during the accumulation tests with activated sludge from Marselisborg and BBR.

4. Feedstock for PHA production at BBR

Production of PHA in wastewater treatment requires a feedstock of VFAs of defined quality and quantity. To this end, the two following organic streams that are available at BBR were assessed as potential future VFA-rich feedstocks in a PHA value chain:

1. The mixture of household waste and surplus sludge after pulping, and
2. The mixture of household waste, surplus sludge and industrial waste after hygienization.

4.1 Materials and methods

Two grab samples were taken from the feed to the anaerobic digester at BBR. One grab sample was taken from the pulper for municipal household waste and surplus sludge. The second sample was taken after hygienization of the mixture of pulped household waste + excess activated sludge and industrial waste.

Samples for VFA were microfiltered (0.45 µm) and analyzed with gas chromatography (GC; Perkin-Elmer). The GC was equipped with a split injector (split 1:20) and flame ionisation detector. The GC had an ELIT FFAP column (30 m, 0.32 mm, 0.25 µm) with helium as carrier gas. The samples were acidified by addition of formic acid. The injector and detector temperatures were 220°C and 250°C, respectively. The column temperature was programmed to be initially 85°C for 0.5 min and then the column temperature was ramped at 25°C/min to 105°C, followed by a second ramp at 7°C/min until 200°C, and then finally by 20°C/min to 240°C and held at 240°C for 1 minute.

Analyses of SCOD, SN, SP and ammonium were conducted on filtered (1.6 µm) samples with HachLange spectrophotometric tests (LCK 114, 138, 350 and 303).

4.2 Feedstock characterization outcomes

The samples taken from the feed to the anaerobic digester were analyzed and assessed as potential feedstocks for PHA accumulation. Focus was on the dissolved fractions of the streams since those are the most relevant for PHA production. The pulper stream had an SCOD concentration of 38 g-COD/L and the mixed waste after hygienization had an SCOD concentration of 23 g-COD/L (Table 1). The fraction of VFA over total soluble COD was 0.28 g-COD/g-COD in the pulper stream and 0.41 g-COD/g-COD in the hygienization stream.

Besides the VFAs, there were also significant concentrations of ethanol in both of the samples, namely 4.6 g-COD/L and 2.7 g-COD/L in the pulper and the hygienization streams, respectively (Table 1). The fractions of total fermentation products (sum of VFAs and ethanol) were 0.40 g-COD/g-COD and 0.52 g-COD/g-COD in the pulper and the hygienization streams, respectively. Ethanol is a fermentation product that occurs from the same RBCOD fraction as the VFAs. Therefore, selective conversion of RBCOD into VFAs rather than ethanol may be possible through optimization of the waste handling and processing.

The concentrations of nutrients were relatively high in both samples resulting in VFA:N:P ratios of 100:15:1.8 for the pulper sample and 100:8.9:2.6 (g-COD:g-N:g-P) in the hygienization sample. Such ratios mean that nutrients are in excess for biomass growth considering only VFAs as carbon source. If the total SCOD was considered as carbon source, the nutrients levels were balanced for biomass growth with a SCOD:N:P ratio in the pulper sample of 100:4.3:0.5 and 100:3.6:1.1 (g-COD:g-N:g-P) in the hygienization sample.

Even though these streams were not fermented, relatively high concentrations of VFAs around 10 gCOD/L were measured. Although such levels mean that it could already be possible to use both streams as substrate for PHA production in a PE3, the accumulation efficiency and overall mass balance would benefit significantly if the streams were fermented to increase the fraction of VFA over COD. Given the nature and origin of these two waste streams, it is expected, based on AnoxKaldnes previous experience (Bengtsson et al., 2008b; Morgan-Sagastume et al., 2015, 2011) that a higher fraction of VFAs is readily obtainable through acidogenic fermentation. In so doing, VFA-rich streams well suitable for PHA accumulation would be established.

The levels of nutrients in the samples mean that biomass growth would occur concurrent with the PHA accumulation. Simultaneous growth and storage can be an opportunity for increased PHA productivities (Valentino et al., 2015; Werker et al., 2014b) and phosphorus levels in the feedstock can be tuned to optimal levels by standard methods of chemical precipitation.

Table 1. Characteristics of the samples from the digester feedstock in BBR.

Parameter		**Pulper** Household waste	**Hygienization** Mixed sludge+ household waste
SCOD	mg-COD/L	38 100	23 170
SN	mg-N/L	1 620	840
NH_4	mg-N/L	760	380
SP	mg-P/L	190	250
PO_4	mg-P/L	170	230
Ethanol	mg-COD/L	4 660	2 710
Acetate	mg-COD/L	9 350	1 610
Propioniate	mg-COD/L	370	1 310
Iso-Buturate	mg-COD/L	20	150
Bututurate	mg-COD/L	640	3 340
Iso-Valerate	mg-COD/L	50	280
Valerate	mg-COD/L	120	2 330
Capronate	mg-COD/L	50	420
Sum VFA	mg-COD/L	10 600	9 430
VFA/SCOD	g-COD/g-COD	0.28	0.41
(VFA+EtOH)/SCOD	g-COD/g-COD	0.40	0.52
COD:N:P	g-COD:g-N:g-P	100:4.3:0.5	100:3.6:1.1
VFA:N:P	g-COD:g-N:g-P	100:15:1.8	100:8.9:2.6

5. PHA production at Marselisborg Renseanlæg and Billund BioRefinery

The PHA accumulation potential of the activated sludge from Marselisborg Renseanlæg and Billund BioRefinery can be considered to be within the typical range for municipal activated sludge. In the case of Marselisborg, the PAP level was nevertheless at the upper end of what we experience to be a typical background levels. A typical background level is a level of PAP where there does not exist a well-defined condition that would create for selection pressure. A well-defined selection pressure is generally required to produce a surplus biomass with enrichment in PAP.

The levels of PAP were determined using a reference assessment procedure that may not reflect a fully optimized potential for PHA accumulation. For example, the results suggested that more than 3 acclimation pulses could have resulted in some improvement of the observed PAP. Nevertheless, the outcomes clearly indicate that these two biomass sources would require some attention to the bioprocess should it be of interest to produce a biomass with PAP exceeding 40 % g-PHA/g-VSS. Above a 40% PAP we estimate that the biomass becomes a suitable raw material for regional economically viable PHA based bioplastic value chains.

But based on our knowledge and experience, only minor process modifications could be applied to improve the PAP in the existing plants to meet the requirements for a PHA producing biomass. At Leeuwarden WWTP, the municipal wastewater was treated in our Cella™ Technologies pilot system while generating a biomass with PAP of 40-50 % g-PHA/g-VSS. As a reference for comparison, the biomass from Leeuwarden's full-scale WWTP treating the same influent wastewater and without well-defined selection environments exhibited a PAP of only 13 % g-PHA/g-VSS. This WWTP has several lanes of carrousel reactors operated two-and-two for pre-denitrification and nitrification. In principle, this is in some ways analogous to the Biodenitro™ process.

There are several established factors that will contribute to the enrichment for PAP in biomass treating municipal wastewater (Section 2.1). For the Biodenitro™ processes at MR and BBR, one effective way to promote PAP in the biomass would be to create zones where the biomass is exposed to more well-defined 'feast' conditions. Such zones can be engineered within or external to the existing process volumes. Under the present operating conditions feast conditions are not achieved because when the influent enters the denitrification reactor under intense mixing, the incoming RBCOD is anticipated to quickly become diluted. 'Feast' conditions are RBCOD concentration dependent and a contact zone with elevated concentrations of RBCOD (initially in pre-denitrification) has been shown to be one effective strategy.

Biomass is stimulated to 'feast' by relatively high concentrations of RBCOD and therefore, the return activated sludge and influent could, for example, be mixed in the 'feast' zone under a relatively short "contact" time. In the Biodenitro™ process, 'feast' conditions may be created either in a separate zone within the carrousel reactors, or in a separate volume up-stream of the main reactor volume. A separate up-stream volume may be associated with the already existing influent distributor.

An anoxic feast zone is anticipated to be the most preferable for the Biodenitro™ process. An anoxic feast zone needs requires a balance of RBCOD and nitrate supply. Such considerations are possible to evaluate based on simulations with well-established activated sludge models.

Other methods and strategies to augment anoxic feast and promote enhanced PAP are also possible to consider. However, a more thorough evaluation needs to be performed for the specific WWTP locations where there are interests of stakeholders to establish a biomass supply for PHA production. In order to make a preliminary investigation to validate the interplay of enrichment strategy and treatment performance, a desk top modelling evaluation is a recommended first step. Pending the outcome of the desk top study, a plan of practical testing and eventual process implementation may be defined.

Estimations were made to assess the quantity of PHA that potentially could be produced from these sources of biomass given a suitable VFA feedstock. The calculations were made based on the assumption that the enrichment of PAP could be improved such that the harvested excess biomass was able to accumulate up to 40 % g-PHA/g-VSS. It was estimated that MR (220 000 PE) could be a source of biomass for the production of in the order of 1 000 tons-PHA/year and that BBR (50 000 PE) could be a source of biomass for around 200 tons-PHA/year.

The feedstocks to the anaerobic digester at BBR have potential to become substrates for PHA production. The levels of VFAs in these feedstocks need to be increased. To this end the controlled evaluation of fermentation of these organic residual sources is a recommended first step. It was estimated that if the fraction of VFAs could be increased up to 80-90 % of the COD by fermentation, in the order of 150 - 250 tons-PHA per year could be produced from these sources given an available harvested biomass.

6. Conclusions

Enrichment and production of biomass with PHA accumulation potential can be achieved under simultaneous biological treatment of municipal wastewater for carbon and nitrogen removal. This potential has been well-documented from investigations carried out at laboratory, pilot and full-scale. Examples in summary have been provided as part of this report.

The PHA accumulation potential (PAP) in activated sludge from Marselisborg Renseanlæg and Billund BioRefinary was shown to be 26 and 18 % g-PHA/g-VSS, respectively. Such levels were expected and are typical of an activated sludge bioprocess where specific attention has not been made to establish conditions to promote selective enrichment of populations of species of PHA storing bacteria.

Environments within the bioprocess of biological nitrogen removal can be readily created to reach PAP levels in excess of 40 % (g-PHA/g-VSS). At these enhanced PAP levels we find that a municipal WWTP can become a raw material supplier and facilitate local biobased networks in regional PHA based bioplastic value chains. Given such improvements in PHA accumulation potential, Marselisborg Renseanlæg and Billund BioRefinary could be sources of biomass for the production of in the order of 1 000 and 200 tons-PHA per year, respectively.

The most suitable strategy is case specific but a common approach to include with both Marselisborg Renseanlæg and Billund BioRefinary would be to engineer a pre-dentrification contact zone that stimulates periodic ‘feast’ conditions for the biomass. Conditions of anoxic feast require a contact zone of biomass together with adequate concentrations of RBCOD and nitrate. As a first step it is recommended to evaluate the implementation of a prospective feast selection strategy by simulations with well-established activated sludge models.

Two digester streams at Billund BioRefinary were evaluated for potential as future feedstocks for PHA production. One of the streams was the mixture of household waste and surplus sludge after pulping. The other stream was the mixture of household waste, surplus sludge and industrial waste after hygienization. These two streams were both found to have relatively high concentrations of soluble COD, 38 and 23 g-COD/L, respectively, of which 28 % and 41 %, respectively, were in form of VFAs. The soluble fractions from both streams have potential to become feedstocks for PHA production. In order to become such feedstocks, improvements to the VFA concentration is recommended which is likely to be achieved through acidogenic fermentation.

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BIOPOL – Udvinding af biopolymer fra spildevand

Hovedformålet med projektet "BIOPOL - Udvinding af biopolymer fra spildevand" har været at undersøge, udvikle og evaluere potentialet for produktion af biopolymerer til bioplastproduktion fra industri- og byspildevand. Dette er blevet realiseret via laboratorieeksperimenter og pilotforsøg, hvorigennem de teknologiske muligheder for fuldskala bioplastanlæg er blevet afdækket.

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